DISSERTATION

MECHANISMS AND ASSOCIATED BIOMARKERS OF EARLY EMBRYO MORTALITY IN HOLSTEIN-FRIESIAN COWS

Submitted by

Carolina L. González-Berríos

Department of Biomedical Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2022

Doctoral Committee:

Advisor: Thomas R. Hansen
Co-Advisor: Milton G. Thomas

Tony Schountz
Hana Van Campen

ABSTRACT

MECHANISMS AND ASSOCIATED BIOMARKERS OF EARLY EMBRYO MORTALITY IN HOLSTEIN-FRIESIAN COWS

Intensive genetic selection for milk yield has declined fertility trait levels that has led to infertility for over 50 years in Holstein-Friesian cows. A substantial contributor to infertility is embryo mortality, but the exact mechanism of how a pregnancy is poorly understood. Recent use of candidate single nucleotide polymorphisms (**SNPs**) in genetic panels has shown to improve genomic estimates of predicted transmitting abilities for fertility traits that are known to be of low heritability. The objectives of this dissertation were then divided into two chapters.

The first chapter had as an objective to elucidate the transcriptomic responses of reproductive tissues (endometrium, peripheral blood mononuclear cells [**PBMC**] and corpus luteum [**CL**]) in normal compared to pregnancies with embryo mortality in lactating Holstein-Friesian cows based upon two follow up experiments (**E1** and **E2**). At day 16, after artificial insemination (pregnant group) or of estrous cycle (non-pregnant group), reproductive tissues (endometrium, **PBMC** and **CL** [only for E2]), serum (E1) or plasma (E2) and uterine flushings were collected. Cows from pregnant group were re-classified based on embryo morphology and appearance [embryo mortality (**EM**) pregnancies had pink, red, restricted in elongation and (or) opaque conceptuses or normal (**N**) pregnancies had translucent and elongated conceptuses]. The main findings for this chapter were that: N conceptuses were longer compared to EM conceptuses. Interferon-tau (IFNT) protein concentrations in uterine flushings were greater in N compared to EM and NP in E1 but not for E2. Western blot analyses for interferon stimulated genes (ISG) 15 protein in endometrium from N cows were greater (E1 and E2) than EM and NP and EM tended

to be greater than non-pregnant in E2. The RTqPCR for *ISG15* mRNA levels in N endometrium were greater (E1 and E2) when compared to EM and NP endometrium but only tended to be greater in EM compared to NP endometrium in E2. Concentrations of progesterone in the radioimmunoassay were only significant on days 7 and 16 in E2. In RNA sequencing, *IFNT* mRNA were greater (E1 and E2) in N conceptuses compared to EM conceptuses. For E1 and E2, IPA revealed for EM compared to N conceptuses the key canonical pathways of T helper 1 (**Th1**) and Th2 to be up-regulated and are adaptive immune response that activated pro-inflammatory cytokines. Within EM compared to N endometrium, E1 had Th1 and Th2 pathways up-regulated while E2 identified differentially expressed genes (**DEGs**) that were up-regulated and associated with estradiol-mediated luteolytic action. Comparison of EM compared to N in PBMC were only significantly in E1 and had down-regulated DEGs associated with tissue growth, remodeling and/or development, cell cycle, conceptus implantation, essential mineral transporters, innate immune system and Th1 activation. The CL of E2 for embryo mortality compared to normal exhibited up-regulation of DEGs associated with inflammation, calcium sequestration/delivery, glucose- and estradiol-metabolism that may be involved in the luteolysis pathway.

The second chapter focused on the identification and validation of candidate SNPs within pregnancies with early embryo mortality that were associated with inferior fertility traits. The RNA sequencing of conceptuses (normal and embryo mortality) in Holstein-Friesian (n=15) cows from the first chapter were used to conduct the SNP discovery phase. Validation of candidate SNPs and genotype to phenotype analysis were conducted in a different cohort of Holstein-Friesian cows (n=500) by collecting blood samples to be genotyped via a genotyping assay panel and collecting cow farms records. Further filtering of candidate SNPs involved removing those that were monomorphic and not in minor allele frequency and a quality control pipeline via pLink software.

The main findings for this chapter were: a total of sixty-nine candidate SNPs were initially discovered but only twenty-three passed the quality control pipeline in pLink software. All candidate SNPs were found explain a higher amount of the R2 variation of each of the models and were in close proximity to SNP that were associated with quantitative trait loci of fertility traits. Out of the twenty-three candidate SNPs, seven (DSC2: age of cows at 1st calving were older with A allele; SREBF1 and UBD: cows took longer to conceive with T or G allele, respectively; UMPS and SREBF1: required longer time to their 1st artificial insemination with C allele; DECR1 and FASN: cows were less likely to become pregnant at 1st artificial insemination with C allele; SREBF1 and BOLA-DMB: cows were less likely to become pregnant at 150 days in milk with T allele) were significantly associated to fertility traits. It was also found that two candidate SNPs (DSC2 gene: 4 SNPs and 2 SNPs] were considered as TAG SNPs. Only two of the seven candidate SNPs had significant allele substitution effects where DSC2 in cows with G allele decreased in the age at 1st calving by 10 days and SREBF1 [rs41912290] in cows with the C allele decreased days to 1st artificial insemination by 5 days and more probability of becoming pregnant at 150 days in milk by 6%.

In summary, the data and results described in this thesis describe mechanisms of why most pregnancies fail, while others succeed in purebred Holstein-Friesian cows. The EM embryos undergo a massive T helper response either as part of or a consequence of dying. These studies may lead to the development of future technologies to improve reproductive efficiency. In addition, the identified candidate SNPs could then be used to genetically screen young heifers to identify the most fertile females while also making progress in milk production. These genetic tools will aid farmers in making decisions of culling reproductively inefficient heifers and cows within a herd.

ACKNOWLEDGEMENTS

I would like to start by thanking my co-mentors, Dr. Thomas R. Hansen and Dr. Milton G. Thomas. The two of you have helped shape me into becoming the scientist I am today but more importantly; you have forever changed my life by offering me the mentorship of a lifetime. You have equally done this through the exposure to grant writing that aided me to acquire pre-doctoral funding and collaborations in a breadth of projects: BVDV life trial (fetus to heifer), open cow test in dairy cows, IFNT pumps in sheep, and the 1000 Bull that provided me with diverse training opportunities. Having also the combination of your mentorship styles, I truly believe that you have made me into a stronger role model and knowledgeable scientist in which I could have never imagined to be. Time and again, you have both demonstrated in action that having care, passion and grit in science can be key when embarking into the unknown. Thank you for being true ambassadors of what the word mentor or in this case co-mentors is because all students should deserve people like yourselves to be part of their graduate academic journey.

To Dr. Tony Schountz and Dr. Hana Van Campen, I appreciate and thank you for having served as part of my committee. Your insights were critical for the development and success of my research during my PhD program in the Animal Reproduction and Biotechnology Laboratory (ARBL) at Colorado State University.

Next, I would like to thank the ARBL staff and faculty for being there for me in times of hurricanes, earthquakes and a pandemic. Thank you for doing above and beyond of what is expected from each and every one of you.

Carlos, Pilar and Guillermo, all of you have provided me with the strength and love that I needed since the start of this journey when all I had to my name were two suitcases and a scholarship that funded a dream. I am grateful each and every day that I can call you, mi familia. You have all been a source for me to become the person that I am today and one that will continue to carry on her Puerto Rican heritage with pride. Because no matter where I am, I know that in my heart as Roy Brown Ramírez said "Yo sería borincana aunque naciera en la luna."

Marcela and Jennifer, it was only a few years ago when the three of us met and became the "The Lunch Club" during our Master's degrees at the University of Puerto Rico-Mayagüez Campus. Thank you for being the sisters that I never had and being present in my life despite the distance, life's curveballs and career changes. Without your words of kindness, inspiration and love to guide me through both graduate journeys, I am sure that I would have not made it to the end. I am forever in your debt.

Zenon, Carolyn, Steve and Devon, despite my Puerto Rican parents and brother being far away, you have all provided the warmth of a family that I needed in Colorado. Words fall short to express the love and support you have all imparted on me during times of hardships. You have all truly adopted me as one of your own and that means everything to me. I cannot wait to officially call you all next year, my second family.

To the Women in Ag Science team, it has been a true privilege to work side by side with each and every one of you. You have all truly sparked within me a fire and passion to be part of outreach to engage with farmers and the non-scientific community. Thank you to the co-founders for creating a space where women can voice their stories to inspire the next generation.

DEDICATION

I dedicate this PhD thesis to all the women in my life and to the future generations to come. They have all been my source of inspiration to never stop moving forward and persist above all adversities even when at times my imposter syndrome took over.

Each woman, as Dr. Clarissa Pinkola Estês describes, holds within a powerful force that is filled with good instincts, passionate creativity, and ageless knowing. I have learned this to be true throughout my life but also during my graduate years and being part of academia because women do play a vital role in science specifically, in agriculture. It is crucial to not carry-on pursuing places that create divisions and keep women out of the picture because it only propels forward the notion that there is only one point of view, that of men. We need to continue to foster ideas that are diverse in nature and enrich work environments. If anything, these steps will spur humanity to attain advancements for progress sooner rather than later.

Therefore, I would like to instill in those reading this dedication as the pioneer justice Ruth Bader Ginsburg said "Women belong in all places where decisions are being made. It shouldn't be that women are the exception."

"Here is that road, maybe a thousand miles long, and the woman walking down it isn't at mile one. I don't know how far she has to go, but I know she's not going backward, despite it all- and she's not walking alone." – *Men Explain Things to Me* by Rebecca Solnit

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

APPENDIX

CHAPTER 1: LITERATURE REVIEW

Introduction

In 2021, there were 9.39 million dairy cattle in the United States of America (**USA**) and 95% of the population was composed of the Holstein-Friesian breed. Superior annual milk yield, estimated at 10,886 kilograms of milk per year per cow, made Holstein-Friesian cows the top choice among US dairy producers (Norman et al., 2007, National Academies of Sciences and Medicine, 2021, Agriculture, 2022). A modern dairy cow's ability to produce substantial amounts of milk was due to farmer's decades-long intensive genetic selection for economically relevant traits such as milk yield and milk fat and protein percentage combined with effective nutritional management (Friggens et al., 2010, Cole and VanRaden, 2018). The main drivers for genetic selection were to enhance milk yield while keeping costs of production within reason and meet the rise of consumer demands for milk and its products (Miglior et al., 2017). However, such actions from farmers caused a continuous decline in fertility traits in dairy cattle since the late 1950s until early 2005 in both the USA and globally (Crowe, 2008, Lonergan et al., 2016a). A combination of implementing genomic selection, changes to the net merit selection index, and improved management to place more emphasis on fertility and less on milk production demonstrated an upward trend in fertility traits (i.e., daughter pregnancy rate) since 2008 (VanRaden et al., 2004, García-Ruiz et al., 2016a).

High milk producing Holstein-Friesian cows still exhibit a reduced duration of estrus (6.2 hours) and related behavior (ie. numbers of times standing to be mounted, walking activity) when compared to a low milk producing cow (10.9 hours) (Kerbrat and Disenhaus, 2004, Lopez et al.,

2004, VanRaden and Miller, 2006). Limited time to detect cattle in heat affects efficient management of non-pregnant cows, when to most appropriately artificially inseminate (**AI**) them and therefore, caused farmers losses in production and profit because of extended calving intervals (Lucy, 2007, Perry, 2012). Additionally, the dairy cattle industry increased culling in Holstein-Friesian cows as a result of not becoming pregnant which then also decreased cow longevity (Ceciliani et al., 2018). Researchers described these high producing Holstein-Frisian cows to suffer from subfertility which is defined as a condition that either delays conception and (or) causes irregular fertility that leads to failure on establishing pregnancy (Royal et al., 2000, Ceciliani et al., 2018). It is estimated that subfertility had an average financial impact of $484 million on the dairy cattle industry (Bellows et al., 2002). Thus, selection for improving fertility traits is crucial to changing the subfertility present in Holstein-Friesian cows.

An example of a Holstein-Friesian cow fertility trait is "conception rate" and is defined as the percentage of cows that become pregnant divided by the number of cows that were inseminated during a twenty-one-day period (Poock, 2009). Recent studies, on annual dairy cow production, considered conception rate at a suboptimal rate of 55-88% in high producing Holstein-Friesian cows when compared to those of moderate production that are at a rate of 80-90% (Santos et al., 2004, Diskin et al., 2011). Moreover, regardless of the cow's production capacity, conception rate was estimated at 34% in primiparous Holstein-Friesian cows and then decreases to a 26% by their fifth breeding during a 21-day period (Pryce et al., 2004, VanRaden et al., 2004, Kuhn et al., 2006). This is further exacerbated by the low heritability of cow and heifer conception rate (0.001 to 0.016) when compared to milk yield traits (~0.30)(Van Tassell et al., 1999). It should be noted that negative correlations between milk yield and fertility traits are estimated to range from -0.35 to -0.60 (VanRaden et al., 2004, Pritchard et al., 2013).

In response to this predicament, modern genetic selection of Holstein-Friesian cows was comprised of reduced milk production traits at 50% of their total net merit index combined with functional traits that improve their reproductive performance (Cole and VanRaden, 2018, Pryce et al., 2018). Researchers also proposed an array of alternatives that aim to reverse the effect of subfertility by using the genomic relationship matrix to more efficiently predict breeding values of fertility traits that are of low hereditability (Meuwissen et al., 2001, Sartori et al., 2010, Walsh et al., 2011). Despite the recent advancements on cattle reproductive performance, it must be acknowledged that fertility is a complex trait that is influenced by not only genetic, environmental and management factors but also by their interactions (Royal et al., 2000, Wiltbank et al., 2016). For this reason, a varied use of strategies should be implemented in the dairy industry in order mitigate subfertility of Holstein-Friesian cows.

This literature review will therefore, focus on an overview of pregnancy in cattle, present factors that are associated with subfertility, and the current strategies that are being used to mitigate it in Holstein-Friesian cows. Further understanding of the underlying developmental biology that contributes to subfertility will aid in identifying novel strategies to improve reproductive management of dairy cattle.

Overview of the Establishment of Pregnancy

Estrous cycle

Cows have, on average, an estrous cycle length of twenty-one days that can range between seventeen to twenty-four days. It is also comprised of four distinct events (pro-estrus, estrus, metestrus, diestrus) and two phases (follicular and luteal). The events of pro-estrus and estrus are part of follicular phase while the events of metestrus and diestrus are part of the luteal phase (Perry et al., 2004). The follicular phase is characterized by four processes (recruitment, selection, dominance and atresia) of follicle growth and degeneration and or atresia that are known as follicular dynamics (Crowe, 1999). Concomitantly, the luteal phase is known for the formation of the transient tissue called corpus luteum (**CL**) on the ovary, synthesis and secretion of progesterone by the CL and the start in the regression, also known as luteolysis, of the CL. Initiation, regulation and conclusion of each of these events/phases heavily rely on the presence of specific hormones (estradiol, progesterone, follicle stimulating hormone [**FSH**], gonadotropin releasing hormone [**GnRH**]), prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) and (or) a conceptus (Kojima et al., 2003).

Pro-estrus, as the first event of the follicular phase, is defined as the moment in which a reset of the estrous cycle takes place, between days 17 to 21 (Driancourt, 1993). Such event can only happen when there is no conceptus present or if the conceptus is unable to establish maternal recognition of pregnancy followed by implantation and placentation (Spencer et al., 2007, Spencer et al., 2008). Luteolysis of the CL transpires via oxytocin-induced pulsatile release of $PGF_{2\alpha}$ from the cow's endometrium (Hansen and Tríbulo, 2019). Production levels of progesterone by the CL are then halted and the borderline-suppression of GnRH in the hypothalamus by progesterone no longer exists (McGee, 2000). Rise of GnRH stimulates the anterior lobe of the pituitary to secrete

two gonadotropins. The first is FSH, which aids the recruited follicles to grow and develop and the second gonadotropin is the luteinizing hormone (**LH**) which causes rupture of the dominant follicle also known as ovulation and formation of the CL (Crowe, 1999). The growing follicles in the ovary start producing estradiol and inhibin via stimulation of FSH and are released into blood circulation. Presence of estradiol in circulation causes positive feedback on the release of GnRH and LH while inhibin causes negative feedback on the release of FSH which assures the selection of a single dominant follicle to ovulate (Lucy, 1992). Also, estradiol increases uterine levels of estrogen receptor alpha (**ESR1**), oxytocin receptor (**OXTR**) and progesterone receptor (**PGR**). ESR1 and OXTR are essential for the activation and release of $PGF_{2\alpha}$ while PGR is responsible for blocking it (Hansen and Tríbulo, 2019). This process is then followed by the selection of the largest follicle becoming both dominant and the main source of estradiol and inhibin release while the other recruited medium-sized follicles undergo atresia (Crowe, 1999). Peak production of estradiol by the dominant follicle marks the estrus event, in the follicular phase, which lasts between twelve hours to eighteen hours and is designated as day zero of the estrous cycle (Wiltbank et al., 1967). During this time, the LH surge occurs and a cow demonstrates visual signs of their reproductive status through inflammation of the vulva, discharge of clear mucus from the vagina and mounting/standing behavior. Rupture of the follicle, also known as ovulation, emerges twenty-five to thirty hours after the onset of estrus and the formation of the corpus hemorrhagic (**CH**) begins (Bernard et al., 1983).

Ovulation of the follicle prompts for the luteal phase and initiates the metestrus event. Metestrus is distinguished by the CH transitioning to become the CL in order to produce progesterone, a decrease in inhibin by the ovulated follicle and a slow release of estradiol and FSH to start the first wave of recruitment of new follicles for the next estrous cycle (Crowe, 1999).

Transition of the CH to the CL originates from being composed of granulosa cells from the ovulated follicle that develop into large luteal cells and theca internal cells that develop into small luteal cells (Alila and Hansel, 1984). Furthermore, small luteal are able to undergo differentiation into large luteal cells by the presence of LH (Farin et al., 1990). Once the threshold of progesterone by the CL is passed, the diestrus event then takes place and borderline-suppression of GnRH by progesterone occurs. The presence of progesterone is also essential for maintaining a competent uterine environment for an implanting conceptus, regulates the reproductive cycle via the PGR and prepares the uterine lining to secrete histotroph (Bazer et al., 1975, Bazer et al., 1997, Spencer et al., 2008, Bauersachs et al., 2009, Spencer and Hansen, 2015, Spencer et al., 2016). A process that continues from the metestrus event is secretion of FSH and estradiol to recruit a second and (or) third wave of follicles. Two scenarios can then occur after the diestrus event. The first is that the recruited follicles will undergo atresia if a conceptus establishes maternal recognition of pregnancy and there is no reset of the estrous cycle. The second scenario is that there is either no presence of a conceptus or a conceptus is unable to establish maternal recognition, the event of proestrus in the follicular phase is prompted and therefore, reset of the estrous cycle occurs (Spencer et al., 2007, Spencer et al., 2008).

Luteal Rescue by the Conceptus

Establishment of pregnancy takes place when the trophectoderm of the conceptus secretes interferon tau (**IFNT**) and prevents luteolysis of the CL through paracrine actions on the endometrial luminal epithelium (Bazer et al., 1992, Spencer et al., 2008, Spencer and Hansen, 2015, Forde et al., 2017, Hansen et al., 2017). Luteal protection by IFNT occurs through the inhibition of ESR1 which in turn prevents up-regulation of OXTR and therefore, attenuates the

pulsatile release of $PGF_{2\alpha}$ (Spencer et al., 1996, McCracken et al., 1999, Spencer and Hansen, 2015, Hansen et al., 2017). The CL can then continue producing progesterone. By understanding the endocrinology and physiology of how all these components work in unison or in a time-dependent axis, are key to reducing the reproductive challenges and improving how to effectively manage the synchronization of dairy cattle.

Factors associated with Subfertility

Embryo Mortality

Profitability in the dairy cattle industry is directly dependent of the reproductive efficacy of each cow because it can impact milk production and the number of calves across their lifetime (Santos et al., 2004). Pregnancies with embryo mortality (**EM**) are considered the main factor responsible for reducing reproductive efficiency in cattle after breeding and it denotes the death of an embryo up to when the stage of differentiation occurs (Evans et al., 2012, Parmar et al., 2016). Furthermore, EM is also divided into two major time points of pregnancy: early (days 7 to 24) and late (days 24 to 45) (Santos et al., 2004). Several studies observed that most pregnancies with EM occur early-on and account for 43 out of 50% of all failed reproductive outcomes in high milk producing Holstein-Friesian cows (Diskin and Morris, 2008, Diskin et al., 2011). Pregnancies with early EM could be due to inappropriate communication between the developing embryo and the maternal endometrium for the establishment of pregnancy to take place on day 16 (Sánchez et al., 2019). Some examples of essential factors that are needed for the establishment of pregnancy are: a sufficient production of histotroph from the uterus (day 7 to 13), proper embryo elongation (days 7 to 15) and sufficient IFNT production from the embryo in order to block $PGF_{2\alpha}$ and maintain pregnancy (day 14 to 18) (Walsh et al., 2011). Failure to maintain pregnancy causes early EM

which can generate an annual loss of $1.6 billion in the US and $1.28 trillion worldwide via repeat breeding costs for artificial insemination procedures, extension of calving intervals, prolonged dry periods and therefore, reducing lifetime milk production (Shah, 2009, Perkel et al., 2015, Parmar et al., 2016). However, it is important to indicate that pregnancies with EM whether early or late can occur due to other factors such as nutrition, environment, endocrine disruptions, infectious disease, immunotolerance and (or) genetics which will be discussed below.

Nutrition

USA Holstein-Friesian cows are often distinguished by their reduced pregnancy rates associated with an excessive energy mobilization when compared to those in New Zealand (Horan et al., 2005, Macdonald et al., 2008). As previously mentioned, USA Holstein-Friesian cows were intensively genetically selected to produce higher milk yield which requires mobilizing a substantial amount of body energy reserves. The expenditure of energy from producing milk then needs to be compensated by dietary feed intake in order to keep body energy in balance rather than in negative. However, output of milk not only increases over time, but it is also often faster than the cow's ability to acquire the appropriate amount of feed intake to prevent excessive energy mobilization (Ingvartsen and Friggens, 2005). Failure to keep energy at a balance increases the chance of delaying first ovulation and (or) start of luteal activity (Garbarino et al., 2004, Berry et al., 2008). In fact, early lactation is classified as the period in which the excessive energy mobilization causes the greatest amount of negative energy balance and excessive uptake of non-esterified fatty acids by the liver. Greater accumulation of fatty acid rather than oxidation can then result in fatty liver disease which decreases both health and reproductive performance (Bobe et al., 2004, Friggens et al., 2010). Consequently, standard use of fat and oil supplementation in the USA

have attempted to reverse the impact of excessive energy mobilization on reproduction (Friggens et al., 2010). Researchers have reported that fat in general can modulate the role of non-esterified fatty acids that can then impact both nutritional and reproduction status in cows (Ambrose et al., 2006, Petit and Twagiramungu, 2006, Friggens et al., 2010). Low body condition score (**bcs**; i.e., $\leq$2.5 from a score of 5) as a result from nutritionally sub-maintained body fatness have also been found to have a negative correlation with fertility in Holstein-Friesian cows (Royal et al., 2000, Pryce et al., 2001, Berry et al., 2003, Berry et al., 2008). Only two studies, in cattle, have been able to clearly separate nutritionally sub-maintained body fatness from excessive energy mobilization in order to asses low body condition score affecting fertility. The first being Wright et al. (1992) documented that feeding a low or high fat diet to thin cows (bcs=2.23) at calving had a longer anoestrus period (89 vs 116 days) and lower LH pulse frequency when compared to those that were fat (bcs=2.85) (Wright et al., 1992). The second study reported that fat heifers with a negative energy balance suppressed progesterone in response to LH (Villa-Godoy et al., 1990). Proper feeding strategies and feed intake before and after calving are then necessary to prevent delay of normal cyclicity and impairment of reproductive performance that could lead to pregnancies with EM (Friggens et al., 2010).

Environment

Heat stress is considered the predominant factor of environmental stressors that affect reproductive performance and fertility in dairy cows (Wolfenson et al., 2000, Parmar et al., 2016). Incidence of heat stress is observed in the summer months which causes a decline in conception rates and thus, longer calving to conception intervals (Cavestany and Foote, 1985, Zeron et al., 2001, Hansen, 2009, Parmar et al., 2016). It's been published that cows who experience heat stress

have a delay on their fall fertility when there is a diminished steroidogenic capacity in their ovaries due to a reduction of androgens from theca cells (Wolfenson et al., 1997). Production of androgens by theca cells are essential to the ovary because they stimulate secretion of estradiol which is a requirement for normal cyclicity to occur in dairy cows (Wolfenson et al., 1997). Similarly, *in vitro* studies of luteal cells retrieved from cows in the summer produce less progesterone when compared to cells obtained in the winter (Wolfenson et al., 1993). Results on progesterone plasma concentrations, however, have been variable in response to heat stress being either acute or chronic (Howell et al., 1994). Altered follicular dynamics, secretions of FSH and progesterone are associated with generating an abnormal endometrial environment for oocyte development (Bilodeau-Goeseels, 2003). Additional components that are also affected by heat stress are oocyte quality which decreases fertilization rates and embryo survival since protective mechanisms occur later in development (Ealy et al., 1993, Edwards et al., 2001). Subsequently, cows that are heat stressed and are AI have a 31% less chance to conceive when compared to non-heat stressed cows (Chebel et al., 2004). These results then indicate that cooling strategies such as fans, sprinklers and barns that provide shade need to utilized by dairy farms to combat heat stress and excessive pregnancies with EM.

Endocrine

A well-studied hormone of early pregnancy (days 4 to 16) of cattle is progesterone. Past evidence suggested that progesterone directly contributes to both the rate of development and growth of an embryo (Garrett et al., 1988). However, *in vitro* culture revealed varied and contradictory results as to progesterone does in fact provide a positive (Ferguson, 2004, Ferguson et al., 2012) or no effect (Reggio et al., 1997, Goff and Smith, 1998, Lonergan et al., 2016a) despite

the mRNA presence of progesterone receptor in embryo (Clemente et al., 2009). It has since then been proposed that progesterone provides a stimulatory effect on trophoblastic elongation mediated through downstream induced changes on the endometrium's cells to produce histotroph for the embryo (Satterfield et al., 2006, Forde et al., 2009, Forde et al., 2011a). Additionally, studies have observed that a reduction of progesterone in the CL can then result in a delay of temporal changes within the endometrium and a setback on the *in vivo* conceptus elongation (Forde et al., 2011a, Forde et al., 2012b, Lonergan et al., 2016a). Another detrimental effect of low concentrations of progesterone is the reduction of fertilization and embryo survival rates due to the CL's inability to suppress increased frequency in pulses of LH that trigger the estradiol cascade and therefore, alter the endometrium's morphology (Diskin et al., 2006). Cows that have early on increases of progesterone during days 4 through 7 of post-insemination have higher chances of maintaining a pregnancy (Diskin and Morris, 2008). This is supported by scientists like Starbuck et al. (2001) and Carter et al. (2008) who reported increased embryo survival and size by supplementing animals with progesterone but found that those with adequate concentrations of progesterone could induce embryo death (Starbuck et al., 2001, Carter et al., 2008). Conversely, excessive secretion of estradiol from a large follicle during the time of maternal recognition can also affect embryo survival (Pritchard and Donnelly, 1999). The relationship between progesterone and estradiol is therefore, of crucial balance in order to maintain CL function and thus, pregnancy itself (Parmar et al., 2016).

Diseases

Periparturient diseases often impact health, compromise the uterine environment and as a result, are associated with reduced reproduction in cattle (Gröhn and Rajala-Schultz, 2000,

Bilodeau-Goeseels, 2003). The length of this period is one that has many interpretations but is generally comprised of three weeks before and after calving (Smith and Risco, 2005). It is also considered a critical period in a cow's production cycle because those that exhibit periparturient diseases are highly prone to develop other types of diseases in the first ten days of milk (Opsomer et al., 2000, Ingvartsen et al., 2003, Williams et al., 2007). According to Barlett et al. (1986), it is estimated that periparturient diseases account for economic losses ranging from $200 to $400 per disease and lactation (C. Bartlett et al., 1986). Among the most common and challenging periparturient diseases is retained fetal membranes (Sandals et al., 1979, Paisley et al., 1986, Correa et al., 1993). It is responsible for predisposing dairy cattle six times more to a uterine infection compared to cows without retained fetal membranes (Dohoo and Martin, 1984, Correa et al., 1993). This is further exacerbated by cows generally experiencing uterine bacterial contamination at parturition compromising uterine function (Griffin et al., 1974). This then makes cows become 1.8 times more likely to experience EM pregnancies, abortions, and hence, a decline in conception rates (Sheldon et al., 2006, Chebel et al., 2016). Dairy cattle also manifest changes in their eating behaviors by reducing the amount of dry matter intake and time spent ingesting feed when in a state of critical decline in health (Urton et al., 2005, Huzzey et al., 2007). The severity of uterine infections can be categorized by the layers of the uterus that are affected and the stage of the infection. Examples of such infections are: metritis, endometritis, pyometra. Studies have also shown that other factors, during the periparturient period, that are responsible for the development of retained fetal membranes are dystocia, sex of the calf, twining, abortion, and hypocalcemia (Dohoo and Martin, 1984, Correa et al., 1993). Periparturient diseases are detrimental to a cow's future reproductive and production cycle and for this reason, need to be prevented by providing special care before and after cows calve.

Immunotolerance

The maternal uterine environment has been found to be a key contributor to the conceptus survival and development during the pre-implantation period in cattle (Berg et al., 2010, Tríbulo et al., 2018). Research evidence has demonstrated that cross-talk between a competent conceptus and a receptive uterine endometrium are essential for establishment and a successful pregnancy to take place (Bazer et al., 2011, Lonergan and Forde, 2014). Any abnormal uterine receptivity and/or suboptimal communication with a conceptus whose development has been impaired has been associated with contributing to a high incidence of pregnancies with EM (Lonergan and Forde, 2014). However, both the uterine environment and conceptus are known to be semi-autonomous up to the moment of pregnancy recognition on day 16 where the conceptus' trophectoderm secretes IFNT to mediate paracrine action on endometrium (Forde et al., 2011a). Further corroboration has been made by studies only finding endometrial transcriptome differences of 'classical' interferon stimulated genes (ISGs: *MX1/2, OAS1, BST2, B2M, CXCL10, STAT1/2, PTX3, EIF4E, USP18*) starting on day 16 of a viable pregnancy between cyclic and pregnant heifers (Forde et al., 2011a, Bauersachs and Wolf, 2012). It has also been argued that the endometrial changes taking place under the influence of hormones (follicular estradiol and luteal progesterone), cytokines and paracrine signals that stimulate growth factor gene expression that drive conceptus elongation are independent of the presence of a conceptus (Clemente et al., 2009, Forde et al., 2011a, Forde et al., 2014). These findings indicate that both a spatial and temporal alteration of the endometrium's transcriptome needs to occur in order for uterine receptivity for implantation to take place (Spencer et al., 1996). It is also thought that such independence between the conceptus and uterine endometrium evolved in ruminants due to the prolonged conceptus elongation and to prevent luteolysis from occurring before implantation (Roberts, 2007). Therefore, it is suggested that the

default mechanism of uterus is to prepare for and/or expect pregnancy regardless of the exposure of an early embryo (Betteridge et al., 1980, Forde et al., 2011a). The previous statements appear true up until day 7 when all embryo transfers occur due to the development of post-hatching and pre-implantation in a conceptus is completely maternally driven and thus, cannot occur *in vitro* or in the absence of uterine glands (Brandão et al., 2004, Vejlsted et al., 2006, Diskin and Morris, 2008). Another type of immunotolerance perspective on the conceptus is it being considered an intruder by the maternal immune system's major histocompatibility complex (**MHC**) within the endometrium due to its paternal inherited proteins (La Rocca et al., 2014). Maternal immunotolerance is thought to partially occur through the signaling and interactions of the conceptus' trophectoderm-derived IFNT, progesterone and the presence of maternal regulatory T cells to downregulate the MHC (Wan et al., 1987, La Rocca et al., 2014). In summary, an effective crosstalk between the uterine environment and the conceptus is needed for pregnancy to not only occur but also to succeed.

Genetics

An approximately 10% of all embryo mortalities that occur within the first two weeks of pregnancy are due to genetic abnormalities (Parmar et al., 2016). Historically, the deficiency of uridine monophosphate synthase (**DUMPS**) has been a major autosomal recessive defect that has impacted embryo survival in the Holstein-Frisian breed (Diskin and Morris, 2008). DUMPS is characterized by the inactivation of the UMPS enzyme, essential for normal growth and development, via a single nucleotide polymorphism (**SNP**) of C/T and therefore, causes abortions to homozygous embryos by day 40 of pregnancy (Schwenger et al., 1993). Research results revealed that DUMPS was mainly spread by semen that was used for AI from selected bull sires

which has prompted for blood testing and eliminated carriers for this condition (VanRaden and Miller, 2006). Apart from DUMPS, the 1/29 Robertsonian translocation is the most studied cattle genetic abnormality where centromeric fusion of chromosomes 1 and 29 occurs during chromosomal re-arrangement and results in a single chromosome rather than two (Bilodeau-Goeseels and Kastelic, 2003). Both female and male carriers that are heterozygous for the mutation have a reduced 3 to 5% in their fertility and can produce more frequently early EM pregnancies than viable ones (Gustavsson, 1979, Bilodeau-Goeseels and Kastelic, 2003). Since the discovery of the 1/29 Robertsonian translocation, various beef cattle breeds and Scandinavian Red breeds have been identified to be carriers of the mutation but not in the Holstein-Friesian breed (Diskin and Morris, 2008). Like DUMPS, blood testing was implemented in Sweden where they were able to eliminate animals that were carriers of the mutation before being enrolled in reproduction and AI programs (Gustavsson, 1979). Other more common genetic abnormalities are errors in the ploidy of chromosomes, loss of genetic material, complex vertebral malformation and maternal inbreeding (Diskin and Morris, 2008, Parmar et al., 2016). In general, genetic variations that do affect embryo survival are often attributed to the genetic differences found between the dam and the embryo or the genetic composition of the embryo itself which could lead to EM (Diskin and Morris, 2008).

Current Strategies to mitigate subfertility

Palpation / Ultrasonography via rectum and blood markers

Palpation and/or ultrasonography via rectum are considered common practices used to determine pregnancy status of a cow (Roberts, 1971, Momont, 1990, Romano and Magee, 2001, Youngquist, 2007). Palpation via rectum is considered a frequent and common practice that has

been found to have a small adverse effect on calving rates when a single veterinarian conducts them (Roberts, 1971, Momont, 1990, Roberts, 2007, Romano et al., 2007, Youngquist, 2007). Therefore, out of the two methods, ultrasonography is considered the gold standard of determining early pregnancy in cows because it provides immediate information on the conceptuses viability and reduces the number of both false positives and negatives (Romano and Magee, 2001). Detection of pregnancies with EM are a challenge due to it taking place during the pre-implantation period, on days 7 to 16 of pregnancy. This means that pregnancies with EM remain undetected until pregnancy status can be determined at day 32 via ultrasound or at day 35 via palpation by a skilled practitioner (Roberts, 1971, Momont, 1990, Youngquist, 2007, Lonergan and Forde, 2014).

Another set of early pregnancy diagnosis tools that have proven to be practical are the use of blood markers (Northrop et al., 2019). Currently, there are two commercially available tests (Biopryn and Alertys) that analyze blood at day 28 after AI for pregnancy but measure different types of antigens (pregnancy-associated glycoprotein [**PAG**] or pregnancy-specific protein B [**PSPB**], respectively) (Sasser et al., 1986, Green et al., 2000). Although both tests have high degrees of accuracy on non-pregnant cows (99%) and pregnant cows (95%) (Northrop et al., 2019), researchers reported that lactating cows could still have PAGs present even after 80 to 100 days after calving and give a false positive result (Green et al., 2005). Another problem that is presented by these blood tests are their inability to be conducted earlier post-AI which could aid with the detection of pregnancies with EM, re-insemination of non-pregnant cows sooner and economic consequences due to delayed return to pregnancy (Lucy et al., 2004). Despite these issues, demand for both blood markers have proven to be advantageous by providing a cost effective and easier method to detect pregnancy rather than having to invest in ultrasound equipment and acquiring skills to master rectal-palpation or -ultrasound (Northrop et al., 2019). Thus, early diagnosis of

non-pregnant cows is imperative to efficiently resynchronizing them in a timely manner and limits the management of a cow's reproductive performance (Zemjanis, 1970, Romano et al., 2007).

Estrous Synchronization Programs and Timed-Artificial Insemination

Detection of cows experiencing estrus has been traditionally performed through visual observation, which is time consuming (Firk et al., 2002). Notably, estrous cycle and visual detection of estrus behavior has shortened due to a combination of factors (i.e., heat stress, genetics, nutrition) in high milk producing cows when compared to a low milk producing cow (Van Eerdenburg et al., 1996, Kerbrat and Disenhaus, 2004, Lopez et al., 2004, Wiltbank and Pursley, 2014). Some of the earliest tools that were used to aid with visual estrus detection were paint or Kamar Heatmount Detectors on a cow's tail (Firk et al., 2002, López-Gatius et al., 2005). Unfortunately, such methods are not considered to successfully and consistently identify all cows experiencing estrus in order to accurately use timed AI and therefore, reducing efficiency and profitability the reproductive performance of a herd (Nowicki et al., 2017). More contemporary and effective tools being used to manipulate the reproductive process in cows and heifers are estrus synchronization programs that are based on using hormones schemes combined with pedometers (Goshen et al., 2015), Eazibreed CIDR (Bartolome et al., 2009) and/or Estrotect patches (Pursley et al., 1995, Thatcher et al., 2002). Advantages of using these types of programs are herds whom experienced estrus at a predicted time frame which permits for follicular development, regression of the CL and facilitates a precise timed embryo transfers or AI (Thatcher et al., 2003). There are a variety of synchronization programs that commonly used in combination with timed AI. Two of the most common examples of such programs that are used in dairy cows are OvSynch and it modified form, Presynch-OvSynch (Goshen et al., 2015). However, each have their pros, cons and

are reliant of what type of reproductive management is wanted to be achieved during a specific time point of the estrous cycle. Ovsynch offers the ability of not having to detect estrus because it can synchronize both follicle maturation and regression of the CL before ovulation and timed AI takes place (Thatcher et al., 2002). The Ovsynch protocol consists of intramuscularly injecting GnRH seven days before followed by forty-eight hours after an intramuscular injection with $PGF_{2\alpha}$ and finally, inseminating cows at twelve to sixteen hours later from the second intramuscular injection of GnRH. Some limitations of Ovsynch are that it can reduce pregnancy rates due if a cow's estrous cycle is not at the specific period of days 5-12. Furthermore, Ovsynch is considered the oldest method out of the two and has been modified as needed to meet the needs for the desired type of reproductive management of a herd (Goshen et al., 2015). The second program is Presynch-OvSynch and is comprised of two $PGF_{2\alpha}$ instramuscular injections that are administered in a fourteen interval and followed by the OvSynch that is initiated twelve days after the second intramuscular injection of $PGF_{2\alpha}$. Therefore, out of the two most common synchronization programs, Presynch-OvSynch provides the most increase of pregnancy rates (25-43%) in dairy lactating cyclic cows due to OvSynch and timed AI being implemented at the most favorable stages of the estrous cycle that could lead towards pregnancy success rather than in EM (Moreira et al., 2001).

In vitro Embryo Production, Selection and Transfer

Cattle have shown to have various patterns of infertility that could occur during the *in vitro* production of bovine embryos (Perkel et al., 2015). According to Lonergan et al. (2003), an approximate 90% of oocytes resume meiosis and undergo maturation *in vitro* but only 30-40% of them develop to the blastocyst stage (Lonergan et al., 2003). Therefore, the previous statement

argues the importance of development taking place during the pre-implantation stage which can influence the blastocyst' outcome (Farin et al., 2001, Rizos et al., 2002, Havlicek et al., 2005, Lonergan et al., 2006). Some have suggested that *in vitro* embryo production is highly responsible for altering lipid content, DNA methylation, metabolism, ultrastructure, gene expression and ultimately, inability to establish pregnancy (Boni, 1999, Crosier et al., 2000, Rizos et al., 2002, Corcoran et al., 2006, Lonergan et al., 2007, McHughes et al., 2009, Pontes et al., 2009, Niemann et al., 2010, Gad et al., 2012, Sudano et al., 2014). While others argue that *in vitro* embryo production is advantageous when compared to both AI and embryo transfer procedures because females who are unresponsive to estrous synchronization programs and/or superovulation treatments can then overcome infertility (Mermillod et al., 1996, Baruselli et al., 2006).

Traditionally, the technique of *in vitro* embryo production has been used for slaughtered cattle which aids in recovering genetic material or during transvaginal ultrasound-guided oocyte retrieval on superior animals to serve as embryo donors (Mermillod et al., 1992, Xu et al., 1992, Van Soom et al., 1994, Bols and Stout, 2018). However, retrieval of cow ovaries from a slaughterhouse tends to be of mixed quality (ill or reproductively inefficient) (Perkel et al., 2015). Another way in which embryos can be used to overcome early EM is by assessing their morphology (fragmentation, symmetry of blastomeres, zona pellucida thickness, cleavage rates) and thus, selecting those with the highest developmental potential for transfer (Ziebe et al., 1997, Stringfellow, 1998, Salumets et al., 2001, Gordon and Melvin, 2003, Salumets et al., 2003, Hansen, 2006, Perkel et al., 2015). The predicament and limitation with the technique of assessing an embryo's quality through visual morphology is that it is highly subjective upon the observer's experience level or bias on the grading scheme being used (Perkel et al., 2015). Some scientists have also found that embryos that were classified as having superior morphology were prevalent

to aneuploidy and could harbor undetectable genetic defects that could later on compromise an embryo's development (Márquez et al., 2000, Khatib et al., 2009, Huang et al., 2010, Nel-Themaat and Nagy, 2011, Perkel et al., 2015). Therefore, time-lapse imaging and embryo metabolism are some of the non-invasive techniques that have been suggested to be used in conjunction with morphological parameters but are not yet widely accepted by the scientific/clinical community (Perkel et al., 2015). Last but not least, the use embryo transfer can also prevent EM pregnancies from happening and are divided into two types of categories. The first is synchronous and is defined as an embryo being in synchrony (development difference of 48 hours or less) to the recipient's uterine environment being transferred (Moore and Shelton, 1964, Rowson and Moor, 1966, Rowson et al., 1972, Pope, 1988). The second is asynchronous and refers to an embryo and uterus being 'out of sync', demonstrating how progesterone has a timely effect on the uterus regulation towards a developing conceptus (Forde and Lonergan, 2017). Research data comparing both types of embryo transfers found that blastocysts that were transferred asynchronously (day 7) vs those synchronously to a uterus (day 5 or 9) resulted in either delayed (day 5 uteri: 5.4 ± 0.4 mm) or advance (day 9 uteri: 50.4 ± 1.5 mm) conceptus growth and a decrease in conceptus survival (20%) (Ledgard et al., 2012). Therefore, each of the previous steps that can be taken to improve embryo quality whether it is through *in vitro* production, selection and/or transfer could potentially aid in mitigating preventing EM pregnancies from occurring.

Biomarkers

With the world's population growth and demand for food production, an increase in cattle efficiency (i.e., reproduction, health, performance) has become a fundamental requirement for farm sustainability and has therefore, driven for the implementation of both novel and precise

strategies in genetic selection (Berry et al., 2013, Mazzoni et al., 2017, Miretti et al., 2020). Rapid improvement in cattle have occurred due to omics and are defined as high-throughput sequencing technologies that can explore either the genome structure (genomics) or -function (transcriptomics, proteomics, metabolomics) (Valour et al., 2015). An example of an omic technology are biomarkers which are a measurable biological indicator such as DNA, mRNA, proteins or metabolites that are within biological fluids and are used as a non-invasive diagnostic tool that predicts the state of an individual's health and reproductive traits (Strimbu and Tavel, 2010, Kadarmideen, 2014). Biomarkers are based on the identification of bovine genetic polymorphisms (SNPs) within genes or quantitative trait loci (**QTL**) regions that are associated with economic relevant traits (**ERTs**) (Valour et al., 2015). Use of biomarkers can also identify the effects of molecules and regulatory networks on ERTs and gives a person the ability to genetically select animals for specific traits of interest with high accuracy (Berry et al., 2014, Suravajhala et al., 2016, Soler et al., 2020). As a result, biomarkers such as those within Clarified Plus have substantially economically impacted the dairy and beef cattle industry by reducing the traditional costs of generational intervals to improve ERTs and overall enhancing the management and productivity of cattle (Witkamp, 2005, Moore et al., 2007, Fessenden et al., 2020). A challenge with biomarkers is that they need large amounts of information in order to accurately predict ERTs and specifically, those associated with low heritability such as reproduction (Berry et al., 2014). Further issues on biomarkers arise due to the QTL regions associated to ERTs being only shared 20% between cattle populations and are mostly found to be in specific cattle breeds (Valour et al., 2015). Despite this, a handful of biomarkers have been previously identified in QTL regions that are associated to non-fertilization, pregnancies with EM, reproductive (i.e., endometritis, sub-clinical mastitis) and non-reproductive diseases (i.e., respiratory) by using large pedigrees and

improved genotyping procedures (Lefebvre, 2011, Hussein et al., 2018, Pereira et al., 2020, Li et al., 2022). Another disadvantage to these QTL regions for pregnancies with EM is that they are centered on *in vitro* embryo transcriptome studies even though they encumber key developmental time points in which there is a maternal to embryonic transition, followed by hatching, elongation and finally the initiation of implantation (Ushizawa et al., 2004, El-Sayed et al., 2006, Hue et al., 2007, Mamo et al., 2011, Sandra et al., 2011, Hue et al., 2012, Khan et al., 2012, Mondou et al., 2012, Graf et al., 2014a, Graf et al., 2014b). Therefore, evaluating *in vivo* embryo development that takes into account both the maternal environment and embryo quality are key to further understanding pregnancies with EM (Valour et al., 2015).

Conclusions

In summary, several factors affect Holstein-Friesian cows during pregnancy that could then lead to EM and numerous types of technologies have tried to mitigate such outcome. However, one of the most vital pieces of pregnancies with EM is on further understanding how it occurs in order to propose more effective alternatives. Current transcriptomic technology in the cattle industry indicates that an evident step towards identifying cows that are reproductively efficient from those that are prone to pregnancies with EM can be accomplished using genomic biomarkers.

Rationale and Significance

Although predisposing factors for the poor fertility in the modern USA Holstein-Friesian cows have been identified, pregnancies with EM cause 43% of all failed pregnancies in high producing USA dairy cows (Diskin and Morris, 2008, Diskin et al., 2011). Such pregnancies with EM occur between days 7 to 19 of pregnancy and there is no detection of it before an ultrasound can be conducted on day 32 after AI (Diskin et al., 2006). It is estimated that pregnancies with EM annually cost the cattle industry $1.4 billion in the USA and $1.28 trillion worldwide due to inefficient management of nonpregnant cows (Perkel et al., 2015). Currently, pregnancies with EM are poorly understood due to the limiting number of studies that have explored the relationship between the amount of IFNT synthesized, embryo morphology (normal or EM) and maternal environment (immune system).

The hypothesis for this dissertation was that pregnancies with EM are associated with impaired conceptus-derived IFNT secretion that may impede action (endocrine and paracrine) on reproductive tissues (endometrium, CL, peripheral blood mononuclear cells) which may lead to luteolysis and disruption of pregnancy. Clarifying the mechanisms involved in pregnancies with EM may lead to the identification of genetic biomarkers that could be used to manage heifers and cows prone to inferior fertility traits.

The objective of chapter 2 of this dissertation was to elucidate the mechanisms from a pregnancy with an EM conceptus and the transcriptome response in reproductive tissues (endometrium, peripheral blood mononuclear cells and corpus luteum) via RNA-Sequencing. Given the transcriptome results of chapter 2, the objective of chapter 3 were to conduct SNP discovery on the RNA Sequencing single end read sequences from the conceptuses (normal vs

EM) in order to identify any candidate SNPs that were associated with EM pregnancies that could cause cows to have inferior fertility traits.

REFERENCES

Agriculture, N. A. S. S.-U. S. D. o. 2022. Production per Cow, 2012-2021. National Agricultural Statistics Service-United States Department of Agriculture.

Alila, H. W. and W. Hansel. 1984. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol Reprod 31(5):1015-1025.

Ambrose, D. J., J. P. Kastelic, R. Corbett, P. A. Pitney, H. V. Petit, J. A. Small, and P. Zalkovic. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in alpha-linolenic acid. J Dairy Sci 89(8):3066-3074.

Bartolome, J. A., J. J. van Leeuwen, M. Thieme, O. G. Sa'filho, P. Melendez, L. F. Archbald, and W. W. Thatcher. 2009. Synchronization and resynchronization of inseminations in lactating dairy cows with the CIDR insert and the Ovsynch protocol. Theriogenology 72(6):869-878.

Baruselli, P. S., M. F. de Sá Filho, C. M. Martins, L. F. Nasser, M. F. Nogueira, C. M. Barros, and G. A. Bó. 2006. Superovulation and embryo transfer in Bos indicus cattle. Theriogenology 65(1):77-88.

Bauersachs, S., E. Ulbrich Susanne, V. Zakhartchenko, M. Minten, M. Reichenbach, H.-D. Reichenbach, H. Blum, E. Spencer Thomas, and E. Wolf. 2009. The endometrium responds differently to cloned versus fertilized embryos. Proceedings of the National Academy of Sciences 106(14):5681-5686.

Bauersachs, S. and E. Wolf. 2012. Transcriptome analyses of bovine, porcine and equine endometrium during the pre-implantation phase. Anim Reprod Sci 134(1-2):84-94.

Bazer, F. W., T. T. Chen, J. W. Knight, D. Schlosnagle, N. J. Baldwin, and R. M. Roberts. 1975. Presence of a progesterone-induced, uterine specific, acid phosphatase in allantoic fluid of gilts. J Anim Sci 41(4):1112-1119.

Bazer, F. W., M. A. Mirando, T. L. Ott, J. P. Harney, D. H. Dubois, T. K. Schalue, C. H. Pontzer, C. Hostetler, H. M. Johnson, and T. Ogle. 1992. Roles of ovine trophoblast protein-1 and oestradiol/prolactin in the establishment of pregnancy in sheep and pigs. Reprod Fertil Dev 4(3):335-340.

Bazer, F. W., T. E. Spencer, G. A. Johnson, and R. C. Burghardt. 2011. Uterine receptivity to implantation of blastocysts in mammals. Front Biosci (Schol Ed) 3(2):745-767.

Bazer, F. W., T. E. Spencer, and T. L. Ott. 1997. Interferon tau: a novel pregnancy recognition signal. Am J Reprod Immunol 37(6):412-420.

Bellows, D. S., S. L. Ott, and R. A. Bellows. 2002. Review: Cost of Reproductive Diseases and Conditions in Cattle11Portions of this research were conducted under a cooperative agreement between USDA, ARS and the Montana Agric. Exp. Stn. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana Agric. Exp. Stn., or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA, ARS, Northern Plains Area, is an equal opportunity/affirmative action employer, and all agency services are available without discrimination. The Professional Animal Scientist 18(1):26-32.

Berg, D. K., J. van Leeuwen, S. Beaumont, M. Berg, and P. L. Pfeffer. 2010. Embryo loss in cattle between Days 7 and 16 of pregnancy. Theriogenology 73(2):250-260.

Bernard, C., J. P. Valet, R. Béland, and R. D. Lambert. 1983. Prediction of bovine ovulation by a rapid radioimmunoassay for plasma LH. J Reprod Fertil 68(2):425-430.

Berry, D. P., F. Buckley, P. Dillon, R. D. Evans, M. Rath, and R. F. Veerkamp. 2003. Genetic parameters for body condition score, body weight, milk yield, and fertility estimated using random regression models. J Dairy Sci 86(11):3704-3717.

Berry, D. P., B. Coughlan, B. Enright, S. Coughlan, and M. Burke. 2013. Factors associated with milking characteristics in dairy cows. J Dairy Sci 96(9):5943-5953.

Berry, D. P., P. Lonergan, S. T. Butler, A. R. Cromie, T. Fair, F. Mossa, and A. C. Evans. 2008. Negative influence of high maternal milk production before and after conception on offspring survival and milk production in dairy cattle. J Dairy Sci 91(1):329-337.

Berry, D. P., E. Wall, and J. E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8 Suppl 1:105-121.

Betteridge, K. J., M. D. Eaglesome, G. C. Randall, and D. Mitchell. 1980. Collection, description and transfer of embryos from cattle 10--16 days after oestrus. J Reprod Fertil 59(1):205-216.

Bilodeau-Goeseels, S. 2003. Effect of oocyte quality on the relative abundance of specific gene transcripts in bovine mature oocytes and 16-cell embryos. Can J Vet Res 67(2):151-156.

Bilodeau-Goeseels, S. and J. P. Kastelic. 2003. Factors affecting embryo survival and strategies to reduce embryonic mortality in cattle. Canadian Journal of Animal Science 83(4):659-671.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J Dairy Sci 87(10):3105-3124.

Bols, P. E. J. and T. A. E. Stout. 2018. Transvaginal Ultrasound-Guided Oocyte Retrieval (OPU: Ovum Pick-Up) in Cows and Mares.

Boni, R., Tosti, E., Roviello, S. and Dale, B. 1999. Intercellular communication in in vivo- and in vitro-produced bovine embryos. Biology of Reproduction 61(4):1050-1055.

Brandão, D. O., P. Maddox-Hyttel, P. Løvendahl, R. Rumpf, D. Stringfellow, and H. Callesen. 2004. Post hatching development: a novel system for extended in vitro culture of bovine embryos. Biol Reprod 71(6):2048-2055.

C. Bartlett, P., J. H. Kirk, M. A. Wilke, J. B. Kaneene, and E. C. Mather. 1986. Metritis complex in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. Preventive Veterinary Medicine 4(3):235-248.

Carter, F., N. Forde, P. Duffy, M. Wade, T. Fair, M. A. Crowe, A. C. Evans, D. A. Kenny, J. F. Roche, and P. Lonergan. 2008. Effect of increasing progesterone concentration from Day 3 of pregnancy on subsequent embryo survival and development in beef heifers. Reprod Fertil Dev 20(3):368-375.

Cavestany, D. and R. H. Foote. 1985. Reproductive performance of Holstein cows administered GnRH analog HOE 766 (Buserelin) 26 to 34 days postpartum. J Anim Sci 61(1):224-233.

Ceciliani, F., C. Lecchi, C. Urh, and H. Sauerwein. 2018. Proteomics and metabolomics characterizing the pathophysiology of adaptive reactions to the metabolic challenges during the transition from late pregnancy to early lactation in dairy cows. J Proteomics 178:92-106.

Chebel, R. C., J. E. Santos, J. P. Reynolds, R. L. Cerri, S. O. Juchem, and M. Overton. 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim Reprod Sci 84(3-4):239-255.

Chebel, R. C., P. R. B. Silva, M. I. Endres, M. A. Ballou, and K. L. Luchterhand. 2016. Social stressors and their effects on immunity and health of periparturient dairy cows1. Journal of Dairy Science 99(4):3217-3228.

Clemente, M., J. de La Fuente, T. Fair, A. Al Naib, A. Gutierrez-Adan, J. F. Roche, D. Rizos, and P. Lonergan. 2009. Progesterone and conceptus elongation in cattle: a direct effect on the embryo or an indirect effect via the endometrium? Reproduction 138(3):507-517.

Cole, J. B. and P. M. VanRaden. 2018. Symposium review: Possibilities in an age of genomics: The future of selection indices. J Dairy Sci 101(4):3686-3701.

Corcoran, D., T. Fair, S. Park, D. Rizos, O. V. Patel, G. W. Smith, P. M. Coussens, J. J. Ireland, M. P. Boland, A. C. Evans, and P. Lonergan. 2006. Suppressed expression of genes involved in transcription and translation in in vitro compared with in vivo cultured bovine embryos. Reproduction 131(4):651-660.

Correa, M. T., H. Erb, and J. Scarlett. 1993. Path analysis for seven postpartum disorders of Holstein cows. J Dairy Sci 76(5):1305-1312.

Crosier, A. E., P. W. Farin, M. J. Dykstra, J. E. Alexander, and C. E. Farin. 2000. Ultrastructural morphometry of bovine compact morulae produced in vivo or in vitro. Biol Reprod 62(5):1459-1465.

Crowe, M. A. 1999. Gonadotropic control of terminal follicular growth in cattle. Reproduction of Domestic Animals 34:157-166.

Crowe, M. A. 2008. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Reprod Domest Anim 43 Suppl 5:20-28.

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

Diskin, M. G., J. J. Murphy, and J. M. Sreenan. 2006. Embryo survival in dairy cows managed under pastoral conditions. Anim Reprod Sci 96(3-4):297-311.

Diskin, M. G., M. H. Parr, and D. G. Morris. 2011. Embryo death in cattle: an update. Reprod Fertil Dev 24(1):244-251.

Dohoo, I. R. and S. W. Martin. 1984. Subclinical ketosis: prevalence and associations with production and disease. Can J Comp Med 48(1):1-5.

Driancourt, M. A., Gougeon, A., Royere, A. and Thibault, C. 1993. Ovarian Function. Reproduction in Mammals and Man. Ellipses, Paris, France.

Ealy, A. D., M. Drost, and P. J. Hansen. 1993. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. J Dairy Sci 76(10):2899-2905.

Edwards, J. L., W. A. King, S. J. Kawarsky, and A. D. Ealy. 2001. Responsiveness of early embryos to environmental insults: potential protective roles of HSP70 and glutathione. Theriogenology 55(1):209-223.

El-Sayed, A., M. Hoelker, F. Rings, D. Salilew, D. Jennen, E. Tholen, M. A. Sirard, K. Schellander, and D. Tesfaye. 2006. Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. Physiol Genomics 28(1):84-96.

Evans, A. C., F. Mossa, S. W. Walsh, D. Scheetz, F. Jimenez-Krassel, J. L. Ireland, G. W. Smith, and J. J. Ireland. 2012. Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. Reprod Domest Anim 47 Suppl 4:31-37.

Farin, C. E., T. M. Nett, and G. D. Niswender. 1990. Effects of luteinizing hormone on luteal cell populations in hypophysectomized ewes. J Reprod Fertil 88(1):61-70.

Farin, P. W., A. E. Crosier, and C. E. Farin. 2001. Influence of in vitro systems on embryo survival and fetal development in cattle. Theriogenology 55(1):151-170.

Ferguson, C. E. 2004. The use of altrenogest to control reproductive function in beef cattle. Louisiana State University and Agricultural & Mechanical College.

Ferguson, C. E., D. J. Kesler, and R. A. Godke. 2012. Progesterone enhances in vitro development of bovine embryos. Theriogenology 77(1):108-114.

Fessenden, B., D. J. Weigel, J. Osterstock, D. T. Galligan, and F. Di Croce. 2020. Validation of genomic predictions for a lifetime merit selection index for the US dairy industry. Journal of Dairy Science 103(11):10414-10428.

Firk, R., E. Stamer, W. Junge, and J. Krieter. 2002. Automation of oestrus detection in dairy cows: a review. Livestock Production Science 75(3):219-232.

Forde, N., M. E. Beltman, P. Lonergan, M. Diskin, J. F. Roche, and M. A. Crowe. 2011. Oestrous cycles in Bos taurus cattle. Anim Reprod Sci 124(3-4):163-169.

Forde, N., F. Carter, T. Fair, M. A. Crowe, A. C. Evans, T. E. Spencer, F. W. Bazer, R. McBride, M. P. Boland, P. O'Gaora, P. Lonergan, and J. F. Roche. 2009. Progesterone-regulated changes in endometrial gene expression contribute to advanced conceptus development in cattle. Biol Reprod 81(4):784-794.

Forde, N. and P. Lonergan. 2017. Interferon-tau and fertility in ruminants. Reproduction 154(5):F33-F43.

Forde, N., P. A. McGettigan, J. P. Mehta, L. O'Hara, S. Mamo, F. W. Bazer, T. E. Spencer, and P. Lonergan. 2014. Proteomic analysis of uterine fluid during the pre-implantation period of pregnancy in cattle. Reproduction 147(5):575-587.

Forde, N., J. P. Mehta, M. Minten, M. A. Crowe, J. F. Roche, T. E. Spencer, and P. Lonergan. 2012. Effects of low progesterone on the endometrial transcriptome in cattle. Biol Reprod 87(5):124.

Forde, N., C. A. Simintiras, R. G. Sturmey, A. Graf, E. Wolf, H. Blum, and P. Lonergan. 2017. Effect of lactation on conceptus-maternal interactions at the initiation of implantation in cattle: I. Effects on the conceptus transcriptome and amino acid composition of the uterine luminal fluid. Biol Reprod 97(6):798-809.

Friggens, N. C., C. Disenhaus, and H. V. Petit. 2010. Nutritional sub-fertility in the dairy cow: towards improved reproductive management through a better biological understanding. Animal 4(7):1197-1213.

Gad, A., M. Hoelker, U. Besenfelder, V. Havlicek, U. Cinar, F. Rings, E. Held, I. Dufort, M. A. Sirard, K. Schellander, and D. Tesfaye. 2012. Molecular mechanisms and pathways involved in bovine embryonic genome activation and their regulation by alternative in vivo and in vitro culture conditions. Biol Reprod 87(4):100.

Garbarino, E. J., J. A. Hernandez, J. K. Shearer, C. A. Risco, and W. W. Thatcher. 2004. Effect of lameness on ovarian activity in postpartum holstein cows. J Dairy Sci 87(12):4123-4131.

García-Ruiz, A., J. B. Cole, P. M. VanRaden, G. R. Wiggans, F. J. Ruiz-López, and C. P. V. Tassell. 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Proceedings of the National Academy of Sciences 113(28):E3995-E4004.

Garrett, J. E., R. D. Geisert, M. T. Zavy, L. K. Gries, R. P. Wettemann, and D. S. Buchanan. 1988. Effect of exogenous progesterone on prostaglandin F2 alpha release and the interestrous interval in the bovine. Prostaglandins 36(1):85-96.

Goff, A. K. and L. C. Smith. 1998. Effect of steroid treatment of endometrial cells on blastocyst development during co-culture. Theriogenology 49(5):1021-1030.

Gordon, R. and C. A. Melvin. 2003. Reverse engineering the embryo: a graduate course in developmental biology for engineering students at the University of Manitoba, Canada. Int J Dev Biol 47(2-3):183-187.

Goshen, T., K. Tsitrin, and M. van Straten. 2015. Reproductive Performance in Dairy Cows Synchronized with the Ovsynch Protocol at Different Stages of the Estrus Cycle. Israel Journal of Veterinary Medicine 70:22-27.

Graf, A., S. Krebs, M. Heininen-Brown, V. Zakhartchenko, H. Blum, and E. Wolf. 2014a. Genome activation in bovine embryos: review of the literature and new insights from RNA sequencing experiments. Anim Reprod Sci 149(1-2):46-58.

Graf, A., S. Krebs, V. Zakhartchenko, B. Schwalb, H. Blum, and E. Wolf. 2014b. Fine mapping of genome activation in bovine embryos by RNA sequencing. Proc Natl Acad Sci U S A 111(11):4139-4144.

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. 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):1624-1631.

Griffin, J. F., P. J. Hartigan, and W. R. Nunn. 1974. Non-specific uterine infection and bovine fertility. I. Infection patterns and endometritis during the first seven weeks post-partum. Theriogenology 1(3):91-106.

Gröhn, Y. T. and P. J. Rajala-Schultz. 2000. Epidemiology of reproductive performance in dairy cows. Anim Reprod Sci 60-61:605-614.

Gustavsson, I. 1979. Distribution and effects of the 1/29 Robertsonian translocation in cattle. J Dairy Sci 62(5):825-835.

Hansen, P. J. 2006. Realizing the promise of IVF in cattle--an overview. Theriogenology 65(1):119-125.

Hansen, P. J. 2009. Effects of heat stress on mammalian reproduction. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 364(1534):3341-3350.

Hansen, P. J. and P. Tríbulo. 2019. Regulation of present and future development by maternal regulatory signals acting on the embryo during the morula to blastocyst transition - insights from the cow. Biol Reprod 101(3):526-537.

Hansen, T. R., L. D. P. Sinedino, and T. E. Spencer. 2017. Paracrine and endocrine actions of interferon tau (IFNT). Reproduction 154(5):F45-f59.

Havlicek, V., F. Wetscher, T. Huber, G. Brem, M. Mueller, and U. Besenfelder. 2005. In vivo culture of IVM/IVF embryos in bovine oviducts by transvaginal endoscopy. J Vet Med A Physiol Pathol Clin Med 52(2):94-98.

Horan, B., P. Dillon, P. Faverdin, L. Delaby, F. Buckley, and M. Rath. 2005. The interaction of strain of Holstein-Friesian cows and pasture-based feed systems on milk yield, body weight, and body condition score. J Dairy Sci 88(3):1231-1243.

Howell, J. L., J. W. Fuquay, and A. E. Smith. 1994. Corpus luteum growth and function in lactating Holstein cows during spring and summer. J Dairy Sci 77(3):735-739.

Huang, W., B. W. Kirkpatrick, G. J. Rosa, and H. Khatib. 2010. A genome-wide association study using selective DNA pooling identifies candidate markers for fertility in Holstein cattle. Anim Genet 41(6):570-578.

Hue, I., S. A. Degrelle, E. Campion, and J. P. Renard. 2007. Gene expression in elongating and gastrulating embryos from ruminants. Soc Reprod Fertil Suppl 64:365-377.

Hue, I., S. A. Degrelle, and N. Turenne. 2012. Conceptus elongation in cattle: genes, models and questions. Anim Reprod Sci 134(1-2):19-28.

Hussein, H. A., K. A. E.-H. A. El-Razik, A. M. Gomaa, M. K. Elbayoumy, K. A. Abdelrahman, and H. I. Hosein. 2018. Milk amyloid A as a biomarker for diagnosis of subclinical mastitis in cattle. Veterinary world 11(1):34-41.

Huzzey, J. M., D. M. Veira, D. M. Weary, and M. A. von Keyserlingk. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J Dairy Sci 90(7):3220-3233.

Ingvartsen, K. L., R. J. Dewhurst, and N. C. Friggens. 2003. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livestock Production Science 83(2):277-308.

Ingvartsen, K. L. and N. C. Friggens. 2005. To what extent do variabilities in hormones, metabolites and energy intake explain variability in milk yield? Domest Anim Endocrinol 29(2):294-304.

Kadarmideen, H. N. 2014. Genomics to systems biology in animal and veterinary sciences: Progress, lessons and opportunities. Livestock Science 166:232-248.

Kerbrat, S. and C. Disenhaus. 2004. A proposition for an updated behavioural characterisation of the oestrus period in dairy cows. Applied Animal Behaviour Science 87(3):223-238.

Khan, D. R., D. Dubé, L. Gall, N. Peynot, S. Ruffini, L. Laffont, D. Le Bourhis, S. Degrelle, A. Jouneau, and V. Duranthon. 2012. Expression of pluripotency master regulators during two key developmental transitions: EGA and early lineage specification in the bovine embryo. PLoS One 7(3):e34110.

Khatib, H., C. Maltecca, R. L. Monson, V. Schutzkus, and J. J. Rutledge. 2009. Monoallelic maternal expression of STAT5A affects embryonic survival in cattle. BMC Genet 10:13.

Kojima, F. N., E. G. Bergfeld, M. E. Wehrman, A. S. Cupp, K. E. Fike, D. V. Mariscal-Aguayo, T. Sanchez-Torres, M. Garcia-Winder, D. T. Clopton, A. J. Roberts, and J. E. Kinder. 2003. Frequency of luteinizing hormone pulses in cattle influences duration of persistence of dominant ovarian follicles, follicular fluid concentrations of steroids, and activity of insulin-like growth factor binding proteins. Anim Reprod Sci 77(3-4):187-211.

Kuhn, M. T., J. L. Hutchison, and G. R. Wiggans. 2006. Characterization of Holstein heifer fertility in the United States. J Dairy Sci 89(12):4907-4920.

La Rocca, C., F. Carbone, S. Longobardi, and G. Matarese. 2014. The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. Immunol Lett 162(1 Pt A):41-48.

Ledgard, A. M., M. C. Berg, W. H. McMillan, G. Smolenski, and A. J. Peterson. 2012. Effect of asynchronous transfer on bovine embryonic development and relationship with early cycle uterine proteome profiles. Reprod Fertil Dev 24(7):962-972.

Lefebvre, R., Fritz, S,Ledoux, D, Gatien, J, Genestout, L, Rossignol, M-N, Grimard, B, Boichard, D, Humblot, P and Ponsart, C. 2011. GENIFER: fine mapping and fertility QTL effects in the Holstein cattle breed. Rencontres Recherche Ruminants 18:415.

Li, J., Y. Zhu, B. Shoemake, B. Liu, P. Adkins, and L. Wallace. 2022. A systematic review of the utility of biomarkers as aids in the early diagnosis and outcome prediction of bovine respiratory disease complex in feedlot cattle. J Vet Diagn Invest:10406387221081232.

Lonergan, P., T. Fair, D. Corcoran, and A. C. Evans. 2006. Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. Theriogenology 65(1):137-152.

Lonergan, P., T. Fair, N. Forde, and D. Rizos. 2016. Embryo development in dairy cattle. Theriogenology 86(1):270-277.

Lonergan, P. and N. Forde. 2014. Maternal-embryo interaction leading up to the initiation of implantation of pregnancy in cattle. Animal 8 Suppl 1:64-69.

Lonergan, P., D. Rizos, A. Gutierrez-Adan, T. Fair, and M. P. Boland. 2003. Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. Reprod Domest Anim 38(4):259-267.

Lonergan, P., A. Woods, T. Fair, F. Carter, D. Rizos, F. Ward, K. Quinn, and A. Evans. 2007. Effect of embryo source and recipient progesterone environment on embryo development in cattle. Reprod Fertil Dev 19(7):861-868.

Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 81(3-4):209-223.

Lucy, M. C. 2007. Fertility in high-producing dairy cows: reasons for decline and corrective strategies for sustainable improvement. Soc Reprod Fertil Suppl 64:237-254.

Lucy, M. C., S. McDougall, and D. P. Nation. 2004. The use of hormonal treatments to improve the reproductive performance of lactating dairy cows in feedlot or pasture-based management systems. Animal Reproduction Science 82-83:495-512.

Lucy, M. C., Savio, J.D., Badiga, L., De La Sota, R.L. and Thatcher, W.W. 1992. Factors that affect ovarian follicular dynamics in cattle. Journal of Animal Science 70:3615-3626.

López-Gatius, F., P. Santolaria, I. Mundet, and J. L. Yániz. 2005. Walking activity at estrus and subsequent fertility in dairy cows. Theriogenology 63(5):1419-1429.

Macdonald, K. A., J. W. Penno, J. A. Lancaster, and J. R. Roche. 2008. Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. J Dairy Sci 91(5):2151-2163.

Mamo, S., J. P. Mehta, P. McGettigan, T. Fair, T. E. Spencer, F. W. Bazer, and P. Lonergan. 2011. RNA sequencing reveals novel gene clusters in bovine conceptuses associated with maternal recognition of pregnancy and implantation. Biol Reprod 85(6):1143-1151.

Mazzoni, G., S. M. Salleh, K. Freude, H. S. Pedersen, L. Stroebech, H. Callesen, P. Hyttel, and H. N. Kadarmideen. 2017. Identification of potential biomarkers in donor cows for in vitro embryo production by granulosa cell transcriptomics. PLoS One 12(4):e0175464.

McCracken, J. A., E. E. Custer, and J. C. Lamsa. 1999. Luteolysis: a neuroendocrine-mediated event. Physiol Rev 79(2):263-323.

McGee, E. A. a. A. J. W. H. 2000. Initial and cyclic recruitment of ovarian follicles. Endocrine Reviews 21(2):200-214.

McHughes, C. E., G. K. Springer, L. D. Spate, R. Li, R. Woods, M. P. Green, S. W. Korte, C. N. Murphy, J. A. Green, and R. S. Prather. 2009. Identification and quantification of differentially represented transcripts in in vitro and in vivo derived preimplantation bovine embryos. Mol Reprod Dev 76(1):48-60.

Mermillod, P., P. Lonergan, C. Carolan, H. Khatir, N. Poulin, and Y. Cognié. 1996. [In vitro oocyte maturation in domestic ruminants]. Contracept Fertil Sex 24(7-8):552-558.

Mermillod, P., A. Massip, and F. Dessy. 1992. In vitro production of cattle embryos: review and Belgian results. Int J Dev Biol 36(1):185-195.

Meuwissen, T. H., B. J. Hayes, and M. E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157(4):1819-1829.

Miglior, F., A. Fleming, F. Malchiodi, L. F. Brito, P. Martin, and C. F. Baes. 2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. J Dairy Sci 100(12):10251-10271.

Miretti, S., C. Lecchi, F. Ceciliani, and M. Baratta. 2020. MicroRNAs as Biomarkers for Animal Health and Welfare in Livestock. Frontiers in veterinary science 7:578193-578193.

Momont, H. 1990. Rectal palpation. The Bovine Practitioner:122-123.

Mondou, E., I. Dufort, M. Gohin, E. Fournier, and M. A. Sirard. 2012. Analysis of microRNAs and their precursors in bovine early embryonic development. Mol Hum Reprod 18(9):425-434.

Moore, N. W. and J. N. Shelton. 1964. EGG TRANSFER IN SHEEP. EFFECT OF DEGREE OF SYNCHRONIZATION BETWEEN DONOR AND RECIPIENT, AGE OF EGG, AND SITE OF TRANSFER ON THE SURVIVAL OF TRANSFERRED EGGS. J Reprod Fertil 7:145-152.

Moore, R. E., J. Kirwan, M. K. Doherty, and P. D. Whitfield. 2007. Biomarker discovery in animal health and disease: the application of post-genomic technologies. Biomarker insights 2:185-196.

Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 84(7):1646-1659.

Márquez, C., M. Sandalinas, M. Bahçe, M. Alikani, and S. Munné. 2000. Chromosome abnormalities in 1255 cleavage-stage human embryos. Reprod Biomed Online 1(1):17-26.

National Academies of Sciences, E. and Medicine. 2021. Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. The National Academies Press, Washington, DC.

Nel-Themaat, L. and Z. P. Nagy. 2011. A review of the promises and pitfalls of oocyte and embryo metabolomics. Placenta 32 Suppl 3:S257-263.

Niemann, H., J. W. Carnwath, D. Herrmann, G. Wieczorek, E. Lemme, A. Lucas-Hahn, and S. Olek. 2010. DNA methylation patterns reflect epigenetic reprogramming in bovine embryos. Cell Reprogram 12(1):33-42.

Norman, H. D., J. R. Wright, R. L. Powell, P. M. VanRaden, F. Miglior, and G. de Jong. 2007. Consistency of maturity rate for milk yield across countries and generations. J Dairy Sci 90(8):3937-3944.

Northrop, E. J., J. J. J. Rich, J. R. Rhoades, and G. A. Perry. 2019. Comparison of two bovine serum pregnancy tests in detection of artificial insemination pregnancies and pregnancy loss in beef cattle. PloS one 14(1):e0211179-e0211179.

Nowicki, A., W. Barański, A. Baryczka, and T. Janowski. 2017. OvSynch Protocol and its Modifications in the Reproduction Management of Dairy Cattle Herds - an Update. J Vet Res 61(3):329-336.

Opsomer, G., Y. T. Gröhn, J. Hertl, M. Coryn, H. Deluyker, and A. de Kruif. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology 53(4):841-857.

Paisley, L. G., W. D. Mickelsen, and P. B. Anderson. 1986. Mechanisms and therapy for retained fetal membranes and uterine infections of cows: A review. Theriogenology 25(3):353-381.

Parmar, S., A. Dhami, K. Hadiya, and C. Parmar. 2016. Early Embryonic Death in Bovines: An Overview. Raksha Technical Review 6:6-12.

Pereira, G., R. Bexiga, J. Chagas e Silva, E. Silva, C. Ramé, J. Dupont, Y. Guo, P. Humblot, and L. Lopes-da-Costa. 2020. Adipokines as biomarkers of postpartum subclinical endometritis in dairy cows. Reproduction 160(3):417-430.

Perkel, K. J., A. K. Tscherner, C. Merrill, J. LaMarre, and P. Madan. 2015. The ART of selecting the best embryo: A review of early embryonic mortality and bovine embryo viability assessment methods. Molecular Reproduction and Development 82.

Perry, G. A. 2012. Physiology and Endocrinology Symposium: Harnessing basic knowledge of factors controlling puberty to improve synchronization of estrus and fertility in heifers. J Anim Sci 90(4):1172-1182.

Perry, G. A., M. F. Smith, and T. W. Geary. 2004. Ability of intravaginal progesterone inserts and melengestrol acetate to induce estrous cycles in postpartum beef cows. J Anim Sci 82(3):695-704.
Petit, H. V. and H. Twagiramungu. 2006. Conception rate and reproductive function of dairy cows fed different fat sources. Theriogenology 66(5):1316-1324.

Pontes, J. H., I. Nonato-Junior, B. V. Sanches, J. C. Ereno-Junior, S. Uvo, T. R. Barreiros, J. A. Oliveira, J. F. Hasler, and M. M. Seneda. 2009. Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore (Bos indicus) donor cows. Theriogenology 71(4):690-697.

Poock, S., Horner, J and Milhollin, R. 2009. Missouri Dairy Growth Council: Dairy cattle reproductive manual. University of Missouri: University of Missouri Extension Commercial Agriculture Program, Missouri, USA.

Pope, W. F. 1988. Uterine asynchrony: a cause of embryonic loss. Biol Reprod 39(5):999-1003.
Pritchard, T., M. Coffey, R. Mrode, and E. Wall. 2013. Genetic parameters for production, health, fertility and longevity traits in dairy cows. Animal 7(1):34-46.

Pritchard, T. J. and C. W. Donnelly. 1999. Combined secondary enrichment of primary enrichment broths increases Listeria detection. J Food Prot 62(5):532-535.

Pryce, J. E., M. P. Coffey, and G. Simm. 2001. The Relationship Between Body Condition Score and Reproductive Performance. Journal of Dairy Science 84(6):1508-1515.

Pryce, J. E., T. T. T. Nguyen, M. Axford, G. Nieuwhof, and M. Shaffer. 2018. Symposium review: Building a better cow-The Australian experience and future perspectives. J Dairy Sci 101(4):3702-3713.

Pryce, J. E., M. D. Royal, P. C. Garnsworthy, and I. L. Mao. 2004. Fertility in the high-producing dairy cow. Livestock Production Science 86(1):125-135.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 44(7):915-923.

Reggio, B., J. Lynn, and R. Godke. 1997. The effect of progesterone on the development of IVF-derived bovine embryos cultured in a semi-defined culture medium. Theriogenology 47:284.

Rizos, D., F. Ward, P. Duffy, M. P. Boland, and P. Lonergan. 2002. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. Mol Reprod Dev 61(2):234-248.

Roberts, R. M. 2007. Interferon-tau, a Type 1 interferon involved in maternal recognition of pregnancy. Cytokine Growth Factor Rev 18(5-6):403-408.

Roberts, S. J. 1971. Veterinary obstetrics and genital diseases (Theriogenology).

Romano, J. and D. Magee. 2001. Applications of trans-rectal ultrasonography in cow/heifer reproduction. Pages 99-104 in Proc. Annual food conference. Conception to parturition: fertility in Texas beef cattle.

Romano, J. E., J. A. Thompson, D. C. Kraemer, M. E. Westhusin, D. W. Forrest, and M. A. Tomaszweski. 2007. Early pregnancy diagnosis by palpation per rectum: Influence on embryo/fetal viability in dairy cattle. Theriogenology 67(3):486-493.

Rowson, L. E. and R. M. Moor. 1966. Non-surgical transfer of cow eggs. J Reprod Fertil 11(2):311-312.

Rowson, L. E., R. Tervit, and A. Brand. 1972. The use of prostaglandins for synchronization of oestrus in cattle. J Reprod Fertil 29(1):145.

Royal, M. D., A. O. Darwash, A. P. F. Flint, R. Webb, J. A. Woolliams, and G. E. Lamming. 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Animal Science 70(3):487-501.

Salumets, A., C. Hydén-Granskog, A. M. Suikkari, A. Tiitinen, and T. Tuuri. 2001. The predictive value of pronuclear morphology of zygotes in the assessment of human embryo quality. Hum Reprod 16(10):2177-2181.

Salumets, A., T. Tuuri, S. Mäkinen, S. Vilska, L. Husu, R. Tainio, and A. M. Suikkari. 2003. Effect of developmental stage of embryo at freezing on pregnancy outcome of frozen-thawed embryo transfer. Hum Reprod 18(9):1890-1895.

Sandals, W. C., R. A. Curtis, J. F. Cote, and S. W. Martin. 1979. The effect of retained placenta and metritis complex on reproductive performance in dairy cattle -- a case control study. The Canadian veterinary journal = La revue veterinaire canadienne 20(5):131-135.

Sandra, O., N. Mansouri-Attia, and R. G. Lea. 2011. Novel aspects of endometrial function: a biological sensor of embryo quality and driver of pregnancy success. Reprod Fertil Dev 24(1):68-79.

Santos, J. E., W. W. Thatcher, R. C. Chebel, R. L. 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., M. R. Bastos, and M. C. Wiltbank. 2010. Factors affecting fertilisation and early embryo quality in single- and superovulated dairy cattle. Reprod Fertil Dev 22(1):151-158.

Sasser, R. G., C. A. Ruder, K. A. Ivani, J. E. Butler, and W. C. Hamilton. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. Biol Reprod 35(4):936-942.

Satterfield, M. C., F. W. Bazer, and T. E. Spencer. 2006. Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. Biol Reprod 75(2):289-296.

Schwenger, B., S. Schöber, and D. Simon. 1993. DUMPS cattle carry a point mutation in the uridine monophosphate synthase gene. Genomics 16(1):241-244.

Shah, V. S. S. a. R. G. 2009. Bovine In vitro Embryo Production: An Overview. Veterinary World 2(12):478-479.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65(8):1516-1530.

Smith, B. I. and C. A. Risco. 2005. Management of periparturient disorders in dairy cattle. Vet Clin North Am Food Anim Pract 21(2):503-521.

Soler, L., S. Uzbekova, E. Blesbois, X. Druart, and V. Labas. 2020. Intact cell MALDI-TOF mass spectrometry, a promising proteomic profiling method in farm animal clinical and reproduction research. Theriogenology 150:113-121.

Spencer, T. E., N. Forde, and P. Lonergan. 2016. The role of progesterone and conceptus-derived factors in uterine biology during early pregnancy in ruminants. J Dairy Sci 99(7):5941-5950.

Spencer, T. E. and T. R. Hansen. 2015. Implantation and Establishment of Pregnancy in Ruminants. Adv Anat Embryol Cell Biol 216:105-135.

Spencer, T. E., G. A. Johnson, F. W. Bazer, R. C. Burghardt, and M. Palmarini. 2007. Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. Reprod Fertil Dev 19(1):65-78.

Spencer, T. E., T. L. Ott, and F. W. Bazer. 1996. tau-Interferon: pregnancy recognition signal in ruminants. Proc Soc Exp Biol Med 213(3):215-229.

Spencer, T. E., O. Sandra, and E. Wolf. 2008. Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. Reproduction 135(2):165-179.

Starbuck, G. R., A. O. Darwash, G. E. Mann, and G. E. Lamming. 2001. The detection and treatment of post insemination progesterone insufficiency in dairy cows. BSAP Occasional Publication 26(2):447-450.

Strimbu, K. and J. A. Tavel. 2010. What are biomarkers? Curr Opin HIV AIDS 5(6):463-466.

Stringfellow, D., Seidel,SM. 1998. Manual of the International Embryo Transfer Society: A Procedural Guide and General Information for the Use of Embryo Transfer Technology Emphasizing Sanitary Procedures. 3rd ed. International Embryo Transfer Society, Savory, IL.

Sudano, M. J., D. M. Paschoal, T. da Silva Rascado, L. F. Crocomo, L. C. Magalhães, A. M. Junior, R. Machado, and F. da Cruz Landim-Alvarenga. 2014. Crucial surviving aspects for vitrified in vitro-produced bovine embryos. Zygote 22(2):124-131.

Suravajhala, P., L. J. A. Kogelman, and H. N. Kadarmideen. 2016. Multi-omic data integration and analysis using systems genomics approaches: methods and applications in animal production, health and welfare. Genetics, selection, evolution : GSE 48(1):38-38.

Sánchez, J. M., D. J. Mathew, S. K. Behura, C. Passaro, G. Charpigny, S. T. Butler, T. E. Spencer, and P. Lonergan. 2019. Bovine endometrium responds differentially to age-matched short and long conceptuses†. Biology of Reproduction 101(1):26-39.

Thatcher, W. W., A. Guzeloglu, A. Meikle, S. Kamimura, T. Bilby, A. A. Kowalski, L. Badinga, R. Pershing, J. Bartolome, and J. E. Santos. 2003. Regulation of embryo survival in cattle. Reprod Suppl 61:253-266.

Thatcher, W. W., F. Moreira, S. M. Pancarci, J. A. Bartolome, and J. E. Santos. 2002. Strategies to optimize reproductive efficiency by regulation of ovarian function. Domest Anim Endocrinol 23(1-2):243-254.

Tríbulo, P., L. G. B. Siqueira, L. J. Oliveira, T. Scheffler, and P. J. Hansen. 2018. Identification of potential embryokines in the bovine reproductive tract. J Dairy Sci 101(1):690-704.

Urton, G., M. A. von Keyserlingk, and D. M. Weary. 2005. Feeding behavior identifies dairy cows at risk for metritis. J Dairy Sci 88(8):2843-2849.

Ushizawa, K., C. B. Herath, K. Kaneyama, S. Shiojima, A. Hirasawa, T. Takahashi, K. Imai, K. Ochiai, T. Tokunaga, Y. Tsunoda, G. Tsujimoto, and K. Hashizume. 2004. cDNA microarray analysis of bovine embryo gene expression profiles during the pre-implantation period. Reprod Biol Endocrinol 2:77.

Valour, D., P. Michot, C. Eozenou, R. Lefebvre, A. Bonnet, A. Capitan, S. Uzbekova, E. Sellem, C. Ponsart, and L. Schibler. 2015. Dairy cattle reproduction is a tightly regulated genetic process: Highlights on genes, pathways, and biological processes. Animal Frontiers 5(1):32-41.

Van Eerdenburg, F. J. C. M., H. S. H. Loeffler, and J. H. van Vliet. 1996. Detection of oestrus in dairy cows: A new approach to an old problem. Veterinary Quarterly 18(2):52-54.

Van Soom, A., P. Mijten, I. Van Vlaenderen, J. Van den Branden, A. R. Mahmoudzadeh, and A. de Kruif. 1994. Birth of double-muscled Belgian Blue calves after transfer of in vitro produced embryos into dairy cattle. Theriogenology 41(4):855-867.

Van Tassell, C. P., G. R. Wiggans, and H. D. Norman. 1999. Method R estimates of heritability for milk, fat, and protein yields of United States dairy cattle. J Dairy Sci 82(10):2231-2237.

VanRaden, P. M. and R. H. Miller. 2006. Effects of nonadditive genetic interactions, inbreeding, and recessive defects on embryo and fetal loss by seventy days. J Dairy Sci 89(7):2716-2721.

VanRaden, P. M., A. H. Sanders, M. E. Tooker, R. H. Miller, H. D. Norman, M. T. Kuhn, and G. R. Wiggans. 2004. Development of a national genetic evaluation for cow fertility. J Dairy Sci 87(7):2285-2292.

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):153-165.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, E. P. Stanisiewski, and R. L. Fogwell. 1990. Influence of Energy Balance and Body Condition on Estrus and Estrous Cycles in Holstein Heifers1. Journal of Dairy Science 73(10):2759-2765.

Walsh, S. W., E. J. Williams, and A. C. O. Evans. 2011. A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science 123(3):127-138.

Wan, D. C., D. Scanlon, B. Power, C. L. Choi, P. Hudson, and B. G. Livett. 1987. Synthesis of a specific oligodeoxyribonucleotide probe and its use for studying proenkephalin A gene expression. Neurosci Lett 76(1):74-80.

Williams, E. J., D. P. Fischer, D. E. Noakes, G. C. England, A. Rycroft, H. Dobson, and I. M. Sheldon. 2007. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68(4):549-559.

Wiltbank, J. N., R. P. Shumway, W. R. Parker, and D. R. Zimmerman. 1967. Duration of estrus, time of ovulation and fertilization rate in beef heifers synchronized with dihydroxyprogesterone acetophenide. J Anim Sci 26(4):764-767.

Wiltbank, M. C., G. M. Baez, A. Garcia-Guerra, M. Z. Toledo, P. L. Monteiro, L. F. Melo, J. C. Ochoa, J. E. Santos, and R. Sartori. 2016. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. Theriogenology 86(1):239-253.

Wiltbank, M. C. and J. R. Pursley. 2014. The cow as an induced ovulator: timed AI after synchronization of ovulation. Theriogenology 81(1):170-185.

Witkamp, R. F. 2005. Genomics and systems biology--how relevant are the developments to veterinary pharmacology, toxicology and therapeutics? J Vet Pharmacol Ther 28(3):235-245.

Wolfenson, D., F. F. Bartol, L. Badinga, C. M. Barros, D. N. Marple, K. Cummins, D. Wolfe, M. C. Lucy, T. E. Spencer, and W. W. Thatcher. 1993. Secretion of PGF2alpha and oxytocin during hyperthermia in cyclic and pregnant heifers. Theriogenology 39(5):1129-1141.

Wolfenson, D., B. J. Lew, W. W. Thatcher, Y. Graber, and R. Meidan. 1997. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. Anim Reprod Sci 47(1-2):9-19.

Wolfenson, D., Z. Roth, and R. Meidan. 2000. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Anim Reprod Sci 60-61:535-547.

Wright, I. A., S. M. Rhind, T. K. Whyte, and A. J. Smith. 1992. Effects of body condition at calving and feeding level after calving on LH profiles and the duration of the post-partum anoestrous period in beef cows. Animal Science 55(1):41-46.

Xu, K. P., B. R. Yadav, W. A. King, and K. J. Betteridge. 1992. Sex-related differences in developmental rates of bovine embryos produced and cultured in vitro. Mol Reprod Dev 31(4):249-252.

Zemjanis, R. 1970. Diagnostic and therapeutic techniques in animal reproduction. Diagnostic and therapeutic techniques in animal reproduction. (2nd edn).

Zeron, Y., A. Ocheretny, O. Kedar, A. Borochov, D. Sklan, and A. Arav. 2001. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. Reproduction 121(3):447-454.

Ziebe, S., K. Petersen, S. Lindenberg, A. G. Andersen, A. Gabrielsen, and A. N. Andersen. 1997. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. Hum Reprod 12(7):1545-1549.

CHAPTER 2: EMBRYO MORTALITY MOUNTS A MASSIVE T HELPER IMMUNE RESPONSE COMBINED WITH AN IMPAIRED INTERFERON-TAU RELEASE AND MATERNAL RECOGNITION ACTION IN LACTATING DAIRY COWS[1]

Summary

Embryo mortality contributes to infertility and occurs between days 7 to 19 of pregnancy in cows. However, the exact mechanism of why a pregnancy with embryo mortality fails is unknown. Our objective was to elucidate the transcriptome response of reproductive tissues (endometrium, corpus luteum and peripheral blood mononuclear cells) to pregnancies with embryo mortality in lactating Holstein-Friesian cows. Two experiments (E1, E2) were conducted with lactating Holstein-Friesian cows (E1: n=20, E2: n=22), randomly divided into pregnant (E1: n=13, E2: n=15) or non-pregnant (E1: n=7, E2: n=7) group and all were subjected to an estrous synchronization. Only the pregnant group was artificially inseminated with the same high-fertile sire's semen. At day 16, after artificial insemination or of estrous cycle, reproductive tissues (endometrium, corpus luteum [only for E2] and peripheral blood mononuclear cells), serum (E1) or plasma (E2) and uterine flushings were collected. Conceptuses were only collected for the pregnant group. Re-classification of the pregnant group was based on embryo morphology and appearance [embryo mortality pregnancies had pink, red, restricted in elongation and (or) opaque conceptuses (E1: n=5, E2: n=6) or normal pregnancies had translucent and elongated conceptuses (E1: n=8, E2: n=9)]. The following procedures were conducted for all cow groups: uterine

[1]Written and formatted with the intention to be submitted to the Journal of Dairy Science in fall of 2022.

flushings to estimate interferon tau (**IFNT**) by ELISA, western blot (only for endometrium) to quantify ISG15 protein, reverse-transcription quantitative polymerase chain reaction (**RTqPCR**; only for reproductive tissues) to semi-quantify *ISG15* mRNA and serum (E1) or (E2) plasma was conducted to estimate estradiol and progesterone concentrations by radioimmunoassay. All procedures previously mentioned were analyzed with Proc GLM-ANOVA in SAS. Reproductive tissues and conceptuses were RNA sequenced, aligned to UMD 3.1 (E1) or ARS-UCD 1.2 (E2) genome assembly and processed within DESeq2 package in R. Gene expression data were submitted into Ingenuity Pathway Analysis (**IPA**) bioinformatic software to generate key canonical pathways and/or upstream regulators associated within group comparisons [Normal vs Non-Pregnant, Embryo Mortality vs Non-Pregnant and Embryo Mortality vs Normal]. Failure to identify pathways, generated a gene list from IPA were submitted into the Search Tool for the Retrieval of Interacting Genes/Protein (**STRING**; http://string-db.org) database to find possible protein-protein interactions and evaluate their gene roles. Normal conceptuses were found to be longer than embryo mortality conceptuses. Additionally, IFNT protein concentrations in uterine flushings were greater in normal compared to embryo mortality and non-pregnant in E1 but not for E2. The ISG15 protein from western blot analyses revealed that endometrium from normal pregnancy cows were greater (E1 and E2) than embryo mortality and non-pregnant and embryo mortality tended to be greater than non-pregnant in E2. RTqPCR for *ISG15* mRNA levels revealed that normal endometrium was greater (E1 and E2) when compared to embryo mortality and non-pregnant endometrium but only tended to be greater in embryo mortality compared to non-pregnant endometrium in E2. The concentrations of both progesterone and estradiol in the radioimmunoassay were only significant by day (7 and 16) in E2 and only for progesterone. RNA sequencing revealed that *IFNT* mRNA was greater (E1 and E2) in normal conceptuses compared

to embryo mortality conceptuses. For E1 and E2, IPA revealed for embryo mortality compared to normal conceptuses that the key canonical pathway T helper 1 (Th1) and Th2 was up-regulated and responsible for an adaptive immune response that activated pro-inflammatory cytokines. Within embryo mortality compared to normal endometrium, E1 had Th1 and Th2 pathways up-regulated while E2 identified differentially expressed genes (**DEGs**) that were up-regulated and associated with estradiol-mediated luteolytic action. Comparison of embryo mortality compared to normal in PBMC were only significantly in E1 and had down-regulated DEGs associated with tissue growth, remodeling and/or development, cell cycle, conceptus implantation, essential mineral transporters, innate immune system and Th1 activation. The corpus luteum of E2 for embryo mortality compared to normal exhibited up-regulation of DEGs associated with inflammation, calcium sequestration/delivery, glucose- and estradiol-metabolism that may be involved in the luteolysis pathway. Our results suggested that pregnancies with embryo mortality were associated with an impaired action (paracrine and endocrine) of IFNT from the trophectoderm of the conceptus on reproductive tissues (endometrium, corpus luteum and peripheral blood mononuclear cells). This may then lead to a disruption in maternal-fetal crosstalk which allows for the activation a massive T helper immune response within the conceptus, genes associated with estradiol and re-modelling of the endometrium, luteolysis cascade in the corpus luteum and therefore, pregnancy loss occurs. Thus, this study was initiated to aid in proposing a mechanism of why most pregnancies fail, while others succeed in purebred Holstein-Friesian cows.

Introduction

Fertility in high-producing dairy cows has been in decline for the past 50 years in the United States of America (USA) and this is attributed to the intensive genetic selection for higher milk yield (García-Ruiz et al., 2016). Such management strategy has resulted in a reduced duration of estrus (6.2 hours vs 10.9 hours) and estrus behavior (i.e.; numbers of times standing to be mounted and walking activity) in high milk producing cows compared to lower milk producing cows (Van Eerdenburg et al., 1996, Kerbrat and Disenhaus, 2004, Lopez et al., 2004). Recent availability and use of the net merit index (i.e., genetic prediction tool) aided both farmers and the dairy cattle industry to efficiently select fertility traits with ascribed low heritability such as daughter pregnancy rate (**DPR**; 0.04 out of 1.0) (García-Ruiz et al., 2016a). This is essential to the dairy industry because DPR is used to predict the ability of a bull's daughter to become pregnant sooner rather than later when compared to another bull's progeny (P.M. VanRaden, 2003). Despite advances in selecting against the declining trend for fertility traits and improved farm management practices for superior health, nutrition and reproduction, calving rates of modern Holstein-Friesian heifers (35-40%) and lactating cows (55-60%) are still considered suboptimal (Lonergan et al., 2016a).

Subfertility, in Holstein-Friesian cows, may be caused by early embryo mortality (**EM**), because it is responsible for 43% of all reproductive failures during the pre-implantation period (days 7 to 19) (Diskin et al., 2006, Walsh et al., 2011, Ribeiro, 2018). Also, about 39% of live embryos during days 5 or 6 after first postpartum artificial insemination (**AI**) fail to implant during the first month of pregnancy (Ribeiro, 2018). Furthermore, there is evidence that a significant proportion embryonic losses happen before or at day 16 of pregnancy in cattle (Diskin et al., 2006). Failure to determine pregnancy status before day 32 by ultrasound, and resynchronizing non-

pregnant (**NP**) cows has limited reproductive performance, breeding management, production and profit (Meadows et al., 2005, De Vries, 2006, Diskin et al., 2006, Ribeiro et al., 2012). Pregnancies with EM are associated with multiple factors, such as uterine infections, negative energy balance, dystocia, heat stress and parental gamete quality (Walsh et al., 2011) but the exact biological causes or mechanisms are poorly understood. Currently, it is estimated that EM pregnancies generate a loss of $1.6 billion in the USA and $1.28 trillion worldwide annually (Shah, 2009, Perkel et al., 2015).

A potential indicator/cause of EM might be restriction in elongation of the conceptus and the amount of interferon tau (**IFNT**) secreted by the trophectoderm on day 16 after AI in order for maternal recognition and establishment of pregnancy to occur (Wiltbank et al., 2016, Moraes et al., 2018). For example, there is a positive correlation between conceptus length and IFNT secretion from the trophectoderm that contributes to the success of a pregnancy (Rizos et al., 2012).

IFNT is also known for its mechanism of preventing luteolysis of the corpus luteum (**CL**) through paracrine action and inhibition of estrogen receptor 1 (**ESR1**) on the endometrial luminal epithelium (Bazer et al., 1992, Spencer et al., 2008, Spencer and Hansen, 2015, Forde and Lonergan, 2017, Hansen et al., 2017). This renders ESR1 unable to up-regulate oxytocin receptors (**OXTR**) and pulsatile release of prostaglandin $F_{2\ alpha}$ (**$PGF_{2\alpha}$**) from endometrial cells. IFNT is thought to alter $PGF_{2\alpha}$ pulses in ewes, and attenuated pulses in cows (Thatcher et al., 1989, Spencer et al., 1996, McCracken et al., 1999, Spencer and Hansen, 2015, Hansen et al., 2017). Regardless, the CL can then continue to produce progesterone which maintains a competent uterine environment for the implanting conceptus, regulates the cow's estrous cycle and prepares the uterine lining to secrete histotroph (Bazer et al., 1975, Bazer et al., 1997, Spencer et al., 2008, Spencer and Hansen, 2015, Spencer et al., 2016).

Other essential paracrine actions of IFNT are to augment the expression of both IFN-stimulated genes (**ISGs**) in the uterus as well as immunodulatory cytokines (Hansen, 2011). Mainly, classical ISGs are known for having a role in antiviral response (MX1/2, OAS1/2, ISG15, IFI6, IFI44) or pathogen recognition receptor (ZBP1, IFI6, DDX58) responses (Shaw et al., 2017). A particular ISG that is highly responsive towards the paracrine action of IFNT in the endometrium is ISG15 and has been suggested to have a role in establishing and maintaining pregnancy (Austin et al., 1996, Johnson et al., 1998, Johnson et al., 1999, Austin et al., 2004). In addition, immunomodulatory cytokines such as GATA3 protect the conceptus from maternal immune responses as early as day 7 of pregnancy (Skopets et al., 1992, Hansen, 2011). This is of importance because studies involving both mice and humans have suggested that an aberrant immune system during pregnancy is associated with recurrent abortions and infertility (Sykes et al., 2012, Piccinni et al., 2015). In ruminants, limited studies have attempted to associate the effect of a conceptus restricted in elongation, production and action of IFNT by the conceptus trophectoderm and the maternal transcriptomic response of reproductive tissues (endometrium, CL) that are essential for pregnancy to succeed (Fair, 2015, Talukder et al., 2017). Hence, additional studies are needed to understand the exact mechanisms that cause EM which may aid in devising strategies to mitigate pregnancies with EM and selecting reproductively efficient cows.

It was hypothesized that pregnancies with EM are associated with impaired conceptus-derived IFNT secretion that may impede action (endocrine and paracrine) on reproductive tissues (endometrium, CL, peripheral blood mononuclear cells [**PBMC**]) which may lead to luteolysis and disrupt pregnancy. The objectives were to discover variation in gene expression for IFNT/ISG15 and transcriptomic changes within conceptuses and reproductive tissues as well as hormonal (estradiol and progesterone) deviations in normal (**N**) pregnancies compared to

pregnancies with EM occurring at the preimplantation stage (day 16). All cow groups were evaluated for RTqPCR (only for reproductive tissues) semi-quantified *ISG15* mRNA, western blot (only for endometrium) to quantitated ISG15 protein, serum or plasma estimated estradiol and progesterone concentrations by radioimmunoassay and uterine flushings (**UF**) to estimate IFNT by ELISA. Pathway analyses from conceptuses and reproductive tissues RNA-Seq aimed to identify key molecular and cellular processes that were associated with pregnancy failure. This study was initiated to aid in proposing a mechanism of why most pregnancies fail, while others succeed in lactating Holst$_e$in-Friesian cows.

Materials and Methods

All procedures and protocols for animal handling and care were approved by the Colorado State University Animal Care and Use Committee (protocol #14-5190 and #17-7539A). The experiments were performed at different commercial dairy farms in Colorado, USA. Experiment #1 (**E1**) was conducted in Galeton during November 2014 while experiment #2 (**E2**) was conducted at Blue Sky Dairy, Meade from November of 2017 to February of 2018. A flow chart of the methodology for both E1 and E2 starting from sample collection to the identification of genes and pathways that influence EM pregnancies in Holstein Friesian cows is presented in Fig. 2.1.

Animal Care

Lactating multiparous Holstein cows (E1: n=20, E2: n=22) were fed and milked twice daily, offered water ad-libitum, and received a total mixed ratio according to the guidelines by the

National Research Council (National Academies of Sciences and Medicine, 2021). Cows were in dairy farm database systems and housed in a free-stall barn equipped with sprinklers and fans, and sand-beds.

Estrous Cycle Synchronization

After the voluntary waiting period (60 days postpartum), healthy multiparous Holstein cows were subjected to synchronization of the estrous cycle and induced ovulation. Synchronization of estrous cycle consisted of an intra-muscular (**IM**) injection of Gonadotropin-releasing hormone (**GnRH**; 0.1 mg/ml of Cystorelin-gonadorelin diacetate tetrahydrate, Merial Ltd., Duluth, GA), followed by seven days later an IM injection of $PGF_{2\alpha}$ (25 mg/mL of Lutalyse sterile solution-dinoprost as tromethamine salt, Zoetis, Florham Park, NJ), and a second IM injection of GnRH fifty-six hours after the $PGF_{2\alpha}$ IM injection. Cows were randomly assigned to either treatment group designated as pregnant (P; exposed to timed artificial insemination & same high-fertile sire's semen; E1: n=13 and E2: n=15) or to the control group designated as non-pregnant (NP; not exposed to semen; E1: n=7 and E2: n=7). Day of timed artificial insemination (**TAI**) was considered day 0 for cows in group P and were artificially inseminated 16 h after the final GnRH injection. Estrus was then considered day 0 of the study for cows in group NP. If no conceptus was recovered on day 16, for E1 after the first TAI, new cows were then assigned to the study.

Tissue and fluid sample collection

Lactating Holstein cows from E1 and E2 had the following fluids and (or) tissues collected on day 16 post-AI: uterine flushing, endometrium, PBMC with the exception of CL which were only collected for E2 (n=22). Also, only cows in group P had conceptuses collected on day 16 of

pregnancy. Ultrasonography (Ibex Lite, E.I. Medical Imaging, Loveland, CO) was performed using an 8.0 MHz linear transducer to determine the presence of CL in ovaries. All cows with CL on day 16 were transported to Genetics West, Berthoud, Colorado to flush embryos and (or) collect UF. Thorough cleansing of vulva and vagina was performed with chlorhexidine (2% solution) before performing any procedures. Subsequently, cows were injected with anesthesia (4 mL of 2% lidocaine hydrochloride solution) into the epidural space to relax the musculature surrounding the rectum for embryo and uterine fluid collection.

Recovery and collection of Conceptus and Uterine Flushing

UF and (or) embryo flushes were performed for E1 by Dr. Kevin McSweeney for (E1; Liebig et al. 2022) and with the assistance of Dr. Thomas Rae, DVM-Theriogenologist for E2. In these efforts, flushing media (30 mL of phosphate buffered saline solution; **PBS**, Sigma, St. Louis, MO and 10 mL of 0.01% of polyvinyl alcohol, Sigma, St. Louis, MO) was infused using a 60 mL plastic syringe (Norm-Ject, Henkes, Saas Wolf) connected to a French silicone-coated latex Foley modified catheter (size 22, Bard, Covington, GA) into the ipsilateral uterine horn to the CL by transcervical catheterization to collect conceptuses. A bovine cervical expander (Ag-Tech, Inc.) was also used, if deemed necessary. Recovery of UF, from infusion, involved using the same 60 mL syringe and placing fluid with conceptus in a sterile petri dish. UF was examined under a stereoscope (Stereo Star Zoom, American Optical) at a 7x magnification to locate the conceptus. This procedure was repeated up to six times to ensure recovery of conceptus, if present. Once the conceptus was located, it was rinsed with fresh drops of sterile PBS and 0.1% (v/v) of polyvinyl alcohol. The conceptuses were then measured (millimeters) in length, photographed beside a ruler, and classified/sorted by their viability as either: N (translucent and elongation; E1: n=8, E2: n=9) or undergoing EM (pink, red, opaque and/or restricted elongation; E1: n=5, E2: n=6). N and EM

conceptuses were snap frozen with liquid nitrogen and stored a -80°C for RNA extraction. After recovering the conceptuses, UF were placed on ice within 2 hours were centrifuged at 2,000 x g for 15 minutes at 4°C upon arrival to laboratory at the Animal Reproduction and Biotechnology Laboratory (**ARBL**) facility at Colorado State University (**CSU**), Fort Collins, Colorado. The uteri of NP cows were also flushed to serve as negative controls. Supernatant was collected, homogenized and aliquoted and were stored at 80°C until processed for protein extraction.

Endometrial Biopsy

Collection of endometrial biopsies were performed using a transcervical Jackson uterine biopsy forceps (Jorvet, Jorgensen Laboratories, Inc.; Loveland, CO) with a cutting area of 4mm x 28mm at the ipsilateral uterine horn to the CL. A protective sheath was introduced with the instrument through the vagina and guided by transrectal palpation. The protective sheath was ruptured once the external cervical orifice was reached. At 10 cm from the bifurcation of the uterine horn, the endometrial tissue was collected, snap frozen with liquid nitrogen and stored at -80°C until processed for RNA and protein extraction.

CL Biopsy

Ultrasound and a biopsy needle were inserted and guided into the vaginal fornix for CL collection (E2: n=22). The ovary containing the CL was positioned using transrectal palpation against the vaginal wall and blade of the biopsy needle was triggered. Tissue biopsies were weighed, washed with PBS, snap frozen with liquid nitrogen and stored at -80°C until processed for RNA and protein extraction.

Blood Collection, Plasma, Serum and Peripheral Blood Mononuclear Cell Isolation

For E1, serum collection tubes and evacuated tubes containing K_2 EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) were used to collect serum and PBMC, respectively. While for E2, only evacuated tubes containing K_2 EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) were used to collect both plasma and PBMC. Two blood samples per each animal from jugular vein were collected and placed on ice. Upon arrival at the laboratory in the ARBL facility, samples were processed for serum (E1: n=40), plasma (E2: n=44) or PBMC (E1: n=40, E2: n=44). Serum was separated by centrifugation at 2,000 x *g* for 15 min at 4°C and stored at -20°C until radioimmunoassay. For plasma samples, tubes were centrifuged at 1,500 x g for 15 min, top layer was removed and frozen at -20°C until radioimmunoassay. For PBMC isolation, tubes were centrifuged at 2,000 x *g* for 10 min at 4°C. For PBMC isolation, tubes were centrifuged at 2,000 x *g* for 10 min at 4°C. White buffy coats were transferred into 15 mL tubes containing 5 mL of ammonium chloride lysis buffer (ACK; 150 mM ammonium chloride, 10 mM potassium bicarbonate, 0.1mM EDTA, pH 7.2 to 7.4) and incubated at room temperature for 15 min. PBMC were pelleted by centrifugation at 2,000 x *g* for 10 min at 4°C and washed in 5 mL of sterile BioWhittaker Hank's balanced salt solution without calcium, but containing magnesium and phenol red (Lonza; Walkersville, MD). PBMC were snap frozen with liquid nitrogen and stored at -80°C until RNA extraction.

Radioimmunoassay

Full details of radioimmunoassays for both progesterone and estradiol 17β (estradiol) are described in in Niswender, 1973 (Niswender, 1973) and England et al., 1974 (England et al., 1974), respectively. Assays were completed in the Endolytics Laboratory in the Animal Reproduction and Biotechnology Laboratory at Colorado State University.

Sandwich Enzyme-Linked Immunosorbent Assay

Description of the procedure used for the quantification of IFNT from UF can be found in Dickinson et al., 2020 (Dickson et al., 2020).

Protein Isolation

Protein pellets from endometrium samples were dissolved in 200-400 μL of 1% SDS, the insoluble material was sedimented via centrifugation at 10,000 x *g* for 10 min at 4°C and the supernatant diluted at 1:10 in PBS for quantification. Protein was quantified utilizing a Pierce BCA Protein Assay Kit (Thermo Scientific; Waltham, MA) as per manufacturer's instructions.

Western Blot

Protein concentrations for endometrial and CL tissue was quantified utilizing a Pierce BCA Protein Assay Kit (Thermo Scientific; Waltham, MA). Tissue samples were loaded into a lane of 12% SDS-PAGE gels and electrophoresed for 1.5 hours at 200 volts. Proteins were then transferred to a nitrocellulose blotting membrane (Amersham Protran 0.2 μm NC; GE Healthcare; Pittsburgh, PA) for 1 hour at 100 volts. Membranes were incubated for 1 h in 5% non-fat dry milk in Tris-buffered saline + Tween 20 (TBST) at room temperature. They were then incubated with rabbit anti-bovine mouse anti-bovine ISG15 primary antibody (1:1000; Austin et al., 2004), diluted in TBST for 5 min and incubated with donkey anti-rabbit or donkey anti-mouse secondary antibody diluted in TBST in 1% non-fat dry milk (1:2000; Santa Cruz Biotechnology Inc.; Santa Cruz, CA) for 1 hour at room temperature. Following three more washes in TBST, proteins were detected using the Amersham ECL Prime Western Blotting Detection Reagent Kit (GE Healthcare; Pittsburgh, PA). Quantifications were performed using the optical densitometry program, Image

Lab 4.1, on a ChemiDoc XRS+System with Image Lab Software (Bio-Rad Life Science; Hercules, CA).

RNA Isolation

Total RNA extractions were performed using TRIzol reagent (Life Technologies; Carlsbad, CA) for tissues (CL, PBMC, endometrium and conceptuses) collected on day 16 following the manufacturer's instructions. Remaining DNA was removed from the RNA fractions by treatment with RNase-free DNase (Qiagen; Valencia, CA) and RNAeasy MinElute Cleanup Kit 50 (Qiagen; Valencia, CA; catalog # 74204). This ensured samples to be of high-quality RNA. A NanoDrop 2000 Spectrophotometer (NanoDrop Technologies; Wilmington, DE; Thermo Scientific, manufacture: ND2000USCAN) was used quantify and determine quality of each RNA sample by dividing absorbance $(A)_{260}$ by A_{280}. Values of 2.0 were considered high quality samples.

Reverse-Transcription Quantitative Polymerase Chain Reaction

Single stranded cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad Life Science; Hercules, CA). Synthesized cDNA was diluted with RNase-free water for the reverse-transcription quantitative polymerase chain reaction (**RT-qPCR**) reaction. Primers for ISG15 (target gene) and RPL19 (housekeeping gene) have been previously validated in our laboratory (Romero et al., 2015). The RT-qPCR were performed in duplicate using a combination of cDNA, iQ SYBR green supermix (Bio-Rad Life Science; Hercules, CA), RNase-free water and solution of each primer set for each well in a 96-well plate and amplified on a CFX384 Touch Real-Time PCR Detection System (Biorad). Amplification of PCR products were performed at 95°C for 3 min for denaturation, followed by 39 cycles of 95°C for 10 seconds, 58°C for 30 seconds, and

72°C for 15 seconds. The reaction products were assessed for quality by melting curve on the CFX384 Touch Real-Time PCR Detection System (Biorad).

RNA-Seq

Preparation and Normalization of Library Files

Total RNA for conceptuses (E1: n=14, E2: n=15), endometrium (E1: n=20, E2: n=22), CL (E2: n=22) and PBMC (E2: n=22) were shipped to and processed by Zoetis Inc. (Kalamazoo, MI, USA) for the preparation cDNA libraries and sequencing using the Illumina HiSeq (2000) System. For E1, raw single-end sequencing data was of 100 base pairs, while for E2, it was 75 base pairs. All sequences were then trimmed and aligned to UMD 3.1 (E1) or ARS-UCD 1.2 (E2) bovine genome (http://bovinegenome.elsiklab.missouri.edu/node/61).

Filtering and Analysis of Raw Sequence Reads on RNA-Seq Data

Single-end reads were tested and verified for normal distribution for each tissue and cow group using a principal component analysis (**PCA**) plot. By having 2 PC in the axes, a model plane was created, which gives a score to each plot. The PCA plots determined if there were sample outliers, but more importantly if the plots, by pregnancy status, were similar or different from each other (see Fig. 2.5).

Bioinformatical Analysis on RNA-Seq Data

To gain additional biological insight into patterns of gene expression from the RNA-Seq data, differentially expressed genes (**DEGs**) were submitted to Ingenuity Pathway Analysis® software (**IPA**; Qiagen, Redwood City, CA) for a core analysis with *p*-adjusted value of ≤ 0.05 and ± 0.585 $\log_2$-fold change which are equivalent to ± 1.5 fold change and were identified as either

up- or down-regulated genes. Only for the comparison of EM vs N conceptus in E2 did we use *p*-adjusted value of ≤0.001 and ±2 log_2-fold change which is equivalent to ±4 fold change. This was necessary to reduce the number of DEGs limit (≥8,000) that IPA can effectively conduct a core analysis. The top canonical pathways and up-stream genes were evaluated for each tissue within both experiments (E1 and E2) when DEGs significantly differed (*p*-adjusted value of ≤0.05). To examine top analysis for ready molecules, top diseases and disorders, and top toxicology functions, see appendices (1-9). Reproductive tissues with no key canonical pathways identified but had DEGs were submitted into the Search Tool for the Retrieval of Interacting Genes/Protein (**STRING**; http://string-db.org) database to find possible protein-protein interactions and their gene roles. If associations were discovered, then cross-referenced Pubmed searches were completed using two or more DEGs in each search for relationships.

Statistical Analysis

Conceptus length was analyzed with Proc GLM using the one-way ANOVA procedure in SAS software OnDemand for Academics Edition. UF data were subjected to a Kruskall-Wallis test using Prism 8 from GraphPad (See Fig. 2.2C) in order to correct for variable volume of flushing media that that was recovered from bovine uteri. For RT-qPCR results, delta cycle threshold (**Ct**) values for each target were obtained after normalization of Ct values of the gene with Ct values from our housekeeping gene, which was RPL19. Housekeeping genes, target genes, and their respective primers are listed in Table 2.1. RT-qPCR data and western blots were analyzed by Proc GLM-ANOVA in SAS software OnDemand for Academics Edition. Treatment differences with $p \leq 0.05$ were considered significant and those with $0.05 > p \leq 0.10$ were considered tendencies. Concentrations of progesterone and estradiol in plasma were analyzed by pregnancy status (NP, EM, N), day of pregnancy/estrous cycle (day 0, 7, 16) and interactions using

Proc Mixed and LSMEANS procedures for repeated measure analyses in SAS software OnDemand for Academics Edition. RNA-Seq data were exported, organized (R package: dplyr and plyr) and filtered (R package: edgeR) to exclude samples with less than 10 mRNA raw counts using R. Use of DESeq2 in R package controlled for potential false discovery using an adjusted p-value and the Benjamini-Hochberg method (Hochberg, 1995). Moreover, within the DESeq2 in R package, comparisons (1x1) were performed between groups (N vs NP, EM vs NP, and N vs EM) for all samples except conceptuses (N vs EM) using a negative binomial distribution model. Conceptus *IFNT* mRNA and endometrial, PBMC and CL *ISG15* mRNA were analyzed by DESeq2 in R to get the DEGs with $\log_2$-fold change and adjusted p-value (Love et al., 2014). All files were exported in Excel spreadsheet format to submit into IPA (Qiagen Catalog # 830018).

Results

Conceptus Morphology and Length, Uterine Flushing IFNT Concentrations and Endometrial Conjugated ISG15 concentrations

Examples of N and EM conceptuses are shown in Fig. 2.2A. N conceptuses were longer (E1: p=0.002, E2: p =0.003; Fig. 2.2B) when compared to EM conceptuses. Likewise, IFNT protein concentrations in UF were greater in N compared to EM and NP in E1, but this was not the case in E2 (Fig. 2.2C). In E2, the IFNT protein concentrations in UF from EM embryos were not different from NP or N UF which may be indicative of an intermediate and compromised release of IFNT. When analyzing endometrial conjugated ISG15 protein concentrations using western blot, they followed the pattern of IFNT release from the conceptus (Fig. 2.2D). For example, in E1, ISG15 concentrations were greater (p <0.001) in N compared to NP and EM,

whereas in E2, ISG15 concentrations were greater ($p < 0.01$) in N compared to NP, but EM concentrations tended ($p = 0.09$) to be greater than NP and were not different from N endometrium.

RTqPCR

Because of limited EM conceptus tissue, all of the EM conceptus RNA samples were submitted for RNA-Seq analysis. For this reason, RTqPCR was only completed for endometrium, PBMC and CL (E2). RTqPCR confirmed that endometrial *ISG15* mRNA levels were greater in N (E1: $p < 0.0003$; E2: $p < 0.0001$-Fig. 2.3A) compared to EM and NP endometrium in E1, and in E2 only tended ($p < 0.07$) to be greater in EM compared to NP endometrium. Based on RTqPCR, there were no differences in PBMC (Fig. 2.3B) or CL (Fig. 2.3C) *ISG15* mRNA levels between NP, EM and N pregnancy status.

Circulating progesterone and estradiol concentrations

In E1, serum progesterone and estradiol (Fig. 2.4) did not change between NP, EM and N pregnancy status on Day 16. In E2, we also examined day 0 to provide a baseline, day 7 to determine if there was an earlier impact on the CL, and day 16 on the day of tissue collection. There was no difference in steroid concentrations between the pregnancy classifications on any day tested. However, as expected there was an increase in progesterone ($p < 0.0001$) on days 7 and 16 relative to day 0 (Fig. 2.4A).

RNA-Seq Experiments

Principal Component Analysis

The PCA plots (Fig. 2.5A) revealed that N conceptus RNA-Seq data clustered more tightly in in the lower left (E1) or the mid to lower left (E2) quadrants, whereas EM data were clearly

separated and more disperse throughout upper-middle and right quadrants. In E1 endometrium samples, NP clustered to the left and was separated from N and EM, which appeared to be more closely aligned to each other, but dispersed through the center of the plot (Fig. 2.5B). In E2 endometrium, there was no clear pattern or localization in the plot regardless of pregnancy status. The PBMC E2 data were more generally scattered across the plot, with no particular region of localization by pregnancy status, whereas in E1 the data were mostly clustered together in the top left quadrant across pregnancy status (Fig. 2.5C). Likewise, in the E2 CL, there was no clear pattern of separation of data across pregnancy status groups (Fig. 2.5D).

Targeted transcript analysis: IFNT in Conceptus and ISG15 in Maternal Tissues

Using RNA-Seq analysis with $\log_2$-fold change, N conceptuses had greater *IFNT1* (p <0.005) in E1 and greater (p <0.001) *IFNT1*, *IFNT2* and *IFNT3* mRNA levels in E2 compared to EM (Fig. 2.6A). Endometrial *ISG15* mRNA levels were also greater in N (E1: p <0.01; E2: p <0.0001) and EM (E1 and E2: p <0.00001) compared to NP (Fig. 2.6B). N endometrial *ISG15* mRNA levels were greater (p <0.05; Fig. 2.6B) than EM in E1, whereas in E2 there were no differences between N and EM cows. In PBMC, N *ISG15* mRNA levels were only greater in N compared to NP in E2 (Fig. 2.6C). All other comparisons of PBMC *ISG15* mRNA levels were not different in E1 and E2. Likewise, no differences in CL *ISG15* mRNA levels across pregnancy status were observed in E2 (Fig. 2.6D).

Conceptus IPA Analysis

When comparing EM to N conceptuses, EM had 2,280 up and 408 downregulated DEGs in E1, and 2,908 up- and 849 downregulated DEGs in E2 (Appendix Fig. 1). Among the top activated canonical pathways for both E1 and E2 in EM compared to N were T helper (**Th**) 1 and

Th2 activation pathways (E1: p =6.38 E^{-22}; E2: p =1.090E^{-13}-Fig. 2.7), and hepatic fibrosis/hepatic stellate cell activation pathway (E1: p =4.35E^{-16}; E2: p =2.30E^{-18}). Out of 172 genes in the Th1/Th2 pathway, there were 68 DEGs (67 up- and 1 down-regulated) in E1, while in E2 there were 67 DEGs (65 up- and 2 down-regulated) (Fig. 2.7). Conversely, out of 194 genes in the hepatic fibrosis/hepatic stellate cell activation pathway, there were 64 DEGs (57 up- and 7 down-regulated) in E1 and 81 DEGs (71 up- and 10 down-regulated) in E2. Analysis of top upstream regulators revelated that lipopolysaccharide, dexamethasone, beta-estradiol and TNF were identified in both E1 and E2 (Appendix Fig. 1). The relationships between pathways and upregulated genes in EM are shown in Fig. 2.8 for the following cytokines: TNF, B2M, IL2, IFNG, IL1B and IL15.

Endometrium IPA and STRING Analysis

For N vs NP endometrium, E1 had 332 up- and 111 down-regulated DEGs, while for E2 there were 274 up- and 13 down-regulated DEGs (Appendix Fig. 2). Within the top activated canonical pathways for both E1 and E2, was IFN signaling (E1: p =3.57E^{-14}; E2: p =5.66E^{-15}), as expected. Out of the 36 genes in IFN signaling, there were 13 DEGs in E, while in E2 there were 12 DEGs and all were up-regulated in both experiments (Fig. 2.9). Analysis of top upstream regulators, revealed that NONO, IRF7, IFNL1 and IFNA were upregulated in N in both E1 and E2. IFNG (E1) and TGFB1 (E2) also were top upstream regulators (Appendix Fig. 2). In E1, when comparing EM to NP endometrium, there were 6 up-regulated and 2 down-regulated genes, whereas in E2 there were 146 up-regulated and 7 down-regulated genes (Appendix Fig. 3). These primary upregulated canonical pathways in EM were Interferon Signaling and Pathogenesis of Influenza, both of which reflect IFNT responses to pregnancy in EM endometrium. In E1, when comparing EM to N endometrium, there were 47 upregulated and 379 downregulated genes

(Appendix Fig. 4). Top canonical pathways were Th1, Th2, pathogenesis of influenza and interferon signaling. In E2, there were 25 upregulated and 1 down-regulated gene in EM compared to N endometrium. Top canonical pathways were metaphase signaling, mitosis, estrogen-mediated s-phase entry, ubiquitination and cell cycle, although with only 26 DEG, there were not a lot of genes in these pathways that were impacted (Appendix Fig. 4). For this reason, protein-protein interactions were examined using STRING database (Fig. 2.10). Twenty out of the twenty-six DEGS were clustered together and many of their functions were within the cell cycle pathway. We suspected that these genes might function in the presence of estradiol in the endometrium. After a PubMed search, using each gene coupled with estradiol, it was discovered that all twenty clustered genes were targeted by estradiol and associated with its action through cell proliferation/mitosis (TROAP, CENPE, CENPF, AURKB, NCAPG, KIF20A, KIF4A, BUB1, BUB1B, CDC20, MTFR2, DEPDC1 and ANLN), meiosis (ASPM, ESPL1 and UBE2C) and (or) DNA damage (TOP2A, ARHGAP11A)/repair (HJURP, E2F7; Table 2.2 and Fig. 2.10).

PBMC IPA Analysis

In E1 there were no DEGs in PBMC examined in N compared to NP. In E2, there were 9 up and 1 down regulated DEGs in N compared to NP PBMC (Appendix Fig. 5). The top canonical pathways were Influenza Pathogenesis and IFN signaling, while top upstream regulators were IRF7, IFNαR2, IRGM, NONO and IFNL1. In E1, there were 2 up- and 30 down-regulated PBMC genes in EM compared to NP, while EM had 2 up- and 18 down-regulated genes compared to N PBMC (Appendix Fig. 6 and 7). Both comparisons (EM vs NP and EM vs N), for E1, yielded only 2 to 3 DEGs in canonical pathway(s), which are not described for these reasons. However, 18 of the DEGs in E1 were identical when comparing EM vs NP and N vs EM (1 up-regulated: C2; 13 down-regulated gene: ADRA2A, ANLN, CEBD, CNTD1, ITGB6, KIAA0044, MPEG1, PAPPA,

PKD2L2, RANBP17, SLC6A10, TACR3, TMC7). Fourteen of these DEGs were associated with tissue growth, remodeling and/or development (ADRA2A, ANLN, CEBPD, PAPPA, RANBP17, TACR3, TMC7), innate immune system (C2, MPEG1), conceptus implantation (ITGB6, KIAA0040), cell cycle (CNTD1) and essential mineral transporters (PKD2L2, SLC4A10). Unique DEGs were associated to Th1 inactivation (TNFAIP3) within EM vs NP and birth complications (CACNB2, EBF1), cytotoxicity (CD300A), cardiovascular control (RGS1), cell growth/control (RADIL, RPL23A, SSH1, SYP3, TBC1D32) and essential mineral transporters (STAC2, SLC16A4, SLC24A5, SLC4A5) within N vs EM. In E2, there were no differences in PBMC gene expression in EM compared to NP and in EM compared to N.

Corpus Luteum IPA and STRING Analysis

When comparing N vs NP CL, there were 3 up-regulated DEGs (ZBP1, OAS1 and MX1; Appendix Fig. 8). Although the number of DEGs were low, IPA identified IFN Signaling (p =6.67E^{-06}) as the top canonical pathway. There were no DEGs in EM vs NP CL. However, when comparing EM vs N CL, there were 6 up- and 1 down-regulated gene (Appendix Fig. 9). No top activated canonical pathway(s) were identified by IPA. Six out of the seven DEGs were clustered using String analysis were found to be involved with inflammation (CA5A, VNN2), calcium sequestration/delivery responses (S100A9/12), glucose- (SDS) and estradiol-metabolism (CYP3A4; Table 2.3).

Discussion

Conceptus Morphology and Length, Uterine Flushing IFNT Concentrations and Endometrial Conjugated ISG15 concentrations

Elongation of the bovine conceptus is important to ensure that proper amounts of IFNT are being secreted in order to establish maternal recognition and maintain pregnancy (Matsuyama et al., 2012, Barnwell et al., 2016, Forde and Lonergan, 2017). Peak synthesis and secretion of IFNT occurs through the conceptus trophectoderm on day 20 of pregnancy (Godkin et al., 1984, Farin et al., 1989, Guillomot et al., 1990). Previously, we classified pregnancies as low (**LP**) or high (**HP**) fertility based on higher DPR and lower services per conception in lactating dairy cows (Liebig et al., 2022). The HP tended to be longer, but had greater IFNT release compared to LP conceptuses. These pregnancies were then sorted based on appearance of the conceptus into N or EM. The EM were shorter than the N conceptuses. A more expanded analysis of these N vs EM conceptuses is described herein as E1. Our current results for both E1 and E2 indicate that N conceptuses were significantly elongated when compared to EM conceptuses.

The uterine response to the conceptus is required for successful establishment of pregnancy (Hansen et al., 2017). One endometrial response is up-regulation of ISG15 in response to IFNT (Austin et al., 1996, Johnson et al., 1998, Johnson et al., 1999, Austin et al., 2004). In endometrium (Austin et al., 2004), CL (Bott et al., 2010, Antoniazzi et al., 2013, Romero et al., 2013) and PBMC (Han et al., 2006a, Gifford et al., 2007, Yang et al., 2010), ISG15 was upregulated in pregnant compared to NP ruminants ISG15 functions in modifying function of proteins, called ISGylation in the endometrium and is hypothesized to not only be involved with ani-luteolytic processes, but also is released and acts as a cytokine (Hansen and Pru, 2014).

The pattern of increase in IFNT concentrations in N compared to EM UF was reflected in increased endometrium ISG15 protein and mRNA in N vs EM and NP in E1. Likewise, in E2 the variable detection of IFNT in EM UF was reflected by EM endometrium ISG15 concentrations that were intermediate and tended to be lower in EM compared to N. Clearly in E2, some of the conceptuses were in earlier stages of mortality compared to E1.

The method of UF collections provided variable concentrations of IFNT in UF because of uterine size, but also variable recovery of media used to recover the conceptus. For this reason, RNA-Seq was completed to determine the amount of *IFNT* mRNA, which revealed that EM conceptuses had lower *IFNT* mRNA concentrations compared to N conceptuses in both E1 and E2. These differences *in IFNT* mRNA may not have been reflected in UF IFNT protein levels at the time that we collected conceptuses in E1 because of a delay in clearing the IFNT protein in uterine fluid (Liu et al., 2016).

Studies in sheep indicate that IFNT is released from the elongating conceptus and enters the uterine vein (Oliveira et al., 2008, Romero et al., 2015), thereby inducing ISGs in maternal tissues and cells including PBMC, liver, and CL (Oliveira et al., 2008, Bott et al., 2010, Ribeiro et al., 2014, Meyerholz et al., 2016, Sinedino et al., 2017). Examination of PBMC revealed that ISGs were up-regulated in response to early pregnancy in both sheep (Yankey et al., 2001) and cattle (Han et al., 2006b, Gifford et al., 2007). In the present experiments, based on RT-qPCR on day 16 of pregnancy, there was no difference in ISG15 mRNA concentrations in the PBMC or in the CL in N compared to EM or NP. RNA-Seq revealed similar responses with the exception in E2 of upregulation of ISG15 mRNA in N compared to NP in PBMC. The lack of an ISG response to pregnancy in E1 may have been caused by collecting tissues on day 16, which may be prior to fully systemic response to IFNT. In E2, pregnancy caused an increase in PBMC ISG15 mRNA

concentrations, whereas EM and NP levels were not different. This lack of a primary ISG response in EM PBMC may have been caused by impaired production and action of IFNT.

Circulating progesterone and estradiol concentrations

Secretion of progesterone by the corpus luteum is a factor associated with the process of implantation and elongation of the conceptus and thus, the likelihood of pregnancy success (Lonergan et al., 2016a, Spencer et al., 2016). Several studies have found that changes in the amount of progesterone secreted during the luteal phase could impair elongation (Forde et al., 2011a, Forde et al., 2012b). However, any direct effects of progesterone on the conceptus size, quality, source (*in vivo* or *in vitro*) and time of recovery are still highly debated (Goff and Smith, 1998, Merlo et al., 2007, Clemente et al., 2009, Ferguson et al., 2012, van Leeuwen et al., 2015).

It was reasoned in the present studies that any insult to the CL in NP and EM would be reflected by decreased progesterone concentrations and possibly increased estradiol concentrations. In E1 and E2, there were no significant change in concentrations of progesterone and estradiol across pregnancy status (NP, EM, N). However, in E2, there was an increase in progesterone and a decrease in estradiol as pregnancy progressed from day 0 of the follicular phase to day 16 of the luteal phase (Sanger, 2012). Both of these responses are typical for an estrous cycle in cattle. It is clear, based on these data, that the CL had not started functional regression from a strong luteolytic response by the endometrium by Day 16. It was probably too early on day 16 to study these peripheral responses in NP and EM cows.

RTqPCR

Upregulation of ISGs such as ISG15 are induced by conceptus derived-IFNT through paracrine action on peripheral tissues in order to regulate uterine functions that are essential for

conceptus elongation, implantation and the establishment of pregnancy in cattle to take place (Hansen et al., 2010). Our E1 and E2 RTqPCR results revealed, as expected, to have greater levels of *ISG15* mRNA in N endometrium when compared to EM and NP endometrium but only tended to be greater in EM compared to NP endometrium only for E2. A possible explanation for a lack of significant differences between EM and NP endometrium in E1 and E2 might be due to the time of tissue collection. Since all tissues were collected on day 16 of pregnancy/estrous cycle, the paracrine action from IFNT might not yet be affecting the uterine environment that induces ISGs to be upregulated.

Conversely, IFNT is less known for its endocrine action on extrauterine tissues such as the CL and PBMC (Han et al., 2006a, Gifford et al., 2007). Studies have found that significant differences of *ISG15* mRNA levels from CL occur early as day 16 in cattle and for PBMC on day 15 pregnant cows when compared to non-pregnant cows (Green et al., 2010, Monteiro et al., 2014). Unfortunately, our CL RTqPCR for E2 only and PBMC results for both E1 and E2 on *ISG15* mRNA were non-significant. Such results might reflect an impaired response of *ISG15* mRNA. For the CL, it might have been the time in which tissue collection was either too early or same day (day 16) as all the other tissues. In the case of PBMC, maximal levels of *ISG15* mRNA often take place on day 20 of pregnancy (Green et al., 2010, Monteiro et al., 2014).

RNA-Seq Analysis

RNA-Seq and microarray procedures have been previously used to understand transcriptome differences in endometrium between pregnant and nonpregnant cows: days 7, 10, 13 (Mamo et al., 2011), 15 (Bauersachs et al., 2012), 16 (Forde et al., 2011b, Forde et al., 2012a, Mamo et al., 2012, Dickson et al., 2022),17 (Cerri et al., 2012), 18 (Klein et al., 2006, Hansen et al., 2010) and 19 (Mamo et al., 2011). Nevertheless, these studies were done in non-lactating dairy

or beef cows or heifers. Both the Bovine HapMap Consortium (Bovine HapMap et al., 2009) resource and the study of Moridi et al. (Moridi et al., 2019) revealed that there are large genetic variations in expressed transcripts between cattle breeds and crossbreeds. To our knowledge, the current studies (E1 and E2) are amongst the first to describe transcriptomic data in conceptuses, endometrium, PBMC and CL on day 16 in N vs EM conceptuses from purebred lactating Holstein Friesian cows.

Principal Component Analysis

The PCA plots for N conceptus in E1 and E2 indicated higher similarity between each of the individual tissue samples (Fig. 2.5A). While in EM conceptuses, in E1 and E2, were very highly dispersed which might be due to the stage in death (beginning or advance) that is demonstrated in our data from the lack of IFNT production from the trophectoderm and conceptus size (Fig. 2.5A). For endometrium, in E1, N and EM were still highly similar which might indicate that the ISG15 response is still sufficient and not yet indicative of pregnancy loss in EM (Fig. 2.5B). On the other hand, NP endometrium in E1 were separated from N and EM endometrium which might indicate absence of pregnancy since there is no ISG15 response by having the absence of a conceptus' trophectoderm producing IFNT (Fig. 2.5B). In the case of E2 endometrium, there were no clear difference between the N, EM and NP. This might suggest that the endometrium of EM are yet not quite reflecting pregnancy loss is taking place from a lack of ISG15 response. While the endometrium of NP may not have yet had a reset the estrous cycle and progesterone is still maintaining the environment to be appropriate for a conceptus to implant (Fig. 2.5B). The PCA plots for PBMC, in E1, demonstrated that N, EM and NP individual tissue samples being highly similar and clustered. For the PCA plots of PBMC and CL in E2 reflected that individual samples were mainly scattered regardless of pregnancy status (Fig. 2.5C and D). Both the PBMC

and CL tissue samples for both E1 and E2 might still be reflecting the peripheral response to either IFNT via ISG15 (N and EM) or progesterone (NP).

Conceptus IPA Analysis

The primary (out of the top 5) shared (E1 and E2) canonical pathway in EM compared to N conceptuses was Th1 and Th2 activation (Appendix Fig. 1; Fig. 2.7). The top pathway in E2 was hepatic fibrosis and hepatic stellate cell activation. Pathogenesis of hepatic fibrosis is often characterized by an injury of epithelial cells which provokes cytokine release and activation of hepatic stellate cells. The environment then becomes pro-inflammatory, contractile, non-proliferative and thus, damages epithelial cells (Friedman, 2015). Inflammatory mediators trigger blood-clot formation, also known as a persistent immune response and an ongoing epithelial injury promotes tissue fibrosis (Wynn, 2008). Th1 cell responses are also related to apoptosis, fibrotic tissues and hepatic stellate cell activation (Sandler et al., 2003). In E2, the top canonical pathway that was massively upregulated in EM compared to N conceptuses was Th1/Th2 Activation, which also was the fifth ranked pathway in E2. Interestingly, the top shared upstream regulators in both E1 and E2 EM conceptuses were lipopolysaccharide (strongly induces innate immune response, dexamethasone (typically thought to suppress Th1; (Elenkov, 2004), TNF (boosts death cell signaling and Th1 and Th2 responses (PMID: 12688591); and beta-estradiol (Th1 and Th2). Because of the massive regulation of the Th1 and Th2 pathways, it is concluded that the EM embryo is mounting innate, humoral and adaptive responses that reflect a dying embryo. To our knowledge, this is the first report of transcriptome changes associated with an inherent trophoblast cell immune response in EM vs N bovine conceptuses.

In human and mouse pregnancies, the Th1/2 paradigm has been heavily debated and many have favored the Th2 cell immune response as an indicator of normal development and survival

of the fetus (Wegmann et al., 1993, Raghupathy, 1997). However, this concept may not be entirely accurate, because each of the Th cell pathways contain specific cytokines that mediate immune responses to pathogens and also are essential in directing events in early pregnancy (Mansouri-Attia et al., 2012). In fact, endometrial transcriptomic expression data of Th1 cytokines have also been reported during the implantation period of the conceptus in mice (Lin et al., 1993, Chaouat et al., 2004). Shirasuna et al. (Shirasuna et al., 2012). Studies in mice (Chaouat et al., 2005) have also demonstrated that by injecting Th1 cytokines (TNF and IFNG), normal pregnancies are terminated and fetuses are reabsorbed during early gestation. This is consistent with action of TNF and IFNG that were identified in this study as upstream regulators of in EM vs N conceptuses (Fig. 2.8). Th1 and Th2 cells are also characterized by the cytokines that they express as either being pro-inflammatory cytokines (TNF, IFNG) and anti-inflammatory cytokines (TGFB1), respectively (Zhang et al., 2015). Furthermore, Th1 cytokines are stimulated by $PGF_{2\alpha}$ synthesis and inhibited by progesterone expression and Th2 cytokines are inversely stimulated and inhibited (Maeda et al., 2013).

The maintenance of pregnancy in cattle has been linked to the up-regulation of Th2 cytokines (Yang et al., 2014, Zhang et al., 2015). The EM vs N conceptus results in the current study also aligned with these reports as per the up-regulation of upstream regulators that were a Th1 cytokine (TNF) and beta-estradiol, which may be stimulated by EM and possibly reflect an ongoing pregnancy loss (Shirasuna et al., 2012, Oliveira et al., 2013, Fair, 2015). It has been hypothesized that the ruminant conceptuses can induce peripheral anti-viral responses to potentially protect the mother and conceptus from viral infection (Hansen et al., 2010) and can regulate cytokine production from both the Th1 and 2 pathways as well as the immune response in the endometrium (Ott, 2020) via IFNT. Certainly a balance most likely exists between the Th1/2

pathways during the progression of a N ruminant pregnancy (Maeda et al., 2013). It is clear that pregnancy is an immune response, but one that is balanced and carefully orchestrated. The massive immune response within the EM conceptus may reflect ongoing mortality, but also signals prompting the uterine lumen to change the environment from nurturing to not only clearing and reabsorbing the conceptus, but also re-setting the endometrium to prepare for a new estrous cycle and ovulation.

Endometrium IPA and STRING Analysis

In N vs NP endometrium, there was a very strong induction of Type I IFN Reponses as reflected by major shared (E1 and E2) Interferon Signaling, Pathogenesis of Influenza, Pattern Recognition Receptors and Activation of IRF Canonical pathways as well as IFN-associated upstream regulators (Appendix Fig. 2 and Fig. 2.9). This endometrial response to conceptus-derived IFNT was expected as described originally by Godkin et al. (Godkin et al., 1984) and Bazer et al. (Bazer et al., 1991) in transcriptome studies completed by many other groups (Klein et al., 2006, Hansen et al., 2010, Forde et al., 2011a, Forde et al., 2011b, Bauersachs and Wolf, 2012, Cerri et al., 2012, Mamo et al., 2012, Dickson et al., 2022). The top upstream regulators in N endometrium are considered part of either a normal immune response (IRF7, IFNA, IFNL1) that ensure a proper uterine environment for pregnancy to take place (Martin et al., 2004, Roberts, 2007).

The activation of the IFN pathway occurs through the secretion of IFNT by the trophectoderm of the conceptus and paracrine action on the luminal epithelium of the endometrium during the pre-implantation period (Spencer and Bazer, 1996). Such action by IFNT inhibits the upregulation of the luteolytic cascade that is mediated by ESR1 induction of OXTR; thereby attenuating the pulsatile release of $PGF_{2\alpha}$ (Bazer et al., 2010). This action of IFNT from the

conceptus allows for maternal recognition to take place by protecting the CL so that it produces adequate progesterone, which prevents pregnancy loss (Bazer et al., 1991). IFNT also induces other genes and also cooperates with progesterone in the uterus to facilitate transport of glucose and amino acids, cell proliferation, migration and attachment, proteases and their inhibitors and intracellular enzymes. These actions of IFNT and progesterone facilitate elongation, survival of the conceptus through endometrial production of histotroph and preparation of the endometrial lining for implantation (Lonergan et al., 2016b, Spencer et al., 2016).

In EM vs NP endometrium, for both E1 and E2, IFN signaling was the primary activated canonical pathway. However, upstream regulators were not similar between E1 and E2. Some of the E1 upstream regulators (IFNA14, RNY3, TREX1) were associated with luteolysis of the CL and a reset of the estrous cycle may occur due an impaired IFNT action and signaling for pregnancy to take place (Pedraza-Alva et al., 2009, Konduri et al., 2010, Kim et al., 2014). Because EM had greater IFN response than NP, this reflects at least some IFNT signaling was occurring in the day 16 EM conceptus that the tissue collected and was indeed conceptus trophoblast. On the other hand, some of the E2 upstream regulators (IFNA, IRF7, IFNL1) were associated with a normal preparation of the endometrium for pregnancy (Martin et al., 2004, Roberts, 2007).

The E1 for EM vs N endometrium found activated the canonical Th1/2 pathway and down-regulation of upstream regulator of IFNA, IFNG, IRF7 which may be possibly indicative of an abnormal immune response that does not ensure a proper uterine environment that may lead to the disruption of pregnancy to take place (Martin et al., 2004, Roberts, 2007). Conversely, E2 was only able to pinpoint a up-regulation of 20 DEGs that are associated with disruption of maternal-embryo crosstalk through the protein-protein interactions and that are associated with cell proliferation and targeted by estradiol action. Previously we mentioned that if IFNT does not

inhibit ESR1 then upregulation of OXTR occurs and pulsatile release of $PGF_{2\alpha}$ causes luteolysis of the CL (Spencer et al., 1996, McCracken et al., 1999, Spencer and Hansen, 2015, Hansen et al., 2017). Production of progesterone is then halted which has been associated with reducing growth and development of the conceptus and also causing an increase in the thickness of the endometrium (Pierson and Ginther, 1987). Such thickening of the uterine wall may be indicative regression of the luteal phase which can cause pregnancy loss, activate ovulation of the dominant follicle to produce estradiol and reset the estrous cycle (Garrett et al., 1988, Griffin and Ginther, 1991, Spencer et al., 2008, Bazer et al., 2010, Forde et al., 2012b). Furthermore, studies have shown that cell cycle control with an increase of cell proliferation is associated to aid increase in the thickness of the endometrium during luteal regression of the estrous cycle (Griffin and Ginther, 1991). Our EM vs N endometrium transcriptome data for E2 further supports this by having 20 up-regulated DEGs that are associated to cell proliferation (TROAP, CENPE, CENPF, AURKB, NCAPG, KIF20A, KIF4A, BUB1, BUB1B, CDC20, MTFR2, DEPDC1 and ANLN), meiosis (ASPM, ESPL1 and UBE2C) and (or) DNA damage (TOP2A, ARHGAP11A)/repair (HJURP, E2F7) and may align with cell cycle control (Fig. 2.6), regression of the luteal phase and reset of the estrous cycle (Sugiura et al., 2018).

PBMC IPA and STRING Analysis

As shown in both the N vs NP endometrium and CL for E2, N vs NP PBMC in E2 had 9 up-regulated DEGs (*ISG15, MX2, OAS1, OAS2, IFI44, IFI6, DDX58, ZBP1* and *RTP4*) that were classical ISGs that are up-regulated by the IFN signaling pathway and may indicate progression of a normal pregnancy (Han et al., 2006a, Gifford et al., 2007). The remaining down-regulated DEG was FAT3 which has been associated with cell-cell adhesion (e-cadherin) and may be altered for implantation to occur (Stephenson et al., 2010). For the upstream regulators, in N vs NP PBMC

for E2, IFNαR2 was expected since they mediate the actions of IFNT in the endometrium (Hansen et al., 1989, Stark et al., 1998, Dorniak et al., 2013). To our understanding, there are no studies that might help explain and suggest IFNT having an endocrine action on PBMC to increase the expression of IFNαR2. The other up-stream regulators, for N vs NP PBMC, were IRF7 and IFNL1. A study on day 16 of pregnancy, observed *IRF7* mRNA overexpression in N endometrium as a normal immune response taking place to pregnancy but not in PBMC (Forde et al., 2011a). For IFNL1, it has been found to be representative of modulating the maternal immunity against the implanting conceptus but has also not been shown for PBMC (Syedbasha and Egli, 2017). Other studies have also suggested that PBMCs may be able to reflect anti- and luteolytic signaling which are representative of conceptus-derived IFNT endocrine action on regulating maternal systemic immune response (Shirasuna et al., 2012, Yang et al., 2014, Yang et al., 2016). Such claims are further corroborated by others who also found an abundance of DEGs in PBMCs that were either associated with IFNT modulating the immune system during early pregnancy (day 18) or may be used as pregnancy biomarkers in *Bos indicus* heifers (Rocha et al., 2020).

Our results for E1 PBMC on EM vs NP and EM vs N demonstrated that within the 14 DEGs shared between both comparisons: 13 were down-regulated and 1 up-regulated. The roles of these 14 DEGs (tissue growth, remodeling and/or development, innate immune system, conceptus implantation and essential mineral transporters) demonstrated an association with an intermediate/failed attempt of a pregnancy taking place for both comparisons (EM vs N; EM vs NP) (Consortium, 2021). The unique DEGs, for EM vs N, that were associated with Th1 being up-regulated while those with fetal growth were being down-regulated also confirmed that a loss of pregnancy was taking place. For EM vs NP, the unique DEGs were associated with birth complications, cytotoxicity, cell growth/control and essential mineral transporters were down-

regulated while those associated with cardiovascular control were up-regulated that may also indicate failure of pregnancy(Consortium, 2021). In contrast, lack of DEGs and significant differences within PBMC for E1 on N vs NP and for E2 on N vs EM and EM vs NP might be due to a peripheral response and endocrine action from IFNT not yet occurring.

Corpus Luteum IPA and STRING Analysis

Likewise, to N vs NP endometrium, the N vs NP CL tissue in E2 had 3 up-regulated classical ISGs (ZBP1, OAS1 and MX1) and some upstream regulators (IFNα1/13, STAT2) may be activated due to the IFN signaling pathway. This has been suggested by only a few bovine studies that mention that IFNT may also have an endocrine role particularly on the CL during maternal recognition through the up-regulation of ISGs (Han et al., 2006a, Gifford et al., 2007, Shirasuna et al., 2015). It was then theorized by results of E2 that IFNT or an IFNT-induced cytokine was released into the uterine vein to then reach the CL and up-regulate ISGs. The EM vs N CL for E2 had six up-regulated DEGs that are associated with inflammation (CA5A, VNN2), calcium sequestration/delivery (*S100A9/12)* and glucose- (SDS) and E_2 -metabolism (CYP3A4). All of the DEGs that are being up-regulated align which may be able to activate the luteolysis pathway that occurs through estradiol binding to ESR1 in the endometrium, activation of OXTR signaling cascade and pulsatile release of $PGF_{2\alpha}$.This pathway is also known as the two-cell gonadotropin theory and it involves both large and small luteal cells in the corpus luteum and explains how they are luteolyzed to restart the estrous cycle in cows (McCracken et al., 1970, Niswender et al., 2007). Activation of the luteolysis pathway may only start through $PGF_{2\alpha}$ binding to its receptor in large luteal cells and opening calcium channels that cause inflammation and apoptosis. Moreover, $PGF_{2\alpha}$ activates protein kinase-C (**PK-C**) which inhibits conversion of cholesterol to progesterone in large luteal cells but also, brings oxytocin into large luteal cells to

be delivered to small luteal cells. There the oxytocin binds to its receptor and activates PK-C which inhibits adenylate cyclase from converting cholesterol to progesterone. Additionally, the PK-C also produces calcium which causes both apoptosis and inflammation of the small luteal cells. Apoptosis of both large and small luteal cells then cause luteolysis of the CL and resets the estrous cycle (Niswender et al., 2007).

Conclusions

Results of two experiments were interpreted to reveal that EM pregnancies were associated with a disruption of maternal-fetal crosstalk when IFNT fails to achieve maternal recognition responsible for inhibiting ESR1 from up-regulating OXTR signaling pathway and pulsatile release of $PGF_{2\alpha}$. Hence, EM conceptuses exhibit a Th1 adaptive immune response that up-regulates pro-inflammatory cytokines through the potential stimulation of $PGF_{2\alpha}$. The peripheral responses of reproductive tissues (endometrium and CL) to an EM conceptus may also change functions in context of an impaired IFNT action (paracrine and endocrine). Within the endometrium, of a pregnancy with EM, genes are targeted and associated with estradiol-mediated luteolytic action were up-regulated and may indicate an increase in endometrial thickness which is unfavorable for conceptus survival. This may be due to a changing uterine lumen that stops producing histotroph to nurture the conceptus to clearing it in order to reset and prepare for a new estrous cycle and ovulation. In the PBMC, of a pregnancy with EM, down-regulated DEGs were associated with tissue growth, remodeling and/or development, innate immune system, conceptus implantation and essential mineral transporters demonstrating an intermediate and failed attempt of a pregnancy taking place. For the CL, of a pregnancy with EM, it exhibited up-regulated DEGs that were

associated the activation of the luteolysis cascade through inflammation, increase in calcium and estradiol steroidogenesis. To our understanding, the transcriptome results from this study are the first to describe day 16 *in vivo* tissues including conceptus, endometrium, CL and PBMC from purebred Holstein-Friesian cows. Thus, these new data may then contribute to the crucial knowledge of what occurs during EM pregnancies (Fig. 2.11) and what reproductive practices may be used to mitigate them.

Table 2.1: Oligonucleotide primer sequences used for semiquantitative reverse-transcription quantitative polymerase chain reaction (RTqPCR) in both experiment 1 (E1) and 2 (E2).

Target	Accession no.	Primer Sequence[1]
ISG15	NM_174366	F: ggtatccgagctgaagcagtt
		R: acctccctgctgtcaaggt
RPL19	XM_004012837.1	F:tcgccggaagggcaggcata
		R:ggctgtgatacatgtgggggtc

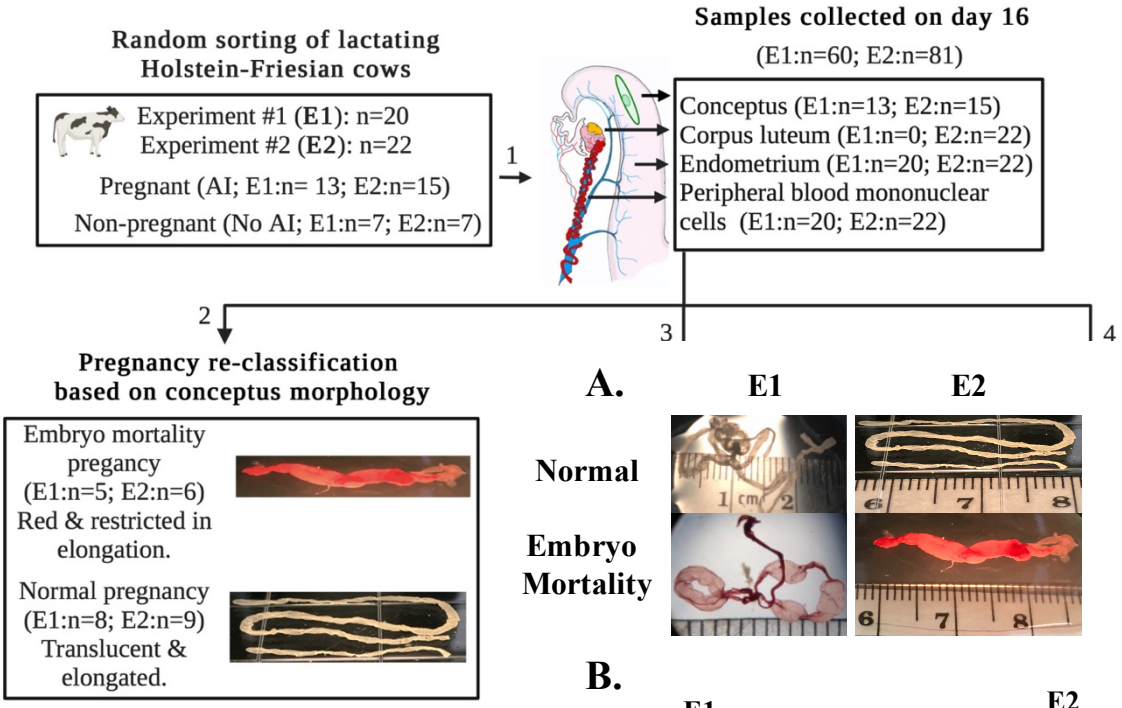
[1]F= forward; R=reverse

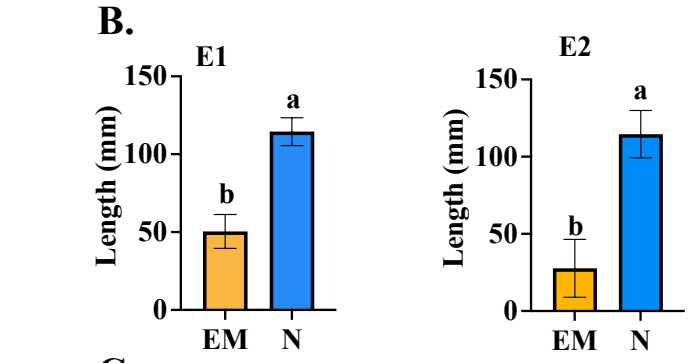
Table 2.1: Embryo Mortality vs normal endometrium and up-regulated differentially expressed (DEGs) from Ingenuity Pathway Analysis and their gene roles for experiment 2.

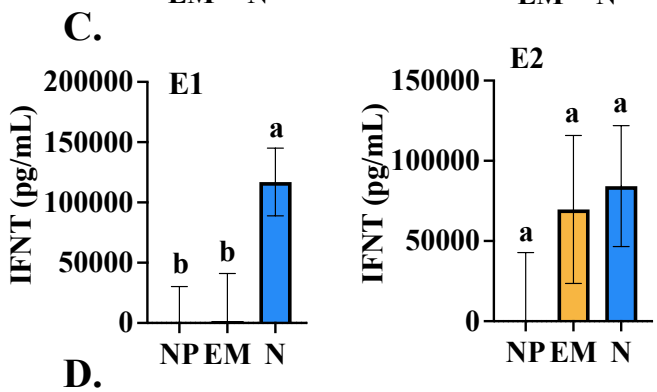
Role of DEGs	Log_2 Fold Change
DNA Damage	
ARHGAP11A	+3.08
TOP2A	+3.82
Meiosis	
ASPM	+3.92
ESPL1	+2.52
UBE2C	+2.89
DNA Repair	
HJURP	+4.18
E2F7	+2.53
Cell proliferation	
ANLN	+1.86
AURKB	+3.32
BUB1	+2.18
BUB1B	+3.23
CDC20	+3.17
DEPDC1	+3.61
CENPE	+2.27
CENPF	+3.26
KIF4A	+4.01
KIF20A	+2.58
MTFR2	+2.45
NCAPG	+3.42
TROAP	+2.72

Table 2.2: Embryo mortality vs normal corpus luteum and up-regulated differentially expressed (DEGs) from Ingenuity Pathway Analysis and their gene roles for experiment 2.

Role of DEGs	Log_2 Fold Change
Calcium Sequestration	
S100A9	+4.78
S100A12	+5.22
Estradiol-Metabolism	
CYP3A4	+4.05
Glucose-Metabolism	
SDS	+2.96
Inflammation	
CA5A	+6.64
VNN2	+2.67







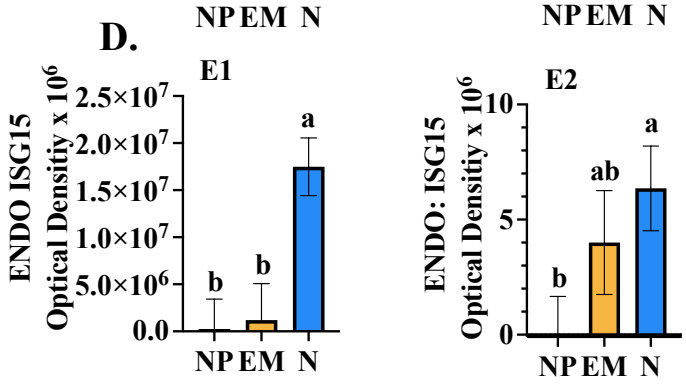


Figure 2.1: Flowchart of analytical st
collections to conducting experimenta
Conceptus, ENDO=Endometrium,
Mononuclear Cells. Created with Bio

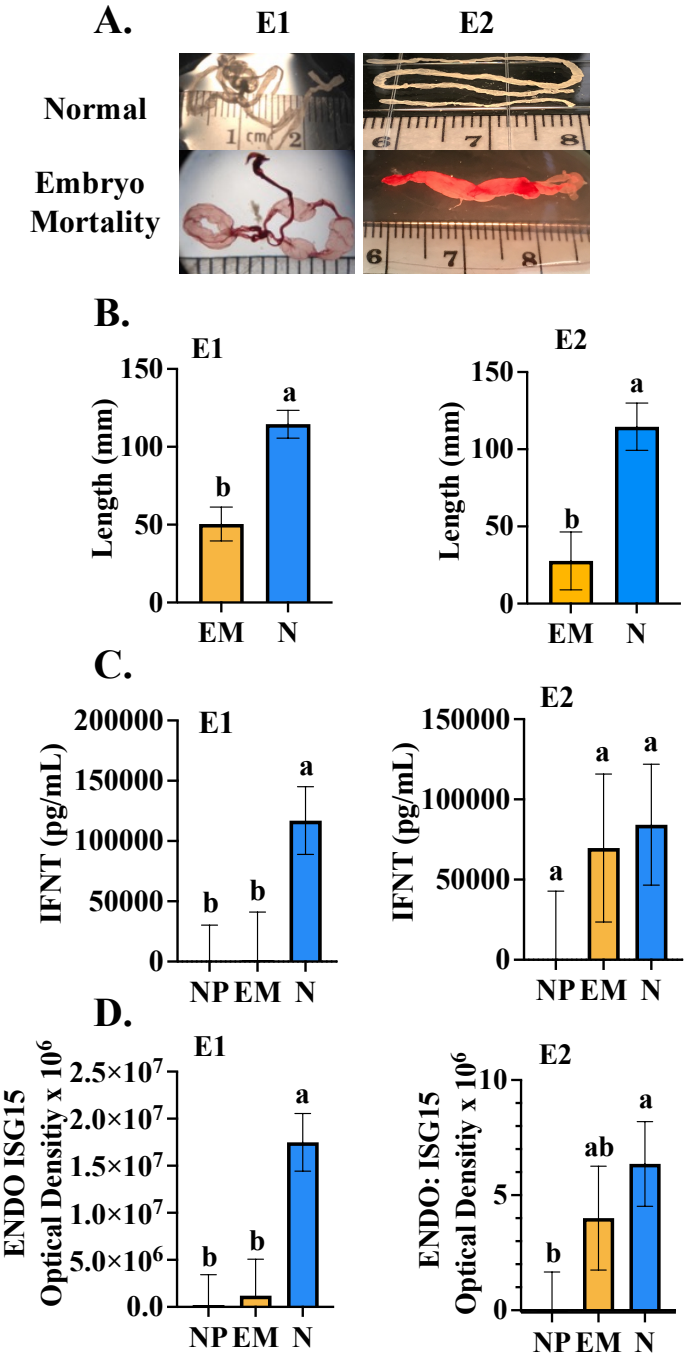


Figure 2.2: Conceptus morphology (A), length (B), IFNT concentrations for uterine flushings (C) and (D) endometrium ISG15 protein in normal embryo compared to embryo mortality.

Pregnant groups were classified based on conceptus morphology: Normal embryo (upper images) which were translucent and elongated and embryo mortality (lower images) which were pink, red, opaque and/or restricted elongation. For (C), N and EM had a separate statistic performed when compared to NP. NP=non-pregnant (yellow), EM=embryo mortality pregnancy (orange), N=normal pregnancy (blue) are the cow's pregnancy status or classification. Means marked with different superscript letters differ (p<0.05).

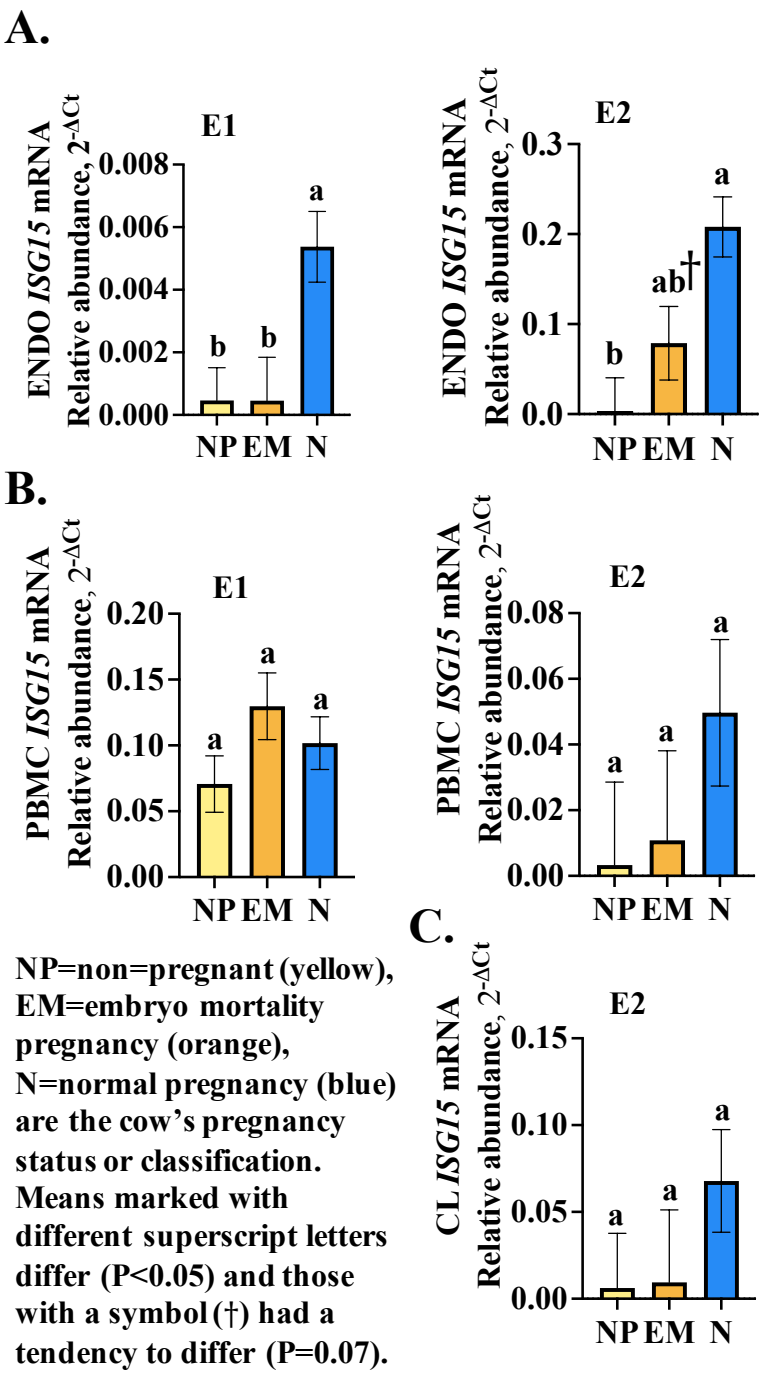


Figure 2.3: Endometrium (ENDO; A), peripheral blood mononuclear cell (PBMC; B) & corpus luteum (CL; C) ISG15 mRNA concentrations from RT-qPCR.

Means marked with different superscript letters differ ($p<0.05$).

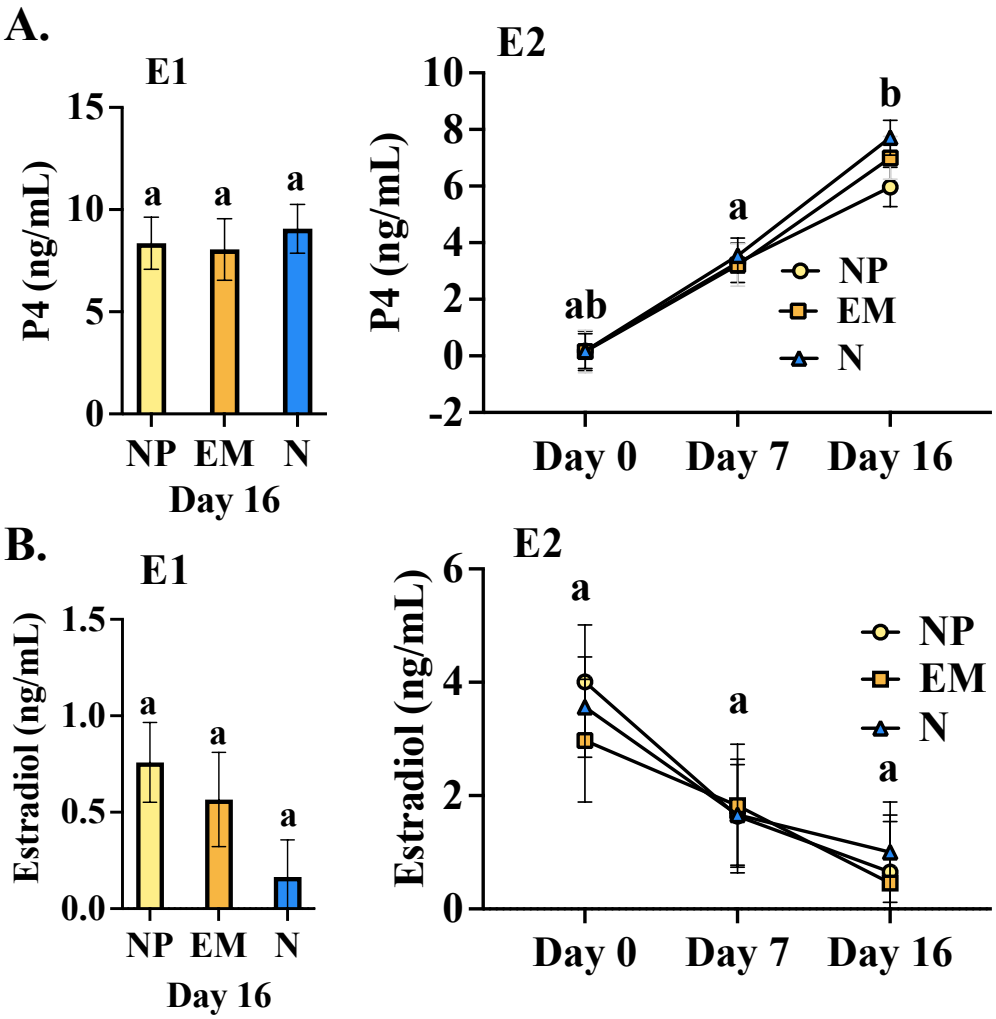


Figure 2.4: Circulating progesterone (A) and estradiol (B) concentrations in Holstein-Friesian cows.
In E1, blood samples were collected on day 16, whereas in E2, samples were collected on days 0, 7 and 16 of the estrous cycle or AI. NP=non-pregnant (yellow, circle), EM=embryo mortality pregnancy (orange; square), N=normal pregnancy (blue; triangle); are the cow's pregnancy

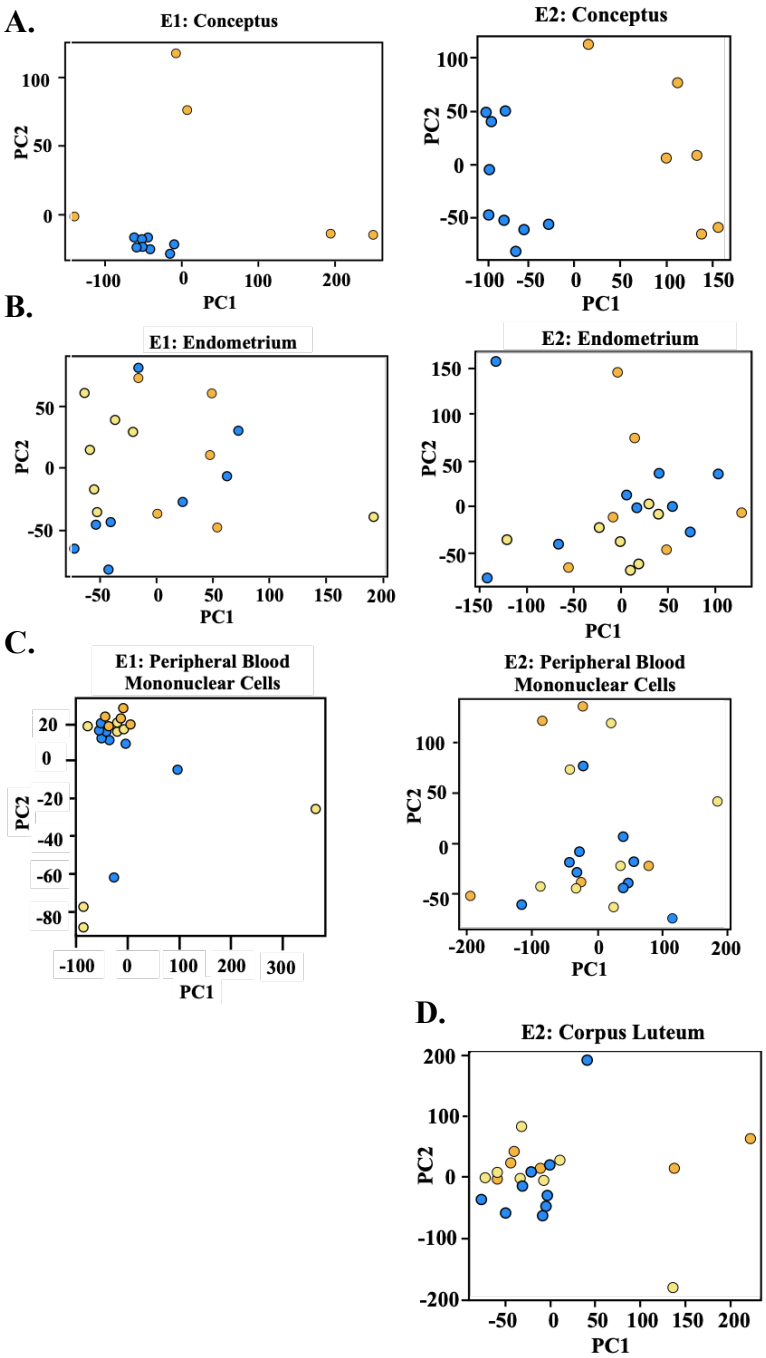


Figure 2.5: Principal Component Analysis (PCA) plot representing tissue and pregnancy state for experiments 1 (E1) and 2 (E2).
Conceptus (A), endometrium (B), peripheral blood mononuclear cells (C), Corpus Luteum (D). Pregnancy state of the cow is differentiated by colors (non-pregnant = yellow, embryo mortality pregnancy = orange and normal pregnancy = blue).

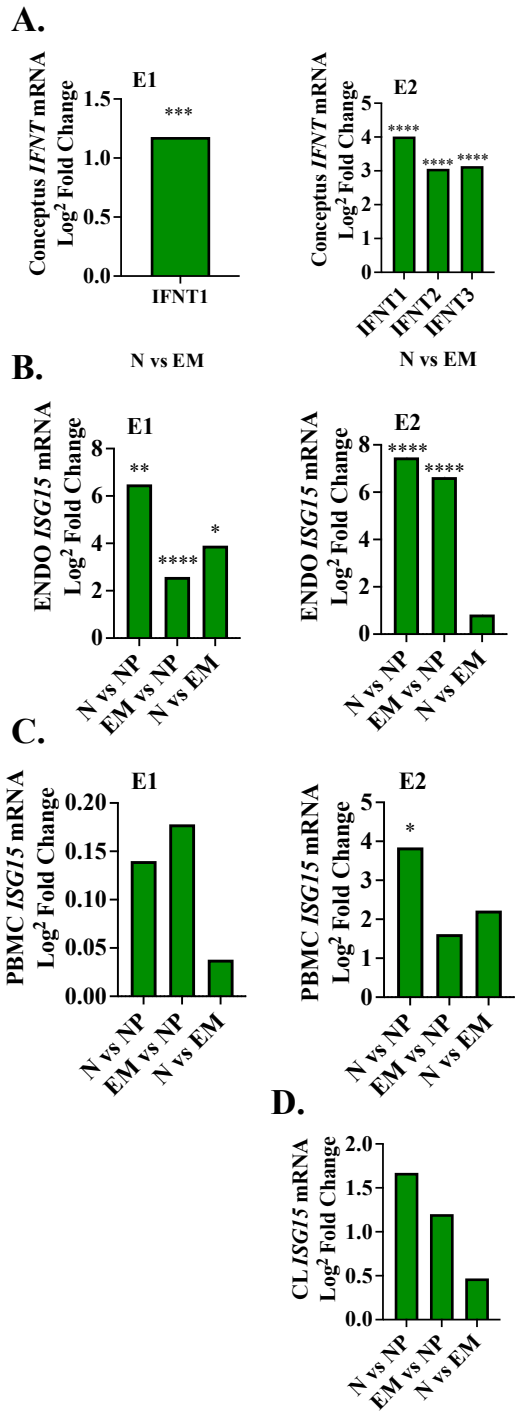


Figure 2.6: Analysis of IFNT mRNA in conceptus (A), and ISG15 mRNA in endometrium (ENDO; B), peripheral blood mononuclear cells (PBMC; C) and corpus luteum (CL; D) by using RNA-Seq in experiment 1 (E2) and 2 (E2). NP=non-pregnant EM=embryo mortality, N=normal pregnancy are the cow's pregnancy status or classification. Bars marked with different asterisk (s) differ (p<0.0001****, p<0.001, p<0.01**, p<0.05*).

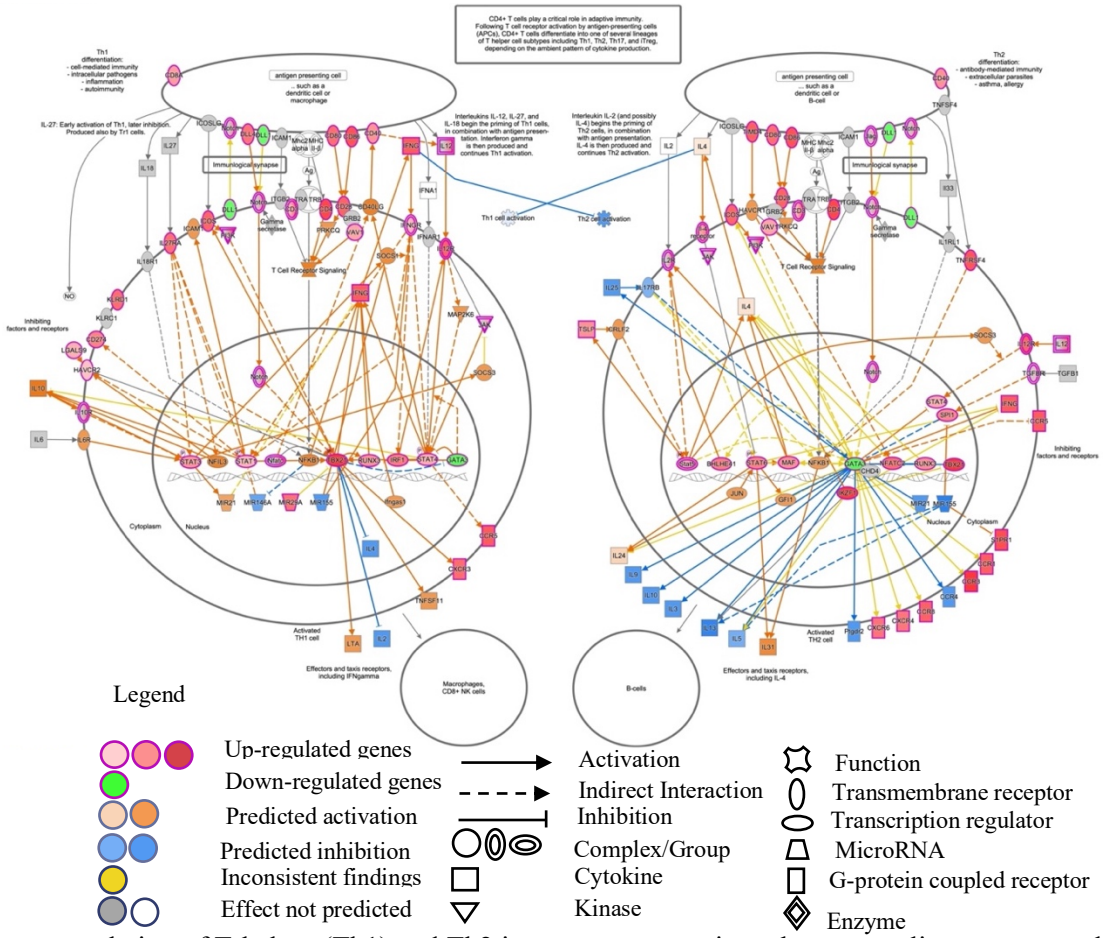


Figure 2.7: Massive up-regulation of T-helper (Th1) and Th2 immune response in embryo mortality vs to normal conceptuses. Experiment 1 is shown as representative of both experiments.Darker shades of color signify a higher extreme/confidence in the regulation/prediction of a gene.

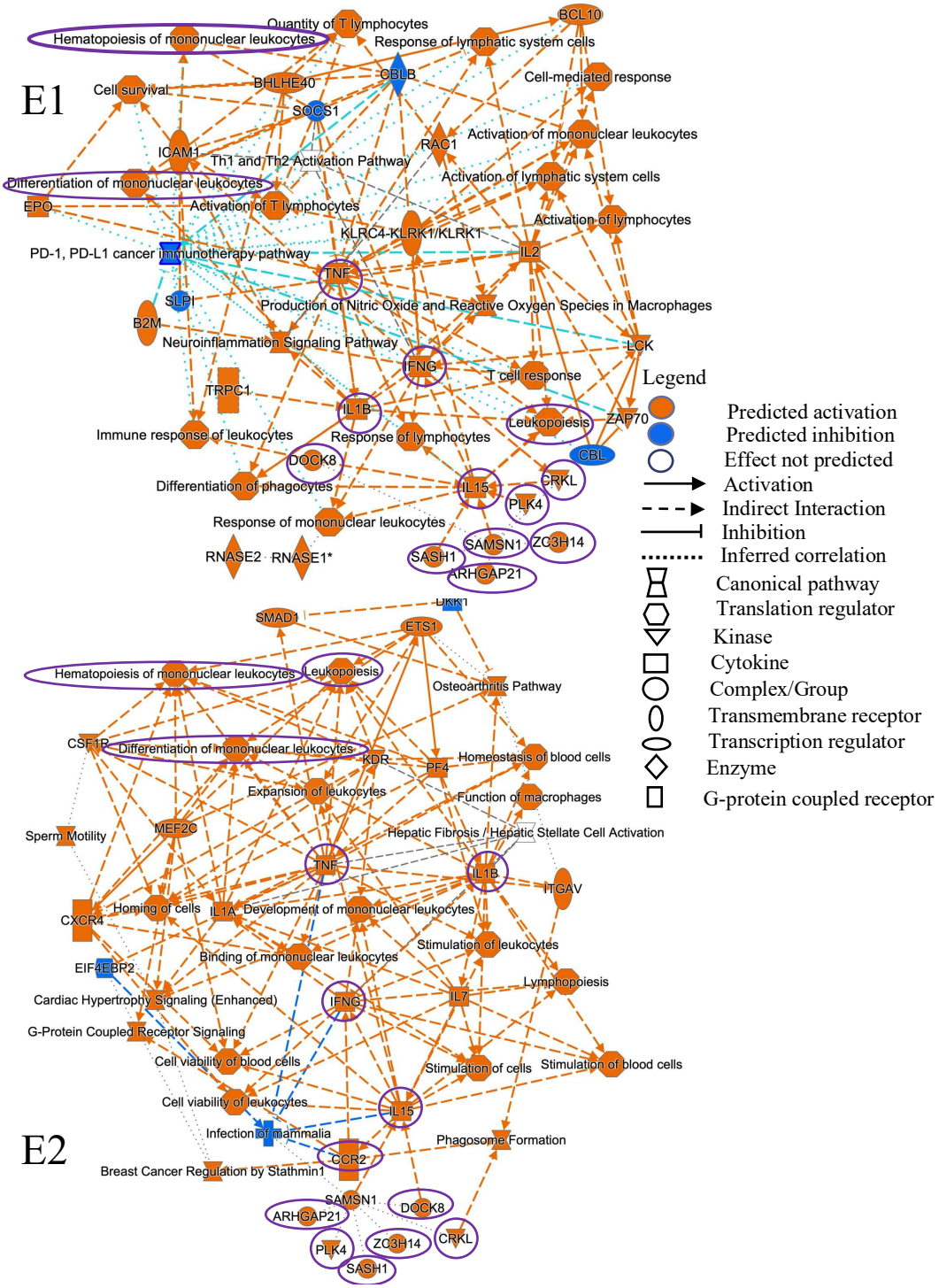


Figure 2.8: Embryo mortality vs normal Conceptus (E1: 0.585 $\log_2$-fold change; E2: 2 $\log_2$-fold change) summary from Ingenuity Pathway Analysis.

Circled in purple are shared commonalities between E1 and E2.

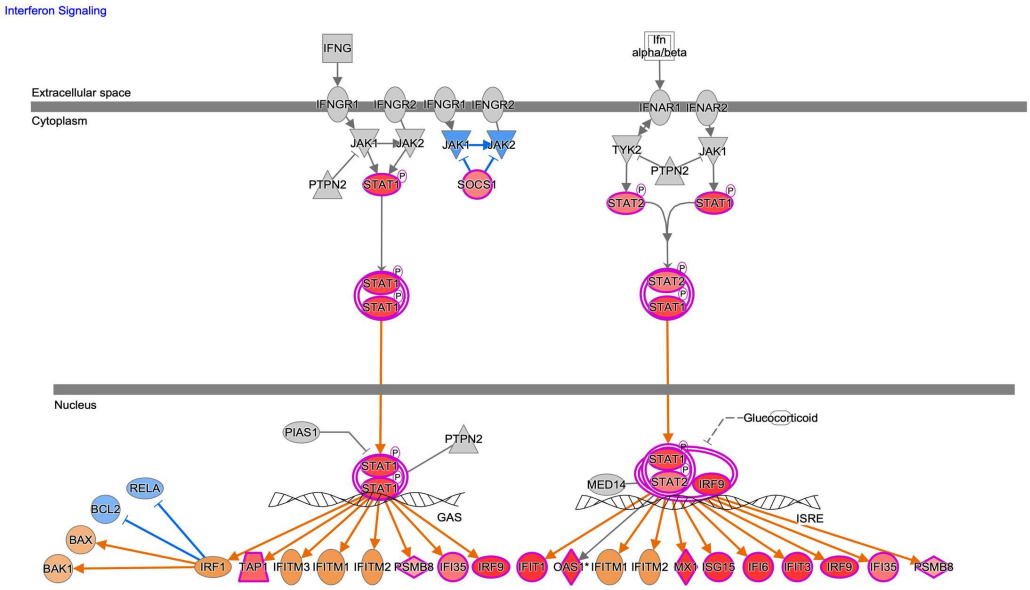


Figure 2.9: Activation of IFN signaling pathway in normal vs non-pregnant endometrium. Experiment 1 is shown as representative of both experiments. Darker shades of color signify a higher extreme/confidence in the regulation/prediction of a gene.

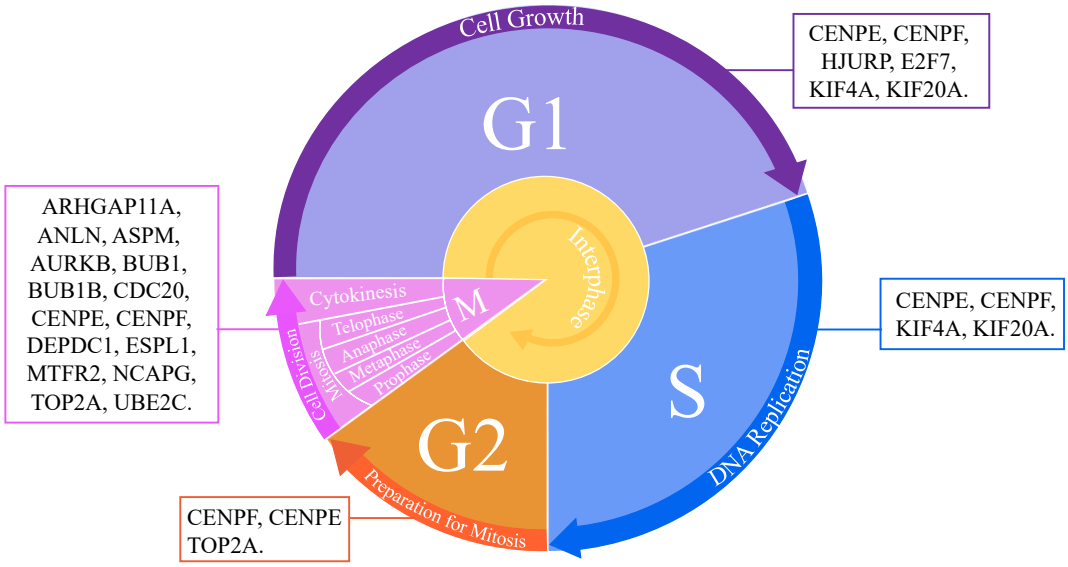


Figure 2.10: Functions of estrogen-associated proteins for embryo mortality vs normal endometrium in experiment 2.
Results were found to be related via STRING protein analysis and PubMed search of gene function.

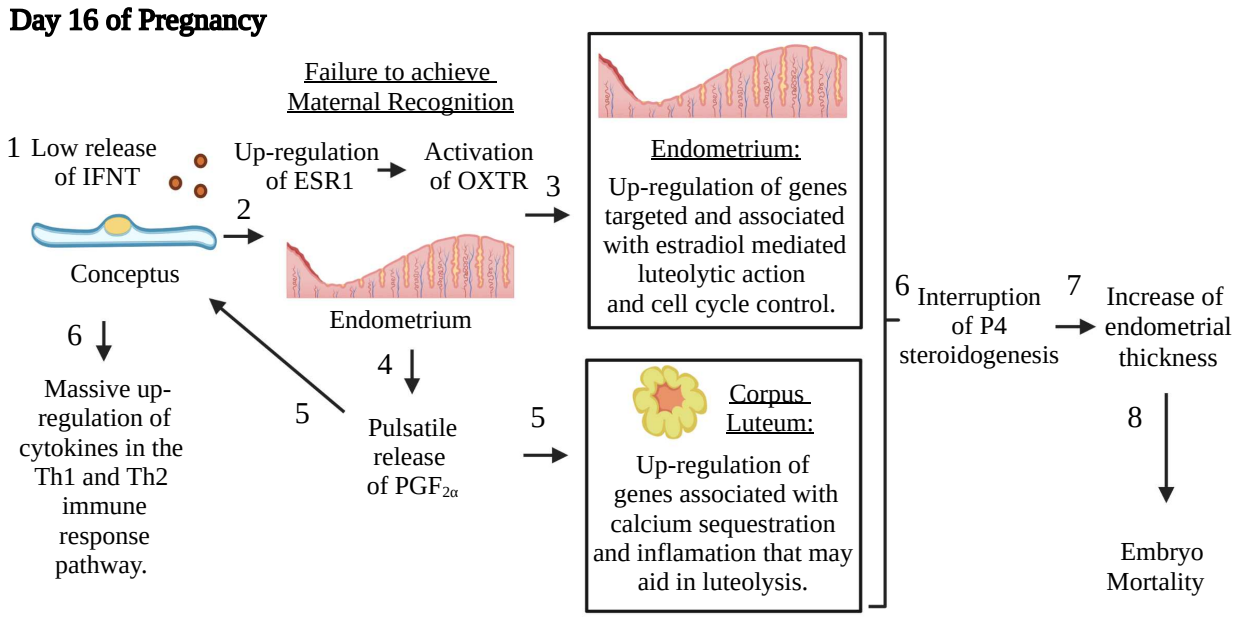


Figure 2.11: Proposed mechanism for pregnancies with embryo mortality conceptuses in lactating Holstein-Friesian cows. Created with Biorender.com.

REFERENCES

Antoniazzi, A. Q., B. T. Webb, J. J. Romero, R. L. Ashley, N. P. Smirnova, L. E. Henkes, R. C. Bott, J. F. Oliveira, G. D. Niswender, F. W. Bazer, and T. R. Hansen. 2013. Endocrine delivery of interferon tau protects the corpus luteum from prostaglandin F2 alpha-induced luteolysis in ewes. Biol Reprod 88(6):144.

Austin, K. J., A. L. Carr, J. K. Pru, C. E. Hearne, E. L. George, E. L. Belden, and T. R. Hansen. 2004. Localization of ISG15 and Conjugated Proteins in Bovine Endometrium Using Immunohistochemistry and Electron Microscopy. Endocrinology 145(2):967-975.

Austin, K. J., S. K. Ward, M. G. Teixeira, V. C. Dean, D. W. Moore, and T. R. Hansen. 1996. Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. Biol Reprod 54(3):600-606.

Barnwell, C. V., P. W. Farin, C. M. Ashwell, W. T. Farmer, S. P. Galphin, Jr., and C. E. Farin. 2016. Differences in mRNA populations of short and long bovine conceptuses on Day 15 of gestation. Mol Reprod Dev 83(5):424-441.

Bauersachs, S., S. E. Ulbrich, H. D. Reichenbach, M. Reichenbach, M. Buttner, H. H. Meyer, T. E. Spencer, M. Minten, G. Sax, G. Winter, and E. Wolf. 2012. Comparison of the effects of early pregnancy with human interferon, alpha 2 (IFNA2), on gene expression in bovine endometrium. Biol Reprod 86(2):46.

Bauersachs, S. and E. Wolf. 2012. Transcriptome analyses of bovine, porcine and equine endometrium during the pre-implantation phase. Anim Reprod Sci 134(1-2):84-94.

Bazer, F. W., T. T. Chen, J. W. Knight, D. Schlosnagle, N. J. Baldwin, and R. M. Roberts. 1975. Presence of a progesterone-induced, uterine specific, acid phosphatase in allantoic fluid of gilts. J Anim Sci 41(4):1112-1119.

Bazer, F. W., M. A. Mirando, T. L. Ott, J. P. Harney, D. H. Dubois, T. K. Schalue, C. H. Pontzer, C. Hostetler, H. M. Johnson, and T. Ogle. 1992. Roles of ovine trophoblast protein-1 and oestradiol/prolactin in the establishment of pregnancy in sheep and pigs. Reprod Fertil Dev 4(3):335-340.

Bazer, F. W., T. E. Spencer, and T. L. Ott. 1997. Interferon tau: a novel pregnancy recognition signal. Am J Reprod Immunol 37(6):412-420.

Bazer, F. W., W. W. Thatcher, P. J. Hansen, M. A. Mirando, T. L. Ott, and C. Plante. 1991. Physiological mechanisms of pregnancy recognition in ruminants. J Reprod Fertil Suppl 43:39-47.

Bazer, F. W., G. Wu, T. E. Spencer, G. A. Johnson, R. C. Burghardt, and K. Bayless. 2010. Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. Mol Hum Reprod 16(3):135-152.

Bott, R. C., R. L. Ashley, L. E. Henkes, A. Q. Antoniazzi, J. E. Bruemmer, G. D. Niswender, F. W. Bazer, T. E. Spencer, N. P. Smirnova, R. V. Anthony, and T. R. Hansen. 2010. Uterine vein infusion of interferon tau (IFNT) extends luteal life span in ewes. Biol Reprod 82(4):725-735.

Bovine HapMap, C., R. A. Gibbs, J. F. Taylor, C. P. Van Tassell, W. Barendse, K. A. Eversole, C. A. Gill, R. D. Green, D. L. Hamernik, S. M. Kappes, S. Lien, L. K. Matukumalli, J. C. McEwan, L. V. Nazareth, R. D. Schnabel, G. M. Weinstock, D. A. Wheeler, P. Ajmone-Marsan, P. J. Boettcher, A. R. Caetano, J. F. Garcia, O. Hanotte, P. Mariani, L. C. Skow, T. S. Sonstegard, J. L. Williams, B. Diallo, L. Hailemariam, M. L. Martinez, C. A. Morris, L. O. C. Silva, R. J. Spelman, W. Mulatu, K. Zhao, C. A. Abbey, M. Agaba, F. R. Araujo, R. J. Bunch, J. Burton, C. Gorni, H. Olivier, B. E. Harrison, B. Luff, M. A. Machado, J. Mwakaya, G. Plastow, W. Sim, T. Smith, M. B. Thomas, A. Valentini, P. Williams, J. Womack, J. A. Woolliams, Y. Liu, X. Qin, K. C. Worley, C. Gao, H. Jiang, S. S. Moore, Y. Ren, X.-Z. Song, C. D. Bustamante, R. D. Hernandez, D. M. Muzny, S. Patil, A. San Lucas, Q. Fu, M. P. Kent, R. Vega, A. Matukumalli, S. McWilliam, G. Sclep, K. Bryc, J. Choi, H. Gao, J. J. Grefenstette, B. Murdoch, A. Stella, R. Villa-Angulo, M. Wright, J. Aerts, O. Jann, R. Negrini, M. E. Goddard, B. J. Hayes, D. G. Bradley, M. Barbosa da Silva, L. P. L. Lau, G. E. Liu, D. J. Lynn, F. Panzitta, and K. G. Dodds. 2009. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. Science 324(5926):528-532.

Cerri, R. L., I. M. Thompson, I. H. Kim, A. D. Ealy, P. J. Hansen, C. R. Staples, J. L. Li, J. E. Santos, and W. W. Thatcher. 2012. Effects of lactation and pregnancy on gene expression of endometrium of Holstein cows at day 17 of the estrous cycle or pregnancy. J Dairy Sci 95(10):5657-5675.

Chaouat, G., N. Ledée-Bataille, K. B. Chea, and S. Dubanchet. 2005. Cytokines and implantation. Chem Immunol Allergy 88:34-63.

Chaouat, G., N. Ledée-Bataille, S. Dubanchet, S. Zourbas, O. Sandra, and J. Martal. 2004. Th1/Th2 Paradigm in Pregnancy: Paradigm Lost? International Archives of Allergy and Immunology 134(2):93-119.

Clemente, M., J. de La Fuente, T. Fair, A. Al Naib, A. Gutierrez-Adan, J. F. Roche, D. Rizos, and P. Lonergan. 2009. Progesterone and conceptus elongation in cattle: a direct effect on the embryo or an indirect effect via the endometrium? Reproduction 138(3):507-517.

Consortium, T. U. 2021. UniProt: the universal protein knowledgebase in 2021. 2021 ed, Uniprot.

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

Dickson, M. J., J. V. Bishop, T. R. Hansen, I. M. Sheldon, and J. J. Bromfield. 2022. The endometrial transcriptomic response to pregnancy is altered in cows after uterine infection. PLoS One 17(3):e0265062.

Dickson, M. J., R. L. Piersanti, R. Ramirez-Hernandez, E. B. de Oliveira, J. V. Bishop, T. R. Hansen, Z. Ma, K. C. C. Jeong, J. E. P. Santos, M. I. Sheldon, J. Block, and J. J. Bromfield. 2020. Experimentally Induced Endometritis Impairs the Developmental Capacity of Bovine Oocytes†. Biology of reproduction 103(3):508-520.

Diskin, M. G., J. J. