REGULATION OF PROSTAGLANDIN $F_{2\alpha}$ BIOSYNTHESIS BY LONG CHAIN FATTY ACIDS IN CATTLE

By

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ABBREVIATION KEY

AA	Arachidonic acid
Ang	Angiotensin
ANPT	Angiopoietin
BEND cells	Bovine endometrial cells
BHBA	Beta hydroxybutyric acid
CAT-1	Carnitine acyl transferase
CL	*Corpus luteum*
CLA	Conjugated linoleic acid
DHA	Docosahexaenoic acid
E_2	Estrogen
EC	Endothelial cells
EPA	Eicosapentaenoic acid
ER	Estrogen receptor
ET-1	Endothelin-1
FGF	Fibroblast growth factor
FSH	Follicle-stimulating hormone
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
IGF	Insulin-like growth factor
IFN-γ	Interferon gamma

IFN-τ	Interferon tau
IL-1	Interleukin
JAK/STAT	Janus kinase signal transducer and activator of transcription
LA	Linoleic acid
LCFA	Long-chain fatty acid
LH	Luteinizing hormone
LNA	Linolenic acid
MUFA	Monounsaturated fatty acid
NEB	Negative energy balance
NEFA	Non-esterified fatty acid
OT	Oxytocin
OTR	Oxytocin receptor
P_4	Progesterone
PDBu	Phorbol-12,13-dibutyrate
PG	Prostaglandin
PGG	Prostaglandin G
PGE_2	Prostaglandin E_2
PGES	Prostaglandin E synthase
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGFS	Prostaglandin F synthase
PGFM	$PGF_{2\alpha}$ metabolite (13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$)

PGH_2	Prostaglandin H_2
PGHS	Prostaglandin endoperoxide synthase
PGI_2	Prostaglandin I_2 (Prostacyclin)
PKC	Protein kinase C
$cPLA_2$	Cytosolic phospholipase A_2
PLC	Phospholipase C
PPARs	Peroxisome proliferators-activated receptors
PPRE	PPAR response element
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TAG	Triacylglycerol
TNF-α	Tumor necrosis factor α
VEGF	Vascular endothelial growth factor

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A series of *in vitro* experiments and an *in vivo* experiment were conducted to examine the effects of long-chain fatty acids (LCFA) on $PGF_{2\alpha}$ biosynthesis in cattle. Treatment of bovine endometrial (BEND) cells with phorbol 13, 14-dibutyrate (PDBu) resulted in induction of $PGF_{2\alpha}$ secretion. The PDBu-induced $PGF_{2\alpha}$ secretion coincided with increased PGHS-2 mRNA and protein expression. There was no evidence for PDBu modulation of PPARδ mRNA or protein synthesis in cultured BEND cells.

Priming of BEND cells with ST, LNA, and EPA reduced $PGF_{2\alpha}$ response to PDBu by 17%, 14%, and 66%, respectively. Both saturated and unsaturated fatty acids had no detectable effects on PGHS-2, PGES or PPARδ mRNA response to PDBu. Similarly, supplementation of BEND cells with *cis*-9, *trans*-11 or *trans*-10, *cis*-12 CLA isomers greatly decreased $PGF_{2\alpha}$ response to PDBu. Co-incubation with both CLA isomers increased PGHS-2 and PPARδ mRNA abundance in PDBu-stimulated BEND cells, suggesting that these fatty acids alter $PGF_{2\alpha}$ production through a mechanism that does

not require repression of PGHS-2 or PPARδ gene expression. Priming of BEND cells with *cis*-9, *trans*-9, *cis*-11, and *trans*-11 isomers of octadecenoic acid further enhanced $PGF_{2\alpha}$ and PGHS-2 mRNA response to PDBu. Pre-incubation of BEND cells with *cis* and *trans* monounsaturated fatty acids decreased PGES mRNA response to PDBu. None of the fatty acids studied altered PPARδ mRNA or protein levels.

Feeding *trans* fatty acids to primiparous or multiparous Holstein cows did not induce any significant alterations in production or metabolic responses when compared to supplementation with saturated fatty acids. Dietary supplementation of cows with *t*FA significantly increased plasma PGFM concentration within the first week of lactation. Whether this augmentation in $PGF_{2\alpha}$ production results in improved fertility and immune competency warrants further research.

CHAPTER 1
INTRODUCTION

In recent years, the effect of nutrition on reproduction has generated much attention and has become increasingly important in the dairy industry. In the past decade, genetic selection for high milk production has been associated to a decrease in reproductive efficiency in lactating dairy cows (Butler, 2000). Poor reproductive efficiency includes early embryonic loss (Thatcher et al., 1995), impaired ovarian cyclicity and low fertility rates (Butler, 2000), which collectively result in reduced milk production (Plaizier et al., 1997). Early lactating dairy cows have higher energy requirements than can be supported by dietary energy intake, which creates a negative energy state and leads to impaired reproductive function (Butler, 2000).

To discuss the effects of nutrition on reproduction during early pregnancy, it is very important to understand the changes brought about by lactation. The most critical period of lactation is considered to be the transition period, which extends from 3 weeks before calving to 3 weeks after parturition. During this period, dramatic metabolic changes (homeorhesis) take place in order to support lactation. Parturition and the onset of lactation cause an abrupt shift in nutritional requirements. Essentially all of the energy that is consumed by the lactating animal is used by mammary tissue for milk production, leaving an insignificant amount of energy to be distributed for other physiological processes (Bell, 1995). Hence, the lactating animal experiences a state of negative energy balance (NEB). This NEB represents a state of undernutrition, which results in massive mobilization of fat from adipose tissue, increasing plasma levels of non-

esterified fatty acids (NEFA). This massive fat mobilization, in combination with reduced energy intake (dry matter intake), results in loss of body condition of lactating animals. Extensive data have shown that the aforementioned conditions (NEB and loss of body condition) invariably affect reproductive efficiency in lactating dairy cows.

The mechanisms by which the periparturient metabolic upsets result in reduced reproductive efficiency are not well understood. However, available evidence indicates that reproductive efficiency is dependent on the growth and development of a viable oocyte that can then be fertilized. This is directly dependent on a normal estrous cycle characterized by folliculogenesis, ovulation, corpus luteum (CL) formation and regression (~21 days). It has been shown that with inappropriate nutrition, ovarian cyclicity may cease. The estrous cycle consists of two major phases: the follicular phase (formation of preovulatory follicles) and luteal phase (CL formation and regression). These two phases are controlled through the action of gonadotropins (FSH and LH), which in turn are regulated by the action of hypothalamic gonadotropin releasing hormone (GnRH) on the pituitary. Therefore, nutrition can affect reproductive efficiency through modulation of the hypothalamic-pituitary axis and/or direct effects at the ovarian level.

The hypothalamic-pituitary axis is essential in the modulation of gonadotropins secretion, which in turn plays a pivotal role in control of the estrous cycle. Even though FSH is critical for follicular growth, LH pulsatility is responsible for normal ovarian activity since it is critical for ovulation. However, results from numerous studies have been inconsistent establishing the relationship between nutritional status and secretion of gonadotropins.

Metabolic upsets, like NEB, have been shown to change the profile of metabolic hormones such as growth hormone (GH), insulin, and insulin-like growth factor I (IGF-I), all of which play an important role in control of follicular development in cattle. Therefore, it is likely that changes in any of these factors due to NEB could alter the pattern of ovarian follicular growth and development during early postpartum period resulting in reduced reproductive efficiency. Several studies have shown that animals in a less severe NEB state have increased levels of plasma insulin and IGF-I, which coincide with a gonadotropin-independent enhancement of follicular growth. These hormones can also influence steroidogenic activity of growing follicles, which is critical for recruitment, selection, dominance and ovulation.

Long-chain fatty acids (LCFA) are generally added to dairy rations to increase the energy density of the diet. It is expected that supplementation of the diet with fatty acids may enhance reproductive efficiency by enhancing the energy status of the dairy cow. However, recent studies indicate that dietary fatty acids may affect reproductive efficiency in farm animals through an energy-independent mechanism. One way that fatty acids could enhance reproductive efficiency would be through regulation of prostaglandin biosynthesis. Prostaglandin production can be influenced by nutrition since the precursor for the biologically active prostaglandin of the two series is arachidonic acid (AA), an n-6 fatty acid synthesized from elongation/desaturation of linoleic acid (LA). Prostaglandins (PG) of the 2 series ($PGF_{2\alpha}$, PGE_2) have been implicated in the process of reproduction, including ovulation, follicular development, and corpus luteum functions. Hence, any effects of fatty acids on $PGF_{2\alpha}$ synthesis are likely to affect overall reproductive performance.

In summary, it is well established that the energy state of the cow during early lactation can influence reproductive efficiency by modulating the estrous cycle. This could be through the neuroendocrine modulation of gonadotropin secretion, specifically LH pulsatility (hypothalamic-pituitary axis), although more consistent evidence is needed. Alternatively, local ovarian effects could take place through modulation of follicular growth and development by metabolic hormones (IGF-I and insulin), which could determine oocyte quality and viability and steroidogenic activity of the CL. However, energy-independent effects may also be observed after fatty acid supplementation, which could involve prostaglandin biosynthesis. Therefore, nutrition can influence reproductive efficiency in dairy cows not only by altering the energy status of the animal but also by influencing factors involved in the regulation of reproductive processes like follicular dynamics, ovulation, CL function and embryo survival among others.

The goal of this research project was to examine the physiological effects of supplemental fatty acids on endometrial $PGF_{2\alpha}$ production in cattle. This dissertation begins with a brief overview of the physiology of reproduction and how dietary fatty acids affect energy balance and reproductive processes in cattle (Chapter 2). Experiments described in Chapters 3 and 4 were designed to elucidate endometrial $PGF_{2\alpha}$ responses to omega-6 and omega-3 fatty acids (Chapter 3) and to *cis* and *trans* conjugated isomers of linoleic acid (Chapter 4). Experiments described in Chapters 5 and 6 evaluated the effects of *trans* fatty acids on bovine endometrial $PGF_{2\alpha}$ production *in vitro* (Chapter 5) and *in vivo* (Chapter 6). The dissertation is concluded with a general discussion of the major findings of this research project (Chapter 7).

CHAPTER 2
LITERATURE REVIEW

Estrous Cycle

The onset of puberty is regarded as the start of the reproductive life of a female, when she enters a period of reproductive cyclicity that continues throughout most of her productive life. This period of reproductive cyclicity is known as the estrous cycle and its importance is stressed by the fact that it provides the female with several opportunities throughout life to be bred and become pregnant in order to perpetuate the existence of her species.

The estrous cycle consists of a series of predictable events, which in general terms are estrus, ovulation, and the beginning of a new cycle with the onset of a subsequent estrus. At the beginning of the estrous cycle, the female enters a state of sexual receptivity, known as estrus, which is followed by mating. Usually, mating takes place prior to ovulation to increase the chances of the newly released oocyte to be fertilized. However, if conception does not occur, another cycle begins, providing the female with another opportunity to reproduce. On the other hand, if conception does occur, the female enters a period in which she will not exhibit regular estrous cycles during pregnancy. This is known as a period of gestational anestrus, which allows for fetal growth and development, and ends after parturition and uterine involution. This and other examples of anestrus or the absence of a regular estrous cycle will be discussed later in this chapter.

The estrous cycle and the events governing reproductive cyclicity are tightly regulated by a series of hormones and factors that will eventually determine normal reproductive efficiency.

Neuro-Endocrine System

All reproductive processes, including the estrous cycle, are under the control of two systems: the central nervous system and the endocrine system. These two systems interact with each other through the hypothalamus, resulting in a neuro-endocrine network responsible for the initiation, coordination and regulation of the functions of the reproductive system (Senger, 1997; Hafez and Hafez, 2000).

The hypothalamus consists of clusters of nerve cell bodies, which are responsible for production of gonadotropin releasing hormone (GnRH), and the paraventricular nucleus, which produces oxytocin (Senger, 1997). Each set of hypothalamic nuclei has different functions and is stimulated under different conditions. However, the communication between the hypothalamus and the pituitary is necessary for these hormones to exert their action. This communication between the hypothalamus and the pituitary is referred to as the hypothalamic-pituitary axis.

The pituitary gland is comprised of the posterior and the anterior lobe. The hypothalamus communicates with the posterior lobe through neural connections, and therefore the posterior pituitary is referred to as neurohypophysis. For example, oxytocin is synthesized in neurons in the paraventricular nucleus and it is transported down the axon to the posterior pituitary where it is released into the blood (Senger, 1997). On the other hand, the hypothalamus communicates with the anterior lobe (adenohypophysis) through the hypothalamo-hypophyseal portal system, which prevents hormones synthesized in the hypothalamus from entering and being diluted by the systemic

circulation. Gonadotropin-releasing hormone is synthesized in either the surge or tonic center of the hypothalamus and is released into the primary capillary plexus at the stalk of the pituitary. Blood enters this capillary system from the superior hypophyseal artery and transports GnRH to a secondary capillary plexus in the anterior pituitary where it acts on target cells to release either follicle stimulating hormone (FSH) or luteinizing hormone (LH) (Senger, 1997; Hafez and Hafez, 2000).

Hormones that are secreted by the pituitary are then transported through systemic circulation to the target tissue to elicit the physiological response. This is where the neural and the endocrine systems interact to modulate reproductive functions such as the estrous cycle.

Stages of the Estrous Cycle

The estrous cycle is divided into four stages: proestrus, estrus, metestrus and diestrus. Proestrus begins when circulating concentration of progesterone (P_4) declines as a result of the regression of the corpus luteum from the previous cycle. It lasts from 2 to 5 days, depending on species, and terminates with the onset of estrus. Proestrus is characterized by an endocrine transition from a period of P_4 dominance to a period of estradiol (E_2) dominance under the control of FSH and LH. During this period, recruitment of follicles takes place and the reproductive tract prepares for the next stage, estrus (Senger, 1997).

Estrus is the stage of the estrous cycle during which the female undergoes behavioral as well as endocrine changes characterized by sexual receptivity. These behavioral and physiological changes in the female are induced by E_2, which is the dominant hormone during this stage. Sexual receptivity during estrus increases gradually

until the animal reaches the typical standing estrus or lordosis. In cattle, estrus can extend from 6 to 24 h, with an average of 15 h (Senger, 1997).

Metestrus is characterized by the transition from E_2 dominance to P_4 secretion as a result of the formation of a functional CL. At the beginning of this stage, concentration of both E_2 and P_4 is relatively low. However, the newly ovulated follicle undergoes cellular and structural changes that result in luteinization of the follicle and formation of the CL, which starts producing P_4 soon after ovulation (Senger, 1997).

Diestrus is the longest stage of the estrous cycle, encompassing maximal CL function and sustained P_4 secretion, and ending with the regression of the CL. High P_4 levels prepare the uterus and its microenvironment for early embryo development and implantation. The duration of diestrus (10 to 14 days) is directly related to CL function and P_4 secretion.

Under certain physiological conditions the female does not exhibit regular estrous cycles and is said to be in anestrus. Anestrus, or lack of estrous cyclicity, is the result of insufficient GnRH release from the hypothalamus to stimulate and maintain gonadotropin secretion. This can be caused by pregnancy, lactation, season or stress (Senger, 1997).

Follicular Development during the Estrous Cycle

The follicular phase is the stage of the estrous cycle when preovulatory follicle development is stimulated by increasing concentrations of FSH and LH in plasma. This phase coincides with a marked reduction in plasma P_4, which leads to significant increase in tonic GnRH secretion. Increased basal concentration of GnRH stimulates FSH and LH secretion from the pituitary and promotes follicular development and E_2 production.

In cattle, growth of early antral follicles (~3 mm) is considered to be gonadotropin-independent (Scaramuzzi et al., 1993). The transient rise in FSH at the time of luteal

regression initiates the process of recruitment, which consists of stimulation of a cohort of small antral follicles to grow beyond 4 mm in diameter around days 1-2 of the cycle (Adams et al., 1992; Sunderland et al., 1994; Ginther et al., 1997). Follicle-stimulating hormone stimulates ovarian follicles to acquire key properties associated with E_2 synthesis such as increased cytochrome P450 side-chain cleavage and cytochrome P450 aromatase enzyme activities (Bao and Garverick, 1998; Garverick et al., 2002).

The process of recruitment is then followed by a decline in FSH due to the negative feedback by E_2 and inhibin from the dominant follicle (Ginther et al., 1998; Hunter et al., 2004). This decline in FSH has been identified as an important component of the selection process (Mihm et al., 1997). Follicle selection then results in a decrease in the number of growing follicles and is thought to end with growth divergence or deviation of a dominant follicle from the subordinate follicles (Evans, 2003). During this time, the dominant follicle can use LH for its continued growth, and this is supported by the fact that there is a switch from FSH- to LH-dependency as the follicle matures and FSH concentration declines in cattle (Gong et al., 1996; Webb et al., 2003). Additionally, there are reports of a transient increase in circulating LH (Kulick et al., 1999, 2001) as well as increased expression of LH receptors in granulosa cells (Xu et al., 1995; Goudet et al., 1999; Beg et al, 2001) surrounding deviation of the dominant follicle in cattle. Acquisition of LH receptors in granulosa cells might allow the transient increase in LH to have a functional effect in follicle selection, since LH is known to increase E_2 concentration in follicular fluid, thus facilitating the establishment of dominance (Ginther et al., 2000b). The freshly selected dominant follicle grows to a much larger size (12-20 mm) than all other subordinate follicles, resulting in enhanced E_2 secretion (Ginther et al.,

1997). This enhanced E_2 secretion is associated with increased expression of the genes encoding aromatase, 3β-hydroxy-steroid dehydrogenase and FSH-receptor, as well as the acquisition of LH-receptors in granulosa cells (Ireland and Roche, 1983; Xu et al., 1995; Bao et al., 1997a, b; Evans and Fortune, 1997). High E_2 secretion is also responsible for maintaining low FSH concentrations to prevent growth of another cohort of follicles (Ireland et al., 1984, Ginther et al., 1999, 2000a, b).

The growth of the first wave dominant follicle does not take place for more than 3-4 days, as P_4 secreted from the developing CL inhibits LH pulse frequency and the LH-dependent dominant follicle becomes atretic (Sunderland et al., 1994; Evans et al., 1997). As a result, the first wave dominant follicle loses its ability to produce E_2 between days 7 and 9 of the estrous cycle. The loss of dominance is followed by another transient rise in FSH, resulting in the emergence of a new follicular wave (Sunderland et al., 1994). If the CL fails to regress, the second dominant follicle will undergo atresia and a third wave of follicles emerges. However, if luteolysis occurs during this second wave of follicular development, then the dominant follicle will ovulate (Cooke et al., 1997).

In cattle, most estrous cycles consist of two to three waves of follicular development (Fortune et al., 1988; Savio et al., 1988; Ginther et al., 1989a) that emerge on about days 2 and 11, or days 2, 9 and 11 for animals with two or three follicular waves, respectively (Sirois and Fortune, 1988). Lactating Holstein cows tend to have two waves per cycle (Taylor and Rajamahendran, 1991, Townson et al., 2002), whereas beef and dairy heifers tend to have two or three waves per cycle (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989c). Cattle with two follicular waves tend to have

shorter estrous cycles, ovulate larger and older follicles and to be less fertile than those with three waves (Townson et al., 2002).

Ovulation and Development of the Corpus Luteum

The luteal phase encompasses about 80% of the estrous cycle and covers the period of time that includes ovulation of the dominant preovulatory follicle, CL formation, and regression of the CL.

A landmark event that precedes ovulation is the preovulatory LH surge, which is stimulated by increased concentrations of E_2 in plasma (Senger, 1997). The LH surge triggers a cascade of events that cause biochemical and structural changes in the preovulatory follicle that lead to the rupture of the follicle wall, resulting in the release of the oocyte and subsequent development of the CL (Berisha and Schams, 2005; Senger, 1997). Ovarian blood flow increases at the time of ovulation and subsequent CL formation (Senger, 1997), which emphasizes the importance of angiogenesis during metestrus and diestrus.

Angiogenesis is defined as the generation of new blood vessels through sprouting from already existing ones. This process involves degradation of the capillary vessel membrane, through which endothelial cells (EC) from pre-existing vessels migrate and proliferate to create a new lumen and further vessel maturation (Abulafia and Sherer, 2000). Angiogenesis is critical for final development and differentiation of the preovulatory follicle as an increase in both vascular area and blood flow has been demonstrated during this stage (Acosta et al., 2003). Acosta et al. (2003) showed that there was a marked increase in blood flow and volume in the subsequent CL, which is closely associated with increased plasma P_4 concentrations. Moreover, it has been reported that follicular diameter, E_2 concentration in the follicular fluid, and vascular area

are highly correlated (Mattioli et al., 2001). Thus, ovulation is the result of an interaction between LH surge and local factors that include steroids, prostaglandins and vasoactive peptides (Acosta and Miyamoto, 2004). Luteinizing hormone has been shown to increase ovarian blood flow in rats (Varga et al., 1985), rabbits (Janson, 1975), and sheep (Niswender et al., 1976). Thus, increased vascular dilatation and permeability, together with degradation of collagen layers that provide strength to the follicular wall, are necessary for facilitating follicular rupture (Murdoch et al., 1986; Abisogun et al., 1988) and oocyte release.

Increased ovarian blood flow may increase the availability of gonadotropins, nutrients, hormonal substrates and other blood components that are necessary for ovulation (Acosta and Miyamoto, 2004). Hence, vasoactive peptides released within the follicular wall may modulate local changes in blood flow observed in the ovulatory follicle (Brannstrom et al., 1998; Acosta et al., 2003). This cascade could then mediate LH action to increase prostaglandin production during ovulation. Vasoactive peptides have been shown to play important roles in the ovulation process as well as early luteal development, through modulation of local secretion of prostaglandins and steroid hormones (Acosta et al., 1999, 2000; Kobayashi et al., 2002). Although several promoters of angiogenesis have been identified, vascular endothelial growth factors (VEGF), fibroblast growth factors (FGF), insulin-like growth factors (IGF) and angiopoietin (ANPT) are thought to be the most important factors modulating follicle maturation and CL formation (Berisha and Schams, 2005).

Normal development of the CL and its capacity to produce P_4, growth factors, angiogenic factors, and vasoactive substances is dependent on its vascularization (Acosta

and Miyamoto, 2004). Ovarian blood flow decreases shortly after ovulation, but gradually increases from day 2 to day 5 of the estrous cycle. This period also coincides with marked increases in CL volume and peripheral P_4 concentration (Acosta et al., 2003). Angiogenesis within the CL reaches a peak 2-3 days after ovulation (Reynolds et al., 2000) and appears to be locally modulated by angiotensin (Ang II) and growth factors, which also support P_4 synthesis in luteal cells (Kobayashi et al., 2001b).

Immediately after ovulation, the follicle walls collapse, allowing theca interna and granulosa cells to mix with one another. At the same time, these cells undergo a dramatic transformation during which they become luteinized after stimulation by preovulatory LH surge (Senger, 1997). Theca interna cells become the small luteal cells, which contain numerous lipid droplets, while granulosa cells become the large luteal cells with secretory granules containing oxytocin (Senger, 1997). Within the developing CL, large luteal cells increase in size, while small luteal cells increase in number (Senger, 1997).

The process of luteinization is characterized by increased synthesis and activity of enzymes responsible of switching steroid production from E_2 to P_4 (Juengel and Niswender, 1999). Progesterone exerts a strong negative feedback on the hypothalamus, reducing GnRH pulses, thus preventing development of the dominant follicle, E_2 synthesis and ovulation (Senger, 1997). It also promotes endometrial secretions and quiescence, which ultimately favors embryo implantation.

In cattle, LH and growth hormone (GH) are the primary hormones regulating development and function of the CL, and this is supported by expression of mRNA for both LH and GH receptors in the CL during the estrous cycle (Schams and Berisha, 2004). Luteinizing hormone is the principal hormone stimulating P_4 synthesis by the

small luteal cells (Niswender and Nett, 1988) and this is further supported by the fact that most of LH receptors are found in these cells (Schams and Berisha, 2004). On the other hand, receptors for growth hormone are mainly located on large luteal cells (Lucy et al., 1993; Kirby et al., 1996; Koelle et al., 1998) which are responsible for 80% of total P_4 production by the CL (Niswender et al., 1985). Growth hormone has been shown to stimulate P_4 and oxytocin secretion by the bovine CL *in vitro* (Liebermann and Schams, 1994) and to support CL development *in vivo* (Lucy et al., 1994; Juengel et al., 1997). Furthermore, GH is a more powerful stimulator of prostaglandin $F_{2\alpha}$ and P_4 production than LH *in vitro* (Kobayashi et al., 2001a).

There are many other local autocrine and paracrine regulators that play an important modulatory role during the lifespan of the CL. Growth factors involved in angiogenesis, such as IGFs and FGFs, induce CL function by stimulating secretion of P_4 and oxytocin (Einspanier et al., 1990; Sauerewein et al., 1992). Insulin-like growth factor-1 has been localized in the cytoplasm of both small and large luteal cells (Amselgruber et al., 1994). Fibroblast growth factor-2 gene and protein expression also have been demonstrated in large luteal cells of the bovine CL (Schams et al., 1994). Ovarian peptides such as oxytocin (OT), angiotensin II (Ang II), endothelin-1 (ET-1), progesterone and prostaglandin also are important in CL function. It is well established that oxytocin is highly expressed in the ruminant CL (Wathes et al., 1983; Schams, 1992), and it has been localized in both small and large luteal cells in cattle (Kruip et al., 1985). The LH surge appears to stimulate ovarian production and secretion of OT together with P_4 (Schams and Berisha, 2004). There are reports that support a potent luteotropic role of OT during the developing phase of the bovine CL (Schams, 1989;

Schams, 1996). Moreover, OT potentiates the luteotrophic effects of LH on P_4 secretion *in vitro* (Schams et al., 1995). On the other hand, the vasoactive peptides Ang II and ET-1 have been shown to directly inhibit P_4 production in bovine luteal cells (Girsh et al., 1996; Miyamoto et al., 1997; Hayashi and Miyamoto, 1999).

Prostaglandin Biosynthesis and Luteolysis in Cattle

Prostaglandins are members of the eicosanoid family of fatty acids. They are derived from 20 carbon polyunsaturated fatty acids, such as arachidonic acid (AA). Prostaglandins of the 2-series, namely prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2), are the most biologically active eicosanoids and are involved in key reproductive processes such as follicular development (Wallach et al., 1975), ovulation (Espey, 1980), luteolysis (Wathes and Lamming, 1995), and parturition (Challis, 1980). Prostaglandin $F_{2\alpha}$ is an important factor modulating luteal function in cattle. There are two different sources of $PGF_{2\alpha}$ within the reproductive tract. Temporal expression and site of synthesis then determine its modulatory role during luteal function. In contrast to the luteolytic effects of endometrium-derived $PGF_{2\alpha}$, luteal $PGF_{2\alpha}$ seems to be luteotrophic during early and mid-luteal phases (Miyamoto et al., 1993). Bovine CL produces high amounts of $PGF_{2\alpha}$ during early luteal phase, but $PGF_{2\alpha}$ concentrations decrease as the luteal cycle progresses (Milvae and Hansel, 1983; Schams et al., 1995; Skarzynski and Okuda, 1999). Furthermore, in an *in vitro* study, infusion of Ang II stimulated P_4 and $PGF_{2\alpha}$ release from the developing CL (Kobayashi et al., 2001b). Moreover, Kobayashi et al. (2001a) showed that Ang II together with $PGF_{2\alpha}$ highly stimulated P_4 secretion from developing bovine CL. In mid-cycle luteal cells, P_4 inhibits luteal $PGF_{2\alpha}$ secretion (Pate, 1988; Skarzynski and Okuda, 1999). The other source of $PGF_{2\alpha}$ biosynthesis and

secretion is the endometrial tissue of the bovine uterus and this is associated with the luteolytic cascade.

Prostaglandin Biosynthesis

Prostaglandins of the 2-series are synthesized from arachidonic acid (AA), which uses linoleic acid as the primary precursor. Arachidonic acid is store in an esterified form at the *sn*2 position of the membrane phospholipids bilayer (Crofford, 2001). The first and rate limiting step of prostaglandin biosynthesis is the hydrolytic release of AA by the action of cytosolic phospholipase A_2 ($cPLA_2$) enzyme (Lapetina, 1982). This enzyme has a preference for phospholipids containing AA at the *sn*2 position. Upon cell activation, release of intracellular Ca^{2+} stimulate cPLA translocation and binding to the membrane, which is a prerequisite for its enzymatic activity (Murakami et al., 1997; Leslie, 1997).

Following its release, AA is converted to prostaglandin H_2 (PGH_2) by the action of prostaglandin H synthase (PGHS), also known as cyclooxygenase (COX). Prostaglandin H synthase has cyclooxygenase and peroxidase activities that convert prostaglandin G (PGG) to PGH_2 (Goff, 2004). This enzyme consists of two isomers (PGHS-1 and PGHS-2) (Goff, 2004), which are primarily located on the luminal surface of the endoplasmic reticulum and the inner and outer membranes of the nuclear envelope (Spencer et al., 1998). The constitutively expressed PGHS-1 is considered to play a housekeeping role, whereas PGHS-2 is the inducible form, hence stimulated by hormones, growth factors, etc. in a variety of tissues (Goff, 2004).

After synthesis of PGH_2, this endoperoxide is converted to one of several possible prostanoids by the action of specific terminal enzymes. Biosynthesis of prostaglandin E_2

(PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are catalyzed by the action of prostaglandin E and prostaglandin F synthases, respectively (Goff, 2004).

Luteolysis in Cattle

In ruminants, ovarian E_2, oxytocin (OT), and P_4 seem to be the physiological regulators of synthesis and secretion of uterine $PGF_{2\alpha}$ during the estrous cycle. Prostaglandin $F_{2\alpha}$ released from the endometrium is the principal luteolytic agent in ruminants (Lukaszewska and Hansel, 1970; McCracken, 1971; McCracken et al., 1972). There is clear evidence that luteal regression at the end of the estrous cycle is caused by episodic release of endometrial $PGF_{2\alpha}$ that reaches the CL through a counter current mechanism between the uterine vein and ovarian artery (Senger, 1997, Krzymowski and Stefańczyk-Krzymowska, 2004; Schams and Berisha, 2004).

It has been demonstrated that P_4 regulates the lifespan of the CL (Ottobre et al., 1980; Schams et al., 1998; Schams and Berisha, 2001, 2002c) through inhibition of $PGF_{2\alpha}$ secretion from the endometrium. The large amplitude pulses of $PGF_{2\alpha}$, responsible for initiation of luteolysis, result from decreasing P_4 and increasing E_2 concentrations (Goff, 2004). This is preceded by the increase in E_2 and estrogen receptors (ER) which in turn up-regulates oxytocin receptor in the endometrium (Goff, 2004). At the end of the luteal phase, the number of ER increases, presumably due to E_2 up-regulation of its own receptor in endometrial cells (Spencer et al., 1996; Xiao and Goff, 1999; Ing and Tornesi, 1997). This increase in ER is thought to initiate luteolysis by increasing oxytocin receptors (OTR) (Spencer et al., 1996; Xiao and Goff, 1999; Ing and Tornesi, 1997; Spencer and Bazer, 2002); however, up-regulation of OTR in the bovine endometrium precedes that of the ER (Robinson et al., 2001). Thus, it has been

proposed that, even though increased concentration of OTR (~days 13-16) are primarily due to withdrawal of P_4 inhibitory effects (Fairclough and Lau, 1992), increasing levels of E_2 during this time can facilitate up-regulation of OTR gene expression (Vallet et al., 1990; Leavitt et al., 1985; Zhang et al., 1992). Therefore, luteolysis is brought about by coordinated changes in both OTR and prostaglandin production, which are regulated by changes in P_4 and E_2 concentrations.

Acosta et al. (2002) demonstrated that the transitory decrease of P_4 may trigger the luteolytic cascade. Earlier studies have also shown a close relationship between the decrease in ovarian blood flow and systemic P_4 concentration in the cow (Ford and Chenault, 1981; Wise et al., 1982). One of the main luteolytic actions of $PGF_{2\alpha}$ is to decrease ovarian blood flow (Knickerbocker et al., 1988).

Locally produced growth factors also mediate the complex process of luteolysis. Members of the IGF system have been shown to play an important role in the process of $PGF_{2\alpha}$-induced luteolysis in cattle (Neuvians et al., 2003). Gene and protein expression of VEGF are known to decline after prostaglandin secretion (Neuvians et al., 2004a), while FGFs and their receptors are up-regulated during functional luteolysis (Berisha and Schams, 2005). Therefore, it has been suggested that cessation of VGEF-support of the CL plays a role during structural luteolysis, whereas FGFs seem to have a major impact in functional luteolysis (Berisha and Schams, 2005).

There is also evidence that vasoconstrictive peptides, such as Ang II and ET-1 may play a role during physiological and induced luteolysis in cows (Girsh et al., 1996; Miyamoto et al., 1997; Hayashi and Miyamoto, 1999; Hayashi et al., 2000). *In vitro*, $PGF_{2\alpha}$ potentiates the inhibitory activity of ET-1 on P_4 secretion and stimulates Ang II

release (Miyamoto et al., 1997). Hence, ET-1 and Ang II may act as vasoconstrictors during functional luteolysis, as well as apoptosis inducers during functional and/or structural luteolysis (Schams and Berisha, 2004).

In the bovine ovary, cytokines such as tumor necrosis factor (TNFα), interferon (IFNγ), and interleukin (IL-1β) are up-regulated during induced luteolysis (Neuvians et al., 2004b). There also is evidence that the combination of TNFα and IFNγ are extremely cytotoxic (Petroff et al., 2001). Therefore, cytokines may be involved not only in structural, but also functional luteolysis in cattle (Berisha and Schams, 2005).

Pregnancy Establishment in Domestic Ruminants

The process of maternal recognition of pregnancy refers to the physiological window when the mother becomes cognizant of the embryo within her reproductive tract and prevents its elimination. If an embryo is present in the bovine uterus between days 14 and 17 of the cycle, luteolysis does not take place and P_4 secretion is maintained, resulting in establishment of pregnancy (Northey and French, 1980). This is achieved by a signal from the embryo that prevents the regression of the corpus luteum (CL) through inhibition of pulsatile $PGF_{2\alpha}$ secretion from the endometrium (Bazer, 1992; Demmers et al., 2001).

In domestic ruminants, the embryonic signal responsible for pregnancy establishment is a cytokine called interferon (IFN)-τ (Lafrance and Goff, 1985; Spencer and Bazer, 1995). Interferon-τ is synthesized by trophoblastic cells of bovine blastocyst (Lafrance and Goff, 1985; Roberts et al., 1990; Spencer and Bazer, 1995), and its secretion is highest between days 15 and 17 of pregnancy (Stojkovic et al., 1995; Bazer et al., 1998).

Pregnancy establishment is accomplished through several mechanisms. Interferon-τ can prevent luteolysis by down-regulation of endometrial oxytocin receptor, which prevents oxytocin-stimulated $PGF_{2\alpha}$ secretion (Lafrance and Goff, 1985). Secretion of INF-τ in ewes has been reported to reduce estradiol receptors, which prevents E_2-stimulation of OTR (Spencer and Bazer, 1995). Moreover, production of $PGF_{2\alpha}$ in cattle can be further suppressed by decreasing the expression of PGHS-2 as well as PGFS (Lafrance and Goff, 1990; Binelli et al., 2000), enzymes which play key roles in the synthesis of this prostaglandin. Binelli et al. (2000) showed that INF-τ blocked $PGF_{2\alpha}$ production by reducing PGHS-2 and PLA2 gene expression. The suppression of PGHS-2 and PLA2 mRNA synthesis appears to be independent of oxytocin-induced intracellular events (Pru et al., 2001).

Another mechanism by which IFN-τ may inhibit luteolysis in cattle is by shifting prostaglandin biosynthesis from the luteolytic $PGF_{2\alpha}$ to the luteotropic PGE_2 (Okuda et al., 2002). Results from studies by Xiao et al. (1998), showed that IFN-τ inhibited PGHS-2 mRNA and attenuated prostaglandin secretion from epithelial cells, which are known to be the primary source of $PGF_{2\alpha}$, while enhancing PGHS-2 mRNA and prostaglandin biosynthesis in stromal cells, which are the primary source of PGE_2 (Kim and Fortier, 1995; Asselin et al., 1996, 1998; Skarzynski et al., 2000). Therefore, achieving an optimal PGE_2 to $PGF_{2\alpha}$ ratio is essential for endometrial receptivity, myometrial quiescence, and maintenance of a functional CL and P_4 secretion, which are critical for successful establishment of pregnancy (Bazer et al., 1998).

Energy Balance and Fertility Responses in Postpartum Dairy Cows

In mammals, nutrition is critical for sustaining important biological processes that allows the animal to grow, survive and reproduce. Food is consumed, digested, and broken down into nutrients, which are then absorbed and partitioned throughout the body for utilization. Nutrients are utilized by tissues involved in maintenance of basic physiological processes, as well as establishing energy stores in the form of lipids and glycogen. The process of maintenance of physiological equilibrium or constant conditions of the internal milieu balance within a given physiological state is under homeostatic control (Bauman and Currie, 1980). However, in an animal's life cycle, there are a series of physiological states through which it must go and adjust adequately. The orchestrated or coordinated changes in metabolism of body tissues necessary to support the transition to a particular physiological state are under homeorhetic regulation (Bauman and Currie, 1980). An example of such dramatic metabolic changes is represented by the onset of lactation in dairy cows. It is clear then, that overall biological functions are governed by food intake, nutrient absorption and partitioning, and the resulting energy status of the animal within a given physiological state.

Energy Balance in Transition Dairy Cows

Energy balance of an animal is the difference between energy intake and energy requirements within a given physiological state (Beam and Butler, 1999; Butler et al., 1981; Canfield and Butler, 1990). In dairy cows, the onset of lactation cause an abrupt shift in nutritional requirements in order to support milk production (Butler, 2000). This rapid increase in energy requirements and changes in the metabolic as well as endocrine status of the cow come about during the transition period (Bauman and Currie, 1980;

Grummer, 1995). This is the result of the prioritized status of lactation which allows it to proceed at the expense of any other physiological processes (Bauman and Currie, 1980).

The transition period extends from three weeks prepartum until three weeks postpartum, and refers to the period during which endocrine and metabolic changes accommodate parturition and the onset of lactation (Grummer, 1995). A reduction in feed intake occurs during the final weeks of pregnancy when nutrient demands for support of fetal growth and initiation of milk synthesis are increasing (Grummer, 1995). As a result, there is a higher energy requirement than can be met or supported by dietary energy intake (Bell, 1995). The dietary energy that is consumed by the lactating animal is almost entirely used by the mammary tissue for milk production, leaving no energy for maintenance (Bell, 1995). To offset this energy deficit, the lactating animal mobilizes body energy reserves, which leads ultimately to a state of negative energy balance.

Since the energy required for lactation and maintenance far exceeds energy intake, the resulting NEB promotes a massive mobilization of fat from the adipose tissue and enhanced nutrient partitioning to the mammary gland for milk synthesis (Bauman and Currie, 1980). Consequently, the transition period is characterized by increased plasma levels of non-esterified fatty acids (NEFA), indicative of onset of lactation. Once free fatty acids are released into blood, they are bound to albumin and other blood proteins to be transported to hepatic and non-hepatic tissues. The uptake of NEFA into the liver takes place as blood flows though the liver. Once inside the liver, NEFAs can undergo three of the following metabolic fates as outlined by Drackley (1999): 1) complete oxidation to carbon dioxide to provide energy for the liver, 2) incomplete oxidation to

produce ketone bodies as an alternate energy source, and 3) re-esterification into triacylglycerol (TAG).

Normally, complete oxidation of fatty acid takes place, but first fatty acids must be translocated into the mitochondria. Mitochondrial uptake of FA is regulated by the activity of carnitine acyl transferase (CAT-1). In spite of the central role of CAT-1 in liver lipid metabolism, little is known about its regulation. However, studies have shown that high malonyl-CoA intracellular concentrations inhibit CAT-1 enzymatic activity (Brindle et al., 1985). Malonyl-CoA is produced by the enzyme acetyl-CoA carboxylase and is an intermediate for FA synthesis. Zammit (1996) demonstrated that the sensitivity of CAT-1 to malonyl-CoA inhibition is lessened during times of low circulating insulin or insulin resistance in rodents. Similarly, Brindle et al. (1985) found that malonyl-CoA concentrations were influenced by insulin and glucagon.

Thus, it would appear that energy balance may play a role in CAT-1 regulation. Cows in NEB exhibit decreased capacity to metabolize fat, which may be linked to CAT-1. Drackley et al. (1991) conducted a study with non-lactating cows to test the effects of carnitine and propionate on liver lipid metabolism. Fasting decreased oxidation of palmitate to CO_2 and decreased palmitate esterification by bovine liver slices. Addition of carnitine *in vitro* increased oxidation of palmitate and also increased total utilization of palmitate. Similar results were obtained in early lactating cows (Drackley et al., 1991).

An alternate pathway for hepatic NEFA metabolism is through partial oxidation that takes place in the peroxisome. This oxidative pathway is similar to that in the mitochondria, with some exceptions. The initial oxidation step is catalyzed by an oxydase, which results in production of hydrogen peroxide rather than reduced NAD as

seen in mitochondrial oxidation (Drackley, 1999). The next key difference is that peroxisomes do not contain respiratory chain linked to ATP formation. As a result, peroxisomal oxidation is not subject to control by energy demands for the cell. Hence, these differences make the peroxisome well suited to partially oxidize fatty acids that are poor substrate for mitochondrial oxidation (Drackley, 1999).

The third metabolic fate of NEFAs entering the liver is reesterification into TAG. Resultant TAGs are either stored in the liver or packaged into very low density lipoproteins and exported into circulation.

Fertility Responses in Postpartum Dairy Cows

Negative energy balance during the first 3 weeks postpartum has been associated with extended interval to first ovulation (Beam and Butler, 1999; Butler, 2001). The first ovulation occurs on average about 30 d postpartum, with a range of 14-42 d (Butler and Smith, 1989; Staples et al., 1990). Conception rates in lactating dairy cows increases when the period of ovarian activity preceding insemination is longer and thus the number of preceding ovulatory cycles is greater (Butler and Smith, 1989; Thatcher and Wilcox, 1973). Since the number of ovulatory estrous cycles preceding insemination influences conception rate, the length of postpartum interval to first ovulation provides an important measure for assessing the effects of NEB on reproductive performance (Butler, 2003). The severity and duration of NEB is variable among cows and relates primarily to differences in dry matter intake and its rate of increase during early lactation (Villa-Godoy et al., 1988; Staples et al., 1990). For example, cows overconditioned at calving exhibit decreased appetite and thus develop a more severe NEB than cows of moderate conditioning. These overconditioned animals mobilize more fat from the adipose tissue and exhibit significant accumulation of TAG in the liver (Rukkwamsuk et al., 1999).

Accumulation of TAG in the liver is, in turn, associated with a longer interval to first ovulation and reduced fertility (Butler and Smith, 1989; Rukkwamsuk et al., 1999; Jorritsma et al., 2000).

Fertility in postpartum cows is directly dependent on a normal estrous cycle characterized by folliculogenesis, ovulation, corpus luteum (CL) formation and regression. However, the mechanisms by which endocrine and metabolic upsets result in reduced reproductive efficiency are not well understood. Many of the hormonal and metabolic changes that occur during the transition period can affect reproductive function by interacting with the hypothalamic-pituitary axis (Butler, 2000).

The hypothalamic-pituitary axis is essential in the modulation of gonadotropin secretion, which in turn plays a pivotal role in control of the estrous cycle. For example, in pubertal heifers, the establishment of LH pulsatility is responsible for the initiation of cyclicity (Schillo et al., 1992). Among the factors associated with onset of puberty, attainment of a critical level of body fat is important (Schillo et al., 1992), further stressing the importance of nutritional and metabolic status for normal reproductive function. The NEB experienced by the early postpartum cow decreases pulsatile LH secretion, which results in delayed resumption of ovarian cyclicity (Butler and Smith, 1989; Beam and Butler, 1999; Butler, 2000).

The first ovulation postpartum reflects the resumption and completion of preovulatory ovarian follicular development and recovery from the hormonal conditions of late pregnancy (Butler, 2000). Delayed time to first ovulation associated with NEB is presumably through inhibition of LH pulse frequency and low levels of blood glucose, insulin, and insulin-growth factor-I (IGF-I) that collectively prevent E_2 production by

dominant ovarian follicles (Butler, 2000). However, Beam and Butler (1999) reported that initiation of a follicular wave and formation of a large dominant follicle during NEB was not a limitation for first ovulation. In fact, following parturition, a wave of follicular development takes place within 5-7 d in response to elevated plasma FSH concentrations and regardless of NEB (Beam and Butler, 1999). Nonetheless, ovulation of a dominant follicle during early lactation is dependent on the re-establishment of pulsatile LH secretion that is conducive to terminal preovulatory growth and E_2 production (Butler, 2000). Beam and Butler (1997) described three possible outcomes of follicular development during early postpartum period: 1) ovulation of the first dominant follicle around days 16-20 postpartum; 2) non-ovulation of the first dominant follicle followed by turnover and a new follicular wave; 3) failure of the dominant follicle to ovulate, becoming cystic and prolonging the interval to first ovulation to 40-50 days postpartum. As discussed by Jolly et al. (1995), the NEB experienced by the postpartum dairy cow represents a physiological state of undernutrition which impairs LH secretion and prevents ovulation. Consistent with this concept, Beam and Butler (1997) observed that follicles emerging after the NEB nadir, rather than before, had greater diameter, enhanced E_2 production, and were more likely to ovulate.

NEB has also been shown to change the profile of metabolic hormones which may play an important local role in control of follicular development in cattle. It is likely that these changes could alter the pattern of ovarian follicular growth and development, and subsequent CL function during the early postpartum period. During early NEB period, the ability of ovarian follicles to produce sufficient E_2 for ovulation appears to depend on the availability of insulin and IGF-I in serum and the changing EB profile (Beam and

Butler, 1999). Both insulin and IGF-I plasma concentrations are directly related to the energy status of the cow, and these hormones are essential for normal follicular development (Spicer et al., 1993; Simpson et al., 1994; Beam and Butler, 1999).

Plasma glucose and insulin levels are decreased by energy deficit (Beam and Butler, 1999; Butler, 2000). In lactating dairy cows, there is high demand for glucose as the primary substrate for mammary lactose synthesis (lactogenesis) (Diskin et al., 2003). Thus, reduced availability of glucose as a result of NEB may affect LH pulsatility, since it influences both tonic and surge modes of secretion, presumably through effects on GnRH (Diskin et al., 2003). Insulin also serves as a metabolic signal influencing LH release from the pituitary (Monget and Martin, 1997). However, it also has been shown to increase ovarian response to gonadotropins and to stimulate recruitment of small follicles and enhanced follicular growth (Gong et al., 2001), suggesting a direct effect at the ovarian level. Moreover, insulin is known to stimulate bovine follicular cells *in vitro* (Spicer et al., 1993) and *in vivo* (Simpson, et al., 1994).

Energy balance also influences plasma levels of insulin-like growth factor-I (IGF-I), which is important for normal ovarian follicular development and activity (Beam and Butler, 1999). In postpartum dairy cows, IGF-I levels were 40-50% higher during the first two weeks in cows in which the dominant follicle would ovulate as compared to levels in cows with non-ovulatory follicles (Beam and Butler, 1997; 1998). Additionally, plasma E_2 concentrations were highly correlated with plasma IGF-I levels (Beam and Butler, 1998). It has been shown that IGF-I directly stimulate proliferation and steroidogenic capacity of thecal (Spicer and Stewart, 1996) and granulosa cells (Spicer et al., 1993) *in vitro*. Consequently, during the postpartum period, the ability of follicles to

produce sufficient E_2 for ovulation seems to be dependent on availability of both insulin and IGF-I (Butler, 2000).

In cattle, peripheral P_4 concentration increases during the first two to three postpartum ovulatory cycles (Villa-Godoy et al., 1988; Spicer et al., 1990; Staples et al., 1990), and the rate of the increase in P_4 levels is attenuated by NEB early postpartum (Villa-Godoy et al., 1988; Spicer et al., 1990). In this regard, Villa-Godoy and coworkers (1988) reported that cows with the most negative energy balance during the first 9 days still had decreased P_4 during their third estrous cycle, which corresponded to the start of the breeding period. The ability of a cow to produce and secrete optimum levels of P_4 is important for fertility because plasma P_4 concentrations are highly correlated with pregnancy outcomes in lactating dairy cows (Folman et al., 1990; Larson et al., 1997).

