INTERACTION OF PRE- AND POSTNATAL NUTRITION ON AGE AT PUBERTY AND SELECTED ASPECTS OF REPRODUCTIVE PHENOTYPE IN SEXUALLY MATURE HEIFERS

A Thesis

by

TATIANE SILVA MAIA

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee, Gary L. Williams
Co-Chair of Committee, Rodolfo C. Cardoso
Committee Member, Thomas H. Welsh, Jr.
Head of Department, G. Cliff Lamb

May 2021

Major subject: Physiology of Reproduction

Copyright 2021 Tatiane Silva Maia

ABSTRACT

Two experiments were conducted to test if pre- and postnatal nutrition in the bovine female, independently or interactively, affect 1) age at puberty and 2) selected phenotypic characteristics of the estrous cycle of sexually mature offspring. Brangus and Braford (n=108) beef cows bearing a female fetus were fed to achieve body condition scores of 7.5-8 (H, obese), 5.5-6 (M, moderate) or 3-3.5 (L, very thin) by the start of the third trimester and maintained until parturition. Heifer offspring were weaned and fed to gain at either a high (H; 1 kg/d) or low (L; 0.5 kg/d) rate between 4 and 8 months of age, then fed a common growth diet until puberty, resulting in a 3x2 factorial arrangement of maternal x postnatal treatments (L/L, L/H, M/L, M/H, H/L, H/H). For Exp. 2, estrous cycles of a subgroup of postpubertal heifers (n = 53) from Exp. 1 were synchronized to evaluate selected ovarian, uterine, estrous behavior and hormonal characteristics, including antral follicle count (AFC), rate of growth and size of the pre-ovulatory follicle, size of corpus luteum (CL) and ovary, endometrial thickness, luteal phase concentrations of progesterone (P4), and follicular phase concentrations of estradiol-17β (E2). Heifers (n = 95) from the H postnatal dietary treatment reached puberty two months earlier (12 ± 0.4 months; $P = 0.0002$) than those from the L postnatal diet (14 ± 0.4 months). However, neither maternal diets nor their interaction with postnatal diets of offspring influenced age at puberty. In Exp. 2, maternal, postnatal and maternal x postnatal interactions failed to affect physiological or hormonal variables selected for

evaluation, although there was a trend for AFC (P = 0.09) and peak concentrations of E2 before estrus (P = 0.08) to be greater in H than in L groups. Results confirm that early-weaned heifers fed a high-gain diet during the juvenile period reach puberty much earlier than those fed a low-gain (L) diet. However, neither maternal dietary treatments during the second and third trimesters of pregnancy nor their interaction with postnatal dietary treatments affected age at puberty or selected physiologic, morphologic, or behavioral characteristics of the estrous cycle.

DEDICATION

This thesis is dedicated to my parents who have always supported me and are my source of inspiration in both professional and personal life.

ACKNOWLEDGMENTS

Although words are not enough to express my eternal gratitude, I would first like to thank my advisor Dr. Williams, for his continuous support and mentoring. According to the dictionary, a mentor is “an experienced and trusted advisor”, but more than a mentor, Dr. Williams became a friend who has taught me how to work hard, be persistent, and showed how fascinating science can be. Thank you for your infinite patience and for being my source of inspiration since first coming to Beeville. I feel blessed to be your graduate student in such an extraordinary program and focus of research. Although I am finalizing one important path of my life, I will carry all the knowledge and lessons that I acquired during these 2 years for the rest of my life. I appreciate everything you have done for me and I will make sure to not forget to “massage my data”.

I would also like to thank Dr. Cardoso for all of his guidance and lessons. The memory of my first visit to his laboratory to see a variety of brain sections and kisspeptin neurons through the microscope still lingers in my mind. It was very exciting! He was the first contact that allowed me to be part of this program. Thanks for your trust and for always being there when needed. As my committee member, thank you to Dr. Welsh for always being available as well as for his immeasurable effort throughout the course of this research. I also had the pleasure of being his student and I am thankful for his wisdom and insight to look at science from a unique perspective. Thanks to Dr. Ky

Pohler and his laboratory team, especially Gessica Franco and Sydney Reese, for your guidance and providing me with my first exposure to a graduate student environment.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience. In special I would like to thank everybody that helped me in this project, including Higor Rezende, Sterling Fahey, Julie Kellerman, Viviana Garza, TJ Gomez and Ernest Soto. Thanks for all the patience. Your help was essential for this work. In special I would also like to thank Sarah West who more than a lab mate, became a very close friend who has made Texas and my graduate student life enjoyable.

Finally, thanks to my parents and my two lovely sisters for their encouragement. Thanks for always believing in my potential and for providing all the support that I needed. I appreciate all the effort my parents have made to allow great opportunities to happen, including coming to Texas, and for reminding me how grateful I am to have them. I would also like to thank my friends from Brazil that kept me virtual company and made hard times so much easier.

To all, my sincere gratitude!

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Dr. Gary Williams [advisor], Dr. Rodolfo Cardoso [co-advisor] of the Department of Animal Science, and Dr. Thomas Welsh of the Department of Veterinary Integrative Biosciences.

Funding Sources

This work was made possible by Agriculture and Food Research Initiative Competitive Grant no. 2013-67015-20960 and 2018-67015-27595 from the USDA National Institute of Food and Agriculture.

NOMENCLATURE

ADG	Average Daily Gain
AFC	Antral Follicle Count
AI	Artificial Insemination
AMH	Anti-Müllerian Hormone
ARC	Arcuate Nucleus
AVPV	Anteroventral Periventricular Nucleus
αMSH	Alpha-Melanocyte Stimulating Hormone
BCS	Body Condition Score
BrW	Birth Weight
BW	Body Weight
CIDR	Controlled Internal Drug Release
CL	Corpus Luteum
DM	Dry Matter
E2	Estradiol
EDTA	Disodium Ethylenediaminetetraacetic acid
ESRα	Estrogen Receptor α
FSH	Follicle-stimulating Hormone
FTAI	Fixed-time Artificial Insemination
GnRH	Gonadotropin Releasing Hormone
H	High

L	Low
LH	Luteinizing Hormone
M	Moderate
NPY	Neuropeptide Y
P4	Progesterone
PGC	Primordial Germ Cells
PGF2α	Prostaglandin F2α
POA	Preoptic Area
POMC	Proopiomelanocortin
RIA	Radioimmunoassay
WW	Weaning Weight

TABLE OF CONTENTS

Page

ABSTRACT ii

DEDICATION iv

ACKNOWLEDGMENTS v

CONTRIBUTORS AND FUNDING SOURCES vii

NOMENCLATURE viii

TABLE OF CONTENTS x

LIST OF FIGURES xii

LIST OF TABLES xiv

CHAPTER I INTRODUCTION 1

CHAPTER II REVIEW OF LITERATURE 4

Fetal programming 4
Maternal nutrition during pregnancy 5
The postnatal environment 12
Neuroendocrine axis 13
Characteristics of bovine reproductive development 13
Processes involved in pubertal maturation and estrous cyclicity in the female 15
Potential biomarkers to predict reproductive performance 25
Expected impact of this research 30

CHAPTER III INFLUENCE OF THE PERINATAL NUTRITIONAL ENVIRONMENT ON AGE AT PUBERTY AND FUNCTIONAL CHARACTERISTICS OF THE BOVINE ESTROUS CYCLE IN HEIFERS 31

Introduction 31
Materials and Methods 32
Results 43
Discussion 54

CHAPTER IV CONLUSIONS 64

LITERATURE CITED 65

LIST OF FIGURES

Page

Fig. 2.1. During gestation there are important events regulating fetal development. The fetal programming concept states that an insult during pregnancy can result in negative outcomes in the offspring 6

Fig. 3.1. Maternal and postnatal nutritional treatments. The 3x2 factorial design represents female offspring born to cows fed to calve in obese, moderate and thin body condition, and fed during the juvenile period to gain BW at high or low rate at 8 months of age 36

Fig. 3.2. Overall timeline of the animal model beginning from onset of maternal dietary treatments through the postnatal dietary treatments and subsequent experimental protocols. Cows were synchronized followed by FTAI and placed 10 days later with a clean-up bull. Final pregnancy status and fetal gender determination occurred between 45 and 70 days after removal from breeding pastures. During the second trimester of gestation (day 90 of gestation), cows were assigned randomly to three dietary treatment groups to achieve a BCS of either 3.5-4 (thin), 5.5-6 (moderate), or 7.5-8 (obese) by the end of the second trimester of gestation (day 180 of gestation). After calving, heifer calves were weaned at approximately 3.5 months of age and assigned randomly to receive one of two dietary treatments (low-gain and high-gain) from approximately 4 to 8 months of age. Following confirmation of puberty (n = 95), experiments evaluating characteristics of selected reproductive variables was performed in a subset (n = 53) of heifers representing all dietary treatments...38

Fig. 3.3. Experimental design for evaluation of selected aspects of reproductive phenotype in nutritionally programmed heifers. Two injections of PGF2α were given to all heifers 11 days apart. On the day of the second injection, blood samples and ultrasound measurements were conducted. PGF Cycle represents the period between the day of the second injection of PGF2α until ovulation. Ultrasound measurements were taken in the PGF Cycle and Cycle 1. Blood samples were collected daily throughout PGF Cycle, Cycle 1 and Cycle 2. Estrous cycle length was evaluated from Cycle 1, 2 and 3. Estrus expression was evaluated based on PGF Cycle, Cycle 1, 2 and 3. E, onset of estrus; OV, day of ovulation; D14, 14 days after ovulation 40

Fig. 3.4. Least squares mean (±SEM) BCS of dams from the 2nd trimester of gestation (week 0) until parturition (270-285 days represented by week 24). Cow BCS did not differ (*P* > 0.1) among high (H; n = 31), moderate (M; n = 33) or low (L; n = 33) maternal dietary groups at onset of the experiment. Differences in BCS (*P* < 0.0001) were detected between H and L groups beginning at week 4 and among all dietary groups beginning at week 6 (*P* ≤ 0.0001) 45

Fig. 3.5. Least squares mean (±SEM) BW of dams in high (H; n = 31), moderate (M; n = 33) and low (L; n = 33) maternal dietary groups from the 2nd trimester of gestation (week 0) until parturition (270-285 days represented by week 24). Cow BW did not differ (*P* > 0.1) at onset of the experiment but differed between H and L beginning at week 4 (*P* = 0.001) and among all dietary groups beginning at week 12 (*P* < 0.0001) 45

Fig. 3.6. Least squares mean (±SEM) BW of heifers fed high (H; n = 48) and low (L; n = 49) postnatal diets after weaning at approximately 3.5 months of age (week 0) and fed until 8 months of age (week 18). Body weight of H heifers was greater (*P* = 0.01) than L heifers beginning at week 2 and continuing through week 18 (*P* < 0.0001) 47

Fig. 3.7. (A) Least squares mean (±SEM) ages (months) at pubertal onset in heifers fed high gain (H; n = 47) and low-gain (L; n = 48) diets postnatally and (B) in heifers representing the 3x2 maternal x postnatal treatment combinations. Heifers fed the high gain (H) diet postnatally reached puberty 2 months earlier (**P* = 0.0002) than heifers fed the low gain diet (L). There was no maternal or maternal x postnatal dietary effect (*P* = 0.76) on age at puberty.. 49

Fig. 3.8. (A) Least squares mean (±SEM) plasma concentrations of P4 during the luteal phase and (B) mean peak concentrations of P4 over two complete estrous cycles in heifers representing the 6 dietary group combinations (n = 8-9/group). Neither the overall mean (*P* = 0.92) nor peak (*P* = 0.63) concentration differed due to maternal x postnatal diet combinations 52

Fig. 3.9. Least squares mean (±SEM) plasma concentrations of E2 between days -5 and -1 before ovulation in heifers representing the 6 dietary group combinations (n = 8-9/group). There was no effect of maternal x postnatal diet combinations on E2 concentrations (*P* = 0.80) 53

Fig. 3.10. (A) Least squares mean (±SEM) peak concentrations of E2 during the follicular phase in heifers fed low gain (L) or high gain (H) postnatal diets, and (B) maternal x postnatal combinations. Heifers fed H diets postnatally tended (**P* < 0.08) to have a greater peak concentration of E2 compared to L heifers (A). Maternal x postnatal diet combinations had no effect (*P* = 0.49) on any E2 variable (B) 54

LIST OF TABLES

Page

Table 3.1. Ingredients and chemical composition of the two diets (Diet A and Diet B) fed to prepubertal heifers starting from approximately 4 to 8 months of age. Heifers received diet A during the first 10 weeks of the experiments and diet B was provided to heifers from week 11 until the end of the study (week 18) 35

Table 3.2. Least squares mean (± SEM) birth weights (BrW), weaning weights (WW) and average daily gains (ADG) in heifers (n = 93) born to cows fed to calve in thin (L group), moderate (M group) and obese (H group) body condition.. 46

Table 3.3. Ultrasound measurements from heifers during two estrous cycles* 50

CHAPTER I

INTRODUCTION

In order to achieve optimal lifetime efficiency, it is extremely important that replacement heifers, which represent the next generation of the beef cow herd, become pregnant early in their first breeding season, calve at 2 years of age, and maintain their productivity over a long-term period [1]. According to USDA National Agricultural Statistics Service (2020), replacement beef heifers represent approximately 29% of all heifers that are 500 pounds (~ 227 kg) and greater. However, 40% of these heifers are not expected to calve [2] due to several factors including inadequate nutrition prior to or during the breeding season [3] or because puberty was not achieved early enough to become pregnant [4]. Selection of heifers prior to the start of the breeding season, based on body condition and reproductive tract scores, the latter of which correlates uterine horn size and ovarian follicular development with pubertal status, demonstrated that approximately 15.3% of the heifers pre-selected as replacements did not conceive in their first breeding season [5]. Thus, other factors appear to influence postpubertal reproductive performance that are unaccounted for by physiological markers [6].

During fetal development, the exposure of the fetus to an insult can induce differential outcomes depending on the nature, severity, and duration of the insult as well as the stage of pregnancy at which it is encountered [7–9]. Notably, the period in which organogenesis and tissue differentiation occur, defined as the perinatal period,

represents a window of susceptibility to the impacts of an adverse environment [10]. In ruminants, irregularities such as reduced maternal nutrient intake during pregnancy can impair normal fetal development with implications seen in postnatal life that can negatively affect birth weight (BrW), postnatal growth, health of the offspring [11], and metabolism [12]. Nutrient deficiency during gestation is a common occurrence in cattle managed in forage-based production systems globally [13]. Importantly, during periods of reduced dry matter (DM) availability, managerial actions such as adoption of early weaning of calves are needed to reduce nutritional requirements of the dams. However, if early weaning is followed by periods of rainfall abundance, non-lactating cows can become obese. Therefore, cattle can experience both under and overnutrition that coincides with their pregnancy, and such conditions [7,14] may result in physiological changes in offspring that negatively affect postnatal and adult wellbeing, and performance.

Considerable effort has been made to understand the effects of maternal nutrition on growth, health, and productivity of offspring. However, among livestock species, the scope of studies that has related maternal nutrition to fertility and prediction of reproductive longevity is limited [15]. In sheep, it has been proposed that nutritional insults during gestation may be mitigated by interventions during early postnatal life [16]. Thus, a better understanding of the interaction of pre- and postnatal nutrition on adult reproductive phenotype is essential for developing strategies to optimize lifetime reproductive performance.

The current study tested the hypothesis that maternal nutrition during the second and third trimesters of pregnancy in the bovine female interacts with postnatal nutrition to affect 1) age at puberty and 2) selected aspects of ovarian, uterine, and behavioral characteristics of the estrous cycle in female offspring.

CHAPTER II

REVIEW OF LITERATURE

FETAL PROGRAMMING

Epidemiological data from the Dutch famine indicated that maternal malnutrition during pregnancy is associated with a marked increase in the risks of the adult offspring to develop obesity [17]. Subsequent studies explored the association between low BrW due to malnutrition during pregnancy and the increased risk of cardiovascular disease [18], as well as type 2 diabetes in adulthood [19]. These observations led to the fetal programming hypothesis known as "thrifty phenotype", which theorizes that inadequate nutrition during fetal development and early infant life induces permanent modifications in both structure and function of specific organs and tissues resulting in metabolic syndrome and other diseases in adulthood [19, 20]. Hence, the term "fetal programming" has been adopted to describe the prenatal adaptation in physiology, endocrinology, and metabolism that enhance postnatal survival under adverse conditions during critical periods of development [21, 22]. Although this concept emerged from epidemiological studies in humans, earlier studies in livestock species, such as horses, had already provided evidence that the maternal uterine environment during pregnancy could permanently influence offspring growth during early postnatal and adult life [23].

During development in mammals, there are a series of critical periods when developmental processes are plastic or sensitive to the environment [24]. For example,

the hypothalamic-pituitary axis exhibits a large degree of developmental plasticity to maternal environmental insults that contribute to alterations in reproductive function postnatally. Such insults include exposure to endocrine disrupting compounds, nutritional status, stress, and a variety of stressors [25]. There is evidence that in ruminants, such as cattle and sheep, maternal undernutrition during different stages of gestation may alter pituitary-gonadal function in the offspring and include effects on pituitary responsiveness to gonadotropin releasing hormone (GnRH), a reduced number of pre-antral follicles (i.e., primordial and primary) [26], as well as decreased concentration of plasma progesterone (P4) during the luteal phase of the estrous cycle [27]. In sheep, maternal malnutrition during late gestation has been associated with changes in metabolic and endocrine functions postnatally that interfere with glucose-insulin homeostasis, hepatic functions, leptin response, and other endocrine functions [28]. A summary of the major events regulating fetal development and the consequences on the offspring based on the concept of fetal programming is represented in **Figure 2.1**.

MATERNAL NUTRITION DURING PREGNANCY

During pregnancy, maternal-conceptus interactions in mammals involve nutrient partitioning to several body tissues that rely on two processes: homeostasis and homeorhesis. The former regulates the internal environment to maintain a physiological equilibrium and the latter maintains a physiological state through coordinated modifications in metabolism of tissues in the body to assure a constant flow of nutrients [187]. The nutrient supply to the fetus can be achieved through different mechanisms with the involvement of the placentomes, the uterine glands through the secretion of

histotroph, and the placenta by the metabolism of substrates [190]. The metabolic substrates from the placenta to the fetus, such as glucose, lactate, amino acids,

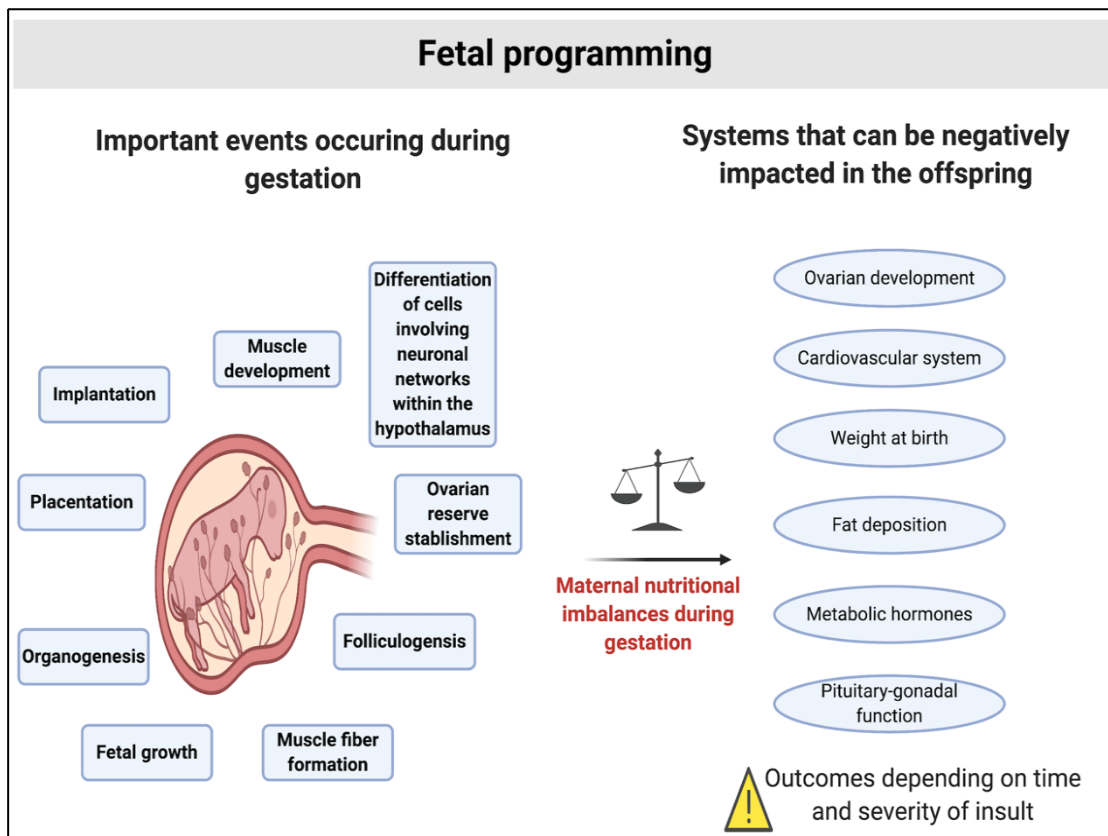

Fig. 2.1. During gestation there are important events regulating fetal development. The fetal programming concept states that an insult during pregnancy can result in negative outcomes in the offspring.
**Created with BioRender.com*

and fatty acids, serve as major energy sources to maintain fetal oxidative metabolism and to form new tissues [188]. The fetus also has its own metabolism that allows the synthesis of metabolic substrates such as glucose, fructose, and lipids [187,188, 191]. In cattle, energy requirements during pregnancy increase rapidly during late gestation

which is associated with the rapid increase in fetal growth [189]. The net energy requirement for maintenance increases 30 to 50% by the end of gestation and approximately 50% of the metabolizable energy is used by the gravid uterus, 25% to the fetus, and 25% to the maternal metabolic activities associated with pregnancy [195]. Thus, the nutritional state of the dam is essential to meet the growing demands of the fetus and maternal adaptations observed during pregnancy appears to be regulated by homeorhetic control from the fetus [187]. Each tissue will have a different priority in terms of nutrient partitioning in response to challenges to the growing fetus. For example, brain and heart will have a greater priority compared to skeletal muscle [11]. In humans, it has been shown that maternal adaptations during pregnancy are characterized by anabolic (i.e., increased deposition of lipids in maternal tissues) and a catabolic (i.e., enhanced lipolytic activity in the adipose tissue) phases which predominant during early to mid and late gestation, respectively [191]. Moreover, in sheep, an adequate level of maternal plasma glucose is sufficient to sustain 50-70% of fetal oxidative metabolism, whereas in the fasted state, the glucose supply to the fetus is reduced to 50% of the normal value which can interfere with fetal metabolism (i.e., require less glucose exogenously) [188].

In ruminants, restricted nutrient intake affects nutrient partitioning, which in turn is modulated by changes in endocrine signals in important tissues and can be associated with reproductive failure [29]. In sheep, maternal dietary restriction during mid to late gestation reduced the cellular proliferation rate of primordial follicles in fetal ovaries compared with ovaries of fetuses from adequately fed ewes [30]. However, during early

gestation, follicular development in offspring was affected by a delay in the transition from primordial to early primary follicles [31]. Similarly, in cattle, offspring born from dams that experienced restricted nutrition during the first third of gestation had a smaller antral follicle count (AFC) as determined by ultrasonography before and after puberty and lower plasma anti-Müllerian hormone (AMH) concentrations relative to control heifers. This indicates that undernutrition during the first trimester results in a smaller ovarian reserve [32, 33]. Feeding dams a restricted amount of protein in the first trimester followed by exposure to a high protein diet in the second trimester, resulted in a reduction in ovarian density and in the diameter of the largest follicle at 23 months of age [34]. In addition, beef heifers born to cows that experienced a total dietary nutrient restriction from early to mid-gestation had lighter ovaries and luteal tissue compared with heifers born from dams that received adequate nutrition [35]. Thus, negative effects caused by maternal undernutrition during different stages of pregnancy may affect the offspring's reproductive performance during their first breeding season and subsequent seasons.

It is well established that, depending on the timing and intensity of nutrient restriction during gestation, offspring can exhibit different physiological outcomes [11]. For example, when beef cows experienced a restriction in total amount of feed during mid to late gestation, heifer offspring BrW, weaning weight (WW), age at puberty and AFC did not differ from controls [36]. These results were not in accordance with studies in rodents and cattle that showed a decrease in BrW [37] and a delay in the onset of puberty [38, 39] of the offspring when the dams were exposed to restricted feed intake

during mid and late gestation. These adverse impacts may be explained by the fact that in the study conducted by Cushman et al. [36], total DM was restricted by only 75% of the estimated requirements. Furthermore, in sheep, moderate undernutrition during mid and late gestation had minimal long-term effects on growth [9]. With regard to the timing of nutrient restriction relative to BrW, it is known that fetal growth in cattle occurs predominantly during the last trimester of pregnancy [40]; consequently, when total dietary intake was restricted during the first or second trimester of gestation in cattle [33, 35] and sheep [31, 41], there was no effect on BrW and postnatal growth rate of the offspring.

Results of several studies demonstrated that offspring tend to "catch-up" in growth after birth in response to nutrient deprivation during gestation. In one study, heifers that had an accelerated growth rate during the rearing period required more services per conception, although they were younger at first calving than those with reduced growth rates [42]. Also, this catch-up in growth was associated with an increase in general adiposity, which led to insulin resistance [37, 43, 44] and leptin resistance [38]. Leptin, which is mainly produced by adipose tissue, interacts with neuronal pathways in the hypothalamus and regulates food intake as well as stimulates energy expenditure [45]. However, in lambs subjected to placental nutrient restriction by removal of endometrial caruncles, plasma leptin was correlated with increased feeding activity [46] that may represent a form of leptin resistance [44]. Since metabolic hormones such as insulin and leptin are also involved in ovarian function [47], animals

with deficient regulation of these hormones may exhibit negative repercussions related to ovarian follicular development.

Although maternal overnutrition is less frequently encountered than undernutrition in beef cattle production, the study of overnutrition in domestic animals has also been used as a model for humans due to the increase in incidence of obesity in pregnant women [22]. Fetuses from overnourished ewe lambs sacrificed on day 103 of gestation had a reduced number of primordial follicles [48]. Similarly, ovarian follicle population and genes involved in follicular development were altered in fetuses from dairy cows fed to achieve 190% of requirements for maintenance during gestation. These fetuses had a lower number of both primordial and total number of ovarian follicles compared with fetuses from moderate intake cows. These results were also associated with a greater expression of follicle-stimulating hormone (FSH)β in the pituitary gland and FSH receptors in the ovarian tissue, as well as upregulation of pro-apoptotic genes [49]. Thus, overnutrition during gestation may negatively interfere with normal reproductive development and function in the offspring [50]. Additionally, with respect to alterations in BrW in offspring exposed to an obesogenic diet *in utero*, in some studies this alteration does not seem to occur [51], while others reported the opposite. Lower BrW, "catch-up" in growth, and early onset of puberty was observed in offspring born from rats that were fed a high-fat (HF) diet with 45% of total calories from fat during gestation and/or lactation compared to offspring born from dams fed a standard control chow (CON). Once sexually mature, offspring from HF-fed dams

presented estrous cycle irregularities characterized by an atypical frequency of a given stage of their cycle or prolonged estrus during one or more cycles [50].

Obesity effects on reproductive function can also be related to an imbalance of endocrine factors that can negatively impact fertility [52]. Diet-induced maternal obesity in the ewe established before and maintained throughout pregnancy led to leptin resistance in the offspring with corresponding increases in appetite, thereby increasing the risk for obesity [53]. In ewes, overnutrition during a similar period altered not only hormonal profiles and adiposity in F1offspring, but also metabolic functions in the granddaughters (F2 generation). Pregnant F1 ewes born from obese dams (OBF1) had greater blood glucose and insulin concentrations compared to pregnant F1 ewes born from dams fed a moderate diet (CONF1). During early postnatal life, lambs from OBF1 (OBF2) had hyperinsulinemia, hyperglycemia, and a greater percentage of body fat compared with lambs born from CONF1 (CONF2). Interestingly, in CONF2 lambs, during postnatal days 5–9, there was a peak of leptin concentration which was not observed in OBF2 lambs [54]. The absence of a neonatal leptin surge in OBF2 lambs may interfere with the normal formation of hypothalamic pathways involved in the regulation of appetite later in life [55]. Additionally, experiments in mice have demonstrated that offspring exposed to a maternal HF diet *in utero* and early in life experienced weight gain, hyperphagia, an increase in adiposity [56], and have a greater risk for developing cardiovascular disease [57]. These outcomes indicate that both over and undernutrition during pregnancy can lead to similar metabolic disorders in the offspring.

THE POSTNATAL ENVIRONMENT

Developmental programming is not limited to the *in utero* environment (prenatal period), but extends into postnatal life, where different organ systems remain sensitive to environmental influence and continue to adapt [44]. This is followed by the loss of plasticity and permanent changes in functional capacity [24]. Indeed, in heifers, different planes of nutrition during early postnatal life can influence the onset of puberty [15], which is important since puberty is the developmental stage when the animal has acquired normal ovarian cyclicity that supports the ability to become pregnant [58]. The acceleration of pubertal maturation by a high plane of nutrition during early life is also observed in steers due to a positive effect on testicular growth, steroidogenesis and spermatogenesis [192,193].

In rodents, a 50% reduction of *ad libitum* intake during the period from late gestation until lactation (embryonic day 14 until day 21 postnatally), or only during lactation, delayed the onset of puberty in both males and females [59]. In contrast, feeding a high concentrate diet in early-weaned *Bos taurus* heifers from 4 to 6.5 months of age decreased age at puberty [60]. Similarly, in crossbred *Bos indicus*-influenced beef heifers, the use of a stair-step program that involves the alternation of dietary periods of restricted and *ad libitum* consumption differentially affected age at onset of puberty [53]. This and other studies have demonstrated that during the early (4 to 6.5 months of age) and later juvenile (6.5 to 9 months) periods, heifers remain sensitive to nutritional programming that can influence timing of pubertal onset [61]. Feeding a high concentrate diet during the early juvenile period altered metabolic hormones, such as

IGF-1 and leptin, and expression of key regulatory genes in the arcuate nucleus (ARC) [62].

NEUROENDOCRINE AXIS

The hypothalamus is extremely sensitive to changes in metabolic status [44]. It is composed of several functional nuclei, including the ARC that perceives metabolic status through signals transmitted via circulating energy metabolites (glucose, volatile fatty acids) and metabolic hormones (insulin, leptin, ghrelin). The ARC is a vital region in the hypothalamus that expresses a variety of genes regulated by energy intake [38, 62, 63]. The ARC also expresses a neuronal network involving inhibitory and excitatory signals towards GnRH neurons that regulate reproductive function [6, 64].

Prepubertal heifers exposed to a restricted diet *in utero*, followed by a relatively low energy diet during early development, showed alterations in hypothalamic neuronal organization [65]. It is possible that these alterations brought about through pre and early postnatal nutritional programming are associated with consistent physiological events that may be displayed only later in life [64]. Thus, developing new strategies that mitigate the effects of adverse environmental and nutritional impacts during early development may serve to help improve efficiency of ruminant livestock production [12].

CHARACTERISTICS OF BOVINE REPRODUCTIVE DEVELOPMENT

Gonadal development and folliculogenesis

The migration and proliferation of primordial germ cells (PGC) to the genital ridge initiates the process of differentiation of the mammalian bipotential gonad. In

females, the PGC differentiate into oogonia that develop further to become primary oocytes and ultimately primordial follicles [66, 67]. The ovaries of all mammals can be described as endocrine structures crucial for female reproductive function [68]. In female fetuses, during the second trimester of gestation, the ovary exists as a cortical band of germinal tissue and a prominent medulla. During this period, most of the surviving primary oocytes become surrounded by a single layer of flattened pre-granulosa cells that give rise to the formation of primordial follicles [69, 70]. Primordial follicles are in a resting state but continuously depart the resting pool to begin the early stages of growth that yields primary follicles [70, 71]. During this process, granulosa cells are transformed from a flattened to a cuboidal shape [71]. Subsequently, there is an increase in the number of granulosa cells with a consequent increase in the surface of the granulosa layer around the oocyte (secondary follicle). The formation of more than 6 layers of granulosa cells and a fluid-filled antrum results in an antral follicle [71, 72]. By late gestation, small developing antral follicles are first seen in fetal ovaries [62] and the fluid-filled antrum is composed of a serum-like fluid abundant in hormones and growth factors. Prepubertally, follicular growth occurs in recurring waves (2 or 3 per cycle), with the largest follicle developing during each wave undergoing atresia. Postpubertally, follicular waves either undergo atresia (occurring coincident with a functional CL) or ovulation in cyclic females following luteal regression [68]. The process of folliculogenesis is complex and most primordial follicles fail to ovulate [73]. Moreover, the total duration of development from primordial to ovulatory size takes about 4-6 months in cattle [68] and most of this period involves pre-antral stages [74].

PROCESSES INVOLVED IN PUBERTAL MATURATION AND ESTROUS CYCLICITY IN THE FEMALE

GnRH secretion

During the estrous cycle in mammals, gonadal steroids modulate the secretion of GnRH in two distinct ways. These two modes are independent neuronal pathways that operate in parallel and that involve the "GnRH pulse generator" and "GnRH surge" [75]. Secretion of GnRH from the hypothalamus occurs in pulses due to a synchronized and rhythmical depolarization of GnRH neurons. The frequency of GnRH pulses depends on the hormonal milieu and thus in intact females on the stage of estrous cycle [76]. Moreover, each pulse of luteinizing hormone (LH) from the pituitary is coincident with the tonic release of GnRH [75]. In the bovine female, the frequency of LH pulses is relatively high, with low amplitude during the follicular phase when circulating P4 is low, whereas during the luteal phase the frequency of LH pulses is low, and amplitude is markedly increased when P4 is greatly increased [77].

In mammals with spontaneous ovulation, such as cattle and sheep, the GnRH surge is controlled by a sustained rise in peripheral E2 during the follicular phase which exerts a positive feedback at both hypothalamic and pituitary levels [76]. In the hypothalamus, E2 stimulates the pulsatile secretion of GnRH possibly by changes in the amount of GnRH discharged in every pulse [76]. Whereas, in the pituitary, E2 stimulates the production of GnRH receptors and enhance responsiveness to GnRH that contribute to the greater amplitude of LH pulses observed during the preovulatory surge [76, 124]. In ovariectomized ewes, approximately 22–24 hours after the insertion of E2 implants

designed to induce a physiological rise of circulating E2 (approximately 8 pg/mL), a GnRH/LH surge is observed. The duration of the GnRH surge appears to extend beyond that of the LH surge [78], indicating that termination of the LH surge is not related to a lack of GnRH [79]. Instead, it occurs due to a temporary desensitization of LH response that decreases the signal between the receptor and the G protein-linked effector, and due to down-regulation of LH receptor expression to avoid a repeated stimulation in the ovary [124, 170]. Further studies in the ewe have demonstrated that GnRH plays a deterministic role in eliciting the surge of LH needed for ovulation to occur in contrast to a permissive role observed in both humans and Rhesus monkey. In the latter species, the actions of gonadal steroid hormones at the anterior pituitary level are enough to induce a pre-ovulatory surge of LH [79].

Physiological mechanisms involved in pubertal maturation

Attainment of puberty in heifers can be defined as the first estrus followed by ovulation with a normal length luteal phase [80]. In terms of pituitary secretory activity, prepubertal heifers as early as 3 months of age are already capable of responding to exogenous GnRH [81] and E2 [82] followed by a surge release of LH in a manner similar to that of sexually mature heifers. Thus, the positive E2 feedback mechanism functions properly long before puberty. Clearly, this is not the limiting factor that leads to the final sexual maturation in the heifer or in other species, such as the ewe [79]. Instead, one of the key factors for the failure of the onset of puberty is related to the hypersensitivity to the inhibitory feedback action of E2 [80] which results in the inability of the hypothalamic-pituitary axis to generate high-frequency pulses of GnRH

[83]. In addition, the main estrogen receptors mediating this negative feedback is estrogen receptor α (ESRα) [64]. As puberty approaches and negative feedback sensitivity to E2 declines, there is an increase in secretion of GnRH by GnRH neurons in the hypothalamus. This results in the release of GnRH/LH pulses approximately every 40 to 50 minutes followed by the growth and selection of a dominant follicle. As ovarian follicular growth is stimulated, there is an increase in E2 production. When E2 reaches a threshold capable of stimulating the surge center in the hypothalamus, a GnRH/LH surge leads to ovulation [84, 85]. Although GnRH secretion stimulates the release of both gonadotropins, LH and FSH, the latter does not seem to be a limiting factor for pubertal maturation. No changes in the pulsatile pattern of FSH was observed from birth up to 10 months of age [85, 86] which is not the case for LH. The onset of a pulsatile release of LH begins at 1 to 2 months of age, followed by increased secretion of LH until 3 to 5 months of age which coincides with a rapid increase in the number of antral follicles on the ovaries. Thereafter, the secretion of LH starts to decrease and reaches a static level until initiation of the peripubertal increase [84].

In prepubertal heifers, approximately 126 days prior to the onset of puberty, there is a gradual decrease in sensitivity to E2 negative feedback. This allows a gradual increase in the mean concentration and frequency of pulses of serum LH needed for final sexual maturation and first ovulation to occur [87]. The increase in frequency and decrease in amplitude of LH pulses is more definitive beginning about 50 days before puberty and this period is known as the peripubertal period [84, 85, 88]. From approximately 60 to 40 days preceding puberty (day -60 to -40) in intact heifers, there is

a 35% decrease in the overall concentration of E2 receptors in the hypothalamus and even further decline after day -50 in the anterior pituitary [88].

Mechanisms involved in the regulation of the GnRH pulse generator as puberty approaches remained a “black box” until the discovery of kisspeptin in the early 2000s [6]. Despite the effect of E2 on secretion of GnRH, GnRH neurons do not contain ESRα [6, 64]. Studies in rodents have shown that kisspeptin neurons within the hypothalamus are expressed in the ARC and anteroventral periventricular nucleus (AVPV) and these neurons express ESRα and P4 receptors. In species such as sheep, in addition to the presence of kisspeptin neurons in the ARC, there is a prominent population of these neurons in the preoptic area (POA) [89]. Importantly, kisspeptin colocalizes with GnRH neurons [90] and appears to function as a gatekeeper between GnRH and E2 in their roles in pubertal maturation. In sheep, almost all of the kisspeptin neurons found in the ARC express both the endogenous opioid dynorphin and the tachykinin, neurokinin B [91]. Thus, this population of neurons have been termed KNDy neurons [89]. In ewes, this single neuronal population is involved in the control of the secretion of GnRH (both in pulses and surge patterns) and it is negatively and positively regulated by gonadal E2. The basis of inhibitory and stimulatory effects of E2 on GnRH release in the same set of neurons may be due to a different response of KNDy neurons to low or high concentration of E2 linked with different intracellular signaling pathways [92]. Moreover, in ewes, it appears that neurokinin B signaling from another region in the hypothalamus (retrochiasmatic area) interact with kisspeptin released from KNDy neurons to enhance the amplitude of the GnRH/LH surge induced by E2 [179]. In

contrast, in rodents the E2 positive feedback does not appear to involve KNDy neurons [92] and the surge of GnRH is regulated by actions of E2 towards kisspeptin neurons in the AVPV [89]. Interestingly, studies in E2-replaced, ovariectomized ewes demonstrated a heightened expression of ESRα mRNA abundance in kisspeptin neurons located in the ARC which was associated with the peripubertal increase in pulses of LH [94]. This contrasts with studies in heifers reported earlier [88]. Thus, the precise mechanism that regulates timing of puberty in ruminants remains mysterious and changes in negative feedback responsiveness to E2 preceding puberty fail to be explained consistently by changes in expression of ESRα in the ARC [6, 64].

Nutritional influence on timing of pubertal onset

In cattle, pubertal maturation is influenced by several factors including body weight (BW), plane of nutrition, breed [80], and season [95]. In general, *Bos indicus* and *Bos indicus*-influenced heifers reach puberty at later ages (14 to 27 months) compared to *Bos taurus* heifers that reach puberty at between 12 and 15 months of age [4, 88, 96]. Ideally, replacement heifers should achieve puberty at 12–14 months of age and conceive at 14 to 16 months of age [95]. Calving for the first time as a 2-year-old resulted in cows that produced 0.7 more calves by 6.5 years of age [183] and more kg (~ 138 kg) of weaned calf in their lifetime [97, 184] compared to heifers calving first as 3-year-olds which impact positively lifetime productivity. Moreover, the pregnancy rate in heifers bred at the third postpubertal estrus was greater (78 vs 57%) than that observed in heifers bred at the pubertal estrus [98]. Thus, age at puberty has a large impact on reproductive and economic efficiencies [99] and management strategies

should be employed to optimize chances of replacement heifers reaching puberty prior to their first breeding season.

As mentioned previously, nutritional status plays a pivotal role in timing the onset of puberty and influences the timing of the increase in tonic release of GnRH/LH [95, 100]. A critical level of body fatness is needed to initiate the endocrine events associated with pubertal development [95] and in which leptin functions as a metabolic signal [101]. During the wintering period, heifers fed to gain at a rate of 0.68 kg per day reached puberty earlier, had their first estrus before or during the breeding season, conceived earlier and had a greater pregnancy rate compared to heifers fed to gain 0.23 kg per day [102]. A variety of studies have also shown that pubertal onset is hastened in heifers weaned earlier and fed a high-concentrate diet during the juvenile period when compared to a control diet [60, 61]. Increased rates of BW gain during the juvenile period resulted in heifers reaching puberty before 300 days of age (precocious puberty) [61]. As expected, this approach was associated with an earlier prepubertal increase in frequency of LH pulses [103], as well as advanced ovarian follicular development [104]. Prepubertal heifers exposed to a high-concentrate diet from 4 to 8.5 months of age had a decreased expression of neuropeptide Y (NPY), in which inputs inhibits GnRH secretion, and greater circulating leptin concentration as compared to heifers fed a low-concentrate diet [105]. Thus, nutritional programming appears to mediate neuroendocrine changes within the hypothalamus that will influence the timing of pubertal maturation.

Estrous cycle

The average length of the bovine estrous cycle is 21 days [106] and usually is composed of a two- or three-wave pattern of follicular development that is mainly determined by the time of luteolysis [106, 107]. During the estrous cycle, final maturation and ovulation of the dominant follicle occurs after the second or third wave [67]. The emergence of follicular waves is initiated by the process known as follicular recruitment, followed by selection, deviation, and growth of the dominant follicle [47, 108].

Antral follicles are recruited continuously during the estrous cycle [106] and occur in groups or cohorts in response to increased plasma concentrations of FSH [108]. The number of recruited follicles growing in the cohort in cattle ranges from 5 to 10 [109]. The appearance of aromatase activity within the granulosa layer is associated with recruitment of individual follicles. Aromatase activity becomes detectable when follicle size reaches 3 to 4 mm and this is a key maturational step enabling the follicle to produce estradiol (E2) from testosterone produced by thecal cells of the follicle [109].

Follicular dominance is characterized by selection and deviation (continued growth) of the dominant follicle which prevents further growth of subordinate follicles [73, 109]. The dominant follicle develops LH receptors on its granulosa cells [109], which is essential for deviation to occur at approximately 8 mm in cattle [110]. During each wave, a single follicle usually becomes dominant, whereas secondary follicles in the same wave undergo regression at the time of deviation. In some cycles, secondary and primary dominant follicles switch position and the secondary follicle becomes

dominant [111]. As the dominant follicle increases its capacity to secrete E2 and inhibin, a decrease in circulating concentrations of FSH is observed coincident with atresia of secondary follicles [74, 108, 110]. The increased capacity to secrete E2 is associated with an increase in the ability of thecal cells to respond to LH by secreting androgen and increased capacity of granulosa cells to aromatize testosterone to E2 [108]. The dominant follicle of each follicular wave is characterized by three phases: 1) linear growth for 6 days (growing phase), 2) static growth for 6 days (static phase) and either 3) regression for early and mid-cycle follicles (regressing phase) or ovulation when associated with luteal regression [74, 112]. Whether the dominant follicle present at the time of CL regression ovulates or regresses is dependent upon its stage of maturation. Therefore, the induction of luteolysis with prostaglandin F2α (PGF2α) at random stages of the estrous cycle can result in intervals to next ovulation ranging from 1 to 6 days or more depending upon the phase of the dominant follicle.

Prior to the LH surge, there is a rapid increase in E2 that reaches a plateau and approximately 12–18 hours later results in the LH surge [113]. Studies in rodents have shown that the preovulatory surge of LH is characterized by an increase in LH pulse frequency that, along with increased release of other hormones, such as the prostaglandins, and local production of proteolytic enzymes, result in follicular rupture and ovulation [114]. In cattle, ovulation occurs approximately 30 hours after the onset of estrus and the LH surge, which is the period that the animal is sexually receptive [115].

Estradiol

Estradiol (estradiol-17β; E2) is the primary biologically active form of estrogen

synthesized by ovarian follicles [116]. Preovulatory concentrations of plasma E2 are involved in a variety of physiological events that are essential for reproductive success [73], such as estrous behavior, the preovulatory gonadotropin surges [117], facilitation of sperm transport in the female reproductive tract [118], regulation of the uterine environment [119], and regulation of granulosa cells and P4 receptors after ovulation [120].

Estrous behavior is influenced by the amount of E2 produced by the dominant follicle. Perry et al. [121] reported that beef cows that exhibited estrus had a greater peak in E2 concentrations (9.9 ± 1.0 pg/mL) compared to cows that did not exhibit estrus (6.2 ± 0.67 pg/mL). This greater peak was correlated positively with follicle size [114]. In beef cows, dominant follicle diameter at the time of artificial insemination (AI) and body condition score (BCS, 4.81 ± 0.06) have been positively associated with estrus expression and intensity [117]. A meta-analysis showed that cows expressing estrus had a 27% overall increase in pregnancies per AI compared to animals that did not show estrus in fixed-time AI (FTAI) protocols [122]. This positive effect on fertility could be associated with direct effects of E2 on the uterine environment and on the cells in close contact with the oocyte, or an indirect effect of E2 on gamete transport [120]. However, follicle size in cows that have a spontaneous ovulation is not related to fertility because spontaneous ovulations are inherently associated with high fertility. In contrast, cows that are induced to ovulate follicles ≤ 11 mm have lesser concentrations of E2, lesser luteal phase concentrations of P4, and a greater rate of late embryonic mortality [123].

Progesterone

During the periovulatory period, there is a shift in the steroidogenic pattern of the ovulatory follicle from E2 to P4 as the CL starts to form [114]. The CL is a transient endocrine gland composed of two types of steroidogenic cells known as small and large luteal cells that are derived, respectively, from thecal and granulosa cells of the preovulatory follicle [125]. The formation of this gland during the luteal phase is influenced by the episodic release of LH prior to the preovulatory surge during the follicular phase. Indeed, inhibition of episodic release of LH in cows 48 h before the preovulatory surge resulted in a smaller CL diameter compared to control cows [126].

The relationship between CL size and production of P4 is not consistent between studies. Mann et al. [127] reported that there is a positive relationship between CL weight and plasma production of P4 only up to day 8 of the luteal phase in non-lactating dairy cows; whereas, once the CL has reached a mature size during mid-luteal phase, that relationship no longer exists. However, in beef heifers, there was no association between CL diameter and circulating P4 on days 5, 7 and 16 of the estrous cycle [128]. Heifers with a greater AFC had greater circulating P4 concentrations from day 3 up to day 14 of their estrous cycle compared to heifers with a lower AFC and had lower circulating P4; however, this difference was not associated with size of the CL. There was also an indication that lower circulating concentrations of P4 are associated with a decreased capacity of granulosa cells isolated from dominant follicles to luteinize and produce P4 in response to LH [129]. In contrast, in non-lactating Holstein cows, size of the preovulatory follicle, the corresponding CL, and plasma P4 concentration were

correlated, where a larger CL had a greater growth rate, greater blood flow and greater plasma concentration of P4 than a smaller CL [130]. Despite the lack of a clear correlation between CL size and circulating P4 concentration, it is well known that P4 plays a pivotal role in the establishment and maintenance of pregnancy [131] and regulates uterine receptivity to implantation of the blastocyst [132]. In sheep, P4 treatment during follicular growth increased the capacity of the oocyte to undergo cleavage and embryo development [133], and, in beef heifers, P4 supplementation on Day 3 of pregnancy increased embryo length [128]. In addition, a delayed rise in P4 in ruminants during the beginning of the luteal phase appears to interfere with the establishment of pregnancy [120]. Dairy cows without an embryo 16 days after insemination had a delayed increase in P4 concentration associated with delayed ovulation relative to cows with an embryo on day 16. In the same study, a delayed increase in P4 was associated with poor embryonic development and production of undetectable amounts of interferon tau [134] which is the pregnancy recognition signal in ruminants [132].

POTENTIAL BIOMARKERS TO PREDICT REPRODUCTIVE PERFORMANCE

Antral follicle count

In Hereford female fetuses, the peak number of germ cells is attained at day 110 of gestation. By around day 150, once oogonial mitosis is ceased, the number of germ cells in the fetal ovaries is fixed [69]. Despite the existence of hypotheses of ovarian self-renewal (neo-oogenesis) in mice, most reports support a model in which there is a

depletion of ovarian reserve throughout the female's reproductive life [66]. In addition, alterations during development that affect the recruitment of the pool of primordial follicles can lead to a depletion of the pool of ovarian antral follicles [66]. For example, the size of the ovarian reserve in cattle may be influenced by maternal nutrition during early gestation [33] and by factors that alter fetal steroid levels or signaling, such as fetal exposure to endocrine-disrupting chemicals during early pregnancy [67].

The ovarian follicular reserve, which is often referred to as the pool of primordial follicles in each pair of ovaries, is established during fetal life and has been used as a predictor of fertility in humans and domestic animals [67]. Danielle Monniaux et al. [135] proposed two different ovarian reserves: 1) the former reserve, which is the pre-established reserve of primordial follicles that occurs during fetal life, and 2) the dynamic reserve, which is the reserve of small gonadotropin-dependent antral follicles that can be estimated by the process known as AFC. Furthermore, the size of the ovarian follicular reserve can be estimated directly through ovarian ultrasonography and indirectly using endocrine markers such as FSH and AMH [135].

In both humans and animals, ultrasound can be used as a tool to access and count the number of antral follicles, which varies considerably (8 to 56) among individuals, but is highly repeatable (0.84) within individuals [136]. This variation may be due to differences in the depletion rates of the ovarian reserve [137]. Even in neonatal bovine ovaries collected at necropsy, the number of visible antral follicles ranges from 0 to 63 in the paired ovaries [138]. In addition, cows can be classified into three different groups according to their average number of antral follicles (≥ 3 mm) during a wave: low (≤15

follicles), intermediate (16 to 24 follicles) and high (≥ 25 follicles) [136]. The number of antral follicles begins to decline around 5 years of age in crossbred beef cows [138], which coincides with the decline in the number of primordial follicles [139]. Follicles counted through ultrasonography include both growing follicles under the influence of FSH as well as nongrowing and/or atretic follicles [140]. Crossbred heifers with relatively high AFC had a greater number of morphologically healthy oocytes and follicles than those with low AFC. This trait was positively associated with ovarian size and weight, as well as plasma AMH concentration [141]. Importantly, data comparing AMH concentration and AFC in infertile women exhibited a disagreement between the two measurements in 20% of these women. In this case, data suggested that AFC is more accurate than AMH in its ability to predict ovarian responses to exogenous stimulation and clinical pregnancy rate [142].

Antral follicle count can be used as an important tool for predicting reproductive performance in cattle involved in superovulation protocols [137]. Beef cows with high AFC are more responsive to superovulation and produce more high-quality embryos for transfer to recipients than cows with low AFC [136]. Beef heifers that gave birth in the first 32 days of the calving season had a greater number of AFC and produced calves with greater WW relative to heifers that calved later [143]. Moreover, a smaller percentage of beef heifers classified as low AFC were pregnant at the end of the breeding season compared to high AFC heifers [138]. Additionally, external and genetic factors can affect AFC [144]. As noted previously, maternal nutrition can influence follicular development [31] and number of healthy follicles found on the ovary [33],

which indicates that the intra-uterine hormonal environment during gestation may program ovarian function later in life [135]. Importantly, the second trimester of bovine gestation coincides with the period during which the pool of primordial follicles is established, and the size of the ovarian follicular reserve could be influenced by the environment of the fetus before and during that period [67].

Although several studies have estimated the value of AFC as a predictor of fertility, most of those conducted in cattle have involved *Bos taurus* females. A comparison of the results of experiments involving *Bos indicus* and *Bos taurus* beef cows have yielded conflicting results, which may be due to differences in AFC classification methods [145] or the physiological differences between these breed types [146]. In other studies, ovaries collected at abattoirs from *Bos taurus* (Aberdeen Angus) and *Bos indicus* (Nellore) cows were evaluated by ultrasonography to count the number of antral follicles (≥ 3 mm) and then evaluated histologically to evaluate the number of pre-antral follicles. The number of pre-antral follicles per ovary was similar between breeds, independent of the number of AFC and there was a positive correlation between AFC and the number of primordial ($r = 0.43$) and total pre-antral follicles ($r = 0.38$) also irrespective of breed [147]. In contrast, results of a recent study indicated the total number of antral follicles (≥ 1 mm) counted on the surface of ovaries was greater for Brahman compared to Angus cows and, despite the fact there were no differences between the breeds for total number of primordial follicles per ovary, the primordial follicle density (follicles/gram of tissue) in ovaries from Brahman cows was less than in

Angus cows. In the same study, Brangus (*Bos indicus*-influenced) cows had ovarian follicular morphology more like Brahman than Angus cows [146].

Endometrial thickness

The use of ultrasonography as a tool to evaluate the female reproductive tract can provide inferences about uterine status as well [148]. For example, imaging a transverse section of the uterine horn makes it possible to visualize the uterine lumen, which accumulates endometrial mucus during estrus, and the endometrium which is surrounded by the myometrium [149]. Early studies described the morphological changes that occur in the uterus during the bovine estrous cycle. During the late follicular phase and estrus (3–4 days prior to ovulation), endometrial scores, such as endometrium thickness [150] and echotexture [151] increase. During the early luteal phase, endometrial thickness and echotexture decrease and remain relatively constant throughout diestrus. In ewes, uterine weight increases at estrus, then decreases until the midluteal phase concomitant with increasing plasma concentrations of P4 [152]. Similarly, in lactating dairy cows, endometrial thickness reflects changes in plasma concentrations of ovarian steroids. Cows treated with an intravaginal P4 releasing device (CIDR) and PGF2α injection to induce estrus had a faster increase in endometrial thickness compared to those evaluated during a natural estrus. This may be explained by the more rapid changes in P4 and E2 concentrations observed in the induced estrus group [153]. In lactating dairy cows, endometrial thickness was negatively correlated ($r = -0.28$) with serum P4 concentration and positively correlated ($r = 0.33$) with E2 48 h after induction of luteolysis in a FTAI protocol. In the same study, cows with an

endometrial thickness greater than 8 mm were more likely to express estrus, had larger ovulatory follicles, and more pregnancies per AI 64 days after insemination compared to cows that had a thinner endometrium [148]. Furthermore, during the early to midluteal phase, heifers classified as having a high compared to low AFC presented a thicker endometrium from the day of ovulation up to 6 days later concurrent with a precipitous decrease thereafter. However, in low AFC heifers, the fluctuations in endometrial size were not observed, which may be correlated with the lower P4 concentrations detected in this group [129]. In humans, supplementation of P4 7 days prior to embryo transfer increased endometrial thickness on the day of embryo transfer [154]. The assessment of endometrial thickness in humans has been used in patients undergoing *in vitro* fertilization [155] and embryo transfer [156] protocols and a thicker endometrium has been associated with greater reproductive outcomes.

EXPECTED IMPACT OF THIS RESEARCH

Determining whether perinatal nutrition influences important traits related to reproduction in replacement heifers can help to improve nutritional strategies to optimize global economic and reproductive efficiency of beef production.

CHAPTER III

INFLUENCE OF THE PERINATAL NUTRITIONAL ENVIRONMENT ON AGE AT PUBERTY AND FUNCTIONAL CHARACTERISTICS OF THE BOVINE ESTROUS CYCLE IN HEIFERS

INTRODUCTION

The achievement of optimal performance in beef cattle commences from the onset of puberty in heifers and impacts subsequent events including age at first calving and lifetime productivity. Adequate nutritional management is essential during all stages of the production cycle [58, 157]. A variety of studies have focused on nutritional interventions during the postnatal period that can lead to hastened pubertal onset [61]. Ideally, heifers are expected to reach puberty at 12–14 months of age and conceive at 14–16 months of age during their first breeding season [157]. Managerial approaches have the potential to accelerate the timing of pubertal onset, however, should be adjusted to avoid precocious puberty because it leads to a greater risk of undesired pregnancies, dystocia, longer postpartum recovery periods and later calving in subsequent years [12, 157].

In forage-based production systems, cows often experience conditions that compromise feed quality and quantity that coincide with gestation [12]. Maternal dietary imbalances during pregnancy, particularly obesity and severe weight loss, in non-livestock species have been shown to result in metabolic diseases in the offspring, such

as insulin resistance, elevated blood pressure [158], and leptin resistance [44]. However, much less is known about the effects of prenatal nutrition on the physiology of offspring in livestock, including timing of puberty and adult reproductive performance [15]. The perinatal period (prenatal and early postnatal life), during which important events related to organogenesis and tissue differentiation occur, is a favorable period for programming adult phenotype [16]. In the studies reported herein, we tested the hypothesis that pre- and postnatal nutritional extremes in the bovine female, either individually or interactively, alter age at puberty and reproductive phenotype in heifers.

MATERIALS AND METHODS

The research outlined in the present study was submitted to and approved by the Institutional Agricultural Animal Care and Use Committee of Texas A&M University (AUP# 2016-010A and 2017-036A). The experiment for data collection was conducted at the Texas A&M Agricultural Research Station, Beeville, Texas.

Animal model and background

The experiments presented in this research were dependent on the availability of a group of heifers nutritionally programmed in a larger study that was part of two funded projects from 2013 and 2018. Brangus and Braford beef cows were inseminated using semen from a single Hereford (Brangus cows) or Angus (Braford cows) sire after synchronization using the 5-Day Bee Synch + CIDR protocol followed by FTAI [185, 186]. Cows were exposed to single Hereford or Angus clean-up bulls 7–10 days after AI for 80 days. Cows were evaluated on day 30–32 after FTAI by transrectal ultrasonography (Honda HS 2100; 5 MHz probe) to determine AI pregnancies and again

45–70 days following removal from breeding pastures to assess final pregnancy status and fetal gender. Cows confirmed pregnant with a female fetus (n=108) were assigned randomly to one of three dietary energy groups: low (L), moderate (M) and high (H) to achieve target BCS of 3-3.5 (very thin), 5.5-6 (moderate) or 7.5-8 (obese) by the end of the second trimester of gestation and maintained at experimental targets until calving, respectively. To attain the target BCS, cows from the L group were placed in individual pens and cows from the M and H groups were placed in dry lots measuring 25 x 9.6 m with 5 cows in each pen. The L group was fed a low energy diet that consisted of restricted amounts (initially ~ 50% of estimated total DM intake) of coastal Bermuda grass hay plus a protein, vitamin and mineral supplement. The M group received *ad libitum* access to mixed hay, a vitamin and protein supplement, and energy supplementation in the form of corn only if needed. The H group received a diet consisting of a 65-70% concentrate diet with *ad libitum* access to mixed grass hay. Cows were weighed every 2 weeks and diets were adjusted as needed to achieve BW gain or loss targets. Initially, the 3x2 factorial experiment was designed to produce 108 female offspring. However, the final number of heifers available was 97 due to pregnancy loss, dystocia, and errors in ultrasound identification of female fetuses. As noted later, the final number of heifers evaluated was 95 because of two statistical outliers.

