THE INFLUENCE OF *BOS INDICUS* GENETICS ON EARLY FETAL DEVELOPMENT AND PLASMA PREGNANCY-ASSOCIATED GLYCOPROTEIN CONCENTRATIONS

By

PAULA MORELLI MERCADANTE

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2012

To my entire family

ACKNOWLEDGEMENTS

The completion of this work has been made possible due to the excellent guidance of my major advisor, Dr. Alan Ealy. He has taught me so much and was so patient with me that I can't thank enough. He was always an amazing PI, researcher and person. Thank you for letting me, an international student, enter your lab and have a great experience that I will carry for life.

Also, I would like to thank the members of my committee, Dr. Risco, Dr. Rae and Dr. Mortensen. Each of them provided me great support and inputs throughout my master's program, and was always very open for discussion. I also thank Dr. Sally Johnson for all the input on my experiments and support in my career and overall future, my sincere thanks to you.

I would like to thank all my lab mates that helped me and made me feel at home when I first came to the United States. Thank you Dr. Pennington, Dr. Yang, Dr. Zhang, Dr. Ozawa, Ashley Grapes, Dr. Esterman and my dear friend Susan Rodgers for all the help, love and support.

No words can be found to describe my extreme thankfulness to my family. My parents, Waleria and Hans, have been an unwavering source of support, and taught me at a young age that hard work and dedication will take you far in life. As my mother use to say: "If you are not going to give your best, don't even start it!". They are my biggest fans and heroes, and I know that I will always have a place to call home. My sister, Luiza, have always been there for me, laughing and crying together. My husband, Vitor, have been my source of support and strength for the past seven years. He shares of all my dreams and expectations and is my best friend. They help me to achieve my goals in life without forgetting what is most important: family.

The friends that have supported me, laughed with me and made my life the joy that it is, are too numerous to mention, I have been blessed immensely to have these people sharing my life with me and for that I am eternally grateful.

In addition, I would like to thank my fist advisor in Brazil, Zequinha, for always believing in my potential and pushing me to do my very best. He was my mentor and second father, who taught me how to overcome the challenges in life. Also, I would like to thank the CONAPEC Jr. (Botucatu, Brazil), that gave me the opportunity to work with farms and build valuable connections and friendship. It opened my eyes and the doors for my future.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AI	Artificial Insemination
Bfgf	Basic Fibroblast Growth Factor
BSP	Binder of Sperm Protein
BNC	Binucleate Trophoblast Cell
BMP15	Bone Morphogenic Protein 15
bPAG-1	Bovine PAG-1
CL	Corpus Luteum
CNL	Crown-nose Length
CRL	Crown-rump Length
cAMP	Cyclic Adenocine Monophosphate
ELISA	Enzyme-linked Immunosorbant Assay
EGF	Epidermal Growth Factor
FSH	Follicle-stimulating Hormone
GAL-15	Galectin-15
GLIMMIX	Generalized Linear Mixed Model Procedure
GlyCAM-1	Glycosylated Cell Adhesion Molecule 1
GDF9	Growth Differentiation Factor 9
GH	Growth Hormone
ICM	Inner Cell Mass
IGF-1	Insuline-like Grwth Factor-1
IFNT	Interferon-tau
LH	Luteinizing Hormone
MPF	Maturation-promoting Factor
MNC	Mononucleate Trophoblast Cells

N	Number
OD	Optical Density
OSP	Osteopotin
PMN	Polymorphonuclear Neutrophil Leukocytes
PAG	Pregnancy-associated Glycoprotein
PSPA	Pregnancy-specific Protein A
PSPB	Pregnancy-specific Protein B
PE	Primitive Endoderm
P4	Progesterone
PR	Progesterone Receptor
PRP-1	Prolactin-related Protein 1
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
RIA	Radioimmunoassay
THI	Thermal Humidity Index
WT	Weight
ZP	Zona Pellucida

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

THE INFLUENCE OF *BOS INDICUS* GENETICS ON EARLY FETAL DEVELOPMENT AND PLASMA PREGNANCY-ASSOCIATED GLYCOPROTEIN CONCENTRATIONS

By

Paula Morelli Mercadante

August 2012

Chair: Alan. D. Ealy
Major: Animal Molecular and Cellular Biology

Cross-breeding beef cattle with *Bos indicus* is being used to mitigate many of the adverse environmental effects on beef production in the Southern United States. Several reproductive events are altered due to the *Bos indicus* genetics, including embryo/conceptus growth and placental development. Three experiments were conducted in Florida to determine whether fetal and placental development is influenced by *Bos indicus* genetics. All cows were synchronized and artificially inseminated. Transrectal ultrasonography and plasma PAG concentrations were used to examine fetal and placental development in early gestation. Also, calving data was collected and plasma P4 was quantified to examine a linkage between parameters. In experiment 1, genotypes were comprised of Angus and Brahman cross-breeds that spanned from >80% Angus to >80% Brahman. Blood samples and fetal CRL were collected at mean gestation of 53 days. Plasma PAG concentrations were reduced in cows with ≥80% Angus genotype than other cows (6.0±1.5 vs. 9.6±1.5 ng/ml, respectively). Fetus length was greater in cows with ≥80% Angus than the other cows (34.0±2.8 vs. 28.5±2.8 mm, respectively). *Bos indicus* genotype had a linkage with longer gestation length. Calf birth

weight was not affected by maternal genotype. There was a tendency for linear correlantion between plasma PAG concentrations and calf birth weight. In experiment 2, Angus, Brangus and Braford cows were used. Blood samples, and fetal CRL and CNL were collected at days 35 and 62 of gestation, respectively. Plasma PAG concentrations tended to be greater in Angus cows than Brangus/Braford cows at day 35 of gestation (8.6±0.9 vs. 7.8±0.9 ng/ml, respectively) and tended to be reduced in Angus cows than Brangus/Braford cows at day 62 of gestation (2.0±0.5 vs. 2.9±0.5 ng/ml, respectively). Cow genotype did not affected fetal size at day 35 of gestation. Angus cows contained larger fetuses than Brangus/Braford cows at day 62 of gestation (29.6±0.3 vs. 28.2±0.3 mm, respectively). Also, there was an effect of plasma PAG concentrations and cow genotype; and plasma P4 concentrations and cow genotype in CNL. Plasma PAG and P4 concentrations were correlated at day 35 of gestation. Gestation length was shorter (P<0.01) in Angus than Brangus/Braford cows (279±1.9 vs. 285.3±1.9 days, respectively). In experiment 3, Angus and Brangus cows were used. Fetal measurements were not collected. Blood samples were collected at days 33/34, 40/41, 47/48 and 54/55 of gestation. Plasma PAG concentrations were greater in Brangus than Angus cows. Plasma P4 concentrations tended to be greater in Brangus than Angus cows at day 33/34 of gestation. Plasma PAG and P4 concentration were correlated at days 40/41 and 54/55 of gestation. Angus cows had shorter (P=0.01) gestation lengths than Brangus cows (280.4±0.7 vs. 282.8±0.7 days, respectively). In summary, *Bos indicus*-based animals had greater plasma PAG concentrations and smaller fetuses than *Bos taurus* animals in early gestation after days 50-60 of gestation. These results could indicate that *Bos indicus* animals may suffer from placental incompetency in early

gestation. It's clear that more studies are needed to clarify exactly how *Bos indicus* genetics is influencing PAGs concentrations and why. Also, maternal/fetal interactions deserve more attention in subsequent studies.

CHAPTER 1
INTRODUCTION

It is estimated that the world's population will increase by 34% (6.8 to 9.1 billion people) over the next 40 years. Global agriculture will need to increase by 70% to accommodate this increased population. Agriculture currently represents 6% of the world's gross domestic product and occupies 41.4 million square kilometers of land worldwide. Increases in efficiency of production are, therefore, crucial to meet these impending demands for food. Several factors will provide challenges to improving agriculture production efficiency. For example, global warming is predicted to decrease global animal and plant production by 9 to 21% in the near future (FAO, 2009).

The United States has an estimated 91,450 million head of cattle in 2012. It is ranked number one in the world in beef production, with a yearly production of 11.5 million tons red meat. The United States dairy industry contains 8.5 million lactating cows that produce 182.7 billion pounds of milk yearly.

In the beef industry infertility is the most costly factor on a cow-calf operation decreasing revenue and increasing the culling rate of beef production in United States. Cows that fail to become pregnant during the breeding season do not give producers an opportunity to sell a calf. Therefore, reducing infertility will ensure that more females calve in the beginning of the calving season and with a greater chance of weaning heavier calves. Several factors take part in reducing fertility in beef cattle. The impact of the environment on reproduction is one of the most devastating in tropical and sub-tropical areas such as Florida. Therefore the use of *Bos indicus* genetics is a need on these regions.

The modern lactating dairy cow is considered sub-fertile in the United States. The increase in milk production per cow over the past 50+ years has caused corresponding decreases in fertility. The degree to which production affects fertility is multifactorial. Increased milk yield is accompanied by an increase in feed intake and overall metabolic rate, which generates a plethora of physiological changes that adversely affect reproductive processes. There are several reproductive issues that still need to be addressed in the dairy industry to sustain dairy farm viability in the future.

Considering the increase demand in food and the unfavorable conditions for increase in cattle production area, it becomes clear that we need to improve overall cattle production. Fertility is a major problem in the dairy or beef industries. Therefore increases in milk and meat production by improving fertility are needed. Increase in fertility can be achieved by better management, nutrition and the use of crossbreeding (in tropical and sub-tropical environments). Overcoming reproduction problems and increasing production will enable the challenge of feeding a growing population in face of the global warming.

CHAPTER 2
LITERATURE REVIEW

Cattle Industries in the United States

Profitable milk production relies upon a careful, efficient and cost-effective management of dairy herds. In order to optimize dairy profitability cows need to become pregnant as early as possible after calving. That will lead to closer peaks of milk production and overall higher milk production in a lifetime. The inability to become pregnant and loss of established pregnancies accounts for substantial loss of income due to decreasing total milk production (Santos et al., 2004; Whitlock and Maxwell, 2008). It is estimated that each pregnancy loss costs the producer $555.00 in lost lifetime milk production and rebreeding costs (De Vries, 2006).

Revenue in the beef industry is based on the number of calves produced each year. To maximize production a cow-calf operation should produce one healthy calf per cow each year. This requires that cows get pregnant during the breeding season and carry the pregnancy to term. Also, to optimize beef production and increase reproduction life, ideally heifers need to calve at two years of age (Lamb et al., 2008).

Reproductive Problems in Cattle

There are several aspects of metabolism that adversely affect fertility and benefit milk production in dairy cattle. High producing dairy cows have a faster clearance in peripheral ovarian steroids (Santos et al., 2004), higher susceptibility to heat stress due to an elevated internal heat production associated with lactation (Wilson et al., 1998), reduced embryo quality when lactating compared to non lactating dairy cows (Sartori et al., 2002), and they undergo a negative energy balance postpartum blocking the negative feedback of insulin like growth factor-1 (IGF-1) and increasing concentrations

of growth hormone (GH) (Leroy et al., 2008). The current decline in reproductive efficiency in dairy cows is also a result of poor estrus expression associated with concrete floor housing and heat stress (Lucy, 2001; Sartori et al., 2002).

The reproductive success of a dairy cow also depends on how well and fast they recover from the postpartum negative energy balance they experience during early lactation (Stevenson, 2001). Cows with slow recovery of reproductive competence after parturition are less fertile, resulting in more days open (due to delay in first ovulation), more inseminations per conception, and higher probability of abortion (Lucy, 2001; Thatcher et al., 2006). It is interesting that higher producing cows and herds with higher overall milk production actually have better reproductive performance then those with moderate or low production. In most cases the high producing cows experience better management, feed and overall health (Lucy, 2001; Vasconcelos et al., 2011).

In the beef industry reproductive problems play a big role in decreased revenue. Beef cows are considered infertile when they fail to become pregnant during the breeding season (usually 60–120 days), fail to maintain the pregnancy or when they become pregnant late in the breeding season (Lamb et al., 2011). A review of reproductive costs in United States (Bellows et al., 2002) concluded that infertility is the most costly factor on a cow-calf operation. Both infertility (i.e. failure to become pregnant) and pregnancy loss are important contributors to this problem. Approximately 4.5% of United States beef cows are culled annually because of fertility problems (Bellows et al., 2002). Also, Lamb et al. (2011) determined that annual costs with infertility in the beef industry exceed U$86 million and U$2.8 billion for Florida and United States beef producers, respectively. Several factors take part in reducing fertility

in beef cattle. These include genetics, nutrition, maternal age, environment, semen quality, time of insemination, hormonal imbalance, disease and traumatic events (Maurer and Chenault, 1983; Lamb et al., 2008).

The impact of the environment on reproduction is even more pronounced in tropical and sub-tropical areas, such as Florida. Adverse environmental factors include climatic conditions (high temperature and humidity) and secondary conditions including low quality forages, and high level of diseases and parasites (Turner, 1980). One strategy for improving beef production in tropical and sub-tropical climates is to utilize *Bos indicus*-based breeds (Chenoweth, 1994). To follow is an overview of these cattle and the benefits they hold in Florida and other hot, humid regions throughout the world.

Bos Indicus Beef Cattle

Domestic cattle are classified phylogenetically within the *Bovidae* family. This classification lists both humped and non-humped cattle into the same genus/species (*Bos taurus*) but as distinct subspecies (*Bos taurus taurus and Bos taurus indicus)* (Sanders, 1980). Out of convention we will not include the true species classification but instead will use the subspecies description for each (e.g. *Bos taurus* and *Bos indicus*).

The *Bos taurus* genotype is indigenous to Europe and is now found throughout the world. *Bos taurus* genetics, such as Angus and Holstein breeds, have provided a foundation for American beef and dairy industries, respectively. In the beef industry *Bos taurus* breeds generally contain reproductive and productive traits that make them ideal for temperate conditions (Chenoweth, 1994). This is not surprising given that these breeds have been selected for optimal reproduction and production traits over 500+ years in Europe. It also is not surprising that these breeds experience severe reduction of feed intake, growth rate, milk yield and reproductive function as consequence of

exposure to heat stress. Managing cows in tropical and sub-tropical regions presents itself with several new challenges (Cartwright, 1980; Hansen, 2004). About 60% of the cow-calf operations in the United Sates sell their calf at weaning and are localized in the tropical and sub-tropical regions of the country. These regions have the advantage of a longer grazing season and less need for supplemental forage to support beef cattle during the winter, which results in lower feed costs (Mcbride and Mathews, 2011). In contrast to cow-calf sector, the feedlot industry in the United States is compressed in temperate climates (Galyean et al., 2011).

One solution to problems with trying to produce calves for feedlots is to incorporate *Bos indicus* genetics into *Bos taurus*-based breeds. The *Bos indicus*, often referred as zebu or humped cattle, is indigenous to Asia and Africa, and are adapted to tropical and sub-tropical environments (Chenoweth, 1994). The Brahman is an American *Bos indicus* developed in the 1930's (Sanders, 1980) from Brazilian imported zebu breeds (Guzerat, Gir and Nelore). Brahman females normally have greater reproductive longevity and life time productivity in tropical and subtropical areas than Angus females (Cartwright, 1980). The most common use for Bos indicus genetics in the United States is crossbreeding. Crossbred animals incorporate the ruggedness of the *Bos indicus* cattle while maintaining the characteristics of better production and reproduction traits of *Bos taurus* breeds. The Brangus is an example of a beef crossbreed and consists in three eights of Brahman (*Bos indicus*) and five eights of Angus (*Bos taurus*) (Johnson et al., 1990; Bidner et al., 2002; Riley et al., 2007).

Bos indicus cattle are best known by their superior thermoregulatory ability. In one study, Gaughan et al. (1999) determined that Brahman steers had a less pronounced

increase in body temperature than Herefords (a *Bos taurus* breed) in environmental chambers with a Thermal humidity index (THI) of >90. In outdoor conditions (75>THI<84), Brahman body temperatures ranged from 38.2 to 38.6^{0}C whereas Herefords ranged from 38.8 to 39.3^{0}C throughout the day. Some of the physiological adaptations that improve heat tolerance for *Bos indicus* breeds include properties of skin, such as the smooth and shiny hair coats and light color of the hair that reduce heat exchange via radiation; lower metabolic rate and higher density of sweat glands than *Bos taurus* breeds (Hansen, 2004). They also have local system of cooling of blood entering the testis that improve fertility in hot climates (Hansen, 2004).

Besides their great thermotolerance *Bos indicus* breeds also present other characteristics of adaptations for tropical and sub-tropical environments. In a review of *Bos indicus* adaptability to harsh environments, Turner (1980) indicated that they are parasites and disease resistant. *Bos indicus* were found to be more tick resistant than *Bos taurus* animals in South Africa (Rechav, 1987). In a study looking at tick fever it was concluded that *Bos indicus* cattle are resistant to *Babesia bovis* and more resistant to *Babesia bigemina* and *Anaplasma marginale* than *Bos taurus* and crossbreed cattle (Bock et al., 1997).

Further considerations for incorporating *B*os indicus genotypes into the United States beef production system must be made when considering the differences in reproduction and production traits between *Bos indicus* and *Bos taurus*. To follow is an overview of some of these benefits and pitfalls.

Feed efficiency of *Bos indicus*

When placed in temperate conditions or hot climates, *Bos indicus* animals are more feed efficient than *Bos taurus* steers, and have greater average daily gain.

Conversely, zebu breeds have their performance compromised in temperate zones during winter. The low temperatures of those environments induce cold stress in zebu, leading to a less feed efficient animal with lower average daily gain then *Bos taurus* in the same cold condition (Coleman et al., 2012).

Bos indicus breeds have greater average daily gain then *Bos taurus* breeds when consuming low quality forages. This improved utilization of low quality forage likely reflects their lower maintenance requirement (Varel and Kreikemeier, 1999) and their faster rate of fiber degradation than *Bos taurus* breeds (Hunter et al., 2004). Also, mature zebu cows are able to utilize more low quality forage than zebu heifers due to their lower maintenance energy requirements, lack of growth requirements and increased digestive function (Varel and Kreikemeier, 1999). In tropical and sub-tropical climates forage quality tends to be poor; which provides an advantage to utilize *Bos indicus*-based breeds for maximal profitability.

Meat quality of *Bos indicus*

Bos indicus animals have poor meat quality based on United States standards. In a study with a *Bos taurus* breed (Angus) and different *Bos indicus*-based breeds, it was observed that European animals had a greater overall meat quality, with more fat thickness and intramuscular fat (marbling). Also, *Bos taurus* breeds contained meat that was more tender with 3 days of aging. Interestingly, *Bos taurus* meat was more tender with 10 days of aging, indicating that *Bos indicus*-based breeds age at a slower rate (Bidner et al., 2002).

Crossbreeding is being used to minimize the effects of poor meat quality in *Bos indicus* carcasses. Steaks from steers with 25% or less of Brahman breeding were more tender than those that contained 50% or more of Brahman breeding (Johnson et al.,

1990). Also, ribeye area and marbling were significantly lower in steers with 50% or more of Brahman breeding than the animals with more *Bos taurus* breeding (Huffman et al., 1990). Also, Bidner et al. (2002) concluded that crossbred *Bos indicus* and *Bos taurus* animals, such as Brangus, produced desirable beef carcass with small differences in quality. Therefore, they could be used in the Gulf Coast Region instead of *Bos taurus* without sacrificing beef quality. And *Bos indicus*-based breeds require additional aging time than Angus meat.

Birth and postnatal growth of *Bos indicus*

The Brahman birth weight has been changing over the last decades, it was well established that Brahman gave birth to smaller calves (25.8kg) than the Angus (26.3kg)(Reynolds et al., 1980), but more recently studies showed that the Brahman were bigger at birth (30.7±0.6) than Angus (29.3±0.6) leading to more dystocia (Riley et al., 2007). The Brangus calves are always heavier at birth than the purebred *Bos indicus* and *taurus* calves.

As stated earlier *Bos indicus* animals are more feed efficient, therefore they have a greater average daily gain when compared to *Bos taurus*. This characteristic is present even in the new calf, resulting in a heavier calf at weaning (Riley et al., 2007). In the other hand their calf vigor and survival rate is significantly lower than Bos taurus calves, with reports of up to 25% of calf death from birth to weaning (Reynolds et al., 1980; Riley et al., 2007).