In summary, it is well established that the energy state of the cow during early lactation can influence reproductive efficiency by modulating the estrous cycle. This may occur through modulation of gonadotropin secretion, specifically LH pulsatility (hypothalamic-pituitary axis). Alternatively, local ovarian effects may involve modulation of follicular growth and development by metabolic hormones (i.e. IGF-I and insulin), which could determine oocyte quality and viability and steroidogenic activity of the CL. However, energy-independent effects also have been observed after fatty acid supplementation. Thus, nutrition may influence reproductive efficiency in dairy cows not only by altering the energy status of the animal but also by influencing factors involved in the regulation of reproductive processes like follicular dynamics, ovulation, CL function and embryo survival among others.

Effects of Dietary Fats on Reproductive Response in Cattle

Fat supplementation of dairy rations is commonly used to alleviate a portion of the dietary energy deficit experienced by early postpartum dairy cows (Butler, 2003). Supplemental long-chain fatty acids (LCFA) have been shown to increase conception rates (Schneider et al., 1988; Sklan et al., 1989; Ferguson et al., 1990), enhance pregnancy rates (Schneider et al,. 1988; Sklan et al., 1991), and reduce the interval to first estrus (Sklan et al., 1991). Dietary fats have also been shown to regulate eicosanoid synthesis (Abayasekara and Wathes, 1999; Cheng et al., 2001), modulate plasma P_4 concentration (Carrol et al., 1990; Lucy et al., 1993b; Garcia-Bojalil et al., 1998), stimulate ovarian follicular development (Lucy et al., 1993b; Thomas and Williams, 1996; Beam and Butler, 1997) and improve fertility (Staples et al., 1998).

To understand the interaction of dietary fats and reproduction, it is essential to understand the basic nature and biology of fatty acids and their metabolism. Fatty acids belong to the family of lipids, which consist of biological compounds that are soluble in organic solvents, such as cholesterol, TAG and phospholipids. Fatty acids are present in all cell types and contribute to cellular structure, provide fuel storage and participate in many biological processes ranging from gene transcription to regulation of key metabolic pathways and physiological responses (Van Bilsen et al., 1997, 1998; Gurr et al., 2002).

Fatty acids consist of a carbon chain that ends with a carboxyl group, varying in the chain length and the degree of unsaturation or number of double bonds. Naturally occurring fatty acids can be saturated (no double bonds) or unsaturated, consisting of one or more double bonds. Saturated fatty acids (SFAs) have all the carbons holding the maximum number of hydrogens possible, thus referred as to be saturated with hydrogen. Some naturally occurring SFAs are palmitic acid (16:0), found in palm oil, and stearic

acid (18:0), commonly present in animal fat (Jenkins, 2004). Unsaturated fatty acids can contain one (monounsaturated fatty acids; MUFA) or more (polyunsaturated fatty acids; PUFA) double bonds.

Fatty acids are generally abbreviated by listing the number of carbons with the number of double bonds (i.e. 18:0; 18 carbons with no double bonds) (Jenkins, 2004). For unsaturated fatty acids, the omega system is used to identify the location of the terminal double bond relative to the methyl end of the carbon chain. In the omega system, carbon atoms in the chain are identified with Greek letters, with the last carbon of the chain, or the one farthest from the carboxyl group, known as the omega (n)-carbon (Gurr et al., 2002). Thus, for PUFAs only the position closest to the omega carbon is given. For instance, a member of the n-6 family such as linoleic acid (18:2, n-6) has its first double bond at the Δ6 position (carbon number 6) counting from the omega end (Gurr et al., 2002).

Fatty acids in mammals are either generated by *de novo* synthesis or provided by the diet. Dietary fatty acids may undergo elongation and desaturation to generate isomers which may have different properties. Elongation involves the addition of two-carbon units to a chain through the action of enzymes known as elongases. Desaturation, on the other hand, is catalyzed by desaturase enzymes that insert a double bond into the acyl chain. These desaturase enzymes are classified according to the position of insertion of the double bond, while the newly created double bonds are almost invariably separated from each other by a methylene group.

However, desaturation of fatty acids in mammals does not occur at positions greater than Δ9 since the required desaturases are absent (Fischer, 1989; Cook, 1996;

Mayes, 1996). Hence, the parent molecules for the n-6 and n-3 families, linoleic (LA; 18:2) and linolenic (LNA; 18:3) acids, respectively, can't be synthesized by the tissues and, therefore, must be supplied in the diet. Mammals have been shown to have an absolute requirement for LA and LNA; therefore, these fatty acids are regarded as essential fatty acids (Burr and Burr, 1929; Aaes-Jorgensen, 1961; Holman et al., 1982). Deficiency in these essential PUFAs may result in a variety of pathophysiologic effects in mammals that include reproductive inefficiency (Burr and Burr, 1929). Nonetheless, the body has different requirements for n-6 and n-3 PUFAs, as they are involved in several, yet varied, essential functions.

Even though the diet of ruminants contains predominantly PUFAs, fatty acids in blood, tissues and milk are highly saturated (Abayasekara and Wathes, 1999). This is the result of extensive biohydrogenation of PUFAs that takes place in the rumen through the activity of ruminal microorganisms (Ward et al., 1964). Biohydrogenation of unsaturated fatty acids consists of addition of hydrogen by microbial enzymes to the double bonds resulting in the saturation of dietary fatty acids. The principal product is stearic acid, a saturated fatty acid (18:0). However, even though ruminal biohydrogenation is extensive, it ranges from 60 – 90% (Murphy et al., 1987). In other words, incomplete biohydrogenation of unsaturated fatty acids results in the formation of several isomers which will depend on the source of the dietary fatty acid fed. Some of the most common products of incomplete biohydrogenation are oleic acid (18:1), conjugated linoleic acid (CLA), and *trans*-vaccinic acid (*trans*-11; 18:1). Thus, in order for intact PUFAs to reach the small intestine for absorption and being transported to target tissues, they need to escape ruminal microbial hydrogenation process.

Essential PUFAs may be made available for absorption by feeding ruminally inert fats. A number of techniques have been developed to protect fats which involve chemical treatment processes such as formaldehyde or calcium salts of fatty acids (Palmquist and Jenkins, 1980; Ashes et al., 1992). Inclusion of calcium soaps of long-chain fatty acids (LCFA) in dairy cattle rations is commonly used to alleviate the dietary energy deficit experienced during early postpartum. Cows fed calcium salts of LCFA have produced more milk and experienced improved fertility (Staples et al., 1998).

Linoleic acid is abundant in nearly all commonly available unprocessed plant oils (e.g. corn, sunflower, safflower, and rape seed) (Sargent, 1977). In cows, AA is provided by dietary intake of LA (Urich, 1994; Gurr et al., 2002). However, dietary LA deprivation causes a general decrease in AA levels, although the proportion in different tissues can be diverse, suggesting tissue-specific uptake of PUFA (Lefkowith et al., 1985). On the other hand, PUFAs of the n-3 family, such as eicosapentaenoic acid (EPA; 20:5) and DHA (22:6), are also essential for many bodily functions (Innis, 1991; Abayasekara and Wathes, 1999). They can be provided in the diet or synthesized in the tissues from the parent molecule LNA. Linolenic acid is the predominant PUFA in most forage lipids (Palmquist and Jenkins, 1980) and high levels are also found in linseed oil, however, it also contains significant amount of LA (Sargent, 1997). Fish oils, which contain low amounts of LNA, offer the most readily available dietary source of EPA and DHA (Neuringer et al., 1988).

The potential mechanisms by which LCFAs affect reproductive responses in cattle include indirect effects of high energy intake on the overall energy state of the cow, as well as direct effects of dietary fatty acids on the pituitary, ovaries, and uterus (Staples et

al., 1998; Mattos et al., 2000). The association between fat content in the diet and fertility in lactating cows has being documented in a variety of studies.

Improvement of the overall energy state provided by fatty acid supplementation (Staples et al., 1998; Jenkins and Palmquist, 1984) may lead to re-establishment of LH pulsatility and ovarian cyclicity in the lactating cow (Palmquist and Jenkins, 1980; Lucy et al., 1991). In fact, Sklan et al. (1994) reported that the energy provided by dietary fats increased LH secretion in dairy cows that consumed less energy than required. However, the mechanism by which LCFAs may affect LH secretion has not been described in ruminants.

There is evidence that increase in consumption of dietary fatty acids stimulates ovarian follicular growth in cattle through a mechanism that is independent from energy intake and weight gain (Staples et al., 1998). Increasing the dietary content of LCFAs in cattle increased both the number and size of follicles present in the ovary and shortened the interval to first ovulation postpartum (Hightshoe et al., 1991; Lucy et al., 1991, 1992; Ryan et al., 1992; Thomas and Williams, 1996; Lammoglia et al., 1997; Beam and Butler, 1997). Lucy et al. (1993b) reported greater numbers of medium-sized ovarian follicles (1.5 to 2.3 mm) in postpartum dairy cows fed a diet containing 2.2% calcium salts of LCFA compared to cows receiving an isocaloric control diet without calcium salts of LCFA. Several studies reported that supplemental fat increased not only the total number of ovarian follicles (Thomas and Williams, 1996; Beam and Butler, 1997; Lammoglia et al., 1997), but also the size of preovulatory follicles in cattle (Lucy et al., 1993b; Beam and Butler, 1997; Oldick et al., 1997). Follicular growth also has been shown to be stimulated by LCFAs in crossbred beef cattle (Thomas et al., 1997).

Moreover, lactating dairy cows fed calcium salts of fat enriched with LA or fish oil (high in n-3 PUFAs) had increased size of the dominant follicle compared to those fed calcium salts of oleic acid (18:1) (Mattos et al., 2000). Whether increased size of preovulatory follicles is due to exogenous LCFA-induced LH secretion from the pituitary, and how the altered ovarian follicular dynamics may impact pregnancy outcome in dairy cows warrants further investigation.

It is possible that increased serum concentrations of insulin in response to feeding LCFAs to cattle (Palmquist and Moser, 1981; Thomas and Williams, 1996; Ryan et al., 1995) may play a role in mediating increased follicular growth. This insulin-mediated event may be by stimulation of granulosa cell IGF-I production (Yoshimura et al., 1994).

Stimulatory effects of dietary LCFAs on follicular development may be through enhanced steroidogenesis via increased cholesterol, thus increased substrate availability for increased follicular steroid synthesis (Wehrman et al., 1991). In mature heifers and dairy cows, elevating fat intake increased both serum and follicular fluid cholesterol concentrations (Park et al., 1983; Talavera et al., 1985; Wehrman et al., 1991). Carroll and coworkers (1990) detected a 21% increase in plasma cholesterol concentrations in dairy cows fed ruminally inert fat compared to control cows. Similarly, beef heifers supplemented with soybean oil had greater concentration of total cholesterol in serum and HDL cholesterol in follicular fluid when compared with control heifers (Ryan et al., 1992). Wehrman et al. (1991) showed that cows fed a high lipid diet had increased intrafollicular levels of androstendione as well as increased P_4 output from the granulosa cells collected and cultured *in vitro*. In addition, several studies have detected subtle increases in plasma P_4 concentrations in cows fed high-fat diets (Carroll et al., 1990;

Lucy et al., 1993b; Garcia-Bojalil et al., 1998). Dietary intake of LCFAs in ruminants during luteal phase increased serum concentrations of P_4 (Talavera et al., 1985; Carrol et al., 1990; Hawkins et al., 1995; Burke et al., 1996).

Recent studies suggested that increase in plasma P_4 in cows fed fat-supplemented diets may not be due to increased synthesis but rather to reduced clearance of P_4 from circulation. In one study, when the CL of cows were removed by ovariectomy, P_4 was cleared from blood at a much slower rate in cows fed supplemental fat compared to cows fed the control diet (Hawkins et al., 1995). In addition, Sangsritavong et al. (2002) showed that fatty acids and/or TAG could increase circulating P_4 and E_2 concentrations by directly inhibiting liver cell metabolism of these steroid hormones. Because CL function and P_4 is crucial for establishment and maintenance of pregnancy in ruminants, increased plasma P_4 may result in improved pregnancy rates in postpartum dairy cows fed supplemented fats. In fact, supplementation of LCFA has been shown to enhance luteal function as confirmed by reduced incidence of short cycles (Williams, 1989). Dietary LCFAs may increase AA in the phospholipids pool of granulosa cells. The AA released upon gonadotropin stimulation (Cooke et al., 1991) had a direct effect on steroidogenesis in goldfish (Van der Kraak and Chang, 1990) and hens (Johnson and Tilly, 1990). It can be used as the precursor for prostaglandin production, which in turn may stimulate steroidogenesis as reported in granulosa cells for marmosets (Michael et al., 1993). This hypothesis is supported by the observation that gonadotropins stimulate prostaglandin production *in vitro* (Tsang et al., 1988).

Fertility responses may also be related to the effects of LCFAs on uterine eicosanoid production. Eicosanoids (i.e. prostaglandins, thromboxanes, leukotrienes and

lipoxins) are synthesized from AA, which uses LA as the primary precursor (Kinsella et al., 1990). Prostaglandins (PG) of the 2 series ($PGF_{2\alpha}$, PGE_2) have been implicated in many reproductive processes including ovulation (Espey, 1980), follicular development (Wallach et al., 1975), corpus luteum function (Bazer and Thatcher, 1977; Auletta and Flint, 1988; Abayasekara et al., 1995; Poyser, 1995; Wathes and Lamming, 1995), parturition (Thorburn and Challis, 1979; Challis, 1980) and uterine involution (Hafez and Hafez, 2000). Hence, any influence that fatty acids might exert on $PGF_{2\alpha}$ synthesis may affect overall reproductive performance.

Ovulation was blocked by inhibition of PG synthesis in monkeys and restored by administration of $PGF_{2\alpha}$ (Wallach et al., 1975; Tsafriri et al., 1972). Experiments performed on rats showed that a diet high in n-3 PUFAs increased ovulation rate, whereas a diet high in n-6 PUFAs resulted in reduction of ovulation rate (Trujillo and Broughton, 1995). Since both diets resulted in increased levels of total PGE, the authors suggested that n-3 PUFAs may increase ovulation by augmentation of the less biologically active PGE_3 at the expense of PGE_2.

Long chain fatty acids may also influence luteal activity and function via modulation of uterine $PGF_{2\alpha}$ production, which causes luteolysis (Auletta and Flint, 1988; Bazer and Thatcher, 1977; Poyser, 1995). Essential PUFAs have been shown to inhibit PG secretion in several cell types (Levine and Worth, 1984; Achard et al., 1997) including bovine endometrial (BEND) cells (Mattos et al., 2003). Manipulation of dietary LCFA content may also influence synthesis of $PGF_{2\alpha}$ as demonstrated by different studies. For example, dietary n-6 and n-3 PUFA have the ability to alter gestational length and time of parturition through modulation of PG synthesis in rats

(Holman, 1971; Leaver et al., 1986), ewes (Baguma-Nibasheka et al., 1999), and humans (Olsen et al., 1986, 1992; Allen and Harris, 2001). Although there is some evidence indicating that n-6 PUFA, LA specifically, enhances PG production by providing more substrate for conversion to AA (Connolly et al., 1996; Nakaya et al., 2001; Elmes et al., 2004; Petit et al., 2004), other studies have found an inhibitory effect (Elattar and Lin, 1989; Pace-Asciak and Wolfe, 1968; Cheng et al., 2001). Abomasal infusion of cycling cattle with yellow grease (72% PUFAs and 17% LA) resulted in significant attenuation of oxytocin-induced secretion of 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM), a metabolite of $PGF_{2\alpha}$ (Oldick et al., 1997). A number of studies have demonstrated inhibitory effects of n-3 PUFAs on $PGF_{2\alpha}$ production (Bezard et al., 1994; Staples et al., 1998; Abayasekara and Wathes, 1999; Mattos et al., 2000, 2001, 2002, 2004). Supplementation of dairy cattle with fish oil (high in n-3 PUFAs) reduced plasma concentrations of PGFM in early postpartum as well as in oxytocin-induced cycling cows (Mattos et al., 2002, 2004). It also has been suggested that fish oil may reduce the sensitivity of the CL to $PGF_{2\alpha}$ since cycling cows fed fish meal had higher P_4 plasma concentrations after injection of a luteolytic dose of $PGF_{2\alpha}$ (Burke et al., 1997). Attenuation of uterine $PGF_{2\alpha}$ secretion and decreased sensitivity of the CL to this PG caused by PUFAs may lead to improved fertility through enhanced luteal function, reduced embryonic loss and increased pregnancy rates.

Positive effects of dietary fats on fertility response are not exclusively related to inhibition of PG biosynthesis. Prostaglandins are key hormones in terms of cervical ripening and myometrial contractility, which are essential for parturition (Challis, 1980). Following parturition, fertility resumes after uterine involution takes place, resulting in

resumption of normal estrous cycles (Kiracofe, 1980). This process of uterine involution is caused by myometrial contractions stimulated by $PGF_{2\alpha}$. A massive and sustained release of $PGF_{2\alpha}$ takes place during the first two weeks postpartum and is essential to reduce the uterine size and increase its tone (Hafez and Hafez, 2000). Plasma PGFM concentrations increase prior to calving (Schindler et al., 1990), and generally decreases to basal levels within 21 d postpartum (Lewis et al., 1984; Del Vecchio et al., 1992a). Since the uterus seems to be the primary source of PGFM in postpartum cows, PGFM is considered to be an indicator of uterine $PGF_{2\alpha}$ production in postpartum cows (Guilbault et al., 1984). Moreover, the duration of this postpartum $PGF_{2\alpha}$ sustained release is negatively correlated with the number of days to complete uterine involution and the interval between parturition and resumption of normal ovarian activity (Lindell et al., 1982; Madej et al., 1984). For conception to occur after calving, the uterus must return to its normal size. Thus, modulation of PG production by dietary fats may affect the timing of rebreeding postpartum.

After parturition, the reproductive tract is also subject to massive bacteriologic insult, and re-establishment of a sterile uterus is one of the requirements for pregnancy to take place in the subsequent postpartum period (Hafez and Hafez, 2000). Uterine infections, such as metritis, reduce reproductive efficiency by increasing calving interval and preventing resumption of ovarian activity (Griffin et al., 1974; Arthur et al., 1989; Lewis, 1997). The incidence of uterine infections ranges from approximately 10-40% of postpartum dairy cattle (Arthur et al., 1989; Lewis, 1997). Multiparous cows may be more resistant to some uterine infections than are primiparous cows (Hussain, 1989).

However, the physiological characteristics that make some animals more susceptible than others are not well understood.

Eicosanoids are also known to have important immunomodulatory effects (Lewis, 2004). Recent studies have confirmed the effects of *in vivo* exposure to PGs on the *in vitro* response of lymphocytes (Ramadan et al., 1997; Lewis, 2003; Wulster-Radcliffe et al.,. 2003). $PGF_{2\alpha}$ has been reported to enhance immune function *in vitro* (Hoedemaker et al., 1992). In addition, $PGF_{2\alpha}$ increased *in vitro* bactericidal activity of neutrophils from ovariectomized mares (Watson, 1988).

Induced and spontaneous uterine infections increase of plasma PGFM concentrations (Del Vecchio et al., 1992a, 1994). However, Seals and coworkers (2002) reported that postpartum concentrations of PGFM were inversely related to emergence of uterine infections. They also reported that postpartum cows with depressed PGFM concentrations were more likely to develop uterine infections. In addition, aberrant $PGF_{2\alpha}$ and PGE_2 production has been associated with retained placenta (Gross et al., 1987; Heuwieser et al., 1992), which in turn is associated with increased incidence of uterine infections (Curtis et al., 1985). Thus, PGFM concentration in early postpartum cows may enhance uterine immune functions and be a good indicator of the likelihood of a cow to develop metritis.

Regulation of Prostaglandin $F_{2\alpha}$ Synthesis by Polyunsaturated Fatty Acids

Evidence is accumulating that dietary manipulations of PUFAs can have major effects on eicosanoid production. Depending on the amount of particular fatty acids reaching the target tissues, supplemental PUFAs can either stimulate or inhibit prostanoids synthesis. In a human study, feeding diets rich in AA increased plasma

phospholipid levels of AA and urinary excretion of the stable metabolites of prostacyclin (PGI_2) and TA_2 (Sinclair and Mann, 1996). Consistent with these findings, ewes infused with either soybean oil (50% LA) or olive oil (16% LA) had greater serum PGFM concentrations than ewes infused with saline (Burke et al., 1996). In postpartum beef heifers, infusion of lipid (20% soybean oil) through the jugular vein increased systemic concentrations of LA and PGFM after oxytocin injection (Filley et al., 1999).

Recent reports in mammalian systems have shown reduced eicosanoid synthesis when PUFAs of the n-3 or n-6 families were fed in the diet. In a study with lactating dairy cows, Oldick et al. (1997) reported that OT-induced PGFM concentrations were greatly reduced in cows infused abomasally with yellow grease, compared with infusion of tallow, glucose or water. Additionally, it was demonstrated that supplementation of postpartum dairy cows with fish meal containing EPA and DHA considerably attenuated PGFM response to OT induction (Mattos et al., 2002). In beef cows, whole cottonseed added to the diets doubled the average lifespan of a GnRH-induced CL, compared with cows fed an isocaloric control diet (Williams, 1989). Although PG concentrations were not measured in the latter study, a lipid-induced suppression of $PGF_{2\alpha}$ would be compatible with a longer CL life (Staples et al., 1998). These observations provide strong evidence for PUFA involvement in modulation of PG biosynthesis.

It has been proposed that PUFAs may reduce $PGF_{2\alpha}$ production through modulation of one or more steps of the PG biosynthetic pathway. These include decreasing availability of the precursor AA, competing for PGHS-2 activity and directly inhibiting synthesis and/or activity of PGHS-2 (Mattos et al., 2000; Cheng et al., 2001).

Reduced availability of AA as a precursor for conversion to $PGF_{2\alpha}$ may result from the inhibition of AA synthesis or its displacement from the membrane phospholipid pool. It has been reported that dietary PUFAs may inhibit AA synthesis during the elongation and desaturation processes of LNA in the liver (Bezard et al., 1994). In fact, supplementation of rats with n-3 PUFAs resulted in reduced desaturation of LA and LNA by liver microsomes and lowered concentration of AA in liver phospholipids (Garg et al., 1988; Christiansen et al., 1991). Incubation of rat hepatoma cells with n-3 PUFAs resulted in reduced Δ6 desaturase activity (Larsen et al., 1997). Sprecher (1981) observed that in rats there was a preferential processing of n-3 PUFAs by Δ6 desaturase at the expense of n-6 PUFAs. This suggested that high levels of LNA in the diet could compete with LA for Δ6 desaturase activity, thus attenuating the conversion of LA to AA. Reduced availability of AA will result in greater incorporation of other fatty acids into the phospholipid pool of the plasma membrane and decreased availability of the substrate for PGHS-2 enzyme (German et al., 1988).

Dietary n-3 PUFAs can also reduce $PGF_{2\alpha}$ synthesis through competition with AA for PGHS-2 activity. The n-3 PUFAs, such as EPA, can serve as substrate for PGHS-2, thus leading to the synthesis of PGs of the 3 series at the expense of PGs of the 2 series. Indeed, feeding rats with diets rich in n-3 PUFAs resulted in increased secretion of PGs of the 3 series from uterine tissues cultured *in vitro* (Leaver et al., 1991).

Direct inhibition of PGHS-2 synthesis and/or activity by PUFAs may also contribute to attenuation of $PGF_{2\alpha}$ synthesis following dietary fatty acids supplementation. When bovine aortic endothelial cells were incubated with n-3 PUFAs (EPA and DHA), PGHS-1 mRNA expression was reduced (Achard et al., 1997). In

cultures of rat hepatoma cells, PGHS enzyme was almost completely inactivated with addition of EPA 30 seconds before addition of AA as substrate (Larsen et al., 1997). Furthermore, PUFAs such as AA, EPA, and DHA, have been also found to inhibit PGHS activity (Smith and Marnett, 1991).

In addition to inhibiting PGHS-2 activity, dietary PUFAs can also modulate the expression of PGHS-2 gene. The proposed mechanism for regulation of gene expression by PUFAs involves activation of nuclear transcription factors such as peroxisome proliferator-activated receptors (PPARs). Peroxisome proliferator-activated receptors belong to a family of nuclear receptor transcription factors activated by specific fatty acids, eicosanoids, and peroxisome proliferators (Desvergne and Wahli, 1999). Three different isoforms, PPARα, PPARγ, and PPARδ, have been identified and are encoded by different genes (Desvergne and Wahli, 1999). Upon activation, PPARs dimerize with the ubiquitous retinoid X receptor (RXR) and binds to a prescribed DNA sequence referred to as PPAR response element (PPRE) (Desvergne and Wahli, 1999). Previous studies have shown PPARs to be involved in regulation of genes modulating steroid and prostaglandin synthesis as well as mediating some of the growth hormone effects in hepatocytes (Lim et al., 1999; Zhou and Waxman, 1999; Schopee et al., 2002). In general, all the n-3 and n-6 PUFAs activate the three PPAR isoforms. However, their affinities for a given receptor vary, suggesting a role for tissue-specific availability and metabolism of particular fatty acids and differences in their affinity for a specific PPAR subtype (Sampath and Ntambi, 2005). Furthermore, it has been concluded that PUFAs are potent endogenous ligands since they have been shown to activate PPARs at micromolar concentrations (Lehmann et al., 1997). MacLaren et al. (2003) documented

expression of PPARα, PPARγ, and PPARδ in endometrium from cyclic and pregnant Holstein cows at day 17 following estrus. These isoforms also have been detected in an immortalized bovine endometrial epithelial cell line.

Collectively, these findings suggest that select dietary n-3 fatty acids can reduce $PGF_{2\alpha}$ synthesis by decreasing AA concentration in tissue phospholipids by increasing the concentration of fatty acids that compete with AA for processing by PGHS-2, and by inhibiting PGHS-2 synthesis and/or activity. Nutritional management that alters endometrial $PGF_{2\alpha}$ biosynthesis may improve reproductive efficiency in high producing dairy cows in which fertility is impaired due to high metabolic demands associated with milk production.

Conjugated Linoleic Acid and Reproduction

Conjugated linoleic acid (CLA) refers to a group of geometrical and positional isomers of LA resulting from incomplete biohydrogenation in the rumen (Chin et al., 1992; Ma et al., 1999; Griinari et al., 2000). The number of double bonds remains the same as in the parent LA, but one of the double bonds is shifted to a new position by microbial isomerases. One or both of the double bonds are either in the *cis* or *trans* configurations separated by a single carbon-to-carbon linkage rather than by the normal methylene group. A broad number of *cis*- and *trans*- CLA isomers have been identified in food; however, the most commonly occurring CLA isomer is the *cis*-9, *trans*-11-octadecadienoic acid with minor but significant proportions of *trans*-10, *cis*-12-18:2 (Parodi, 1977; 1997; Chin et al., 1992; McGuire et al., 1998).

After ruminal synthesis of *cis*-9, *trans*-11 CLA, it may be absorbed in the small intestine or further biohydrogenated into *trans*-11-octadecenoic acid (*trans* vaccenic acid)

by ruminal microorganisms. The *trans*-11 18:1 can be reduced to stearic acid (18:0) or transported to peripheral tissues as MUFA. In the peripheral tissues (i.e. mammary gland, muscle), this *trans*-isomer may be converted back into *cis*-9, *trans*-11 CLA by the action of Δ9 desaturase (Pollard et al., 1980; Holman and Mahfouz, 1981). This conversion appears to be a major source of *cis*-9, *trans*-11 CLA in the cow's milk (Corl et al., 1998; Griinari and Bauman, 1999; Santora et al., 2000).

The presence of *trans*-10, *cis*-12 CLA in milk (Griinari and Bauman, 1999) suggests the existence of endogenous ruminal bacteria with the capability for synthesis of this isomer. In support of this hypothesis, LA has been shown to be converted to *trans*-10, *cis*-12 CLA by *Propionobacter in vitro* (Verhulst et al., 1987). However, the *trans*-10, *cis*-12 CLA detected in ruminal tissues (Griinari and Bauman, 1999; Dhiman et al., 1999) seem to originate solely from ruminal synthesis, since mammalian tissues do not have the Δ12 desaturase necessary for conversion of *trans*-10-octadecenoic acid (elaidic acid) back to *trans*-10, *cis*-12 CLA.

A large number of beneficial effects have been attributed to CLA ranging from enhancing feed efficiency and growth in rats (Chin et al., 1994) and pigs (Bee, 2000), decreasing body fat in mice (DeLany et al., 1999) and pigs (Dugan et al., 1997; Ostrowska et al., 1999), and reduced milk fat synthesis in lactating dairy cows (Griinari et al., 1998; Romo et al., 2000; Baumgard et al., 2001). Although it is likely that some biological effects of CLA may be induced and/or enhanced synergistically by these isomers, there is evidence suggesting that numerous effects of CLA are due to separate actions of these biologically active isomers (Pariza et al., 2000). Uruquhart et al. (2002) observed that after treatment of human vein endothelial cells with 100 μM of individual

CLA isomers, differential effects were observed with *cis*-9, *trans*-11 CLA inhibiting, while *trans*-10, *cis*-12 CLA inducing Ca ionophore-stimulated eicosanoid production. It seems that *trans*-10, *cis*-12 works preferentially through modulation of apoptosis and cell cycle, whereas *cis*-9, *trans*-12 may work through AA metabolism (Ochoa et al., 2004).

Nonetheless, beneficial actions of CLA have been linked to modulation of eicosanoid production (Sugano et al., 1998). Recent studies detected an inhibitory effect of CLA on eicosanoid synthesis in various animal and cell systems (Liu and Belury, 1998; Kavanaugh et al., 1999; Uruquhart et al., 2002; Eder et al, 2003). Feeding pregnant rats with dietary CLA resulted in inhibition of uterine $PGF_{2\alpha}$ independent of PUFA content (Harris et al., 2001). In addition, Cheng et al. (2003) reported that treatment of endometrial cells isolated from late pregnant ewes with CLA suppressed $PGF_{2\alpha}$ in a dose dependent manner, while low doses of CLA stimulated PGE_2 generation. Cheng and coworkers (2003) showed that treatment of intercotyledonary endometrial cells with CLA resulted in a dose-dependent inhibition of $PGF_{2\alpha}$ production. Similarly, CLA also has been shown to inhibit $PGF_{2\alpha}$ synthesis in rat placental and uterine tissues (Harris et al., 2001). Although the mechanism underlying this inhibition is not clear, it has been suggested that CLA may compete with AA for PGHS-2 activity as well as for incorporation to the membrane phospholipids. Alternatively, CLA may modulate conversion of $PGF_{2\alpha}$ to PGE_2 as reported by Gross and Williams (1988) in bovine placental cells. Therefore, CLA may regulate reproductive processes through modulation of prostaglandin biosynthesis.

CHAPTER 3
EFFECTS OF POLYUNSATURATED FATTY ACIDS ON PROSTAGLANDIN $F_{2\alpha}$ PRODUCTION BY BOVINE ENDOMETRIAL CELLS

Introduction

In the past decade, genetic selection for high milk production has been associated with a decrease in reproductive efficiency in lactating dairy cows (Butler, 2000). Poor reproductive efficiency includes early embryonic loss (Thatcher et al., 1995), impaired ovarian cyclicity and low fertility rates (Butler, 2000), which collectively result in reduced life-long milk production (Plaizier et al., 1997). Early postpartum dairy cows have higher energy requirements than can be supported by dietary energy intake, which creates a negative energy state that can lead to impaired reproductive function (Butler, 2000).

Polyunsaturated fatty acids (PUFA) are generally added to dairy rations to increase the energy density of the diet. In mammals, the parent molecules for the n-6 and n-3 families, linoleic (LA; 18:2) and linolenic (LNA; 18:3) acids, respectively, cannot be synthesized by the tissues and, therefore, must be supplied in the diet. Since there is an absolute requirement for these PUFAs, they are regarded as essential fatty acids (Burr and Burr, 1929; Aaes-Jorgensen, 1961; Holman et al., 1982).

Supplemental polyunsaturated fatty acids (PUFA) have been reported to increase conception rates (Schneider et al., 1988; Sklan et al., 1989; Ferguson et al., 1990), enhance pregnancy rates (Schneider et al,. 1988; Sklan et al., 1991), and reduce the interval to first estrus (Sklan et al., 1991) of lactating dairy cows. Select dietary fats have

been also shown to regulate eicosanoid synthesis (Abayasekara and Wathes, 1999; Cheng et al., 2001), modulate plasma P_4 concentration (Carrol et al., 1990; Lucy et al., 1993b; Garcia-Bojalil et al., 1998), stimulate ovarian follicular development (Lucy et al., 1993b; Thomas and Williams, 1996; Beam and Butler, 1997) and improve fertility (Staples et al., 1998) of lactating dairy cows.

Prostaglandins are members of the eicosanoid family synthesized from 20 carbon PUFAs, such as arachidonic acid (AA). Arachidonic acid is, in turn, synthesized from elongation and desaturation of LA. The first and rate limiting step of prostaglandin biosynthesis is the hydrolytic release of AA by the action of cytosolic phospholipase A_2 ($cPLA_2$) enzyme (Lapetina, 1982). Following its release, AA is converted to prostaglandin H_2 (PGH_2) by the action of prostaglandin H synthase-2 (PGHS-2), also known as cyclooxygenase-2 (COX-2). Prostaglandin H synthase has cyclooxygenase and peroxidase activities that convert prostaglandin G (PGG) to PGH_2 (Goff, 2004). After synthesis of PGH_2, this endoperoxide is converted to one of several possible prostanoids by the action of specific terminal enzymes. Biosynthesis of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) are catalyzed by the action of prostaglandin E and prostaglandin F synthases, respectively (Goff, 2004). Prostaglandins of the 2-series, $PGF_{2\alpha}$ and PGE_2, are the most biologically active eicosanoids and are involved in key reproductive processes such as follicular development (Wallach et al., 1975), ovulation (Espey, 1980), luteolysis (Wathes and Lamming, 1995), and parturition (Challis, 1980).

Essential PUFAs have been shown to inhibit PG secretion in several cell types (Levine and Worth, 1984; Achard et al., 1997) including bovine endometrial (BEND) cells (Mattos et al., 2003). The BEND cells are a line of spontaneously replicating

endometrial cells originating from cows in their 14th day of their estrous cycle (Staggs et al., 1998). Nutritional studies have shown that manipulation of dietary PUFA content may influence circulating concentrations of $PGF_{2\alpha}$. For example, dietary n-6 and n-3 PUFA have the ability to alter gestational length and time of parturition through modulation of PG synthesis in rats (Holman, 1971; Leaver et al., 1986), ewes (Baguma-Nibasheka et al., 1999), and humans (Olsen et al., 1986; 1992; Allen and Harris, 2001). Although there is some evidence indicating that n-6 PUFAs, LA specifically, enhance PG production by providing more precursors for conversion to AA (Connolly et al., 1996; Nakaya et al., 2001; Elmes et al., 2004; Petit et al., 2004), other studies have found an inhibitory effect of n-6 PUFAs on PG synthesis (Elattar and Lin, 1989; Pace-Asciak and Wolfe, 1968; Cheng et al., 2001). A number of studies have demonstrated inhibitory effects of n-3 PUFAs on $PGF_{2\alpha}$ production (Bezard et al., 1994; Staples et al., 1998; Abayasekara and Wathes, 1999; Mattos et al., 2000, 2001, 2002, 2004). Supplementation of dairy cattle with fish oil (high in n-3 PUFAs) greatly reduced plasma PGFM response to oxytocin (Mattos et al., 2002, 2004). It also has been suggested that fish oil may reduce the sensitivity of the CL to $PGF_{2\alpha}$ since cycling cows fed fish meal had higher P_4 plasma concentrations after injection of a luteolytic dose of $PGF_{2\alpha}$ (Burke et al., 1997). Attenuation of uterine $PGF_{2\alpha}$ secretion and decreased sensitivity of the CL to this PG caused by PUFAs may lead to improved fertility through enhanced luteal function, reduced embryonic loss and increased pregnancy rates.

The objective of this investigation was to examine the effects of n-6 and n-3 PUFAs on phorbol 13, 14-dibutyrate (PDBu)-induced $PGF_{2\alpha}$ biosynthesis in BEND cells.

We hypothesized that PUFAs may be more effective in inhibiting $PGF_{2\alpha}$ production when compared to saturated fatty acids.

Materials and Methods

Materials

Polystyrene tissue culture dishes (100 x 20 mm) were purchased from Corning (Corning Glass Works, Corning, NY). The Ham F-12 medium, antibiotic/antimycotic (ABAM), phorbol 13, 14-dibutyrate (PDBu), horse serum, D-valine, insulin, fatty acid-free bovine serum albumin (BSA), stearic acid (ST, $C_{18:0}$), aprotinin, leupeptin, and pepstatin were from Sigma Chemical Co. (St. Louis, MO). The Minimum Essential Medium (MEM) and fetal bovine serum (FBS) were from US Biologicals (Swampscott, MA) and Atlanta Biologicals (Norcross, GA), respectively. Linoleic acid ($C_{18:2}$n-6), LNA ($C_{18:3}$n-3), eicosapentaenoic acid (EPA, $C_{20:5}$n-3), PGHS-2 and PGES antibodies, and $PGF_{2\alpha}$ standard were from Cayman Chemicals (Ann Arbor, MI). The PPARδ and secondary anti-rabbit IgG antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The enhanced chemiluminescence (ECL) kit was purchased from Perkin Elmer (Boston, MA). Hanks Balanced Salt Solution (HBSS) and TriZol reagent were from GIBCO BRL (Carlsbad, CA). Isotopically-labelled $PGF_{2\alpha}$ (5, 6, 8, 9, 11, 12, 14, 15[n-^{3}H] $PGF_{2\alpha}$; 208 Ci/mmol) was from Amershan Biosciences (Piscataway, NJ). The anti-$PGF_{2\alpha}$ antibody was purchased from Oxford Biomedicals (Oxford, MI). BioTrans nylon membrane and [α-^{32}P]deoxycitidine triphosphate (SA 3000 Ci/nmol) were from MP Biomedicals (Atlanta, GA). The PGHS-2 cDNA probe was cloned from an ovarian follicular cDNA library (Liu et al., 1999), the PGES cDNA probe was cloned from

endometrial cDNA (Guzeloglu et al., 2004), whereas the PPARδ probe was generated from bovine endometrial RNA (Balaguer et al., 2005).

Cell Culture and Treatment

Bovine endometrial (BEND; American Type Culture Collection # CRL-2398; Manassas, VA) cells were plated on 100-mm tissue culture plates in complete culture medium (40% Ham F-12, 40% MEM containing 10 ml ABAM/L, 0.0343g D-valine/L, 200 U insulin/L, 10% fetal bovine serum and 10% horse serum) and grown to confluence at 37°C in a humidified atmosphere containing 95% O_2 and 5% CO_2. Cells then were rinsed twice with Hanks balanced salt solution (HBSS), and cultured in fresh serum-free medium containing appropriate treatments for an additional 24 h.

To examine the effects of PDBu on $PGF_{2\alpha}$ production, subconfluent BEND cells were incubated with medium alone (control, n=2) or medium supplemented with 100 ng/ml PDBu (n=2) for 6 h. After incubation, aliquots (1 ml) of cell-conditioned media were collected and stored at -20°C for subsequent analysis of $PGF_{2\alpha}$ using RIA. The entire experiment was repeated five times.

To investigate the effects of supplemental PUFAs on uterine $PGF_{2\alpha}$ synthesis, BEND cells were treated with PDBu alone (100 ng/ml) or with a combination of PDBu and stearic (ST; 100 μM), LA (100 μM), LNA (100 μM) or EPA (100 μM). Subconfluent cells were incubated with serum-free medium alone (PDBu) or with appropriate treatments (listed above) complexed with BSA (1:3 ratio) for a period of 24 h. Cells then were rinsed twice with HBSS, and challenged with phorbol ester 12, 13-dibutyrate (PDBu, 100 ng/ml) for an additional 6 h. Control plates were cultured in the absence of PDBu. Samples of conditioned media (1 ml/plate) were collected after PDBu

challenge and stored at -20°C until analyzed for $PGF_{2\alpha}$ concentration. The remaining cell monolayer was rinsed with HBSS, lysed in TriZol reagent, and stored at -80°C for subsequent mRNA analysis. For analysis of protein abundance, cells were collected and lysed as described later in the section for Western blot analysis. These experiments were repeated two times.

$PGF_{2\alpha}$ Radioimmunoassay

The concentration of $PGF_{2\alpha}$ in cell-conditioned media was measured as described by Danet-Desnoyers et al. (1994) and modified by Binelli et al. (2000). One hundred and fifty microliters of Tris buffer (50 mM Tris-HCl, 1 g/l sodium azide, pH 7.5), 50 µl of conditioned media or standards (range 10 – 1000 pg/tube), 100 µl of Tris buffer/serum-free medium, 100 µl of anti-$PGF_{2\alpha}$ antibody (1: 30,000 in Tris buffer) and 100 µl of ^{3}H-$PGF_{2\alpha}$ (18,000 dpm) were added sequentially to 12x75 mm disposable glass tubes, vortexed and incubated for 24 h at 4°C. After incubation, 500 µl of dextran-coated charcoal solution (25 mg dextran and 250 mg charcoal into 100 ml RIA buffer) was added, and the mixture was incubated for an additional 4 min at 4°C. Assay tubes were then centrifuged (3200 rpm for 15 min at 4°C), decanted and counted in a beta counter. Assay sensitivity was 0.5 ng/ml and intra- and inter-assay coefficients of variation were 8.2 and 15.8%, respectively. Final $PGF_{2\alpha}$ concentrations were expressed as picograms per milliliter.

RNA Isolation and Analysis

Total RNA was isolated using TriZol reagent, following the manufacturer's instructions. Ten µg of total RNA was fractioned in a 1.5 % agarose formaldehyde gel using the MOPS buffer (Fisher Scientific, Pittsburgh, PA) (Ing et al., 1996) and

transferred to a Biotrans nylon membrane by downward capillary transfer. The RNA was cross-linked to the nylon membrane by exposure to a UV light source for 90 sec and baked for 1 h. Membranes were prehybridized for 2 h at 42°C in ultrasensitive hybridization buffer (ULTRAhyb; Ambion, Austin, TX) followed by an overnight incubation at 42°C in the same ULTRAhyb solution containing the ^{32}P-labeled bovine prostaglandin H synthase 2 (PGHS-2), prostaglandin E synthase (PGES), and peroxisome proliferator-activated receptor δ (PPARδ) cDNA probes. Filters were sequentially washed in 2X SSC (1X= 0.15 M sodium chloride, 0.015 M sodium citrate)-0.1% SDS and in 0.1x SSC-0.1% SDS two times each at 42°C and then exposed to X-ray film to detect radiolabeled bands. Equal loading of total RNA for each experimental sample was verified by comparison to 18S rRNA ethidium bromide staining.

Western Blot Analysis of PGHS-2, PGES and PPARδ

For Western blot analyses, whole cell lysates were prepared as described by Binnelli et al. (2000). Five hundred microliters of ice-cold whole cell extract buffer (50 mM Tris, pH 8.0, 300 mM NaCl, 20 mM NaF, 1 mM Na_3VO_4, 1 mM $Na_4P_2O_7$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.5 mM PMSF, 10% v/v glycerol, 0.5% v/v NP-40, and 10 μg/ml each of Aprotinin, Leupeptin, and Pepstatin), were added to each dish and cells were collected in 1.5 ml microcentrifuge tubes and incubated for 30 min at 4°C. Cell lysates then were centrifuged at 4°C for 10 min (13000 rpm) to remove cell debris. Protein concentration in the supernatant was determined by the Lowry method (Lowry et al., 1951). Protein (25 μg) from each dish was resolved on a 7.5% SDS-PAGE, and electrophoretically transferred to a nitrocellulose membrane. The Membrane was blocked for 2 h in 5% (w/v) nonfat dried milk in Tris-buffered saline (TBS)

containing 0.1% Tween-20 (TBST, pH 7.4), rinsed with TBST and hybridized with antibodies against either PGHS-2, PGES, or PPARδ diluted (1:500) in 5% nonfat dried milk in TBS. The secondary antibody was anti-rabbit IgG (1:3000 in 5% nonfat dried milk in TBS). Target proteins were detected by enhanced chemiluminescence.

Statistical Analyses

Data were analyzed by Least-Squares analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2001). For $PGF_{2\alpha}$ response, the mathematical model included fixed effects of treatment (n = 5), experiment (n = 5), treatment by experiment interaction, and random effect of dish (n = 2/treatment) nested within treatment by experiment interaction. The variance of dish nested within treatment by experiment interaction was used as the error term for all effects. For Northern and western blot data, hybridization volumes obtained from densitometric analysis were subjected to ANOVA using the GLM procedure. For Northern blot analysis, the mathematical model included independent fixed effects of treatment (n = 5), experiment (n = 2), treatment by experiment interaction, and random residual error. Results are presented as ratios of densitometric values for the target mRNA over those for 18S rRNA ethidium bromide staining, and are presented as LS means ± SEM. For Western blot analysis, the statistical model included only the main effect of treatment. Treatment effects were further analyzed using preplanned orthogonal contrasts. These contrasts were constructed to compare PDBu vs. fatty acids responses; SFA vs. PUFA; n-6 vs. n-3 PUFA; and n-3 LNA vs. longer chain n-3 EPA.

Results

To test the hypothesis that PUFAs may be more effective in inhibiting endometrial $PGF_{2\alpha}$ secretion, we examined endometrial $PGF_{2\alpha}$ response to PDBu in the presence of n-6 (LA) and n-3 (LNA and EPA) PUFAs in comparison to saturated fatty acid (ST).

In vitro systems designed to evaluate endometrial $PGF_{2\alpha}$ secretion involved stimulation of cells with PDBu, an activator of protein kinase C which induces PGHS-2 gene expression and secretion of $PGF_{2\alpha}$ (Binelli et al., 2000). Treatment of BEND cells with PDBu resulted in a 25-fold induction ($P < 0.0001$) of $PGF_{2\alpha}$ secretion (Figure 3-1). The PDBu-induced $PGF_{2\alpha}$ secretion coincided with increased abundance of PGHS-2 mRNA ($P < 0.0001$; Figure 3-2) and protein ($P = 0.0003$; Figure 3-3). Stimulation of BEND cells with PDBu also resulted in induction ($P < 0.0001$) of PGES gene expression (Figure 3-4), but no differences were observed for PGES protein abundance (Figure 3-5).

Co-incubation of BEND cells with fatty acids decreased ($P < 0.0001$) $PGF_{2\alpha}$ response to PDBu (Figure 3-6). Analysis of individual fatty acid effects revealed that EPA greatly reduced $PGF_{2\alpha}$ induction by PDBu. The other fatty acids had minimal effects on PDBu-induced prostaglandin production.

To determine the molecular mechanism by which fatty acids altered $PGF_{2\alpha}$ production in BEND cells, we examined PGHS-2, PGES and PPARδ mRNA and protein responses to PDBu in the presence of various fatty acids. On average, long-chain fatty acids had no detectable effects on PGHS-2, PGES and PPARδ mRNA responses to PDBu (Figures 3-7, 3-9 and 3-11). The long-chain fatty acids stimulated ($P = 0.0001$) PGES protein expression, but failed to alter PGHS-2 or PPARδ protein response to PDBu (Figures 3-8, 3-10 and 3-12).

Discussion

Polyunsaturated fatty acids have been associated with improvement of reproductive efficiency in cattle, and modulation of prostaglandin synthesis has being suggested as a potential mechanism. In the present study, we examined the effects of selected long-chain fatty acids on PDBu-induced $PGF_{2\alpha}$ synthesis in BEND cells. On average, the long-chain fatty acids tested in this study decreased $PGF_{2\alpha}$ response to PDBu in BEND cells. EPA decreased $PGF_{2\alpha}$ production to a greater extent than did the other fatty acids. However, both saturated and unsaturated fatty acids had no detectable effects on PGHS-2, PGES and PPARδ mRNA responses to PDBu.

Reports on the effects of feeding n-6 PUFA on prostaglandin synthesis *in vivo* have been inconsistent. Recent studies showed that *in vitro* supplementation of LA to maternal intercotyledonary endometrial cells isolated from late pregnant ewes caused a significant reduction of 2-series prostaglandins (Cheng et at., 2003, 2004). Similarly, cyclic dairy cows fed a diet high in LA had reduced endometrial prostaglandin production (Cheng et al., 2001). On the other hand, Cheng and others (2005) showed that late pregnant ewes fed a diet high in LA had increased endometrial and placental prostaglandin production. In the present study, incubation of BEND cells with EPA caused a reduction of $PGF_{2\alpha}$ secretion in response to PDBu stimulation. These findings are in agreement with reports indicating that n-3 PUFAs inhibit prostaglandin secretion *in vitro* (Achard et al., 1997; Levine and Worth, 1984; Mattos et al., 2001, 2003; Caldari-Torres et al., 2006) and *in vivo* (Staples et al., 1998; Abayasekara and Wathes, 1999; Mattos et al., 2000, 2002, 2004).