At calving, cows were removed from feedings pen and placed in grass paddocks with *ad libitum* access to grass hay. In addition, cows in the L group were segregated and supplemented with a small amount of concentrate to allow recovery from the nutrient restriction diet imposed during pregnancy. All calves were weighed at birth.

Heifer calves born to dams assigned to the three nutritional regimens were weaned at 3–3.5 months of age. Following a dietary acclimation period of 2 weeks, heifers were stratified by age and BW and randomly assigned to one of two dietary treatments low-gain (L) or high-gain (H) designed to promote average daily gains (ADG) of 0.5 and 1.0 kg/day, respectively, from approximately 4 to 8 months of age. To accommodate changes in nutrient requirements of heifers during the experiment, two diets were formulated. After the acclimation period, Diet A was offered for the first 10 weeks of the experiment; afterwards, animals started to receive Diet B until the end of the experiment. The transition from Diet A to Diet B occurred by a gradual increase in the proportion of Diet B in the total feed. Ingredients and nutritional compositions of these two diets are presented in **Table 3.1**. Weight gains were controlled by adjusting pounds of feed fed to these heifers in the two dietary treatments every two weeks.

Table 3.1. Ingredients and chemical composition of the two diets (Diet A and Diet B) fed to prepubertal heifers starting from approximately 4 to 8 months of age. Heifers received diet A during the first 10 weeks of the experiments and diet B was provided to heifers from week 11 until the end of the study (week 18).

	Diet A (first 10 weeks)	**Diet B** (after 10 weeks)
Ingredients (%DM)		
Rolled Corn	38.75	52.35
Distillers Gold	18.00	5.25
Cottonseed Hulls	16.25	16.25
Cottonseed Meal	11.25	10.50
Alfalfa Pellets	10.00	10.00
Calcium Carbonate	0.90	0.90
12:12 Mineral	0.50	0.50
Molasses	4.35	3.50
Chemical composition		
Crude protein (%)	15.7	14.0
Degradable intake protein (%)	11.1	10.2
Metabolizable energy (Mcal/kg DM)	2.00	2.00

After the completion of the experimental dietary period (from approximately 4 until 8 months of age), all heifers were then placed on common growth, forage-based diet plus a supplement to permit a rate of gain of approximately 0.6 kg/day until puberty. Heifers represented a 3x2 factorial arrangement of maternal and postnatal treatments as depicted in **Figure 3.1**.

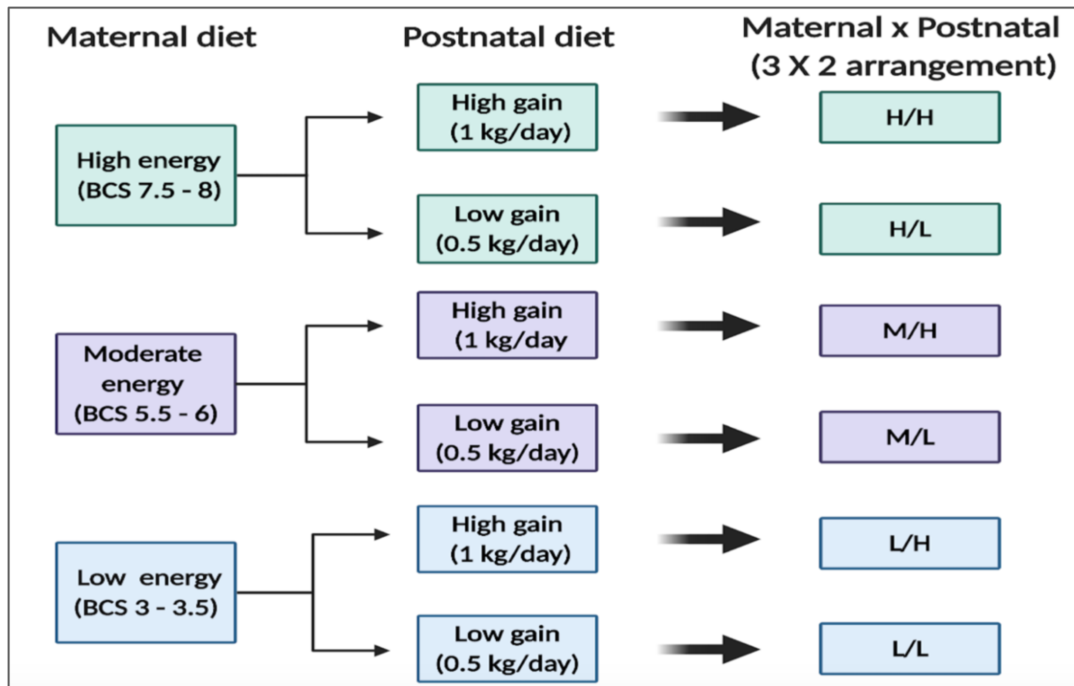

Fig. 3.1. Maternal and postnatal nutritional treatments. The 3x2 factorial design represents female offspring born to cows fed to calve in obese, moderate and thin body condition, and fed during the juvenile period to gain BW at high or low rate at 8 months of age.
**Created with BioRender.com*

Experiment 1: Age at puberty

Following completion of the experimental dietary treatment period in heifers, blood samples (10 ml) were collected twice weekly (n = 97) to determine pubertal status. Body weight was recorded every 14 days until confirmation of puberty. Both blood samples and BW were obtained before the morning feeding until puberty was confirmed or 19.5 months of age, whichever occurred first. Two heifers failed to reach puberty by 19.5 months and were considered outliers. Therefore, a total of 95 heifers were used to evaluate age at puberty (H/H, n = 15; H/L, n = 14; M/H, n = 16; M/L, n =

17; L/H, n = 16; L/L, n = 17).

Determination of pubertal onset. Onset of puberty was defined as at least 3 consecutive samples with concentrations of P4 ≥ 1 ng/mL or 2 consecutive samples of P4 ≥ 1 ng/mL and an ultrasonographically visible CL [87]. After each collection, blood samples were placed on ice immediately. Plasma was obtained by centrifugation (2,200 X g for 20 min at 4°C) of blood samples and stored at -20°C until analysis of circulation concentrations of P4. Upon pubertal confirmation, heifers were placed on pasture and supplemented with hay and a concentrate (common growth period) as described earlier.

Experiment 2: Evaluation of selected aspects of reproductive characteristics in heifers

A total of 53 pubertal heifers (averaging approximately 21 months of age) were used in this experiment, representing all the treatment combinations (H/H, n = 8; H/L, n = 9; M/H, n = 9; M/L, n = 9; L/H, n = 9; L/L, n = 9). Prior to the initiation of the experiment, heifers were brought in from pastures, maintained in pens, and fed Coastal Bermuda grass hay and an energy/protein (20%) supplement as needed to maintain normal BCS (5–6). The overall timeline of the animal model is represented in **Figure 3.2**. Because of timing of puberty in various groups of heifers, 60% of the data collection of this study was conducted from April until August of 2019, and the remaining 40% was conducted from May until August of 2020.

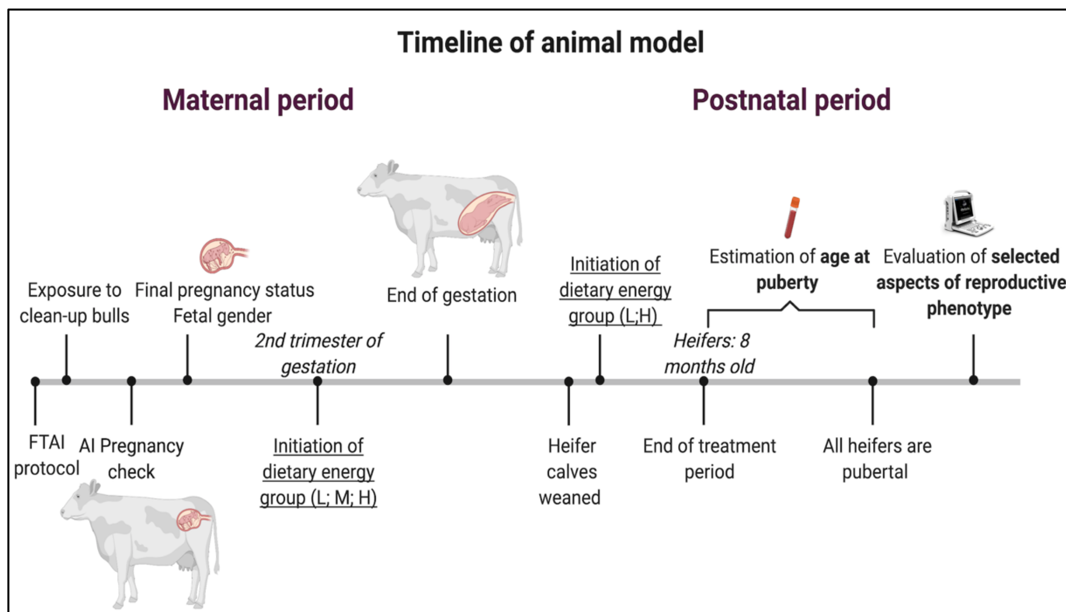

Fig. 3.2. Overall timeline of the animal model beginning from onset of maternal dietary treatments through the postnatal dietary treatments and subsequent experimental protocols. Cows were synchronized followed by FTAI and placed 10 days later with a clean-up bull. Final pregnancy status and fetal gender determination occurred between 45 and 70 days after removal from breeding pastures. During the second trimester of gestation (day 90 of gestation), cows were assigned randomly to three dietary treatment groups to achieve a BCS of either 3.5-4 (thin), 5.5-6 (moderate), or 7.5-8 (obese) by the end of the second trimester of gestation (day 180 of gestation). After calving, heifer calves were weaned at approximately 3.5 months of age and assigned randomly to receive one of two dietary treatments (low-gain and high-gain) from approximately 4 to 8 months of age. Following confirmation of puberty (n = 95), experiments evaluating characteristics of selected reproductive variables was performed in a subset (n = 53) of heifers representing all dietary treatments.
**Created with BioRender.com*

Experimental design. Heifers (n = 53) were administered two injections of PGF2α; (Lutalyse, Zoetis Animal Health) 11 days apart to induce luteolysis and synchronize occurrence of estrus. Following the second injection, ovaries of all heifers were examined daily using a Honda HS 2100 high resolution ultrasound scanner with a 7.5 MHz probe until ovulation was detected. Transrectal ultrasonography was performed

during the follicular phase of two consecutive estrous cycles (PGF Cycle and Cycle 1) to record size of largest (dominant) and second largest (subordinate) follicle, follicle growth rate, number of antral follicles (AFC), CL size, ovarian size (determined by length and height for both ovaries), and endometrium thickness. If the CL had a cavity (lacuna), the average size of the cavity was subtracted from the total diameter. In addition, ovarian size was recorded for all heifers on the day of ovulation. Day of ovulation (Day 0) was defined as the disappearance of the largest follicle from an ovary between two consecutive days of ultrasonographic evaluation. Additionally, following the second injection of PGF2α, heifers were observed visually with the aid of estrus detection patches (Estrotec™) for 30 minutes twice daily for signs of estrus.

Concurrent with ultrasonography and through two consecutive estrous cycles (PGF Cycle, Cycles 1 and 2), blood samples were collected daily by jugular or caudal vessel puncture and dispensed into 10-ml Vacutainer tubes (Monoject™, Covidien, Mansfield, MA) containing EDTA. Plasma was separated by centrifugation (2,200 X g) for 20 min at 4°C and frozen at -20°C for subsequent evaluation of plasma concentrations of E2 and P4.

Estrous cycle length was determined as the interval between two ovulations for each heifer. Three consecutive estrous cycles (Cycles 1, 2 and 3) were monitored to determine average estrous cycle lengths (**Figure 3.3**).

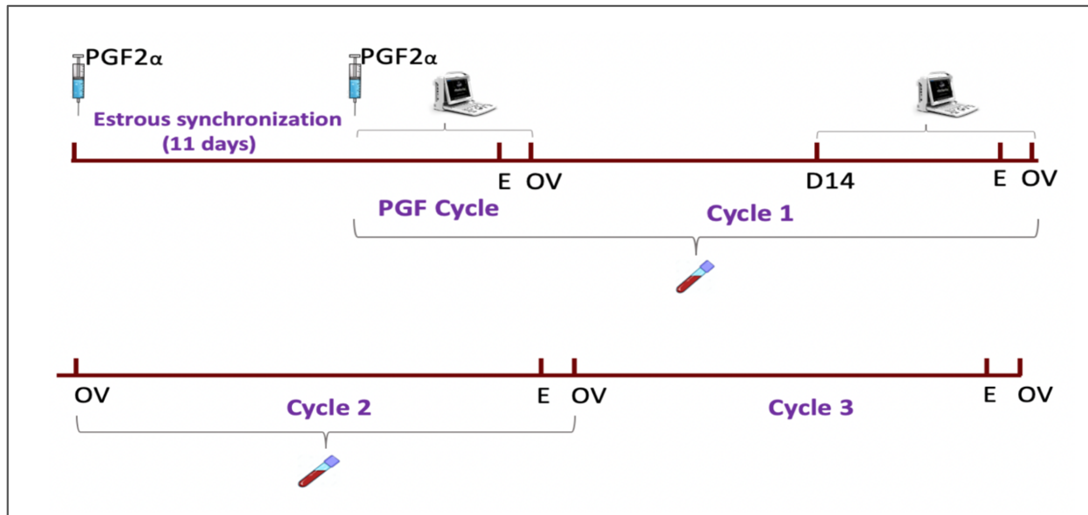

Fig. 3.3. Experimental design for evaluation of selected aspects of reproductive phenotype in nutritionally programmed heifers. Two injections of PGF2α were given to all heifers 11 days apart. On the day of the second injection, blood samples and ultrasound measurements were conducted. PGF Cycle represents the period between the day of the second injection of PGF2α until ovulation. Ultrasound measurements were taken in the PGF Cycle and Cycle 1. Blood samples were collected daily throughout PGF Cycle, Cycle 1 and Cycle 2. Estrous cycle length was evaluated from Cycle 1, 2 and 3. Estrus expression was evaluated based on PGF Cycle, Cycle 1, 2 and 3. E, onset of estrus; OV, day of ovulation; D14, 14 days after ovulation.

Ultrasound scanning of antral follicles. Each ovary was scanned using the transrectal probe from end to end, and video images from different ovarian sections were captured on a computer monitor. The average AFC was determined using measurements obtained during the follicular phases of two cycles and used as an assessment of ovarian follicular reserve. The videos were recorded on a random day during the follicular phase of the estrous cycle for each animal.

Ultrasound scanning for endometrial thickness. Endometrial thickness was assessed through ultrasonography, ideally on the day of estrus for each animal or on the day of ovulation. Endometrial thickness was estimated as reported [153] by averaging

the major and minor axes of the endometrial area. If an intraluminal cavity was detected, the average of the major and minor axes of the cavity area was subtracted from endometrial thickness. Each of the uterine horns in a separate frozen image was analyzed, and then averaged to determine the final endometrium thickness for each heifer.

Hormone assays

Radioimmunoassay (RIA) was performed on plasma samples to determine concentrations of P4 and E2. Circulating concentrations of P4 for Expts. 1 (twice weekly) and 2 (during the luteal phase of two estrous cycles) were determined using a commercial RIA kit (ImmuChem™ Coated Tube, MP Biomedicals, Santa Ana, CA) with modifications for assay of bovine plasma. For Exp. 2, P4 was assayed in samples collected from the day of ovulation (D0) through day 14 of each of two cycles. Day 14 was chosen because a functional CL would be expected to exist 14 days after ovulation [74] regardless of estrous cycle length. Sensitivity of the assay was 0.2 ng/ml, and intra-assay and inter-assay coefficients of variation (CV) averaged 11.5% and 5.6%, respectively. Recovery of added mass for high (10 ng/mL), mid (5 ng/mL) and low (2 ng/mL) references was 101.9%, 95% and 92.3%, respectively. Because of the assay precision, samples were assayed as singles. Circulating concentrations of E2 representing the follicular phase of two estrous cycles were measured using a modification of a RIA protocol as reported earlier [93] using E2 antiserum and [^{125}I] estradiol-17β labeled Tracer from MP Biomedicals (Santa Ana, CA). Briefly, duplicate samples (300 μl) were extracted in 4 ml of methyl-tert-butyl ether for 2 min on a

multitube vortexer. Samples were then allowed to sit for 5 min at room temperature. Extracts were frozen using dry ice in methanol. The solvent portion was decanted into 12 x 75 mm borosilicate glass tubes and dried at 37°C under air. The extracts and E2 standards (0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10, and 20 pg/tube) were hydrated using 100 µl of 1% BSA buffer followed by the addition of 100 µl of E2-17β antiserum. Tubes were incubated for 1 hour at 4°C, followed by addition of 100 µl of [^{125}I] estradiol-17β labeled tracer to each tube followed by incubation at 4°C for 20 hours. A dextran-coated charcoal suspension (500 µl) was added to the tubes, followed by centrifugation at 3000 X g for 10 min at 4°C. Supernatants were decanted into new 12 x 75 mm tubes and counted in a gamma counter for 2 min. Plasma samples used in this assay included those collected from 5 days before the day of ovulation (Day -1 up through Day -5) during two estrous cycles (PGF Cycle and Cycle 1). In some cases, specifically during the PGF Cycle, there were less than 5 samples per heifer. The sensitivity of the assay averaged 0.65 pg/mL. Intra-assay and inter-assay CV for E2 were 10.5% and 19.6%, respectively. Recovery of added mass for high (10 ng/mL), mid (5 ng/mL) and low (2 ng/mL) references was 103%, 112% and 106%.

Statistical analysis

Statistical analyses of data were conducted using JMP Software® and SAS Software® as for a completely randomized design with all possible interactions. Body weights, BCS, hormone concentrations and follicle size were analyzed as for a 3x2 factorial by general mixed models for repeated measures in JMP Software®. To account

for repeated measure autocorrelation in variables measured over time (BW, BCS, P4, E2, daily follicle size and dominant follicle growth rate), time was used as the repeated variable, and heifer within dietary group as the subject in the model. Test for normal distribution for each variable was performed using the Goodness of Fit test (Shapiro-Wilk). When data were non normally distributed, a log transformation was employed before further analyses. Birth and WW, age at puberty, estrous cycle lengths, maximum size of ovulatory follicles, AFC, endometrium thickness, ovarian size and CL size were analyzed in JMP Software® and SAS Software® by analysis of variance (one-way ANOVA). Sources of variation included maternal diet, postnatal diet, time and their interactions. Data were reported as least squares means contrasted using the Tukey modification as the post-hoc analysis when a significant F was detected. For all analyses, significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Student's *t*-test was used to compare means between the H and L postnatal diets.

RESULTS

Maternal BW and BCS, BrW and growth performance of heifer offspring, and age at puberty

Body weight and BCS of dams during the second and third trimesters of pregnancy. The BW and BCS of cows from H, M and L diets were recorded every two weeks from 90 days of gestation (beginning of the second trimester of gestation) until parturition (day 270-285 of gestation). Because no replicate effect was observed for BW and BCS, data were pooled by treatment. Dams that aborted or had a calf that died

before the completion of the postnatal dietary period were not included in the statistical analysis. Thus, the analyses reported herein included a total of 97 cows (H, n = 31; M, n = 33; L, n = 33). A dietary group x week interaction ($P < 0.0001$) was detected for both BW and BCS. Mean (±SEM) BCS (5.1 ± 0.02) and BW (562 ± 8.59 kg) at the beginning of the experiment did not differ among dietary groups ($P \geq 0.86$). However, both BW and BCS began to differ ($P = 0.001$) between H (5.5 ± 0.13) and L (4.8. ± 0.12) groups beginning at week 4 and differed ($P < 0.0001$) among all dietary groups starting at week 6 (BCS) and 12 (BW), respectively (H > M > L ($P < 0.0001$; **Figure 3.5**). By the end of the second trimester of gestation (~ 14 weeks after the start of the experimental dietary period), mean BCS were 6.8 ± 0.14, 5.2 ± 0.14 and 3.8 ± 0.14 for the H, M and L group, respectively ($P < 0.0001$; **Figure 3.4)**. At parturition, mean BCS were 7.6, 5.1, and 3.3 and mean BW were 722.7 kg, 625.6 kg and 514.8 kg for H > M > L, respectively ($P < 0.0001$).

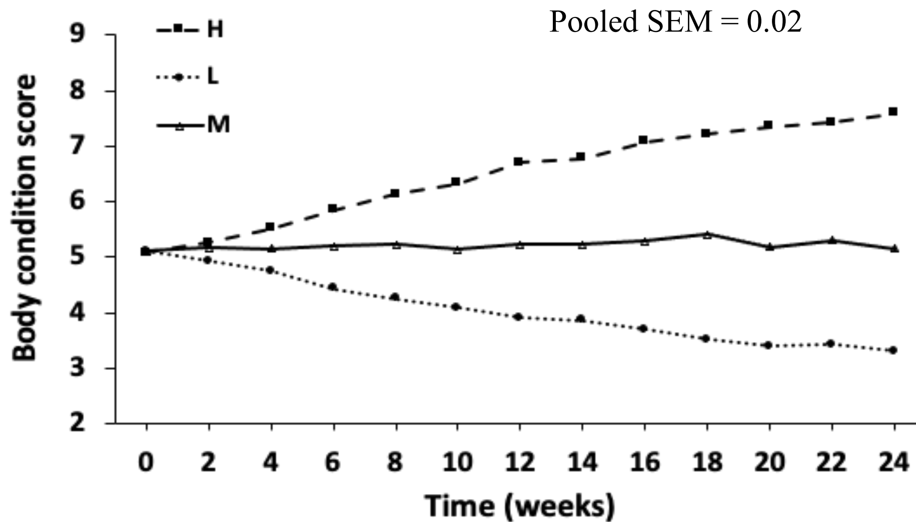

Fig. 3.4. Least squares mean (±SEM) BCS of dams from the 2nd trimester of gestation (week 0) until parturition (270-285 days represented by week 24). Cow BCS did not differ ($P > 0.1$) among high (H; n = 31), moderate (M; n = 33) or low (L; n = 33) maternal dietary groups at onset of the experiment. Differences in BCS ($P < 0.0001$) were detected between H and L groups beginning at week 4 and among all dietary groups beginning at week 6 ($P \leq 0.0001$).

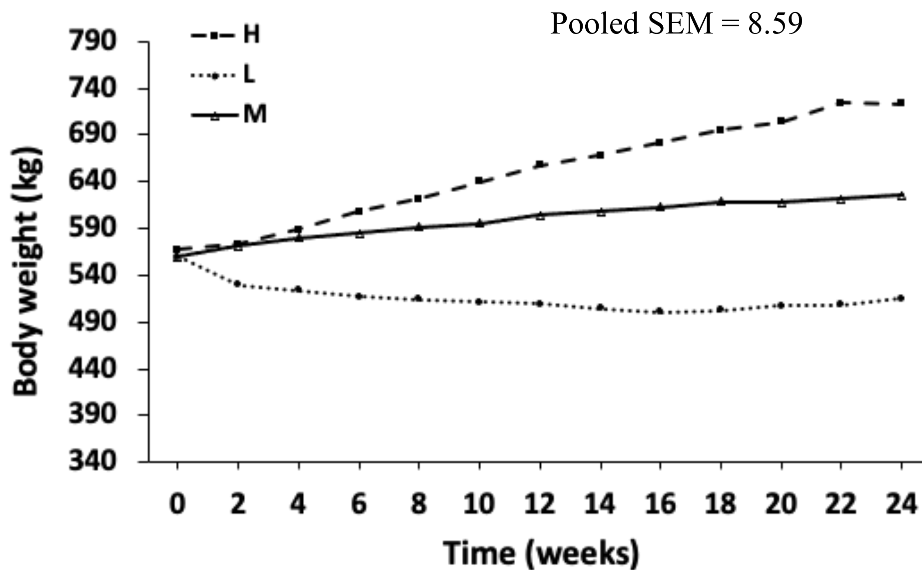

Fig. 3.5. Least squares mean (±SEM) BW of dams in high (H; n = 31), moderate (M; n = 33) and low (L; n = 33) maternal dietary groups from the 2nd trimester of gestation (week 0) until parturition (270-285 days represented by week 24). Cow BW did not differ ($P > 0.1$) at onset of the experiment but differed between H and L beginning at week 4 ($P = 0.001$) and among all dietary groups beginning at week 12 ($P < 0.0001$).

Birth weight and WW of heifers. Birth weight and WW of heifers (n = 97) were not normally distributed and thus were log transformed before further analyses. However, after log transformation, BrW distribution remained non-normal. Goodness of fit analysis of log transformed data revealed the presence of 4 outliers (1 H, 1 L and 2 M). When excluded, BrW and WW from the remaining population (n = 93) were normally distributed. Heifers born to H dams were approximately 3.5 kg **(Table 3.2)** heavier at birth than heifers born to the L dams ($P = 0.02$). However, WW at 3.5 months of age did not differ ($P = 0.14$) among dietary groups. Similarly, ADG from birth to weaning did not differ among groups ($P = 0.52$), with all averaging about 1 kg/day (**Table 3.2**).

Table 3.2. Least squares mean (± SEM) birth weights (BrW), weaning weights (WW) and average daily gains (ADG) in heifers (n = 93) born to cows fed to calve in thin (L group), moderate (M group) and obese (H group) body condition.

Dietary group	**BrW (kg)**	**WW (kg)**	**ADG (kg/day)**
Low	31.69 ± 0.86^{a}	129.69 ± 3.11^{a}	0.95 ± 0.03^{a}
Moderate	34.06 ± 0.86^{ab}	136.88 ± 3.11^{a}	0.99 ± 0.03^{a}
High	35.10 ± 0.89^{b}	137.77 ± 3.21^{a}	0.99 ± 0.03^{a}

Superscripts with different values in the same column differ (P < 0.05)

Body weight during the experimental dietary feeding period of heifers. A dietary group x week interaction ($P < 0.0001$) for BW was observed during the experimental feeding period of heifers, with no replicate or dietary group x replicate effect. Mean (±SEM) BW at the beginning of the experiment did not differ ($P = 0.97$) between groups

(138.7 ± 2.1 kg). Body weight increased linearly throughout the experiment in both dietary groups as observed in **Figure 3.6**. Heifers fed to gain 1 kg/day had greater (*P* = 0.01) BW (152 ± 3.0 Kg) than heifers fed to gain 0.5 kg/day (135.4 ± 3.0 kg) starting from the second week of feeding and continuing until the end of the experimental feeding period (*P* < 0.0001). Mean (±SEM) ADG for H and L groups was 0.92 ± 0.02 kg/day and 0.47 ± 0.02 kg/day, respectively. At 8 months of age (end of experimental feeding period), heifers fed the H postnatal diet were heavier (257 ± 5.2 kg; *P* < 0.0001) than those from the L postnatal diet (188 ± 5.2 kg).

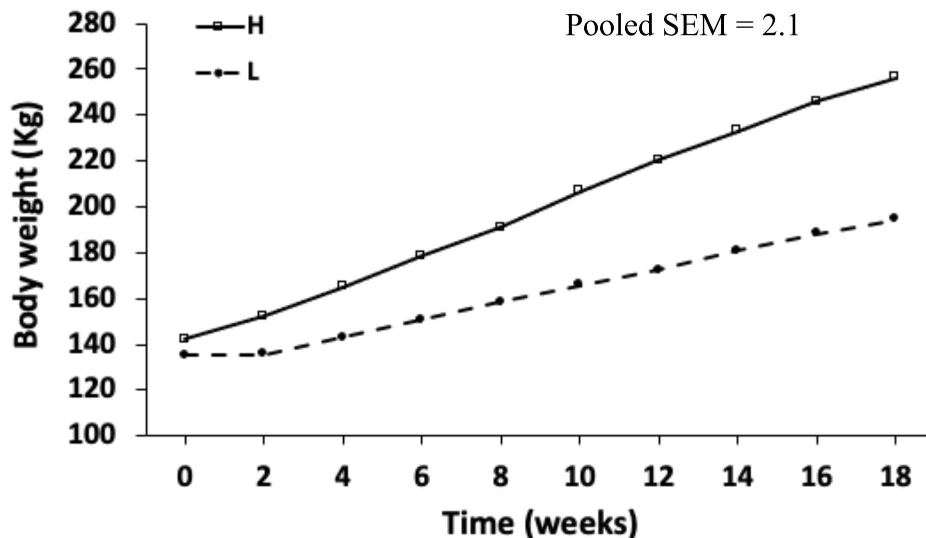

Fig. 3.6. Least squares mean (±SEM) BW of heifers fed high (H; n = 48) and low (L; n = 49) postnatal diets after weaning at approximately 3.5 months of age (week 0) and fed until 8 months of age (week 18). Body weight of H heifers was greater (*P* = 0.01) than L heifers beginning at week 2 and continuing through week 18 (*P* < 0.0001).

Body weight during common growth period, and age and BW at puberty. At the start of the common growth period, heifers in the L postnatal groups (188.0 ± 5.2 kg)

were lighter ($P = 0.001$) than heifers in the H postnatal groups (257 ± 5.2 kg). However, the L heifers exhibited compensatory gain and by week 34 (~ 15 months of age), BW did not differ ($P = 0.15$) between these groups. The ADG up to this period differed ($P = <0.0001$) between groups and was 0.58 ± 0.04 kg/day and 0.79 ± 0.04 kg/day for the H and L heifers, respectively. At this time, approximately 84 % of postnatal H and 59% of postnatal L had already reached puberty.

Age at pubertal onset is summarized in **Figure 3.7**. Age at puberty was not affected by maternal diet ($P = 0.66$) or maternal x postnatal diet interaction ($P = 0.76$). However, heifers from the H postnatal diet reached puberty 2 months earlier (12 ± 0.4 months) than heifers from the L postnatal diet (14 ± 0.4 months; **Fig. 3.7A**). Mean BW at puberty was 341.5 ± 5.13 kg and was not influenced by dietary treatments ($P = 0.83$).

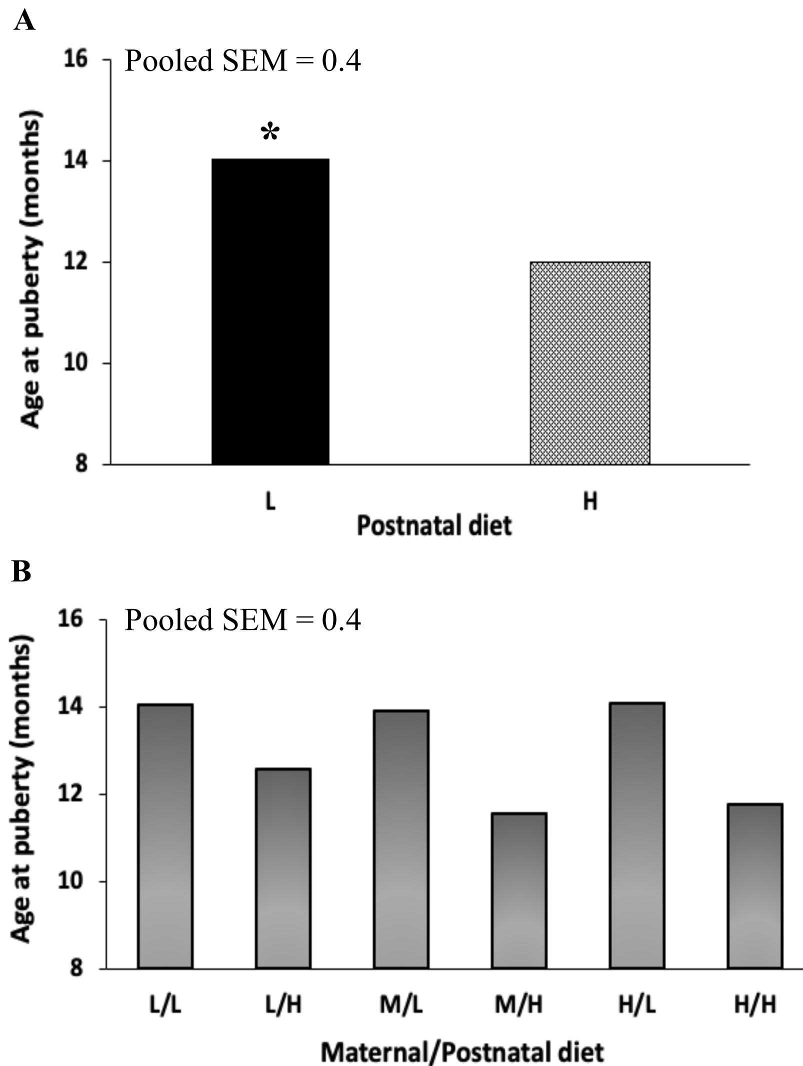

Fig. 3.7. (A) Least squares mean (±SEM) ages (months) at pubertal onset in heifers fed high gain (H; n = 47) and low-gain (L; n = 48) diets postnatally and (B) in heifers representing the 3x2 maternal x postnatal treatment combinations. Heifers fed the high gain (H) diet postnatally reached puberty 2 months earlier (*P = 0.0002) than heifers fed the low gain diet (L). There was no maternal or maternal x postnatal dietary effect (P = 0.76) on age at puberty.

Experiment 2: Ovarian, uterine and behavioral characteristics of heifers

Body weight and BCS of dams and BW of heifers. Body weight and BCS of dams and BW of heifers representing the subgroup used in this experiment were virtually

identical to those observed for the entire group as summarized above. Therefore, BW and BCS data specific to this subgroup are not shown.

Ovarian and uterine morphology. Neither maternal diets, postnatal diets nor their interaction affected size of the pre-ovulatory follicle (*P* = 0.93), growth rate of the dominant follicle (*P* = 0.76), CL size (*P* = 0.6) or endometrial thickness (*P* = 0.56; **Table 3.3**). A prominent proportion (35.8%) of heifers had a CL with a lacuna (fluid-filled cavity) but was unrelated to maternal or postnatal dietary treatments or their interaction.

Table 3.3. Ultrasound measurements from heifers during two estrous cycles*

Variable	**Mean**	**SEM**	***P*-value**
Pre-ovulatory follicle size	12.4 mm	0.15	0.93
Growth rate (dominant follicle)	0.9 mm/day	0.05	0.76
CL size	16.8 mm	0.42	0.6
Endometrial thickness	14.6 mm	0.22	0.56

* Ovaries and uterus were assessed in sexually mature female offspring (n = 53) that were nutritionally programmed in utero and postnatally during the juvenile period. There were no significant main effects or interaction for any of the variables listed.

Maternal dietary treatment during the second and third trimester of gestation influenced ovarian size of the offspring (*P* = 0.03). Ovaries from heifers born to cows fed the M maternal diet were larger (24.2 ± 0.55 mm) compared to heifers born to cows fed the H maternal diet (22.1 ± 0.57 mm). There were no differences in this characteristic due to postnatal diets (*P* = 0.93) or the maternal x postnatal interaction (*P* = 0.12). Moreover, neither maternal diets (*P* = 0.65) nor their interaction with postnatal

diets (*P* = 0.96) affected AFC. However, there was a trend (*P* = 0.09) for the number of AFC in heifers fed the H diet postnatally to be greater (25 ± 1.3) than those fed the L diet (21 ± 1.4). As expected, regardless of dietary group, AFC was associated positively (*P* < 0.0001) with ovarian size (r = 0.57).

Estrus expression and estrous cycle length. Estrous cycle length was recorded for three cycles (Cycles 1, 2 and 3). The mean estrous cycle length of heifers in this experiment was 20.7 ± 0.1 days and this was not affected (*P* = 0.58) by maternal or postnatal diets. Similarly, analyses of four estrous cycles (PGF Cycle, Cycles 1, 2 and 3) failed to show any dietary effects on expression of estrus. The overall incidence of estrus (full standing heat) across all estrous cycles was 87%.

Concentrations of luteal P4 and follicular phase E2. Neither mean (±SEM) concentrations of plasma P4 (3.2 ± 0.1 ng/mL) from day 0 (day of ovulation) through day 14 of the cycle (Cycles 1 and 2) nor mean peak P4 (6.2 ± 0.16 ng/mL) differed (*P* = 0.92 and *P* = 0.63, respectively) among dietary treatments (**Fig. 3.8A and B**).

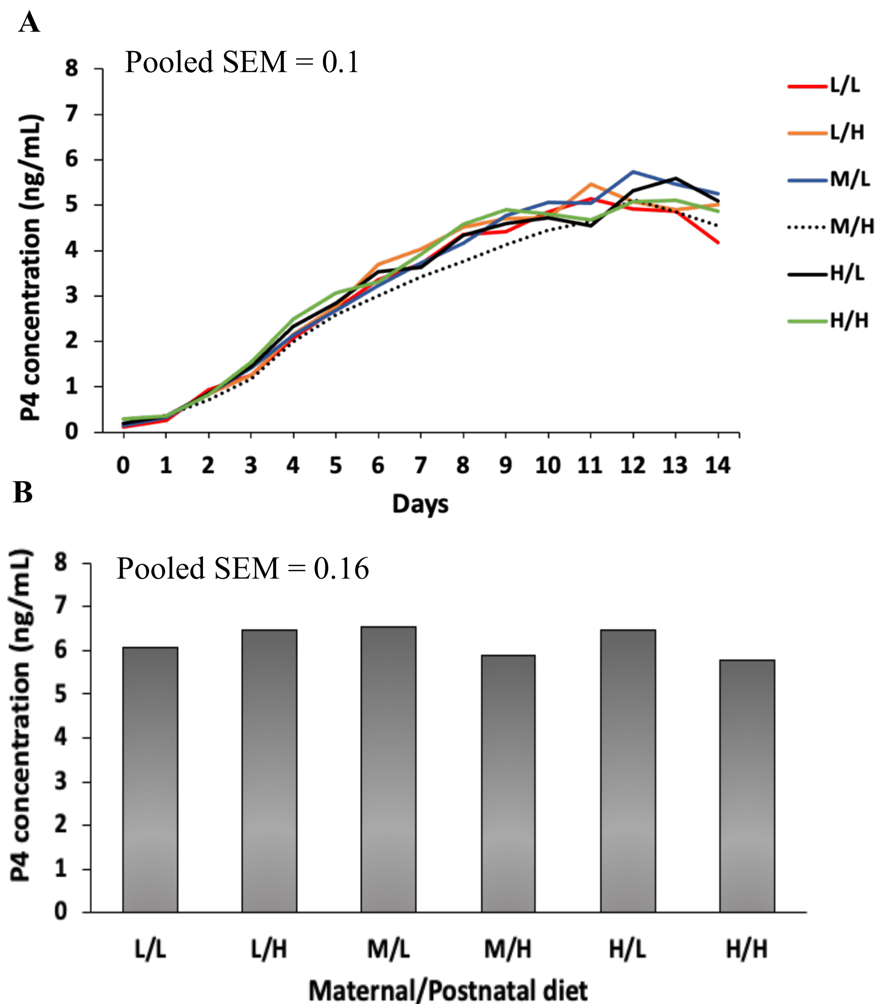

Fig. 3.8. (A) Least squares mean (±SEM) plasma concentrations of P4 during the luteal phase and (B) mean peak concentrations of P4 over two complete estrous cycles in heifers representing the 6 dietary group combinations (n = 8-9/group). Neither the overall mean (P = 0.92) nor peak (P = 0.63) concentration differed due to maternal x postnatal diet combinations.

Mean (±SEM) plasma concentrations of E2 (4.9 ± 0.15 pg/mL) during the follicular phase (Day -5 to Day -1) of two estrous cycles (PGF Cycle and Cycle 1) did not differ among dietary groups (P = 0.80; **Figure 3.9)**. As expected, day of cycle

had a significant ($P < 0.0001$) effect on mean concentrations, with greatest values on day -2. There was a trend ($P < 0.08$) for heifers in the postnatal H groups to have greater (8.3 ± 0.4 pg/mL) mean peak concentrations of E2 than postnatal L heifers (7.2 ± 0.4 pg/mL; **Fig. 3.10A**) but maternal x postnatal diet combinations had no effect (**Fig. 3.10B**).

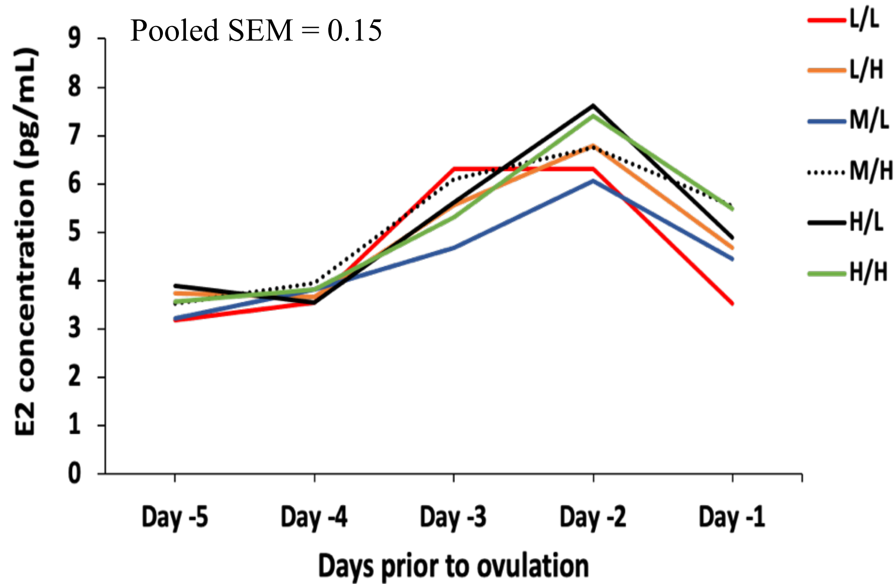

Fig. 3.9. Least squares mean (±SEM) plasma concentrations of E2 between days -5 and -1 before ovulation in heifers representing the 6 dietary group combinations (n = 8-9/group). There was no effect of maternal x postnatal diet combinations on E2 concentrations ($P = 0.80$).

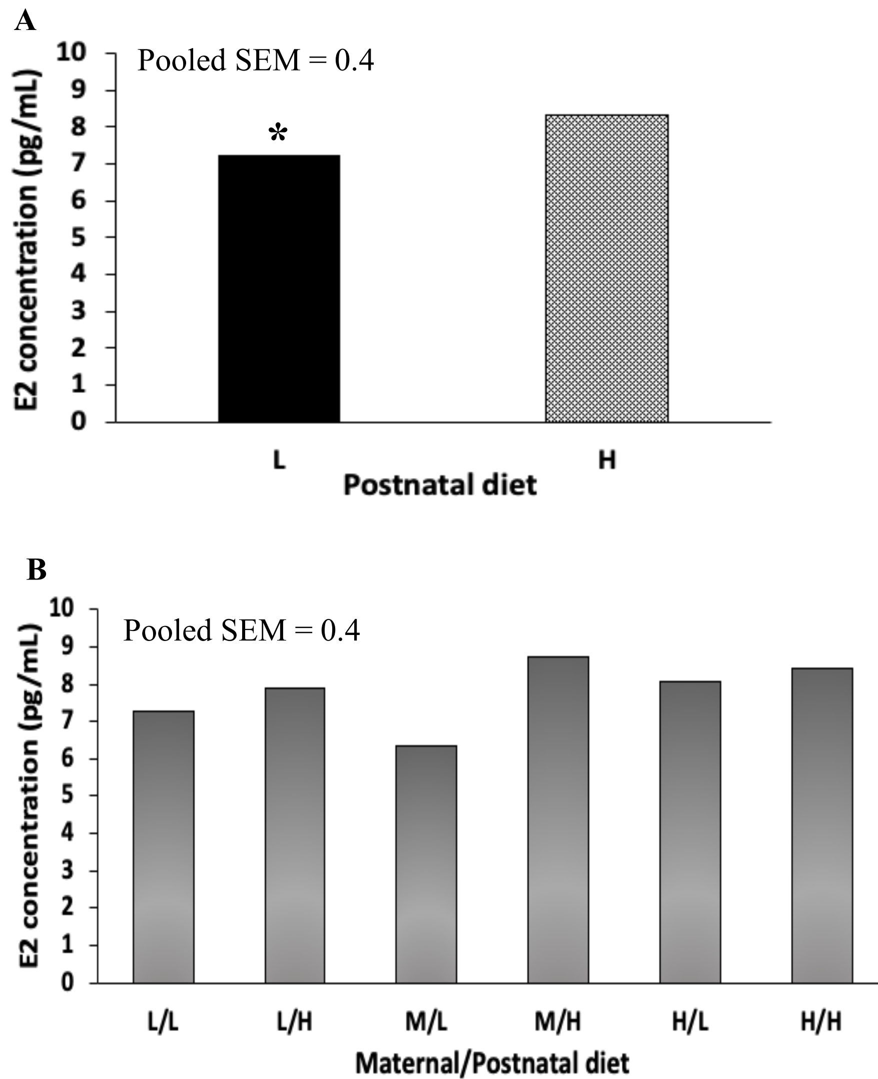

Fig. 3.10. (A) Least squares mean (±SEM) peak concentrations of E2 during the follicular phase in heifers fed low gain (L) or high gain (H) postnatal diets, and (B) maternal x postnatal combinations. Heifers fed H diets postnatally tended (**P* < 0.08) to have a greater peak concentration of E2 compared to L heifers (A). Maternal x postnatal diet combinations had no effect (*P* = 0.49) on any E2 variable (B).

DISCUSSION

In Exp.1, we investigated whether nutritional extremes during the second and third trimester of gestation in beef cows, and during the postnatal juvenile period of their

female offspring, interact to affect BrW and age and BW at puberty. In cattle, the influence of maternal nutrition on BrW of calves has been reported in earlier studies in which a restricted diet depending on the severity, may or may not reduce BrW [165, 180, 181]. There was a 10.7% difference in BrW in calves born to dams that were nutrient restricted (L) compared to calves born to dams that were overfed (H). However, there was no evidence that maternal nutrient restriction during mid-late gestation affected BrW in the offspring when compared to a normal (moderate nutrition) diet during the same period. This observation is similar to an earlier report [36] in *Bos taurus* cows demonstrating that BrW was not affected in calves whose dams had total DM intake restricted to 75% of National Research Council recommendations during the same period of gestation. Notably, both breed type and the severity of restriction imposed during gestation could potentially result in different outcomes in the offspring. In the current study, experimental animals were *Bos indicus*-influenced crossbreds and were exposed to a more severely restricted diet (~50% of NRC requirements until target body condition was reached) than dams in the study referenced above [36]. However, it has been suggested that genetic factors associated with dietary energy metabolism in *Bos-indicus* influenced cattle may result in an enhanced ability to cope with extreme dietary energy restrictions which would result in lesser or no effects on BrW of offspring [164]. Earlier studies in humans have associated nutrient restriction during pregnancy with low BrW which increases the risk for metabolic diseases in the offspring later in life [18, 19, 171]. However, the influence of maternal nutritional imbalances during pregnancy on BrW has not been consistent among studies [158] and maternal nutrient

status during pregnancy does not predictably influence BrW [161]. Moreover, BrW alone does not necessarily reflect alterations in body composition and adiposity (i.e., lean-to-fat ratios) of offspring due to prenatal nutritional perturbations [28]. Laboratory animal studies have demonstrated that similar physiological characteristics can arise (e.g., hypertension) in response to such perturbations in offspring born with or without low BrW [158]. Moreover, studies have shown that animals born with reduced BrW tend to overcome this difference later [42–44, 165]. In the current study, heifers born to the L maternal group had compensated for their previous lighter BrW at the time of weaning at 3 to 3.5 months of age.

The results of the present work indicated that, while there was a significant postnatal dietary energy effect on age at puberty, neither maternal nutritional status nor its interaction with postnatal nutrition influenced this variable. Other experimental examples in cattle have also demonstrated that neither reduced feed intake during pregnancy [36] nor protein supplementation [161] affect pubertal maturation in heifer offspring. Ideally, one of the goals of management should be to produce replacement heifers that reach puberty by 12–14 months of age and that become pregnant within the first few weeks of their first breeding season as yearlings [15, 157]. In southern regions of the US, *Bos indicus*-influenced cattle (Brahman crossbred and composites) are the predominant maternal breed types utilized for beef production [159]. However, because the average age at puberty occurs later in these breed types [4], many heifers still calve for the first time at 2.5 to 3 years of age [157]. This phenomenon is not restricted solely to *Bos indicus*-influenced breed types [160] but is often more pronounced compared to

Bos taurus cattle [172]. In the present study, ¼ of the heifer's genotype came from Brahman and, despite the 2 months difference in pubertal onset between heifers fed the H and L postnatal diets, both groups ultimately reached puberty at ages that can impact positively their lifetime productivity and performance [97, 98, 157, 160].

Cells involved in the neuronal network regulating GnRH neurons in the hypothalamus differentiate during fetal development [163] and dams fed restricted energy diets during gestation produced offspring with marked changes in leptin receptor expression in the choroid plexus [65]. However, studies examining the influence of maternal nutrition in cattle on reproductive performance of offspring are limited [15], with most having utilized rodents and sheep to address issues related to fetal programming. Similar to our findings, Martin et al. [161] reported that age at puberty in the offspring was not affected by maternal nutrition; however, it appears that maternal nutrition may influence reproductive performance in the mature heifer. A greater percentage of heifers born to cows supplemented with protein had greater overall pregnancy rate and more calved within the first 3 weeks of their calving season compared with heifers born to unsupplemented cows. In contrast to current and previous reports in heifers, a maternal high fat diet (45% in fat) fed to female rats led to irregular estrous cycles in the offspring [50]. Moreover, maternal undernutrition (50% of daily food intake) in rats delayed the onset of puberty [38] and protein-restricted diets fed during gestation have been shown to affect brain development and occurrence of a leptin surge early in life [162]. In addition, experiments in sheep have shown that exposure to both prenatal under- and overnutrition alter fetal ovarian follicular development (i.e.,

reduced cellular proliferation in primordial follicles) [48, 30, 31, 49] which can negatively impact overall reproductive function [28] as observed by decreased ovulation rate [176] and reduced peripheral concentrations of P4 during the luteal phase of the estrous cycle in the ewe [27]. In examining the effects of maternal nutrition on reproductive characteristics of offspring postweaning, it should be noted that there is some potential for maternal effects to be confounded by the early postnatal nutritional environment of the suckling offspring before weaning. In the current work, variation in maternal traits that might affect offspring growth and health [9], such as colostrum and milk production, were not evaluated.

In the present study, regardless of perinatal diet, Bos *indicus*-influenced beef heifers attained puberty at approximately 60% of their expected mature weight. However, an accelerated rate of gain during the juvenile period hastened pubertal attainment by 2 months relative to heifers with a slower rate of gain. Most beef heifers are weaned from their dams at 6 to 8 months of age in the US. A common approach is to manage replacement heifers to attain 60 to 65% of their expected mature BW by the onset of the breeding season [157, 166]. Rate of gain up to ~8 months of age appears to have a more significant impact on pubertal onset than postweaning gains (growth up to the start of breeding) [166, 167]. Moreover, previous studies from our laboratory and others have shown that the juvenile period (ranging from 4 to 9 months of age) represents a period of significant hypothalamic plasticity in terms of nutritional programming which can influence pubertal attainment [60, 61]. Although there is evidence that both over- and undernutrition during pregnancy can negatively impact the

offspring's metabolic functions, it appears that relative to onset of puberty in females, the juvenile period is more sensitive to dietary manipulation in which a faster rate of gain more readily advances pubertal onset.

Although the exact physiological mechanisms involved in the nutritional acceleration of puberty are not completely clear, an earlier decrease in the sensitivity of the negative feedback effects of E2 is associated with a decreased inhibition of kisspeptin neurons in the ARC [168, 182]. This change may involve leptin and inhibitory (NPY)/excitatory (proopiomelanocortin; POMC) outputs towards GnRH neurons which in turn will influence the pulsatile release of GnRH/LH for pubertal maturation [6, 64]. It appears that the period of early postnatal life is sensitive to changes in neuronal projections in the hypothalamus. Genes involved in axon elongation were abnormally expressed and density of alpha-melanocyte stimulating hormone (αMSH) fibers in the hypothalamus (paraventricular nucleus) was reduced in 12 day-old rats exposed to a pre and early postnatal low-protein diet, which was not the case in pups that were exposed to a normal protein diet postnatally [162]. Importantly, a positive energy balance during the postnatal period to accelerate puberty in heifers may require increased signaling of αMSH [64]. According to Amstalden et al. [168], the establishment of structural and functional modifications in the NPY-POMC-kisspeptin pathways is initiated a few to several months before puberty and these changes are influenced by rate of gain and adiposity. This hypothesis is further supported by findings that an elevated BW gain during the juvenile period in heifers is associated with increased circulating concentrations of leptin, increased expression of *POMC* mRNA,

and decreased NPY innervation towards GnRH neurons in the ARC, as well as an earlier decline of E2 negative feedback [60, 62, 103, 105, 169].

In Exp. 2, we examined the effects of perinatal nutrient extremes on selected aspects of reproductive phenotype. The present results indicate that nutritional status during mid-late gestation and in early postnatal life, based on the nutritional perturbations employed, do not affect major aspects of ovarian-uterine morphology or function, including size of the pre-ovulatory follicle, growth rate of the dominant follicle, CL size, endometrial thickness during the period of estrogen dominance, estrus expression, or estrous cycle length. Moreover, we failed to observe any effects on circulating concentrations of P4 during the luteal phase or E2 during the follicular phase of the estrous cycle after sexual maturation. Thus, our hypothesis that maternal nutrition during second and third trimesters, postnatal nutrition between 4 and 8 months of age, or their interaction would affect certain aspects of ovarian-uterine reproductive phenotype was rejected. However, similar to results reported previously [141, 173], ovarian size was positively correlated with AFC, irrespective of nutritional status. Gunn et al. [174] reported no influence of maternal nutrition on ovarian size from dams fed an excessive amount of dietary crude protein during late gestation and early lactation. In contrast, ovaries of heifers from the M maternal dietary group were larger than ovaries from H maternal group, but similar in size to the L maternal group. Nonetheless, this difference was not reflected by a difference in AFC among maternal groups. Although AFC and the peak of E2 before estrus tended to be affected by postnatal diets, and ovarian size was affected by maternal diets, physiologically, these differences would not likely have

a major impact on overall reproductive performance. Irrespective of maternal diets, heifers from the H postnatal group were classified as having high AFC (≥ 25 follicles), whereas heifers from the L postnatal diet were classified as having an intermediate AFC classification (16 to 24 follicles) [136]. These results are not in accordance with studies in the rat model where maternal plane of nutrition affected reproductive characteristics in the female offspring, including CL number, E2 concentration and estrous cyclicity [175].

The reproductive characteristics selected for evaluation in the current experiment represent physiological variables that have a strong influence on reproductive function and efficiency; thus, if impacted negatively by the perinatal environment could in turn impact lifetime productivity. During the follicular phase, especially the pre-ovulatory period, elevated concentrations of E2 produced by the dominant estrogen-active follicle potentiate uterine contractions to facilitate sperm transport in the female tract. Estradiol also regulates the uterine environment through changes in uterine protein expression during specific times of the estrous cycle. This coordinates endometrial remodeling that is involved in nutritional support to the embryo which influences embryo survival and thus reproductive success [73, 119, 177]. Size of the preovulatory follicle and expression of estrus are associated with greater amounts of E2 produced by the dominant follicle and reflect physiological maturity of the follicle and oocyte. Plasma concentrations of E2 before ovulation increase the likelihood of estrus and both serve as positive influences on the subsequent concentration of luteal P4 and rate of embryonic survival [123].

Progesterone is also involved in the regulation of the uterine environment, as well as expression of genes that regulate important cell functions, including cell growth and differentiation, immune response [177], and growth factors to support conceptus development and pregnancy maintenance [178]. Moreover, because endometrial thickness changes with stage of the estrous cycle [152] and correlates positively with E2 concentration [153], it has been used as a potential predictor of reproductive success [148]. Although the depletion of the ovarian reserve is not as important in livestock species as in humans, since most leave the herd before this event occurs, the ovarian reserve has been associated with fertility [67]. A greater number of antral follicles present on the ovary (high AFC) has been associated with an enhanced response to superovulation protocols, improved embryo quality [136], and a greater percentage of cows pregnant at the end of the breeding season compared to low AFC animals [138].

In cattle, approximately 75% of fetal growth occurs during the third trimester of gestation [40]. Although the first trimester of gestation is the period when nutrient requirements for fetal growth are minimal [48], it represents the period when critical events regulating normal conceptus development and reproduction occur, such as organogenesis, maximal placental growth [11], as well as folliculogenesis [108]. In ruminants, there is evidence that maternal nutritional status during early-mid gestation alters ovarian development [49], AFC [33], maximum size of ovarian follicles [34] and circulating steroid hormone concentrations in the offspring [27]. Since we did not ovariectomize heifers in this study, we could not determine whether any of the dietary treatments influenced the number of pre-antral follicles. Because the maternal diets

employed in the current study were applied during the second and third trimesters of pregnancy, it appears that the greatest influence of nutrition on AFC and other ovarian characteristics occurs earlier in gestation. Thus, the timing of nutritional manipulations is a key factor in determining morphological and functional effects in the offspring. However, such effects appear to be selective as opposed to universal. Mossa et al. [33] reported no influence of maternal nutrition in the first trimester of gestation on CL size, maximum follicular diameter, or estrous cycle length in cattle [33] and any such effects will likely be dependent upon the nature of the dietary alteration [174]. Although we failed to identify any physiologically significant effects on selected aspects of ovarian, uterine, or behavioral phenotype in this experiment, the possibility that other more subtle alterations caused by the nutritional extremes imposed could still alter long-term reproductive performance. However, in cattle and sheep it appears that nutritional status of the dam will not result in any significant functional impact on reproductive performance during adulthood [194]. In summary, all of the reproductive physiological, morphological, hormonal, and behavioral characteristics observed in this study exhibited values that were well within the normal range of those reported earlier in bovine females [73, 74, 108, 119, 127, 138] and none were affected by the dietary treatments imposed in these experiments.

CHAPTER IV

CONCLUSIONS

In summary, our results indicate that, of the perinatal nutritional factors studied, postnatal dietary energy status (and thus growth rate) was the only factor that influenced age at puberty. No significant effects of maternal nutrition during second and third trimesters or its interaction with postnatal nutrition was observed. Moreover, none of the maternal or postnatal nutritional treatments imposed had measurable impacts on important ovarian, uterine and behavioral characteristics selected for this study. The results reported here did not support our primary hypothesis that postnatal diets during the juvenile period would mitigate the negative effects of nutritional imbalances during pregnancy. The effects of the current perinatal nutritional model on the adult secretion pattern of LH, the negative and positive feedback sensitivity to E2, responsiveness of kisspeptin neurons to an important regulatory secretagogue (neurokinin B), and several aspects of hypothalamic gene expression and neuronal development remain under investigation. Longer-term studies will be required to determine whether perinatal nutrition or their interaction affect other unexamined variables including lifetime reproductive performance in cattle.

LITERATURE CITED

1. Engelken TJ. Developing replacement beef heifers. Theriogenology 2008; 70:569–572.

2. National Agricultural Statistics Service (NASS). Cattle inventory 2020.

3. Perry G, Walker J, Wright C, Olson K. Impact of method of heifer development and post-AI management on reproductive efficiency. Range Beef Cow Symp XXI 2009:263.

4. Randel RD. Reproduction of *Bos Indicus* breeds and crosses. Proc Appl Reprod Strateg Beef Cattle 2005:104–124.

5. Dickinson SE, Elmore MF, Kriese-Anderson L, Elmore JB, Walker BN, Dyce PW, Rodning SP, Biase FH. Evaluation of age, weaning weight, body condition score, and reproductive tract score in pre-selected beef heifers relative to reproductive potential. J Anim Sci Biotechnol 2019; 10:18:1–7.

6. Cardoso RC, West SM, Maia TS, Alves BRC, Williams GL. Nutritional control of puberty in the bovine female: prenatal and early postnatal regulation of the neuroendocrine system. Domest Anim Endocrinol 2020; 73:106434.

7. Wu G, Bazer FW, Wallace JM, Spencer TE. Board-invited review: intrauterine growth retardation: implications for the animal sciences. J Anim Sci 2006; 84:2316–2337.

8. Ramammorthy TG, Begun G, Harno E, White A. Developmental programming of hypothalamic neuronal circuits: impact on energy balance control. Front Neurosci 2015; 9:1–16.

9. Greenwood P, Bell A, Vercoe P, Viljoen G. Managing the prenatal environment to enhance livestock productivity. In: Greenwood P, Thompson A, Ford S (eds). Postnatal consequences of the maternal environment and of growth during prenatal life for productivity of ruminants, Springer Netherlands; 2010:3–36.

10. Padmanabhan V, Cardoso RC, Puttabyatappa M. Developmental programming, pathway to disease. Endocrinology 2016; 157:1328–1340.

11. Funston RN, Larson DM, Vonnahme KA. Effects of maternal nutrition on conceptus growth and offspring performance: implications for beef cattle production. J Anim Sci 2010; 88:205–215.

12. Caton JS, Crouse MS, Reynolds LP, Neville TL, Dahlen CR, Ward AK, Swanson KC. Maternal nutrition and programming of offspring energy requirements. Transl Anim Sci 2019; 3:976–990.

13. Du M, Ford SP, Zhu M. Optimizing livestock production efficiency through maternal nutritional management and fetal developmental programming. Anim Front 2017; 7:5–11.

14. Reynolds LP, Borowicz PP, Caton JS, Vonnahme KA, Luther JS, Hammer CJ, Carlin KRM, Grazul-Bilska AT, Redmer, DA. Developmental programming: the concept, large animal models, and the key role of uteroplacental vascular development. J Anim Sci 2010; 88:61–72.

15. Cushman RA, Perry GA. Developmental programming of fertility in livestock. Vet Clin N Am-Food Anim Pract 2019; 35:321–330.

16. Cardoso RC, Padmanabhan V. Developmental programming of PCOS traits: insights from the sheep. Med Sci 2019; 7:79.

17. Ravelli G, Stein Z, Susser M. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med 1976; 295:349–353.

18. Barker DJP. The effect of nutrition of the fetus and neonate on cardiovascular disease in adult life. Proc Nutr Soc 1992; 51:135–144.

19. Hales CN, Barker DJP. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 1992; 35:595–601.

20. Hales CN, Barker DJP. The thrifty phenotype hypothesis. Br Med Bull 2001; 60:5–20.

21. Lucas A. Programming by early nutrition in man. Ciba Found Symp 1991; 156:38–50.

22. Mossa F, Walsh SW, Ireland JJ, Evans ACO. Early nutritional programming and progeny performance: is reproductive success already set at birth? Anim Front 2015; 4:18–24.

23. Walton A, Hammond J. The maternal effects on growth and conformation in shire horse-shetland pony crosses. Proc R Soc London Ser B - Biol Sci 1938; 125:311–335.

24. Barker DJP. The origins of the developmental origins theory. J Intern Med 2007; 261:412–417.

25. Evans NP, Bellingham M, Robinson JE. Prenatal programming of neuroendocrine reproductive function. Theriogenology 2016; 86:340–348.

26. Dupont C, Cordier AG, Junien C, Mandon-Pépin B, Levy R, Chavatte-Palmer P. Maternal environment and the reproductive function of the offspring. Theriogenology 2012; 78:1405–1414.

27. Long NM, Nijland MJ, Nathanielsz PW, Ford SP. The effect of early to mid-gestational nutrient restriction on female offspring fertility and hypothalamic-pituitary-adrenal axis response to stress. J Anim Sci 2010; 88:2029–2037.

28. Khanal P, Nielsen MO. Impacts of prenatal nutrition on animal production and performance: a focus on growth and metabolic and endocrine function in sheep. J Anim Sci Biotechnol 2017; 8:75:1–14.

29. Meikle A, de Brun V, Carriquiry M, Soca P, Sosa C, Adrien ML, Chilibroste P, Abecia JA. Influences of nutrition and metabolism on reproduction of the female ruminant. Anim Reprod 2018; 15:899–911.

30. Grazul-Bilska AT, Gaton JS, Arndt W, Burchill K, Thorson C, Borowczyk E, Bilski JJ, Redmer DA, Reynolds LP, Vonnahme KA. Cellular proliferation and vascularization in ovine fetal ovaries: effects of undernutrition and selenium in maternal diet. Reproduction 2009; 137:699–707.

31. Rae MT, Palassio S, Kyle CE, Brooks AN, Lea RG, Miller DW, Rhind, SM. Effect of maternal undernutrition during pregnancy on early ovarian development and subsequent follicular development in sheep fetuses. Reproduction 2001; 122:915–922.

32. Mossa F, Kenny D, Jiemenez-Krassel F, Smith G, Berry D, Butler S, Fair T, Lonergan P, Ireland JJ, Evans ACO. Undernutrition of heifers during the first trimester of pregnancy diminishes size of the ovarian reserve in female offspring. Biol Reprod 2009; 81:135.

33. Mossa F, Carter F, Walsh SW, Kenny DA, Smith GW, Ireland JLH, Hildebrandt TB, Lonergan P, Ireland JJ, Evans ACO. Maternal undernutrition

in cows impairs ovarian and cardiovascular systems in their offspring. Biol Reprod 2013; 88:1–9.

34. Sullivan TM, Micke GC, Greer RM, Irving-Rodgers HF, Rodgers RJ, Perry VEA. Dietary manipulation of *Bos indicus* heifers during gestation affects the reproductive development of their heifer calves. Reprod Fertil Dev 2009; 21:773–784.

35. Long NM, Tousley CB, Underwood KR, Paisley SI, Means WJ, Hess BW, Du M, Ford SP. Effects of early- to mid-gestational undernutrition with or without protein supplementation on offspring growth, carcass characteristics, and adipocyte size in beef cattle. J Anim Sci 2012; 90:197–206.

36. Cushman RA, McNeel AK, Freetly HC. The impact of cow nutrient status during the second and third trimesters on age at puberty, antral follicle count, and fertility of daughters. Livest Sci 2014; 162:252–258.

37. Ellis-Hutchings RG, Zucker RM, Grey BE, Norwood J, Richards JH, Lau C, Rogers JM. Altered health outcomes in adult offspring of Sprague Dawley and Wistar rats undernourished during early or late pregnancy. Birth Defects Research Part B - Dev Reprod Toxicol 2010; 89:396–407.

38. Iwasa T, Matsuzaki T, Murakami M, Fujisawa S, Kinouchi R, Gereltsetseg G, Kuwahara A, Yasui T, Irahara M. Effects of intrauterine undernutrition on hypothalamic Kiss1 expression and the timing of puberty in female rats. J Physiol 2010; 588:821–829.

39. O’Neil M, West S, Maia T, Cardoso RC, Williams GL. Effects of maternal nutrition on secretion of leptin in the neonatal heifer and interaction of maternal and postnatal nutrition on age at puberty and postpubertal secretion of luteinizing hormone. J Anim Sci 2019; 97:140–141.

40. Eley RM, Thatcher WW, Bazer FW, Wilcox CJ, Becker RB, Head HH, Adkinson RW. Development of the conceptus in the bovine. J Dairy Sci 1978; 61:467–473.

41. Gardner DS, Tingey K, van Bon BWM, Ozanne SE, Wilson V, Dandrea J, Keisler DH, Stephenson T, Symonds ME. Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition. Am J Physiol - Regul Integr Comp Physiol 2005; 289:947–954.

42. Brickell JS, Bourne N, McGowan MM, Wathes DC. Effect of growth and development during the rearing period on the subsequent fertility of nulliparous Holstein-Friesian heifers. Theriogenology 2009; 72:408–416.

43. Swali A, Wathes DC. Influence of primiparity on size at birth, growth, the somatotrophic axis and fertility in dairy heifers. Anim Reprod Sci 2007; 102:122–136.

44. Calkins K, Devaskar S. Fetal origins of adult disease. Curr Probl Pediatr Adolesc Health Care 2011; 41:158–176.

45. Zhu Z, Cao F, Li X. Epigenetic programming and fetal metabolic programming. Front Endocrinol 2019; 10:764.

46. de Blasio MJ, Blache D, Gatford KL, Robinson JS, Owens JA. Placental restriction increases adipose leptin gene expression and plasma leptin and alters their relationship to feeding activity in the young lamb. Pediatr Res 2010; 67:603–608.

47. Webb R, Garnsworthy PC, Gong JG, Armstrong DG. Control of follicular growth: local interactions and nutritional influences. J Anim Sci 2004; 82: E63–74.

48. da Silva P, Aitken RP, Rhind SM, Racey PA, Wallace JM. Effect of maternal overnutrition during pregnancy on pituitary gonadotrophin gene expression and gonadal morphology in female and male foetal sheep at day 103 of gestation. Placenta 2003; 24:248–257.

49. Weller MMDCA, Fortes MRS, Marcondes MI, Rotta PP, Gionbeli TRS, Valadares Filho SC, Campos MM, Silva FF, Silva W, Moore S, Guimaraes SEF. Effect of maternal nutrition and days of gestation on pituitary gland and gonadal gene expression in cattle. J Dairy Sci 2016; 99:3056–3071.

50. Connor KL, Vickers MH, Beltrand J, Meaney MJ, Sloboda DM. Nature, nurture or nutrition? Impact of maternal nutrition on maternal care, offspring development and reproductive function. J Physiol 2012; 590:2167–2180.

51. Shankar K, Harrell A, Liu X, Gilchrist JM, Ronis MJJ, Badger TM. Maternal obesity at conception programs obesity in the offspring. Am J Physiol - Regul Integr Comp Physiol 2008; 294:528–538.

52. Silvestris E, de Pergola G, Rosania R, Loverro G. Obesity as disruptor of the female fertility. Reprod Biol Endocrinol 2018; 16:1–13.

53. Long NM, George LA, Uthlaut AB, Smith DT, Nijland MJ, Nathanielsz PW, Ford SP. Maternal obesity and increased nutrient intake before and during

gestation in the ewe results in altered growth, adiposity, and glucose tolerance in adult offspring. J Anim Sci 2010; 88:3546–3553.

54. Shasa DR, Odhiambo JF, Long NM, Tuersunjiang N, Nathanielsz PW, Ford SP. Multigenerational impact of maternal overnutrition/obesity in the sheep on the neonatal leptin surge in granddaughters. Int J Obes 2015; 39:695–701.

55. Bouret SG, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. Science 2004; 304:108–110.

56. Parlee S, MacDouglad O. Maternal nutrition and risk of obesity in offspring: the trojan horse of developmental plasticity. Biochim Biophys Acta 2014; 1842:495–506.

57. Cerf ME. High fat programming and cardiovascular disease. Medicina (Lithuania) 2018; 54:1–13.

58. Diskin MG, Kenny DA. Optimising reproductive performance of beef cows and replacement heifers. Animal 2014; 8:27–39.

59. Léonhardt M, Lesage J, Croix D, Dutriez-Casteloot I, Beauvillain JC, Dupouy JP. Effects of perinatal maternal food restriction on pituitary-gonadal axis and plasma leptin level in rat pup at birth and weaning and on timing of puberty. Biol Reprod 2003; 68:390–400.

60. Gasser CL, Behlke EJ, Grum DE, Day ML. Effect of timing of feeding a high-concentrate diet on growth and attainment of puberty in early-weaned heifers. J Anim Sci 2006; 84:3118–3122.

61. Cardoso RC, Alves BRC, Prezotto LD, Thorson JF, Tedeschi LO, Keisler DH, Amstalden M, Williams GL. Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers. J Anim Sci 2014; 92:2942–2949.

62. Allen CC, Alves BRC, Li X, Tedeschi LO, Zhou H, Paschal JC, Riggs PK, Braga-Neto UM, Keisler DH, Williams GL, Amstalden M. Gene expression in the arcuate nucleus of heifers is affected by controlled intake of high-and low-concentrate diets. J Anim Sci 2012; 90:2222–2232.

63. Breton C. The hypothalamus-adipose axis is a key target of developmental programming by maternal nutritional manipulation. J Endocrinol 2013; 216:19–31.

64. Cardoso RC, Alves BRC, Williams GL. Neuroendocrine signaling pathways and the nutritional control of puberty in heifers. Anim Reprod 2018; 15:868–878.

65. Zhang Y, O'Neil M, Scarpa JO, Alves BRC, Welsh Jr. TH, Cardoso RC, Williams GL. Perinatal nutrition alters NPY neuronal projections to GnRH neurons and leptin receptor expression in the choroid plexus of prepubertal heifer. Proc 50th Annu Meet Soc Study Reprod 2017:27.

66. Monget P, Bobe J, Gougeon A, Fabre S, Monniaux D, Dalbies-Tran R. The ovarian reserve in mammals: a functional and evolutionary perspective. Mol Cell Endocrinol 2012; 356:2–12.

67. Fortune JE, Yang MY, Allen JJ, Herrick SL. Triennial Reproduction Symposium: the ovarian follicular reserve in cattle: what regulates its formation and size? J Anim Sci 2013; 91:3041–3050.

68. Evans ACO, Mossa F, Walsh SW, Scheetz D, Jimenez-Krassel F, Ireland JLH, Smith GW, Ireland JJ. Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. Reprod Domest Anim 2012; 47:31–37.

69. Erickson B. Development and radio-response of the prenatal bovine ovary. J Reprod Fertil 1966; 11:97–105.

70. Fortune JE, Yang MY, Muruvi W. *In vitro* and *in vivo* regulation of follicular formation and activation in cattle. Reprod Fertil Dev 2011; 23:15–22.

71. Braw-Tal R, Yossefi S. Studies *in vivo* and *in vitro* on the initiation of follicle growth in the bovine ovary. J Reprod Fertil 1997; 109:165–171.

72. Lussier JG, Matton P, Dufour JJ. Growth rates of follicles in the ovary of the cow. J Reprod Fertil 1987; 81:301–307.

73. Ireland JJ. Control of follicular growth and development. J Reprod Fertil 1987; 34:39–54.

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

75. Karsch FJ. Central actions of ovarian steroids in the feedback regulation of pulsatile secretion of luteinizing hormone. Rev Physiol 1987; 49:365–382.

76. Herbison AE. A simple model of estrous cycle negative and positive feedback regulation of GnRH secretion. Front Neuroendocrinol 2020; 57:100837.

77. Rahe CH, Owens RE, Fleeger JL, Newton HJ, Harms PG. Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. Endocrinology 1980; 107:498–503.

78. Moenter SM, Caraty A, Karsch FJ. The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990; 127:1375–1384.

79. Karsch FJ, Bowen JM, Caraty A, Evans NP, Moenter SM. Gonadotropin-releasing hormone requirements for ovulation. Biol Reprod 1997; 56:303–309.

80. Moran C, Quirke JF, Roche JF. Puberty in heifers: a review. Anim Reprod Sci 1989; 18:167–182.

81. Barnes MA, Bierley ST, Halman RD, Henricks DM. Follicle stimulating hormone, luteinizing hormone and estradiol-17β response in GnRH treated prepuberal Holstein heifers. Biol Reprod 1980; 22:459–465.

82. Schillo KK, Dierschke DJ, Hauser ER. Regulation of luteinizing hormone secretion in prepubertal heifers: increased threshold to negative feedback action of estradiol. J Anim Sci 1982; 54:325–336.

83. Wildt L, Marshall G, Knobil E. Experimental induction of puberty in the infantile female rhesus monkey. Science 1980; 207:1373–1375.

84. Day ML, Anderson LH. Current concepts on the control of puberty in cattle. J Anim Sci 1998, 76:1–15.

85. Kinder JE, Day M, Kittok R. Endocrine regulation of puberty in cows and ewes. J Reprod Fertil 1987; 34:167–186.

86. Schams D, Schallenberger E, Gombe S, Karg H. Endrocine patterns associated with puberty in male and female cattle. J Reprod Fertil 1981; 30:103–110.

87. Day ML, Imakawa K, Garcia-Winder M, Zalesky D, Schanbacher BD, Kittok RJ, Kinder JE. Endocrine mechanisms of puberty in heifers: estradiol negative feedback regulation of luteinizing hormone secretion. Biol Reprod 1984; 31:332–341.

88. Day ML, Imakawa K, Wolfe PL, Kittok RJ, Kinder JE. Endocrine mechanisms of puberty in heifers. Role of hypothalamo-pituitary estradiol receptors in the negative feedback of estradiol on luteinizing hormone secretion. Biol Reprod 1987; 37:1054–1065.

89. Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 2012; 92:1235–1316.

90. Mayer C, Acosta-Martinez M, Dubois SL, Wolfe A, Radovick S, Boehm U, Levine E. Timing and completion of puberty in female mice depend on estrogen receptor α-signaling in kisspeptin neurons. Proc Natl Acad Sci USA 2010; 107:22693–22698.

91. Goodman RL, Lehman MN, Smith JT, Coolen LM, de Oliveira CVR, Jafarzadehshirazi MR, Pereira A, Iqbal J, Caraty A, Ciofi P, Clarke IJ. Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. Endocrinology 2007; 148:5752–5760.

92. Lehman MN, Coolen LM, Goodman RL. Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 2010; 151:3479–3489.

93. Kirby CJ, Smith MF, Keisler DH, Lucy MC. Follicular function in lactating dairy cows treated with sustained-release bovine somatotropin. J Dairy Sci 1997; 80:273–285.

94. Bedenbaugh MN, D'Oliveira M, Cardoso RC, Hileman SM, Williams GL, Amstalden M. Pubertal escape from estradiol negative feedback in ewe lambs is not accounted for by decreased ESR1 mRNA or protein in kisspeptin neurons. Endocrinology 2018; 159:426–438.

95. 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.

96. Chenoweth PJ. Aspects of reproduction in female *Bos indicus* cattle: a review. Aust Vet J 1994; 71:422–426.

97. Lesmeister JL, Burfening PJ, Blackwell RL. Date of first calving in beef cows and subsequent calf production. J Anim Sci 1973; 36:1–6.

98. Byerley DJ, Staigmiller RB, Berardinelli JG, Short RE. Pregnancy rates of beef heifers bred either on puberal or third estrus. J Anim Sci 1987; 65:645–650.

99. Maquivar M, Day LM. Nutritional regulation of precocious puberty in beef heifers. The Ohio State University 2010;1–24.

100. Foster DL, Olster DH. Effect of restricted nutrition on puberty in the lamb: patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. Endocrinology 1985; 116:375–381.

101. Williams GL, Amstalden M, Garcia MR, Stanko RL, Nizielski SE, Morrison CD, Keisler DH. Leptin and its role in the central regulation of reproduction in cattle. Domest Anim Endocrinol 2002; 23:339–349.

102. Short RE, Bellows RA. Relationships among weight gains, age at puberty and reproductive performance in heifers. J Anim Sci 1971; 32:127–131.

103. Gasser CL, Grum DE, Mussard ML, Fluharty FL, Kinder JE, Day ML. Induction of precocious puberty in heifers I: enhanced secretion of luteinizing hormone. J Anim Sci 2006; 84:2035–2041.

104. Gasser CL, Burke CR, Mussard ML, Behlke EJ, Grum DE, Kinder JE, Day ML. Induction of precocious puberty in heifers II: advanced ovarian follicular development. J Anim Sci 2006; 84:2042–2049.

105. Alves BRC, Cardoso RC, Prezotto LD, Thorson JF, Bedenbaugh M, Sharpton SM, Caraty A, Keisler DH, Tedeschi LO, Williams GL, Amstalden M. Elevated body weight gain during the juvenile period alters neuropeptide Y-gonadotropin-releasing hormone circuitry in prepubertal heifers. Biol Reprod 2015; 92:1–10.

106. Adams GP, Jaiswal R, Singh J, Malhi P. Progress in understanding ovarian follicular dynamics in cattle. Theriogenology 2008; 69:72–80.

107. Jaiswal RS, Singh J, Marshall L, Adams GP. Repeatability of 2-wave and 3-wave patterns of ovarian follicular development during the bovine estrous cycle. Theriogenology 2009; 72:81–90.

108. Fortune JE. Ovarian follicular growth and development in mammals. Biol Reprod 1994; 50:225–232.

109. Driancourt MA. Regulation of ovarian follicular dynamics in farm animals. Implications for manipulation of reproduction. Theriogenology 2001; 55:1211–1239.

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

111. Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K. Selection of the dominant follicle in cattle. Biol Reprod 1996; 55:1187–1194.

112. 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 1989; 87:223–230.

113. Chenault JR, Thatcher WW, Kalra PS, Abrams RM, Wilcox CJ. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J Dairy Sci 1975; 58:709–717.

114. Fortune JE, Willis EL, Bridges PJ, Yang CS. The periovulatory period in cattle: progesterone, prostaglandins, oxytocin and ADAMTS proteases. Anim Reprod 2009; 6:60–71.

115. White FJ, Wettemann RP, Looper ML, Prado TM, Morgan GL. Seasonal effects on estrous behavior and time of ovulation in nonlactating beef cows. J Anim Sci 2002; 80:3053-3059.

116. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during ageing: from periphery to brain. Trends Mol Med 2013; 19:197–209.

117. Rodrigues AD, Cooke RF, Cipriano RS, Silva LGT, Cerri RLA, Cruppe LH, Meneghetti M, Pohler KG, Vasconcelos JLM. Impacts of estrus expression and intensity during a timed-ai protocol on variables associated with fertility and pregnancy success in *Bos indicus*-influenced beef cows. J Anim Sci 2018; 96:236–249.

118. Hawk HW. Sperm survival and transport in the female reproductive tract. J Dairy Sci 1983; 66:2645–2660.

119. Perry GA, Cushman RA, Perry BL, Schiefelbein AK, Northrop EJ, Rich JJJ, Perkins SD. Role of preovulatory concentrations of estradiol on timing of conception and regulation of the uterine environment in beef cattle. Syst Biol Reprod Med 2020; 66:12–25.

120. Dickinson SE, Geary TW, Monnig JM, Pohler KG, Green JA, Smith MF. Effect of preovulatory follicle maturity on pregnancy establishment in cattle: the role of oocyte competence and the maternal environment. Anim Reprod 2016; 13:209–216.

121. Perry GA, Swanson OL, Larimore EL, Perry BL, Djira GD, Cushman RA. Relationship of follicle size and concentrations of estradiol among cows exhibiting or not exhibiting estrus during a fixed-time AI protocol. Domest Anim Endocrinol 2014; 48:15–20.

122. Richardson BN, Hill SL, Stevenson JS, Djira GD, Perry GA. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Anim Reprod Sci 2016; 166:133–140.

123. Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, Roberts AJ, Geary TW. Relationship between follicle size at insemination and pregnancy success. Proc Natl Acad Sci USA 2005; 102:5268–5273.

124. Kesner JS, Convey EM, Anderson CR. Evidence that estradiol induces the preovulatory LH surge in cattle by increasing pituitary sensitivity to LHRH and then increasing LHRH release. Endocrinology 1981; 108:1386–1391.

125. Alila H, Hansel W. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol Reprod 1984; 31:1015–1025.

126. Quintal-Franco JA, Kojima FN, Melvin EJ, Lindsey BR, Zanella E, Fike KE, Wehrman ME, Clopton DT, Kinder JE. Corpus luteum development and function in cattle with episodic release of luteinizing hormone pulses inhibited in the follicular and early luteal phases of the estrous cycle. Biol Reprod 1999; 61:921–926.

127. Mann GE. Corpus luteum size and plasma progesterone concentration in cows. Anim Reprod Sci 2009; 115:296–299.

128. Carter F, Forde N, Duffy P, Wade M, Fair T, Crowe MA, Evans ACO, Kenny DA, Roche JF, Lonergan P. Effect of increasing progesterone concentration from Day 3 of pregnancy on subsequent embryo survival and development in beef heifers. Reprod Fertil Dev 2008; 20:368–375.

129. Jimenez-Krassel F, Folger JK, Ireland JLH, Smith GW, Hou X, Davis JS, Lonergan P, Evans ACO, Ireland JJ. Evidence that high variation in ovarian reserves of healthy young adults has a negative impact on the corpus luteum

and endometrium during estrous cycles in cattle. Biol Reprod 2009; 80:1272–1281.

130. Miura R, Haneda S, Matsui M. Ovulation of the preovulatory follicle originating from the first-wave dominant follicle leads to formation of an active corpus luteum. J Reprod Dev 2015; 61:317–323.

131. Lonergan P, O'Hara L, Forde N. Role of diestrus progesterone on endometrial function and conceptus development in cattle. Anim Reprod 2013; 10:223–227.

132. Bazer FW, Wu G, Johnson GA. Pregnancy recognition signals in mammals: the roles of interferons and estrogens. Anim Reprod 2017; 14:7–29.

133. Menchaca A, Cuadro F, dos Santos-Neto PC, Bosolasco D, Barrera N, de Brun V, Crispo M. Oocyte developmental competence is improved by relatively greater circulating progesterone concentrations during preovulatory follicular growth. Anim Reprod Sci 2018; 195:321–328.

134. Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction 2001; 121:175–180.

135. Monniaux D, Clément F, Dalbiès-Tran R, Estienne A, Fabre S, Mansanet C, Monget P. The ovarian reserve of primordial follicles and the dynamic reserve of antral growing follicles: what is the link? Biol Reprod 2014; 90:1–11.

136. Ireland JJ, Ward F, Jimenez-Krassel F, Ireland JLH, Smith GW, Lonergan P, Evans ACO. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. Hum Reprod 2007; 22:1687–1695.

137. Evans ACO, Mossa F, Fair T, Lonergan P, Butler ST, Zielak-Steciwko AE, Smith GW, Jimenez-Krassel F, Folger JK, Ireland JLH, Ireland JJ. Causes and consequences of the variation in the number of ovarian follicles in cattle. Soc Reprod Fertil Suppl 2010; 67:421–429.

138. Cushman RA, Allan MF, Kuehn LA, Snelling WM, Cupp AS, Freetly HC. Evaluation of antral follicle count and ovarian morphology in crossbred beef cows: investigation of influence of stage of the estrous cycle, age, and birth weight. J Anim Sci 2009; 87:1971–1980.

139. Erickson B. Development and senescence of the postnatal bovine ovary. J Anim Sci 1966; 25:800–805.

140. Burns DS, Jimenez-Krassel F, Ireland JLH, Knight PG, Ireland JJ. Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. Biol Reprod 2005; 73:54–62.

141. Ireland JLH, Scheetz D, Jimenez-Krassel F, Themmen APN, Ward F, Lonergan P, Smith GW, Perez GI, Evans ACO, Ireland JJ. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. Biol Reprod 2008; 79:1219–1225.

142. Zhang Y, Xu Y, Xue Q, Shang J, Yang X, Shan X, Kuai Y, Wang S, Zeng C. Discordance between antral follicle counts and anti-Müllerian hormone levels in women undergoing in vitro fertilization. Reprod Biol Endocrinol 2019; 17:1–6.

143. McNeel AK, Cushman RA. Influence of puberty and antral follicle count on calving day in crossbred beef heifers. Theriogenology 2015; 84:1061–1066.

144. Cushman RA, Perry GA, Britt JH. Current understanding of factors influencing antral follicle count and applications to reproductive management in cattle. J Anim Sci 2018; 96:21–22.

145. Morotti F, Zangirolamo AF, da Silva NC, da Silva CB, Rosa CO, Seneda MM. Antral follicle count in cattle: advantages, challenges, and controversy. Anim Reprod 2017; 14:514–520.

146. Cushman RA, Soares ÉM, Yake HK, Patterson AL, Rosasco SL, Beard JK, Northrop EJ, Rich JJJ, Miles JR, Chase Jr. CC, Gonda MG, Perry GA, McNeel AK, Summers AF. Brangus cows have ovarian reserve parameters more like Brahman than Angus cows. Anim Reprod Sci 2019; 209:106170.

147. Silva-Santos KC, Santos GMG, Siloto LS, Santos JT, Oliveira ER, Machado FZ, Rosa CO, Seneda MM. The correlation between the number of antral follicles and ovarian reserves (preantral follicles) in purebred *Bos indicus* and Bos taurus cows. Anim Reprod Sci 2014; 151:119–125.

148. Souza AH, Silva EPB, Cunha AP, Gümen A, Ayres H, Brusveen DJ, Guenther JN, Wiltbank MC. Ultrasonographic evaluation of endometrial thickness near timed AI as a predictor of fertility in high-producing dairy cows. Theriogenology 2011; 75:722–733.

149. DesCôteaux L, Chastant-Maillard S, Gnemmi G, Colloton J, Bollwein H. Bovine uterus. Practical atlas of ruminant and camelid reproductive ultrasonography, 2010; 61–80.

150. Pierson RA, Ginther OJ. Ultrasonic imaging of the ovaries and uterus in cattle. Theriogenology 1988; 29:21–37.

151. Bonafos LD, Kot K, Ginther OJ. Physical characteristics of the uterus during the bovine estrous cycle and early pregnancy. Theriogenology 1995; 43:713–721.

152. Johnson ML, Redmer DA, Reynolds LP. Uterine growth, cell proliferation, and c-fos proto-oncogene expression throughout the estrous cycle in ewes. Biol Reprod 1997; 56:393–401.

153. Sugiura T, Akiyoshi S, Inoue F, Yanagawa Y, Moriyoshi M, Tajima M, Katagiri S. Relationship between bovine endometrial thickness and plasma progesterone and estradiol concentrations in natural and induced estrus. J Reprod Dev 2018; 64:135–143.

154. Bu Z, Yang X, Song L, Kang B, Sun Y. The impact of endometrial thickness change after progesterone administration on pregnancy outcome in patients transferred with single frozen-thawed blastocyst. Reprod Biol Endocrinol 2019; 17:1–6.

155. Bu Z, Sun Y. The impact of endometrial thickness on the day of human chorionic gonadotrophin (hCG) administration on ongoing pregnancy rate in patients with different ovarian response. PLoS ONE 2015; 10:1–8.

156. Zhang T, Li Z, Ren X, Huang B, Zhu G, Yang W, Jin L. Endometrial thickness as a predictor of the reproductive outcomes in fresh and frozen embryo transfer cycles: a retrospective cohort study of 1512 IVF cycles with morphologically good-quality blastocyst. Medicine (Baltimore) 2018; 97:e9689.

157. Day ML, Nogueira GP. Management of age at puberty in beef heifers to optimize efficiency of beef production. Anim Front 2013; 3:6–11.

158. Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? J Physiol 2004; 561:355–377.

159. McGrann J, Leister K. The United States beef cattle industry: production, structure and trends. Leister, K. (ed.) 2010; 1–21.

160. Perry RC, Corah LR, Cochran RC, Brethour JR, Olson KC, Higgins JJ. Effects of hay quality, breed, and ovarian development on onset of puberty and reproductive performance of beef heifers. J Prod Agric 1991; 4:13–18.

161. Martin JL, Vonnahme KA, Adams DC, Lardy GP, Funston RN. Effects of dam nutrition on growth and reproductive performance of heifer calves. J Anim Sci 2007; 85:841–847.

162. Coupé B, Amarger V, Grit I, Benani A, Parnet P. Nutritional programming affects hypothalamic organization and early response to leptin. Endocrinology 2010; 151:702–713.

163. Aujla PK, Naratadam GT, Xu L, Raetzman LT. Notch/Rbpjκ signaling regulates progenitor maintenance and differentiation of hypothalamic arcuate neurons. Development 2013; 140:3511–3521.

164. Fontes PLP, Oosthuizen N, Ciriaco FM, Sanford CD, Canal LB, Pohler KG, Henry DD, Mercadante VRG, Timlin CL, Ealy AD, Johnson SE, DiLorenzo N, Lamb GC. Impact of fetal vs. maternal contributions of *Bos indicus* and *Bos taurus* genetics on embryonic and fetal development. J Anim Sci 2019; 97:1645–1655.

165. Hight GK. The effects of undernutrition in late pregnancy on beef cattle production. New Zealand J Agric Res 1966; 9:479–490.

166. Roberts AJ, Geary TW, Grings EE, Waterman RC, Macneil MD. Reproductive performance of heifers offered ad libitum or restricted access to feed for a one hundred forty-day period after weaning. J Anim Sci 2009; 87:3043–3052.

167. Funston RN, Summers AF. Epigenetics: setting up lifetime production of beef cows by managing nutrition. Annu Rev Anim Biosci 2013; 1:339–363.

168. Amstalden M, Cardoso RC, Alves BRC, Williams GL. Reproduction Symposium: hypothalamic neuropeptides and the nutritional programming of puberty in heifers. J Anim Sci 2014; 92:3211–3222.

169. Gasser CL, Bridges GA, Mussard ML, Grum DE, Kinder JE, Day ML. Induction of precocious puberty in heifers III: hastened reduction of estradiol negative feedback on secretion of luteinizing hormone. J Anim Sci 2006; 84:2050–2056.

170. Menon KMJ, Munshi UM, Clouser CL, Nair AK. Regulation of luteinizing hormone/human chorionic gonadotropin receptor expression: a perspective. Biol Reprod 2004; 70:861–866.

171. Barker DJP, Osmond C, Winter PD, Margetts B, Simmonds SJ. Weight in infancy and death from ischemic heart disease. The Lancet 1989; 334:577–580.

172. Nogueira GP. Puberty in South American *Bos indicus* (zebu) cattle. Anim Reprod Sci 2004; 82–83:361–372.

173. Eborn DR, Cushman RA, Echternkamp SE. Effect of postweaning diet on ovarian development and fertility in replacement beef heifers. J Anim Sci 2013; 91:4168–4179.

174. Gunn PJ, Schoonmaker JP, Lemenager RP, Bridges GA. Feeding distiller's grains as an energy source to gestating and lactating beef heifers: impact on female progeny growth, puberty attainment, and reproductive processes. J Anim Sci 2015; 93:746–757.

175. Khorram O, Keen-Rinehart E, Chuang T-D, Ross MG, Desai M. Maternal undernutrition induces premature reproductive senescence in adult female rat offspring. Fertil Steril 2015; 103:291–298.

176. Rae MT, Kyle CE, Miller DW, Hammond AJ, Brooks AN, Rhind SM. The effects of undernutrition, *in utero*, on reproductive function in adult male and female sheep. Anim Reprod Sci 2002; 72:63–71.

177. Pedersen HS, Mazzoni G, Stroebech L, Kadarmideen HN, Hyttel P, Callesen H. Basic and practical aspects of pregnancy establishment in cattle. Anim Reprod 2017; 14:581–588.

178. Bazer FW, Song G, Thatcher WW. Roles of conceptus secretory proteins in establishment and maintenance of pregnancy in ruminants. Asian Austral J Anim Sci 2012; 25:1–16.

179. Goodman RL, He W, Lopez JA, Bedenbaugh MN, McCosh RB, Bowdridge EC, Coolen LM, Lehman MN, Hileman SM. Evidence that the LH surge in ewes involves both Neurokinin B-dependent and -independent actions of Kisspeptin. Endocrinology 2019; 160:2990–3000.

180. Holland MD, Odde KG. Factors affecting calf birth weight: a review. Theriogenology 1992; 38:769–798.

181. Carstens GE, Johnson DE, Holland MD, Odde KG. Effects of prepartum protein nutrition and birth weight on basal metabolism in bovine neonates. J Anim Sci 1987; 65:745–751.

182. Nestor CC, Briscoe AMS, Davis SM, Valent M, Goodman RL, Hileman SM. Evidence of a role for kisspeptin and neurokinin B in puberty of female sheep. Endocrinology 2012; 153:2756–2765.

183. Withycombe, R, Potter EL, Edwards FM. Deferred breeding of beef cows. Oregon Agric Exp Sta Bull 1930; 271:1–18.

184. Núnez-Dominguez R, Cundiff LV., Dickerson GE, Gregory KE, Koch RM. Lifetime production of beef heifers calving first at two *vs* three years of age. J Anim Sci 1991; 69:3467–3479.

185. Williams GL, Stanko RL, Allen CC, Cardoso RC, Prezotto LD, Thorson JF, Amstalden M. Evidence that luteal regression at the onset of a 5-Day CO-Synch + CIDR synchronization protocol markedly improves fixed-time AI pregnancy rates in *Bos indicus*-influenced cattle. J Anim Sci 2011;89:251.

186. Williams GL, Stanko RL, Amstalden M. Bee synch for successful fixed-time AI of Brahman-influenced cattle. Proc. 59th Ann. Texas A&M Beef Cattle Short-course 2013; G6e9.

187. 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.

188. Battaglia FC, Meschia G. Principal substrates of fetal metabolism. Physiol Rev 1978; 58:499–527.

189. Ferrell CL, Garrett WN, Hinman N. Growth, development and composition of the udder and gravid uterus of beef heifers during pregnancy. J Anim Sci 1976; 42:1477–1489.

190. Dunlap KA, Brown JD, Keith AB, Satterfield MC. Factors controlling nutrient availability to the developing fetus in ruminants. J Anim Sci Biotechnol 2015; 6:1–10.

191. Zeng Z, Liu F, Li S. Metabolic adaptations in pregnancy: a review. Ann Nutr Metab 2017; 70:59–65.

192. David A Kenny, Kate Keogh. Early life nutrition on the molecular control of sexual development in the bull. J Anim Sci 2020; 98:187-188.

193. Bollwein H, Janett F, Kaske M. Effects of nutrition on sexual development of bulls. Anim Reprod 2017; 14:607–613.

194. Gardner DS, Ozanne SE, Sinclair KD. Effect of the early-life nutritional environment on fecundity and fertility of mammals. Philos Trans R Soc B: Biol Sci 2009; 364:3419–3427.

195. Caton JS, Crouse MS, Reynolds LP, Neville TL, Dahlen CR, Ward AK, Swanson KC. Maternal nutrition and programming of offspring energy requirements. Transl Anim Sci 2019; 3:976–990.