In summary, fertility is a big problem in dairy and beef industry in the United States. Revenue of dairy farms depends on the milk production, which depends on the ability of the cow to get pregnant and keep this pregnancy. Beef farmers need to produce one healthy calf per cow per year to maximize profitability. European breeds

have great production and reproduction traits, but they struggle with the harsh environment conditions of tropical and sub-tropical regions. Conversely, *Bos indicus* cattle exposed to heat stress experience less alteration in feed intake, growth rate, production and reproduction performance than *Bos taurus* breeds (Hansen, 2004). Also, they present disease and parasite tolerance and ability to efficiently utilize low quality forage. In the other hand they have other genetics characteristics that limit their usefulness in the American beef industry. To minimize performance loss due to harsh environment conditions in tropical and sub-tropical areas crossbreeding is becoming largely used. Investigations into the unique characteristics, emphasizing the reproductive performance, of *Bos indicus* genetics are necessary to allow them to become more viable in the beef industry.

Ovarian Dynamics and Fetal and Placental Development

Although *Bos indicus* and *Bos taurus* cattle undergo the same reproductive processes to generate viable offspring, they have different gestation length, 291±1 and 282±1 days, respectively (Paschal et al., 1991). In most mammals, the developmental processes begin with the maturation of an oocyte and follicle dynamics, then fertilization occurs and the zygote begins dividing and generating cell types that will produce the placenta and fetus. To follow is a brief overview of specific developmental events that are crucial for pregnancy success in cattle. Differences between *Bos indicus* and *Bos taurus* will be mentioned when there is any.

Oocyte Maturation

Oocyte maturation is the first of several elements that dictate the success of fertilization and early embryonic development. In cows the oocyte stock is developed during fetal life, approximately on day 90 of gestation (Aerts & Bols, 2010a). Follicular

recruitment occurs early in fetal development, and antral follicles already appear before birth. It's estimated that a healthy calf at birth has 120,000 to 150,000 primordial and primary follicles, 200 to 500 growing follicles, and 20 to 50 antral follilces (Yang et al., 1998).

Oocytes are encased in ovarian cells to provide a 'niche' for oocyte maturation. These cells include granulosa cells and theca cells. In primordial follicles, a single layer of flattened granulosa cells is surrounding the oocyte that is arrested in Prophase I. Those follicles are in the quiescent state. Once they begin growing they will become primary follicles. A hallmark feature of this stage is the cuboidal nature of the surrounding granulosa cells. Secondary follicles have two or more layers of granulosa cells around the oocyte. Tertiary follicles, also called antral follicles, are characterized by several layers of granulosa cells (cumulus and mural cells), a well formed zona pellucida, a theca cell layer and a fluid-filled cavity (antrum). Mature antral follicles are also known as Graafian follicles (Gosden and Lee, 2010; Aerts and Bols, 2010a). The total duration of the development from a primordial to ovulatory follicle is 180 days in the cow. It generally takes 42 to 60 days for an early antral stage follicle to become an ovulatory follicle (Aerts and Bols, 2010a).

Subsets of activated primordial follicles are recruited to develop and potentially ovulate. This process is dependent on a balance between local inhibitors like anti-mullerian hormone (AMH) (Gigli et al., 2005) and activators such as basic fibroblast growth factor (bFGF) (Nilsson et al., 2001), growth differentiation factor 9 (GDF9) and bone morphogenic protein 15 (BMP15) (Knight and Glister, 2001). Estrogen and progesterone also show inhibitory effects on the activation of primordial follicles

(Fortune et al., 2010) whereas gonadotropins do not appear important for preantral follicle development (Knight and Glister, 2001).

The post-antral phase of oocyte maturation requires that oocytes acquire meiotic and developmental competence. The oocyte will resume meiosis after induction by the luteinizing hormone (LH) surge that occurs prior ovulation (Aerts and Bols, 2010a). Epidermal growth factor (EGF)-like molecules are required for meiosis reentry of the oocyte and for the LH action in preovulatory follicles (Park et al., 2012). The primary regulator of this process is the maturation-promoting factor (MPF). Inhibitory factors in the mural granulosa cells maintain a high level of cyclic adenosine monophosphate (cAMP) in the oocyte resulting in a low level of MPF. With the activation by LH surge the oocyte is released from these inhibitory factors, which will reduce cAMP in oocytes and upregulate MPF, resulting in meiosis resumption (Jones, 2004). Meiosis I occurs, yielding a small structure called polar body by asymmetric division. In the cow, the oocyte is arrested again in Metaphase II until fertilization occurs (Aerts and Bols, 2010a).

Developmental competence refers to the ability of the oocyte to mature and support initial embryogenesis. During its growth the oocyte needs to accumulate enough mitochondria, mRNA, ribosomes and proteins, that will enable a successful fertilization and initial cell division in the zygote (Gosden and Lee, 2010; Aerts and Bols, 2010a). During the first few cleavage divisions the embryo must rely on maternally-derived RNA that were stored in the oocyte before ovulation for translation of new proteins (Telford et al., 1990; Goossens et al., 2007).

The LH surge also induces other events that are required for fertilization success. This includes the initiation of cumulus expansion. Expansion of cumulus is required for normal ovulation and fertilization (Aerts and Bols, 2010b). The LH surge stimulates synthesis and organization of hyluronan into an extracellular matrix by the cumulus cells. The accumulation of hyaluronan expands the COC and promotes detachment of the COC from the follicle wall in preparation for ovulation. This secretion makes the cumulus cells sticky and appears to be controlled at the mRNA transcription level (Saito et al., 2000). The LH surge also regulates follicle rupturing, or ovulation. This process is controlled by several transcription regulators. Progesterone receptor (PR) expression is initiated in the mural granulosa cells with the LH surge. And the ovulation process seems to be regulated, in part, by the action of progesterone produced within the oocyte (Natraj and Richards, 1993).

Puberty is determined as the time of first ovulation or estrus and it marks the initiation of reproductive life. *Bos indicus* and *Bos taurus* present differences in age of puberty. The estimated age at puberty (first ovulation or estrus) for Zebu in the tropics and subtropics ranges from 16 to 40 months (Nogueira, 2004), which is much longer than the average of 15 months for *Bos taurus* breeds (Randel, 1990). This is attributed to both genetic and environmental factors including nutrition, disease, temperature, humidity and season of birth. To attenuate this effect the crossbreeding is used. Brangus heifers have an average of 17 to 18 months of age at puberty (Randel, 1990).

Another interesting difference in oocyte maturation process is that oocytes from *Bos indicus* animals are less subjected to heat stress and more likely to develop into an

embryo after fertilization than oocytes from *Bos taurus* animals in tropical and sub-tropical conditions during the hot seasons (Camargo et al., 2007).

Follicle Dynamics

In the cow, estrous cycle is characterized by follicle growth waves, where only the last wave will result in ovulation. After the development of the antral oocyte, the follicle requires the pituitary gonadotropins follicle-stimulating hormone (FSH) and LH for growth and development. In every growth wave, several antral follicles are recruited in a cohort. Only one follicle will acquire dominance, grow, and will undergo atresia or ovulation depending in which follicular wave it is.

A cohort of antral follicles is recruited with the increase in levels of FSH. This hormone will promote the proliferation and prevent atretic degeneration of the early antral follicles. The follicles will continue to grow with the FSH influence until they reach 3mm. By the size of 5mm they produce estradiol and inhibin, which are FSH inhibitors. At this period one follicle will be more developed than the others and will acquire dominance, whereas the remaining follicles will undergo atresia (Aerts and Bols, 2010b).

The dominant follicle is the primary FSH inhibitor and can optimize the utilization of low concentrations of FSH. Also, the dominant follicle undergoes a transition in gonadotropin dependency from FSH to LH, enabling to survive and mature despite the low concentrations of FSH. Increased estradiol by the dominant follicle will positively affect the pulsatility of LH leading to its surge. With the absence of a functional corpus luteum (CL), this surge will stimulate the ovulation process (Ginther et al., 2001).

The follicular dynamics of *Bos indicus* and *Bos taurus* cows is overall very similar with only a few differences. Ovarian follicular dynamics in *Bos indicus* cattle is

characterized by the occurrence of two, three or sometimes four waves of follicular development versus the predominantly two follicular waves observed in *Bos taurus* breeds (Bó et al., 2003). The dominance period is similar between breeds, but *Bos indicus* cows have smaller follicular size and greater follicular number than *Bos taurus* (Bó et al., 2003). Also, Brahman cows have greater plasma IGF-1 concentrations and reduced FSH concentrations than Angus cows (Alvarez et al., 2000). It was hypothesized that the increased IGF-I is responsible for the greater follicular number in *Bos indicus*-based breeds (Alvarez et al., 2000). The plasma LH concentrations during the preovulatory LH surge is also reduced in Brahman than *Bos taurus*-based breeds (Randel, 1990; Bó et al., 2003).

Bos indicus cows often present decreased pregnancy rate than *Bos taurus* females due to their reduced ovulation rate (Carvalho et al., 2008). However, if they show estrus, *Bos indicus* fertility tend to be very similar to *Bos taurus* animals (Randel, 1990). Estrus is the period that the female accepts to be mounted and its behavior is induced by high levels of estrogen. It was reported that Brahman and Brahman-based cows have shorter and less intense estrous behavior and they ovulate earlier than Angus cows (Randel, 1990). Also, the zebu cows take the longest to show estrus after exogenous estradiol injection than Hereford cows. This explain the fact that Brahman cows have a shorter period from the onset of estrus to ovulation than Hereford cows (Randel, 1990). However, more recent data using modern heat detection system didn't observed differences in interval from estrus to ovulation between cow genotype (Bó et al., 2003). Therefore, further studies are required to confirm this observation. Estrus is also affected by social hierarchy in *Bos indicus* cows (Chenoweth, 1994). Dominant

cows tend to delay estrus expression after induction of luteolyses and are less likely to stand to be mounted (Bó et al., 2003). Therefore heat detection is a challenge in *Bos indicus* cows.

Bos indicus cows were also reported to have their cyclicity affected by seasonality (Chenoweth, 1994). Anestrus and anovulatory estrus were reported to be increased during winter in Brahman cows. Also, conception rates are greater in summer than in fall in Brahman cows (Bó et al., 2003).

In order to overcome the difficulty of estrus detection in *Bos indicus* cows, protocols of estrous synchronization is an important tool to improve pregnancy rates in zebu herds. When cows are synchronized with prostaglandin F2, duration of estrus does not differ between Angus and Brahman cows (Bó et al., 2003). However, variability in response to hormonal treatments and the time and effort required to perform treatments, particularly in *Bos indicus* cattle, limit the widespread application and success of these technologies. Synchronization protocols done to date have still not solved the variability in response with *Bos indicus* and since their concentrations of reproductive hormone are significantly decreased than *Bos taurus* cows, protocols using reduced doses or splitting the hormone dose might be needed (Bó et al., 2003; Bridges et al., 2005).

Fertilization

Fertilization is an orchestrated process where female and male gametes fuse and generate a new organism. For natural mating, the cow needs to be receptive to the bull by showing estrus behavior. Estrus expression is triggered by the actions of estradiol on the hypothalamus. Estrogen's relationship with the LH surge synchronizes estrus behavior and ovulation so that semen deposition occurs prior to or during

ovulation. Its intensity and duration depends on the individual behavior and social interactions among cows (Lucy, 2001). For the past 60 years artificial insemination (AI) has been used in place of natural mating to a large extent in the dairy industry and a limited degree in the beef industry. This procedure improves genetic selection for specific traits and can provide a vast improvement in genetic potential of the offspring. Much work has been completed to maximize fertilization rates in cattle, which requires timely sperm deposition. Timed AI has been extensively used in order to improve fertilization rates in cattle. Timed AI programs consist in synchronize follicular cycle and ovulation by using extrinsic hormone therapy. This reproductive tool facilitates fertilization by giving the opportunity to inseminate cows in a fixed time without estrous detection.

Once spermatazoa reach the oviduct in the female reproductive tract, they interact with a binder of sperm protein (BSP) in the epithelium surface where they undergo biochemical processes that provides them with the capacity to fertilize, a process known as capacitation (Suarez, 2008; Sutovsky, 2009; Ikawa et al., 2010). Spermatazoa are stored in the oviduct for several hours to several days depending on the species. Normally, spermataozoa are released gradually to limit polyspermy.

After ovulation of the cumulus oocyte complex, spermatozoa will swim through the cumulus cells. An acrosome reaction ensues once the sperm reach the zona pellucida (ZP). The acrosome is a Golgi-derived organelle that covers the tip of the sperm head, and its activation releases enzymes that degrade the acrosome and cause an inner border of sperm membrane specific set of antigens to present themselves to the ZP (Ikawa et al., 2010). Sperm antigens bind to glycoproteins in the

ZP that triggers the completion of the acrosome reaction leading to the ability of penetrating the ZP and fuse to the egg's membrane and posterior haploid nucleus fusion of egg and sperm (Wassarman and Litscher, 2008). Once the sperm succeed in bind and fuse with the oocyte membrane, cortical granules within the oocyte are activated to prevent further spermatozoa penetration, or polyspermy. The resumption of Meiosis II also is induced by sperm fusion.

Early Embryo Development

After fertilization, the zygote undergoes a series of developmental steps before it attaches to the uterine lumen, which occurs on or after day 19 of gestation in cattle (Ealy and Yang, 2009). The 1-cell embryo begins cleaving to form multiple cells, termed blastomeres. After 2 to 3 cleavage events (i.e. 8-16-cell stage), the embryo is transported from the oviduct to the uterus for further development (Telford et al., 1990).

At the 8-16 cell stage, the bovine embryonic genome begins to be transcribed, and this embryonic genome activation than allows the newly formed organism to orchestrate its own fate. This event involves degradation of the maternal RNA and proteins that was accumulated during oocyte maturation, demethylation of the maternal and paternal DNA, remodeling of the embryonic new DNA and, finally, activation of the embryonic genome by transcription factors (Li et al., 2010).

Blastocyst Formation

As additional cleavage divisions continue, the embryo will develop into a clump of uniform cells known as the morula. Blastomeres then begin to compact and differentiate and eventually form a blastocyst comprised of trophoblast cells along the outer border (trophectoderm) and non-differentiated cells within the inner cell mass (ICM) (Watson et al., 1999). This differentiation process is a result of increase in blastomeres polarity by

cell-to-cell contact and asymmetric division that will lead to the first cell fate decision and set the two populations of cells (Zernicka-Goetz et al., 2009).

The trophectoderm is the outer cells layer that will develop in the fetal part of the placenta once implantation occurs. It is the first differentiated cell type of development (Duranthon et al., 2008). During the blastocyst formation the water has an osmotic movement into the extracellular space of the embryo caused by the Na/K-ATPase confined in the trophectoderm basolateral membrane and facilitated by basal and apical molecular water channels called aquaporins (Watson et al., 1999).These events combined with the establishment of a trophectoderm tight junctional seal makes possible the blastocoel cavity formation (Duranthon et al., 2008).

The ICM is a population of cells positioned inside of the embryo that will retain the pluripotency of the cells. It will give rise to the entire fetus and extraembryonic tissues (Zernicka-Goetz et al., 2009). In the bovine species, blastocysts are usually formed on day 7 of gestation (Lindneri and Wright Jr., 1983).

The next stage in conceptus development is blastocyst hatching. This occurs on day 8-9 of gestation (Rodríguez-Alvarez et al., 2009). This process consists in the loss of the zona pellucida by rupture and hatching after blastocyst growth (Spencer et al., 2004). This period also coincides with a second cell fate decision, where the ICM cells in contact with the blastocoel cavity tend to differentiate into primitive endoderm (PE). The PE is a monolayer of cells on the surface of the ICM that will further differentiate into visceral and parietal endoderm that will form the extraembryonic membranes (Gasperowicz and Natale, 2011). The inner ICM cells, or cells that make up the epiblast,

maintain their pluripotency and become progenitors of other cell types that make up the fetus (Zernicka-Goetz et al., 2009; Gasperowicz and Natale, 2011).

The process of embryo development is similar to all bovine breeds. Some differences can be seen in blastocyst development rate between *Bos indicus* and *Bos taurus* under heat stress conditions. Due to their heat tolerance, zebu breeds have greater blastocyst development in heat stress conditions than *Bos taurus* breeds (Hernández-Cerón et al., 2004). The oocyte plays a more important role in the thermotolerance of the *Bos indicus* blastocyst than the spermatozoid (Block et al., 2002). It was speculated that the thermotolerance of a crossbreed embryo is due to the beneficial effect of heterosis, which can be found in vivo but not always in vitro development (Barros et al., 2006).

Gastrulation and Conceptus Elongation

Around day 14 post-fertilization the bovine epiblast differentiates further as gastrulation takes place. In this stage the pluripotent epiblast differentiates into three cell layers: endoderm, mesoderm, and ectoderm. Each cell germ layer will give rise to specific tissues and organs in the developing calf (Vejlsted et al., 2006). The ectoderm will differentiate into surface ectoderm and the major part will transform in neural ectoderm beginning the neurulation process. The neural ectoderm formation progresses gradually in a cranial-caudal direction.

The mesoderm germ layer will differentiate into somites and extraembryonic mesoderm. The inner layer of extraembryonic mesoderm will form an extraembryonic membrane called yolk sac with the endoderm, and the outer layer will form the chorion with the trophectoderm. The chorion will rise up from around the embryo creating a fluid-filled extraembryonic space to protect the embryo called amnion. The mesoderm

also forms the allantois that is the extraembryonic membrane that will vascularize the chorion and amnion (Schlafer et al., 2000). The endoderm germ layer will differentiate into primitive guts and portions of allantois (Vejlsted et al., 2006).

Concomitant with gastrulation the embryo starts the elongation process, which is the lengthening and morphological transition from ovoid to filamentous stage. This expansion of the trophoblast begins around day 13 to 15 of gestation in cattle. During this period the bovine conceptus size increases more than 100 fold and can extend to the entire length of both unterine horn (Blomberg et al., 2008). This extensive surface contact with the uterine lining increases placental surface area that is important for feto-maternal communication and exchange of nutrients essential for conceptus well-being (Blomberg et al., 2008).

Maternal Recognition of Pregnancy

Concomitant with the expansion of trophectoderm maternal recognition of pregnancy have to occur around day 16 of gestation in cattle. To enable maternal recognition the conceptus trophectoderm has to produce and release interferon-tau (IFNT) in sufficient amounts (Bazer et al., 2009). The expression of IFNT occurs during pre- and peri-implantation, with an increase surge at day 14-15 of gestation in cattle. This period is coincident with conceptus elongation, therefore the extensive trophectoderm mass is important not only for conceptus attachment and placental formation but, also, for large amounts of IFNT production. The expression of IFNT rapidly decreases after day 21 of gestation in cattle, coincident with trophectoderm attachment to the maternal uterus (Ealy and Yang, 2009).

In order to maintain the pregnancy IFNT will act as antiluteolytic factor. In cattle, IFNT will down-regulate oxytocin receptors in the uterus endometrium, which are up-

regulated by the follicular secretion of estradiol. The down-regulation of oxytocin receptor will inhibit the CL released oxytocin to bind to its receptor that is up-regulated in the endometrium by the release of estradiol by the follicles. This inhibition will prevent pulses of uterine prostaglandin (PG) $F_{2\alpha}$ release to occur, sustaining the CL function. The $PGF_{2\alpha}$ is a luteolytic factor that is normally released by the endometrium of non pregnant cows at days 17 to 20 post-estrus to cause CL regression and ovulation of the dominant follicle (Thatcher et al., 2001).

IFNT also acts as a luteotrophic factor by promoting endometrial production of PGE_2 without impacting $PGF_{2\alpha}$ production, therefore increasing the $PGE_2/PGF_{2\alpha}$ ratio. PGE_2 is a luteotrophin and luteoprotectant prostaglandin that is produced by endometrium and conceptus to maintain luteal function. The luteotrophic and antiluteolytic actions of IFNT are indispensable for establishing and maintaining pregnancy in cattle (Ealy and Yang, 2009).

Fetal Development

After the conceptus is fully elongated the chorion will extend throughout the whole uterine lumen at day 23 of gestation. At this period the allantois develops visibly with an initial vasculature formation and the yolk sac which arises from the ventral part of the embryo becomes prominent. Between days 20 to 30 of gestation the embryo shows a typical C-shape body form and optic and otic vesicles, as well as first branchial arc and heart prominence are formed. At day 25-26 of gestation three brain vesicles, bud of forelimb and bud hindlimb appear in cattle embryo (Assis Neto et al., 2010).

Around day 30 of gestation the bovine embryo acquires a sausage-like appearance. The embryonic eyes begin to acquire pigmentation, and the olfactory pits and hand plate are present with subsequent forelimb digits formation. At this period the

bundle that comprises the allantois, yolk sac and ventral amnion wrapping them, becomes longer, thereby forming the umbilical cord. Short after that the eyelids are formed and genital tubercle is present. Amniotic growth begins and is more distinct after day 40 of gestation, when embryo sexual differentiation occurs (Assis Neto et al., 2010).

After day 42 of gestation the nomenclature changes from embryo to fetus with the organogenesis. From day 42 to 50 of gestation the tongue and digits become visible and the eyelids begin to cover eyes. Around day 50 of gestation the fetus acquires an elongated neck that bends by about 90^0 to the axis of the head. Also, most allantoic vessels are observed near the fetus and progressively become thinner towards the gestational sac extremities (Assis Neto et al., 2010).

From days 50 to 70 of gestation the amnion does not change in size in relation to the fetus. The umbilical cord becomes as long as the hind leg with the umbilical vessels (allantoic arteries and veins) being evident. In most cases, the yolk sac disappears completely by day 70 of gestation in cattle (Assis Neto et al., 2010).

Although all bovines have the same process of intrauterine development, some differences can be observed according to their genotypes. O'Rourke et al., 1991 observed that *Bos indicus* fetuses develop more slowly during mid-gestation but faster on later gestation than *Bos taurus* fetuses, suggesting a phenomenon of 'catch up' on growth. Also, Ferrell, 1991 in an experiment with *Bos indicus* and *Bos taurus* cows pregnant with both fetal genotypes concluded that fetal genotype primarily dictates fetal growth and that maternal genotypes plays a greater role later in gestation.

Corpus Luteum and Progesterone

In order to maintain pregnancy the formation of a functional CL that will produce sufficient amount of progesterone is indispensable. The CL is a transient endocrine

gland formed following ovulation from the secretory cells of the follicle (Rekawiecki et al., 2008). Before ovulation the LH production and release by the hypothalamus will prepare follicular granulosa and theca cells for the luteinization process after ovulation of the oocyte. In this process cumulus cells begin synthesis of hyaluronic acid and hydration that will enable cumulus expansion through enlargement of the space between granulosa cells. The LH pulses before its surge are necessary for CL formation in cows, but are not required for maintenance of luteal function (Aerts and Bols, 2010b).

Progesterone is transferred locally from the ovarian/oviductal venous drainage to the uterine artery in cattle. This local transfer results in higher concentrations of P4 within the uterus ipsilateral to the CL (Lucy, 2001). Sufficient amounts of P4 produced by the CL are required for establishing and maintaining pregnancy. Elevated concentrations of circulating P4 in the immediate post-conception period are associated with conceptus elongation by affecting uterine tissues and secretion, increase in IFNT production through supporting the elongation process, maternal recognition by the decrease in P4 receptors in the endometrium and higher pregnancy rates in cattle. Also, the high expression of P4 during the luteal phase of the estrous cycle is responsible to inhibit any further ovulation of a dominant follicle (Lonergan, 2011).

Although follicular dominance is similar between *Bos indicus* and *Bos taurus* cows, maximum diameters of the dominant follicle and CL are smaller in *Bos indicus* than in *Bos taurus*. The smaller CL size is probably due to a decreased capacity for LH secretion. This leads to reduced progesterone produced by the CL in zebu animals (Segerson et al., 1984; Bó et al., 2003). However, effects of cow genotype in CL size and plasma P4 concentrations were shown to be also seasonal. *Bos indicus* animals

have bigger CL and greater P4 concentrations during summer and spring, and smaller CL and reduced plasma P4 concentrations during fall and winter (McNatty et al., 1984; Alvarez et al., 2000).

Placental Development

The placenta is an amazing organ that serves for the sole purpose of supporting pregnancy to term and provide nutrients and immune components for fetal development and birth (Schlafer et al., 2000). The ruminant placenta is classified as cotyledonary and synepitheliochorial (Wooding, 1992). Implantation begins once the conceptus is fully elongated and the maternal recognition has occurred. This usually occurs on or after day 19 of gestation (Blomberg et al., 2008; Rodríguez-Alvarez et al., 2009). Development of the placenta, a process known as placentation, is diverse among mammals but most mammals undergo the same series of events. These include trophoblast appostion to the uterine lumen followed by trophoblast adhesion and eventually trophoblast invasion (Schlafer et al., 2000).

Once the conceptus is fully elongated implantation will be achieved by apposition and further adhesion and invasion of the trophectoderm cells to the endometrial luminal epithelium followed by binucleate cells formation. The apposition process is possible due progesterone actions downregulating mucin-1 in the endometrium, which will, in turn, express other endometrial proteins involved in the trophoblast adhesion process, such as glycosylated cell adhesion molecule 1 (GlyCAM-1), galectin-15, osteopotin (OSP) and integrins. Galectin-15, GlyCAM-1 and OSP are adhesion proteins secreted by the epithelium that will mediate the adhesion between trophoblast and endometrial cells. Integrins act as membrane receptors of maternal and conceptus cells that will bind adhesion proteins (Spencer et al., 2004). Once adhesion is completed the invasion

phase begins. In the ruminant, this last phase is not very invasive. Binucleate cells will form in the trophectoderm and migrate forming a hybrid trinucleate cell with the fetus trophectoderm and maternal endometrium (Bazer et al., 2009).

Binucleate trophoblas cells (BNC), also called trophoblast giant cells, are cells of the trophectoderm first seen at the beginning of implantation and continue throughout pregnancy in all ruminants. They represent 20% of trophoblast cells throughout gestation. BCS are produced by mononuclear trophoblast cells that went through a mitotic process without cytokinesis leading to a cell with two nuclei (Igwebuike, 2006). Also, about 15-20% of these BNCs are mature (containing a large rough endoplasmic reticulum, Golgi body and granules occupying more than 50% of their volume) and migrating at any time point of gestation until right before parturition (Wooding, 1992). Whereas the remainder are in the maturation process with the whole cytoplasma re-arranged in a way that it doesn't have any contact with the basement membrane or the apical trophectoderm tight junction (Wooding, 1982).

Once mature all BNC migrate from the chorionic epithelium and through the apical trophectoderm tight junction, they fuse with uterine cells of the maternal side of the placenta and form a feto-maternal syncytium. At implantation the maternal caruncles are covered by feto-maternal derived syncytium. In the cow, this syncytium doesn't persist and eventually is replaced by uterine epithelial cells and transient trinucleate cells (Wooding, 1992). Rather, BNCs fuse with single epithelial cells to form trinuclear feto-maternal hybrid cells that will eventually die and be reabsorbed after releasing their granules in the maternal circulation (Wooding, 1982; Wooding, 1992). This migration

and syncytium formation are thought to be important in the establishment and maintenance of the placenta (Igwebuike, 2006).

The second main function of the BNC is the endocrine role that they play during gestation. Migration and fusion of BNC with maternal epithelial cells are important mechanisms for the purpose of delivering the BNC granules contents into the maternal system. It is thought that this is probably the primary function of the BNC, since they die right after releasing their granules (Wooding, 1982). These granules contain hormones associated with pregnancy that are produced by the BNC, such as placental lactogen, prolactin-related protein-1 (PRP-1), progesterone, estradiol and pregnancy-associated glycoprotein (PAG) (Wooding, 1982; Igwebuike, 2006).

The trophectoderm is formed by binucleate and mononucleate trophoblast cells. The mononucleate cells (MNCs) are the precursors for the BNC. They comprise approximately 80% of the bovine trophectoderm throughout gestation and serve as the proliferating cell type within the placenta. These cells are located in the basal lamina. They have their apical surface modified to form the interdigitations with the maternal epithelium and are crucial in fetal nutrition, by phagocyting the uterus histotroph (also called uterine milk), which is a complex mix of enzymes, growth factors, cytokines, hormones transport proteins, and other substances necessary for the nourishment of fetus (Igwebuike, 2006).

Bovine conceptus trophoblast cells begin to differentiate around day 17 of gestation into MNC and eventually BNC. This histological morphology is different for other animals and is used as a differentiation marker. Rodents and primates have a haemochorial placenta where maternal blood comes in direct contact with the

trophoblast whereas many carnivores have an endotheliochoral placenta characterized by complete erosion of endometrial epithelium with maternal capillaries directly exposed to epithelial cells of the fetal chorio. By contrast, pigs and horses contain an epitheliochoral placenta, which involves a complete intact layer of epithelium in both maternal and fetal components. Ruminants have a placenta that is best characterized as being synepitheliochoral. This placenta endometrial epithelium cells are displaced by a syncytium in early placentation that will be later displaced by regrowth of the mononuclear cell uterine epithelium, leading to a co-existence between epithelium and syncytium (Wooding, 1992; Senger, 2003; Igwebuike, 2006).

Placenta is also classified based on the gross anatomy. The ruminant placenta is classified as cotyledonary, because is formed by localized special round structures (cotyledons) where the fetal-maternal interaction occurs. In the cow, cotyledons formation begins at about four weeks of gestation. The extraembryonic membrane called chorioallantois, which has as its epithelial cell layer, the trophectoderm, attaches in the uterus and starts to form cotyledons that form villous projections that interdigitate in the irregular caruncle surface, enhancing the contact area between these two membranes. This adhesion of fetal and maternal tissues, cotyledon and caruncle respectively, is called placentome. The placentome is the functional unit of feto-maternal exchange that enables supporting the increasing fetal metabolic demands. In the cow, the number of placentomes varies between 70 to 120 during the whole gestation, they spread throughout the whole uterus and appear in different sizes, being the largest ones around the fetus (Schlafer et al., 2000). Other species have different organization of the villi, i.e. pig and horse have a diffuse placenta, carnivores have the

villi tightly grouped into a band (zonary), and primates and rodents have one single big cotyledon and is classified as discoid (Cross et al., 2003).

Bos indicus and *Bos taurus* animals also present some differences in the placenta and placentation process. Ferrell (1991) descibed differences in contributions of the maternal and fetal genotypes on intra-uterine development of *Bos taurus* and *Bos indicus* cattle. It was transferred day 18 Charolais and Brahman embryos into Charolais and Brahman cows to get the four possible combinations between fetus and cow breeds. Half the animals were slaughter at day 232 and half at day 271 of gestation. It was concluded that maternal uterine environment may constrain fetal growth, particularly in late gestation. Also, *Bos indicus* genotype had greater placentome mass than *Bos taurus* genotype. This difference was not observed in placentome number. Another study determined that uterine luminal proteins as well as uterine myometrium and endometrium thickness, number of glands and uterine luminal epithelial cell height were greater in in Angus than Brahman cows at the same gestational age (Segerson et al., 1984). All these data combined with the fact that the mortality rate of the Brahman new born tends to be much higher than *Bos taurus* (Reynolds et al., 1980) could be an indicative that *Bos indicus* animals may suffer from placental incompetency in early gestation.

The placenta is the unit of fetal-maternal exchange, and its failure to properly form and maintain throughout the whole pregnancy is one of the main cause of pregnancy loss. To follow is a review of pregnancy loss in different time points of gestation.

Pregnancy Maintenance and Loss

The previous emphasis on describing events relating the establishment and maintenance of pregnancy in cattle is important because pregnancy losses have devastating economic impacts on livestock producers, and especially those in the United States. In a recent review, Santos et al. (2004) estimated that up to 60% of all fertile matings will not develop to term in lactating dairy cattle. Also, cows that abort a pregnancy are 5 times more likely to abort subsequent pregnancies (De Vries, 2006). Therefore is extremely important to understand the nature of these losses so that schemes to revert this process can be developed.

The Committee on Bovine Reproductive Nomenclature (1972) established definitions for the various forms of pregnancy loss in cattle. The embryonic period of gestation extends from conception to the end of the differentiation stage, at approximately 42 days of gestation in cattle, and that the fetal period extends from gestation day 42 to the delivery of the calf. Therefore pregnancy loss is classified into embryonic loss or fetal loss. The first one is responsible for the higher rate of pregnancy loss in cows and is subdivided into early embryonic death that includes the period of conception to implantation (day 24 of gestation in cattle) and late embryonic death, which is from day 24 to 42 of pregnancy (Santos et al., 2004; López-Gatius et al., 2007b).

Several factors are responsible for the high pregnancy loss in cattle in the United States. Bacteria, viruses, fungi and protozoa are potential infectious sources of conceptus loss, whereas toxins, genetic defects and stress can be noninfectious inducers of pregnancy loss (Whitlock and Maxwell, 2008). This following review will focus on noninfectious factors that impact pregnancy failure in cattle.

Early Embryonic Loss

A large portion of these losses occur during the first week of gestation and likely are caused by issues relating to oocyte competency (Lucy, 2001; Santos et al., 2004), genetic abnormalities of the zygote/embryo, and uterine insufficiency (King, 1991). In dairy cattle, only 65% of the fertilized eggs are considered viable at days 5-6 of gestation (Santos et al., 2004).

Substantial pregnancy losses also occur in the second and third week of gestation. Thatcher et al. (2001) estimated that 40% of all fertile matings are lost at this time. This is not surprising given that IFNT signaling must occur at this time and conceptus- or uterine-related issues that retard conceptus elongation and IFNT production will prompt return to estrus and pregnancy loss. Also, the epiblast is beginning to form germ cells and undergo gastrulation during this period, and issues with this development will induce pregnancy loss (Santos et al., 2004; Ealy and Yang, 2009).

Late Embryonic Loss

In cattle, late embryonic death is not numerically high, but consists in a big economical loss to producers, because it is often too late to rebreed the females in a breeding season system (Diskin and Morris, 2008). Therefore, pregnancy rates by the end of this period are, normally, a good estimative of calving rates (King, 1991). Santos et al., 2004, in an analyses of several studies, observed that late embryonic loss after day 27 of gestation was very variable, ranging from 3.2% in dairy cows producing 6000–8000 kg of milk per year in Ireland to up to 42.7% in high producing cows under heat stress, but it normally stays around 10% of pregnancy loss (Whitlock and Maxwell, 2008).

Losses within this period usually are a result of failure in the attachment of the developing placenta to the uterine wall. However, since many fetal developmental events occur at this stage, namely organogenesis, it is possible that a fair amount of this loss may be caused by lethal embryonic abnormalities (Stevenson, 2001).

Fetal Loss

In cattle fetal loss rate is not very great. Dairy and beef cows have a fetal loss rate of up to 11%. In heifers this rate drops to 4.2% and 2.5%, respectively (Santos et al., 2004; Whitlock and Maxwell, 2008). Approximately half of the losses after day 42 are caused by trauma or infections. The remaining losses are largely unexplained.

The cost of fetal losses increases dramatically with increase of gestation. De Vries (2006) observed that for an average milk production cow in the first lactation the cost of a pregnancy loss in the first, fourth, and seventh month of pregnancy were U$110, 279 and 578, respectively. These numbers were found to increase with greater milk production cows, greater lactation number and greater days open, leading to a revenue loss of U$1,055 in a high producing cow in the third lactation that lost the pregnancy at seven months of gestation.

Diagnosis of Pregnancy Losses

In order to increase herd efficiency and optimize cattle production revenue, pregnancy diagnosis need to be implemented in beef and dairy farms. Due to the high incidence of pregnancy loss, diagnosis of pregnancy should be done at least twice. Ideally, the first should be performed as early as possible to identify pregnant and, more importantly, open cows and re-enlist them to minimize revenue loss and the second at some point after day 60 of pregnancy, since a low number of cows will undergo fetal loss (Stevenson, 2001).

As stated above, the key component in reproductive management is the diagnosis of open cows following insemination. Several different methods can be used to achieve such purpose, including transrectal palpation, transrectal ultrasonography and pregnancy-associated glycoproteins (PAGs) detection. The following section will describe PAGs molecules, their classification and profile during pregnancy as well as fluctuations within the bovine species. Also, it will be discussed their potential functions and utilization in the cattle industry.

Pregnancy-Associated Glycoproteins

The first members of the PAG family of proteins were identified by Butler et al. (1982). For that work bovine cotyledons were homogenized and protein lysates were used to inoculate rabbits. The antisera produced reacted with two pregnancy-specific antigens: pregnancy-specific protein A (PSPA) and pregnancy-specific protein B (PSPB). PSPA turned out to be alpha fetal protein. PSPB represented a novel protein that was not similar with other known pregnancy-associated molecule. Later on a different group of researchers (Zoli et al., 1991) purified what turned out to be a similar antigen with the same plasma profile during gestation. This molecule was named pregnancy-associated glycoprotein or PAG. Eventually it was discovered that the PAG-1 molecule had very similar properties when compared with PSPB (Green et al., 1998). Therefore PAG is a better term to use instead of PSPB. Additional antigens were later identified in the blood of pregnant sheep, mule deer, white tail deer, and muskoxen, suggesting they exist in all ruminants (Green et al., 1998).

The eventual cDNA sequence determination and inferred amino acid analysis of PAGs indicate that they are part of a large group of placental aspartic proteinases (Zoli et al., 1991) that are related to pepsinogens. In fact, bovine PAG-1 (bPAG-1) is 50%

similar in amino acid sequence to pepsinogen-A at the amino acid level (Green et al., 1998). Although PAGs have conserved the substrate binding cleft responsible for the catalytic activity throughout evolution, many of them, and especially those that are released into the maternal bloodstream are enzymatic inactive and incapable of acting as classical proteinases because of mutations within the conserved catalytic site (Green et al., 1998; Szafranska et al., 2006).

Phylogenetic analyses of PAG and PAG-like genes indicated that the PAG family originates from an ancient PAG-like precursor that underwent duplication and positive selection approximately 86 million years ago (Szafranska et al., 2006).They represent one of the major trophoblast secretory products in *Artiodactyla* family (cattle, swine, ovine, deer, goat etc.). PAG-like molecules were also found in other families, including the horse (*Perissodactyla*), and canines and potentially other members of the *Carnivora* family. The discovery in this last family, which is a group that have diverged from ungulates 100 million years ago, shows the ancient origin of this placental molecules (Green et al., 1998). The great degree of sequence conservation of these genes among ungulates and other species suggests they may contain vital functions during pregnancy (Szafranska et al., 2006). Potential functions for PAGs will be discussed later.

The PAGs found in ruminants can be categorized into two main groups based on their site of expression within the placenta. One group is referred to as the 'ancient PAGs'. When the evolutionary pathway is tracked back it's noticed that the 'ancient PAGs' have arisen more than 80 million years ago, suggesting that it was created with the appearance of the Artiodactyla family (Telugu et al., 2009). These PAGs are expressed in both MNCs and BNCs. Some of these PAGs include PAG-2, -8, -10, -11,

-12 and -13. Most of PAGs in the ancient group contain enzymatic activity (Telugu et al., 2009).

The second PAG grouping is termed the ‘modern PAG’ group. The ‘modern PAGs’ have arisen by 50-55 million years ago. This could suggest that they were created with the ruminant divergence from swine (Telugu et al., 2009). These PAGs are expressed primarily by BNCs and as such make up the majority of PAGs found in maternal blood during pregnancy. Some of these PAGs include PAG-1, -3 to -7, -9 and -14 to -21. It appears that all of the modern PAGs are inactive proteases. Although many of them may be able to bind certain proteins they appear unable to cleave proteins (Green et al., 2000; Telugu et al., 2009).

Plasma PAG Profiles in Cattle

Following the initial discovery of PSPB by Butler et al. (1982), the same laboratory determined the profile of PSPB concentration in serum of Holsteins cows after developing an RIA specific for PSPB and several other PAGs (Sasser et al., 1986). PSPB was detectable in all animals by day 24 post-breeding and could be detected throughout gestation and into the early and mid postpartum period. The PSPB profile is interesting. Its plasma concentrations increase fairly rapidly between day 24 and 40 post-breeding, then they decrease until around day 60 before beginning to increase again. At parturition there is a rapid surge in PSPB concentrations that peaks between 10 before to the day of parturition and averages approximately 500 ng/ml.

In 1991, Zoli et al., classified the PSPB molecule as PAG. Further Xie et al. (1994) identified another trophectoderm molecule similar to the PAG found by Butler and Zoli, but different enough to be named PAG-2. After this study the PSPB begun to be called

PAG-1. In the last 15 to 20 years a lot of research was done to better understand these molecules and several others PAGs were discovered (Green et al., 1998).

In ruminants, PAGs constitute a large and diverse gene family that is expressed in the trophectoderm throughout the entire pregnancy (Szafranska et al., 2006). It has become clear that there are probably more than 100 PAG genes and cattle have 22 distinct PAG cDNAs in the Genbank (Telugu et al., 2009). PAGs have long half-life and are expressed differently throughout gestation (Green et al., 2000; Szafranska et al., 2006), i.e. Telugu et al. (2009) showed that bPAG-8 and bPAG-10 had relative temporal expression profiles exactly opposite from each other.

In a relatively new study from Haugejorden et al. (2006), unbred Norwegian Red cows were bled between day 0 and 138 postpartum to look at the prevalence of PAG in the cow circulation after parturition. Blood samples from newborn calves were collected before and after colostrum intake to determine if maternal PAG is transferred to the calf. Plasma PAG half-life was estimated 8.9 days, similarly to a previous report from Kiracofe et al., 1993. It was concluded that pregnancy diagnosis can be made with accuracy using PAG concentration by day 28 post AI as long as the voluntary waiting period is at least 60 days, and that PAG seems to be transferred to the fetus and to the newborn calf through colostrum. The assay used in both studies was based on bPAG-1 concentrations. Bovine PAG-1 is highly expressed late in gestation and during parturition explaining the extremely long half-life (Green et al., 2000).

The concentration of circulating PAGs is affected by several factors (Sasser et al., 1986; Green et al., 2005). Concentrations of PAG are greater in twin-bearing than singleton cows (Patel et al., 1995). An interaction effect between cow breed and fetus

gender on plasma PAG concentrations is also observed. Zoli et al., 1992 observed that Hereford cows carrying female fetuses had greater PAG concentrations than cows carrying males. Also, Holstein cows and heifers carrying male fetuses had greater PAG profiles than the ones carrying females. Cows producing more milk have lower PAG concentration, especially on day 63 of gestation (López-Gatius et al., 2007a). Chavatte-Palmer et al., 2006 found greater PAG concentrations by day 35 of gestation in cows carrying nuclear transfer clones embryos then cows carrying normal embryos. Constant et al., 2011 observed the same effect only later in gestation. It was also suggested that concentration of PAGs can probably serve as a marker of placental function by reflecting fetal well-being, since PAG values rapidly decrease as soon as an embryo/fetal death occurs (Szenci et al., 2003; Giordano et al., 2012) and indicating the probability of difficulty in parturition(Dobson et al., 1993; Kindahl et al., 2002; Kornmatitsuk et al., 2002).

There is also some evidence that genotype may influence PAG production (Sousa et al., 2002, 2003). In one study, plasma PAG concentrations in zebu cattle were similar to *Bos taurus* breeds during first two trimesters of pregnancy but were less than *Bos taurus* breeds during the peripartum period. Also, the zebu PAGs had a longer PAG half-life (9.2-10.1 days) than *Bos taurus*. However, this work used a PAG RIA that used antibodies not utilized by other PAG assays. It is uncertain if the same outcomes may exist when using one of these well-validated PAG assays.

Potential Functions of PAGs

According to evolutionary theories, most duplicated genes usually either are quickly lost due to lack of positive selection or accumulate as pseudogenes unless they undergo positive selection for a new biological activity. By this argument PAGs not only

serve critical functions but individual PAGs must contain slightly different and essencial activities (Xie et al., 1997). Although definitive PAG activities have not been described, their ability to interact with peptides likely is an essential component of their activity (Xie et al., 1991). Because of this, they have been proposed to possibly serve as carrier and/or adhesive molecules (Green et al., 1998).

Additional activities have been proposed for these proteins. The 'modern PAG' group has two potential functions. They may act as immunosuppressive factors, speculated that they could be involved in the maintenance of the histoincompatible feto-maternal unit (Wooding et al., 2005; Szafranska et al., 2006). Also, two studies were conducted in United States and Europe, where plasma PAG concentrations of dairy cows with mastitis were investigated from 3 weeks pre- to 5 weeks post-calving. In both studies the oxidative burst activity of polymorphonuclear neutrophil leukocytes (PMN) decreased immediately after peak of bPAG peri-parturition (Dosogne et al., 1999). Furthermore, the proliferation of bone marrow cells was inhibited by bPAG at concentrations of 2,400ng/ml and up *in vitro* (Hoeben et al., 1999).

A second proposed function for modern PAGs may be their ability to act as luteotrophic hormones. Del Vecchio et al., 1990, 1995 observed that bPAG-1 treatment *in vitro* increases PGE_2 production in bovine cells but didn't affect $PGF_{2\alpha}$ concentrations or progesterone. They also suggested that bPAG-1 could be an indirect stimulator of luteal progesterone production through stimulating PGE_2 production. Two studies conducted in ewe endometrium and luteal cells, respectively, was observed the same outcome than in cattle but only later in gestation, suggesting that PAG plays a critical role to maintain pregnancy after 40-50 days of gestation in ewes (Weems et al., 2003,

2007). The ability of PAGs to impact progesterone concentrations is debatable since some studies observe effects in progesterone and others not (Del Vecchio et al., 1996). Interestingly enough auto-immunization of ewes against PAGs didn't produce an impact on the pregnancy or fetus, suggesting that a systemic role may be secondary to their local role at the feto-maternal interface (Egen et al., 2009).

Use of PAGs for Diagnosing Pregnancy

Pregnancy-associated glycoproteins are a direct placental product that are present in the maternal system, and can be quantified by RIA and enzyme-linked immunosorbant assay (ELISA) (Szafranska et al., 2006). Several studies showed that PAG plasma measurement is a very easy method of pregnancy diagnosis with very good accuracy, varying from 86 to 94.8% between day 30 to 35 of pregnancy, depending on the RIA (Humblot et al., 1988; Szenci et al., 1997). Most assays show an accuracy of 90-95% for diagnosing pregnancy by day 27 of pregnancy when a polyclonal ELISA is used (Green et al., 2005, 2009; Silva et al., 2007; Thompson et al., 2010).

Most of the studies utilizing RIA as a quantification method based on the work of Zoli et al., 1992. This assay recognizes and quantifies plasma concentration of bPAG-1, which is greatly expressed late in gestation resulting in confounding pregnancy tests in cows bred early. It was speculated that this assay has a cross-reaction with bPAG-4 explaining the concentrations of PAG around day 25 of gestation (Green et al., 2000). In 2005 a 'sandwich' ELISA was developed. A pool of monoclonal antibodies that recognizes 'modern' bPAG-4, -6, -7, -16, -20 and -21were used as a trapping reagent and a polyclonal antiserum as the primary antibody (Green et al., 2005). There is another bPAG-1 ELISA that is commercial available and it's called BioPRYN

(BioTracking LLC, Moscow, ID, USA). The BioPRYN assay gives a qualitative pregnancy status using three threshold optical density (OD) values (high, low and cut-off) (Piechotta et al., 2011).

According to the company BioPRYN can be used as early as 28 days of gestation with an accuracy of 99% to identify open cows and a 5% of false-positive if used after 90 days post calving (BioTracking, 2012). The great advantage of the 'sandwich' polyclonal ELISA is that since it recognizes several different 'modern PAGs', and not PAG-1, it reduces the problem with detecting the antigens from the last pregnancy by 90 days (RIA and BioPRYN) postpartum to 60 days postpartum (Green et al., 2005). Silva et al., 2007 concluded that pregnancy outcomes based on the PAG ELISA had a high negative predictive value, indicating that the probability of re-synchronize a pregnant cow is very low when using this approach for pregnancy diagnosis.

PAG concentrations also may be used as a predictor of pregnancy loss in cattle (Humblot, 2001; López-Gatius et al., 2007b; Thompson et al., 2010). In one study in Florida, high producing Holsteins that were verified pregnant by ultrasound at day 32 but were not pregnant at day 60 had reduced PAG concentrations by day 30 of gestation when compared with cohorts that maintained pregnancies to term (Thompson et al., 2010). In another study (López-Gatius et al., 2007b), high producing dairy cows with reduced PAG concentrations at day 35 of gestation were 10 times more likely to lose their pregnancy and cows with greater PAG concentrations were 6.8 times more likely to lose their pregnancy than cows with average PAG levels. Moreover, reduced PAG concentrations could predict subsequent pregnancy loss in 57% of the cows, and

the combination of reduced PAGs and reduced progesterone concentrations predicted 92% of the losses (Gábor et al., 2007).

Overall, pregnancy-associated glycoproteins are present in the bovine placenta throughout the whole gestation. It's affected by several different factors and their functions are still not clear. Furthermore PAGs can be used as an early pregnancy diagnosis method to determine the pregnant and, more importantly, the open cows and identify probable future pregnancy loss, therefore re-enlisting in re-breed programs when needed and minimize financial loss by decreasing feed cost of an open cow into the calving season, allowing the producers to make the decision whether to cull or not an open cow.

In summary, revenue of dairy and beef industry relies on milk production and number of calves yearly, respectively. To optimize profitability in both industries cows need to become pregnant as early as possible and maintain pregnancy to term. In both dairy and beef industry fertility is a challenge and is the most costly problem. Heat stress plays a big role in decreasing fertility in tropical and sub-tropical regions, such as Florida. Therefore, the use of *Bos indicus* genetics as a tool to improve fertility in beef production is being largely implemented in such areas of the United States. The *Bos taurus* genetics is the foundation for the dairy and beef industry. It provides great reproductive and productive traits. However those animals struggle in tropical and sub-tropical environments, reducing drastically their performances in such conditions. Conversely the *Bos indicus* genetics are adapted to tropical and sub-tropical environments, with great parasite/disease and heat tolerance. However they have poor reproductive and productive traits. Therefore the most common use for this genetics in

the United States is the crossbreeding, which can incorporate the toughness of the *Bos indicus* with the better traits of the *Bos taurus* genetics. The most used crossbred in United States is the Brangus, which consists in three eights of Brahman (*Bos indicus*) and five eights of Angus (*Bos taurus*). Although both genotypes undergo the same reproductive processes, such as ovarian dynamics and fetal/placental development, some differences are observed affecting fertility per se.

Placenta formation is crucial for maintenance of pregnancy and can only begin once the conceptus is fully elongated. The ruminant placenta has areas of feto-maternal exchange called cotyledons and is a synepitheliochorial with BNC forming the syncytium units. BNC migrate from the fetal to maternal part of the placenta forming a hybrid trinucleate cell that will release hormones in the maternal circulation, such as lactogen, PRP-1, P4, estradiol and PAGs. The placentation process and, therefore, the fetal development are slightly delayed in *Bos indicus* genotype when comparing to *Bos taurus*. This could be an indicative of a placental incompetency of the zebu animals. Placenta formation and maintenance is crucial for carrying a pregnancy to term. The failure of any of the previous reproductive processes will result in failure of establishing and maintaining pregnancy to term.

In order to minimize economic losses with open cows due to either failure of getting pregnant or maintaining the pregnancy, their early detection for management is indispensable. Plasma PAG concentrations is a useful tool for this purpose. PAGs are inactive members of the aspartic family. The role of PAGs in maintaining pregnancies to term is not understood but it is clear that PAG plasma concentrations are correlated with factors that impact pregnancy loss in dairy cattle. These molecules are affected by

several factors and are being used as a pregnancy diagnosis tool and possibly pregnancy loss predictor. Therefore the understanding of these molecules, their actions and variability are necessary in order to overcome infertility and improve the cattle industry.

Rationale

This overview of the literature determines that the establishment and maintenance of pregnancy is a complex process that is prone to mistakes. Problems with various facets of these processes lead to infertility and reproductive inefficiencies in beef and dairy cattle. The *Bos indicus* genotype is needed to improve reduced fertility of *Bos taurus* in tropical and sub-tropical environments. PAGs are molecules that are important for pregnancy and can be used as pregnancy diagnosis. Up to date we are still lacking on information of how PAG concentrations correlates with *Bos indicus* genotype. This thesis provides insights into how these genotypes impact PAG profiles and fetal development by using transrectal ultrasonography and PAG concentrations in *Bos taurus* and *Bos indicus*-based animals in early gestation.

CHAPTER 3
THE INFLUENCE OF *BOS INDICUS* GENETICS ON EARLY FETAL DEVELOPMENT AND PLASMA PREGNANCY-ASSOCIATED GLYCOPROTEIN CONCENTRATIONS

Many cow-calf producers located in hot climates throughout the United States utilize *Bos indicus* genetics to limit the impacts of heat stress, parasites and low forage quality on reproductive efficiency and resulting calf production (Turner, 1980; Hansen, 2004). Various *Bos taurus* x *Bos indicus* crossbreeding strategies and *Bos indicus*-based cattle breeds (*e.g.* Brangus, Braford) are being utilized in the Gulf Coast region (Gregory and Cundiff, 1980). Predicted increases in global temperatures over the next several decades and centuries likely will increase the use of *Bos indicus* genetics. Therefore, continued efforts to understand *Bos indicus* biology and learn how these differences impact beef production is imperative for sustaining beef production systems in the United States and other countries located with hot climates.

Several reproductive events are altered in *Bos indicus* cattle, including pregnancy and embryonic/fetal development (Chenoweth, 1994). Breeds containing *Bos indicus* genetics usually have longer gestation lengths than their *Bos taurus* counterparts (Randel, 1990). Fetuses develop at different rates during mid- and late-gestation between *Bos indicus* and *Bos taurus* (Ferrell, 1991; O'Rourke et al., 1991). Placental development also differs between these subspecies in mid- and late-gestation (Ferrell, 1991). Differences in fetal and placental development between *Bos indicus* and *Bos taurus* during early gestation remains largely undefined. This work describes three experiments completed to examine whether fetal and placental development is influenced by *Bos indicus* genotypes during early pregnancy in beef cattle. Transrectal ultrasonography and plasma pregnancy-associated glycoprotein (PAG) concentrations (Green et al., 1998; Szafranska et al., 2006) were used to examine fetal and placental

development status, respectively. The linkage of these early gestational parameters to gestation length and newborn calf weight also was examined.

Materials and Methods

All animal use was completed in accordance with and was approved by the Institute of Food and Agricultural Sciences Animal Care and Use Committee at the University of Florida.

Experimental Design

Experiment 1: The study was completed at the University of Florida Beef Research Unit (Gainesville, Florida). Ninety primiparous and multiparous beef cows were used. Genotypes were comprised of Brangus cows (n=15) and Angus and Brahman cross-breeds that spanned from >80% Angus to >80% Brahman (n=75). Cows were maintained on Bahiagrass pasture with Coastal Bermudagrass hay. Cows were supplemented with wet brewers grains, soyhulls and millet silage from the time estrous synchronization began until pregnancy diagnosis.

Cows were separated into three groups according to calving date. Estrous synchronization began an average of 86 days postpartum (range: 55 to 397 days) by administering GnRH (100 μg; Cystorelin, Merial, Duluth, GA) and inserting an intravaginal CIDR^{TM} device (Pfizer Animal Health, New York, NY). After 5 days the CIDR was removed and $PGF_{2\alpha}$ (25 mg; Lutalyse, Pfizer Animal Health) was administered. Estrus was observed for 3 days. Cows exhibiting estrus were inseminated 12 h later. Cows not exhibiting estrus at 72 h post-CIDR removal were given 100 μg GnRH and inseminated. Multiple sires contained varying degrees of Angus and Brahman genetics were used in the crossbred herd. In most cases Brangus cows were inseminated with semen from Brangus bulls and cows containing ≤20% on Angus

genotype were inseminate with pure Brahman bulls. Cows returning to estrus within 25 days of insemination were inseminated again.

At a mean gestational age of 53 days (range 48 to 56 days; d 0 = day of insemination), transrectal ultrasonography was completed by a single technician with an Ibex portable ultrasound equipped with a linear 8-5MHz multi-frequency transducer (E.I. Medical Imaging, Loveland, CO, USA). Fetal crown-rump length (CRL) was measured (Chavatte-Palmer et al., 2006) (Figure 3-1 for example). A blood sample was collected at the time of transrectal ultrasonography by coccygeal venipuncture using EDTA-treated Vacutainers (BD Diagnostics, Franklin Lakes, NJ, USA). Blood was placed on ice until centrifugation at 1500g at 4^0C for 15 minutes. Plasma was collected and stored at -20^0C until progesterone (P4) and PAG analysis. At birth, calf weight and gender was recorded and gestation length was calculated.

Experiment 2: The study was completed at the University of Florida North Florida Research and Education Center (Marianna, Florida). Fifty-one primiparous and multiparous Angus (n=17), Brangus (n=25) and Braford (n=9) cows were used. Cows were maintained on Ryegrass and Bahiagrass pasture with *ad libitum* access to mineral supplement (Southern States, Marianna, FL) throughout the study period.

Cows were separated into two groups based on calving date. Estrous synchronization began an average of 67.7 days postpartum (range: 34 to 94 days) by administering 100 µg GnRH and CIDR insertion. After 7 days the CIDR was removed and 25mg $PGF_{2\alpha}$ was administered. After 72h 100 ug GnRH was administered and cows were bred using multiple sires within each breed.

Transrectal ultrasonography was completed at day 35 of pregnancy for CRL measurement and at day 62 for crown to nose length (CNL) measurement (Riding et al., 2008) by using Ibex portable ultrasound equipped with a linear 8-5MHz multi-frequency transducer (Figure 3-1 for example). A blood sample was collected at each transrectal ultrasonography event and processed as described previously. At birth, calf weight and gender was recorded and gestation length was calculated.

Experiment 3: This study was completed at the University of Florida Santa Fe River Ranch Unit (Alachua, Florida). Seventy-seven primiparous and multiparous Angus (n=44) and Brangus (n=33) pregnant cows were used. Cows were provided corn gluten feed and access to Bahaigrass pasture and either Bermudagrass hay or stock piled forage throughout the study period.

These cows were enrolled in one of two TAI protocols based on parity, days postpartum (34 to 132 days, average of 81 days postpartum) and genotype. All cows were provided 100μg GnRH and a CIDR was inserted for 5 or 7 days. At implant removal, two 25mg $PGF_{2\alpha}$ injections were given 8 h apart. Estrous detection was completed for 72 h after CIDR removal. Cows were inseminated 8 to 12 h after detecting estrus. Cows not detected in estrus at 80 h post-CIDR removal received 100μg GnRH and were inseminated. Multiple sires were used within each breed.

Transrectal ultrasonography was completed over two days each week for four weeks at days 33/34, 40/41, 47/48 and 54/55 of pregnancy by using an Aloka 500V machine equipped with a 5.0 MHz transducer (Corometrics Medical Systems, Wallingford, CT, USA) for pregnancy diagnosis. A blood sample was collected at each

transrectal ultrasonography event and processed as described previously. At birth, calf weight and gender was recorded and gestation length was calculated.

Progesterone Quantification

Plasma P4 concentrations were determined by a solid-phase RIA (Coat-A-Count Progesterone kit, DPC Diagnostic Products Corp., Los Angeles, CA) (Seals et al., 1998). The standard curve dilution consisted of duplicate uncoated tubes for total counts and nonspecific binding. A 100µL aliquot of increasing progesterone concentrations (0.1, 0.25, 0.5, 1, 2, 5, 10 and 20 ng/mL) was used to establish the standard curve. The intra-assay coefficient of variation was 1.3% for samples analyzed in experiments 1 and 2 and 1.0% for samples analyzed in experiment 3.

Quantification of Pregnancy-Associated Glycoprotein

Plasma PAG concentrations were determined by ELISA as described previously (Green et al., 2005) with slight modifications. A pool of three anti-PAG monoclonal antibodies recognizing different binucleate cell-specific PAGs (bPAG-4, -6, -7, -16, -20 and -21) was used as trapping antibodies. A polyclonal antiserum with broad specificity for PAGs was used as the primary antibody. An alkaline phosphatase-conjugated anti-rabbit antibody was used as the detector. Samples were completed in duplicates. Serial dilutions of PAG standards (in non-pregnant heifer serum) were added to duplicate wells for the standard curve. Intra- and inter-assay coefficients of variation were 8.9 and 12.0%, respectively in experiment 1, 8.4 and 10.4%, respectively in experiment 2 and 8.2 and 18.6%, respectively in experiment 3.

Statistical Analyses

Experiment 1: Data for the Angus-Brahman crossbred cattle were grouped according to the amount of Angus genetics (see Table 1). Brangus cows and fetuses

obtained from mating Brangus cattle with Brangus sires were maintained as their own group. The effects of estrous synchronization/TAI group, genotype, calf gender and their interactions on CRL, P4 concentrations, PAG concentrations, calving weight and gestation length were determined by using the general linear model of the Statistical Analysis System (version 9.2; SAS Institute Inc., Cary, NC). The day of pregnancy at transrectal ultrasonography was used as a covariate in CRL, P4 and PAG analyses. Effects of genotype were examined further by completing Orthogonal Contrasts 1) >80% Angus vs. all other groups, 2) Brangus vs. ≤20 to 78% Angus, 3) 65 to 78% vs. ≤20 to 59% of Angus , 4) 40 to 59% vs. ≤20 to 38% Angus and 5) ≤20% vs. 21 to 38% Angus. Regression analyses were completed to examine associations between genotype, PAG concentrations, P4 concentrations and fetal measurements. All breed groups, including the Brangus were included in these analyses.

Experiment 2: Effects of estrous synchronization/TAI group, breed, calf gender, day of gestation and their interactions on PAG, P4, fetal measurements, calf weight and gestation length was determined using the general linear model of SAS (version 9.2; SAS Institute Inc., Cary, NC). Orthogonal Contrasts were used to further partition differences (Brangus versus Braford; Angus versus Brangus and Braford). Regression analyses were completed to examine whether PAG concentrations were associated with P4 concentrations or fetal measurements independent of breed.

Experiment 3: The effects of breed, day of gestation, calf gender and their interactions on PAG, P4, calf weight and gestation length were analyzed using the general linear model of SAS (version 9.2; SAS Institute Inc., Cary, NC). Data with repeated measurements over time within the same experimental unit was analyzed with

a generalized linear mixed model procedure (GLIMMIX) of SAS software version 9.2 and with cow (genotype) as the random effect in the model. Several covariate structures were tested and the one that resulted in the lowest Akaike information criterion was used (first-order autoregressive). Estrous synchronization protocol (5 vs. 7 d CIDR exposure) was not included in the model. The general linear model was used to examine effects of breed, calf gender and their interaction on PAG and P4 concentrations that were averaged across weeks. Regression analyses were completed to examine if PAG concentrations were associated with P4 concentrations or fetal measurements.

A subset of the cows was used for a feeding trial during the last third of gestation (n=6 Angus and 6 Brangus). Diets influenced calf birth weight but not gestation length (data not shown). No diet x breed interaction existed with either variable. These treatments were included as a covariate in the birth weight and gestation length analyses.

Results

Experiment 1

Fetal measurements and blood samples were collected once in early gestation from cows with various degrees of Angus and Brahman crossbreeding to provide an initial assessment of whether cow and fetal genotype is associated with PAG and P4 concentrations and fetal development.

The effects of maternal genotype on plasma PAG and P4 concentrations and CRL, gestation length and birth weight are presented in Table 1. An overall effect of genotype on plasma PAG concentrations was observed (P=0.06). Plasma PAG concentrations were less in cows with >80% Angus genetics than other cows (P<0.01)

(6.0±1.5 vs. 9.6±1.5 ng/ml, respectively). Also, a linear correlation was detected between plasma PAG concentrations and Angus genotype, and PAG concentrations were reduced as the percentage of Angus genetics increased in crossbred cattle (*P*= 0.01) (Figure 3-2).

An overall trend effect of cow genotype on plasma P4 concentrations was observed (*P*=0.1). There was no difference in P4 concentrations between cows with >80% Angus genetics versus other cows. However, Brangus cows had lower plasma P4 concentration than cows containing <78% Angus genetics (*P*=0.02) (7.2±1.0 vs. 9.9±1.0ng/ml, respectively) (Table 1).

An overall effect of maternal genotype on CRL was not observed (*P*=0.11). However, CRL was greater in >80% Angus than other groups (*P*<0.01) (34.0±2.8 vs. 28.5±2.8 mm, respectively). There was an overall effect of maternal genotype on gestation length (*P*<0.01). Cows with >80% Angus had reduced gestation lengths when compared with other groups (*P*<0.01) (281.3±1.8 vs. 286.8±1.8 days, respectively). Also, Brangus cows tended to have shorter gestation lengths than cows containing <20 to 78% Angus genetics (*P*=0.07) (283.6±1.8 vs. 287.6±1.8 days, respectively). In addition, cows containing between 65 and 78% Angus genetics had shorter gestation lengths than cows with <60% Angus genetics (*P*<0.01) (283.1±1.8 vs. 289.3±1.8 days, respectively). Moreover, there was a linear correlation between gestation length and percentage of Angus genetics (*P*<0.01; y=-0.1597x+293.67; R^2=0.29). No effects of maternal genotype on birth weight were detected.

Fetal genotype did not always mirror that of maternal genotype, therefore an additional set of analyses was completed to examine how this parameter impacted

plasma PAG and P4 concentrations, CRL, gestation length and birth weight (Table 2). Fetal genotype did not affect plasma PAG concentrations and orthogonal contrasts failed to detect any significant differences among genotype groups. There was a tendency for an overall effect of fetal genotype on P4 concentrations (*P*=0.09). Plasma P4 concentrations were decreased in fetuses containing >80% Angus genetics when compared with other groups (*P*=0.04) (7.7±1.1 vs. 9.7±1.1ng/ml, respectively). Also, there was a tendency for decreased P4 concentrations in Brangus fetuses when compared with fetuses containing <78% Angus genetics (*P*=0.09) (7.8±1.1 vs. 10.1±1.1ng/ml, respectively).

An overall effect of fetal genotype on CRL was not observed but there was almost a tendency (*P*=0.12) for fetuses with >80% Angus genetics to be larger than fetus in other genotypes (31.1±2.1 vs. 28.9±2.1 mm, respectively). Also, a tendency for linear trend was observed between fetal genotype and CRL (*P*=0.07; y=0.0234x+28.339; R^2=0.02). An overall effect of fetal genotype on gestation length was observed (*P*<0.01). Fetuses containing >80% Angus genetics had shorter gestation length than other genotypes (*P*<0.01) (281.2±2.1 vs. 287.4±2.1days, respectively). Also, fetuses with 65 to 78% Angus genetics had shorter gestation lengths than fetuses with <60% Angus genetics (*P*<0.01) (280.3±2.1 vs. 288.6±2.1days, respectively). Birth weight was not affected by fetal genotype. A linear correlation was observed between gestation length and percentage of fetal genotype (*P*=0.05; y=-0.1272x+291.33; R^2=0.29).

A linear correlation was observed between plasma PAG concentrations and CRL (Figure 3-2) (*P*=0.01). There was a tendency for a linear relationship between PAG concentrations and calf birth weight (*P*=0.09; y=-0.0894x+15.856; R2=0.04). Calf

gender did not affect P4 concentrations, CRL and gestation length. However, female calves were lighter at birth than male calves (P=0.04) (32.6±1.0 vs. 35.7±1.0kg, respectively). Also, there were tendencies for relationships between cow genotype, calf gender and plasma PAG concentrations (P=0.08) and between fetus genotype, calf gender and plasma P4 concentrations (P=0.06) (Data not shown). There was no correlation between plasma PAG and P4 concentrations (P=0.21). Also, no pregnancy losses occurred after ultrasonography and blood collection in this study.

Experiment 2

This study compared PAG and P4 concentration and fetal measurements at days 35 and 62 of gestation as well as gestation length and birth weight between two Brahman-based breeds (Brangus and Braford) and Angus cattle (Table 3). No overall effects were detected for breed and day of measurement on plasma PAG concentrations. Orthogonal contrasts did not detect differences in PAG concentrations between Brangus and Braford. However, PAG concentrations tended to be greater in Angus cows than Brangus/Braford cows at day 35 of gestation (P<0.09) and tended to be reduced in Angus cows than Brangus/Braford cows at day 62 of gestation (P<0.09).

No overall effects of breed on P4 concentrations were detected at days 35 or 62 of gestation. Plasma P4 concentrations tended to be greater in Brangus than Braford cattle at day 35 of gestation (P<0.09). No other effects on P4 concentrations were observed at either day of gestation.

No overall effect of breed on fetal measurements was detected at day 35 of gestation. However, an overall effect of breed on fetal measurements was detected at day 62 of gestation (P<0.01). Angus cows contained larger fetuses than Brangus/Braford cows at day 62 of gestation (P<0.01). Also, there was an effect of

plasma PAG concentrations and cow genotype; and plasma P4 concentrations and cow genotype in CNL (P<0.01 for both). No overall effect of fetus length at days 35 or 62 of gestation and birth weight was detected. There was an overall effect of breed on gestation length (P<0.01). Gestation length was shorter in Angus than Brangus/Braford cows (P<0.01) and tended to be longer in Braford than Brangus cows (P=0.09).

There was a tendency for relationship between cow genotype, calf gender and plasma PAG concentrations (P=0.06; 2.1±0.7 and 1.8±0.7ng/ml for males and females Angus, respectively; 3.6±0.7 and 2.4±0.7ng/ml for males and females Brangus/Braford, respectively) on day 62 of gestation. No further effects of calf gender were observed on PAG and P4 concentrations, CRL at day 35, CNL at day 62 of gestation and gestation length. There was an overall effect of calf gender on calf birth weight (P=0.03), with males being heavier at birth than females (36.1±1.0 vs. 32.8±1.0kg, respectively).

Regression analyses identified an association between plasma PAG concentrations and P4 concentrations at day 35 of gestation (Fig. 3). This association was not evident at day 62. Plasma PAG concentrations were not associated with fetus size, birth weight and gestation length. Plasma P4 concentrations were not associated with fetus size, birth weight and gestation length.

Two Brangus cows lost their pregnancies between days 35 and 62 of gestation. The low number of animals prevented detection of statistical differences in PAG and P4 concentrations and fetal length. However, the Brangus cows that lost their pregnancies had lower PAG (2.8±1.5ng/ml) and P4 (5.8±1.0ng/ml) concentrations than comparable measurements for Brangus cows that maintained their pregnancies (4.1±1.5 and 8.5±1.0 ng/ml, respectively). The fetus for one of the failed pregnancies was very small

at day 35 and could not be measured, but the fetus from the other failed pregnancy was no different than the other fetuses at day 35 of gestation (16 mm).

Experiment 3

A final study was completed on a large group of Angus and Brangus cattle to further examine the potential influence of *Bos indicus* genetics on PAG and P4 concentrations, gestation length and birth weight. Fetal measurements are not presented here.

Plasma PAG concentrations were greater in Brangus than Angus cows at each weekly measurement ($P<0.05$) (Fig. 4). Also, PAG concentrations were greater in Brangus than Angus cows after being averaged across the four-week collection period ($P<0.01$) (Table 4). There was a main effect of day of pregnancy on plasma PAG concentrations ($P<0.01$), and PAG concentrations decreased in each breed across the four-week collection period (Fig. 4).

Plasma P4 concentrations were not affected by breed or week. Pair-wise comparisons within week detected a tendency for greater P4 concentrations in Brangus versus Angus cows at day 33/34 of gestation ($P = 0.07$) (9.4±0.4 vs. 8.5±0.4ng/ml, respectively) but not at other time-points.

Angus cows had shorter gestation lengths than Brangus cows ($P=0.01$) (Table 4). Also, Angus calves weighed less at birth than Brangus cows ($P=0.03$) (Table 4). There was a tendency for calf gender to affect overall mean plasma PAG concentrations ($P0=0.09$) (5.7±0.8 vs. 7.5±0.8 ng/ml PAG for males vs. females, respectively). This effect was not detected when examining PAG concentrations within day. Male calves had greater birth weight than female calves ($P<0.01$) (38.0±0.8 vs. 33.9±0.8kg, respectively). Also, there was relationship between cow genotype, calf gender and

plasma PAG concentrations (P=0.02) (4.6±1.13 and 6.0±1.13ng/ml for males and females Angus, respectively; 6.8±1.13 and 9.7±1.13ng/ml for males and females Brangus, respectively).

Regression analysis identified a linear relationship between PAG and P4 concentrations at days 40/41 (P<0.01) and days 54/55 of gestation (P=0.05) (Figure 3-5) but not at the other time points. Plasma PAG concentrations were not associated with eventual calf birth weight and gestation length at any time point.

One Brangus and one Angus cow lost their pregnancy in this experiment. Statistical examinations of differences between these cows and their herd mates were not completed, but some interesting observations were made nonetheless. The Angus cow lost its pregnancy between days 33/34 and 40/41 of gestation and appeared to have greater PAG concentrations (11.8ng/ml), decreased plasma P4 concentration (7.6ng/ml) and slightly smaller fetus size (11.9mm) at days 33/34 when compared with the other Angus cows (6.5±0.9ng/ml, 8.4±0.3ng/ml and 12.9±0.02mm, respectively). The Brangus cow lost its pregnancy between days 40/41 and 47/48 of gestation. At days 33/34 this cow had a reduced PAG concentration (5.5ng/ml), a normal plasma P4 concentration (9.1ng/ml) and a normal sized fetus (13mm) when compared to the other Brangus cows (9.2±1.4ng/ml, 9.4±0.4ng/ml and 13.8±0.3 mm, respectively). At days 40/41 this cow had a more dramatic reduction in PAG concentrations (3.5ng/ml), an increased P4 concentration (12.1ng/ml) and smaller fetal size (12mm) than other Brangus cows (8.8±1.4ng/ml, 9.7±0.4ng/ml and 19±0.05mm, respectively).

Discussion

Infertility is a tremendous cost in the United States cattle industry. Harsh environmental conditions in tropical and sub-tropical areas, such as Florida, further add

to challenges associated with reproduction and limit the success of achieving one healthy calf per cow per year in beef production systems. The incorporation of *Bos indicus* genetics is being used to minimize the impacts of the environment on production. The most common *Bos indicus* crossbred in the United States is the Brangus. This breed consists in three eights of Brahman (*Bos indicus*) and five eights of Angus (*Bos taurus*) genotype. The combination of beneficial traits shared from both subspecies combined with the high rate of heterosis has made this and other *Bos indicus* x *Bos taurus* crossbreeding schemes very popular (Johnson et al., 1990; Bidner et al., 2002; Riley et al., 2007).

The overall focus of this work was to describe how *Bos indicus*-based genetics influences placental and embryonic/fetal development. Investigating placental development is challenging because these development events are difficult to monitor in the live animal. The placentomes are the exchange unit of the placenta and they have a unique cell type called binucleate trophoblast cells (BNC) that will migrate from the chorionic epithelium to fuse with uterine cells of the maternal side of the placenta forming a hybrid trinucleate cell (Schlafer et al., 2000; Igwebuike, 2006). Once they fuse the BNC release their granules containing hormones in the maternal circulation. These hormones include placental lactogen, prolactin-related protein-1, progesterone and estradiol. The BNCs also produce a large group of factors known as PAGs, which are inactive aspartic proteases found in the maternal circulation from the third or fourth weeks throughout the remainder of pregnancy (Sasser et al., 1986). The functional roles for PAGs throughout gestation remains speculative but their association with pregnancy permits their utilization for diagnosing pregnancy and examining placental fitness. PAG

concentrations are influenced by several physiological factors during gestation, including fetus number, fetus gender, lactation status and transgenics (Sasser et al., 1986; Green et al., 1998). However, information on how *Bos indicus* breed may affect PAG concentration is still lacking.

Each of the three experiments examined plasma PAG concentrations during early pregnancy in cattle containing *Bos indicus* genetics. An ELISA was used to determine concentrations of PAG, as described previously (Green et al., 2005). This assay was chosen because it has a high specificity and sensitivity like the PAG RIA used in previous experiments (Zoli et al., 1992) but it targets modern PAGs that are predominantly expressed in early gestation, therefore it doesn't compromise early pregnancy diagnosis by the persistence of circulating antigen in the post-partum period.

A herd of cross-bred cattle with different degrees of *Bos indicus* genetics were used in the first study to initially examine whether plasma PAG concentrations were dependent on genotype. Having as little as 20% Brahman genetics was sufficient to detect increases in PAG concentrations. This also held true when examining Brangus cows, which provided the impetus for using Brangus and Braford breeds in subsequent studies. Plasma PAG concentrations were numerically and/or statistically greater in cows containing *Bos indicus* genetics in each study. One exception to this was observed in the second experiment, where Angus cows had greater PAG concentrations than Brangus/Braford cows on day 35 of gestation. This observation cannot be explained by day of testing and therefore must be associated with some other variables that require further testing (e.g. genetic pool, location, diet/management). Also, plasma PAG concentrations of Angus cows were very similar between the second

and third experiments. Brangus cows in the third experiment had much greater PAG concentrations than the second experiment, supporting the theory that PAG concentrations are influenced by other variables, such as genetic pool. However, animals from the first experiment had much greater overall plasma PAG concentrations. This variation could be a result of the great range of gestation periods and the variance from the ELISA, once the samples of this experiment were ran by themselves and in a different laboratory. It is interesting to note that Brangus and Angus calves in experiment 2 were more similar in birth weights than Brangus and Angus calves in experiment 3. The genetic and/or environmental factors that influence this outcome may also have impacted plasma PAG concentrations during early pregnancy.

According to Sasser et al. (1986) and Green et al. (2005) by day 35 of gestation the plasma PAG concentration peaks in the first small surge, then it decreases prior to the linear increase that will peak right before or on set parturition. This decrease was showed to be true for the *Bos taurus* and *Bos indicus* breed with the third experiment (Figure 3-4). That explain why PAG values are greater in the first blood sample collections than the subsequent ones in experiment 2 and 3. In experiment 3 plasma PAG concentrations were greater in Brangus than Angus cows throughout all collections. This could be either a delay in PAG expression, since *Bos indicus* influenced animals have delayed fetal and placental development (Ferrell, 1991; O'Rourke et al., 1991), as the inverse relationship of genotype and PAG concentrations is observed between days 35 and 62 of gestation; or a matter of elevated plasma PAG concentrations in *Bos indicus* when compared to *Bos taurus*, as indicated in experiment 3. The non repeatable results between experiment 2 and 3 and some differences in the

literature (Green et al., 2005; Thompson et al., 2010) indicates that a better establishment of the plasma PAG profile in early gestation is needed.

The days selected for data collection varied between experiments. In the first experiment animals were inseminated by heat detection and fixed time. On top of that, pregnancy diagnosis was performed only once at this facility. Therefore, animals presented a wide range of gestation length at the day of transrectal ultrasonography and pregnancy losses could not be evaluated. In order to further evaluate the effect of maternal genotype in the early gestation length and to study the differences in cows that underwent pregnancy loss, the second experiment was designed with data collection on two time points: days 35 and 62 of gestation. Due to differences in plasma PAG expression between Angus and Brangus/Braford in both days of gestation of the second experiment, a third experiment was conducted to evaluate the changes in plasma PAG profiles on four time points from days 33 to 55 of gestation for Angus and Brangus genotypes.

Effects of genotype on plasma P4 concentration were generally not observed. In experiment 1 an increase in the proportion of Brahman genetics was linked with greater P4 concentrations, but this phenomenon was not detected in other experiments other than at one of the four weeks of sample collections in experiment 3. Previous studies found that *Bos indicus*-based breeds have smaller CLs size and reduced plasma P4 concentrations (Segerson et al., 1984; Bó et al., 2003). However, this effect of genotype on plasma P4 concentrations is seasonal. *Bos indicus*-based genotypes tend to have higher plasma P4 concentrations in the spring and summer and reduced plasma P4 concentrations on winter and fall than *Bos taurus* breeds (McNatty et al., 1984; Bó et al.,

2003). The three experiments described herein were conducted during late spring/early summer. Therefore *Bos indicus*-based breeds would be expected to contain equivalent or greater plasma P4 concentrations than *Bos taurus* breeds.

Correlations between plasma PAG and P4 concentrations were detected in two of the three experiments. No such correlation was detected in experiment 1, possible because of large range in genotypes and limitations in animal numbers within genotype groups. In experiment 2 this correlation was observed at d 35 and not d 62 and in experiment 3 it was detected at d 40/41 and d54/55 but not at other days. Several previous studies in dairy cattle examined interactions between plasma PAG and P4 concentrations (Del Vecchio et al., 1996; Weems et al., 2007). The results are very contradictory and debatable, such as the results showed herein. Del Vecchio et al. (1996) observed that treatment of bovine luteal cells with PAG increased progesterone concentrations, but no correlation between PAG and P4 concentrations were observed on their previous studies. Also, Weems et al. (2007) observed that PAG affected P4 secretion by luteal cells only from days 40 to 90 of gestation in ewes. It was hypothesized that PAGs indirectly influence P4 production by increasing PGE_2 secretion. Therefore, longer exposure to elevated levels of PAGs is needed to observe a solid effect on plasma P4 concentration.

The incidence of pregnancy loss was low in all of the experiments, and this prevented examination of whether plasma PAG concentrations associated with pregnancy failures in beef cattle. Such association have been observed in dairy cattle, where most cows that lose their pregnancy contain reduced PAG concentrations prior to abortion (Mialon et al., 1993; Thompson et al., 2010). López-Gatius et al., 2007

established that the odds of failure to maintain a pregnancy increases in dairy cows within the lowest and highest quartile of plasma PAG concentrations at day 35 of gestation. Also, animals that have decreased plasma PAG concentrations tend to have decreased plasma P4 concentrations (Ayad et al., 2007), and detecting reductions in both PAG and P4 concentrations predicted 90% of impending pregnancy failures in one study (Gábor et al., 2007). Given the low incidence of pregnancy loss after day 35 in this work, greater animal numbers will be needed to establish similar linkages between PAGs, P4 and late embryonic and fetal pregnancy losses.

Another important focus of this work was to examine how *Bos indicus* genetics impacts embryonic and early fetal growth. In both experiment 1 and 2 Angus cows had larger fetuses than *Bos indicus*-based cows. Detecting this effect at day 53 in experiment 1 and at day 62 and not day 35 in experiment 2 suggests that genotype-dependent differences in embryonic/fetal development may not be evident until 50-60 days of gestation. Embryonic and fetal measurements are not presented for experiment 3. Later in gestation *Bos indicus*-based breeds contain smaller fetuses and appear to develop more slowly in early and mid gestation then undergo a period of 'catch up' growth at the end of gestation (O'Rourke et al., 1991) to be born at similar or greater birth weights than *Bos taurus*-based breeds (Reynolds et al., 1980; Riley et al., 2007). This work presented herein support the notion that fetal growth rates are delayed in *Bos indicus* genotypes and that this effect is not detected at day 35 of gestation but is evident by days 50-60.

Fetus measures were influenced by maternal genotype. Plasma PAG concentrations were also affected by maternal genotype. Therefore it would make

sense that fetus measures were affected by the correlation between plasma PAG concentrations and maternal genotype, such as we saw at day 62 of gestation in experiment 2. This correlation was not observed at day 35 of gestation. Such association was not evaluated in the first experiment (there were too many genetic variation). The same outcome was observed for correlations between plasma P4 concentrations, fetal measurements and cow genotype.

Calf gender also showed some effects in plasma P4 and PAG concentrations, and calf birth weight. Numerically, female fetuses had greater plasma P4 concentrations than male fetuses in every day of analysis from the three experiments. Also, there was an effect in the third experiment and a trend in the first and second experiments of interaction between cow genotype and fetus gender in PAG concentration. Female fetuses had numerically greater plasma PAG concentration than male fetuses in every time point of data collection, besides on day 62 of gestation of the second experiment, where male fetuses had greater PAG concentrations. Zoli et al. (1992) observed that in Hereford cows, plasma concentrations of PAG were greater in female calves than males in the pre- and post-partum period. This effect wasn't the same in Holstein cows and heifers. In that study they used a bPAG-1 RIA. The bPAG-1 is highly expressed late in gestation but not in early gestation. Therefore this could explain why this effect wasn't observed earlier in gestation. In this present paper the ELISA approach targeting multiple modern PAGs expressed early in gestation enabled the detection of an effect of gender in plasma PAG concentration. Also, males were heavier at birth than females in all experiments, as expected (Riley et al., 2007).

Fetus genotypes didn't have the same effects as maternal genotypes. There was a tendency for overall effect of fetus genotype on plasma P4 concentrations; no such effect was seen in correlation with maternal genotype. This effect was more significant when looked at correlation of fetus genotype with gender on plasma P4 concentration in the first experiment. However fetus genotype did not affected plasma PAG concentrations or CRL as maternal genotype did. Ferrell (1991) conducted a study where he transferred Charolais and Brahman embryos into Charolais and Brahman cows to get the four possible combinations. The animals were slaughtered at days 232 and 271 of gestation in order to determine differences in gestation between *Bos taurus* and *Bos indicus*. It was observed that fetal genotype is a primary regulator of fetal growth at day 232 of gestation and maternal genotype regulated fetal growth at day 271 of gestation. Also, fetal membranes and placentome weight were regulated by both fetal and maternal genotype. Therefore it was concluded that fetal and maternal genotypes contribute differently and in different time-points of gestation for fetal development. With these results is clear that maternal/fetal interactions deserve more attention in subsequent studies.

As anticipated, *Bos indicus* genetics was associated with greater gestation lengths. This agrees with previous reports (Randel, 1990; Paschal et al., 1991; Riley et al., 2007). Interestingly, a linear trend was observed between plasma PAG concentrations and calf birth weight only in the first experiment. This effect was previously observed by Patel et al. (1995) and Lobago et al. (2009). In the latter study, such as in the work presented here, cows with heavier calves at birth had reduced plasma PAG concentrations in early gestation. This difference could be due to

difference in placental development or to a difference in metabolic clearance. In contrast, Kiracofe et al. (1993) didn't observe effect of calf birth weight in maternal plasma PAG concentration postpartum.

CHAPTER 4
SUMMARY AND CONCLUSIONS

In summary, plasma PAG concentrations were affected by maternal genotype and not fetal genotype. Conversely, plasma P4 concentrations were affected by fetal and not maternal genotype. Also, fetus gender in correlation with genotype seems to affect plasma PAG concentration. Correlations between plasma PAG concentrations, fetal measures and birth weight are still very debatable. Even in this report, different results were observed within the three studies. *Bos indicus*-based animals had greater plasma PAG concentrations and smaller fetuses than *Bos taurus* animals in early gestation. These results combining with the fact that *Bos indicus* calf mortality rate tends to be higher than *Bos taurus* (Reynolds et al., 1980) and their intrauterine growth rate is delayed (O'Rourke et al., 1991) could be an indicative that *Bos indicus* animals may suffer from placental incompetency in early gestation. It's clear that more studies are needed to clarify exactly how *Bos indicus* genetics is influencing PAGs concentrations and why. By the knowledge of the authors this work is a pioneer on trying to determine plasma PAG differences on *Bos indicus* and *Bos taurus* animals.

Table 3-1. The effects of maternal genotype on plasma PAG and P4 concentrations and fetal size at d47 to 58 of pregnancy and on gestational measurements at term.

Maternal Angus Genetics[a], %	# cows	PAG, ng/ml	P4, ng/ml	CRL, mm (N)	Gestation length., d	Birth wt., kg
≥ 80	17	6.0	8.0	34.0 (9)	281.3	35.0
65-78	15	9.7	9.2	28.0 (9)	283.1	33.4
40-59	21	8.4	10.7	29.8 (14)	287.3	35.6
21-38	9	8.4	10.3	29.7 (6)	286.9	29.8
≤ 20	13	11.5	9.6	28.4 (7)	293.8	34.1
Brangus	15	9.9	7.2	26.4 (7)	283.6	34.3
SE	-	1.5	1.0	2.8	1.8	1.9
P-value Contrast 1[b]	-	<0.01	0.2	<0.01	<0.01	0.44
P-value Contrast 2[b]	-	0.77	0.02	0.26	0.07	0.65
P-value Contrast 3[b]	-	0.36	0.42	0.54	<0.01	0.91
P-value Contrast 4[b]	-	0.54	0.56	0.72	0.22	0.14
P-value Contrast 5[b]	-	0.13	0.65	0.68	0.06	0.14

[a] Cows were grouped according to the percentage of Angus genetics. Measurements were collected once on a single day.

[b] Effects of genotype were examined further by completing Orthogonal Contrasts 1) >80% Angus vs. all other groups, 2) Brangus vs. ≤20 to 78% Angus, 3) 65 to 78% vs. ≤20 to 59% of Angus , 4) 40 to 59% vs. ≤20 to 38% Angus and 5) ≤20% vs. 21 to 38% Angus.

Table 3-2. The influence of fetal genotype on maternal plasma PAG and P4 concentrations and fetal size and on gestational measurements at term.

Fetal Angus Genetics[a], %	# fetuses	PAG, ng/ml	P4, ng/ml	CRL, mm (N)	Gestation length., d	Birth wt., kg
≥ 80	25	8.0	7.7	31.1 (15)	281.2	34.4
65-78	7	10.1	9.3	26.0 (3)	281.6	30.8
40-59	16	7.0	9.7	31.6 (9)	286.5	35.4
21-38	9	10.2	11.6	29 (4)	288.7	35.1
≤ 20	23	11.6	10.0	29.1 (15)	290.9	32.7
Brangus	10	11.1	7.8	25.8 (6)	284.4	36.2
SE	-	1.6	1.1	2.1	2.1	2.0
P-value Contrast 1[b]	-	0.17	0.04	0.12	<0.01	0.89
P-value Contrast 2[b]	-	0.53	0.09	0.24	0.42	0.32
P-value Contrast 3[b]	-	0.85	0.52	0.26	<0.01	0.22
P-value Contrast 4[b]	-	0.05	0.37	0.31	0.2	0.45
P-value Contrast 5[b]	-	0.57	0.31	0.97	0.56	0.45

[a] Fetuses were grouped according to the percentage of Angus genetics. Measurements were collected once on a single day.

[b] Effects of genotype were examined further by completing Orthogonal Contrasts 1) >80% Angus vs. all other groups, 2) Brangus vs. ≤20 to 78% Angus, 3) 65 to 78% vs. ≤20 to 59% of Angus , 4) 40 to 59% vs. ≤20 to 38% Angus and 5) ≤20% vs. 21 to 38% Angus.

Table 3-3. The impact of breeds containing Bos indicus genetics on plasma PAG and P4 concentrations and fetal size at d35 and 62 of gestation and on gestation length and calf birth weight.

Parameter[a]	Brangus	Braford	Angus	Pooled SE	*P*-value[b]	*P*-value[c]
# cows	25	9	17	-	-	-
PAG, ng/ml						
d35	4.3	3.5	5.0	0.5	0.3	0.09
d62	2.5	3.3	2.0	0.5	0.2	0.09
P4, ng/ml						
d35	8.9	6.6	8.6	0.9	0.09	0.45
d62	7.5	9.2	7.9	0.9	0.24	0.72
Fetus size, mm						
CRL d35	15.4	14.6	15.5	0.4	0.2	0.32
CNL d62	28.0	28.4	29.6	0.3	0.4	<0.01
Gestation length, d	283.4	287.1	279.8	1.9	0.09	<0.01
Birth wt., kg	35.3	32.8	34.8	1.4	0.3	0.65

[a] Blood samples and ultrasonographic data were collected at d35 and 62 of gestation.
[b] Orthogonal Contrast between Brangus and Braford.
[c] Orthogonal Contrast between Angus and Brangus/Braford cows.

Table 3-4. The influence of Angus versus Brangus breeding on mean plasma PAG and P4 concentrations across several time-points and on gestation length and calf birth weight.

Traits[a]	Angus	Brangus	SE	*P*-value
# cows	44	33	-	-
Pooled PAG, ng/ml	4.8	8.0	0.8	<0.01
Pooled P4, ng/ml	8.7	8.9	0.3	0.68
Gestation length, d	280.4	282.8	0.7	0.01
Birth wt., kg	35.0	37.6	0.8	0.03

[a] Mean plasma PAG and P4 concentrations are shown from samples collected at d33/34, 40/41 and 47/48, and 54/55 of pregnancy.

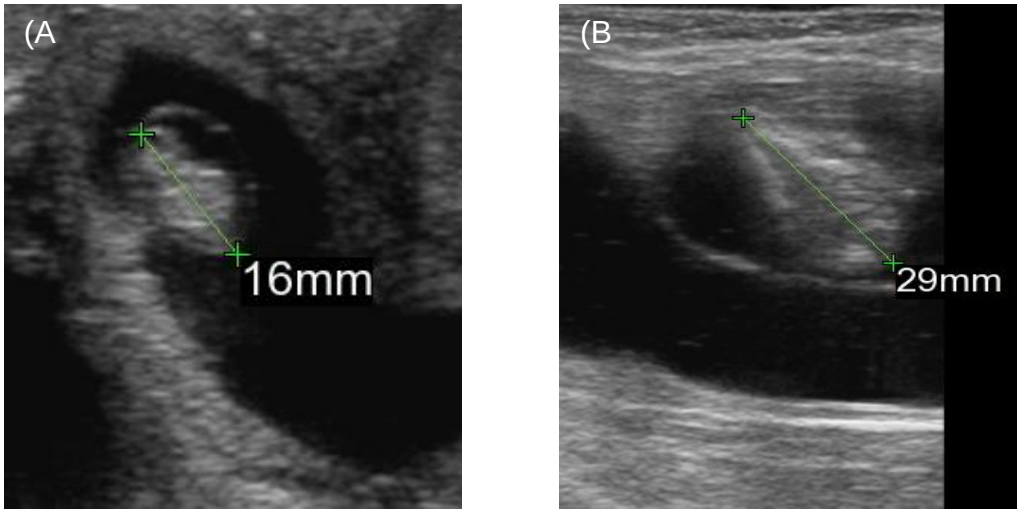

Figure 3-1. Examples of fetal ultrasonographic measurements collected for CRL around day 53 in experiment 1 and at day 35 in experiment 2 (A) and CNL at day 62 in experiment 2 (B).

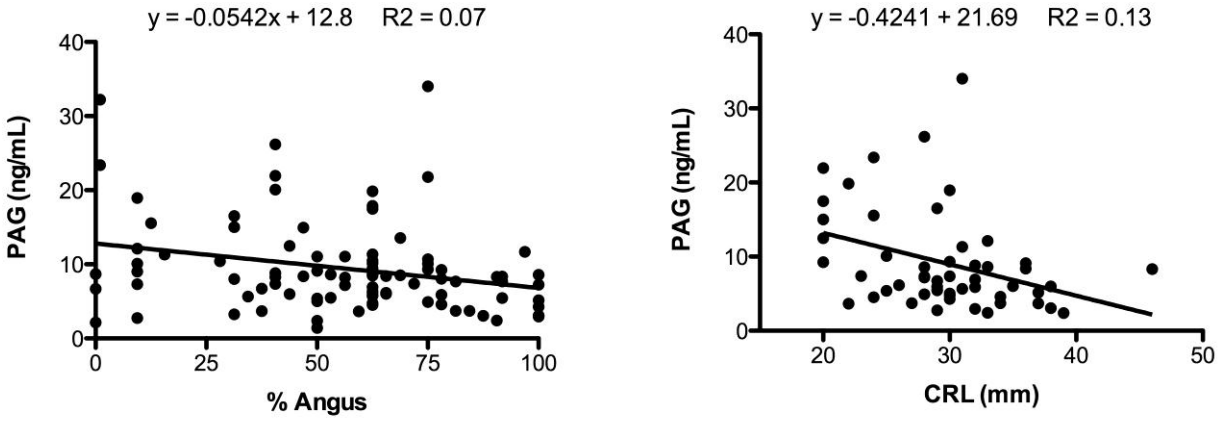

Figure 3-2. The linear relationship between plasma PAG concentrations and percentage of Angus genetics in cows (left panel; *P*=0.01). The percentage of Angus genetics in each cow was plotted against the corresponding plasma PAG value obtained from a single sample at d48 to 56 of gestation in experiment 1. The linear relationship between plasma PAG concentrations and embryo length (right panel; *P*<0.01). The CRL of each embryo was plotted against the corresponding plasma PAG value at d48 to 56 of gestation.

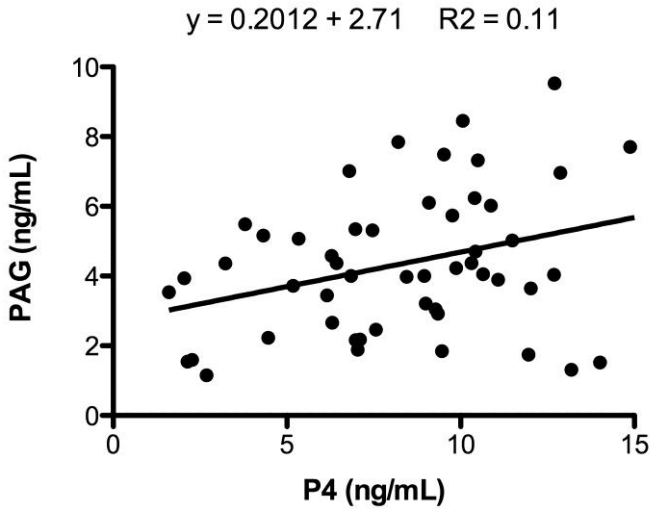

Figure 3-3. The positive linear relationship between plasma PAG and P4 concentrations in Angus, Brangus and Braford cows at day 35 of pregnancy in experiment 2 (*P* = 0.01).

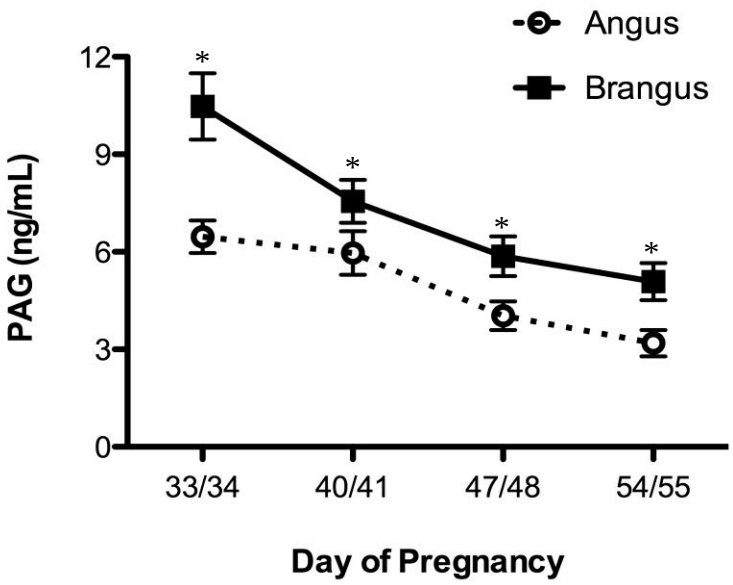

Figure 3-4. Differences in plasma PAG concentrations between Angus and Brangus cows throughout early gestation in experiment 3. Plasma PAG concentrations were determined weekly from d32/33 to d54/55 of pregnancy. The * indicates differences between breeds ($P<0.05$).

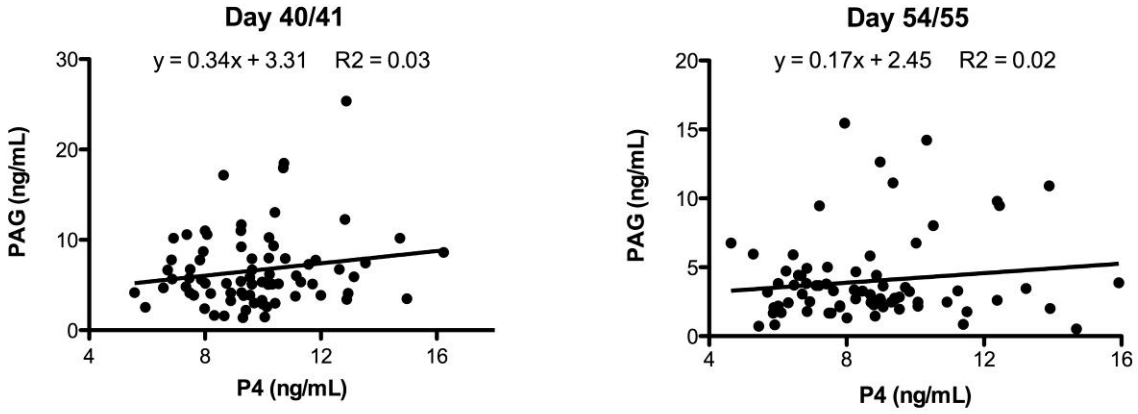

Figure 3-5. The linear relationship between plasma PAG and P4 concentrations between Angus and Brangus cows at d40 (left panel; *P*<0.01) and 54 (right panel; *P*= 0.05) of pregnancy in experiment 3.

APPENDIX A
DETERMINATION OF GASTRULATION MARKERS FOR THE BOVINE CONCEPTUS

Early conceptus development is different in ruminants than other well-studied mammals, such as the mouse and human, due to a prolonged pre-attachment period. The bovine conceptus hatches from the zona pellucida on day 8-9 of gestation (Rodríguez-Alvarez et al., 2009), and the trophectoderm begins to elongate around days 13 to 15 (Ealy and Yang, 2009). During this period the conceptus increases more than 100 fold in size and acquires a filamentous shape (Blomberg et al., 2008). Gastrulation takes place concomitant with conceptus elongation. During gastrulation the pluripotent epiblast will differentiate into the endoderm, mesoderm and ectoderm germ cell layers that eventually give rise to specific tissues and organs (Vejlsted et al., 2006). Gastrulation markers for the bovine conceptus remain largely unknown. This work identified primer pairs that amplified several lineage-specific transcripts from bovine conceptuses collected at day 17 of pregnancy.

Materials and Methods

All animal use was completed in accordance with and was approved by the Institute of Food and Agricultural Sciences Animal Care and Use Committee at the University of Florida.

In Vitro Production of Bovine Embryo

Bovine embryos were generated by *in vitro* production (IVP) by oocyte maturation, fertilization and culture procedures described previously (Loureiro et al., 2009). Bovine ovaries were collected from Central Beef Packing Co. (Center Hill, Fl) and transported in 0.9% (w/v) NaCl at room temperature to the Animal Sciences department at University of Florida (Gainesville, Fl). Cumulus oocyte complexes (COCs)

were collected by slashing 2 to 10mm follicles on the surface of ovaries. The COCs were washed and placed in maturation medium for 20-22 hours at 38.5^0C in a humidified atmosphere of 5% (vol/vol) CO_2. Mature COCs were fertilized with $1x10^6$ Percoll-purified spermatozoa of frozen and thaw semen from three different bulls and incubated for 8-10 hours in the same conditions. Putative zygotes were denuded of cumulus cells by vortexing then they were placed in groups of 30 and incubated at 38.5^0C in a humidified atmosphere with 5% (v/v) O_2, 5% (vol/vol) CO_2 and 90% (vol/vol) N_2. At day seven of maturation embryos classified as grade 1 or 2 were harvested and loaded into 0.25ml straws for consecutive transfer into the cow.

Animals

Cows from the University of Florida Dairy Research Unit, Hague, FL were used as recipients. Synchronization was conducted by administering 100 µg GnRH (Cystorelin, Merial, Duluth, GA) and an intravaginal CIDR device (Pfizer Animal Health, New York, NY) insertion. After 7 days the CIDR was removed and 25mg $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health) was administered. After 56 hours 100 µg GnRH was administered. After 7 days cows were examined for the presence of a CL by transrectal ultrasonography using Ibex portable ultrasound equipped with a linear 8-5MHz multi-frequency transducer. Cows containing a CL were given an epidural anesthesia (5ml of 2% lidocaine) and a single embryo was non-surgically transferred into the uterine horn ipsilateral to the ovary. After 10 days conceptuses were recovered non-surgically by inserting 50ml of Dulbecco's phosphate-buffered saline (DPBS) and flushing it back with a syringe. Embryos were then collected with a transfer pipette into cryovials and snap-frozen in liquid nitrogen and stored at -80^0C for further analyses.

Quantitative RT-PCR

Bovine conceptuses had their RNA extracted using the PureLink RNA Mini Kit. The concentration and quality of the tcRNA (A_{260}/A_{280}≥1.8) was determined using a NanoDrop 2000 Spectrophotometer (Thermo Scientific). Samples were incubated with RNase-free DNase for 30 min at 37^0C with later inactivation by heat (75^0C for 10 min). Then RNA was reverse transcribed by using a High Capacity cDNA Reverse Transcription Kit with random hexamers. Primer pairs were used in combination with SyberGreen Detection System to determine different tissues in the conceptus. The relative abundance of specific transcripts was quantified by 7300 Real Time PCR System (Applied Biosystems). A dissociation curve was used to verify the amplification of a single product. Levels of RNA were normalized using *GAPDH* mRNA. Transcripts efficiency were expressed in percentage and calculated using the standard curve by the formula: E = (10^(-1/slope)-1)*100

Results and Discussion

All primer pairs that successfulling amplified products in day 17 bovine conceptuses are listed in Table 1.

Trophectoderm markers included interferon-tau (*IFNT*), caudal type homeobox 2 (*CDX2*), bovine pregnancy-associated glycoprotein 9 (*PAG9*), trophoblast Kunitz domain protein 5 (*TKDP5*) and placental prolactin-related protein 1 (*PRP1*). All transcripts were detected in bovine conceptuses. Primer efficiencies were within the acceptable range.

Nanog homeobox (*NANOG*) was examined as the epiblast marker. Previous work determined it is produced in bovine blastocysts (Yang et al., 2011) and present work also identified transcripts for this marker in elongated bovine conceptuses.

Acceptable markers of endoderm identified in bovine conceptuses included Sex determining Y box 2 (*SOX2*) and GATA binding protein 4 (*GATA4*). Identification of SOX2 in bovine conceptuses is novel but *GATA4* had been associated with primitive endoderm in previous work examining endoderm outgrowths derived from bovine blastocysts (Yang et al., 2011). Transcripts for another SOX variant, *SOX17* could not be identified in bovine conceptuses (data not shown). It remains unclear whether the primers examined were not sufficient to amplify *SOX17* or if it is not expressed at this stage of development.

This work determined that mesoderm development candidate 2 (*MESDC2*) transcripts were present in elongated bovine conceptuses. This was the only mesoderm marker identified. Other mesoderm markers used in other species (Brachyrury, Guscoid) could not be amplified with several primer sets (data not shown).

The ectoderm marked identified in bovine conceptus mRNA was zic family member 1 (*ZIC1*). Other putative ectoderm markers (e.g. *SOX1*) could not be amplified (data not shown).

These findings determined that each of the major extraembryonic and embryonic cell lineages are present in elongated bovine concetpuses collected during the peri-implantation period. It is our hope that others may use this information to examine differences in the degree of conceptus development that may occur under various physiological events (e.g. heat stress, nutritional manipulations and TAI programs).

Table A-1. Primers used to specify lineages in day 17 bovine conceptus mRNA.

Gene		Primer Sequence	Efficeinecy (%)
Trophectoderm			
IFNT	Forward:	TGCAGGACAGAAAAGACTTTGGT	Determined in (Cooke et al., 2009)
	Reverse:	CCTGATCCTTCTGGAGCTGG	
CDX2	Forward:	CCTGTGCGAGTGGATGCGGAA	118
	Reverse:	CCTTTGCTCTGCGGTTCT	
PAG9	Forward:	AGCAGGCGAATGGAGAGTACA	92
	Reverse:	CACAGCCATCAGAACAAGCAA	
TKDP5	Forward:	GAGCTCCCAACAGCAGTACTCCA	88
	Reverse:	ACATGTCCTCACGGGGACCCT	
PRP1	Forward:	CAGACAGGTTTATGAATGCCGC	87
	Reverse:	CGCAGGCAGTAGAACAGGTTAT	
Epiblast			
NANOG	Forward:	GACACCCTCGACACGGACAC	Determined in (Yang et al., 2011)
	Reverse:	CTTGACCGGGACCGTCTCTT	
Endoderm			
GATA4	Forward:	ATGAAGCTCCATGGCGTCCC	89
	Reverse:	CGGCTTCAGCTCCGTCTCCATC	
SOX2	Forward:	TAACAATCATCGGCGGCGGT	119
	Reverse:	CGGCTTCAGCTCCGTCTCCATC	
Mesoderm			
MESDC2	Forward:	CCGCGCCGCCGTGATTTTTC	112
	Reverse:	GAAGACGGGCCATGTCGGCG	
Ectoderm			
ZIC1	Forward:	AACATGGCCGCTCACCACGG	94
	Reverse:	GGGTTGGCCAGCTGCTAGG	

LIST OF REFERENCES

Aerts, J.M.J., and P.E.J. Bols. 2010a. Ovarian follicular dynamics: a review with emphasis on the bovine species. Part I: Folliculogenesis and pre-antral follicle development. Reprod. Dom. Anim. 45(1): 171-179.

Aerts, J.M.J., and P.E.J. Bols. 2010b. Ovarian follicular dynamics: a review with emphasis on the bovine species. Part II: Antral development, exogenous influence and future prospects. Reprod. Dom. Anim. 45(1): 180-187.

Alvarez, P., L.J. Spicer, C.C. Chase, M.E. Payton, T.D. Hamilton, R.E. Stewart, A.C. Hammond, T.A. Olson, and R.P. Wettemann. 2000. Ovarian and endocrine characteristics during an estrous cycle in Angus , Brahman , and Senepol cows in a subtropical environment. J. Anim. Sci. 78: 1291-1302.

Assis Neto, A.C., F.T.V. Pereira, T.C. Santos, C.E. Ambrosio, R. Leiser, and M.A. Miglino. 2010. Morpho-physical recording of bovine conceptus (Bos indicus) and placenta from days 20 to 70 of pregnancy. Reprod. Dom. Anim. 45(5): 760-772.

Ayad, a, N.M. Sousa, J. Sulon, J.L. Hornick, J. Watts, F. Lopez-Gatius, M. Iguer-Ouada, and J.F. Beckers. 2007. Influence of progesterone concentrations on secretory functions of trophoblast and pituitary during the first trimester of pregnancy in dairy cattle. Theriogenology 67(9): 1503-1511.

Barros, C.M., M.F. Pegorer, J.L.M. Vasconcelos, B.G. Eberhardt, and F.M. Monteiro. 2006. Importance of sperm genotype (indicus versus taurus) for fertility and embryonic development at elevated temperatures. Theriogenology 65(1): 210-218.

Bazer, F.W., T.E. Spencer, G. a Johnson, R.C. Burghardt, and G. Wu. 2009. Comparative aspects of implantation. Reproduction 138(2): 195-209.

Bellows, D.S., S.L. Ott, and R.A. Bellows. 2002. Review: Cost of Reproductive and Conditions in Cattle. The Professional Animal Scientist 18: 26-32.

Bidner, T.D., W.E. Wyatt, P.E. Humes, D.E. Franke, and D.C. Blouin. 2002. Influence of Brahman-derivative breeds and Angus on carcass traits , physical composition , and palatability. J. Anim. Sci. 80: 2126-2133.

BioTracking. 2012. BioPRYN. Available at http://www.biotracking.com/LAB (verified 28 May 2012).

Block, J., C.C. Chase, and P.J. Hansen. 2002. Inheritance of resistance of bovine preimplantation embryos to heat shock: relative importance of the maternal versus paternal contribution. Mol. Reprod. Dev. 63(1): 32-37.

Blomberg, L., K. Hashizume, and C. Viebahn. 2008. Blastocyst elongation, trophoblastic differentiation, and embryonic pattern formation. Reproduction 135(2): 181-195.

Bock, R.E., A.J. de Vos, T.G. Kingston, and D.J. McLellan. 1997. Effect of breed of cattle on innate resistance to infection with Babesia bovis, B bigemina and Anaplasma marginale. Aust. Vet. J. 75(5): 337-340.

Bridges, G. a, G.E. Portillo, J.W. de Araujo, W.W. Thatcher, and J.V. Yelich. 2005. Efficacy of either a single or split treatment of PGF2alpha after a 14 day melengestrol acetate treatment to synchronize estrus and induce luteolysis in Bos indicus x Bos taurus heifers. Theriogenology 64(2): 344-362.

Butler, J.E., W.C. Hamilton, R.G. Sasser, G.M. Ruder, C. A.Hass, and R.J. Williams. 1982. Detection and partial characterization of two bovine pregnancy-specific proteins. Biol. Reprod. 26: 925-933.

Bó, G. a., P.S. Baruselli, and M.F. Martínez. 2003. Pattern and manipulation of follicular development in Bos indicus cattle. Anim. Reprod. Sci. 78(3-4): 307-326.

Camargo, L.S. a, J.H.M. Viana, a a Ramos, R.V. Serapião, W.F. de Sa, a M. Ferreira, M.F.M. Guimarães, and V.R. do Vale Filho. 2007. Developmental competence and expression of the Hsp 70.1 gene in oocytes obtained from Bos indicus and Bos taurus dairy cows in a tropical environment. Theriogenology 68(4): 626-632.

Cartwright, T.C. 1980. Prognosis of Zebu Cattle : Research and Application. J. Anim. Sci. 50: 1221-1226.

Carvalho, J.B.P., N. a T. Carvalho, E.L. Reis, M. Nichi, a H. Souza, and P.S. Baruselli. 2008. Effect of early luteolysis in progesterone-based timed AI protocols in Bos indicus, Bos indicus x Bos taurus, and Bos taurus heifers. Theriogenology 69(2): 167-175.

Chavatte-Palmer, P., N. de Sousa, P. Laigre, S. Camous, a a Ponter, J.-F. Beckers, and Y. Heyman. 2006. Ultrasound fetal measurements and pregnancy associated glycoprotein secretion in early pregnancy in cattle recipients carrying somatic clones. Theriogenology 66(4): 829-840.

Chenoweth, P.J. 1994. Aspects of reproduction in female Bos indicus cattle: a review. Aust. Vet. J. 71(12): 422-426.

Coleman, S.W., C.C. Chase, W. a Phillips, D.G. Riley, and T. a Olson. 2012. Evaluation of tropically adapted straightbred and crossbred cattle: Postweaning gain and feed efficiency when finished in a temperate climate. Journal of animal science: 1-29.

Constant, F., S. Camous, P. Chavatte-Palmer, Y. Heyman, N. de Sousa, C. Richard, J.F. Beckers, and M. Guillomot. 2011. Altered secretion of pregnancy-associated glycoproteins during gestation in bovine somatic clones. Theriogenology 76(6): 1006-1021.

Cooke, F.N.T., K. a Pennington, Q. Yang, and A.D. Ealy. 2009. Several fibroblast growth factors are expressed during pre-attachment bovine conceptus development and regulate interferon-tau expression from trophectoderm. Reproduction 137(2): 259-69.

Cross, J.C., D. Baczyk, N. Dobric, M. Hemberger, M. Hughes, D.G. Simmons, H. Yamamoto, and J.C.P. Kingdom. 2003. Genes, Development and Evolution of the Placenta. Placenta 24(2-3): 123-130.

DelVecchio, R.P., R.G. Sasser, and R.D. Randel. 1990. Effect of pregnancy-specific protein B on prostaglandin F2a and prostaglandin E2 release by day 16-perifused bovine endometrial tissue. Prostaglandins 40(3): 271-282.

DelVecchio, R.P., W.D. Sutherland, and R.G. Sasser. 1995. Effect of pregnancy-specific protein B on luteal cell progesterone, prostaglandin, and oxytocin production during two stages of the bovine estrous cycle. J. Anim. Sci. 73(9): 2662-2668.

DelVecchio, R.P., W.D. Sutherland, and R.G. Sasser. 1996. Bovine luteal cell production in vitro of prostaglandin E2, oxytocin and progesterone in response to pregnancy-specific protein B and prostaglandin F2 alpha. J. Reprod. Fert. 107(1): 131-136.

DeVries, A. 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89(10): 3876-3885.

Diskin, M.G., and D.G. Morris. 2008. Embryonic and early foetal losses in cattle and other ruminants. Reprod. Dom. Anim. 43 Suppl 2: 260-267.

Dobson, H., I.S. Kippax, and P. Humblot. 1993. Assessment of fetal number, and fetal and placental viability throughout pregnancy in cattle. Theriogenology 40: 411-425.

Dosogne, H., C. Burvenich, a E. Freeman, M.E. Kehrli, J.C. Detilleux, J. Sulon, J.F. Beckers, and D. Hoeben. 1999. Pregnancy-associated glycoprotein and decreased polymorphonuclear leukocyte function in early post-partum dairy cows. Vet. Immunol. Immunopathol. 67(1): 47-54.

Duranthon, V., A.J. Watson, and P. Lonergan. 2008. Preimplantation embryo programming: transcription, epigenetics, and culture environment. Reproduction 135(2): 141-150.

Ealy, A.D., and Q.E. Yang. 2009. Control of interferon-tau expression during early pregnancy in ruminants. Am. J. Reprod. Immunol. 61(2): 95-106.

Egen, T.E., A.D. Ealy, L.A. Landon, R.M. Roberts, and J.A. Green. 2009. Autoimmunization of ewes against pregnancy-associated glycoproteins does not interfere with the establishment and maintenance of pregnancy. Animal 3(6): 850-857.

FAO. 2009. How to feed the world 2050: Global agriculture towards 2050. Food and Agriculture Organization of the United NationsAvailable at http://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050_Global_Agriculture.pdf.

Ferrell, C.L. 1991. Maternal and fetal influences on uterine and conceptus development in the cow: I. Growth of tissues of the gravid uterus. J. Anim. Sci. 69: 1945-1953.

Fortune, J.E., M.Y. Yang, and W. Muruvi. 2010. In vitro and in vivo regulation of follicular formation and activation in cattle. Reprod. Fertil. Dev. 23: 15-22.

Gabor, G., F. Tóth, L. Ozsvári, Z. Abonyi-Tóth, and R.G. Sasser. 2007. Early detection of pregnancy and embryonic loss in dairy cattle by ELISA tests. Reprod. Dom. Anim. 42(6): 633-636.

Galyean, M.L., C. Ponce, and J. Schutz. 2011. The future of beef production in North America. Animal Frontiers 1(2): 29-36Available at http://animalfrontiers.fass.org/cgi/doi/10.2527/af.2011-0013 (verified 2 June 2012).

Gasperowicz, M., and D.R.C. Natale. 2011. Establishing three blastocyst lineages--then what? Biol. Reprod. 84(4): 621-630.

Gaughan, J.B., T.L. Mader, S.M. Holt, M.J. Josey, and K.J. Rowan. 1999. Heat tolerance of Boran and Tuli crossbred steers. J. Anim. Sci.: 2398-2405.

Gigli, I., R. a Cushman, C.M. Wahl, and J.E. Fortune. 2005. Evidence for a role for anti-Mullerian hormone in the suppression of follicle activation in mouse ovaries and bovine ovarian cortex grafted beneath the chick chorioallantoic membrane. Mol. Reprod. Dev. 71(4): 480-488.

Ginther, O.J., M.A. Beg, D.R. Bergfelt, F.X. Donadeu, and K. Kot. 2001. Minireview: Follicle selection in monovular species. Biol. Reprod. 647: 638-647.

Goossens, K., A. Van Soom, M. Van Poucke, L. Vandaele, J. Vandesompele, A. Van Zeveren, and L.J. Peelman. 2007. Identification and expression analysis of genes associated with bovine blastocyst formation. BMC Dev. Biol. 7: 64-76.

Gosden, R., and B. Lee. 2010. Portrait of an oocyte : our obscure origin. J. Clin. Invest. 120(4): 973-983.

Green, J. a, T.E. Parks, M.P. Avalle, B.P. Telugu, A.L. McLain, a J. Peterson, W. McMillan, N. Mathialagan, R.R. Hook, S. Xie, and R.M. Roberts. 2005. The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers. Theriogenology 63(5): 1481-1503.

Green, J.C., D.H. Volkmann, S.E. Poock, M.F. McGrath, M. Ehrhardt, a E. Moseley, and M.C. Lucy. 2009. Technical note: A rapid enzyme-linked immunosorbent assay blood test for pregnancy in dairy and beef cattle. J. Dairy Sci. 92(8): 3819-3824.

Green, J.A., S. Xie, X. Quan, B. Bao, X. Gan, N. Mathialagan, J.F. Beckers, and R.M. Roberts. 2000. Pregnancy-associated bovine and ovine glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy. Biol. Reprod. 62(6): 1624-31.

Green, J.A., S. Xie, and R.M. Roberts. 1998. Pepsin-related molecules secreted by trophoblast. Rev. Reprod. 3(1): 62-9.

Gregory, K.E., and L.V. Cundiff. 1980. Crossbreeding in beef cattle : evaluation of systems. J. Anim. Sci. 51: 1224-1242.

Hansen, P.J. 2004. Physiological and cellular adaptations of zebu cattle to thermal stress. Anim. Reprod. Sci. 82-83: 349-360.

Haugejorden, G., S. Waage, E. Dahl, K. Karlberg, J.F. Beckers, and E. Ropstad. 2006. Pregnancy associated glycoproteins (PAG) in postpartum cows, ewes, goats and their offspring. Theriogenology 66(8): 1976-1984.

Hernández-Cerón, J., C.C. Chase, and P.J. Hansen. 2004. Differences in Heat Tolerance Between Preimplantation Embryos from Brahman, Romosinuano, and Angus Breeds. J. Dairy Sci. 87(1): 53-58.

Hoeben, D., C. Burvenich, a M. Massart-Leën, M. Lenjou, G. Nijs, D. Van Bockstaele, and J.F. Beckers. 1999. In vitro effect of ketone bodies, glucocorticosteroids and bovine pregnancy-associated glycoprotein on cultures of bone marrow progenitor cells of cows and calves. Vet. Immunol. Immunopathol. 68(2-4): 229-240.

Huffman, R.D., S.E. Williams, and T.T. Marsha. 1990. Effects of percentage Brahman and Angus breeding , age-season of feeding and slaughter end point on feedlot performance and carcass characteristics. J. Anim. Sci. 68: 2243-2252.

Humblot, P. 2001. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology 56(9): 1417-1433.

Humblot, P., S. Camous, J. Martal, J. Charlery, N. Jeanguyot, M. Thibier, and G. Sasser. 1988. Diagnosis of pregnancy by radioimmunoassay of a pregnancy-specific protein in the plasma of dairy cows. Theriogenology 30(2): 257-267.

Hunter, M.G., R.S. Robinson, G.E. Mann, and R. Webb. 2004. Endocrine and paracrine control of follicular development and ovulation rate in farm species. Anim. Reprod. Sci. 82-83: 461-77Available at http://www.ncbi.nlm.nih.gov/pubmed/15271473 (verified 2 March 2012).

Igwebuike, U.M. 2006. Trophoblast cells of ruminant placentas--A minireview. Anim. Reprod. Sci. 93(3-4): 185-198.

Ikawa, M., N. Inoue, A.M. Benham, M. Okabe, M.C. Chang, and C.R. Austin. 2010. Fertilization : a sperm ' s journey to and interaction with the oocyte. J. Clin. Invest. 120(4): 984-994.

Johnson, D.D., R.D. Huffman, S.E. Williams, and D.D. Hargrove. 1990. Effects of percentage Brahman and Angus breeding , age-season of feeding and slaughter end point on meat palatability and muscle characteristics. J. Anim. Sci. 68: 1980-1986.

Jones, K.T. 2004. Turning it on and off: M-phase promoting factor during meiotic maturation and fertilization. Mol. Hum. Reprod. 10(1): 1-5.

Kindahl, H., B. Kornmatitsuk, K. Königsson, and H. Gustafsson. 2002. Endocrine changes in late bovine pregnancy with special emphasis on fetal well-being. Domest. Anim. Endocrinol. 23(1-2): 321-328.

King, W.A. 1991. Embryo-mediated pregnancy failure in cattle. Can. Vet. J. 32: 99-103.

Kiracofe, G.H., J.M. Wright, R.R. Schalles, C. a Ruder, S. Parish, and R.G. Sasser. 1993. Pregnancy-specific protein B in serum of postpartum beef cows. J. Anim. Sci. 71(8): 2199-2205.

Knight, P.G., and C. Glister. 2001. Review Potential local regulatory functions of inhibins , activins and follistatin in the ovary. Reproduction: 503-512.

Kornmatitsuk, B., M.C. Veronesi, A. Madej, E. Dahl, E. Ropstad, J.F. Beckers, M. Forsberg, H. Gustafsson, and H. Kindahl. 2002. Hormonal measurements in late pregnancy and parturition in dairy cows-possible tools to monitor foetal well being. Anim. Reprod. Sci. 92(3-4): 153-164.

Lamb, G.C., C. Dahlen, and M. Maddox. 2008. What is the Economic Impact of Infertility in Beef Cattle ? Univ. Florida IFAS Ext. AN208: 1-3.

Lamb, G.C., C. Dahlen, V. Mercadante, and K. Bischoff. 2011. What Is the Impact of Infertility in Beef Cattle ? Univ. Florida IFAS Ext. AN208: 1-2.

Leroy, J.L.M.R., T. Vanholder, a T.M. Van Knegsel, I. Garcia-Ispierto, and P.E.J. Bols. 2008. Nutrient prioritization in dairy cows early postpartum: mismatch between metabolism and fertility? Reprod. Dom. Anim. 43 Suppl 2: 96-103.

Li, L., P. Zheng, and J. Dean. 2010. Maternal control of early mouse development. Development 137(6): 859-870.

Lindneri, G.M., and R.W. Wright Jr. 1983. Bovine embryo morphology and evaluation. Theriogenology 20: 407-416.

Lobago, F., M. Bekana, H. Gustafsson, J.F. Beckers, G. Yohannes, Y. Aster, and H. Kindahl. 2009. Serum profiles of pregnancy-associated glycoprotein, oestrone sulphate and progesterone during gestation and some factors influencing the profiles in Ethiopian Borana and crossbred cattle. Reprod. Dom. Anim. 44(4): 685-692.

Lonergan, P. 2011. Influence of progesterone on oocyte quality and embryo development in cows. Theriogenology 76(9): 1594-1601.

Lopez-Gatius, F., J.M. Garbayo, P. Santolaria, J. Yániz, A. Ayad, N.M. de Sousa, and J.F. Beckers. 2007a. Milk production correlates negatively with plasma levels of pregnancy-associated glycoprotein (PAG) during the early fetal period in high producing dairy cows with live fetuses. Domest. Anim. Endocrinol. 32(1): 29-42.

Lopez-Gatius, F., R.H.F. Hunter, J.M. Garbayo, P. Santolaria, J. Yániz, B. Serrano, A. Ayad, N.M. de Sousa, and J.F. Beckers. 2007b. Plasma concentrations of pregnancy-associated glycoprotein-1 (PAG-1) in high producing dairy cows suffering early fetal loss during the warm season. Theriogenology 67(8): 1324-1330.

Loureiro, B., L. Bonilla, J. Block, J.M. Fear, A.Q.S. Bonilla, and P.J. Hansen. 2009. Colony-stimulating factor 2 (CSF-2) improves development and posttransfer survival of bovine embryos produced in vitro. Endocrinology 150(11): 5046-5054.

Lucy, M.C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84(6): 1277-1293.

Maurer, R.R., and J.R. Chenault. 1983. Fertilization Failure and Embryonic Mortality in Parous and Nonparous Beef Cattle. J. Anim. Sci. 56: 1186-1189.

McBride, W.D., and K. Mathews. 2011. ERS Report Summary: The Diverse Structure and Organization of U.S. Beef Cow-Calf Farms. Economic Research Service: 1-2Available at http://www.ers.usda.gov/Publications/EIB73/EIB73_ReportSummary.pdf.

McNatty, K.P., N. Hudson, M. Gibb, K.M. Henderson, S. Lun, D. Heath, and G.W. Montgomery. 1984. Seasonal differences in ovarian activity in cows. J. Endocr. 102: 189-198.

Mialon, M.M., S. Camous, G. Renand, J. Martal, and F. Ménissier. 1993. Peripheral concentrations of a 60-kDa pregnancy serum protein during gestation and after calving and in relationship to embryonic mortality in cattle. Reprod. Nutr. Dev. 33: 269-282.

Natraj, U., and J.S. Richards. 1993. Hormonal regulation, localization, and functional activity of the progesterone receptor in granulosa of rat preovulatory. Endocrinology 133(2): 761-769.

Nilsson, E., J. a Parrott, and M.K. Skinner. 2001. Basic fibroblast growth factor induces primordial follicle development and initiates folliculogenesis. Mol. Cell Endocrinol. 175(1-2): 123-130.

Nogueira, G.P. 2004. Puberty in South American Bos indicus (Zebu) cattle. Anim. Reprod. Sci. 82-83: 361-372.

O'Rourke, P.K., K.W. Entwistle, C. Arman, C.R. Esdale, and B.M. Bums. 1991. Fetal development and gestational changes in Bos taurus and Bos indicus genotypes in the tropics. Theriogenology 36(5): 839-853.

Park, J.-young, Y.-qiang Su, M. Ariga, L. Evelyn, S.L. Catherine Jin, and M. Conti. 2012. EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science 303: 682-684.

Paschal, J.C., J.O. Sanders, and J.L. Kerr. 1991. Calving and weaning characteristics of Angu-, Gray Brahman-, Gir-, Indu-Brazil-, Nellore-, and Red Brahman-sired F1 calves. J. Anim. Sci. 69: 2395-2402.

Patel, O.V., I. Domeki, N. Sasaki, T. Takahashi, M. Hirako, R.G. Sasser, and P. Humblots. 1995. Effect of Fetal Mass, Number and Stage of Gestation on Pregnancy-Specific Protein B Concentrations in the Bovine. Theriogenology 44: 827-833.

Piechotta, M., J. Bollwein, M. Friedrich, T. Heilkenbrinker, C. Passavant, J. Branen, G. Sasser, M. Hoedemaker, and H. Bollwein. 2011. Comparison of commercial ELISA blood tests for early pregnancy detection in dairy cows. J. Reprod. Dev. 57(1): 72-75.

Randel, R.D. 1990. Unique Reproductive Traits of Brahman and Brahman Based Cows. p. 60-82. *In* 39th Ann. Beef Cattle Short Course.

Rechav, Y. 1987. Resistance of Brahman and Hereford cattle to African ticks with reference to serum gamma globulin levels and blood composition. Exp. Appl. Acarol. 3(3): 219-232.

Rekawiecki, R., M.K. Kowalik, D. Slonina, and J. Kotwica. 2008. Review article: Regulation of Progesterone Synthesis and Action in Bovine Corpus Luteum. J. Physiol. Pharmacol. (59): 75-89.

Reynolds, W.L., T.M. DeRouen, S. Moin, and K.L. Koonce. 1980. Factors Influencing Gestation Length , Birth Weight and Calf Survival of Angus , Zebu and Zebu Cross Beef Cattle. J. Anim. Sci. 51: 860-867.

Riding, G. a, S. a Lehnert, A.J. French, and J.R. Hill. 2008. Conceptus-related measurements during the first trimester of bovine pregnancy. Vet. J. 175(2): 266-72.

Riley, D.G., C.C. Chase, S.W. Coleman, and T. a Olson. 2007. Evaluation of birth and weaning traits of Romosinuano calves as purebreds and crosses with Brahman and Angus. J. Anim. Sci. 85(2): 289-98Available at http://www.ncbi.nlm.nih.gov/pubmed/17235015 (verified 14 March 2012).

Rodríguez-Alvarez, L., J. Cox, F. Navarrete, C. Valdés, T. Zamorano, R. Einspanier, and F.O. Castro. 2009. Elongation and gene expression in bovine cloned embryos transferred to temporary recipients. Zygote 17(4): 353-365.

Saito, H., T. Kaneko, T. Takahashi, S. Kawachiya, T. Saito, and M. Hiroi. 2000. Hyaluronan in follicular fluids and fertilization of oocytes. Fertil. Steril. 74(6): 1148-1152.

Sanders, J.O. 1980. History and Development of Zebu Cattle in the United States. J. Anim. Sci. 50: 1188-1200.

Santos, J.E.P., W.W. Thatcher, R.C. Chebel, R.L. a Cerri, and K.N. Galvão. 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim. Reprod. Sci. 82-83: 513-535.

Sartori, R., R. Sartor-Bergfelt, S. a Mertens, J.N. Guenther, J.J. Parrish, and M.C. Wiltbank. 2002. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J. Dairy Sci. 85(11): 2803-2812.

Sasser, R.G., C.A. Ruder, K.A. Ivani, J.E. Butler, and W.C. Hamilton. 1986. Detection of Pregnancy by Radioimmunoassay in Serum of Cows and a Profile of Serum of a Novel Pregnancy-Specific Protein Concentrations during Gestation. Biol. Reprod. 35: 936-942.

Schlafer, D.H., P.J. Fisher, and C.J. Davies. 2000. The bovine placenta before and after birth: placental development and function in health and disease. Anim. Reprod. Sci. 60-61: 145-160.

Seals, R.C., J.W. Lemaster, F.M. Hopkins, and F.N. Schrick. 1998. Effects of elevated concentrations of prostaglandin F2 alpha on pregnancy rates in progestogen supplemented cattle. Prostaglandins Other Lipid Mediat. 56: 377-389.

Segerson, E.C., T.R. Hansen, D.W. Libby, and R.D. Randel. 1984. Ovarian and uterine morphology and function in Angus and Brahman cows. J. Anim. Sci. 59: 1026-1046.

Senger, P.L. 2003. Placentation, the endocrinology of gestation and parturition. p. 304-325. *In* Pathways to pregnancy and parturition.

Silva, E., R. a Sterry, D. Kolb, N. Mathialagan, M.F. McGrath, J.M. Ballam, and P.M. Fricke. 2007. Accuracy of a pregnancy-associated glycoprotein ELISA to determine pregnancy status of lactating dairy cows twenty-seven days after timed artificial insemination. J. Dairy Sci. 90(10): 4612-4622.

Sousa, N.M., B. Remy, B. El Amiri, J.R. de Figueiredo, H. Banga-Mboko, P.B.D. Gonçalves, and J.-françois Beckers. 2002. Characterization of pregnancy-associated glycoproteins extracted from zebu (Bos indicus) placentas removed at different gestational periods. Reprod. Nutr. Dev. 42: 227-241.

Sousa, N.M., M. Zongo, W. Pitala, H. Boly, L. Sawadogo, M. Sanon, J.R. de Figueiredo, P.B.D. Gonçalves, B. El Amiri, Z. Perènyi, and J.-F. Beckers. 2003. Pregnancy-associated glycoprotein concentrations during pregnancy and the postpartum period in Azawak Zebu cattle. Theriogenology 59(5-6): 1131-1142.

Spencer, T.E., G. a Johnson, F.W. Bazer, and R.C. Burghardt. 2004. Implantation mechanisms: insights from the sheep. Reproduction 128(6): 657-68Available at http://www.ncbi.nlm.nih.gov/pubmed/15579583 (verified 14 March 2012).

Stevenson, J.S. 2001. Reproductive Management of Dairy Cows in High Milk-Producing Herds. J. Dairy Sci. 84: E128-E143.

Suarez, S.S. 2008. Regulation of sperm storage and movement in the mammalian oviduct. Int. J. Dev. Biol. 52(5-6): 455-462.

Sutovsky, P. 2009. Sperm-egg adhesion and fusion in mammals. Expert Rev. Mol. Med. 11: 1-12.

Szafranska, B., G. Panasiewicz, and M. Majewska. 2006. Biodiversity of multiple Pregnancy-Associated Glycoprotein (PAG) family : gene cloning and chorionic protein purification in domestic and wild eutherians (Placentalia) – a review. Reprod. Nutr. Dev. 5: 481-502.

Szenci, O., F.F. Beckers, P. Humblot, J. Sulon, G. Sasser, M.A.M. Taverne, J. Varga, R. Baltusen, and G. Schekk. 1997. Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology 55(98): 77-88.

Telford, N.A., A.J. Watson, and G.A. Schultz. 1990. Review Article: Transition From Maternal to Embryonic Control in Early Mammalian Development : A Comparison of Several Species. Mol. Reprod. Dev. 26: 90-100.

Telugu, B.P.V.L., A.M. Walker, and J. a Green. 2009. Characterization of the bovine pregnancy-associated glycoprotein gene family--analysis of gene sequences, regulatory regions within the promoter and expression of selected genes. BMC Genomics 10: 185-202.

Thatcher, W.W., T.R. Bilby, J. a Bartolome, F. Silvestre, C.R. Staples, and J.E.P. Santos. 2006. Strategies for improving fertility in the modern dairy cow. Theriogenology 65(1): 30-44.

Thatcher, W.W., A. Guzeloglu, R. Mattos, M. Binelli, T.R. Hansen, and J.K. Pru. 2001. Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology 56: 1435-1450.

Thompson, I.M., R.L. a Cerri, I.H. Kim, J. a Green, J.E.P. Santos, and W.W. Thatcher. 2010. Effects of resynchronization programs on pregnancy per artificial insemination, progesterone, and pregnancy-associated glycoproteins in plasma of lactating dairy cows. J. Dairy Sci. 93(9): 4006-4018.

Turner, J.W. 1980. Genetic and Biological Aspects of Zebu Adaptability. J. Anim. Sci. 50: 1201-1205.

Varel, V.H., and K.K. Kreikemeier. 1999. Low- and high-quality forage utilization by heifers and mature beef cows . J. Anim. Sci.: 2774-2780.

Vasconcelos, J.L.M., O.G. Sá Filho, P.L.T. Justolin, P. Morelli, F.L. Aragon, M.B. Veras, and S. Soriano. 2011. Effects of postbreeding gonadotropin treatments on conception rates of lactating dairy cows subjected to timed artificial insemination or embryo transfer in a tropical environment. J. Dairy Sci. 94(1): 223-34.

Vejlsted, M., Y. Du, G. Vajta, and P. Maddox-Hyttel. 2006. Post-hatching development of the porcine and bovine embryo--defining criteria for expected development in vivo and in vitro. Theriogenology 65(1): 153-65.

Wassarman, P.M., and E.S. Litscher. 2008. Mammalian fertilization: the egg's multifunctional zona pellucida. Int. J. Dev. Biol. 52: 665-676.

Watson, A.J., M.. Westhusin, P.A. De Sousa, D.H. Betts, and L.C. Barcroft. 1999. Gene expression regulationg blastocyst formation. Theriogenology 51: 117-133.

Weems, Y.S., L. Kim, V. Humphreys, V. Tsuda, R. Blankfein, A. Wong, and C.W. Weems. 2007. Effect of luteinizing hormone (LH), pregnancy-specific protein B (PSPB), or arachidonic acid (AA) on secretion of progesterone and prostaglandins (PG) E (PGE; PGE(1) and PGE(2)) and F(2alpha) (PGF(2alpha)) by ovine corpora lutea of the estrous cycle or pr. Prostaglandins Other Lipid Mediat. 84(3-4): 163-173.

Weems, Y.S., L. Kim, V. Humphreys, V. Tsuda, and C.W. Weems. 2003. Effect of luteinizing hormone (LH), pregnancy specific protein B (PSPB), or arachidonic acid (AA) on ovine endometrium of the estrous cycle or placental secretion of prostaglandins E2 (PGE2) and F2α (PGF2α) and progesterone in vitro. Prostaglandins Other Lipid Mediat. 71(1-2): 55-73.

Whitlock, B.K., and H.S. Maxwell. 2008. Pregnancy-associated glycoproteins and pregnancy wastage in cattle. Theriogenology 70(3): 550-559.

Wilson, S.J., R.S. Marion, J.N. Spain, D.E. Spiers, D.H. Keisler, and M.C. Lucy. 1998. Effects of controlled heat stress on ovarian function of dairy cattle. 1. Lactating cows. J. Dairy Sci. 81(8): 2124-2131.

Wooding, F.B. 1982. The role of binucleate cell in ruminant placental structure. J. Reprod. Fert. 31 Suppl.: 31-39.

Wooding, F.B. 1992. Current topic: the synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. Placenta 13(2): 101-113.

Wooding, F.B.P., R.M. Roberts, and J. a Green. 2005. Light and electron microscope immunocytochemical studies of the distribution of pregnancy associated glycoproteins (PAGs) throughout pregnancy in the cow: possible functional implications. Placenta 26(10): 807-827.

Xie, S., J. Green, J.B. Bixby, B. Szafranska, J.C. DeMartini, S. Hecht, and R.M. Roberts. 1997. The diversity and evolutionary relationships of the pregnancy-associated glycoproteins, an aspartic proteinase subfamily consisting of many trophoblast-expressed genes. Proc. Natl. Acad. Sci. USA 94(24): 12809-12816.

Xie, S., B.G. Low, R.J. Nagel, J.-francois Beckers, and R.M. Roberts. 1994. A Novel Glycoprotein of the Aspartic Proteinase Gene Family Expressed in Bovine Placental Trophectoderm. Biol. Reprod. 51: 1145-1153.

Yang, Q.E., S.D. Fields, K. Zhang, M. Ozawa, S.E. Johnson, and A.D. Ealy. 2011. Fibroblast growth factor 2 promotes primitive endoderm development in bovine blastocyst outgrowths. Biol. Reprod. 85(5): 946-953.

Yang, X., C. Kubota, H. Suzuki, M. Taneja, P.E.J. Bols, and G.A. Presicce. 1998. Control of oocyte maturation in cows - biological factors. Theriogenoly 49: 471-482.

Zernicka-Goetz, M., S. a Morris, and A.W. Bruce. 2009. Making a firm decision: multifaceted regulation of cell fate in the early mouse embryo. Nat. Rev. Genet. 10(7): 467-477.

Zoli, A.P., J.F. Beckers, P. Wouters-Ballman, J. Closset, P. Falmagne, and F. Ectors. 1991. Purification and characterization of a bovine pregnancy-associated glycoprotein. Biol. Reprod. 45(1): 1-10.

Zoli, A.P., L.A. Guilbault, P. DeLahaut, W.B. Ortiz, and J.-F. Beckers. 1992. Radioimmunoassay of a Bovine Its Application Pregnancy-Associated Glycoprotein for Pregnancy in Serum : Its Application for Pregnacy Diagnosis. Biol. Reprod. 46: 83-92.

BIOGRAPHICAL SKETCH

Paula Morelli was born in Campinas, Sao Paulo, Brazil on 1985. She was raised and completed her High School degree in Campinas, Brazil. In 2005, Paula entered the Veterinary Medicine School at the Sao Paulo State University, where she joined CONAPEC Junior Company to improve her cattle production skills. After graduation she got married and changed her name to Paula Morelli Mercadante. Concomitantly, she moved to Gainesville, FL, US to pursue her master's degree in animal molecular and cellular biology at the University of Florida. She joined Dr. Alan Ealy's research program, where she focused in the placental and embryonic events of early pregnancy in beef and dairy cattle.