The mechanisms by which EPA alters $PGF_{2\alpha}$ response to PDBu are not well understood. It has been proposed that PUFAs may reduce $PGF_{2\alpha}$ production through modulation of one or more steps of the PG biosynthetic pathway. These include decreasing availability of the precursor AA, competing for PGHS-2 activity and directly inhibiting synthesis and/or activity of PGHS-2 (Mattos et al., 2000; Cheng et al., 2001). In the present study, no changes were observed in PGHS-2 mRNA response to PDBu. This is consistent with a previous study (Mattos et al., 2003) and suggests that EPA may be competing with AA for the available binding sites on PGHS-2, thus shifting to production of 3-series prostaglandins ($PGF_{3\alpha}$ and PGE_3). This hypothesis does not rule out the possibility that EPA may also affect the PGHS-2 enzyme activity through posttranslational modifications.

The observation that the long-chain fatty acids tested in this study had no effects on PPARδ gene or protein expression indicates that these fatty acids likely affect $PGF_{2\alpha}$ production through mechanisms which do not involve PPARδ induction. However, whether or not long-chain fatty acids alter PPARδ activity warrants further investigation.

Summary

Phorbol-ester stimulated $PGF_{2\alpha}$ production and up-regulated PGHS-2 gene and protein expression within 6 h in cultured BEND cells. Priming of BEND cells with ST, LNA, and EPA reduced $PGF_{2\alpha}$ response to PDBu by 17%, 14%, and 66%, respectively. Both saturated and unsaturated fatty acids had not detectable effects on PGHS-2, PGES or PPARδ mRNA response to PDBu, suggesting that long-chain fatty acids tested in this study likely affect $PGF_{2\alpha}$ production through PGHS-2-, PGES-, and PPARδ-independent mechanisms. Whether and how these fatty acids affect the activities of various enzymes

and transcription factors involved in the $PGF_{2\alpha}$ biosynthetic cascade warrants further information.

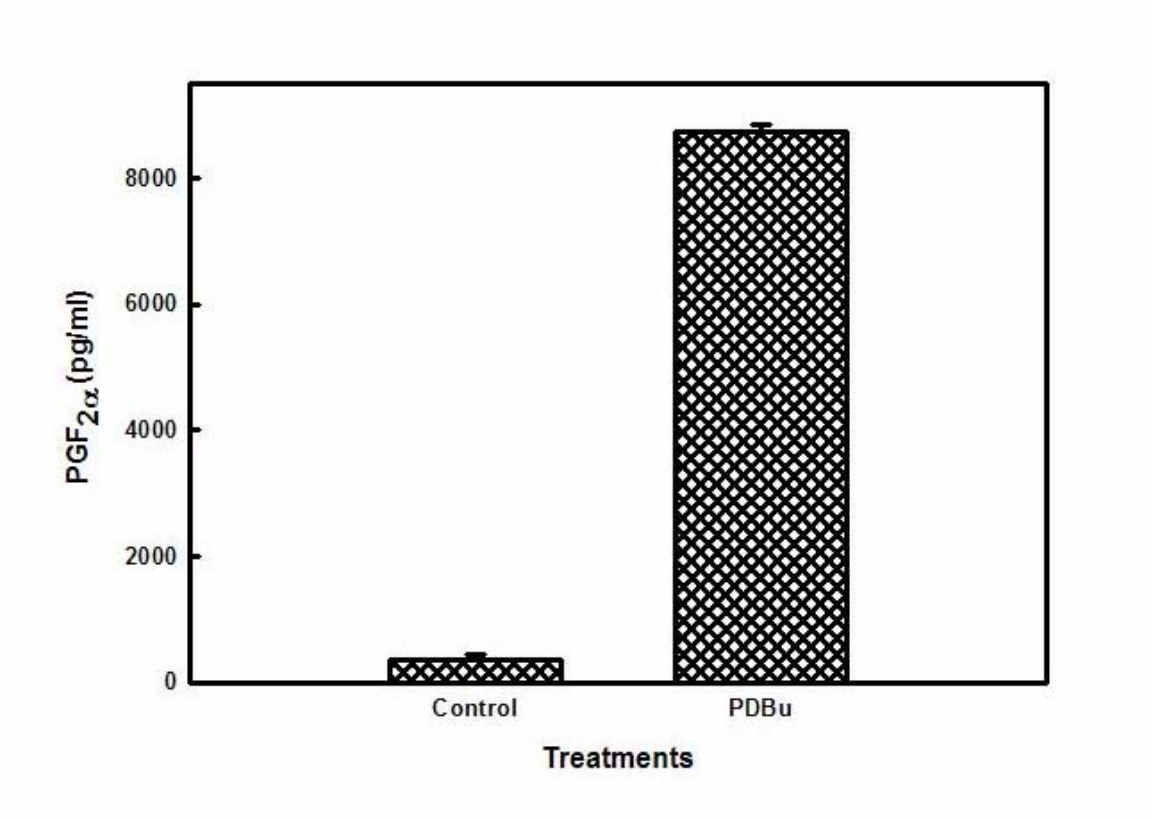

Figure 3-1. Effect of phorbol 12, 13 dibutyrate (PDBu) on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secretion in bovine endometrial (BEND) cells. Treatment by experiment interaction was significant ($P < 0.05$). Data represents least square means ± SEM of five independent experiments (treatment effect, $P < 0.0001$).

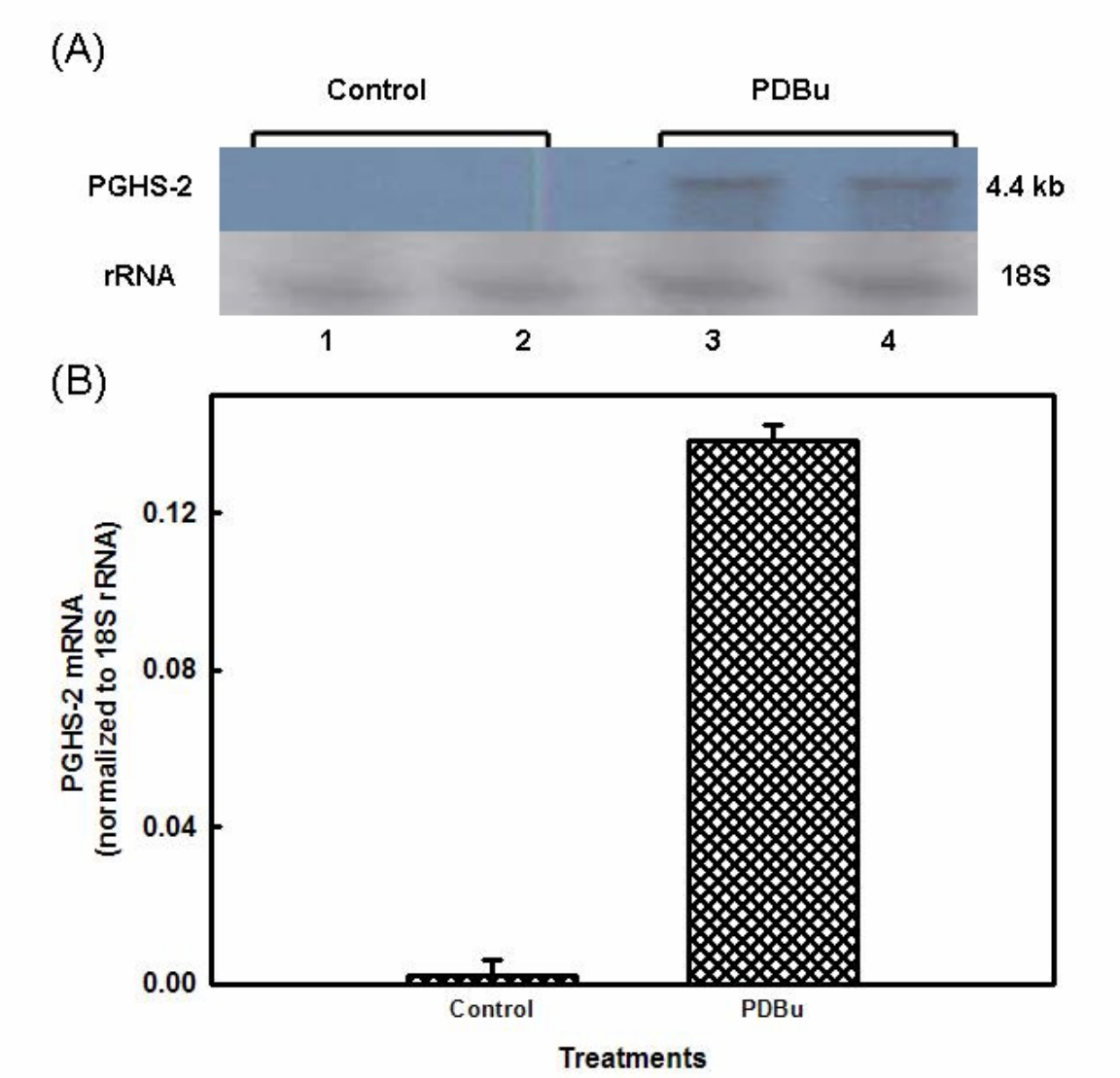

Figure 3-2. Effect of phorbol 12, 13 dibutyrate (PDBu) on prostaglandin endoperoxide synthase (PGHS-2) mRNA abundance in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment, $P < 0.0001$). There was no treatment by experiment interaction ($P = 0.06$).

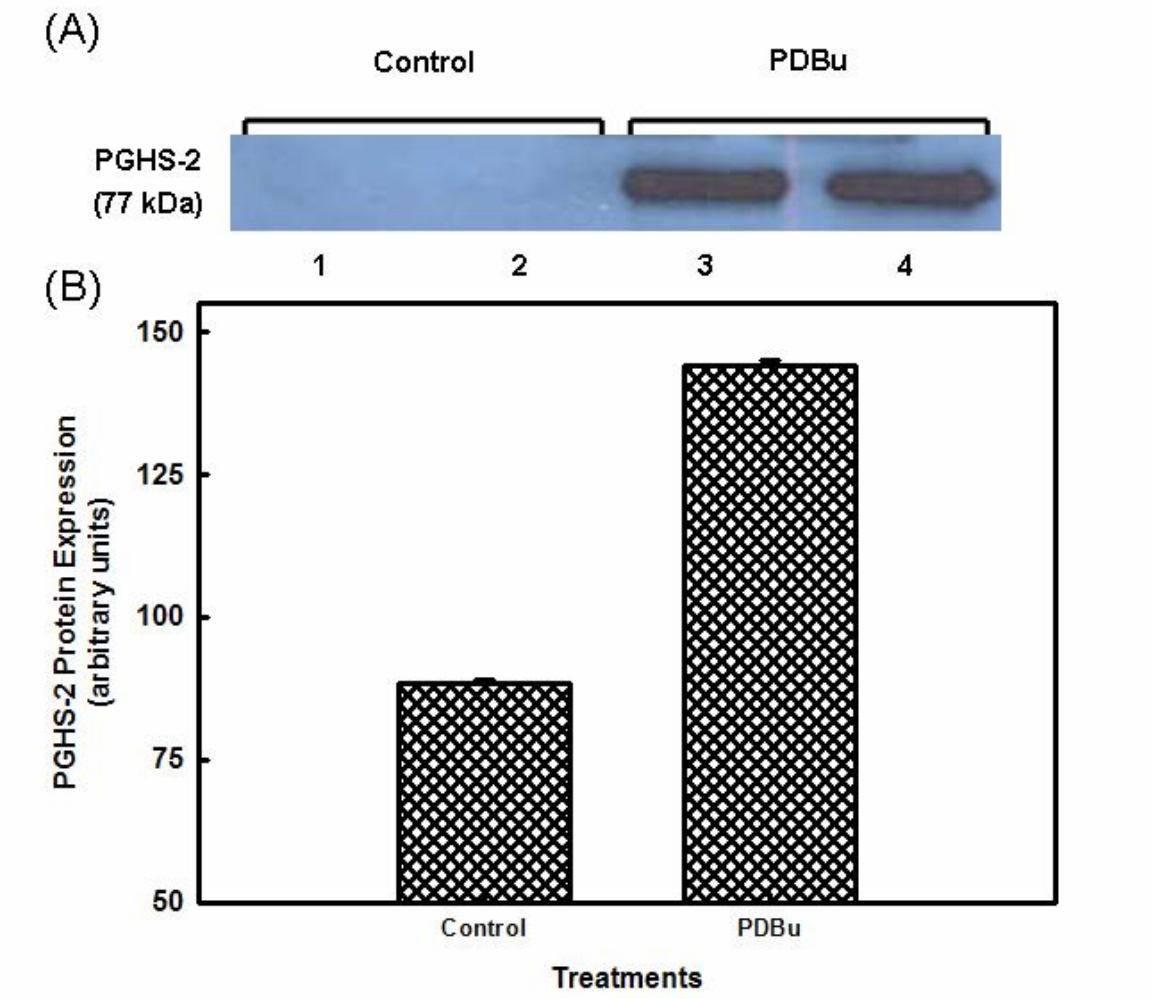

Figure 3-3. Effect of phorbol 12, 13 dibutyrate (PDBu) on prostaglandin endoperoxide synthase (PGHS-2) protein levels in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment, $P = 0.0003$).

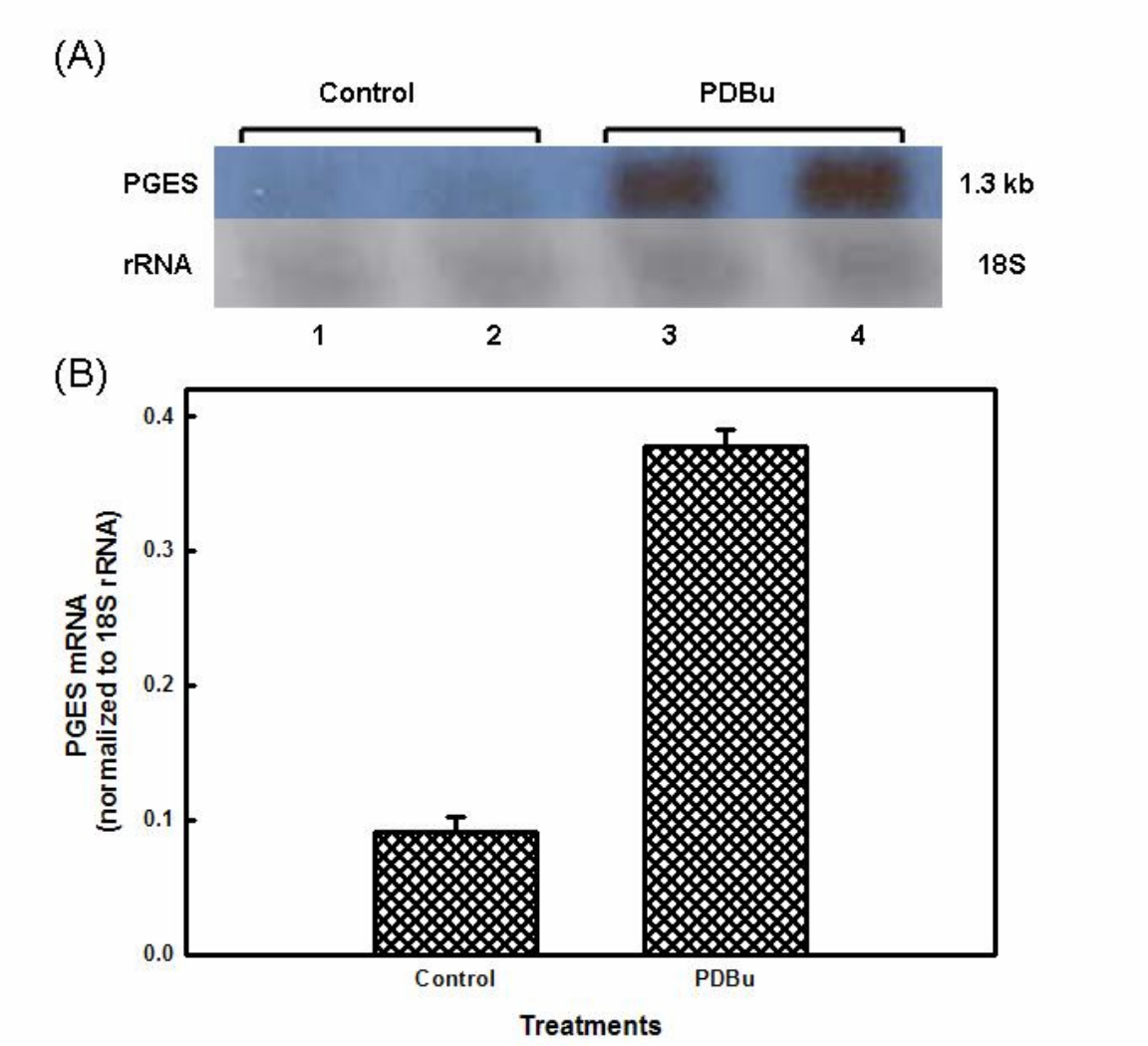

Figure 3-4. Effect of phorbol 12, 13 dibutyrate (PDBu) on prostaglandin E synthase (PGES) mRNA abundance in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment, $P < 0.0001$). Treatment by experiment interaction was significant ($P = 0.009$).

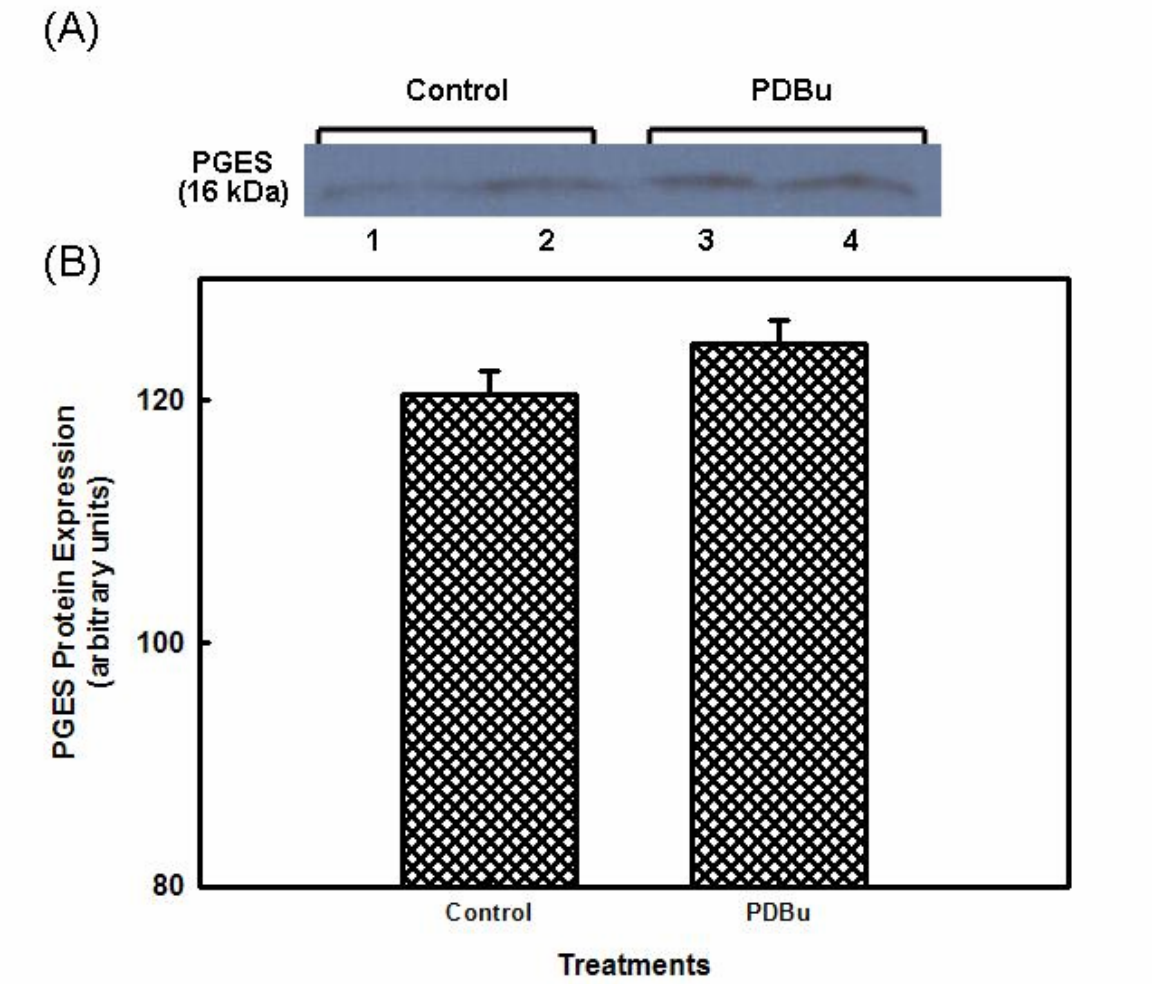

Figure 3-5. Effect of phorbol 12, 13 dibutyrate (PDBu) on prostaglandin E synthase (PGES) protein levels in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment, P = 0.3).

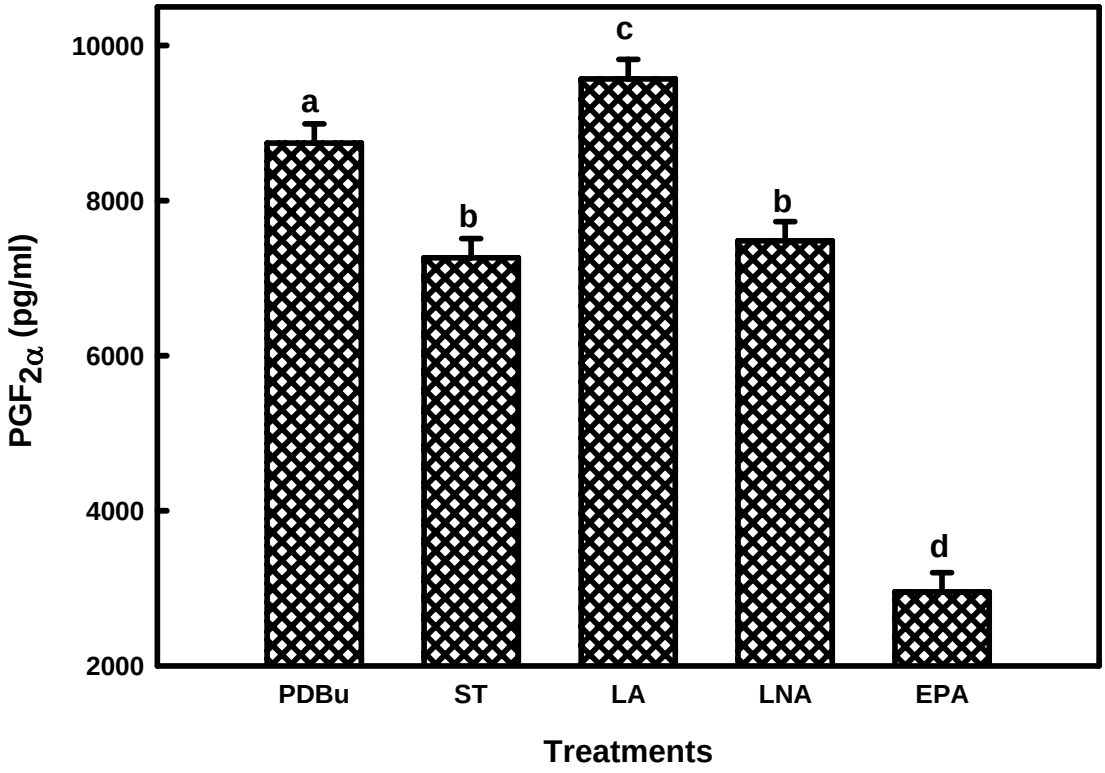

Figure 3-6. Effect of fatty acids on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (ST) + (LA) + (LNA) + (EPA), $P < 0.0001$. Contrast 2: (ST) vs. (LA) + (LNA) + (EPA), $P = 0.04$. Contrast 3: (LA) vs. (LNA) + (EPA), $P < 0.0001$. Contrast 4: (LNA) vs. (EPA), $P < 0.001$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. There was no treatment by experiment interaction ($P = 0.14$).

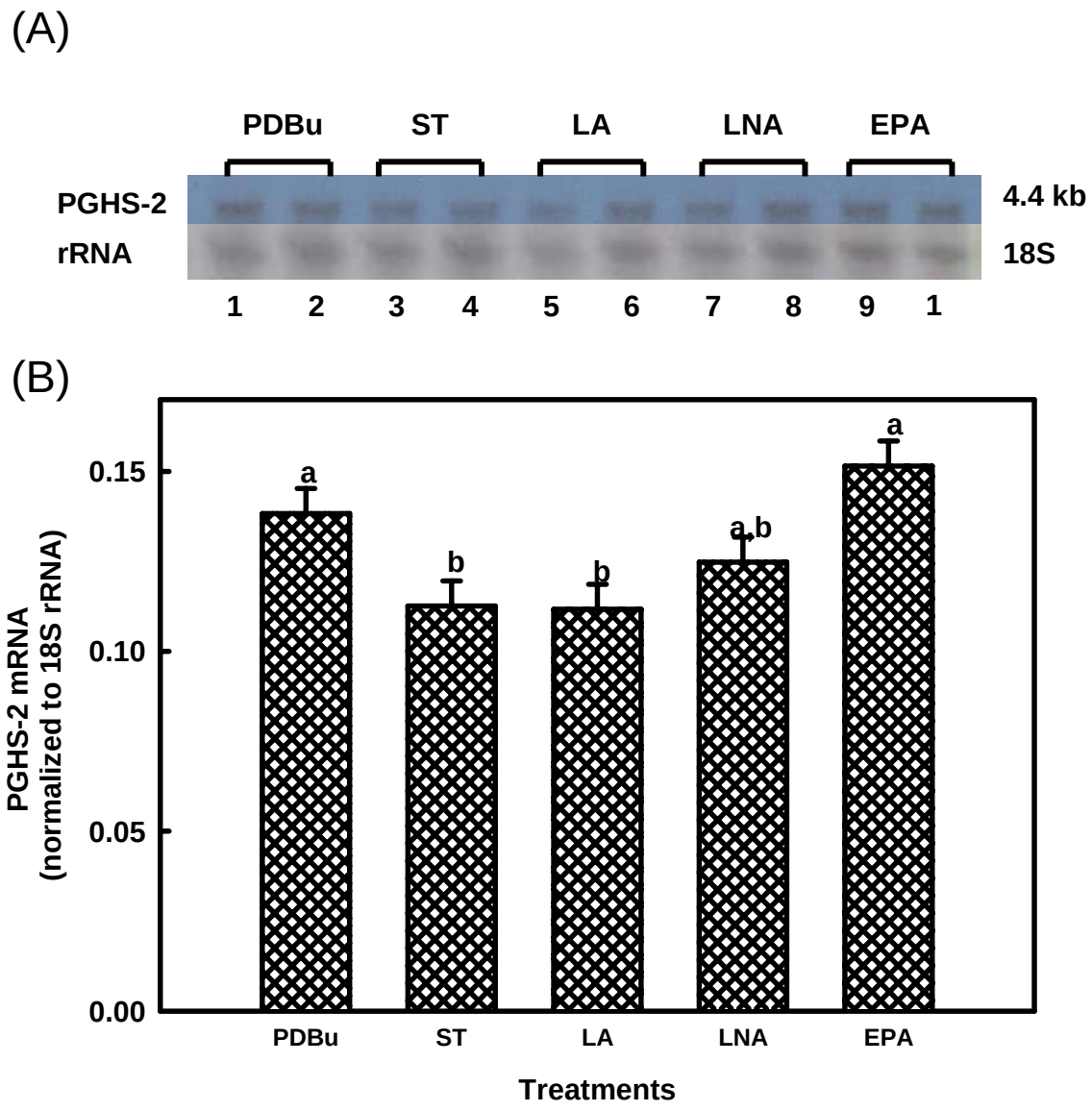

Figure 3-7. Effect of fatty acids on prostaglandin endoperoxide synthase (PGHS-2) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (ST) + (LA) + (LNA) + (EPA), $P = 0.1$. Contrast 2: (ST) vs. (LA) + (LNA) + (EPA), $P = 0.06$. Contrast 3: (LA) vs. (LNA) + (EPA), $P = 0.06$. Contrast 4: (LNA) vs. (EPA), $P = 0.02$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. There was no treatment by experiment interaction ($P = 0.38$).

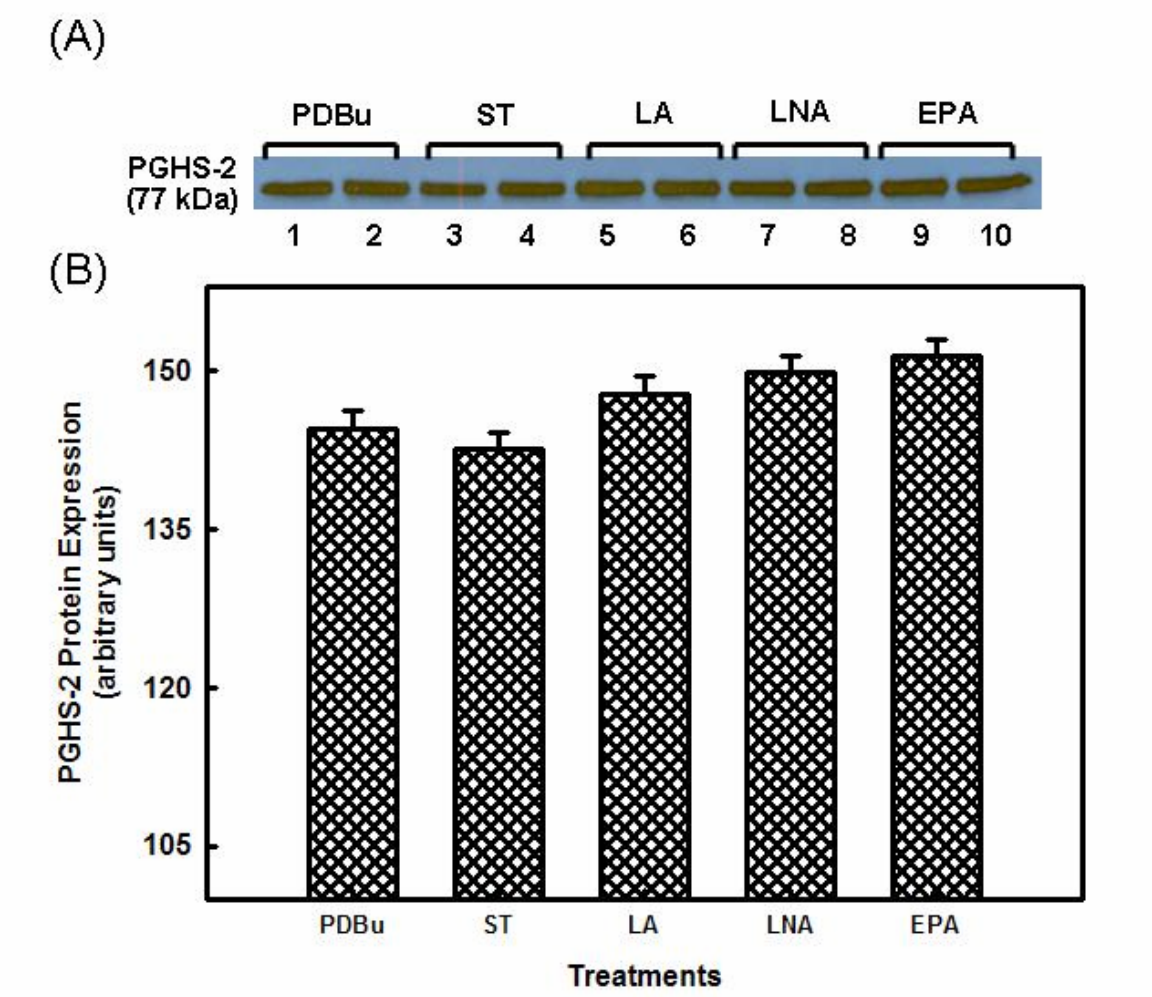

Figure 3-8. Effect of fatty acids on prostaglandin endoperoxide synthase (PGHS-2) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (ST) + (LA) + (LNA) + (EPA), $P = 0.1$. Contrast 2: (ST) vs. (LA) + (LNA) + (EPA), $P = 0.01$. Contrast 3: (LA) vs. (LNA) + (EPA), $P = 0.2$. Contrast 4: (LNA) vs. (EPA), $P = 0.5$.

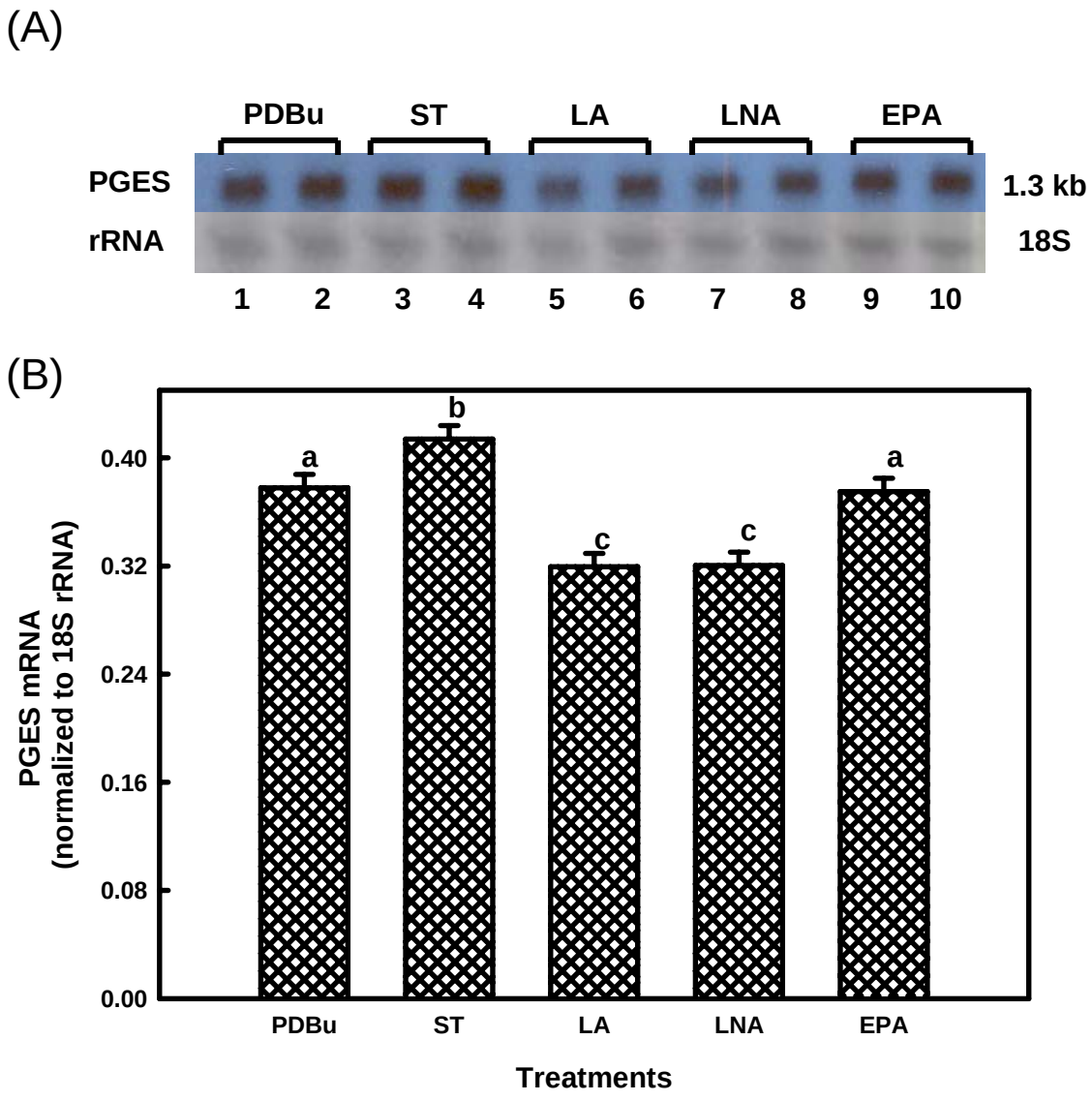

Figure 3-9. Effect of fatty acids on prostaglandin E synthase (PGES) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (ST) + (LA) + (LNA) + (EPA), $P = 0.09$. Contrast 2: (ST) vs. (LA) + (LNA) + (EPA), $P < 0.0001$. Contrast 3: (LA) vs. (LNA) + (EPA), $P = 0.04$. Contrast 4: (LNA) vs. (EPA), $P = 0.003$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. Treatment by experiment interaction was significant ($P = 0.0003$).

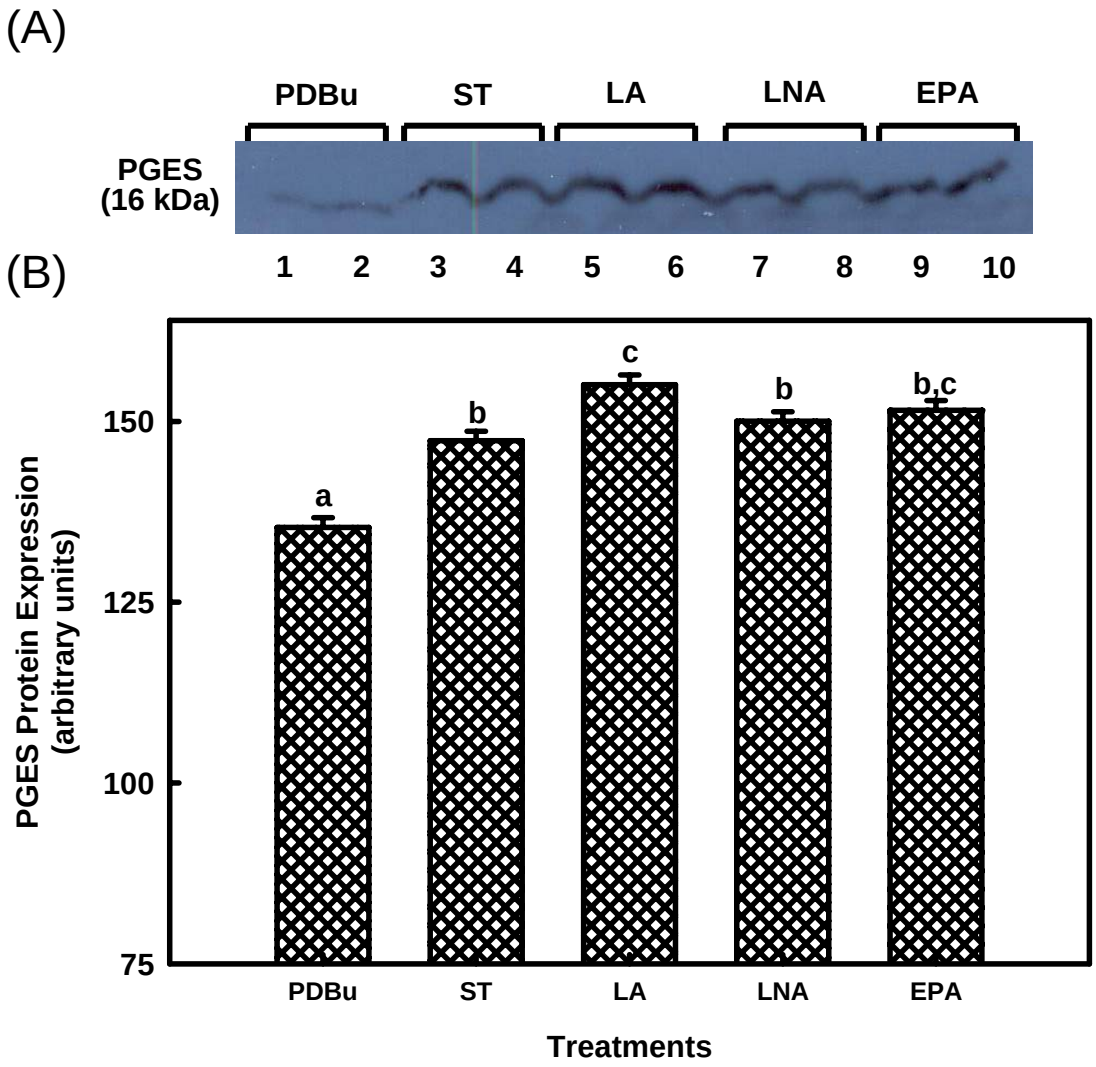

Figure 3-10. Effect of fatty acids on prostaglandin E synthase (PGES) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (ST) + (LA) + (LNA) + (EPA), $P = 0.0001$. Contrast 2: (ST) vs. (LA) + (LNA) + (EPA), $P = 0.02$. Contrast 3: (LA) vs. (LNA) + (EPA), $P = 0.05$. Contrast 4: (LNA) vs. (EPA), $P = 0.5$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters.

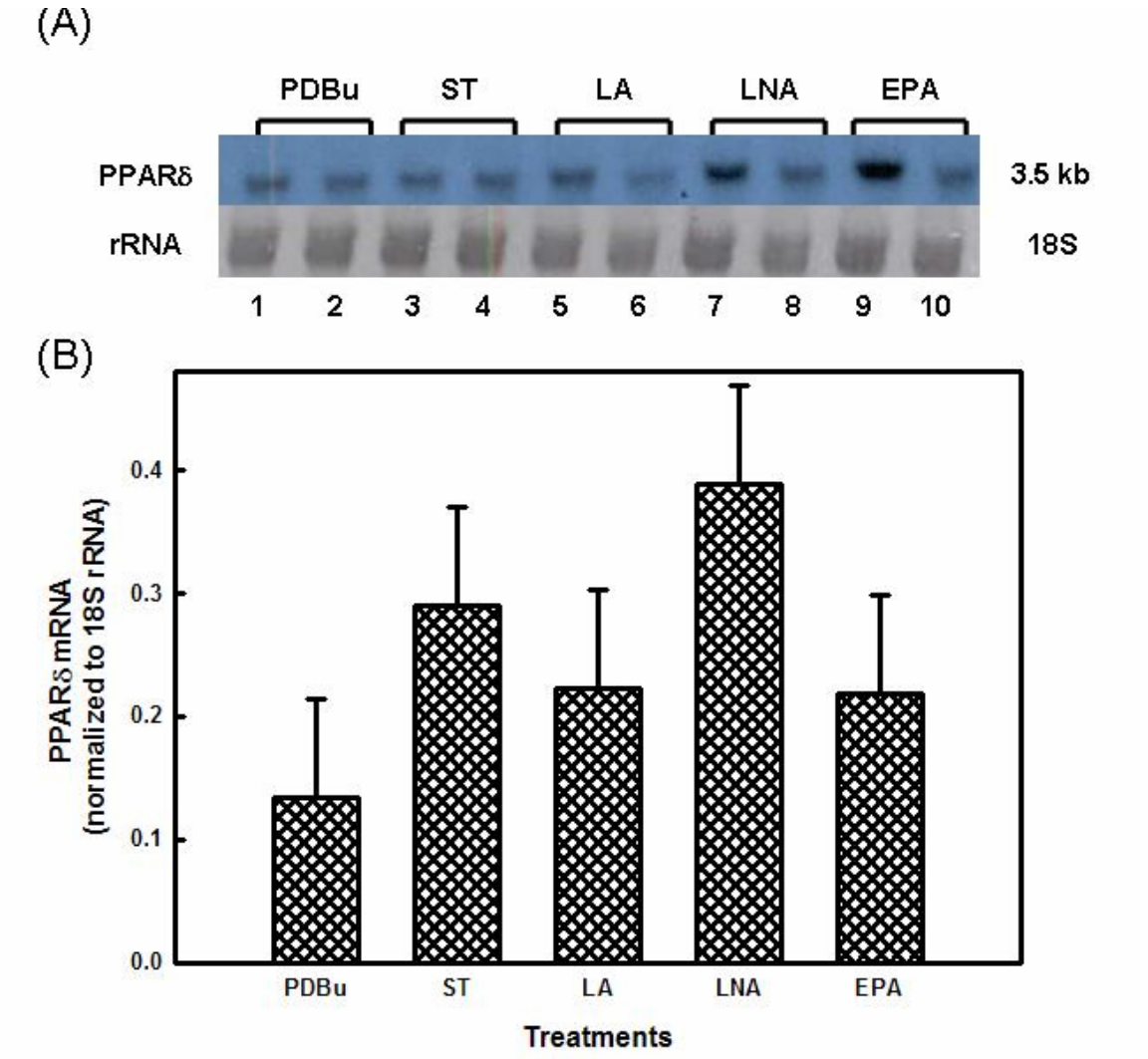

Figure 3-11. Effect of fatty acids on peroxisome proliferator-activated receptor δ (PPARδ) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (ST) + (LA) + (LNA) + (EPA), $P = 0.2$. Contrast 2: (ST) vs. (LA) + (LNA) + (EPA), $P = 0.9$. Contrast 3: (LA) vs. (LNA) + (EPA), $P = 0.5$. Contrast 4: (LNA) vs. (EPA), $P = 0.2$.

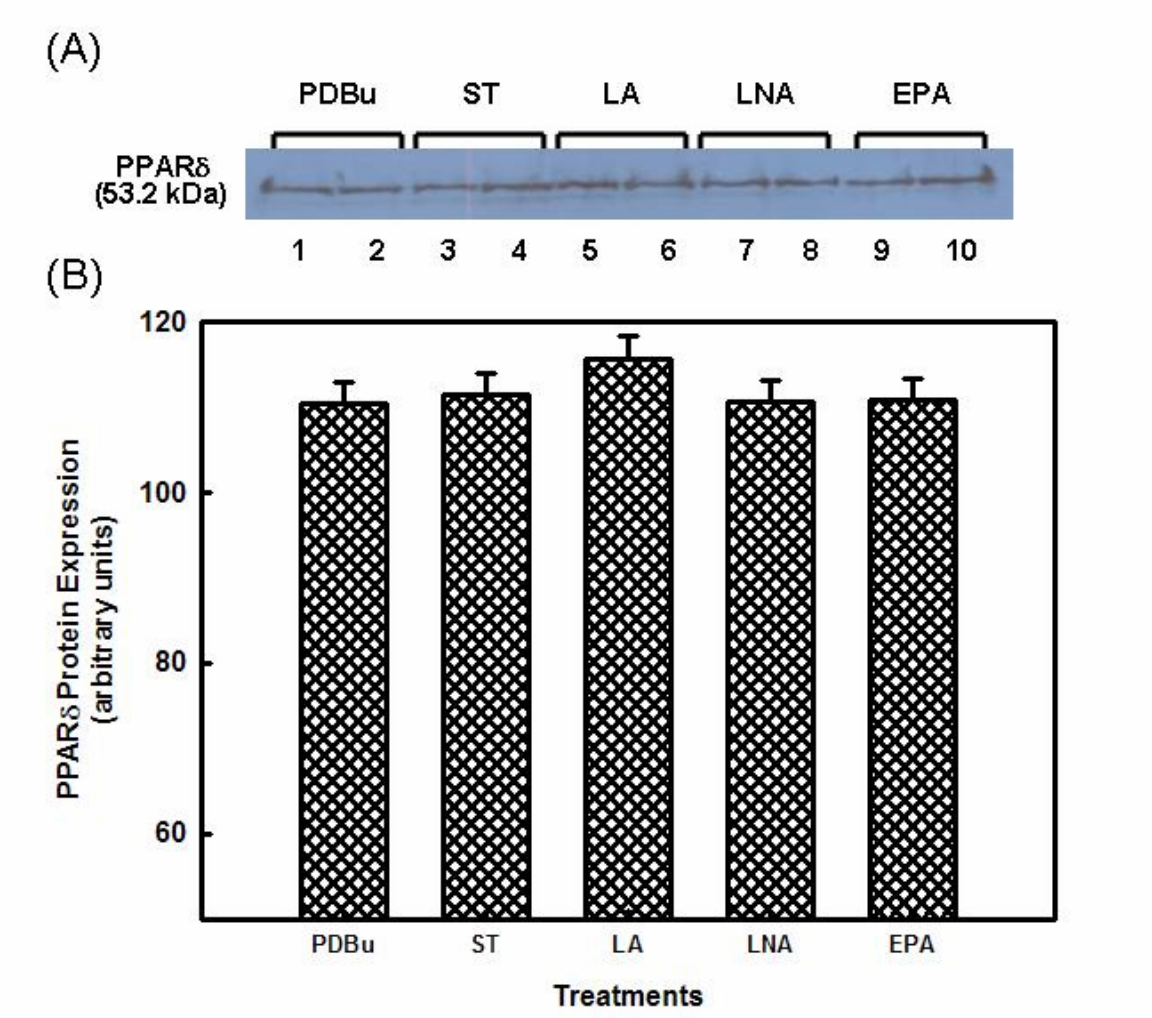

Figure 3-12. Effect of fatty acids on peroxisome proliferator-activated receptor δ (PPARδ) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (ST) + (LA) + (LNA) + (EPA), $P = 0.6$. Contrast 2: (ST) vs. (LA) + (LNA) + (EPA), $P = 0.8$. Contrast 3: (LA) vs. (LNA) + (EPA), $P = 0.2$. Contrast 4: (LNA) vs. (EPA), $P = 0.9$.

CHAPTER 4
EFFECTS OF CONJUGATED LINOLEIC ACIDS ON PROSTAGLANDIN $F_{2\alpha}$ PRODUCTION BY BOVINE ENDOMETRIAL CELLS

Introduction

Conjugated linoleic acid (CLA) refers to a group of geometrical and positional isomers of LA resulting from incomplete biohydrogenation in the rumen (Chin et al., 1992; Ma et al., 1999; Griinari et al., 2000). The number of double bonds remains the same as in the parent LA, but one of the double bonds is shifted to a new position by microbial isomerases. A broad number of *cis*- and *trans*- CLA isomers have been identified in food; however, the most commonly occurring CLA isomer is the *cis*-9, *trans*-11-octadecadienoic acid with minor but significant proportions of *trans*-10, *cis*-12-18:2 (Parodi, 1977, 1997; Chin et al., 1992; McGuire et al., 1998).

After ruminal synthesis of *cis*-9, *trans*-11 CLA, it may be absorbed in the small intestine or further biohydrogenated to *trans*-11-octadecenoic acid (*trans* vaccenic acid) by rumen microorganisms. The *trans*-11 18:1 can be reduced to stearic acid (18:0) or transported to peripheral tissues as MUFA. In the peripheral tissues (i.e. mammary gland, muscle), this *trans*-isomer may be converted back into *cis*-9, *trans*-11 CLA by the action of Δ9 desaturase (Holman and Mahfouz, 1981; Pollard et al., 1980). This appears to be a major source of *cis*-9, *trans*-11 CLA in the cow's milk (Corl et al., 1998; Griinari and Bauman, 1999; Santora et al., 2000). The *trans*-10, *cis*-12 CLA detected in ruminal tissues (Griinari and Bauman, 1999; Dhiman et al., 1999) seem to originate solely from

ruminal synthesis, since mammalian tissues do not have the Δ12 desaturase necessary for conversion of *trans*-10-octadecenoic acid (elaidic acid) back to *trans*-10, *cis*-12 CLA.

A large number of beneficial effects have been attributed to CLA ranging from enhancing feed efficiency and growth (Chin et al., 1994; Bee, 2000), decreasing body fat in mice (DeLany et al., 1999) and pigs (Dugan et al., 1997; Ostrowska et al., 1999), and reducing milk fat synthesis in lactating dairy cows (Griinari et al., 1998; Romo et al., 2000; Baumgard et al., 2001). Although it is likely that some biological effects of CLA may be induced and/or enhanced synergistically by these isomers, there is evidence suggesting that the effects of CLA are due to separate actions of these biologically active isomers (Pariza et al., 2000).

The anticarcinogenic effect of CLA has been linked to its ability to modulate eicosanoid production (Sugano et al., 1998). Recent studies detected an inhibitory effect of CLA on eicosanoid synthesis in various animal and cell systems (Liu and Belury, 1998; Kavanaugh et al., 1999; Uruquhart et al., 2002; Eder et al, 2003). Feeding pregnant rats with dietary CLA resulted in inhibition of uterine $PGF_{2\alpha}$ independent of PUFA content (Harris et al., 2001). In addition, Cheng et al. (2003) reported that treatment of endometrial cells isolated from late pregnant ewes with CLA suppressed $PGF_{2\alpha}$ in a dose dependent manner, while low doses of CLA stimulated PGE_2 generation. Cheng and coworkers (2003) showed that treatment of intercotyledonary endometrial cells with CLA resulted in a dose-dependent inhibition of $PGF_{2\alpha}$ production. Similarly, CLA has also been shown to inhibit $PGF_{2\alpha}$ synthesis in rat placental and uterine tissues (Harris et al., 2001). Prostaglandins of the 2-series are involved in several reproductive processes such as ovulation (Espey, 1980), follicular growth and development (Wallach

et al., 1975), and CL function (Abayasekara et al., 1995; Poyser, 1995; Wathes and Lamming, 1995). Hence, CLA isomers may regulate reproductive processes through modulation of $PGF_{2\alpha}$ biosynthesis.

The objective of this investigation was to examine the effects of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers on PBDu-induced $PGF_{2\alpha}$ production in bovine endometrial (BEND) cells. We hypothesized that both CLA isomers would be equally effective in inhibiting PDBu-induced $PGF_{2\alpha}$ production in BEND cells. Additionally, if these effects are specific to CLA, LA should have minimal effect on $PGF_{2\alpha}$ production.

Materials and Methods

Materials

Polystyrene tissue culture dishes (100 x 20 mm) were purchased from Corning (Corning Glass Works, Corning, NY). The Ham F-12 medium, antibiotic/antimycotic (ABAM), phorbol 13, 14-dibutyrate (PDBu), horse serum, D-valine, insulin, fatty acid-free bovine serum albumin (BSA), stearic acid (ST, $C_{18:0}$), aprotinin, leupeptin, and pepstatin were from Sigma Chemical Co. (St. Louis, MO). The Minimum Essential Medium (MEM) and fetal bovine serum (FBS) were from US Biologicals (Swampscott, MA) and Atlanta Biologicals (Norcross, GA), respectively. Linoleic acid (LA, $C_{18:2}$n-6), *cis*-9, *trans*-11 conjugated linoleic acid (CLA, $C_{18:2}$), *trans*-10, *cis*-12 CLA ($C_{18:2}$), PGHS-2 and PGES antibodies, and $PGF_{2\alpha}$ standard were from Cayman Chemicals (Ann Arbor, MI). The PPARδ and secondary anti-rabbit IgG antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The enhanced chemiluminescence (ECL) kit was purchased from Perkin Elmer (Boston, MA). Hanks Balanced Salt Solution (HBSS) and TriZol reagent were from GIBCO BRL (Carlsbad, CA). Isotopically-labelled $PGF_{2\alpha}$ (5,

6, 8, 9, 11, 12, 14, 15[n-^{3}H] $PGF_{2\alpha}$; 208 Ci/mmol) was from Amershan Biosciences (Piscataway, NJ). The anti-$PGF_{2\alpha}$ antibody was purchased from Oxford Biomedicals (Oxford, MI). BioTrans nylon membrane and [α-^{32}P]deoxycitidine triphosphate (SA 3000 Ci/nmol) were from MP Biomedicals (Atlanta, GA). The PGHS-2 cDNA probe was cloned from an ovarian follicular cDNA library (Liu et al., 1999), the PGES cDNA probe was cloned from endometrial cDNA (Guzeloglu et al., 2004), whereas the PPARδ probe was generated from bovine endometrial RNA (Balaguer et al., 2005).

Cell Culture and Treatment

Bovine endometrial (BEND) cells were plated and cultured as described in chapter 3. To investigate the effects of supplemental CLA acids on uterine $PGF_{2\alpha}$ synthesis, BEND cells were treated with PDBu alone (100 ng/ml) or PDBu in combination with 100 μM linoleic acid (LA), *cis*-9, *trans*-11 CLA or *trans*-10, *cis*-12 CLA. Fatty acids were complexed with BSA (1:3 ratio) for 2 h, and then treatments were applied to cells for a 24 h period. After treatment, cells were rinsed with HBSS and challenged with PDBu for another 6 h. The remaining cell monolayer was rinsed with HBSS, lysed in TriZol reagent, and stored at -80°C for subsequent mRNA analysis. This experiment was repeated two times. Radioimmunoassay of $PGF_{2\alpha}$ and Northern and Western blot analyses were performed as described in chapter 3.

Statistical Analyses

Data were analyzed by Least-Squares analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2001). For $PGF_{2\alpha}$ response, the mathematical model included fixed effects of treatment (n = 4), experiment (n = 4), treatment by experiment interaction, and random effect of dish (n =

2/trt) nested within treatment by experiment interaction. The variance of dish nested within treatment by experiment was used as the error term for all upstream effects. For Northern and western blot data, hybridization volumes obtained from densitometric analysis were subjected to ANOVA using the GLM procedure. For Northern blot analysis, the statistical model included independent fixed effects of treatment (n = 4), experiment (n = 2), treatment by experiment interaction, and random residual error. Results are presented as ratios of densitometric values for target genes over those for 18S rRNA ethidium bromide staining, and are presented as LS means ± SEM. For Western blot analysis, the mathematical model included only the main effect of treatment. Treatment effects were further analyzed using preplanned orthogonal contrasts. These contrasts were constructed to compare PDBu vs. fatty acids responses; LA vs. CLA isomers; and *cis*-9, *trans*-11 CLA vs. *trans*-10, *cis*-12 CLA.

Results

In the present study we used bovine endometrial (BEND) cells as a model to study CLA regulation of $PGF_{2\alpha}$ production. BEND cells are a line of spontaneously replicating endometrial cells originating from d 14 cycling cows (Staggs et al., 1998). Priming of BEND cells with *cis*-9, *trans*-11 or *trans*-10, *cis*-12 CLA isomers resulted in a 1.3-fold inhibition ($P < 0.0001$) of $PGF_{2\alpha}$ response to PDBu (Figure 4-1). Conversely, both CLA isomers stimulated ($P < 0.05$) PGHS-2 mRNA response to PDBu (Figure 4-2). Co-incubation with C18 fatty acids resulted in a significant reduction ($P < 0.003$) in PGES mRNA response to PDBu (Figure 4-3). In contrast with PGES gene expression, PPARδ mRNA levels were increased ($P < 0.0008$) by all the polyunsaturated fatty acids tested in

this study (Figure 4-6). Average PPARδ protein levels did not differ among treatments (Figure 4-7).

Discussion

Prostaglandins of the 2-series affect numerous processes in reproduction, including ovulation (Espey, 1980), follicular development (Wallach et al., 1975), corpus luteum function (Poyser, 1995; Wathes and Lamming, 1995; Abayasekara and Wathes, 1999) and parturition (Challis et al., 1997). The anticarcinogenic effect of CLA has been attributed partially to its inhibitory effect on eicosanoid synthesis (Banni et al., 1999; Gregory and Kelly, 2001).

In the present study, incubation of BEND cells with either *cis*-9, *trans*-11 or *trans*-10, *cis*-12 CLA isomers resulted in a 1.3-fold inhibition ($P < 0.0001$) of PDBu-induced $PGF_{2\alpha}$ secretion. This is in agreement with several studies that have detected inhibitory effect of CLA on eicosanoid synthesis *in vivo* and *in vitro* (Liu and Belury, 1998; Kavanaugh et al., 1999; Uruquhart et al., 2002; Eder et al., 2003). In addition, Cheng et al. (2003) reported that treatment of endometrial cells isolated from late pregnant ewes with CLA suppressed $PGF_{2\alpha}$ production. However, CLA inhibition of endometrial $PGF_{2\alpha}$ response to PDBu in BEND cells was not mediated through repression of PGHS-2 gene expression. On the contrary, priming of BEND cells with CLA isomers resulted in increased PGHS-2 mRNA steady-state levels, though no changes were observed at the protein level.

The mechanism of CLA inhibition of $PGF_{2\alpha}$ has not been fully elucidated. CLA may be competing with AA for incorporation into the membrane phospholipids as well as for PGHS-2 activity (Mattos et al., 2000; Cheng et al., 2001). Banni and others (1999)

showed that CLA supplementation to rat mammary tissue increased accumulation of CLA metabolites and decreased LA metabolites, such as AA. These isomers may also be directly inhibiting the activity of PGHS-2 (Mattos et al., 2000; Cheng et al., 2001). CLA metabolites have been shown to be powerful inhibitors of PGHS enzyme (Nugteren and Christ-Hazelhof, 1987).

Alternatively, CLA may regulate the conversion of $PGF_{2\alpha}$ to PGE_2, as reported by Gross and Williams (1988) in bovine placental cells. However, in this study, incubation of BEND cells with both CLA isomers resulted in reduced PGES mRNA expression, with no changes in protein concentration. This finding provides no evidence that CLA favors PGE_2 synthesis at the expense of $PGF_{2\alpha}$ production. Whether CLA affects PGES activity was not documented in this study.

CLA is a naturally occurring ligand of PPARs (Moya-Camarena et al., 1999). Priming of BEND cells with *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers induced PPARδ gene expression, but no changes were observed in PPARδ protein concentration. There was no correlation between PPARδ mRNA levels and $PGF_{2\alpha}$ concentration in cell-conditioned medium. Collectively, these findings indicate that CLA-induced attenuation of $PGF_{2\alpha}$ secretion in BEND cells is not mediated through modulation of PGHS-2 or PPARδ gene expression. Whether and how CLA isomers affect PGHS-2 enzymatic activity warrants further study.

Summary

Evidence is rapidly accumulating that CLA isomers modulate eicosanoid biosynthesis in various cell systems. In the present study, priming of BEND cells with *cis*-9, *trans*-11 or *trans*-10, *cis*-12 CLA isomers greatly decreased $PGF_{2\alpha}$ response to

PDBu. Interestingly, co-incubation with both CLA isomers increased PGHS-2 mRNA abundance in PDBu-stimulated BEND cells, suggesting that these fatty acids alter $PGF_{2\alpha}$ production through a mechanism that does not require repression of PGHS-2 gene expression. Further studies are needed to test whether or not CLA isomers modulate the activity of various enzymes and transcription factors involved in the prostaglandin biosynthetic cascade.

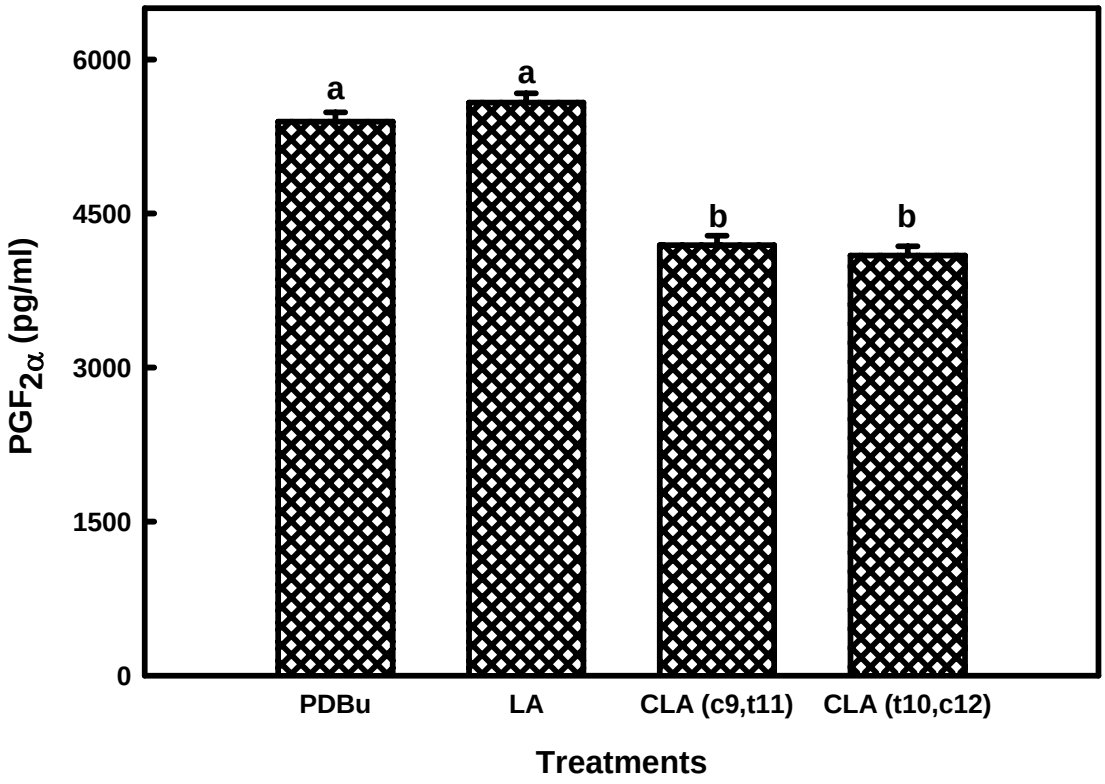

Figure 4-1. Effect of *c*9, *t*11 and *t*10, *c*12 CLA isomers on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (LA) + (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.0001$. Contrast 2: (LA) vs. (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.0001$. Contrast 3: (*cis*-9, *trans*-11) vs. (*trans*-10, *cis*-12), $P = 0.44$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. Treatment by experiment interaction was significant ($P < 0.0001$).

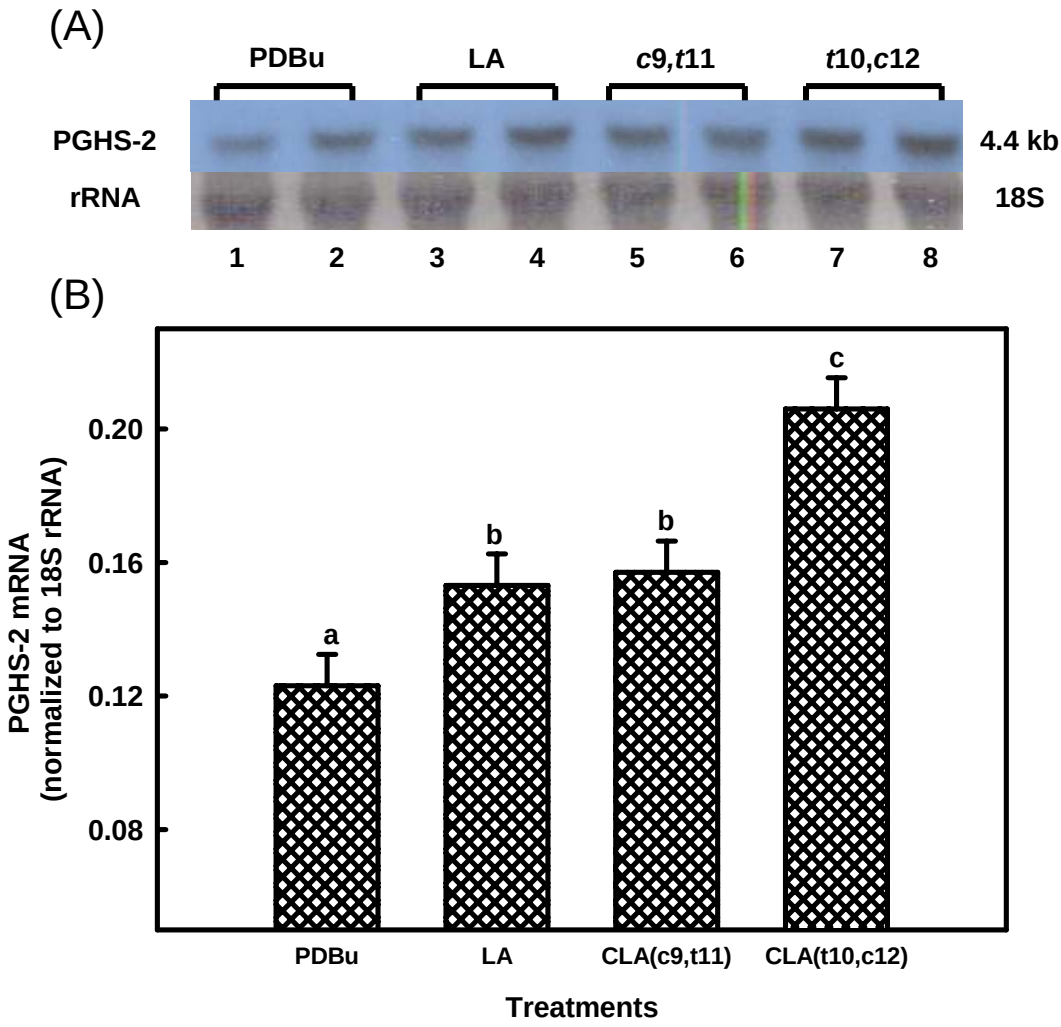

Figure 4-2. Effect of *c*9, *t*11 and *t*10, *c*12 CLA isomers on prostaglandin endoperoxide synthase (PGHS-2) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (LA) + (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.002$. Contrast 2: (LA) vs. (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.04$. Contrast 3: (*cis*-9, *trans*-11) vs. (*trans*-10, *cis*-12), $P = 0.006$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. There was no treatment by experiment interaction ($P = 0.65$).

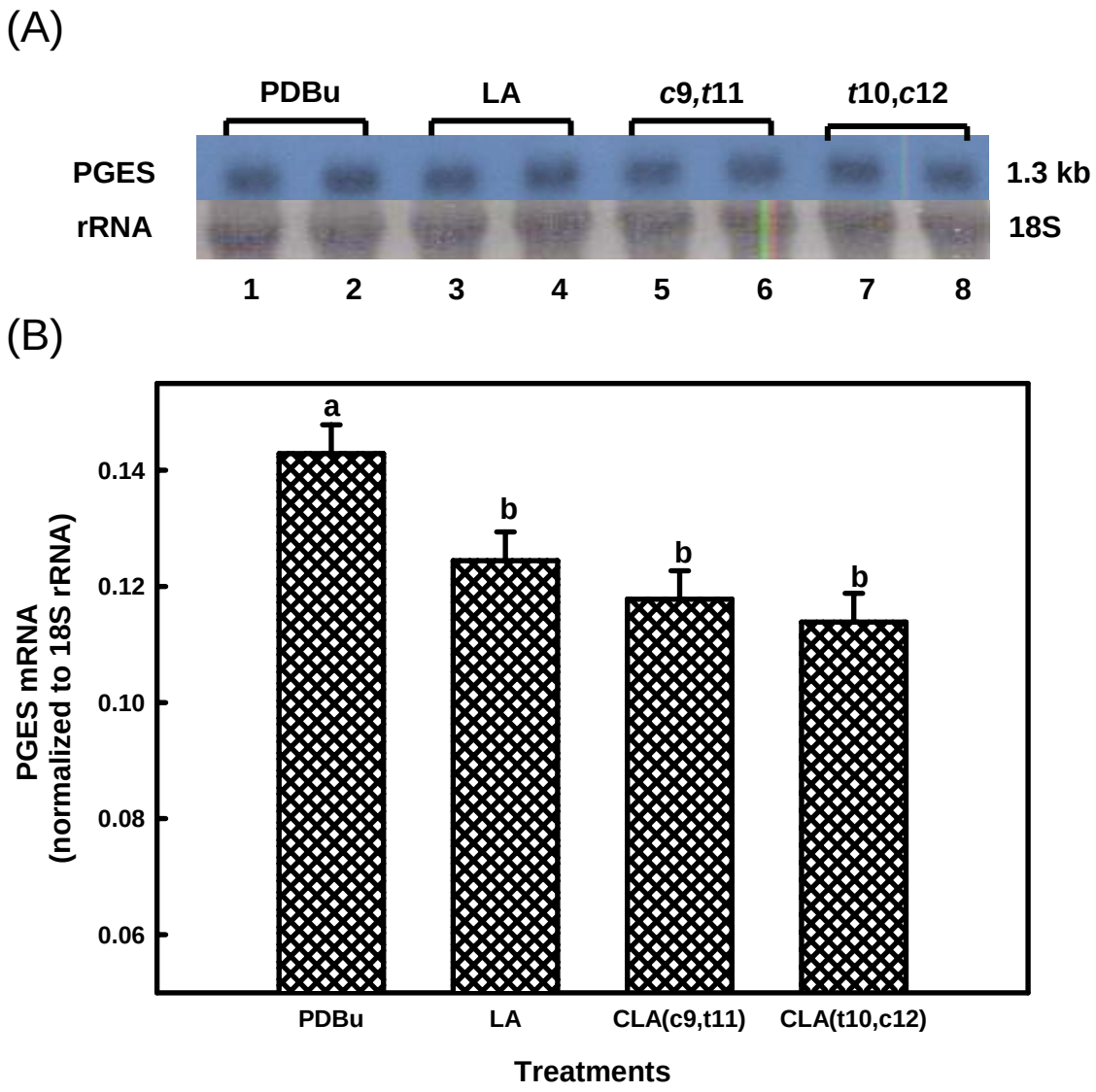

Figure 4-3. Effect of *c*9, *t*11 and *t*10, *c*12 CLA isomers on prostaglandin E synthase (PGES) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (LA) + (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.003$. Contrast 2: (LA) vs. (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.2$. Contrast 3: (*cis*-9, *trans*-11) vs. (*trans*-10, *cis*-12), $P = 0.6$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. There was no treatment by experiment interaction ($P = 0.10$).

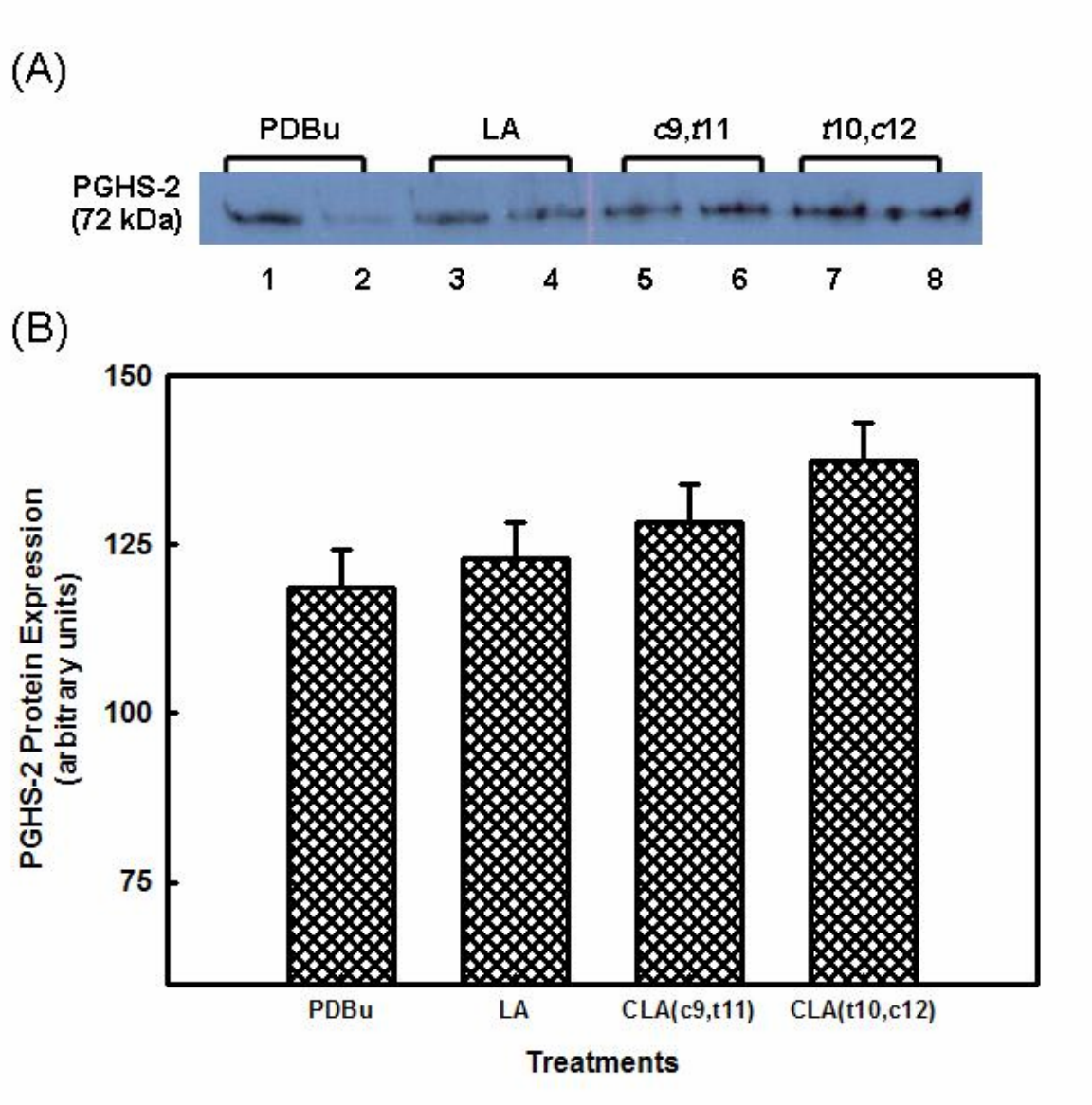

Figure 4-4. Effect of *c*9, *t*11 and *t*10, *c*12 CLA isomers on prostaglandin endoperoxide synthase (PGHS-2) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (LA) + (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.2$. Contrast 2: (LA) vs. (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.2$. Contrast 3: (*cis*-9, *trans*-11) vs. (*trans*-10, *cis*-12), $P = 0.3$.

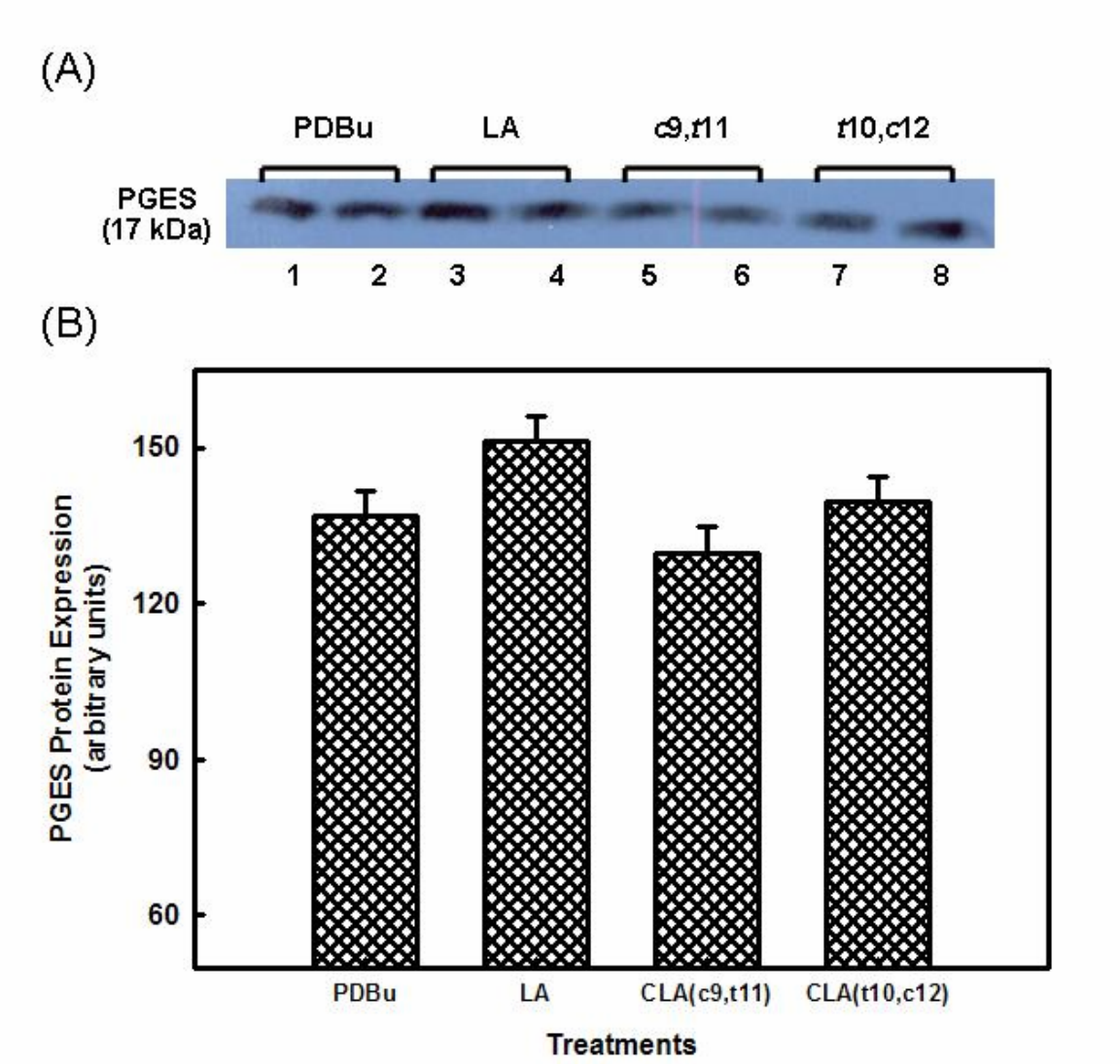

Figure 4-5. Effect of *c*9, *t*11 and *t*10, *c*12 CLA isomers on prostaglandin E synthase (PGES) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (LA) + (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.6$. Contrast 2: (LA) vs. (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P = 0.05$. Contrast 3: (*cis*-9, *trans*-11) vs. (*trans*-10, *cis*-12), $P = 0.2$.

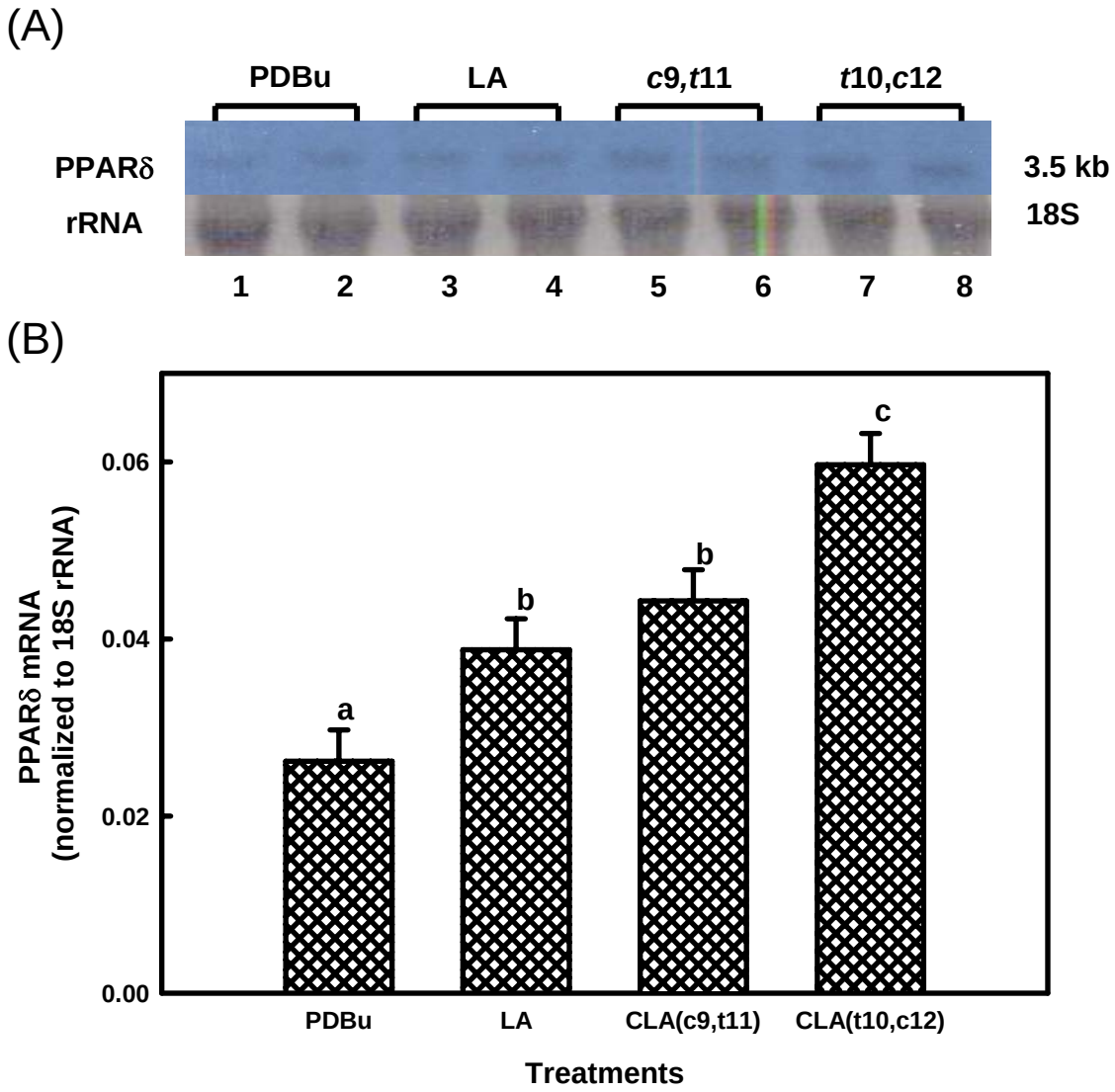

Figure 4-6. Effect of *c*9, *t*11 and *t*10, *c*12 CLA isomers on peroxisome proliferator-activated receptor δ (PPARδ) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (LA) + (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.0008$. Contrast 2: (LA) vs. (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.02$. Contrast 3: (*cis*-9, *trans*-11) vs. (*trans*-10, *cis*-12), $P = 0.01$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. There was no treatment by experiment interaction ($P = 0.08$).

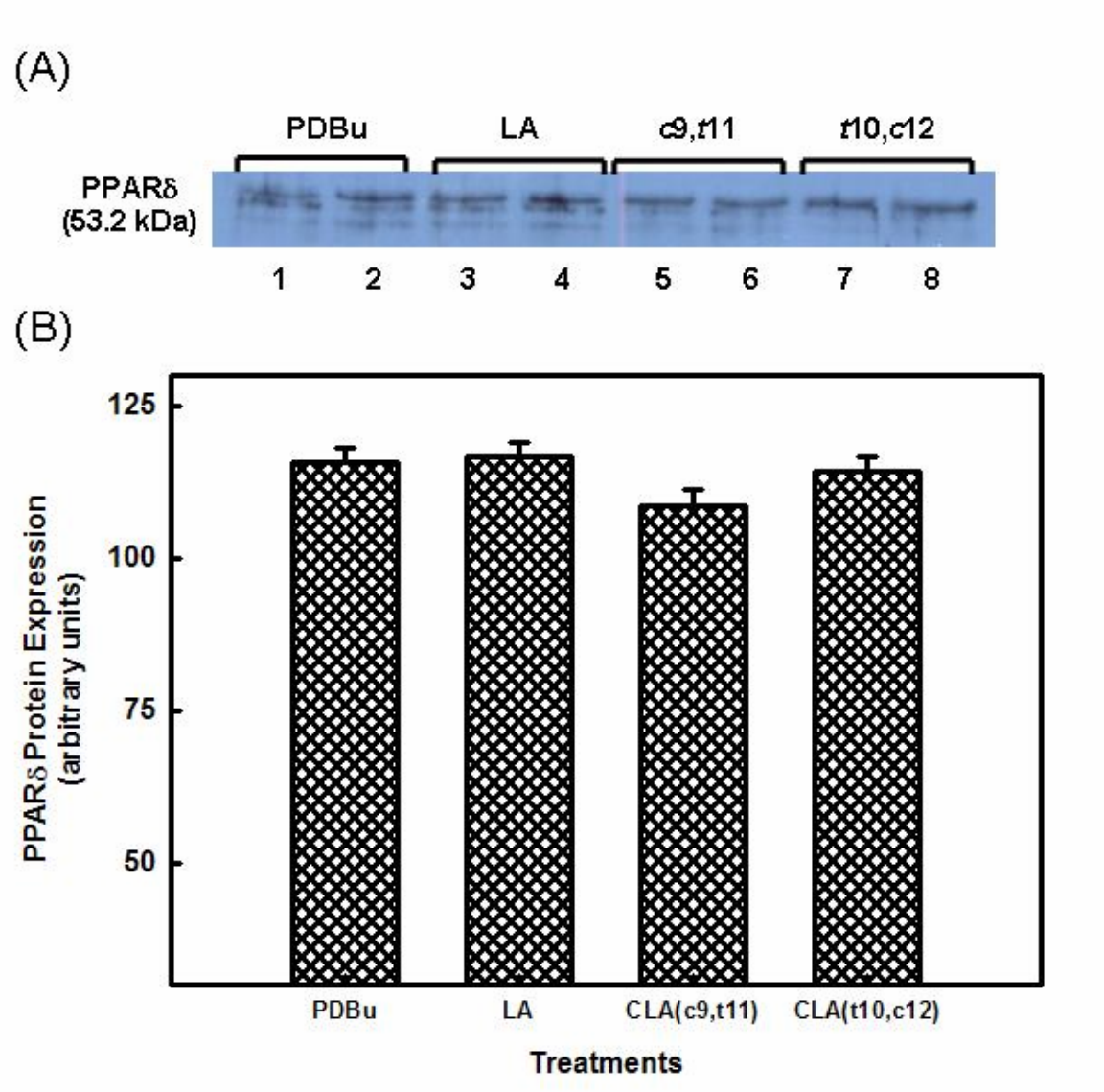

Figure 4-7. Effect of *c*9, *t*11 and *t*10, *c*12 CLA isomers on peroxisome proliferator-activated receptor δ (PPARδ) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (LA) + (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.4$. Contrast 2: (LA) vs. (*cis*-9, *trans*-11) + (*trans*-10, *cis*-12), $P < 0.2$. Contrast 3: (*cis*-9, *trans*-11) vs. (*trans*-10, *cis*-12), $P = 0.2$.

CHAPTER 5
EFFECTS OF *CIS*- AND *TRANS*-OCTADECENOIC ACIDS ON PROSTAGLANDIN $F_{2\alpha}$ PRODUCTION BY BOVINE ENDOMETRIAL CELLS

Introduction

Evidence is rapidly accumulating that dietary manipulations of fatty acids can have major effects on eicosanoid synthesis in domestic mammals. Dietary supplementation of long-chain fatty acids, a method commonly used to increase the energy density of diets for lactating dairy cows, has been shown to attenuate eicosanoid synthesis (Abayasekara and Wathes, 1999; Cheng et al., 2001), increase serum P_4 concentration (Carroll et al., 1990; Lucy et al., 1993; Garcia-Bojalil et al., 1998), stimulate ovarian follicular development (Lucy et al., 1993; Thomas and Williams, 1996; Beam and Butler, 1997), and improve fertility (Staples et al., 1998) in cattle. Preliminary data in our laboratory indicate that *trans*-octadecenoic fatty acids shorten the postpartum interval to estrus in early postpartum Holstein cows, suggesting enhanced ovarian activity and function after parturition.

Naturally occurring monounsaturated fatty acids (MUFA) are generally 16-22 carbons in length and contain the double bond in the *cis* configuration. The most common naturally occurring MUFA is oleic acid ($C_{18:1}$), which contains its double bond in the *cis* configuration. However, as a result of the biohydrogenation process in the rumen, accumulation of *trans* $C_{18:1}$ fatty acids (TFA) takes place by anaerobic microorganisms. Partial biohydrogenation of PUFAs generate ruminal conjugated linoleic acid (CLA) and *trans* vaccinic acid ($C_{18:1}$; *trans*-11). As a result, cows on a

typical forage diet accumulate *trans*-11, the major TFA present in ruminal contents (Jenkins, 2004). Moreover, Mosley et al. (2002) reported that biohydrogenation of oleic acid involves the formation of several TFA isomers rather than direct biohydrogenation to form stearic acid (ST; $C_{18:0}$). In addition, the *trans*-9 isomer of octadecenoic acid can be converted to both ST and a series of TFA isomers (Proell et al., 2002). Since hydrogenation of *trans*-11 $C_{18:1}$ to ST is less rapid than formation of *trans*-11 $C_{18:1}$ (Tanaka and Shigeno, 1976; Singh and Hawke, 1979), it accumulates in the rumen making it more available for absorption (Keeney, 1970).

The physiological mechanism(s) by which supplemental *trans* fatty acids may affect reproductive efficiency have not been characterized. Kummerow and coworkers (2004) observed that dietary supplementation of pigs with TFA resulted in reduced concentration of plasma n-3 and n-6 PUFAs. In this study, TFA also were reported to inhibit conversion of linoleic acid (LA) to longer chain n-6 PUFAs. This agrees with earlier studies by Mahfouz and others, showing that TFAs had an inhibiting effect on Δ6 desaturase in vascular cells *in vitro* (1980) and *in vivo* (1984). Taken together, these studies indicate a possible role of octadecenoic fatty acids in reproduction through modulation of eicosanoid production.

The objective of this study was to examine the effects of the *cis* and *trans* isomers of octadecenoic acid ($C_{18:1}$) on phorbol 13, 14-dibutyrate (PDBu)-induced $PGF_{2\alpha}$ production in cultured BEND cells. We hypothesized that MUFAs may modulate production of $PGF_{2\alpha}$ in endometrial cells, and that these effects may differ among the isomers of octadecenoic acid tested.

Materials and Methods

Materials

Polystyrene tissue culture dishes (100 x 20 mm) were purchased from Corning (Corning Glass Works, Corning, NY). The Ham F-12 medium, antibiotic/antimycotic (ABAM), phorbol 13, 14-dibutyrate (PDBu), horse serum, D-valine, insulin, fatty acid-free bovine serum albumin (BSA), stearic acid (ST, $C_{18:0}$), aprotinin, leupeptin, and pepstatin were from Sigma Chemical Co. (St. Louis, MO). The Minimum Essential Medium (MEM) and fetal bovine serum (FBS) were from US Biologicals (Swampscott, MA) and Atlanta Biologicals (Norcross, GA), respectively. Oleic acid (*cis*-9 $C_{18:1}$), elaidic acid (*trans*-9 $C_{18:1}$), vaccinic acid (*cis*-11 $C_{18:1}$), *trans*-vaccinic acid (*trans*-11 $C_{18:1}$), PGHS-2 and PGES antibodies, and $PGF_{2\alpha}$ standard were from Cayman Chemicals (Ann Arbor, MI). The PPARδ and secondary anti-rabbit IgG antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The enhanced chemiluminescence (ECL) kit was purchased from Perkin Elmer (Boston, MA). Hanks Balanced Salt Solution (HBSS) and TriZol reagent were from GIBCO BRL (Carlsbad, CA). Isotopically-labelled $PGF_{2\alpha}$ (5, 6, 8, 9, 11, 12, 14, 15[n-^{3}H] $PGF_{2\alpha}$; 208 Ci/mmol) was from Amershan Biosciences (Piscataway, NJ). The anti-$PGF_{2\alpha}$ antibody was purchased from Oxford Biomedicals (Oxford, MI). BioTrans nylon membrane and [α-^{32}P]deoxycitidine triphosphate (SA 3000 Ci/nmol) were from MP Biomedicals (Atlanta, GA). The PGHS-2 cDNA probe was cloned from an ovarian follicular cDNA library (Liu et al., 1999), the PGES cDNA probe was cloned from endometrial cDNA (Guzeloglu et al., 2004), whereas the PPARδ probe was generated from bovine endometrial RNA (Balaguer et al., 2005).

Cell Culture and Treatment

Bovine endometrial (BEND) cells were plated and cultured as described in chapter 3. To investigate the effects of *cis*- and *trans*-octadecenoic acids on uterine $PGF_{2\alpha}$ synthesis, BEND cells were treated with PDBu alone (100 ng/ml) or PDBu in combination with 100 μM of *cis*-9, *trans*-9, *cis*-11, or *trans*-11 isomers of octadecenoic acid. Fatty acids were complexed with BSA (1:3 ratio) for 2 h, and then treatments were applied to cells for a period of 24 h. After treatment, cells then were rinsed with HBSS and challenged with PDBu for another 6 h. Samples of cell-conditioned media (1 mL/plate) were collected and stored at -20°C for subsequent analysis for $PGF_{2\alpha}$ using RIA. The remaining cell monolayer was rinsed with HBSS, lysed in TriZol reagent, and stored at -80°C for subsequent mRNA analysis. This experiment was repeated two times. The $PGF_{2\alpha}$ radioimmunoassay and Northern and Western blot analyses were performed as described in chapter 3.

Statistical Analyses

Data were analyzed by Least-Squares analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 2001). For $PGF_{2\alpha}$ response, the mathematical model included fixed effects of treatment (n = 5), experiment (n = 5), treatment by experiment interaction, and random effect of dish (n = 2/trt) nested within treatment by experiment interaction. The variance of dish nested within treatment by experiment was used as the error term for all effects. For Northern and Western blot data, hybridization volumes obtained from densitometric analysis were subjected to ANOVA using the GLM procedure. For Northern blot analysis, the statistical model included independent fixed effects of treatment (n = 5), experiment (n =

2), treatment by experiment interaction, and random residual error. Results are presented as ratios of densitometric values for target genes over those for 18S rRNA ethidium bromide staining, and are presented as LS means ± SEM. For Western blot analysis, the mathematical model included only the main effect of treatment. Treatment effects were further analyzed using preplanned orthogonal contrasts. These contrasts were constructed to compare PDBu vs. fatty acids responses; isomers with double bond in position 9 vs. isomers with double bond in position 11; geometric isomers in position 9 (*cis* vs. *trans*); geometric isomers in position 11 (*cis* vs. *trans*).

Results

To test the hypothesis that MUFAs may modulate endometrial $PGF_{2\alpha}$ secretion, we examined endometrial $PGF_{2\alpha}$ response to PDBu in the presence of *cis*-9, *trans*-9, *cis*-11 and *trans*-11 isomers of octadecenoic acid.

Priming of BEND cells with *cis* and *trans*-fatty acids further enhanced $PGF_{2\alpha}$ ($P < 0.0001$; Figure 5-1) and PGHS-2 mRNA ($P = 0.001$; Figure 5-2) responses to PDBu. Interestingly, priming of BEND cells with the MUFAs decreased ($P = 0.04$) PGES mRNA response to PDBu (Figure 5-4). None of the fatty acid treatments altered PPARδ mRNA (Figure 5-6) or protein (Figure 5-7) expression in cultured BEND cells.

Discussion

Evidence is rapidly accumulating that dietary manipulations of fatty acids can have a major effect on eicosanoid synthesis, such as prostaglandins of the 2-series, in domestic animals. Prostaglandins of the 2-series affect numerous reproductive processes, including ovulation (Espey, 1980), follicular development (Wallach et al., 1975), corpus

luteum function (Poyser, 1995; Wathes and Lamming, 1995; Abayasekara and Wathes, 1999) and parturition (Challis et al., 1997).

In the present study, incubation of BEND cells with *cis* and *trans*-fatty acids resulted in significant augmentation of $PGF_{2\alpha}$ response to PDBu. Stimulation of endometrial $PGF_{2\alpha}$ secretion by supplemental MUFAs coincided with significant induction of PGHS-2 mRNA. To our knowledge, this is the first report of the effects of MUFAs on endometrial $PGF_{2\alpha}$ secretion.

Following parturition, fertility resumes after uterine involution and repair takes place, resulting in resumption of normal oestrous cycles (Kiracofe, 1980). This process of uterine involution is caused by myometrial contractions stimulated by $PGF_{2\alpha}$. A massive and sustained release of $PGF_{2\alpha}$ takes place during the first two weeks postpartum and is essential to reduce the uterine size and increase its tone (Hafez and Hafez, 2000). Moreover, the duration of this postpartum $PGF_{2\alpha}$ sustained release is negatively correlated with the number of days to complete uterine involution and the interval between parturition and resumption of normal ovarian activity (Lindell et al., 1982; Madej et al., 1984). This is in agreement with preliminary data from our lab indicating higher estrous rates in transition Holstein cows fed *trans* fatty acids (Kurt Selberg, unpublished observations).

The mechanism by which these MUFAs may be stimulating endometrial $PGF_{2\alpha}$ is not well understood. Although we observed enhanced response of PGHS-2 mRNA to PDBu after incubation of BEND cells with MUFA, no changes were observed at the protein level (Figure 5-3; $P = 0.32$). Additionally, in the present study we did not measure PGHS-2 enzyme activity. Priming of BEND cells with the MUFAs decreased

($P = 0.04$) PGES mRNA response to PDBu, indicating that MUFAs may favor $PGF_{2\alpha}$ production over PGE_2. However, no changes were observed on PGES protein levels (Figure 5-5; $P = 0.10$) and PGES enzyme activity was not documented in this study.

Alternatively, dietary supplementation with TFA has been reported to reduce concentration of plasma n-3 and n-6 PUFAs in pigs (Kummerow et al., 2004). TFA were also reported to inhibit conversion of LA to longer chain n-6 PUFAs. This agrees with earlier studies by Mahfouz and others, showing that TFAs had an inhibiting effect on Δ6 desaturase in vascular cells *in vitro* (1980) and *in vivo* (1984). Additionally, essential PUFAs have been shown to inhibit PG secretion in several cell types (Levine and Worth, 1984; Achard et al., 1997) including BEND cells (Mattos et al., 2003). Taken together, these studies indicate that isomers of octadecenoic fatty acids may induce $PGF_{2\alpha}$ production through a mechanism involving inhibition of synthesis of PUFAs.

Although fatty acids are naturally occurring ligands of PPARs, it appears that the effect of MUFAs on $PGF_{2\alpha}$ does not require modulation of PPARδ gene or protein since no changes were observed after incubation with the isomers of octadecenoic acid. Whether and how these fatty acids may control activity of this nuclear receptor is yet to be elucidated.

Summary

In the present study, priming of BEND cells with *cis*-9, *trans*-9, *cis*-11, or *trans*-11 isomers of octadecenoic acid further enhanced $PGF_{2\alpha}$ and PGHS-2 mRNA response to PDBu. Interestingly, pre-incubation of BEND cells with the MUFAs decreased PGES mRNA response to PDBu. None of the fatty acids altered PPARδ mRNA or protein levels. Supplemental monoenoic fatty acids appear to increase $PGF_{2\alpha}$ through a

mechanism that does not require induction of PPARδ gene or protein expression. Whether and how these fatty acids may control activity of this nuclear receptor warrants further study.

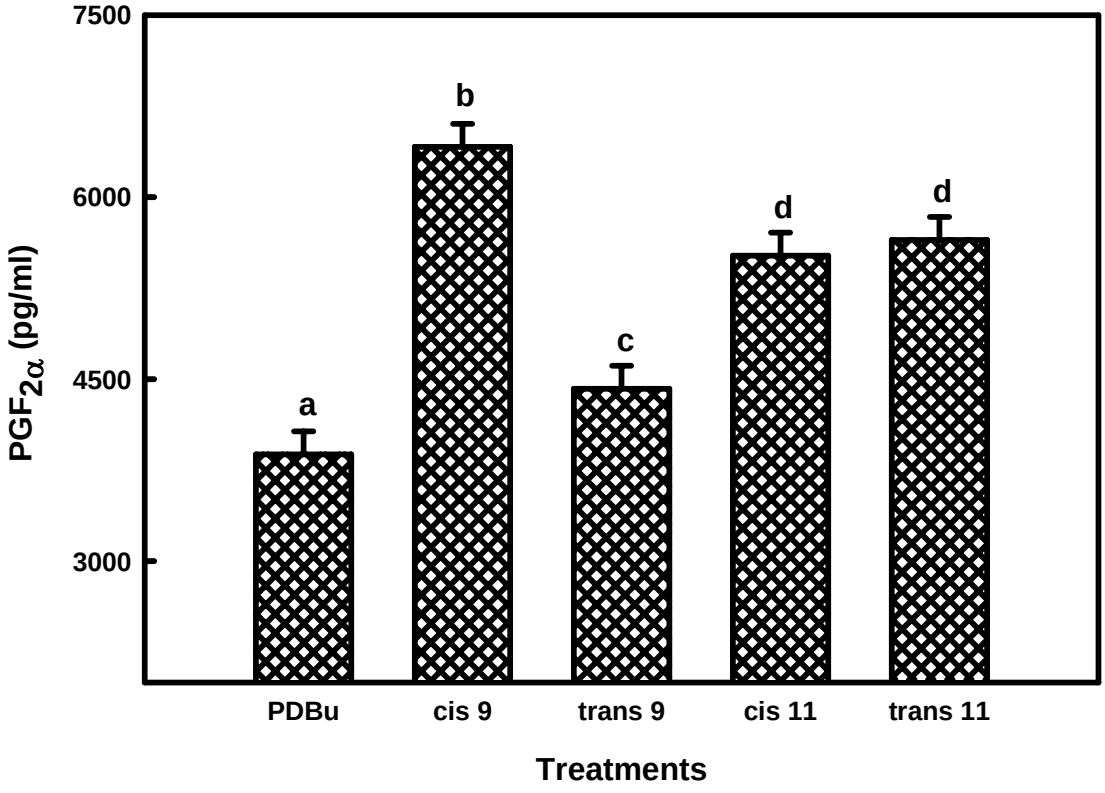

Figure 5-1. Effect of *cis*- and *trans*- isomers of octadecenoic acid on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (*cis*-9) + (*trans*-9) + (*cis*-11) + (*trans*-11), $P < 0.0001$. Contrast 2: (*cis*-9) + (*trans*-9) vs. (*cis*-11) + (*trans*-11), $P < 0.0001$. Contrast 3: (*cis*-9) vs. (*trans*-9), $P < 0.0001$. Contrast 4: (*cis*-11) vs. (*trans*-11), $P = 0.63$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. Treatment by experiment interaction was significant ($P = 0.0002$).

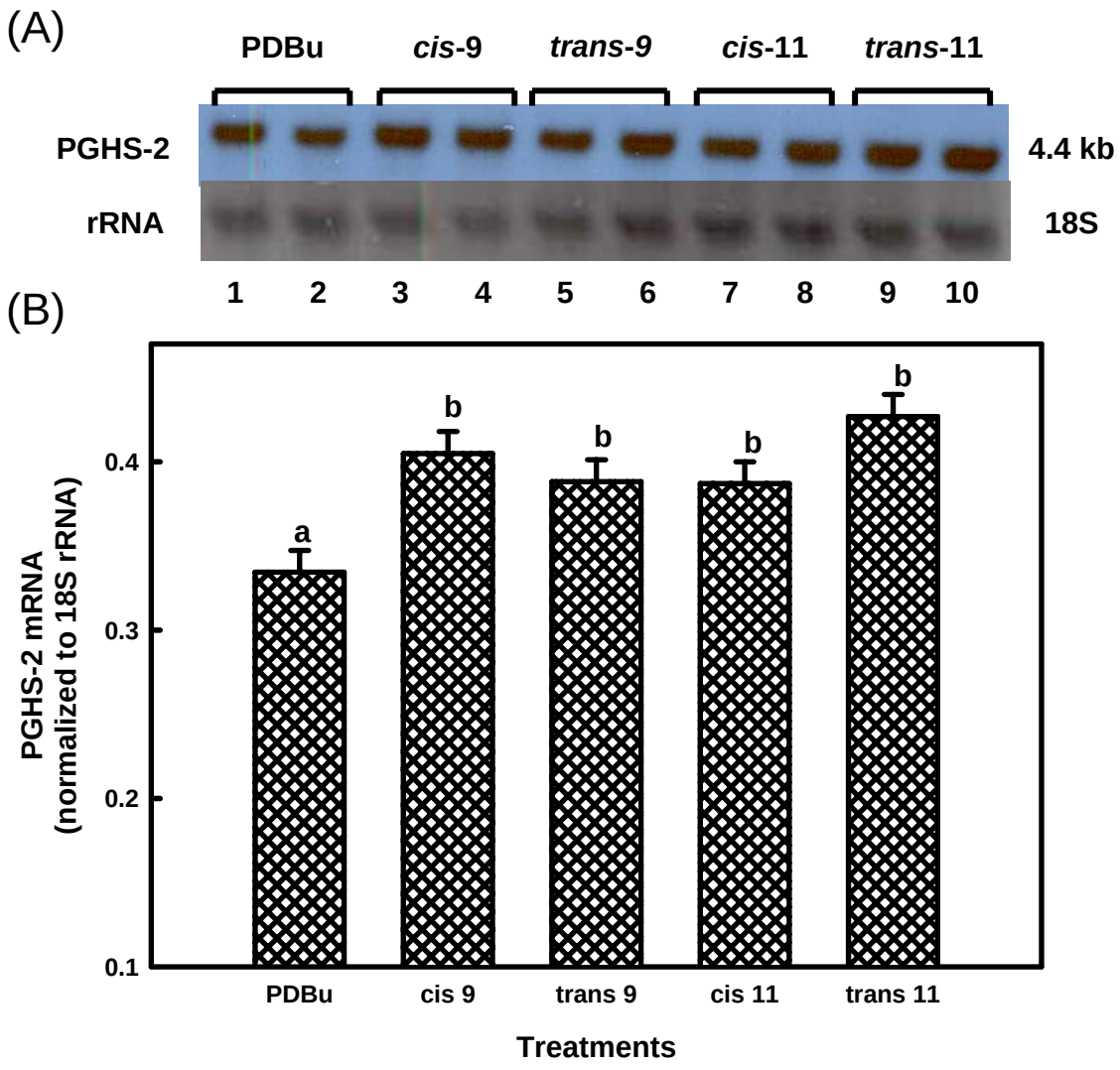

Figure 5-2. Effects of *cis*- and *trans*- isomers of octadecenoic acid on prostaglandin endoperoxide synthase (PGHS-2) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (*cis*-9) + (*trans*-9) + (*cis*-11) + (*trans*-11), $P = 0.001$. Contrast 2: (*cis*-9) + (*trans*-9) vs. (*cis*-11) + (*trans*-11), $P = 0.44$. Contrast 3: (*cis*-9) vs. (*trans*-9), $P = 0.39$. Contrast 4: (*cis*-11) vs. (*trans*-11), $P = 0.06$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. There was no treatment by experiment interaction ($P = 0.17$).

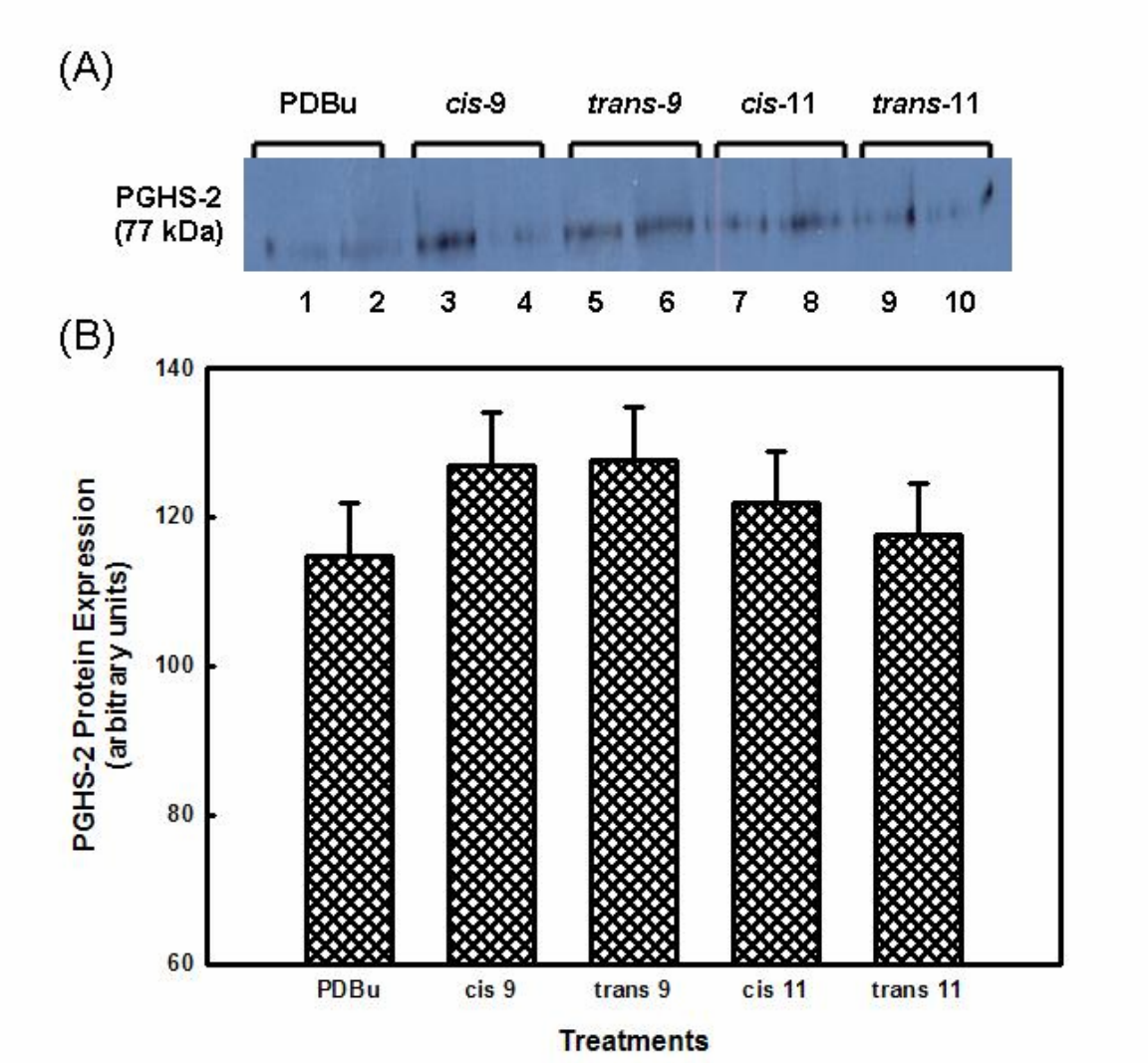

Figure 5-3. Effects of *cis*- and *trans*- isomers of octadecenoic acid on prostaglandin endoperoxide synthase (PGHS-2) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (*cis*-9) + (*trans*-9) + (*cis*-11) + (*trans*-11), $P = 0.32$. Contrast 2: (*cis*-9) + (*trans*-9) vs. (*cis*-11) + (*trans*-11), $P = 0.33$. Contrast 3: (*cis*-9) vs. (*trans*-9), $P = 0.95$. Contrast 4: (*cis*-11) vs. (*trans*-11), $P = 0.68$.

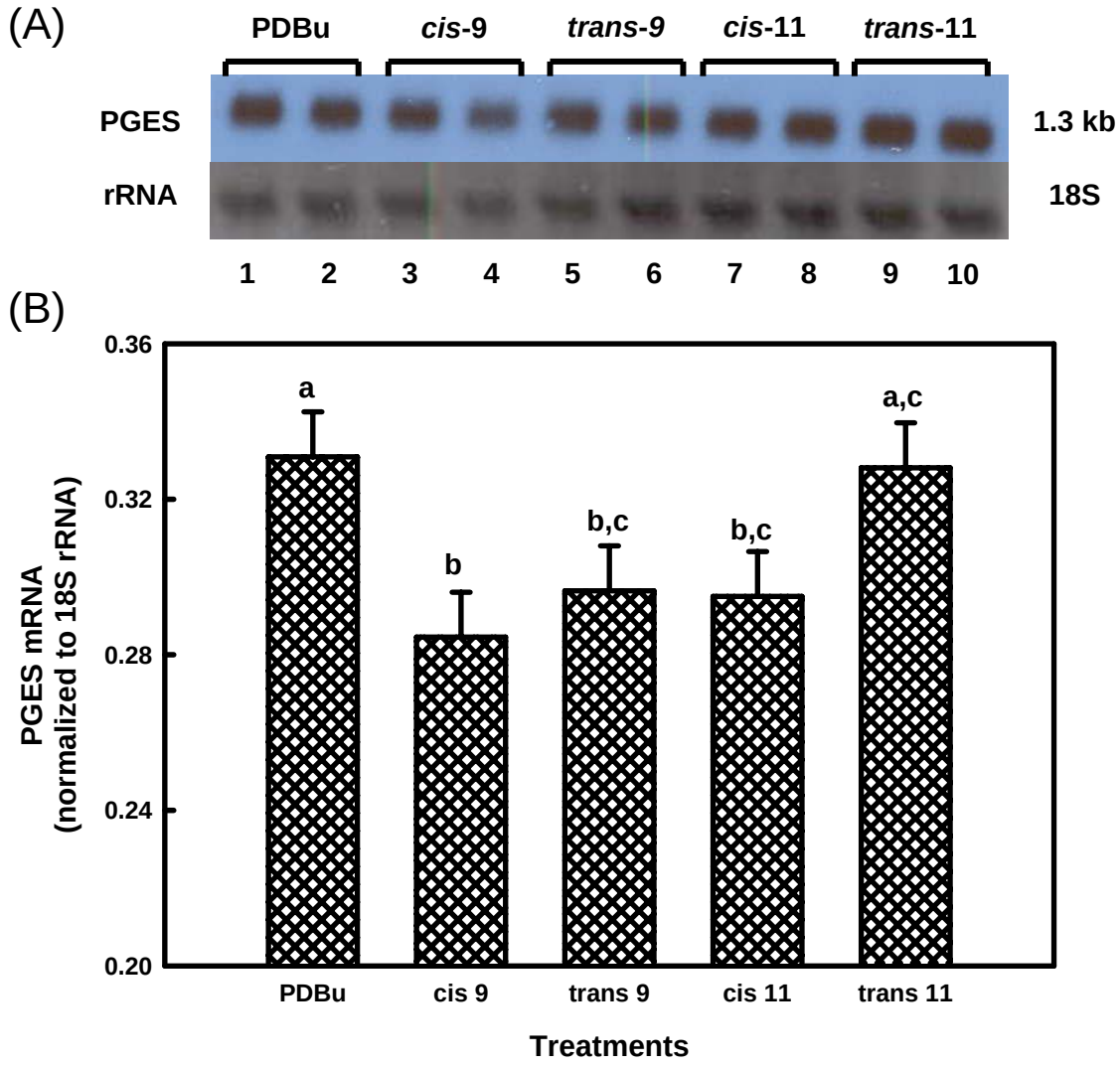

Figure 5-4. Effects of *cis*- and *trans*- isomers of octadecenoic acid on prostaglandin E synthase (PGES) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (*cis*-9) + (*trans*-9) + (*cis*-11) + (*trans*-11), $P = 0.04$. Contrast 2: (*cis*-9) + (*trans*-9) vs. (*cis*-11) + (*trans*-11), $P = 0.10$. Contrast 3: (*cis*-9) vs. (*trans*-9), $P = 0.48$. Contrast 4: (*cis*-11) vs. (*trans*-11), $P = 0.07$. When main treatment effect was significant ($P < 0.05$), then differences between treatments are represented by different letters. There was no treatment by experiment interaction ($P = 0.41$).

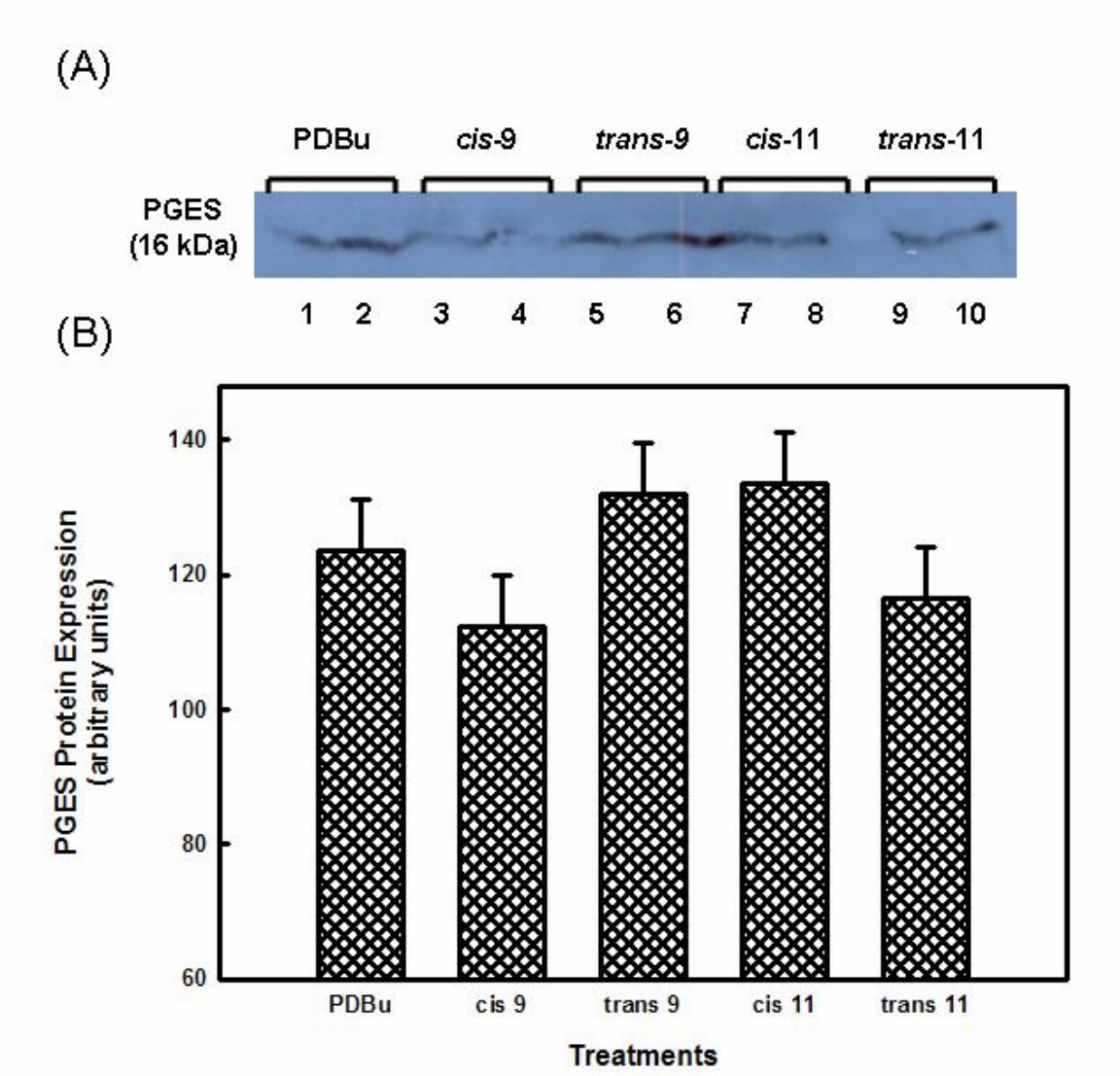

Figure 5-5. Effects of *cis*- and *trans*- isomers of octadecenoic acid on prostaglandin E synthase (PGES) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (*cis*-9) + (*trans*-9) + (*cis*-11) + (*trans*-11), $P = 0.10$. Contrast 2: (*cis*-9) + (*trans*-9) vs. (*cis*-11) + (*trans*-11), $P = 0.72$. Contrast 3: (*cis*-9) vs. (*trans*-9), $P = 0.13$. Contrast 4: (*cis*-11) vs. (*trans*-11), $P = 0.18$.

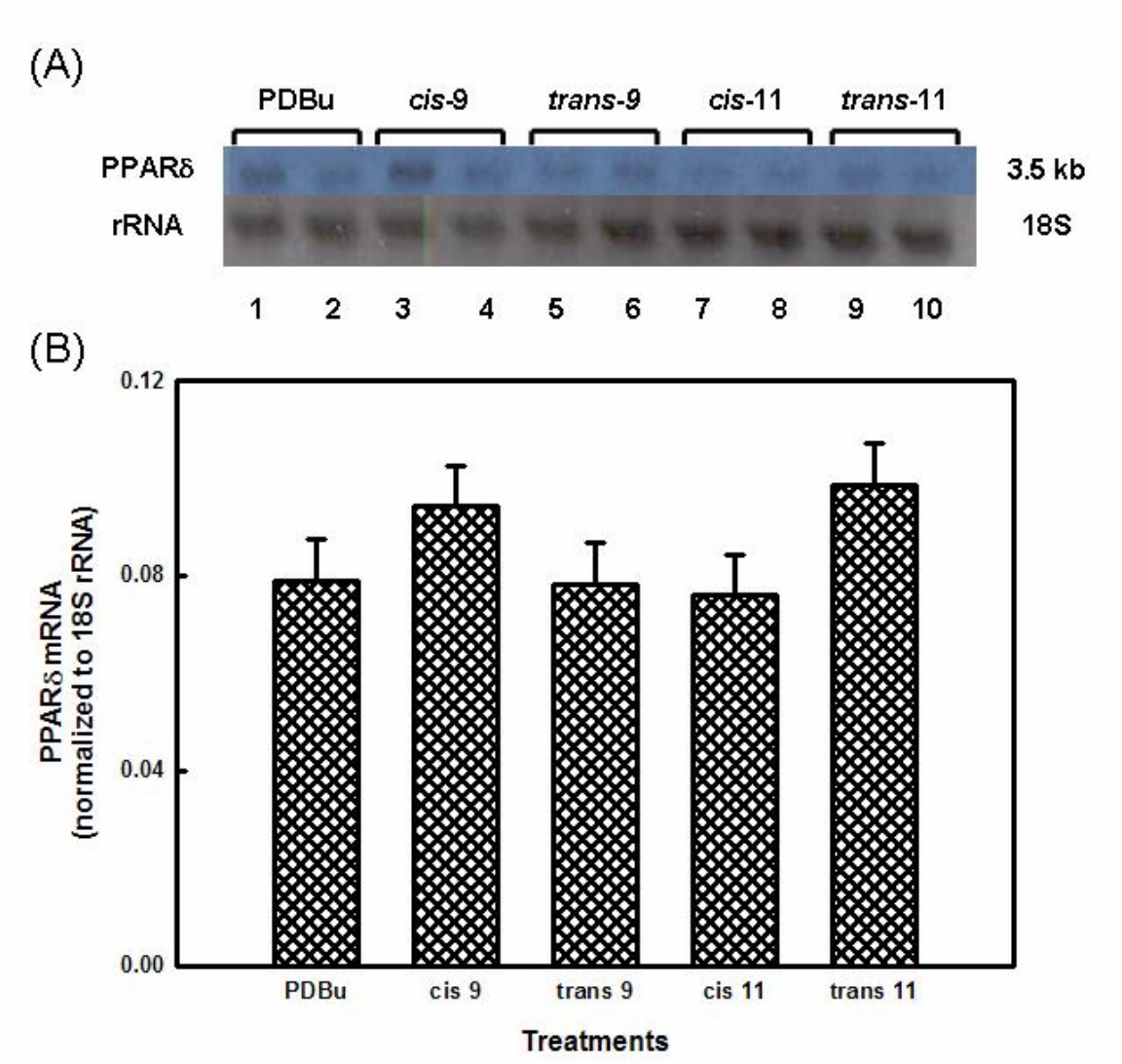

Figure 5-6. Effects of *cis*- and *trans*- isomers of octadecenoic acid on peroxisome proliferator-activated receptor δ (PPARδ) mRNA response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Ten micrograms of total cellular RNA isolated from control and treated BEND cells were subjected to Northern blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Northern blot, whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (*cis*-9) + (*trans*-9) + (*cis*-11) + (*trans*-11), $P = 0.43$. Contrast 2: (*cis*-9) + (*trans*-9) vs. (*cis*-11) + (*trans*-11), $P = 0.90$. Contrast 3: (*cis*-9) vs. (*trans*-9), $P = 0.21$. Contrast 4: (*cis*-11) vs. (*trans*-11), $P = 0.09$. Treatment by experiment interaction was significant ($P = 0.006$).

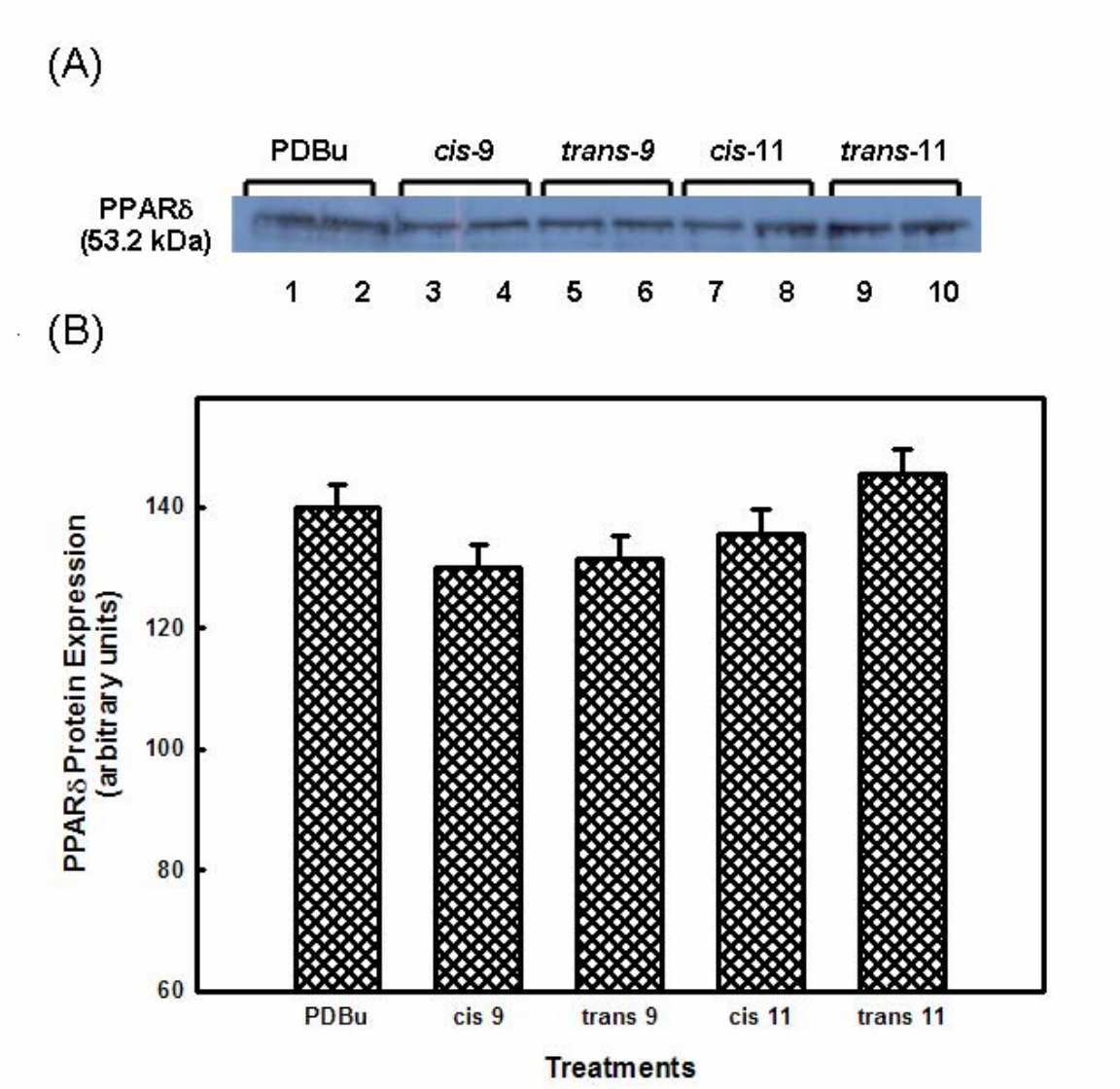

Figure 5-7. Effects of *cis*- and *trans*- isomers of octadecenoic acid on peroxisome proliferator-activated receptor δ (PPARδ) protein response to phorbol 12, 13 dibutyrate (PDBu) in bovine endometrial (BEND) cells. Twenty five micrograms of total cellular protein extracted from control and treated cells were subjected to Western blot analysis (A), and resulting densitometric values were analyzed by the GLM procedure of SAS (B). The top panel shows a representative Western blot whereas the bottom panel represents means ± SEM calculated over two experiments (n = 4 for each treatment). When treatment effects were detected ($P < 0.05$), means were separated using orthogonal contrasts. Contrast 1: (PDBu) vs. (*cis*-9) + (*trans*-9) + (*cis*-11) + (*trans*-11), $P = 0.38$. Contrast 2: (*cis*-9) + (*trans*-9) vs. (*cis*-11) + (*trans*-11), $P = 0.05$. Contrast 3: (*cis*-9) vs. (*trans*-9), $P = 0.82$. Contrast 4: (*cis*-11) vs. (*trans*-11), $P = 0.14$.

CHAPTER 6
EFFECTS OF DIETARY *TRANS* FATTY ACIDS ON PROSTAGLANDIN $F_{2\alpha}$ CONCENTRATIONS IN POSTPARTUM HOLSTEIN COWS

Introduction

Over the last five decades, continuous genetic progress for milk production has been associated to a decrease in reproductive efficiency of dairy cows (Butler, 2000). Poor reproductive performance affects the amount of milk produced per cow per day of herd life, breeding costs, rates of voluntary and involuntary culling, and the rate of genetic progress for traits of economic importance (Plaizier et al., 1997). Poor reproductive efficiency includes early embryonic loss (Thatcher et al., 1995), impaired ovarian cyclicity and low fertility rates (Butler, 2000), which collectively result in reduced milk production (Plaizier et al., 1997). One of the factors contributing to low breeding efficiency in high milking cows is the high energy deficit of early lactation, which delays re-establishment of ovarian cyclicity and impairs reproductive performance after calving (Butler, 2000).

Lactating dairy cows in the first few weeks postpartum have higher energy requirements than can be supported by dietary energy intake, which creates a negative energy state and can lead to impaired reproductive function (Butler, 2000). Energy balance of an animal is the difference between energy intake and energy requirements within a given physiological state (Beam and Butler, 1999; Butler et al., 1981; Canfield and Butler, 1990). In dairy cows, parturition and the onset of lactation cause an abrupt shift in nutritional requirements in order to support milk production (Butler, 2000). This

rapid increase in energy requirements and changes in the metabolic as well as endocrine status of the cow come about during the transition period (Bauman and Currie, 1980; Grummer, 1995). This is the result of the prioritized status of lactation which allows it to proceed at the expense of any other physiological process (Bauman and Currie, 1980).

The transition period extends from three weeks prepartum until three weeks postpartum, and refers to the period during which endocrine and metabolic changes accompany parturition and the onset of lactation (Grummer, 1995). A reduction in feed intake occurs during the final week of pregnancy when nutrient demands for support of fetal growth and initiation of milk synthesis are increasing (Grummer, 1995). The dietary energy that is consumed by the lactating animal is almost entirely used by the mammary tissue for milk production, leaving no energy for maintenance (Bell, 1995). To offset this energy deficit, the lactating animal mobilizes body energy reserves, which leads ultimately to a state of negative energy balance (NEB). This NEB represents a state of undernutrition, which results in massive mobilization of fat from adipose tissue, increasing plasma concentrations of non-esterified fatty acids (NEFA). Massive fat mobilization, in combination with reduced energy intake (dry matter intake), results in loss of body condition of lactating animals. Extensive data has shown that the aforementioned conditions (NEB and loss of body condition) invariably affect reproductive efficiency in lactating dairy cows.

Fertility in postpartum cows is directly dependent on a normal estrous cycle characterized by folliculogenesis, ovulation, corpus luteum (CL) formation and regression. However, the mechanisms by which endocrine and metabolic upsets result in reduced reproductive efficiency are not well understood. Many of the hormonal and

metabolic changes that occur during the transition period can affect reproductive function by interacting with the hypothalamic-pituitary axis (Butler, 2000). Negative energy balance also has been shown to change the profile of metabolic hormones which may play an important local role in control of follicular development in cattle. It is likely that these changes could alter the pattern of ovarian follicular growth and development, and subsequent CL function during the early postpartum period. However, energy-independent effects also have been observed after fatty acid supplementation. Thus, nutrition may influence reproductive efficiency in dairy cows not only by altering the energy status of the animal but also by influencing factors involved in the regulation of reproductive processes like follicular dynamics, ovulation, CL function, and embryo survival among others.

Dietary supplementation of long chain fatty acids (LCFA), commonly used to increase energy density of diets for lactating dairy cows, has been shown to attenuate eicosanoid synthesis (Abayasekara and Wathes, 1999; Cheng et al., 2001), increase serum P_4 concentration (Carroll et al., 1990; Lucy et al., 1993; Garcia-Bojalil et al., 1998), stimulate ovarian follicular development (Lucy et al., 1993; Thomas and Williams, 1996; Beam and Butler, 1997), and improve fertility (Staples et al., 1998) in cattle. The potential mechanisms by which LCFAs affect reproductive responses in cattle include indirect effects of high energy intake on the overall energy state of the cow, as well as direct effects of dietary fatty acids on the pituitary, ovaries, and uterus (Staples et al., 1998; Mattos et al., 2000).

Improvement of the overall energy state provided by fatty acid supplementation (Staples et al., 1998; Jenkins and Palmquist, 1984) may lead to re-establishment of LH

pulsatility and ovarian cyclicity in the lactating cow (Lucy et al., 1991). In fact, Sklan et al. (1994) reported that the energy provided by dietary fats increased LH secretion in dairy cows that consumed less energy than required.

There is evidence that increase in consumption of dietary fatty acids stimulates ovarian follicular growth in cattle through a mechanism that is independent from energy intake and weight gain (Staples et al., 1998). Increasing the dietary intake of LCFAs by cattle increased both the number and size of follicles present in the ovary and shortens the interval to first ovulation postpartum (Hightshoe et al., 1991; Lucy et al., 1991, 1992; Ryan et al., 1992; Thomas and Williams, 1996; Lammoglia et al., 1997; Beam and Butler, 1997). Lucy et al. (1993b) reported greater numbers of medium-sized ovarian follicles (6 – 9 mm) in postpartum dairy cows fed a diet containing 2.2% calcium salts of LCFA compared to cows receiving an isocaloric control diet. Several studies reported that supplemental fat increased not only the total number of ovarian follicles (Thomas and Williams, 1996; Beam and Butler, 1997; Lammoglia et al., 1997), but also the size of preovulatory follicles in cattle (Lucy et al., 1993b; Beam and Butler, 1997; Oldick et al., 1997). Follicular growth also has been shown to be stimulated by LCFAs in crossbred beef cattle (Thomas et al., 1997). Moreover, lactating dairy cows fed calcium salts of fat enriched in LA or fish oil (high in n-3 PUFAs) had increased size of the dominant follicle compared to those fed calcium salts of oleic acid (18:1) (Staples et al., 1998).

Fertility responses may also be related to the effects of LCFAs on uterine eicosanoid production. Eicosanoids (i.e. prostaglandins, thromboxanes, leukotrienes and lipoxins) are synthesized from AA, which uses LA as the primary precursor (Kinsella et al., 1990). Prostaglandins (PG) of the 2 series ($PGF_{2\alpha}$, PGE_2) have been implicated in

many reproductive processes including ovulation (Espey, 1980), follicular development (Wallach et al., 1975), corpus luteum function (Bazer and Thatcher, 1977; Auletta and Flint, 1988; Abayasekara et al., 1995; Poyser, 1995; Wathes and Lamming, 1995), parturition (Thorburn and Challis, 1979; Challis, 1980) and uterine involution (Hafez and Hafez, 2000). Hence, any influence that fatty acids might exert on $PGF_{2\alpha}$ synthesis may affect overall reproductive performance.

Preliminary data in our laboratory indicate that feeding *trans*-octadecenoic fatty acids reduced fat mobilization (Selberg et al., 2004) and shorten the postpartum interval to estrus (Selberg et al., unpublished data) in early postpartum Holstein cows, suggesting enhanced ovarian activity and function after parturition. Moreover, studies from our lab showed that supplementation of bovine endometrial (BEND) cells with *trans*-octadecenoic fatty acids stimulated production of $PGF_{2\alpha}$ (see Chapter 5). These observations collectively suggest that dietary *trans* fatty acids (*t*FA) may improve reproductive efficiency in transition dairy cows by reducing the incidence of postpartum metabolic upsets such as fatty liver and stimulating ovarian function after parturition.

The objective of this investigation was to examine the effects of dietary *t*FAs on ovarian and $PGF_{2\alpha}$ responses in early postpartum Holstein cows. We hypothesized that supplementation of transition dairy cows with dietary *t*FAs may reduce periparturient fat mobilization and enhance PGFM production, thus stimulating ovarian function after parturition.

Materials and Methods

Materials

A highly saturated fat product (ruminal bypass fat; RBF) mixed in the control diet was provided by Cargill (Minneapolis, MN). Calcium salts of *trans*-$C_{18:1}$ fatty acid mix (EnerG TR) was provided by Virtus Nutrition^{TM} LLC (Fairlawn, OH). Kendall Monoject blood collection tubes (10 mL) containing EDTA as anticoagulant, and needles (20GA 1") were purchased from Webster Veterinary (Alachua, FL). Borosilicate glass disposable culture tubes (12 x 75 mm), scintillation vials (7 mL), flat bottom 96-well plates, Scintiverse II scintillation fluid, and serum filters were purchased from Fisher Scientific (Pittsburgh, PA). Polypropylene milk sample vials (2 mL) were from Capitol Vial, Inc (Auburn, AL). Autokit 3-HB, NEFA C, and Glucose C2 Autokit test kits were purchased from Wako Chemicals USA (Richmond, VA). Activated charcoal, dextran, and authentic 13, 14-Dihydro-15-keto $PGF_{2\alpha}$ were from Sigma Chemical Co. (St. Louis, MO). Isotopically-labelled 13, 14-Dihydro-15-keto[n-^{3}H] $PGF_{2\alpha}$; 208 Ci/mmol was from Amershan Pharmacia Biotech (Piscataway, NJ). The anti-13, 14-Dihydro-15-keto $PGF_{2\alpha}$ antibody was kindly provided by Dr. William W. Thatcher (University of Florida, Gainesville, FL).

Cows and Diets

Holstein cows (18 multiparous and 12 primiparous) were utilized in a completely randomized design to determine the effect of feeding calcium salts of *trans*-$C_{18:1}$ fatty acids on production, metabolic and reproductive responses during the transition period. Primiparous heifers (n = 3) and multiparous cows (n = 5) that were diagnosed with displaced abomasum, enteritis, or that had metritis within 10 d after parturition were

removed from production, metabolic and reproductive analyses (Table 6-1). Therefore, a total of nine heifers (4 C, 5 *t*FA) and 13 cows (7 C, 6 *t*FA) were used in statistical analyses. The experiment was conducted from March to July, 2005. All experimental animals were managed according to the guidelines approved by the University of Florida Animal Care and Use Committee.

Two dietary treatments were initiated approximately 28 d prior to estimated calving dates and continued through 21 d postpartum. The control diet (C) contained a highly saturated fat supplement (90 % saturated fatty acids; RBF) at 1.55% of dietary DM. The second experimental diet contained a Ca salt of primarily *trans*-$C_{18:1}$ fatty acid (*t*FA) at 1.8% of dietary DM. Diets were isolipid since the Ca salt product was 15% Ca. The fatty acid profile of each fat supplement is in Table 6-2. Diets were formulated for intakes of approximately 150 to 200 g/d prepartum and 250 to 300 g/d postpartum of supplemental lipid. Fat supplements were mixed with the concentrates and offered as part of the TMR to experimental animals.

Prepartum cows were housed in pens with a sod base equipped with shaded Calan gates (American Calan Inc., Northwood, NH). Postpartum cows were housed in a free-stall barn equipped with fans, sprinklers, and Calan gates. Intake of DM was measured daily. All experimental cows were offered *ad libitum* amounts of TMR to allow for 5 to 10% refusals (Table 6-3). Corn silage was the major forage component and ground corn was the primary concentrate. Dry matter of corn silage was determined weekly and the rations were adjusted accordingly to maintain a constant forage: concentrate ratio on a DM basis. Samples of forages, dried at 55°C and ground to pass 2-mm screen of a Wiley Mill (C.W. Brabender® Instruments, Inc.), and concentrate mixes were collected weekly,

composited monthly, and analyzed by wet chemistry for fat (Soxhlet method) and minerals (Dairy One, Ithaca, NY), and CP (Elementar Analysensysteme, Hanau, Germany). Composite samples also were analyzed for ADF and NDF with heat stable α-amylase (Termamyl 120L, Novo Nordisk Biochem, Franklinton, NC) as described by Van Soest et al. (1991). Detailed ingredient and chemical composition of the experimental diets are listed in Tables 6-3 and 6-4, respectively.

Postpartum cows were milked 3 times per day and milk weights were recorded at each milking. For each experimental cow, samples of milk from 2 consecutive morning (1000 h) and evening (1800 h) milkings were collected one day during wk 3 postpartum and analyzed for fat, protein, and SCC. Daily values were calculated by averaging morning and evening milk values. Body weights were measured and BCS assigned weekly by the same individual.

Prepartum energy balance was calculated using the following equation:

Energy balance (Mcal/d) = net energy of intake – (net energy of maintenance + net energy of pregnancy).

Net energy of intake was calculated by multiplying the weekly average DMI (kg) by the calculated energy value of the diet (Mcal/kg) (NRC, 2001). Energy requirement for body maintenance was computed using the following equation (NRC, 2001):

Net energy of maintenance (Mcal) = $0.08 \times BW^{0.75}$

Pregnancy requirements were estimated using the following equation (NRC, 2001):

Net energy of pregnancy (Mcal) = [(0.00318 x days pregnant – 0.0352) x (calf BW/45)]/0.218

Postpartum energy balance was estimated using the following equation (NRC, 2001):

Energy balance (Mcal/d) = net energy intake – (net energy of maintenance + net energy of lactation)

Milk energy was estimated by the following equation:

Net energy of lactation (Mcal/d) = [(0.0929 x % fat) + (0.0547 x % protein) + 0.192] x milk weight (kg)

Collection of Blood Samples

Blood (~20 mL) was collected once daily at 1730 h from d 14 before calculated due date until parturition and from d 15 until d 21 postpartum. Between the day of parturition (d 0) and d 14 postpartum, blood samples were collected 2x per day at 0800 and 1730 h. Blood was collected via coccygeal arterio-venipuncture into evacuated blood tubes containing EDTA as an anticoagulant (10.5 mg). Samples were placed immediately in ice until plasma was separated by centrifugation at 3000 rpm for 30 min (4°C). Plasma was separated and stored at -20°C for subsequent metabolite and PGFM analyses.

Metabolite and PGFM Assays

Plasma concentrations of NEFA, BHBA, and glucose were measured in samples collected on days -14, -7, 7, 14, and 21, relative to the day of parturition. Plasma

concentration of NEFA, BHBA, and glucose were measured with the NEFA C, Autokit 3-HB, and Glucose C2 Autokit test kits, respectively.

Samples collected between d 5 prepartum and d 14 postpartum were analyzed for concentrations of PGFM using a modification of the radioimmunoassay procedure described by Mitchell et al. (1976). The PGFM standard solutions were made by serial dilutions in a buffer of a stock solution (1 μg/mL in 10% ethanol and 90% PBS buffer) of authentic PGFM. Standards (100 μL) were run in duplicates at the following concentrations: 15.6, 31.2, 62.5, 125, 250, 500, 1000, 2000, 4000, and 8000 pg/mL. The standard curve included 100 μL of plasma containing low concentration of prostaglandin. Low PGFM plasma was obtained by pooling samples corresponding to wk 7 postpartum cows, known to have low to negligible prostaglandin concentrations. The PBS buffer contained 2.3 g/L of $NaH_2PO_4H_2O$, 4.76 g/L of $Na_2HPO_47H_2O$, 1 g/L of sodium azide, and 8.41 g/L of NaCL. The buffer pH was adjusted to 7.5 with NaOH.

Activity and volume of radioactively labeled PGFM used were 18,000 dpm and 100 μL, respectively. For unknown samples, the final assay volume was 400 μL [i.e., 100 μL of sample, 100 μL of rabbit antiserum to PGFM (1:5000), 100 μL of buffer, and 100 μL of labeled PGFM]. After overnight incubation at 4°C, free PGFM was separated using 500 μL of a solution of dextran-coated charcoal (50 mg dextran and 500 mg activated charcoal in 100 mL PBS buffer). After centrifugation at 3500 rpm for 20 min (4°C), the supernatant was transferred to scintillation vials and mixed with 4.5 mL of Scintiverse II. Radioactivity was measured using a liquid scintillation counter (Packard, Canberra Company). Assay sensitivity was 15.6 pg/mL and intra- and inter-assay

coefficients of variation were 6.5 and 5.9%, respectively. Final PGFM concentrations were expressed as picograms per milliliter.

Ultrasonography

Ultrasound examination of the ovaries was performed for each cow at days 7, 14 and 21 postpartum to determine the number of small (C1; 3 to 5 mm), medium (C2; 6 to 9 mm) and large (C3; > 9 mm) follicles. A real-time ultrasound scanner (Aloka SSD 500 V, Aloka Co. Ltd., Tokyo, Japan) equipped with a 7.5 MHz rectal probe was used.

Statistical Analyses

Milk production and intake responses were reduced to weekly means before statistical analysis. Milk composition was analyzed by Least-Square analysis of variance (ANOVA) using the General Linear Model (GLM) procedure. All other dependent responses were evaluated using the MIXED procedure for repeated measurement of the SAS software package (SAS, 2001). Fixed effects included treatment, parity, week relative to calving (for production responses) or days relative to calving (for metabolic and reproductive responses), and treatment by week interaction. The variance for cow nested within treatment by parity was used as random error term to test the main effect of treatment. Differential temporal responses to dietary treatments were further examined using the SLICE option of the MIXED procedure. Mean treatment and time (week or day relative to calving) effects are reported as least squares means.

Results

Production Responses

Multiparous cows had greater ($P < 0.05$) dry matter intake (15.5 ± 0.7 kg/d), body weight (700 ± 15.5 kg), and BCS (3.14 ± 0.04) when compared to primiparous cows (11.9 ± 0.8 kg/d, 539.7 ± 18.7 kg, and 3.00 ± 0.04, respectively) throughout the

experimental period. However, these responses were similar for both treatment groups in either heifers or cows during the prepartum and postpartum periods.

Postpartum DMI in heifers and cows increased ($P < 0.0001$) from 2.1% of BW at wk 1 to approximately 2.9% of BW at wk 3 of lactation, with no differences observed between treatment groups (Figure 6-1A, B). Patterns of BW response did not vary between the two dietary groups (Figure 6-2A, B). Moreover, the amount of BW loss during the experimental period (wk -2 to wk 3) between control and *t*FA groups was similar in heifers (92 vs. 105 kg) and cows (95 vs. 99 kg). Changes in energy balance were similar for heifers and cows for both experimental diets (Figure 6-3A, B).

Heifers and cows fed control and *t*FA had comparable BCS and experienced similar patterns of change throughout the experiment (Figure 6-4A, B). The main loss in BCS occurred between wk 2 prepartum and wk 1 postpartum in both heifers (3.26 vs. 2.98 ± 0.1) and cows (3.40 vs. 3.00 ± 0.1). However, during the postpartum period no detectable changes in BCS were observed.

Average milk production during the 3-wk postpartum treatment period did not differ between dietary groups, although cows consistently produced more milk ($P < 0.0005$) than heifers (35.8 vs. 44.5 kg/d) (Figure 6-5A, B). The amount of milk produced as a function of consumption of DM (feed efficiency) increased with time in both treatment groups in heifers and cows (Figure 6-6). At week 3 of lactation, heifers produced less milk than cows (35.8 ± 2.2 kg/d vs. 44.5 ± 1.8 kg/d) (Table 6-5). Mean milk fat and protein production and concentration and SCC did not differ between dietary treatments (Table 6-5).

Metabolic Responses

Both treatment groups had similar patterns of plasma NEFA concentrations, which were low during the prepartum period, dramatically increased at 1 to 2 wk after calving, then gradually declining over time (Figure 6-7A, B). During the postpartum period, NEFA concentrations in heifers (491.9 ± 93.4 μEq/L) and cows (420.0 ± 70.7 μEq/L) peaked around d 7, then declining towards prepartum concentrations by d 21.

Plasma BHBA concentrations during the prepartum period were similar between treatment groups in heifers (2.03 ± 1.1 mg/dL) and cows (2.66 ± 0.8 mg/dL). Immediately after calving, a dramatic increase in BHBA concentration was observed by d 7 postpartum in both heifers (4.91 ± 1.1 mg/dL) and cows (4.53 ± 0.8 mg/dL) (Figure 6-8A, B). After a plateau was reached between d 7 and 14, heifers from the control group further increased ($P = 0.035$) plasma BHBA concentration at d 21 (6.68 ± 1.1 mg/dL), while BHBA concentration in heifers from the *t*FA group continued to decline until d 21 (3.12 ± 1.1 mg/dL) (Figure 6-8A). On the other hand, plasma BHBA concentration in control cows declined between d 7 and 21 (3.23 ± 0.8 mg/dL). Cows fed *t*FA continued to gradually increase BHBA concentration through d 14 ($P = 0.04$) (5.39 ± 0.8 mg/dL) until reaching its highest concentration at d 21 ($P = 0.005$) postpartum (5.90 ± 0.8 mg/dL) (Figure 6-8B).

Control and *t*FA-fed heifers and cows exhibited similar patterns of change in plasma glucose concentration throughout the experimental period. Plasma glucose concentrations during the prepartum (60.9 ± 3.3 vs. 61.6 ± 2.4 mg/dL) and postpartum (56.0 ± 3.3 vs. 52.4 ± 2.4 mg/dL) periods were similar between treatments in heifers and cows, respectively (Figure 6-9A, B). Concentrations of glucose in plasma were unaffected by dietary treatment (Figure 6-9A, B).

Reproductive Responses

The number and distribution of small (C1), medium (C2), and large (C3) follicles in the ovaries were not different between dietary treatments for heifers and cows at d 7 and d 14 postpartum (Table 6-6). However, at d 21, cows tended ($P = 0.11$) to have more C1 follicles (9.5 ± 1.7) than heifers (6.5 ± 2.0), while heifers had ($P = 0.03$) more C2 follicles (2.8 ± 0.9) when compared to cows (0.8 ± 0.9) (Table 6-6). The number of C3 follicles increased ($P < 0.05$) from d 7 to d 21 postpartum in heifers (0 vs. 1.2 ± 0.4) and cows (0.3 vs. 1.1 ± 0.3) (Table 6-6).

Control and *t*FA-fed heifers had comparable plasma PGFM concentrations and showed similar patterns of change during the experimental period (Figure 6-10A). Pulses of plasma PGFM in heifers were characterized by an initial raise around calving date (1125.7 ± 285.8 pg/mL), persisting and reaching its highest concentration by d 4 (1155.1 ± 285.8 pg/mL), then gradually decreasing and approaching basal concentration around d 10 postpartum (Figure 6-10A).

Circulating PGFM concentration and patterns of change in control cows were similar to those observed in heifers. Initial stimulation of PGFM secretion occurred one day prior to calving, peaking in the afternoon of d 1 postpartum (1501.7 ± 322.3) and gradually decreasing to basal concentration by d 10 postpartum (Figure 6-10B). In *t*FA-supplemented cows, a dramatic increase ($P = 0.04$) in PGFM concentration were observed by d 1 (2106.1 ± 355.5) (Figure 6-10B). The *t*FA-stimulation of PGFM reached peak concentration at d 2 pm (3767.3 ± 347.9), and persisted with higher concentration up to d 4 postpartum (2150.8 ± 347.9) before decreasing to basal concentration by d 10 postpartum (Figure 6-10B). Even though greater PGFM concentrations were detected in *t*FA-fed cows between d 1 and 4 postpartum, the decrease in PGFM concentration was

faster in *t*FA-fed cows (d 2 pm to d 10 pm = 458.2 pg/mL per day) than control cows (d 1 pm to d 10 pm = 152.2 pg/mL per day).

Discussion

During the experimental period, DMI, BW, and BCS responses were similar between dietary groups. However, cows had consistently higher DMI (kg/d) and BW as well as better BCS when compared to heifers. Similarly, cows produced more milk than heifers, which is also related to the difference in DMI between cows and heifers. Moreover, when DMI is expressed as a percentage of BW, no difference between cows and heifers were detected. Additionally, milk production as a function of DMI (calculated feed efficiency) also was similar between cows and heifers. Taken together, these observations further support the fact of increased nutrient needs for growth of heifers taking place simultaneously with the demands of lactation and their lower feed intake capacity as described previously (Rémond et al., 1991).

The higher plasma NEFA and BHBA concentrations detected in heifers and cows after parturition, regardless of dietary treatments, likely reflect the NEB of the animals, as previously observed (Invartsen and Andersen, 2000; Moorby et al., 2000). Additionally, induction of plasma NEFA and BHBA concentrations after parturition in heifers and cows results from decreased DMI prior to calving and periparturient hormonal changes that stimulate fat mobilization from the adipose tissue to provide energy for lactogenesis (Vazquez-Añon et al., 1994; Grum et al., 1996). This is further supported by the observation that plasma NEFA concentration is a reliable index of the magnitude of adipose fat mobilization (Bauman et al., 1988).

In the present study, plasma NEFA concentrations were not affected by either diet ($P = 0.44$) or parity ($P = 0.94$). The lack of parity effect contrasts with several cattle

studies in which heifers mobilized more fat from adipose tissue than cows as indicated by plasma NEFA concentrations (Belyea et al., 1975; Drackley et al., 2003; Meikle et al., 2004; Cavestany et al., 2005). In the aforementioned studies, differences between heifers and cows were attributed to higher energetic demands (e.g. growth) experienced by heifers. Thus, results from the present study indicate that supplementation with either saturated or *t*FA may assist heifers to reduce the dramatic energy deficit generally experienced due to the combination of growth and lactation.

While the patterns of change of NEFA concentration postpartum in the present study were similar to several published reports (Garcia-Bojalil et al., 1998; Mattos et al., 2004; Meikle et al., 2004; Cavestany et al., 2005), NEFA concentrations, in general, appeared to be slightly lower. This difference may be due to different experimental conditions as well as dietary supplementation. However, cows supplemented with *t*FA in this study exhibited a similar profile and NEFA concentrations during the early postpartum period compared to NEFA profile and concentrations observed on *t*FA-fed cows in the study by Selberg et al. (2004). Taken together, these observations indicate that dietary supplementation with either saturated fat or *t*FA may reduce the fat mobilization observed in cows and heifers during the transition period. This is in agreement with the study by Selberg et al. (2004) indicating that dietary *trans*-octadecenoic fatty acids reduced fat mobilization by early postpartum Holstein cows.

Plasma BHBA concentrations in heifers and cows were similar to those previously reported (Meikle et al., 2004; Cavestany et al., 2005; Selberg et al., 2004). The greater BHBA concentration detected in heifers and cows soon after calving likely reflects the NEB experienced by these animals around parturition. Although not statistically

significant, NEFA concentrations were numerically greater in control heifers than *t*FA-supplemented heifers at d 7 (564.0 vs. 419.8 ± 84.2). When compared to the control group, *t*FA-fat cows exhibited numerically greater blood NEFA concentration during the postpartum period. Hence, increased plasma BHBA levels experienced by control heifers and *t*FA cows may be the product of NEFA accumulation in the liver, thus shifting metabolism towards partial oxidation.

The observation that supplemental *t*FA failed to alter peripheral glucose concentration in periparturient animals is consistent with a recent cattle study (Selberg et al., 2004). Although Selberg and coworkers (2004) also found that dietary *trans* fatty acids induced steady state mRNA levels of enzymes involved in hepatic gluconeogenesis, no information on enzyme activity was available. Hence, they concluded that the discrepancy between the amount of an enzyme and the concentration of its metabolic product may reflect differences between peripheral processing of the metabolite and the enzymatic activity.

In the present study, we examined the effects of the dietary supplementation on follicular dynamics by monitoring the proportion of small (C1), medium (C2) and large (C3) ovarian follicles at d 7, 14 and 21 postpartum. There was no parity or diet effect on the number of small- and medium-size follicles (3 to 9 mm) within the first three weeks of lactation. The increased number of large follicles between d 7 and d 21 observed in heifers and cows reflects the normal movement of follicles from class 2 to class 3 between wk 1 and 3 postpartum.

Several studies have shown that manipulations of dietary fatty acids may have major effects on PGFM concentration in ruminants. A number of studies have

demonstrated inhibitory effects of n-3 PUFAs on $PGF_{2\alpha}$ production (Bezard et al., 1994; Staples et al., 1998; Abayasekara and Wathes, 1999; Mattos et al., 2000, 2001, 2002, 2004). Supplementation of dairy cattle with fish meal (high in n-3 PUFAs) reduced basal and OT-induced plasma PGFM concentrations in early postpartum dairy cows (Mattos et al., 2002, 2004). In the present study, cows supplemented with dietary *t*FA had higher plasma PGFM concentration than control cows. The increased PGFM concentration observed in cows supplemented with *t*FA is in agreement with *in vitro* studies from our laboratory showing that supplementation of bovine endometrial (BEND) cells with *trans* isomers of octadecenoic fatty acids resulted in significant augmentation of $PGF_{2\alpha}$ response to PDBu (Chapter 5).

Positive effects of dietary fats on fertility response are not related exclusively to inhibition of PG biosynthesis. For example, preliminary data in our laboratory indicated that *trans*-octadecenoic fatty acids may shorten the postpartum interval to estrus in early postpartum Holstein cows, suggesting enhanced ovarian activity and function after parturition (Selberg et al., unpublished data). Following parturition, resumption of normal estrous cycles is dependent on uterine involution and repair caused by myometrial contractions stimulated by $PGF_{2\alpha}$ (Kiracofe, 1980). In addition, application of exogenous $PGF_{2\alpha}$ early postpartum, increased myoelectrical activity and contraction of the uterus (Patil et al., 1980; Gajewski et al., 1999). Thus, enhanced ovarian activity and function may be related to elevated levels of PGFM since the present study showed that dietary *t*FA greatly increased and sustained higher plasma PGFM concentration between days 1 and 4 postpartum when compared to control cows. In addition, the duration of postpartum $PGF_{2\alpha}$ sustained release is negatively correlated with the number of days to

complete uterine involution and the interval between parturition and resumption of normal ovarian activity (Lindell et al., 1982; Madej et al., 1984). While the uterus reaches the size of the nonpregnant uterus at about three weeks postpartum, Lindell and Kindahl (1983) reported that exogenous application of $PGF_{2\alpha}$ between days 3 and 10 postpartum decreased involution time by about one week.

Increased plasma PGFM also may have beneficial effects on immune competency since, in this study, the proportion of cows with metritis was higher in control compared to *t*FA dietary group (22 % vs. 0 %; Table 6-1). A study by Seals et al. (2002) supports this observation as they reported that postpartum concentrations of PGFM were inversely related to emergence of uterine infections. Since $PGF_{2\alpha}$ stimulates myometrial contraction (Lindell and Kindahl, 1983; Patil et al., 1980; Gajewski et al., 1999), this may be a mechanism to expel debris and micro-organisms that contaminate the uterine lumen after calving (Dhaliwal et al., 2001). Moreover, $PGF_{2\alpha}$ may have a stimulatory effect on the phagocytic activity of uterine polymorphonuclear inflammatory cells (Paisley et al., 1986).

Summary

Feeding *trans* fatty acids did not induce any significant alterations in production as well as metabolic responses when compared to supplementation with saturated fatty acids in either heifers or cows. Although no differences were observed in PGFM levels between dietary groups in heifers, dietary supplementation of cows with *t*FA, increased plasma PGFM concentration within 4 d postpartum when compared to saturated fatty acids. Whether this augmentation in $PGF_{2\alpha}$ production results in enhanced ovarian activity and fertility as well as immune competency, warrants further research.

Table 6-1. Incidence of health disorders of heifers and cows fed diets containing a highly saturated fat (Control) or a Ca salt enriched in *trans* $C_{18:1}$ (*t*FA).

Disorder	Heifers		Cows	
	Control	*t*FA	Control	*t*FA
Calving Difficulty[1]	1/6 (17%)	2/6 (33%)	1/9 (11%)	0/9 (0%)
Retained Placenta[2]	0/6 (0%)	1/6 (17%)	2/9 (22%)	2/9 (22%)
Enteritis[3]	0/6 (0%)	0/6 (0%)	0/9 (0%)	1/9 (11%)
Metritis[4]	2/6 (33%)	1/6 (17%)	2/9 (22%)	0/9 (0%)
Displaced Abomasum[5]	0/6 (0%)	0/6 (0%)	1/9 (11%)	2/9 (22%)

[1] Calving difficulty refers to animals that scored greater than 2 in a 5 point calving difficulty scale.
[2] Retained placenta refers to animals that did not expel fetal membranes within 12 h of calving.
[3] Enteritis refers to inflammation of the intestinal cells (enterocytes).
[4] Metritis refers to inflammation of the uterus with purulent discharge from the vagina and a fever greater than 102 °C for two consecutive days.
[5] Displaced abomasum refers to cases that required surgery.

Table 6-2. Fatty acid profile according to the manufacturers of a highly saturated fat (RBF; Cargill, Minneapolis, MN) and a Ca salt lipid enriched in *trans* $C_{18:1}$ (*t*FA) (Virtus Nutrition LLC, Fairlawn, OH) fed during the prepartum and postpartum periods to Holstein heifers and cows.

Fatty acid	RBF	*t*FA
	--------(g/100 g)--------	
C12:0	…	0.04
C14:0	5.60	0.31
C16:0	37.80	12.21
C16:1	…	0.15
C18:0	48.00	6.70
C18:1, *trans* 6-8	…	20.62
C18:1, *tran*-9	…	10.47
C18:1, *trans*-10	…	10.62
C18:1, *trans*-11	…	7.05
C18:1, *trans*-12	…	8.73
C18:1, *cis*-9	4.80	10.04
C18:1, *cis*-9, *cis*-12	…	1.97
Unknown	3.80	11.07

Table 6-3. Ingredient composition of prepartum and postpartum diets.

Ingredient	Prepartum	Postpartum
	--------% of DM--------	
Corn silage	45.0	37.5
Bermudagrass hay	15.0	…
Alfalfa hay	…	11.9
Corn meal	14.9	20.3
Soy Plus[1]	…	13.4
Soybean meal	12.0	7.6
Citrus pulp	5.0	…
Cottonsead hulls	…	2.5
Fat supplement[2]	1.5 - 1.8	1.5 - 1.8
Mineral and vitamin mix[3]	6.5	…
Mineral and vitamin mix[4]	…	4.8
Trace mineralized salt[5]	0.1	…
Biophos[6]	…	0.3

[1] West Central Soy, Ralston, IA.
[2] Fat source consisted of 1.5% and 1.8% of a highly saturated fat (RBF) and a Ca salt lipid enriched in *trans* $C_{18:1}$ (*t*FA), respectively.
[3] Mineral and vitamin mix contained 22.8% CP, 2.1% fat, 22.89% Ca, 0.16% P, 2.77% Mg, 0.75% Na, 0.20% K, 2.42% S, 8.03% Cl, 150 mg/kg of Mn, 97.0 mg/kg of Zn, 168 mg/kg of Fe, 186 mg/kg of Cu, 11 mg/kg of Co, 8.4 mg/kg of I, 6.9 mg/kg of Se, 268,130 IU/kg of vitamin A, 40,000 IU/kg of vitamin D, and 1,129 IU/kg of vitamin E (DM basis).
[4] Mineral and vitamin mix contained 26.4% CP, 1.74% fat, 10.15% Ca, 0.90% P, 3.1% Mg, 8.6% Na, 5.1% K, 1.5% S, 4.1% Cl, 2,231 mg/kg of Mn, 1,698 mg/kg of Zn, 339 mg/kg of Fe, 512 mg/kg of Cu, 31 mg/kg of Co, 26 mg/kg of I, 7.9 mg/kg of Se, 147,756 IU/kg of vitamin A, 43,750 IU/kg of vitamin D, and 787 IU/kg of vitamin E (DM basis).
[5] Minimum concentrations of 40% Na, 55% Cl, 0.25% Mn, 0.2% Fe, 0.033% Cu, 0.007% I, 0.005% Zn, and 0.0025% Co (DM basis).
[6] IMC-Agrico, Bannockburn, IL.

Table 6-4. Chemical composition of prepartum and postpartum diets.

	Prepartum		Postpartum	
Ingredient	Control	*t*FA	Control	*t*FA
DM, %	47.2	47.2	50.9	50.9
CP, % DM	13.8	14.0	17.2	16.9
ADF, % DM	22.1	22.3	17.8	17.8
NDF, % DM	37.4	37.7	31.1	31.2
Lipid, % DM	3.89	4.05	4.64	5.45
NE_L, Mcal/kg	1.53	1.53	1.67	1.67
Ca, % DM	1.78	1.83	1.02	1.11
P, % DM	0.33	0.34	0.48	0.47
Mg, % DM	0.33	0.34	0.34	0.34
K, % DM	1.30	1.32	1.56	1.57
Na, % DM	0.20	0.19	0.45	0.43
Fe, mg/kg	563	541	417	425
Zn, mg/kg	65	58	92	90
Cu, mg/kg	27	23	32	37
Mn, mg/kg	67	61	92	88
Mo, mg/kg	0.77	0.97	1.4	1.1

Table 6-5. Performance of lactating heifers and cows fed a diet containing a highly saturated fat (Control) or a Ca salt enriched in *trans* $C_{18:1}$ (*t*FA) at wk 3 postpartum.

	Heifers		Cows			*P*-value		
Variable	Control	*t*FA	Control	*t*FA	SEM	Diet	Parity	D x P
Milk, kg/d	37.0	34.6	45.2	43.8	2.50	0.34	0.0001	0.8420
3.5% FCM[1], kg	29.5	27.8	36.7	35.5	2.39	0.55	0.0053	0.95
Milk fat								
%	3.36	3.48	3.48	3.31	0.24	0.90	0.92	0.56
kg/d	0.83	0.79	1.06	1.02	0.11	0.73	0.04	0.98
Milk true protein								
%	2.72	2.60	2.81	2.85	0.09	0.69	0.08	0.39
kg/d	0.65	0.60	0.86	0.88	0.07	0.84	0.001	0.56
SCC (x 1000)	116	135	65	87	29	0.80	0.55	0.94

[1] 3.5% Fat-corrected milk = (0.4324)(kg milk) + (kg milk fat)(16.216)

Table 6-6. Distribution of follicles* in lactating heifers and cows fed a diet containing a highly saturated fat (Control) or a Ca salt enriched in *trans* $C_{18:1}$ (*t*FA).

Variable	Heifers		Cows		SEM	*P*-value		
	Control	*t*FA	Control	*t*FA		Diet	Parity	D x P
Day 7 postpartum								
C1	9.5	9.8	8.4	7.3	1.8	0.83	0.34	0.77
C2	1.3	2.2	0.7	2.2	0.9	0.17	0.74	0.49
C3	0.0	0.0	0.3	0.3	0.3	0.94	0.36	0.84
Day 14 postpartum								
C1	8.0	10.2	8.4	6.5	1.8	0.94	0.38	0.55
C2	1.8	1.2	1.3	1.2	0.9	0.70	0.78	0.97
C3	0.8	0.8	0.7	1.2	0.3	0.46	0.63	0.73
Day 21 postpartum								
C1	5.8	7.2	10.0	9.0	1.8	0.90	0.11	0.39
C2	3.8	1.8	0.4	1.2	0.9	0.49	0.03	0.07
C3	1.3	1.0	1.0	1.2	0.3	0.90	0.90	0.94

C1 = 3 - 5 mm; C2 = 6 - 9 mm; C3 = > 9 mm.

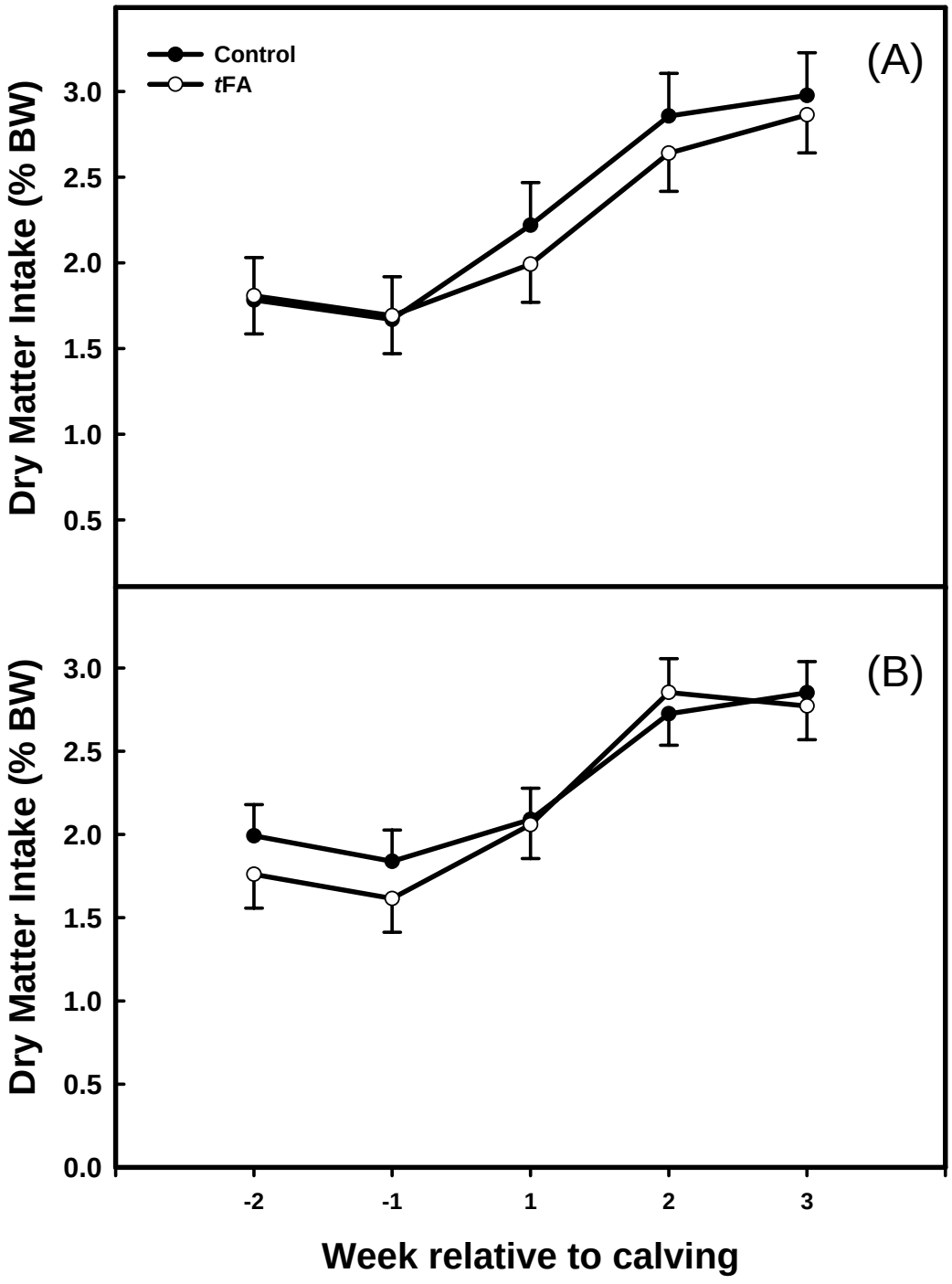

Figure 6-1. Average dry matter intake (DMI) as a percentage of body weight (BW) of periparturient Holstein heifers (A) and cows (B) fed a control (C) or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). There was no trt x par x wk interaction ($P = 0.3750$).

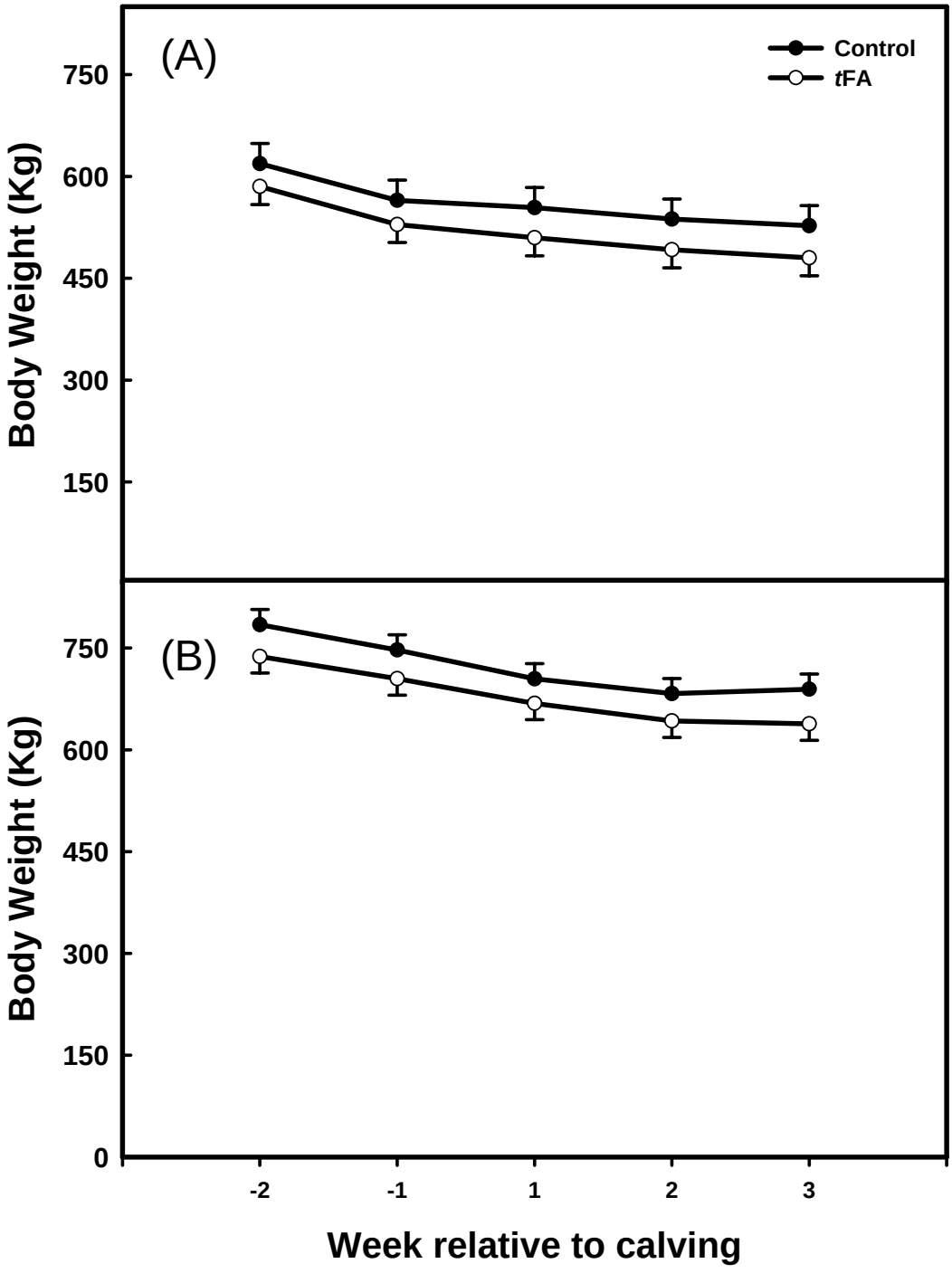

Figure 6-2. Average body weight (BW) of periparturient Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). There was no trt x par x wk interaction ($P = 0.9559$).

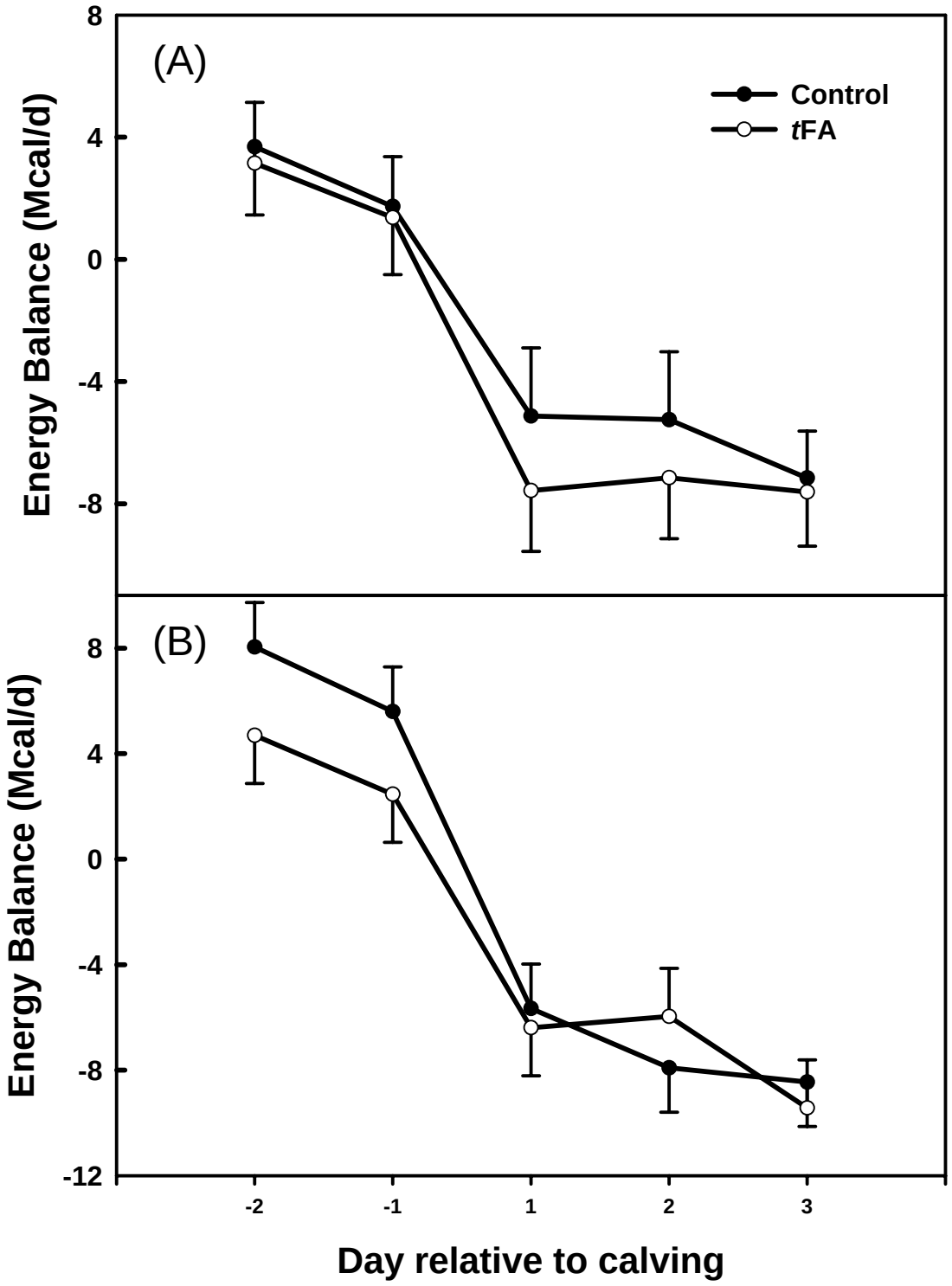

Figure 6-3. Calculated energy balance by week relative to parturition for Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). There was no trt x par x wk interaction ($P = 0.1959$).

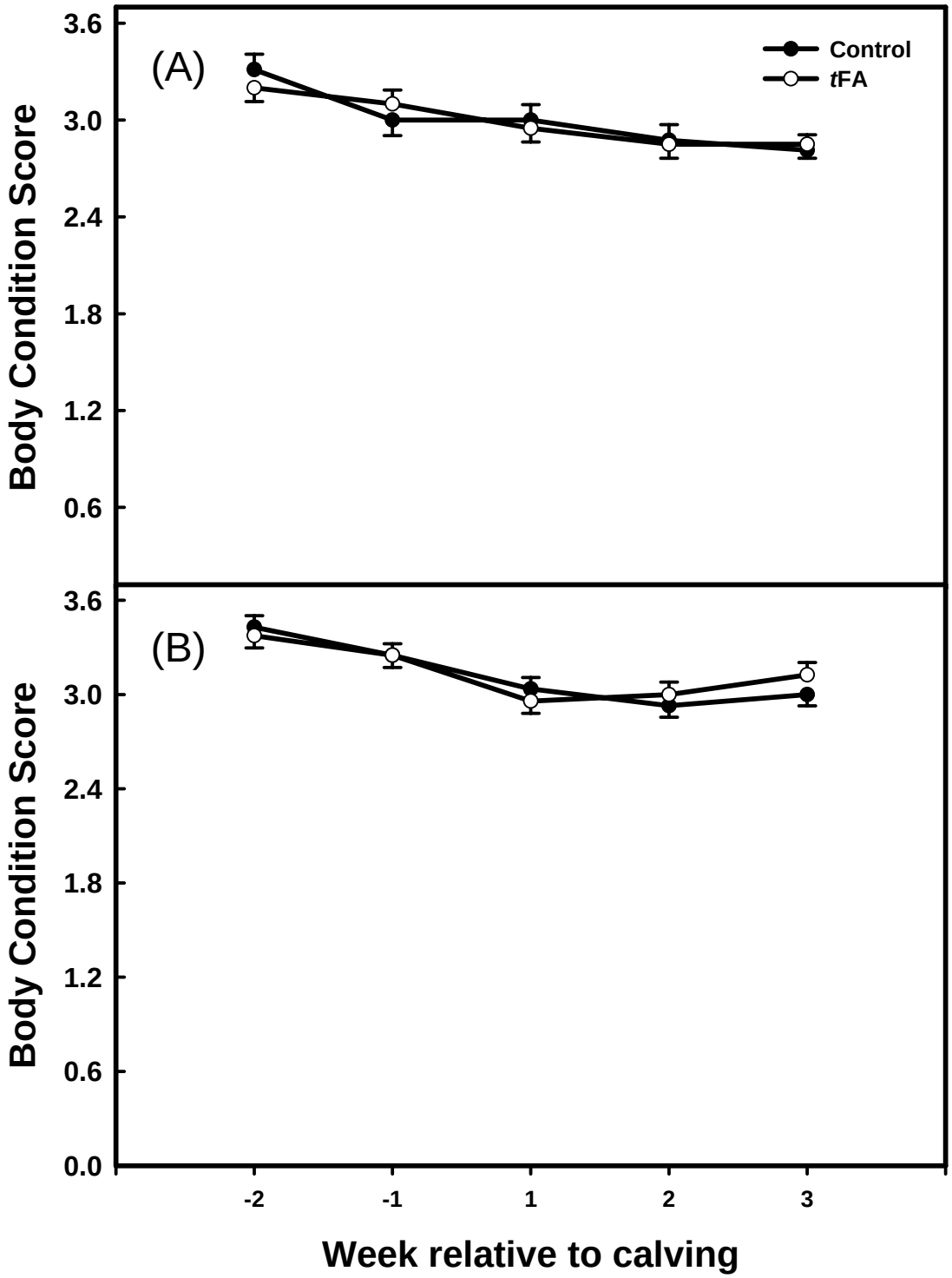

Figure 6-4. Average body condition score (BCS) of periparturient Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). There was no trt x par x wk interaction ($P = 0.8333$).

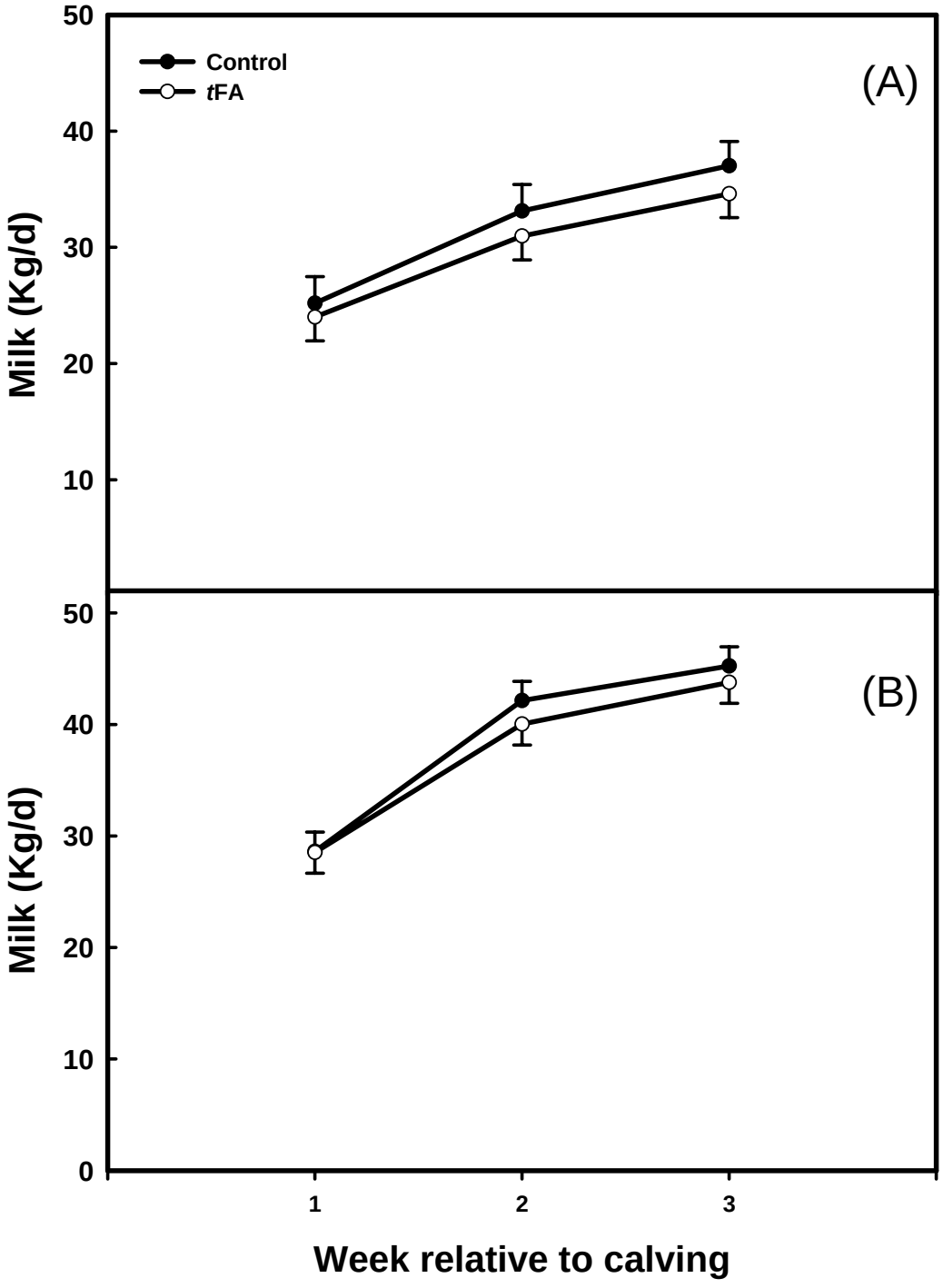

Figure 6-5. Temporal patterns of milk yield by periparturient Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). There was no trt x par x wk interaction ($P = 0.9203$).

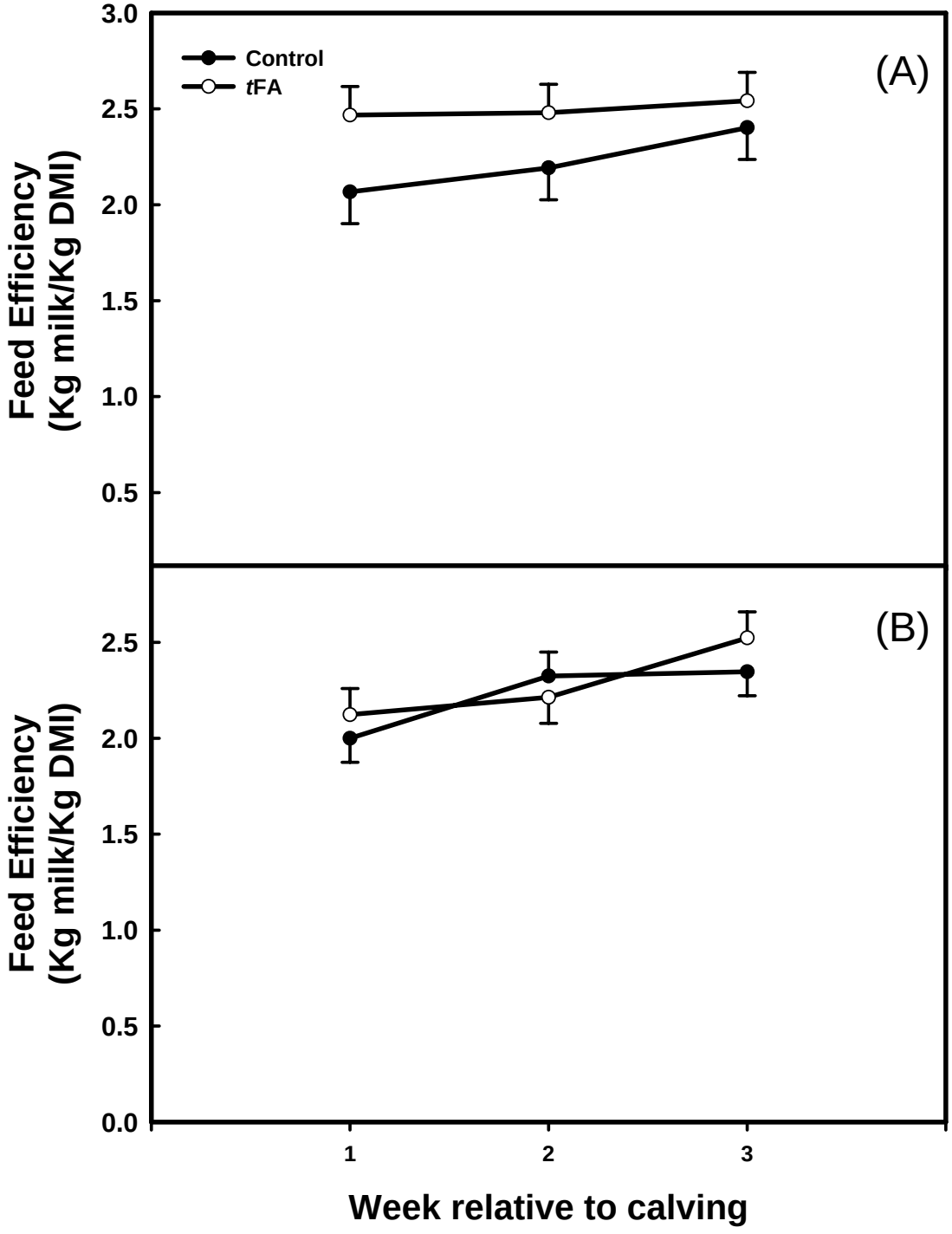

Figure 6-6. Average feed efficiency as a function of milk yield over intake of periparturient Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). There was no trt x par x wk interaction (P = 0.1818).

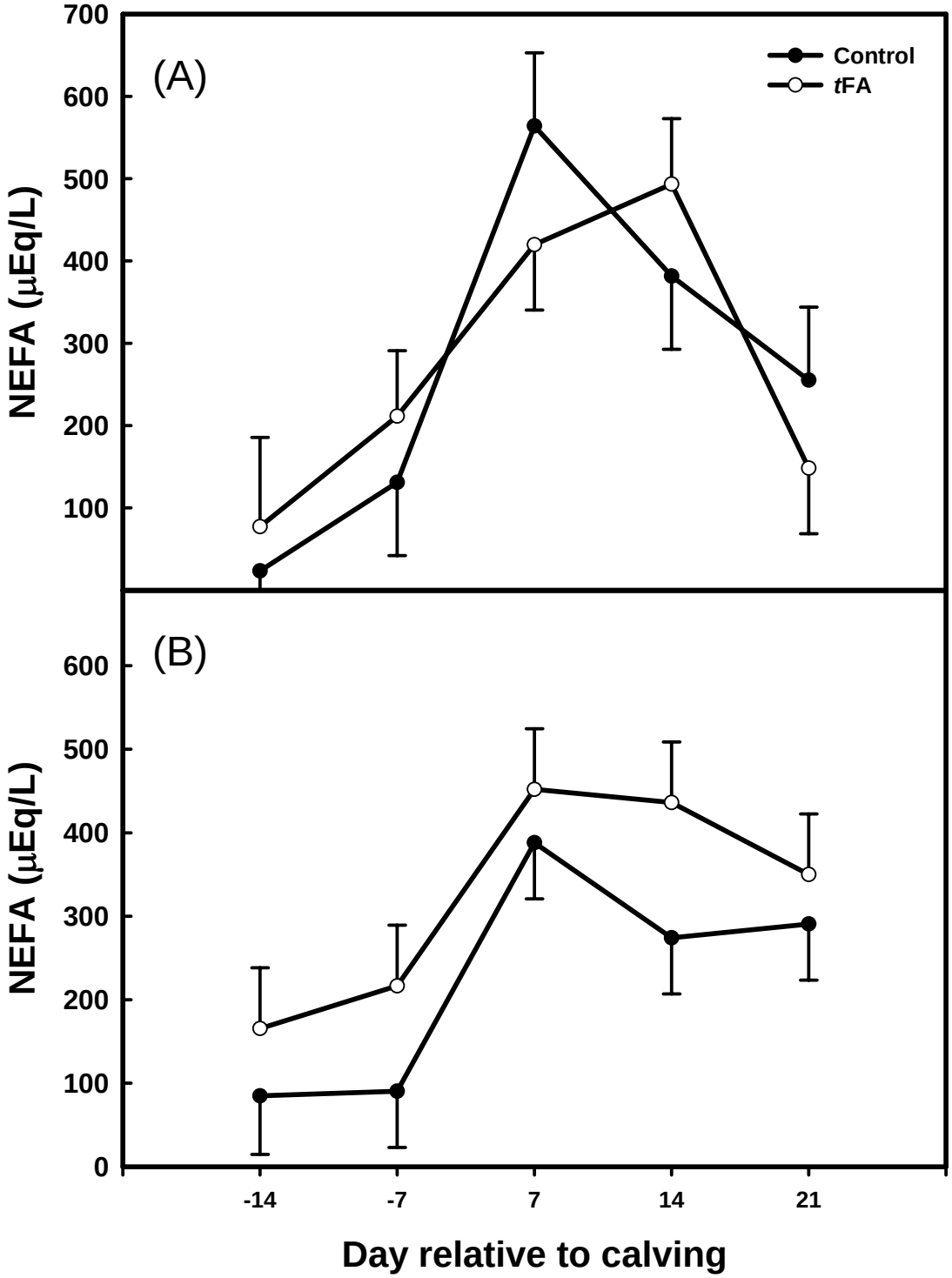

Figure 6-7. Plasma NEFA concentrations by week relative to calving in periparturient Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). There was a par x day interaction ($P = 0.04$).

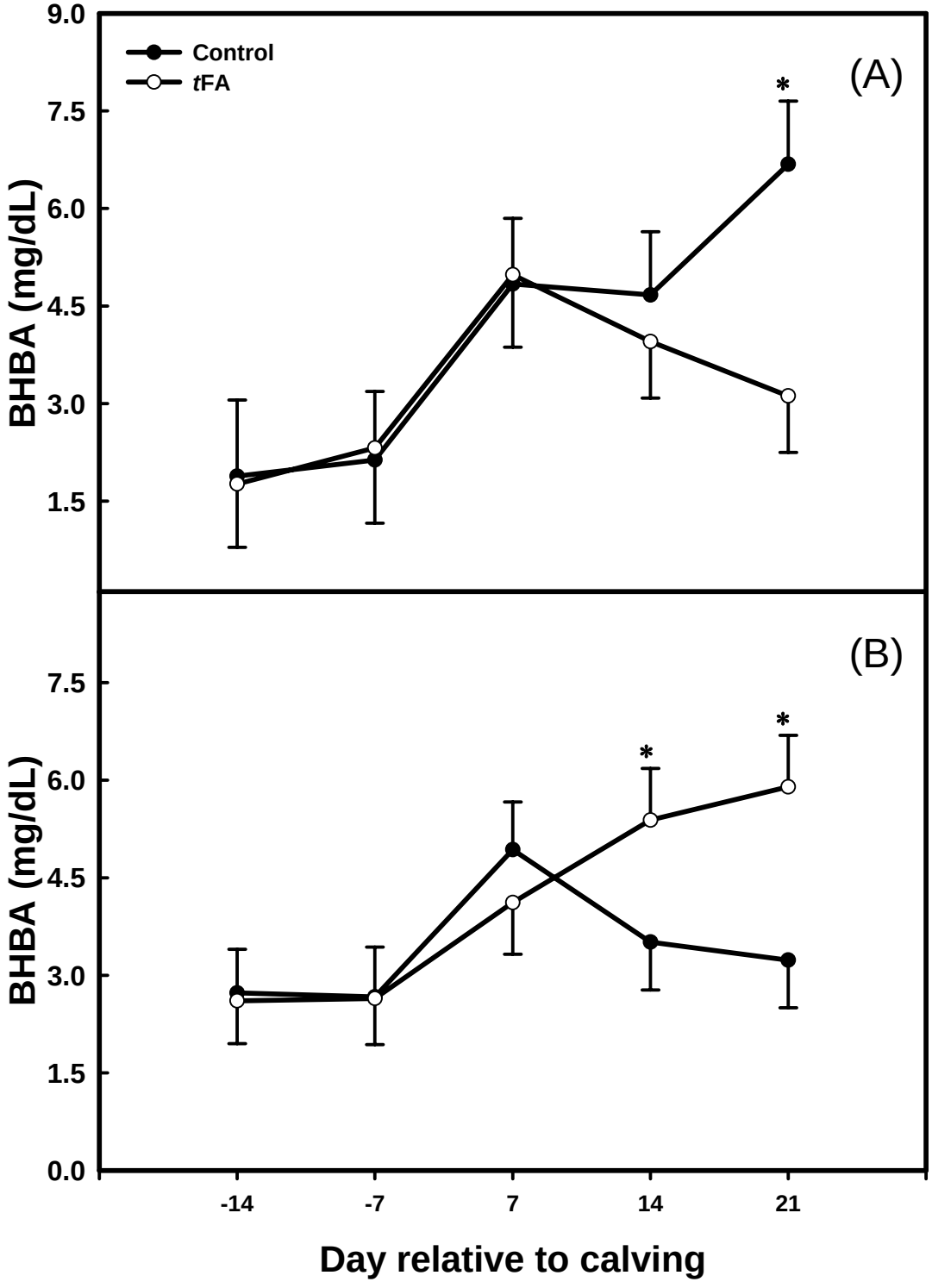

Figure 6-8. Plasma BHBA concentrations by week relative to calving in periparturient Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). Asterisks indicate significant treatment differences ($P < 0.05$). There was a trt x par x day interaction ($P = 0.0244$).

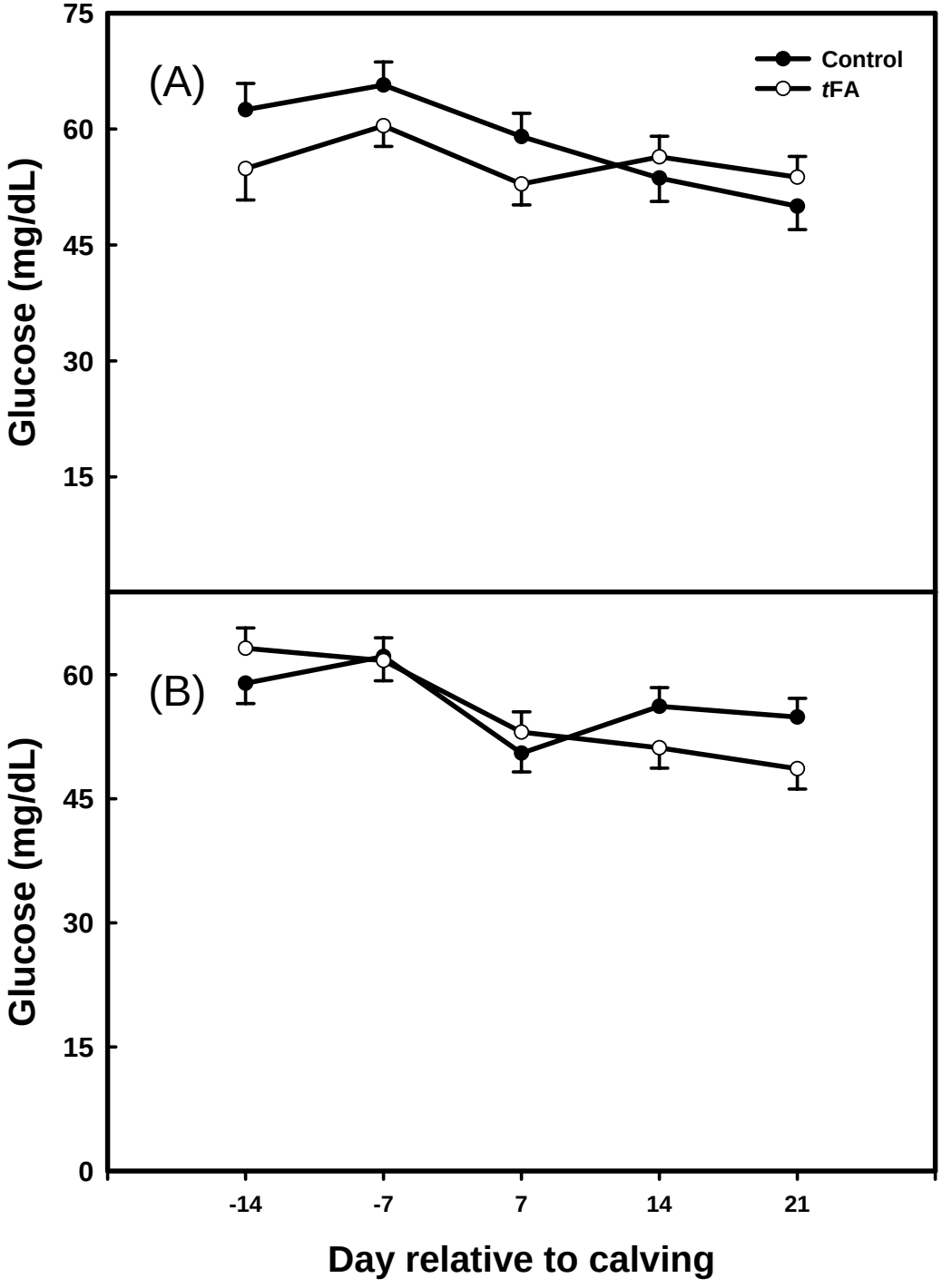

Figure 6-9. Plasma glucose concentrations by week relative to calving in periparturient Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). There was a trt x par x day interaction (P = 0.0124).

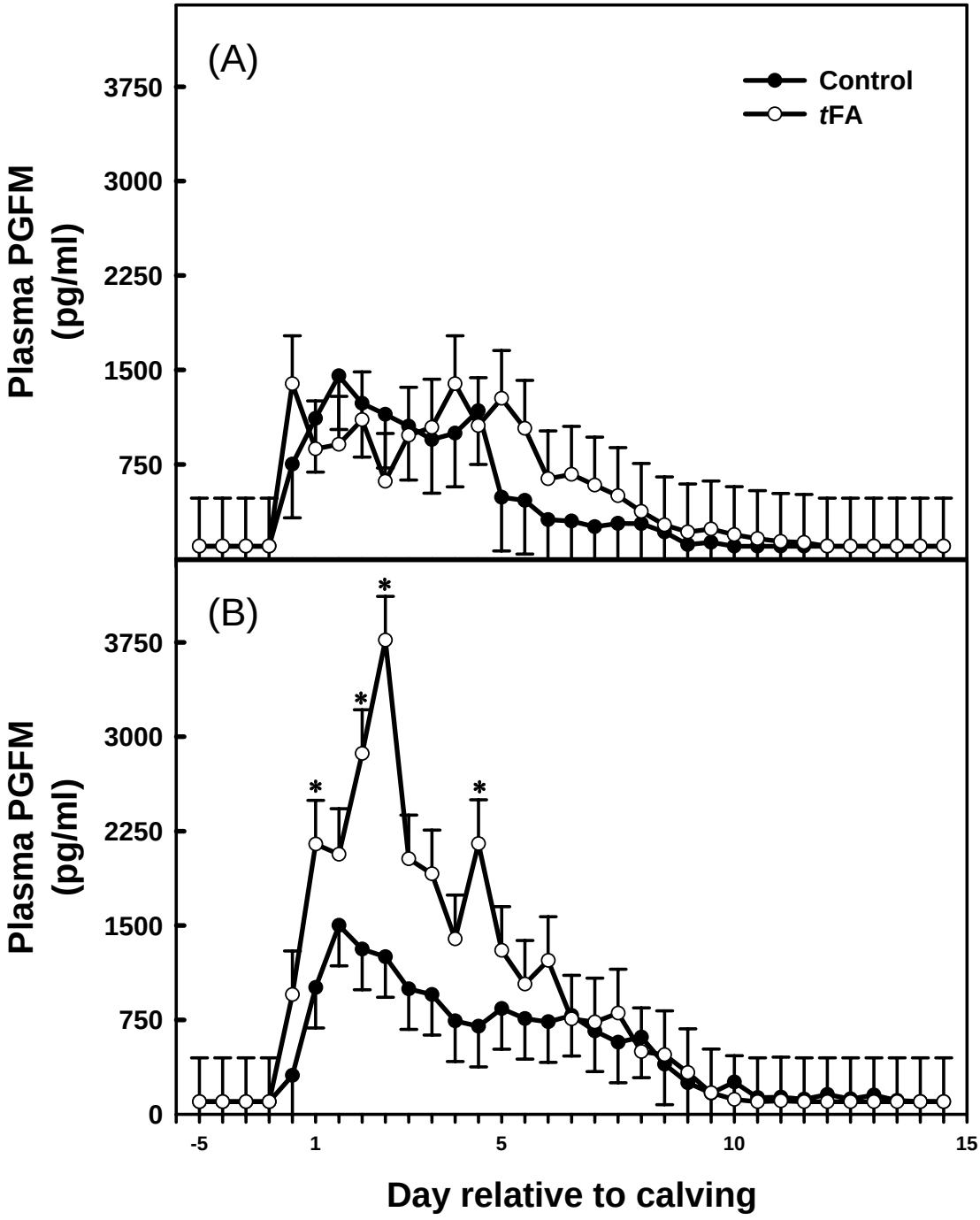

Figure 6-10. Plasma PGFM concentrations by week relative to calving in periparturient Holstein heifers (A) and cows (B) fed a control or *trans*-$C_{18:1}$ (*t*FA)-supplemented diet. Data represents least square means ± SEM (Heifers: 4 C, 5 *t*FA; Cows: 7 C, 6 *t*FA). Asterisks indicate significant treatment differences ($P < 0.05$).

CHAPTER 7
GENERAL DISCUSSION

In the past decade, genetic selection for high milk production has been associated with a decrease in reproductive efficiency in lactating dairy cows. This reduction in reproductive efficiency stems from early embryonic loss, impaired ovarian cyclicity and low fertility rates in high producing dairy cows. The transition to lactation, which extends from 3 weeks before calving to 3 weeks after parturition, is characterized by an abrupt shift in nutritional requirements, followed by dramatic metabolic changes in order to support lactation. Essentially all of the energy that is consumed is used by the mammary tissue for milk production, leaving no energy for maintenance. Hence, lactating dairy cows experience a state of negative energy balance (NEB), resulting in massive mobilization of fat from the adipose tissue.

The mechanisms by which the periparturient metabolic upsets reduce reproductive efficiency are not well understood. However, available evidence indicates that reproductive efficiency is dependent on the growth and development of a viable oocyte that can then be fertilized. This is directly dependent on a normal estrous cycle characterized by folliculogenesis, ovulation, and corpus luteum (CL) formation and regression (~21 days). It has been shown that with inappropriate nutrition, ovarian cyclicity may cease. Thus the nutritional and energy state of the cow may influence reproductive efficiency by modulating the estrous cycle. This could be through the neuroendocrine modulation of gonadotropin secretion, although more consistent evidence is needed. Alternatively, local ovarian effects could take place through modulation of

follicular growth and development, which might determine oocyte quality and viability and steroidogenic activity of the CL. However, energy-independent effects may also be observed after fatty acid supplementation, which could involve prostaglandin biosynthesis. Therefore, nutrition can influence reproductive efficiency in dairy cows not only by altering the energy status of the animal, but also by influencing factors involved in the regulation of reproductive processes like follicular dynamics, ovulation, CL function and embryo survival.

Long-chain fatty acids (LCFA) are generally added to dairy rations to increase the energy density of the diet. It is expected that supplementation of the diet with fatty acids may enhance reproductive efficiency by enhancing the energy status of the dairy cow. However, recent studies indicate that dietary fatty acids may affect reproductive efficiency in farm animals through an energy-independent mechanism. One way that fatty acids could enhance reproductive efficiency would be through regulation of prostaglandin biosynthesis. Prostaglandin production can be influenced by nutrition since the precursor for the biologically active prostaglandin of the two series is arachidonic acid (AA), an n-6 fatty acid synthesized from elongation/desaturation of linoleic acid (LA). Prostaglandins (PG) of the 2 series ($PGF_{2\alpha}$, PGE_2) have been implicated in the process of reproduction; including ovulation, follicular development, and CL function. Hence, any effects of fatty acids on $PGF_{2\alpha}$ synthesis is likely to affect overall reproductive performance.

There are reports that indicate that dietary supplementation of fat has positive effects on reproduction. Many speculate that these positive effects are a result of inhibition of prostagladin biosynthesis, specifically $PGF_{2\alpha}$, which results in prevention of

CL regression and increased progesterone secretion around the time of embryo recognition. The CL function is very important for reproductive efficiency since it is critical for establishment and maintenance of pregnancy. Alternatively, stimulation of $PGF_{2\alpha}$ during the first 10 d of lactation also would be beneficial since this prostaglandin is important for uterine involution after parturition.

We first set out to examine the effects of PUFAs on $PGF_{2\alpha}$ responses to PDBu in cultured bovine endometrial (BEND) cells (Chapters 3 and 4). Phorbol-ester stimulated $PGF_{2\alpha}$ production and up-regulated PGHS-2 gene and protein expression within 6 h in cultured BEND cells. Priming of BEND cells with EPA and both, *cis*-9, *trans*-11 and *trans*-10, *cis*-12, CLA isomers decreased $PGF_{2\alpha}$ response to PDBu by 66% and 24%, respectively. This is in agreement with several *in vivo* and *in vitro* studies that have detected inhibitory effects of EPA (Achard et al., 1997; Levine and Worth, 1984; Mattos et al., 2000, 2001, 2002, 2003, 2004; Caldari-Torres et al., 2006; Staples et al., 1998; Abayasekara and Wathes, 1999) and CLA (Liu and Belury, 1998; Kavanaugh et al., 1999; Uruquhart et al., 2002; Eder et al., 2003) on eicosanoid synthesis.

While EPA did not have detectable effects on PGHS-2, both CLA isomers increased PGHS-2 mRNA abundance in PDBu-stimulated BEND cells, indicating that EPA or CLA likely affected $PGF_{2\alpha}$ production through a mechanism that does not require repression of PGHS-2 gene expression. Moreover, even though CLA increased PPARδ mRNA response to PDBu, no changes were detected at the protein levels. These observations collectively suggest that modulation of endometrial $PGF_{2\alpha}$ production by supplemental fatty acids is through PGHS-2-, PGES-, and PPARδ-independent mechanisms. Whether and how these fatty acids may affect the activities of various

enzymes and transcription factors involved in the $PGF_{2\alpha}$ biosynthetic cascade warrants further information.

Next, we evaluated the effects of monounsaturated fatty acids (MUFA) on bovine endometrial $PGF_{2\alpha}$ production. The most common naturally occurring MUFA is oleic acid ($C_{18:1}$), which contains its double bond in the *cis* configuration. However, as a result of the biohydrogenation process by microorganisms in the rumen, accumulation of *trans* $C_{18:1}$ fatty acids (*t*FA) may occur in ruminants. Moreover, Mosley et al. (2002) showed that biohydrogenation of oleic acid involves the formation of several *t*FA isomers rather than direct biohydrogenation to form stearic acid (ST; $C_{18:0}$). In addition, the *trans*-9 isomer of octadecenoic acid can be converted to both ST and a series of *t*FA isomers (Proell et al., 2002). Results indicated that supplementation with *trans* fatty acids resulted in enhanced endometrial $PGF_{2\alpha}$ production *in vitro* (Chapter 5) as well as *in vivo* (Chapter 6). The mechanism by which *t*FA stimulates endometrial $PGF_{2\alpha}$ is not well understood. Although we observed enhanced response of PGHS-2 mRNA to PDBu after incubation of BEND cells with *t*FA, no changes were observed at the protein level. Additional studies are needed to examine if and to what extent these fatty acids modulate the activity of various enzymes involved in the prostaglandin biosynthetic cascade.

Dietary supplementation with *t*FA has been shown to reduce plasma concentration of n-3 and n-6 PUFAs in pigs (Kummerow et al., 2004). *Trans* fatty acids also have been shown to inhibit conversion of LA to longer chain n-6 PUFAs *in vitro* (Mahfouz et al., 1980) and *in vivo* (Mahfouz et al., 1984). Additionally, essential PUFAs have been reported to inhibit prostaglandin secretion in several cell types (Levine and Worth, 1984; Achard et al., 1997), including BEND cells (Mattos et al., 2003). Taken together, these

studies indicate that isomers of octadecenoic fatty acids may induce $PGF_{2\alpha}$ production through a mechanism involving inhibition of synthesis of PUFAs.

Although fatty acids are naturally occurring ligands of PPARs, it appears that the effect of *t*FA on $PGF_{2\alpha}$ does not require modulation of PPARδ gene or protein since no changes were observed after incubation with *t*FA. Whether and how these fatty acids may control activity of this nuclear receptor is yet to be elucidated.

The present study indicated that supplemental *t*FA induced $PGF_{2\alpha}$ production by BEND cells. However, the physiological significance of this increase in $PGF_{2\alpha}$ production is yet to be elucidated. Following parturition, resumption of normal estrous cycles is dependent on uterine involution and repair resulting from myometrial contractions stimulated by $PGF_{2\alpha}$ (Kiracofe, 1980). In addition, application of exogenous $PGF_{2\alpha}$ early postpartum, increased myoelectrical activity and contraction of the uterus (Patil et al., 1980; Gajewski et al., 1999). Thus, enhanced ovarian activity and function may be related to elevated $PGF_{2\alpha}$ concentration since we showed that dietary *t*FA greatly increased and sustained higher plasma PGFM concentration within the first week of lactation. While the uterus reaches the size of the nonpregnant uterus at about three weeks postpartum, Lindell and Kindahl (1983) showed that exogenous application of $PGF_{2\alpha}$ between days 3 and 10 postpartum decreased involution time by about one week.

Increased plasma PGFM also may have beneficial effects on immune competency because in this study, the proportion of cows with metritis was higher in control when compared to the *t*FA dietary group (22 vs. 0%). A similar observation was made by Seals et al. (2002), who reported that postpartum concentrations of PGFM were inversely related to emergence of uterine infections. Moreover, $PGF_{2\alpha}$ may have a stimulatory

effect on the phagocytic activity of uterine polymorphonuclear inflammatory cells (Paisley et al., 1986). Whether or not this augmentation in $PGF_{2\alpha}$ production results in improved fertility warrants further research.

Further understanding on how *t*FA may affect reproductive performance in postpartum dairy cows will lead to the development of novel nutritional strategies to increase embryo survival and pregnancy rates in high producing dairy cows in which fertility is impaired due to high metabolic demands associated with milk production. For example, modulation of endometrial $PGF_{2\alpha}$ production by manipulation of dietary long-chain fatty acids, such as dietary supplementation with *t*FA (during transition period) or n-3 PUFAs (during the breeding period), may reduce the postpartum interval to estrus and increase pregnancy rates in high producing dairy cows.

LIST OF REFERENCES

Aaes-Jorgensen E. Essential fatty acids. Physiol Rev 1961; 41:1-51.

Abayasekara DRE, Sheldrick EL, Flick-Smith HC, Flint APF. Role of protein kinase C in the inhibitory action of trophoblast interferons on expression of the oxytocin receptor in sheep endometrium. Endocrine 1995; 3:151-158.

Abayasekara DRE, Wathes DC. Effects of altering dietary fatty acid composition on prostaglandin synthesis and fertility. Prostaglandins Leukot Essent Fatty Acids 1999; 61:275-287.

Abisogun AO. Modulatory role of eicosanoids in vascular changes during the preovulatory period in the rat. Biol Reprod 1988; 38:756-762.

Abulafia O, Sherer DM. Angiogenesis of the ovary. Am J Obstet Gynecol 2000; 182:240-246.

Achard D, Gilbert M, Bénistant C, Slama SB, DeWitt DL, Smith WL, Lagarde M. Eicosapentaenoic and docosahexaenoic acids reduce PGH synthase 1 expression in bovine aortic endothelial cells. Biochem Biophys Res Comm 1997; 241:513-518.

Acosta TJ, Berisha B, Ozawa T, Sato K, Schams D, Miyamoto A. Evidence for a local endothelin-agiotensin-atrial natriuretric peptide system in bovine mature follicles in vitro: effects on steroids hormones and prostaglandin secretion. Biol Reprod 1999; 61:1419-1425.

Acosta TJ, Ozawa T, Kobayashi S, Hayashi K, Ohtani M, Kraetzel K, Schams D, Miyamoto A. Periovulatory changes in the local release of vasoactive peptides, prostaglandin F2α, and steroid hormones from bovine mature follicles in vivo. Biol Reprod 2000; 63:1253-1261.

Acosta TJ, Yoshizawa N, Ohtani M, Miyamoto A. Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin F2α injection in the cow. Biol Reprod 2002; 66:651-658.

Acosta TJ. Local changes in blood flow within the pre-ovulatory follicle wall and early corpus luteum in cows. Reproduction 2003; 125:759-767.

Acosta TJ, Miyamoto A. Vascular control of ovarian function: ovulation, corpus luteum formation and regression. Anim Reprod Sci 2004; 82-83:127-140.

Adams GP. Comparative patterns of follicle development and selection in ruminants. J Reprod Fertil Suppl 1999; 54:17-32.

Adams GP, Matteri RL, Kastelic JP, Ko JCH, Ginther OJ. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J Reprod Fertil 1992; 94:177-188.

Allen KGD, Harris MA. The role of n-3 fatty acids in gestation and parturition. Exp Biol Med 2001; 226:498-506.

Amselgruber W, Sinowatz F, Schams D, Skottner A. Immunohistochemical aspects of insulin-like growth factors I and II in the bovine corpus luteum. J Reprod Fertil 1994; 101:445-451.

Arthur GH, Noakes DE, Pearson H. Veterinary Reproduction and Obstetics. 6th Ed. Bailliere Tindall 1989; Philadelphia, PA.

Ashes JR, Siebert BD, Gulati SK, Cuthbertson AZ, Scott TW. Incorporation of n-3 fatty acids of fish oil into tissues and serum lipids of ruminants. Lipids 1992; 27:629-631.

Asselin E, Goff AK, Bergeron H, Fortier MA. Influence of sex steroids on the production of prostaglandin F2α and E2 and response to oxytocin in cultured epithelial and stromal cells of the bovine endometrium. Biol Reprod 1996; 54:371-379.

Asselin E, Drolet P, Fortier MA. In vitro response to oxytocin and interferon-τ in bovine endometrial cells from caruncular and inter-caruncular areas. Biol Reprod 1998; 59:241-247.

Auletta FJ, Flint APF. Mechanisms controlling corpus luteum function in sheep, cows, nonhuman primates, and women especially in relation to the time of luteolysis. Endo Rev 1988; 9:88-105.

Austin EJ, Mihm M, Evans ACO, Knight PG, Ireland JLH, Ireland JJ, Roche JF. Alterations in intrafollicular regulatory factors and apoptosis during selection of follicles in the first follicular wave of the bovine estrous cycle. Biol Reprod 2001; 64:839-848.

Baguma-Nibasheka M, Brenna JT, Nathanielsz PW. Delay of preterm delivery in sheep by n-3 long chain polyunsaturates. Biol Reprod 1999; 60:698-701.

Balaguer SA, Pershing RA, Rodriguez-Sallaberry C, Thatcher WW, Badinga L. Effects of bovine somatotropin on uterine genes related to the prostaglandin cascade in lactating dairy cows. J Dairy Sci 2005; 88:543-552.

Banni S, Angioni E, Casu V, Melis MP, Carta G, Corongiu FP, Thompson H, Ip C. Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. Carcinogenesis 1999; 20:1019-1024.

Bao B, Garverick HA, Smith GW, Smith MF, Salfen BE, Youngquist RS. Changes in messenger ribonucleic acid encoding luteinizing hormone receptor, cytochrome P450-side chain cleavage, and aromatase are associated with recruitment and selection of bovine ovarian follicles. Biol Reprod 1997a; 56:1158-1168.

Bao B, Garverick HA, Smith GW, Smith MF, Salfen BE, Youngquist RS. Expression of messenger ribonucleic acid (mRNA) encoding 3β-hydroxysteroid dehydrogenase Δ^4, Δ^5 isomerase (3β-HSD) during recruitment and selection of bovine ovarian follicles: identification of dominant follicles by expression of within the granulosa cell layer. Biol Reprod 1997b; 56:1466-1473.

Bao B, Garverick HA. Expression of steroidogenic enzymes and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: a review. J Anim Sci 1998; 76:1903-1921.

Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J Dairy Sci 1980; 63:1514-1529.

Bauman DE, Peel CJ, Steinhour WD, Reynolds PJ, Tyrrel HF, Brown AC, Haaland GL. Effects of bovine somatotropin on metabolism of lactating dairy cows: influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J Nutr 1988; 118:1031-1040.

Baumgard LH, Sangster JK, Bauman DE. Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of trans-10, cis-12 conjugated linoleic acid (CLA). J Nutr 2001; 131:1764-1769.

Bazer FW. Mediators of maternal recognition of pregnancy in mammals. Proc Soc Exp Biol Med 1992; 199:373-384.

Bazer FW, Ott TL, Spencer TE. Endocrinology if the transition from recurring estrous cycles to establishment of pregnancy in subprimate mammals. In: Bazer FW, ed. Endocrinology of pregnancy. Totowa, NJ: Humana Press 1998; 1-34.

Bazer FW, Thatcher WW. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F by the uterine endometrium. Prostaglandins 1977; 14:397-401.

Beam SW, Butler WR. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod 1997; 56:133-142.

Beam SW, Butler WR. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J Dairy Sci 1998; 81:121-131.

Beam SW, Butler WR. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J Reprod Fertil 1999; Suppl 54:411-424.

Bee G. Dietary conjugated linoleic acid consumption during pregnancy and lactation influences growth and tissue composition in weaned pigs. J Nutr 2000; 130:2981-2989.

Beg MA, Bergfelt DR, Kot K, Wiltbank MC, Ginther OJ. Follicular fluid and granulosa cell gene expression associated with follicle deviation in cattle. Biol Reprod 2001; 64:432-441.

Bell AW. Regulation of organic nutrient metabolism during transition from pregnancy to early lactation. J Anim Sci 1995; 73:2804-2819.

Belyea RL, Coppock CE, Lake GB. Effects of silage on health, reproduction, and blood metabolites of dairy cattle. J Dairy Sci 1975; 58:1336-1346.

Berisha B, Schams D. Ovarian function in ruminants. Domest Anim Fertil 2005; 29:305-317.

Bezard J, Blond JP, Bernard A, Clouet P. The metabolism and availability of essential fatty acids in animal and human tissues. Reprod Nutr Dev 1994; 34:539-568.

Binelli M, Guzeloglu A, Badinga L, Arnold DR, Sirois J, Hansen TR, Thatcher WW. Interferon-τ modulates phorbol ester-induced production of prostaglandin and expression of cyclooxygenase-2 and phospholipase A_2 from bovine endometrial cells. Biol Reprod 2000; 63:417-427.

Brannstrom M, Zackrisson U, Hagstrom H, Josefsson B, Hellberg P, Granberg S, Collins W, Bourne T. Preovulatory changes of blood flow in different regions of the human follicle. Fertil Steril 1998; 69:435-442.

Brindle NP, Zammit VA, Pogson CI. Regulation of carnitine palmitoyltransferase activity by malonyl-CoA in mitochondria from sheep liver, a tissue with low capacity for fatty acid synthesis. Biochem J 1985; 232:177-182.

Burke JM, Carroll DJ, Rowe KE, Thatcher WW, Stormshak F. Intravascular infusion of lipid into ewes stimulates production of progesterone and prostaglandin. Biol Reprod 1996; 55:169-175.

Burke JM, Staples CR, Risco CA, De La Sota RL, Thatcher WW. Effect of ruminant grade menhaden fish meal on reproductive and productive performance of lactating dairy cows. J Dairy Sci 1997; 80:3386-3398.

Burr GO, Burr MM. New deficiency disease produced by rigid exclusion of fat from diet. J Biol Chem 1929; 82:345-367.

Butler WR, Everett RW, Coppock CE. The relationships between energy balance, milk production and ovulation in post-partum Holstein cows. J Anim Sci 1981; 53:742-748.

Butler WR, Smith RD. Interrelationship between energy balance and postpartum reproductive function in dairy cattle. J Dairy Sci 1989; 72:767-783.

Butler WR. Nutritional interactions with reproductive performance in dairy cattle. Anim Reprod Sci 2000; 60:449-457.

Butler WR. Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. Anim Sci Occas Publ 2001; 26:133-145.

Butler WR. Energy balance relationship with follicular development, ovulation and fertility in postpartum dairy cows. Livestock Prod Sci 2003; 83:211-218.

Caldari-Torres C, Rodriguez-Sallaberry C, Green ES, Badinga L. Differential effects of n-3 and n-6 fatty acids on prostaglandin F 2alpha production by bovine endometrial cells. J Dairy Sci 2006; 89:971-977.

Canfield RW, Butler WR. Energy balance and pulsatile luteinizing hormone secretion in early post-partum dairy cows. Domest Anim Endocrinol 1990; 7:323-333.

Carrol DJ, Jerred MJ, Grummer RR, Combs DK, Rierson RA, Hauser ER. Effects of fat supplementation and immature alfalfa to concentrate ratio on plasma progesterone, energy balance, and reproductive traits of dairy cattle. J Dairy Sci 1990; 73:2855-2863.

Cavestany D, Blanc JE, Kulcsar M, Uriarte G, Chilibroste P, Meikle A, Febel H, Ferraris A, Krall E. Studies of the transition cow under a pasture-based milk production system: metabolic profiles. J Vet Med A Physiol Pathol Clin Med 2005; 52:1-7.

Challis JRG. Endocrinology of late pregnancy and parturition. Intern Rev Physiol 1980; 22:277-324.

Cheng Z, Robinson RS, Pushpakumara PGA, Mansbridge RJ, Wathes DC. Effect of dietary polyunsaturated fatty acids on uterine prostaglandin synthesis in the cow. J Endocrinol 2001; 171:463-473.

Cheng Z, Elmes DRE, Abayasekara DC, Wathes DC. Effects of conjugated linoleic acid on prostaglandins produced from maternal intercotyledonary endometrium, fetal allantochorion and amnion in late pregnant ewes. Biochim Biophys Acta 2003; 1633:170-178.

Cheng Z, Elmes M, Kirkup SE, Abayasekara DR, Wathes DC. Alteration of prostaglandin production and agonist responsiveness by n-6 polyunsaturated fatty acids in endometrial cells from late-gestation ewes. J Endocrinol 2004; 182:249-256.

Cheng Z, Elmes M, Kirkup SE, Chin EC, Abayasekara DR, Wathes DC. The effect of a diet supplemented with the n-6 polyunsaturated fatty acid linoleic acid on prostaglandin production in early- and late-pregnant ewes. J Endocrinol 2005; 184:165-178.

Chin SF, Liu W, Storkson JM, Ha JL, Pariza MW. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Compos Anal 1992; 5:185-197.

Chin SF, Storkson JM, Liu W, Albright KJ, Pariza MW. Conjugated linoleic acid (9,11- and 10, 12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. J Nutr 1994; 124:694-701.

Christiansen EN, Lund JS, Rortveit T, Rustan AC. Effect of dietary n-3 and n-6 fatty acid desaturation in rat liver. Biochim Biophys Acta 1991; 1082:57-62.

Connolly JM, Liu XH, Rose DP. Dietary linoleic acid-stimulated human breast cancer cell growth and metastasis in nude mice and their suppression by indomethacin, a cyclooxygenase inhibitor. Nutr Cancer 1996; 25:231-240.

Cooke BA, Dirami G, Chaudry L, Choi MSK, Abayasekara DRE, Phipp L. Release of arachidonic acid and the effects of corticosteroids on steroidogenesis in rat testis Leydig cells. J Steroid Biochem Mol Biol 1991; 40:465-471.

Cook HW. Fatty acid desaturation and chain elongation in eukaryotes. In Biochemistry of Lipids, Lipoproteins and Membranes 1996; pp 129-152. Eds DE Vance & J Vance; Amsterdam: Elsevier.

Cooke DJ, Crowe MA, Roche JF. Circulating FSH isoform pattern during recurrent increases in FSH throughout the oestrous cycle of heifers. J Reprod Fertil 1997; 110:339-345.

Corl BA, Chouinard PY, Bauman DE, Dwyer DA, Griinari JM, Nurmela KV. Conjugated linoleic acid in milk fat of dairy cows originates in part by endogenous synthesis from trans-11 octadecenoic acid. J Dairy Sci 1998; 81(Suppl 1):233.

Crofford LJ. Prostaglandin biology. Gastroenterol Clin North Am 2001; 30:863-876.

Curtis CR, Erb HN, Sniffen CJ, Smith RD, Kronfeld DS. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J Dairy Sci 1985; 68:2347-2360.

Danet-Desnoyers G, Wetzels C, Thatcher WW. Natural and recombinant bovine interferon tau regulate basal and oxytocin-induced secretion of prostaglandin F2 alpha and E2 by epithelial cells and stromal cells in the endometrium. Reprod Fertil Dev 1994; 6:192-202.

DeLany JP, Blohm F, Truett AA, Scimeca JA, West DB. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. Am J Physiol 1999; 276(4 Pt 2):R1172-1179.

Del Vecchio RP, Matsas DJ, Inzana TJ, Sponenberg DP, Lewis GS. Effects of intrauterine bacterial infusions and subsequent endometritis on prostaglandin $F_{2\alpha}$ metabolite concentrations in postpartum beef cows. J Anim Sci 1992; 70:3158-3162.

Del Vecchio RP, Matsas DJ, Fortín S, Sponenberg DP, Lewis GS. Spontaneous uterine infections are associated with elevated prostaglandin $F_{2\alpha}$ metabolite concentrations in postpartum dairy cows. Theriogenology 1994; 41:413-421.

Demmers KJ, Derecka K, Flint A. Trophoblast interferon and pregnancy. Reproduction 2001; 121:41-49.

Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999; 274:35881-35888.

Dhaliwal GS, Murray RD, Woldehiwet Z. Some aspects of immunology of the bovine uterus related to treatments for endometritis. Anim Reprod Sci 2001; 67:135-152.

Dhiman TR, Anand GR, Satter LD, Pariza MW. Conjugated linoleic acid content of milk from cows fed different diets. J Dairy Sci 1999; 82:2146-2156.

Diskin MG, Mackey DR, Roche JF, Sreenan JM. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim Reprod Sci 2003; 78:345-370.

Drackley JK, Beitz DC, Young JW. Regulation of in vitro metabolism of palmitate by carnitine and propionate in liver from dairy cows. J Dairy Sci 1991; 74:3014-3024.

Drackley JK. Biology of dairy cows during the transition period: the final frontier. J Dairy Sci 1999; 82:2259-2273.

Drackley JK, Cicela TM, LaCount DW. Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. J Dairy Sci 2003; 86:1306-1314.

Dugan MER, Aalhus JL, Schaefer AL, Kramer JKG. The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. Can J Anim Sci 1997; 77:723-725.

Eder K, Schleser S, Becker K, Körting R. Conjugated linoleic acids lower the release of eicosanoids and nitric oxide from human aortic endothelial cells. J Nutr 2003; 133:4083-4089.

Einspanier R, Miyamoto A, Schams D, Muller M, Bren G. Tissue concentration, mRNA expression and stimulation of IGF-I in luteal tissue during the oestrous cycle and pregnancy of cows. J Reprod Fertil 1990; 90:439-445.

Elattar TM, Lin HS. Comparison of the inhibitory effect of polyunsaturated fatty acids on prostaglandin synthesis in oral squamous carcinoma cells. Prostaglandins Leukot Essent Fatty Acids 1989; 38:119-125.

Elmes M, Tew P, Cheng Z, Kirkup SE, Abayasekara DRE, Calder PC, Hanson MA, Wathes DC, Burdge GC. The effect of dietary supplementation with linoleic acid to late gestation ewes on the fatty acid composition of maternal and fetal plasma and tissues and the synthetic capacity of the placenta for 2-series prostaglandins. Biochim Biophys Acta 2004; 1686:139-147.

Espey LL. Ovulation as an inflammatory reaction – a hypothesis. Biol Reprod 1980; 22:73-106.

Evans ACO, Fortune JE. Selection of the dominant follicle in cattle occurs in the absence of differences in the expression of messenger ribonucleic acid for gonadotropin receptors. Endocrinology 1997; 138:2963-2971.

Evans ACO, Komar CM, Wandji S-A, Fortune JE. Changes in androgen secretion and luteinizing hormone pulse amplitude are associated with the recruitment and growth of ovarian follicles during the luteal phase of the bovine estrous cycle. Biol Reprod 1997; 57:394-401.

Evans ACO. Characteristics of ovarian follicle development in domestic animals. Reprod Domest Anim 2003; 38:240-246.

Fairclough RJ, Lau TM. Hormonal control of concentrations of endometrial oxytocin receptors in the ewe. Reprod Fertil Dev 1992; 4:313-320.

Ferguson JD, Sklan D, Chalupa WV, Kronfeld DS. Effects of hard fats on in vitro and in vivo rumen fermentation, milk production and reproduction in dairy cows. J Dairy Sci 1990; 73:2864-2879.

Filley SJ, Turner HA, Stormshak F. Prostaglandin F2α concentrations, fatty acid profiles, and fertility in lipid-infused postpartum beef heifers. Biol Reprod 1999; 61:1317-1323.

Fisher S. Dietary polyunsaturated fatty acids and eicosanoid formation in humans. Adv Lipid Res 1989; 23:169-198.

Folman Y, Kaim M, Herz Z, Rosenberg M. Comparison of methods for the synchronization of estrous cycles in dairy cows 2. Effects of progesterone and parity on conception. J Dairy Sci 1990; 73:2817-2825.

Ford SP, Chenault JR. Blood flow to the corpus luteum-bearing ovary and ipsilateral uterine horn of cows during the oestrous cycle and early pregnancy. J Reprod Fertil 1981; 62:555-562.

Fortune JE, Sirois J, Quirk SM. The growth and differentiation of ovarian follicles during the bovine estrous cycle. Theriogenology 1988; 29:95-109.

Fortune JE, Rivera GM, Yang MY. Follicular development: the role of the follicular microenvironment in selection of the dominant follicle. Anim Reprod Sci 2004; 82-83:109-126.

Gajewski Z, Thun R, Faundez R, Boryczko Z. Uterine motility in the cow during puerperium. Reprod Dom Anim 1999; 34:185-191.

Garg ML, Sebokova E, Thomson AB, Clanclinin MT. Delta 6-desaturase activity in the liver microsomes of rats fed diets enriched with cholesterol and/or n-3 fatty acids. Biochem J 1988; 249:351-356.

Garcia-Bojalil CM, Staples CR, Risco CR, Savio JD, Thatcher WW. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows. J Dairy Sci 1998; 81:1385-1395.

Garverick HA, Baxter G, Gong J, Armstrong DG, Campbell BK, Gutierrez CG, Webb R. Regulation of expression of ovarian mRNA encoding hypogonadotrophic cattle. Reproduction 2002; 123:651-661.

German JB, Lokesh B, Kinsella JE. The effect of dietary fish oils on eicosanoid biosynthesis in peritoneal macrophages is influenced by both dietary n-6 polyunsaturated fats and total dietary fat. Prostaglandins Leukot Essent Fatty Acids 1988; 34:37-45.

Ginther OJ, Kastelic JP, Knopf L. Composition and characteristics of follicular waves during the bovine estrous cycle. Anim Reprod Sci 1989a; 20:187-200.

Ginther OJ, Knopf L, Kastelic JP. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. J Reprod Fertil 1989b; 87:223-230.

Ginther OJ, Knopf L, Kastelic JP. Ovarian follicular dynamics in heifers during early pregnancy. Biol Reprod 1989c; 41:247-254.

Ginther OJ, Kulick LJ, Kot K, Wiltbank MC. Emergence and deviation of follicles during the development of follicular waves in cattle. Theriogenology 1997; 48:75-87.

Ginther OJ, Bergfelt DR, Kulick LJ, Kot K. Pulsatility of systemic FSH and LH concentrations during follicular-wave development in cattle. Theriogenology 1998; 50:507-519.

Ginther OJ, Bergfelt DR, Kulick LJ, Kot K. Selection of the dominant follicle in cattle: establishment of follicle deviation in less than 8 hours through depression of FSH concentrations. Theriogenology 1999; 52:1079-1093.

Ginther OJ, Bergfelt DR, Kulick LJ, Kot K. Selection of the dominant follicle in cattle: role of the two-way functional coupling between follicle-stimulating hormone and the follicles. Biol Reprod 2000a; 62:920-927.

Ginther OJ, Bergfelt DR, Kulick LJ, Kot K. Selection of the dominant follicle in cattle: role of estradiol. Biol Reprod 2000b; 63:383-389.

Girsh E, Wang W, Mamluk R, Arditi F, Friedman A, Milvae RA, Meidan R. Regulation of endothelin-1 expression in the bovine corpus luteum: elevation by prostaglandin F2α. Endocrinology 1996; 137:5191-5196.

Goff AK. Steroid hormone modulation of prostaglandin secretion in the ruminant endometrium during the estrous cycle. Biol Reprod 2004; 71:11-16.

Gong JG, Campbell BK, Bramley TA, Gutierrez CG, Peters AR, Webb R. Suppression in the secretion of follicle stimulating hormone and luteinizing hormone and ovarian follicle development in heifers continuously infused with gonadotropin releasing hormone agonist. Biol Reprod 1996; 55:68-74.

Gong JG, Moghaddam A, Baxter G, Garnsworthy PC, Webb R, Armostrong DG. Effect of feeding a diet to increase circulating insulin concentrations on LH secretion during the early post-partum period in dairy cows. Reprod Abstr Ser 2001; Abstract No 15.

Goudet G, Francois B, Bezard J, Nadine G. Intrafollicular content of luteinizing hormone receptor, α-inhibin, and aromatase in relation to follicular growth, estrous cycle stage, and oocyte competence for in vitro maturation in the mare. Biol Reprod 1999; 60:1120-1127.

Gregory S, Kelly ND. Conjugated linoleic acid: a review. Altern Med Rev 2001; 6:367-382.

Griffin JFT, Hartigan PJ, Nunn WR. Non-specific uterine infection and bovine fertility. I. Infection patterns and endometritis during the first seven weeks postpartum. Theriogenology 1974; 1:91-106.

Griinari JM, Dwyer DA, McGuire MA, Bauman DE, Palmquist DL, Nurmela KVV. Trans-octadecenoic acid and milk fat depression in lactating dairy cows. J Dairy Sci 1998; 81:1251-1261.

Griinari JM, Bauman DE. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: Yurawecz MP, Mossoba MM, Kramer JKG, Pariza MW, Nelson GJ (Ed) Advances in conjugated linoleic acid research 1999; Vol 1:180-200. AOS Press, Champaign, IL.

Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KVV, Bauman DE. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9-desaturase. J Nutr 2000; 130:2285-2291.

Gross TS, Williams WF, Manspeaker JE, Lewis GS, Russek-Cohen E. Bovine placental prostaglandin synthesis in vitro as it relates to placental separation. Prostaglandins 1987; 34:903-917.

Gross TS, Williams WF. Bovine placental prostaglandin synthesis: principal cell synthesis as modulated by the binucleate cell. Biol Reprod 1988; 38:1027-1034.

Grum DE, Drackley JK, Younker RS, LaCount DW, Veenhuizen JJ. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J Dairy Sci 1996; 70:1850-1864.

Grummer RR. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J Anim Sci 1995; 73:2820-2833.

Guilbault LA, Thatcher WW, Drost M, Hopkins SM. Source of F series prostaglandins during early postpartum period in cattle. Biol Repro 1984; 31:879-887.

Guzeloglu A, Binelli M, Badinga L, Hansen TR, Thatcher WW. Inhibition of phorbol ester-induced PGF2alpha secretion by IFN-tau is not through regulation of protein kinase C. Prostaglandins Other Lipid Mediat 2004; 74:87-99.

Gurr MI, Harwood JL, Frayn KN. Lipid Biochemistry: An Introduction. 5th ed, 2002. Blackwell Science Ltd, Oxford, UK.

Hafez ESE, Hafez B. Reproduction in farm animals. 7th ed, 2000. Lippincott Williams & Williams, Philadelphia, PA.

Harris MA, Hansen RA, Vidsudhiphan P, Koslo JL, Thomas JB, Watkins BA, Allen KGD. Effects of conjugated linoleic acids and docosahexaenoic acid on rat liver and reproductive tissue fatty acids, prostaglandins and matrix metalloproteinase production. Prostaglandins Leukot Essent Fatty Acids 2001; 65:23-29.

Hawkins DE, Niswender KD, Oss GM, Moeller CL, Odde KG, Sawyer HR, Niswender GD. An increase in serum lipids increase luteal lipid content and alter disappearance rate of progesterone in cows. J Anim Sci 1995; 73:541-545.

Hayashi K, Miyamoto A. Angiotensin II interacts with prostaglandin F2α and endothelin-1 as a local luteolytic factor in the bovine corpus luteum in vitro. Biol Reprod 1999; 60:1104-1109.

Hayashi K, Miyamoto A, Berisha B, Kosmann MR, Okuda K, Schams D. Regulation of angiotensin II production and angiotensin II receptors in microvascular endothelial cells from bovine corpus luteum. Biol Reprod 2000; 62:162-167.

Heuwieser W, Hoppen HO, Grunert E. Blood levels of prostaglandin metabolites (PGFM, PGEM) after parturition in cows with and without retained placenta considering spontaneous calving and dystocia. Zentbl Vetmed 1992; A 39:509-514.

Hightshoe RB, Cochran RC, Corah LR, Kiracofe GH, Harmon DL, Perry RC. Effects of calcium soaps of fatty acids on post-partum reproductive function in beef cows. J Anim Sci 1991; 69:4097-4103.

Hoedemaker M, Lund LA, Wagner WC. Influence of arachidonic acid metabolites and steroids on function of bovine polymorphonuclear neutrophils. Am J Vet Res 1992; 53:1534-1539.

Holman RT. Essential fatty acid deficiency. Prog Chem Fats Other Lipids 1971; 9:275-348.

Holman RT, Mahfouz MM. Cis and trans-octadecenoic acids as precursors of polyunsaturated acids. Prog Lipid Res 1981; 20:151-156.

Holman RT, Johnson SB, Hatch TF. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr 1982; 35:617-623.

Hunter MG, Robinson RS, Mann GE, Webb R. Endocrine and paracrine control of follicular development and ovulation rate in farm species. Anim Reprod Sci 2004; 82-82:461-477.

Hussain AM. Bovine uterine defense mechanisms: a review. J Vet Med Ser B 1989; 36:641-651.

Ing NH, Spencer TE, Bazer FW. Estrogen enhances endometrial estrogen receptor gene expression by a posttranscriptional mechanism in the ovariectomized ewe. Biol Reprod 1996; 54:591-599.

Ingvartsen KL, Andersen JB. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J Dairy Sci 2000; 83:1573-1597.

Innis SM. Essential fatty acids in growth and development. Prog Lipid Res 1991; 30:39-103.

Ireland JJ, Roche JF. Development of non-ovulatory antral follicles in heifers: changes in steroids in follicular fluid and receptors for gonadotropins. Endocrinology 1983; 112:150-156.

Ireland JJ, Fogwell RL, Oxender WD, Ames K, Cowley JL. Production of estradiol by each ovary during the estrous cycle of cows. J Anim Sci 1984; 59:764-771.

Janson PO. Effects of luteinizing hormone on blood flow in the follicular rabbit ovary, as measured by radioactive microspheres. Acta Endocrinol 1975; 79:122-133.

Jaroszewski JJ, Skarzynski DJ, Hansel W. Nitric oxide as a local mediator of prostaglandin F2α-induced regression in bovine corpus luteum: an in vivo study. Exp Biol Med 2003; 228:1057-1062.

Jenkins TC, Palmquist DL. Effect of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. J Dairy Sci 1984; 67:978-986.

Jenkins TC. Challenges of meeting cow demands for omega fatty acids. Florida Ruminant Nutr Symp 2004; 52-66

Johnson AL, Tilly JL. Evidence that arachidonic acid influences hen granulosa cell steroidogenesis and plasminogen activator activity by a protein kinase C-independent mechanism. Biol Reprod 1990; 43:922-928.

Jolly PD, McDougall S, Fitzpatrick LA, MacMillan KL, Entwistle KW. Physiological effects of undernutrition on postpartum anoestrous cows. J Reprod Fertil 1995; Suppl 49:477-492.

Jones LS, Ottobre JS, Pate JL. Progesterone regulation of luteinizing hormone receptors on cultured bovine luteal cells. Mol Cell Endocrinol 1992; 85:33-39.

Jorritsma R, Jorritsma H, Schukken YH, Wentink GH. Relationships between fatty liver and fertility and some periparturient diseases in commercial Dutch dairy herds. Theriogenology 2000; 54:1065-1074.

Juengel JL, Nett TM, Anthony RV, Niswender GD. Effects of luteotrophic and luteolytic hormones on expression of mRNA encoding insulin-like growth factor I and growth hormone receptor in the ovine corpus luteum. J Reprod Fertil 1997; 110:291-298.

Juengel JL, Niswender GD. Molecular regulation of luteal progesterone synthesis in domestic ruminants. J Reprod Fertil 1999; 54 (Suppl.):193-205.

Kavanaugh CJ, Liu KL, Belury MA. Effect of dietary conjugated linoleic acid on phorbol ester-induced PGE2 production and hyperplasia in mouse epidermis. Nutr Cancer 1999; 33:132-138.

Keeney M. Lipid metabolism in the rumen. Physiology of digestion and metabolism in the ruminant. In: A.T. Philipson (Ed). Oriel Press, Newcastle upon Tyne, UK 1970; pp 489-503.

Kim JJ, Fortier MA. Cell type specificity and protein kinase C dependency on the stimulation of prostaglandin E2 and prostaglandin F2α production by oxytocin and platelet-activating factor in bovine endometrial cells. J Reprod Fertil 1995; 103:239-247.

Kinsella JE, Lokesh B, Broughton S, Whelan J. Dietary polyunsaturated fatty acids and eicosanoids; potential effects on the modulation of inflammatory and immune cells: an overview. Nutrition 1990; 6:24-44.

Kiracofe GH. Uterine involution: its role in regulating postpartum intervals. J Anim Sci 1980; 51:816-821.

Kirby CJ, Thatcher WW, Collier RJ, Simmen FA, Lucy MC. Effects of growth hormone and pregnancy on expression of growth hormone receptor, insulin-like growth factor-I, and insulin-like growth factor binding protein-2 and -3 genes in bovine uterus, ovary and oviduct. Biol Reprod 1996; 55:996-1002.

Knickerbocker JJ, Wiltbank MC, Niswender GD. Mechanisms of luteolysis in domestic livestock. Domest Anim Endocrinol 1988; 5:91-107.

Kobayashi S, Miyamoto A, Berisha B, Schams D. Growth hormone, but not luteinizing hormone, acts with luteal peptides on prostaglandin F2α and progesterone secretion by bovine corpora lutea in vitro. Prostaglandins Lipid Mediat 2001a; 63:79-92.

Kobayashi S, Berisha B, Amselgruber WM, Schams D, Miyamoto A. Production and localisation of angiotensin factors and prostaglandin F2α. J Endocrinol 2001b; 170:369-380.

Kobayashi S, Berisha B, Amselgruber WM, Schams D, Miyamoto A. Production and localization of angiotensin II in the bovine early corpus luteum: a possible interaction with angiogenic factors and prostaglandin F2α. J Endocrionol 2001c; 171:369-380.

Kobayashi S, Acosta TJ, Ozawa T, Hayashi K, Berisha B, Ohtani M, Schams D, Miyamoto A. Intraluteal release of angiotensin II and progesterone in vivo during corpora lutea development in the cow: effect of vasoactive peptides. Biol Reprod 2002; 66:174-179.

Koelle S, Sinowatz F, Boie G, Lincoln D. Developmental changes in the expression of the growth hormone receptor messenger ribonucleic acid and protein in the bovine ovary. Biol Reprod 1998; 59:836-842.

Kruip TA, Vullings HG, Schams D, Jonis J, Klarenbeek A. Immunocytochemical demonstration of oxytocin in bovine ovarian tissues. Acta Endocrinol (Copenh) 1985; 109:537-542.

Krzymowski T, Stefańczyk-Krzymowska S. The oestrous cycle and early pregnancy – a new concept of local endocrine regulation. The Vet J 2004; 168:285-296.

Kulick LJ, Kot K, Wiltbank MC, Ginther OJ. Follicular and hormonal dynamics during the first follicular wave in heifers. Theriogenology 1999; 52:913-921.

Kulick LJ, Bergflet DR, Kot K, Ginther OJ. Follicular selection in cattle: follicle deviation and codominance within sequential waves. Biol Reprod 2001; 65:839-846.

Kummerow FA, Zhou Q, Mahfouz MM, Smiricky MR, Grieshop CM, Schaeffer DJ. Trans fatty acids in hydrogenated fat inhibited the synthesis of the polyunsaturated fatty acids in the phospholipid of arterial cells. Life Sci 2004; 74:2707-2723.

Lafrance M, Goff AK. Effect of pregnancy in oxytocin-induced release of prostaglandin F2α in heifers. Biol Reprod 1985; 33:1113-1119.

Lafrance M, Goff AK. Control of bovine uterine PGF2α release in vitro. Biol Reprod 1990; 42:288-293.

Lammoglia MA, Willard ST, Hallford DM, Randel RD. Effects of dietary fat on follicular development and circulating concentrations of lipids, insulin, progesterone, estradiol-17β, 13,14-dihydro-15-keto-prostaglandin F2α and growth hormone in estrous cyclic Brahman cows. J Anim Sci 1997; 75:1591-1600.

Lapetina EG. Regulation of arachidonic acid production: role of phospholipases C and A2. Trends Pharmacol Sci 1982; 4:115-118.

Larson SF, Butler WR, Currie WB. Reduced fertility associated with low progesterone postbreeding and increased milk urea nitrogen in lactating cows. J Dairy Sci 1997; 80:1288-1295.

Larsen LN, Hovic K, Bremer J, Holm KH, Myhren F, Borretzen B. Heneicosapentaenoate (21:5n-3): its incorporation into lipids and its effects on arachidonic acid and eicosanoid synthesis. Lipids 1997; 32:707-714.

Leaver HA, Lytton FDC, Dyson H, Watson ML, Mellor DJ. The effect of dietary ω3 and 6 polyunsaturated fatty acids on gestation, parturition and prostaglandins in intrauterine tissues and the kidneys. J Lipid Res 1986; 25:143-146.

Leaver HA, Howie A, Wilson NH. The biosynthesis of the 3-series prostaglandins in rat uterus after alpha-linolenic acid feeding: mass spectroscopy of prostaglandins E and F produced by rat uteri in tissue culture. Prostaglandins Leukot Essent Fatty Acids 1991; 42:217-224.

Leavitt WW, Okulicz WC, McCracken JA, Schramm W, Robidoux WF Jr. Rapid recovery of nuclear estrogen receptor and oxytocin receptor in the ovine uterus following progesterone withdrawal. J Steroid Biochem 1985; 22:687-691.

Lefkowith JB, Flippo V, Sprecher H, Needleman P. Paradoxical conservation of cardiac and renal arachidonate content in essential fatty acid deficiency. J Biol Chem 1985; 260:15736-15744.

Lehman JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA. Peroxisome proliferators-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 1997; 272:3406-3410.

Leslie CC. Properties and regulation of cytosolic phospholipase A2. J Biol Chem 1997; 272:16709-16712.

Levine L, Worth N. Eicosapentaenoic acid: its effects on arachidonic acid metabolism by cells in culture. J Allergy Clin Immunol 1984; 74:430-436.

Lewis GS, Thatcher WW, Bliss EL, Drost M, Collier RJ. Effects of heat stress during pregnancy on postpartum reproductive changes in Holstein cows. J Anim Sci 1984; 58:174-186.

Lewis GS. Uterine health and disorders. J Dairy Sci 1997; 80:984-994.

Lewis GS. Steroidal regulation of uterine resistance to bacterial infection in livestock. Reprod Biol Endocrinol 2003; 1:117-124.

Lewis GS. Steroidal regulation of uterine immune defenses. Anim Reprod Sci 2004; 82-83:281-294.

Liebermann J, Schams D. Actions of somatotrophin on oxytocin and progesterone release from microdialysed bovine corpus luteum in vitro. J Endocrinol 1994; 143:243-250.

Lim H, Gupta RA, Wa WG, Paria BC, Moller DE, Morrow JD, DuBois RN, Trzaskos JM, Dey SK. Genes Dev 1999; 13:1561-1574.

Lindell JO, Kindahl H, Edqvist LE, Tufvesson G. Effect of hysterectomy on the postpartum prostaglandin levels in the cow. Acta Vet Scand 1982; 23:144-146.

Lindell JO, Kindahl H. Exogenous prodtaglandin F2 alpha promotes uterine involution in the cow. Acta Vet Scand 1983; 24:269-274.

Liu KL, Belury MA. Conjugated linoleic acid reduces arachidonic acid content and PGE2 synthesis in murine keratinocytes. Cancer Lett 1998; 127:15-22.

Liu KL et al 1999

Lucy MC, Staples CR, Michel FM, Thatcher WW. Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin F2α, luteinizing hormone, and follicular growth. J Dairy Sci 1991; 74:483-489.

Lucy MC, Savio JD, Badinga L, De La Sota RL, Thatcher WW. Factors that affect ovarian follicular dynamics in cattle. J Anim Sci 1992; 70:3615-3626.

Lucy MC, Collier RJ, Kitchell ML, Dibner JJ, Hauser SD, Krivi GG. Immunohistochemical and nucleic acid analysis of somatotropin receptor populations in the bovine ovary. Biol Reprod 1993a; 48:1219-1227.

Lucy MC, De La Sota RL, Staples CR, Thatcher WW. Ovarian follicular populations in lactating dairy cows treated with recombinant bovine somatotropin (sometribove) or saline and fed diets differing in fat content and energy. J Dairy Sci 1993b; 76:1014-1027.

Lucy MC, Byatt JC, Curran TL, Curran DF, Collier RJ. Placental lactogen and somatotropin: hormone binding to the corpus luteum and effects on the growth and functions of the ovary in heifers. Biol Reprod 1994; 50:1136-1144.

Lukaszewska JH, Hansel W. Extraction and partial purification of luteolytic activity from bovine endometrial tissue. Endocrinology 1970; 86:261-270.

Ma DW, Wierzbicki AA, Field CJ, Clandinin MT. Conjugated linoleic acid in Canadian dairy and beef products. J Agric Food Chem 1999; 47:1956-1960.

MacLaren LA, Guzeloglu A, Bilby TR, Michel F, Thatcher WW. Expression of peroxisome proliferators-activated receptors (PPARs) in bovine endometrium. Biol Reprod 2003; 68(Suppl 1):264.

Madej A, Kindahl H, Woyno W, Edqvist LE, Stupnicki R. Blood levels of 15-keto-13, 14-dihydroprostaglandin F2 alpha during the postpartum period in primiparous cows. Theriogenology 1984; 21:279-287.

Mahfouz MM, Johnson S, Holman RT. The effect of isomeric trans-18:1 acids on the desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids by rat liver microsomes. Lipids 1980; 15:100-107.

Mahfouz MM, Smith TL, Kummerow FA. Effect of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat-liver microsomes. Lipids 1984; 19:214-222.

Mattioli M, Barboni B, Turriani M, Galeati G, Zannoni A, Castellani G, Berardinelli P, Scapolo PA. Follicle activation involves vascular endothelial growth factor production and increased blood vessel extension. Biol Reprod 2001; 65:1014-1019.

Mattos R, Staples CR, Thatcher WW. Effects of dietary fatty acids on reproduction in ruminants. Rev Reprod 2000; 5:38-45.

Mattos R, Guzeloglu A, Thatcher WW. Effect of polyunsaturated fatty acids on secretion of $PGF_{2\alpha}$ from bovine endometrial (BEND) cells stimulated with phorbol, 12, 13 dibutyrate (PDBu). Theriogenology 2001; 55:326.

Mattos R, Staples CR, Williams J, Amorocho A, McGuire MA, Thatcher WW. Uterine, ovarian, and production responses of lactating dairy cows to increasing dietary concentrations of menhaden fish meal. J Dairy Sci 2002; 85:755-764.

Mattos R, Guzeloglu A, Badinga L, Staples CR, Thatcher WW. Polyunsaturated fatty acids and bovine interferon-τ modify phorbol ester-induced secretion of prostaglandin $F_{2\alpha}$ and expression of prostaglandin endoperoxide synthase-2 and phospholipase-A_2 in bovine endometrial cells. Biol Reprod 2003; 69:780-787.

Mattos R, Staples CR, Arteche A, Wiltbank MC, Diaz FJ, Jenkins TC, Thatcher WW. The effects of feeding fish oil on uterine secretion of $PGF_{2\alpha}$, milk composition, and metabolic status of periparturient Holstein cows. J Dairy Sci 2004; 87:921-932.

Mayes PA. Metabolism of unsaturated fatty acids and eicosanoids. In: Murray RK, Granner DK, Mayes PA, Rodwell VW (Eds) Harper's Biochemistry, 24th ed. Connecticut: Appleton and Lange, 1996:236-244.

McCracken JA. Prostaglandin F2α and corpus luteum regression. Annals NY Acad Sci 1971; 180:456-472.

McCracken JA, Carlson JC, Glew ME, Goding JR, Baird DT, Green K, Samuelsson B. Prostaglandin F2α identified as a luteolytic hormone in sheep. Nature New Biol 1972; 238:129-134.

McGuire MA, Duckett SK, Andrae JG, Giesy JG, Hunt CW. Effect of high-oil corn on content of conjugated linoleic acid (CLA) in beef. J Anim Sci 1998; 76(Suppl):301.

Meikle A, Kulcsar M, Chilliard Y, Febel H, Delavaud C, Cavestany D, Chilibroste P. Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reproduction 2004; 127:727-737.

Michael AE, Abayasekara DRE, Webley GE. The luteotrophic actions of prostaglandins E_2 and $F_{2\alpha}$ on dispersed marmoset luteal cells are differentially mediated via cyclic AMP and protein kinase C. J Endocrinol 1993; 138:291-298.

Mihm M, Good TEM, Ireland JLH, Ireland JJ, Knight PG, Roche JF. Decline in serum follicle-stimulating hormone concentrations alters key intrafollicular growth factors involved in the selection of the dominant follicle in heifers. Biol Reprod 1997; 57:1328-1337.

Mihm M, Crowe MA, Knight PG, Austin EJ. Follicle wave growth in cattle. Reprod Domest Anim 2002; 37:191-200.

Mihm M, Bleach ECL. Endocrine regulation of ovarian antral follicle development in cattle. Anim Reprod Sci 2003; 78:217-237.

Milvae RA, Hansel W. Prostacyclin, prostaglandin F2α and progesterone production by bovine luteal cells during the estrous cycle. Biol Reprod 1983; 29:1063-1068.

Mitchell MD, Flint APF, Turnbull AC. Plasma concentrations of 13, 14-dihydro-15-keto-prostaglandin F during pregnancy in sheep. Prostaglandins 1976; 11:319-329.

Miyamoto A, von Lutzow H, Schams D. Acute actions of prostaglandin F2alpha, E2, and I2 in microdialyzed bovine corpus luteum in vitro. Biol Reprod 1993; 49:423-430.

Miyamoto A, Kobayashi S, Arata S, Ohtani M, Fukui Y, Schams D. Prostaglandin F2α promotes the inhibitory action of endothelin-1 on the bovine luteal function in vitro. J Endocrinol 1997; 152:R7-11.

Monget P, Martin GB. Involvement of insulin-like growth factors in the interactions between nutrition and reproduction in female mammals. Hum Reprod 1997; Suppl 1:33-52.

Mosley EE, Powell GL, Riley MB, Jenkins TC. Microbial biohydrogenation of oleic acid to trans isomers in vitro. J Lipid Res 2002; 43:290-296.

Moya-Camarena SY, Vanden Heuvel JP, Blanchard SG, Leesnitzer LA, Belury MA. Conjugated linoleic acid is a potent natural occurring ligand and activator of PPARalpha. J Lipid Res 1999; 40:1426-1433.

Moorby JM, Dewhurst RJ, Tweed JK, Dhanoa MS, Beck NF. Effects of altering energy and protein supply to dairy cows during the dry period. 2. Metabolic and hormonal responses. J Dairy Sci 2000; 83:1795-1805.

Murakami M, Nakatani Y, Atsumi G, Inoue K, Kudo I. Regulatory functions of phospholipase A2. Crit Rev Immunol 1997; 17:225-283.

Murdoch W, Peterson T, Van Kirk E, Vincent D, Inskeep E. Interactive roles of progesterone, prostaglandins, and collagenase in the ovulatory mechanism in the ewe. Biol Reprod 1986; 35:1187-1194.

Murphy M, Udén P, Palmquist DL, Wiktorsson. Rumen and total diet digestibilities in lactating cows fed diets containing full-fat rapeseed. J Dairy Sci 1987; 70:1572-1582.

Nakaya A, Wakabayashi H, Imamura L, Fukuta K, Makimoto S, Naganuma K, Orihara T, Minemura M, Shimizu Y, Nagasawa T, Hamazaki T, Watanabe A. Helicobacter pylori alters n-6 fatty acid metabolism and prostaglandin E2 synthesis in rat gastric mucosal cells. J Gastroenterol Hepatol 2001; 16:1197-1205.

Neuringer M, Anderson GJ, Connor WV. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Ann Rev Nutr 1988; 8:517-544.

Neuvians TP, Pfaffl MW, Berisha B, Schams D. The mRNA expression of the members of the IGF-system in bovine corpus luteum during induced luteolysis. Domest Anim Endocrinol 2003; 25:359-372.

Neuvians TP, Berisha B, Schams D. Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) expression during induced luteolysis in the bovine corpus luteum. Mol Reprod Dev 2004a; 67:389-395.

Neuvians TP, Schams D, Berisha B, Pfaffl MW. Involvement of pro-inflammatory cytokines, mediators of inflammation, and basic fibroblast growth factor in prostaglandin F2α-induced luteolysis in bovine corpus luteum. Biol Reprod 2004b; 70:473-482.

Niswender GD, Reimers TJ, Diekman MA, Nett TM. Blood flow: a mediator of ovarian function. Biol Reprod 1976; 14:64-81.

Niswender GD, Schwall RH, Fitz TA, Farin CE, Sawyer HR. Regulation of luteal function in domestic ruminants: new concepts. Recent Prog Horm Res 1985; 41:101-151.

Niswender GD, Nett TM. The corpus luteum and its control. In: Knobil E, Neill J (Eds), The Physiology of Reproduction. Raven Press, New York, 1988; pp. 489-525.

Northey DL, French LR. Effect of embryo removal and intra-uterine infusion of embryonic homogenates on the lifespan of the bovine corpus luteum. J Anim Sci 1980; 50:298-302.

NRC. Nutrient requirements of dairy cattle. 7th rev. ed. Natl Acad Sci 2001; Washington, DC.

Nugteren DH, Christ-Hazelhof E. Naturally occurring conjugated octadecatrienoic acids are strong inhibitors of prostaglandin biosynthesis. Prostaglandins 1987; 33:403-417.

Ochoa JJ, Farquharson AJ, Grant I, Moffat LE, Heys SD, Wahle KWJ. Conjugated linoleic acids (CLAs) decrease prostate cancer cell proliferation: different molecular mechanisms for cis-9, trans-11 and trans-10, cis-12 isomers. Carcinogenesis 2004; 25:1185-1191.

Okuda K, Miyamoto A, Skarzynski DJ. Regulation of endometrial prostaglandin F2α synthesis during luteolysis and early pregnancy in cattle. Domest Anim Endo 2002; 23:255-264.

Oldick BS, Staples CR, Thatcher WW, Gyawu P. Abomasal infusion of glucose and fat: effect of digestion, production, and ovarian and uterine function of cows. J Dairy Sci 1997; 80:1315-1328.

Olsen SF, Hansen HS, Sorensen TI, Jensen B, Secher NJ, Sommer S, Knudsen LB. Intake of marine fat, rich in (n-3)-polyunsaturated fatty acids may increase birthweight by prolonging gestation. Lancet 1986; ii:367-369.

Olsen SF, Sorensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS, Grant A. Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. Lancet 1992; 25:1003-1007.

Ostrowska E, Muralitharan M, Cross RF, Bauman DE, Dunshea FR. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. J Nutr 1999; 129:2037-2042.

Ottobre JS, Lewis GS, Thayne WV, Inskeep EK. Mechanism by which progesterone shortens the estrous cycle of the ewe. Biol Reprod 1980; 23:1046-1053.

Pace-Asciak C, Wolfe LS. Inhibition of prostaglandin synthesis by oleic, linoleic and linolenic acids. Biochim Biophys Acta 1968; 152:784-787.

Paisley LG, Mickelsen WD, Anderson PB. Mechanisms and therapy for retained fetal membranes and uterine infections of cows: a review. Theriogenology 1986; 25:353-381.

Palmquist DL, Jenkins TC. Fat in lactation rations: review. J Dairy Sci 1980; 63:1-14.

Palmquist DL, Moser EA. Dietary fat effects on blood insulin, glucose utilization, and milk protein content of lactating cows. J Dairy Sci 1981; 64:1664-1670.

Pariza MW, Park Y, Cook ME. Mechanisms of action of conjugated linoleic acid: evidence and speculation. Proc Soc Exp Biol Med 2000; 223:8-13.

Park CD, Ralafowski W, Marx GD. Effect of dietary fat supplement on lipid metabolism of Holstein heifers. J Dairy Sci 1983; 66:528-534.

Parodi PW. Conjugated octadecadienoic acids of milk fat. J Dairy Sci 1977; 60:1550-1553.

Parodi PW. Cow's milk fat components as potential anticarcinogenic agents. J Nutr 1997; 127:1055-1060.

Pate JL. Regulation of prostaglandin synthesis by progesterone in the bovine corpus luteum. Prostaglandins 1988; 36:303-315.

Patil RK, Sinha SN, Einarsson S, Settergren I. The effect of prostaglandin F2 alpha and oxytocin on bovine myometrium in vitro. Nord Vet Med 1980; 32:474-479.

Petit HV, Germiquet C, Lebel D. Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. J Dairy Sci 2004; 87:3889-3898.

Petroff MG, Petroff BK, Pate JL. Mechanisms of cytokine-induced death of cultured bovine luteal cells. Reproduction 2001; 121:753-760.

Plaizier JCB, King GJ, Dekkers JCM, Lissemore K. Estimation of economic values of indices for reproductive performance in dairy herds using computer simulation. J Dairy Sci 1997; 80:2775-2783.

Pollard ME, Gunstone FD, Morris LJ, James AT. The delta 5 and delta 6 desaturation of fatty acids of varying chain length by rat liver: a preliminary report. Lipids 1980; 15:690-693.

Poyser NL. The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. Prostaglandins Leukot Essent Fatty Acids 1995; 53:147-195.

Proell JM, Mosley EE, Powell GL, Jenkins TC. Isomerization of stable isotopically labeled elaidic acid to cis and trans monoenes by ruminal microbes. J Lipid Res 2002; 43:2072-2076.

Pru JK, Rueda BR, Austin KJ, Thatcher WW, Guzeloglu A, Hansen TR. Interferon-tau suppresses PGF2α secretion independently of the mitogen-activated protein kinase and nuclear factor κB pathways. Biol Reprod 2001; 64:965-973.

Ramadan AA, Johnson GL III, Lewis GS. Regulation of uterine immune function during the estrous cycle and in response to infectious bacteria in sheep. J Anim Sci 1997; 75:1621-1632.

Rémond B, Cisse M, Ollier A, Chilliard Y. Slow release somatotropin in dairy heifers and cows fed two levels of energy concentrate. 1. Performance and body condition. J Dairy Sci 1991; 74:1370-1381.

Reynolds L, Grazul-Bilska, Redmer D. Angiogenesis in the corpus luteum. Endocrine 2000; 12:1-9.

Roberts RM, Farin CE, Cross JC. Trophoblast proteins and maternal recognition of pregnancy. Oxf Rev Reprod Biol 1990; 12:147-180.

Robinson RS, Mann GE, Lamming GE, Wathes DC. Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. Reproduction 2001; 122:965-979.

Romo GA, Erdman RA, Teter BB, Sampugna J, Casper DP. Milk composition and apparent digestibilities of dietary fatty acids in lactating dairy cows abomasally infused with cis or trans fatty acids. J Dairy Sci 2000; 83:2609-2619.

Rueda BR, Hendry IR, Hendry III WJ, Stormshak F, Slayden OD, Davis JS. Decreased progesterone levels and progesterone receptor antagonists promote apoptotic cell death in bovine luteal cells. Biol Reprod 2000; 62:269-276.

Rukkwamsuk T, Wensing T, Kruip TAM. Relationship between triacylglycerol concentration in the liver and first ovulation in postpartum dairy cows. Theriogenology 1999; 51:1133-1142.

Ryan DP, Spoon RA, Williams GL. Ovarian follicle characteristics, embryo recovery and embryo viability in heifers fed high fat diets and treated with follicle stimulating hormone. J Anim Sci 1992; 70:3505-3513.

Ryan DP, Bao B, Griffith MK, Williams GL. Metabolic and luteal sequelae to heightened dietary fat intake in undernourished, anestrous beef cows induced to ovulate. J Anim Sci 1995; 73:2086-2093.

Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu Rev Nutr 2005; 25:15.1-15.24.

Sangsritavong S, Mashek DG, Gumen A, Haughian JM, Grummer RR, Wiltbank MC. Metabolic clearance rate of progesterone and estradiol-17β is decreased by fat. J Dairy Sci 2002; 85(Suppl 1):142.

Santora JE, Palmquist DL, Roehrig KL. Trans-Vaccenic acid is desaturated to conjugated linoleic acid in mice. J Nutr 2000; 130:208-215.

Sargent JR. Fish oils and human diet. Br J Nutr 1997; 78(Suppl 1):S5-13.

Statistical Analysis System (SAS). SAS user's guide: Statistics. SAS Institute Inc. 2001; Cary, NC.

Sauerwein H, Miyamoto A, Ginther J, Meyer HHD, Schams D. Binding and action of insulin-like growth factors and insulin in bovine luteal tissue during the oestrous cycle. J Reprod Fertil 1992; 96:103-115.

Savio JD, Keenan L, Boland MP, Roche JF. Pattern of growth of dominant follicles during the oestrous cycle of heifers. J Reprod Fertil 1988; 83:663-671.

Scaramuzzi RJ, Adams NR, Baird DT, Campbell BK, Downing JA, Findlay JK, Henderson KM, Martin GB, McNatty KP, McNeilly AS, Tsonis CG. A model for follicle selection and the determination of ovulation rate in the ewe. Reprod Fertil Dev 1993; 5:459-478.

Schams D. Ovarian peptides in the cow and sheep. J Reprod Fertil 1989; 37 (Suppl.):225-231.

Schams D. Regulation of bovine intra-luteal function by peptide hormones. J Physiol Pharmacol 1992; 43:117-129.

Schams D, Amselgruber W, Einspanier R, Sinowatz F, Gospodarowicz D. Localization and tissue concentration of fibroblast growth factor in the bovine corpus luteum. Endocrine 1994; 2:907-912.

Schams D, Liebermann J, Miyamoto A. Luteotropic role of bovine luteal oxytocin in vitro. Adv Exp Med Biol 1995; 395:543-544.

Schams D. Paracrine/autocrine regulation of bovine corpus luteum function. J Reprod Dev 1996; 42 (Suppl.):54-57.

Schams D, Kotwica J, Elger W. The importance of progesterone for the timing of luteolysis and oestrous cycle length in sheep and cow. Exp Clin Endocrinol Diabetes 1998; 106(Suppl):S9.

Schams D, Berisha B. Regulatory factors for luteolysis in ruminants. Arch Tierz 2001; 44:51-53.

Schams D, Berisha B. Steroids as local regulators of ovarian activity in domestic animals. Domest Anim Endocrinology 2002; 23:53-65.

Schams D, Berisha B. Regulation of corpus luteum function in cattle-an overview. Reprod Domest Anim 2004; 39:241-251.

Schindler D, Lewis GS, Rosenberg M, Tadmor A, Ezov N, Ron M, Aizinbud E, Lehrer AR. Vulvar electrical impedance in periparturient cows and its relation to plasma progesterone, oestradiol-17β and PGFM. Anim Reprod Sci 1990; 23:283-292.

Schillo KK, Hall JB, Hileman SM. Effects of nutrition and season on the onset of puberty in the beef heifer. J Anim Sci 1992; 70:3994-4005.

Schneider P, Sklan D, Chalupa W, Kronfeld DS. Feeding calcium salts of fatty acids to lactating cows. J Dairy Sci 1988; 71:2143-2151.

Schopee PD, Garmey JC, Veldhuis JD. Putative activation of the peroxisome proliferator-activated receptor γ impairs androgen and enhances progesterone biosynthesis in primary cultures of porcine theca cells. Biol Reprod 2002; 66:190-198.

Seals RC, Matamoros I, Lewis GS. Relationship between postpartum changes in 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ concentrations in Holstein cows and their susceptibility to endometritis. J Anim Sci 2002; 80:1068-1073.

Selberg KT, Lowe AC, Staples CR, Luchini ND, Badinga L. Production and metabolic responses of periparturient Holstein cows to dietary conjugated linoleic acid and trans-octadecenoic acids. J Dairy Sci 2004; 87:158-168.

Senger PL. Pathways to pregnancy and parturition. Current Conceptions, Inc. 1997. Pullman, WA.

Shirasuna K, Wijayagunawardane MPB, Watanabe S, Yamamoto D, Matsui M, Ohtani M, Miyamoto A. A blood flow in the corpus luteum acutely increases together with endothelin 1 mRNA expression at early stage of regression during spontaneous luteolysis in the cow. Biol Reprod 2004; 71(Suppl.1):A149.

Simpson RB, Chase CC Jr, Spicer LJ, Vernon RK, Hammond AC, Rae DO. Effects of exogenous insulin on plasma and follicular insulin-like growth factor I, insulin-like growth factor binding protein activity, follicular oestradiol and progesterone and follicular growth in superovulated Angus and Brahman cows. J Reprod Fertil 1994; 102:483-492.

Sinclair AJ, Mann NJ. Short-term diets rich in arachidonic acid influence plasma phospholipids polyunsaturated fatty acid levels and prostacyclin and thromboxanes production in humans. J Nutr 1996; 126(Suppl):1110S-1114S.

Singh S, Hawke JC. The in vitro lipolysis and biohydrogenation of monogalactosyldiglyceride by whole rumen contents and its fraction. J Sci Food Agric 1979; 30:603-612.

Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol Reprod 1988; 39:308-317.

Skarzynski DJ, Okuda K. Sensitivity of bovine corpora lutea to prostaglandin F2α is dependent on progesterone, oxytocin, and prostaglandins. Biol Reprod 1999; 60:1292-1298.

Skarzynski DJ, Miyamoto Y, Okuda K. Production of prostaglandin F2α by cultured bovine endometrial cells in response to tumor necrosis factor α: cell type specificity and intracellular mechanisms. Biol Reprod 2000; 62:1116-1120.

Skarzynski DJ, Jarosezewski JJ, Okuda K. Luteotropic mechanism in the bovine corpus luteum: role of oxytocin, prostaglandin F2α, progesterone and noradrenalin. J Reprod Dev 2001; 47:125-137.

Sklan D, Bogin E, Avidar Y, Gur-Arie S. Feeding calcium soaps of fatty acids to lactating cows: effect on production, body condition and blood lipids. J Dairy Res 1989; 56:675-681.

Sklan D, Moallen U, Folman Y. Effect of feeding calcium soaps of fatty acids on production and reproductive responses in high producing lactating cows. J Dairy Sci 1991; 74:510-517.

Sklan D, Kaim M, Moallem U, Folman Y. Effects of dietary calcium soaps on milk yield, body weight, reproductive hormones, and fertility in first parity and older cows. J Dairy Sci 1994; 77:1652-1660.

Smith WL, Marnett LJ. Prostaglandin endoperoxide synthase: structure and catalysis. Biochim Biophys Acta 1991; 1083:1-17.

Smith LC, Olivera-Angel M, Groome NP, Bhatia B, Price CA. Oocyte quality in small antral follicles in the presence or absence of a large dominant follicle in cattle. J Reprod Fertil 1996; 106:193-199.

Spencer TE, Bazer FW. Temporal and spacial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol Reprod 1995; 53:1527-1543.

Spencer TE, Mirando MA, Mayes JS, Watson GH, Ott TL, Bazer FW. Effects of interferon-tau and progesterone on oestrogen-stimulated expression of receptors for oestrogen, progesterone and oxytocin in the endometrium of ovariectomized ewes. Reprod Fertil Dev 1996; 8:843-853.

Spencer TE, Woods JW, Arakawa T, Singer II, Smith WL. Subcellular localization of prostaglandin endoperoxide H synthases-1 and -2 by immunoelectron microscopy. J Biol Chem 1998; 273:9886-9893.

Spencer TE, Bazer FW. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. Front Biosci 2002; 7:d1879-1898.

Spicer LJ, Tucker WB, Adams GD. Insulin-like growth factors in dairy cows: relationships among energy balance, body condition, ovarian activity and estrous behavior. J Dairy Sci 1990; 73:929-937.

Spicer LJ, Alpizar E, Echternkamp SE. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulose cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production in vitro. J Anim Sci 1993; 71:1232-1241.

Spicer LJ, Stewart RE. Interaction among bovine somatotropin, insulin, and gonadotropins on steroid production by bovine granulosa and theca cells. J Dairy Sci 1996; 79:813-821.

Sprecher H. Biochemistry of essential fatty acids. Prog Lipid Res 1981; 20:13-22.

Staggs KL, Austin KJ, Johnson GA, Teixeira MG, Talbott CT, Dooley VA, Hansen TR. Complex induction of bovine uterine proteins by interferon-tau. Biol Reprod 1998; 59:293-297.

Staples CR, Thatcher WW, Clark JH. Relationship between ovarian activity and energy balance during the early postpartum period of high producing cows. J Dairy Sci 1990; 73:938-947.

Staples CR, Burke JM, Thatcher WW. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J Dairy Sci 1998; 81:856-871.

Stojkovic M, Wolf E, Büttner M, Berg U, Charpigny G, Schmitt A, Brem G. Secretion of biologically active interferon tau by in vitro-derived bovine trophoblastic tissue. Biol Reprod 1995; 53:1500-1507.

Sugano M, Tsujita A, Yamasaki M, Noguchi M, Yamada K. Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rat. Lipids 1998; 33:521-527.

Sunderland SJ, Crowe MA, Boland MP, Roche JF, Ireland JJ. Selection, dominance and atresia of follicles during the oestrous cycle of heifers. J Reprod Fertil 1994; 101:547-555.

Tanaka K, Shigeno K. The biohydrogeneation of linoleic acid by rumen micro-organisms. Jpn J Zootech Sci 1976; 47:50-53.

Talavera F, Park CS, Williams GL. Relationship among dietary lipid intake, serum cholesterol and ovarian function in Holstein heifers. J Anim Sci 1985; 60:104-105.

Taylor CC, Rajamahendran R. Follicular dynamics, corpus luteum growth and regression in lactating dairy cattle. Can J Anim Sci 1991; 71:61-68.

Thatcher WW, Wilcox CJ. Postpartum estrus as an indicator of reproductive status in the dairy cow. J Dairy Sci 1973; 56:608-610.

Thatcher WW, Meyer MD, Danet-Desnoyers G. Maternal Recognition of Pregnancy. J Reprod Fertil Suppl 1995; 49:15-28.

Thomas MG, Williams GL. Metabolic hormone secretion and FSH-induced superovulatory responses of beef heifers fed dietary fat supplements containing predominantly saturated or polyunsaturated fatty acid. Theriogenology 1996; 45:451-458.

Thomas MG, Bao B, Williams GL. Dietary fats varying in their fatty acid composition differentially influence follicular growth in cows fed isoenergetic diets. J Anim Sci 1997; 75:2512-2519.

Thorburn GD, Challis JRG. Control of parturition. Physiological Rev 1979; 59:863-918.

Townson DH, Tsang PC, Butler WR, Frajblat M, Griel LC Jr, Johnson CJ, Milvae RA, Niksic GM, Pate JL. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. J Anim Sci 2002; 80:1053-1058.

Trujillo EP, Broughton KS. Ingestation of n-3 polyunsaturated fatty acids and ovulation in rats. J Reprod Fertil 1995; 105:197-203.

Tsafriri A, Lindner HR, Zor U, Lamprecht SA. Physiological role of prostaglandins in the induction of ovulations. Prostaglandins 1972; 2:1-10.

Tsang BK, Arodi J, Li M, Ainsworth L, Srikandakumar A Downey BR. Gonadotrophic regulation of prostaglandin production by ovarian follicular cells of the pig. Biol Reprod 1988; 38:627-635.

Urich K. Lipids. In: Comparative Animal Biochemistry: An introduction. 4th ed. London: Champman & Hall, 1994: 562-576.

Uruquhart P, Parkin SM, Rogers JS, Bosley JA, Nicolaou A. The effect of conjugated linoleic acid on arachidonic acid metabolism and eicosanoid production in human saphenous vein endothelial cells. Biochim Biophys Acta 2002; 1580:150-160.

Vallet JL, Lamming GE, Batten M. Control of endometrial oxytocin receptor and uterine response to oxytocin by progesterone and oestradiol in the ewe. J Reprod Fertil 1990; 90:625-634.

Van Bilsen M, de Vries JE, Van der Vusse GJ. Long-term effects of fatty acids on cell viability and gene expression of neonatal cardiac myocytes. Prostaglandins Leukot Essent Fatty Acids 1997; 57:39-45.

Van Bilsen M, Van der Vusse GJ, Reneman RS. Transcriptional regulation of metabolic processes: implications for cardiac metabolism. Pflugers Arch 1998; 437:2-14.

Van der Kraak G, Chang JP. Arachidonic acid stimulates steroidogenesis in goldfish preovulatory ovarian follicles. Gen Comp Endocrinol 1990; 77:221-228.

Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J

Varga B, Horvath E, Folly G, Stark E. Study of the luteinizing hormone-induced increase of ovarian blood flow during the estrous cycle in the rat. Biol Reprod 1985; 32:480-488.

Vazquez-Añon M, Bertics S, Luck M, Grummer RR, Pinheiro J. Peripartum liver triglyceride and plasma metabolites in dairy cows. J Dairy Sci 1994; 77:1521-1528.

Verhulst A, Janssen G, Parmentier G, Eyssen H. Isomerization of polyunsaturated long-chain fatty acids by propionibacteria. System Appl Microbiol 1987; 9:12-15.

Villa-Godoy A, Hughes TL, Emery RS, Chapin LT, Fogwell RL. Association between energy balance and luteal function in lactation dairy cows. J Dairy Sci 1988; 71:1063-1072.

Wallach EE, Bronson R, Hamada Y, Wright KH, Steversens VG. Effectiveness of prostaglandin F2α in restoration of hMG-hCG induced ovulation in endomethacin treated rhesus monkeys. Prostaglandins 1975; 10:129-138.

Ward PFV, Scott TW, Dawson BA. Dietary and ruminally derived trans-18:1 fatty acids alter bovine milk lipids. J Nutr 1964; 124:556-565.

Wathes DC, Swann RW, Birkett SD, Porter DG, Pickering BT. Characterization of oxytocin, vasopressin, and neurophysin from the bovine corpus luteum. Endocrinology 1983; 113:693-698.

Wathes DC, Hamon M. Localization of oestradiol, progesterone and oxytocin receptors in the uterus during the oestrous cycle and early pregnancy of the ewe. J Endocrinol 1993; 138:479-491.

Wathes DC, Lamming GE. The oxytocin receptor, luteolysis and maternal recognition of pregnancy. J Reprod Fertil 1995; 49(Suppl):53-67.

Watson ED. Eicosanoids and phagocytic and bactericidal activity of equine neutrophils. Equine Vet J 1988; 20:373-375.

Webb R, Nicholas B, Gong JG, Campbell BK, Gutierrez CG, Garverick HA, Armstrong DG. Mechanisms regulating follicular development and selection of the dominant follicle. Reproduction 2003; 61:71-90.

Wehrman ME, Welsh TH Jr, Williams GL. Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics and hastens the onset of postpartum luteal activity. Biol Reprod 1991; 45:514-523.

Williams GL. Modulation of luteal activity in postpartum beef cows through changes in dietary lipid. J Anim Sci 1989; 67:785-793.

Wise TH, Canton D, Thatcher WW, Barron DH, Fields MJ. Ovarian function during the estrous cycle of the cow: ovarian blood flow and progesterone release rate. J Anim Sci 1982; 55:627-637.

Wulster-Radcliffe MC, Seals RC, Lewis GS. Progesterone increases susceptibility of gilts to uterine infections after intrauterine inoculation with infectious bacteria. J Anim Sci 2003; 81:1242-1252.

Xiao CW, Liu JM, Sirois J, Goff AK. Regulation of cyclooxygenase-2 and prostaglandin F synthase gene expression by steroid hormones and interferon-τ in bovine endometrial cells. Endocrinology 1998; 139:2293-2299.

Xiao CW, Goff AK. Hormonal regulation of estrogen and progesterone receptors in cultured bovine endometrial cells. J Reprod Fertil 1999; 115:101-109.

Xu Z, Garverick HA, Smith GW, Smith MF, Hamilton SA, Youngquist RS. Expression of follicle-stimulating hormone and luteinizing hormone receptor messenger ribonucleic acids in bovine follicles during the first follicular wave. Biol Reprod 1995; 53:951-957.

Yoshimura Y, Iwashita M, Karube M, Oda T, Akiba M, Shiokawa S, Ando M, Yoshinaga A, Nakamura Y. Growth hormone stimulates follicular development by stimulating ovarian production of insulin-like growth factor-I. Endocrinology 1994; 135:887-894.

Zammit VA. Role of insulin in hepatic fatty acid partitioning: emerging concepts. Biochem J 1996; 314(Pt 1):1-14.

Zhang J, Weston PG, Hixon JE. Role of progesterone and oestradiol in the regulation of uterine oxytocin receptors in ewes. J Reprod Fertil 1992; 94:395-404.

Zhou Y-C, Waxman DJ. Cross-talk between janus kinase-signal transducer and activator of transcription (JAK-STAT) and peroxisome proliferator-activated receptor-alpha (PPARα) signaling pathways. Growth hormone inhibition of PPARalpha transcriptional activity mediated by stat5b. J Biol Chem 1999; 274:2672-2681.

BIOGRAPHICAL SKETCH

Carlos Jesús Rodríguez Sallaberry was born on December 2, 1971, to Fritz I. Rodríguez Munoz and Lourdes Sallaberry Morciglio in Mayagüez, Puerto Rico. He is the youngest of seven children. He received his bachelor's degree in molecular genetics from The Ohio State University, Columbus, OH, in December 1994. He attended the University of Puerto Rico, Mayagüez Campus for a year to complete a postbachalaurate degree in the Department of Animal Industry, and subsequently entered the Graduate School in the Department of Animal Sciences at the University of Florida in 1997 where he completed his master's program under the supervision of Dr. Rosalia C.M. Simmen in the Fall of 1999. He continued his PhD program with Dr. Simmen, which he was forced to postpone for personal reasons. During the period between March of 2001 and April of 2003 he worked as a technician in the laboratory of Dr. Karen Moore, Department of Animal Sciences, University of Florida. He then resumed his PhD degree under the supervision of Dr. Lokenga Badinga. After completion of the PhD degree, he plans to work as a postdoctoral fellow in the laboratory of Dr. Andrés Kowalski in Universidad Centrooccidental Lisandro Alvarado, Barquisimeto, Venezuela.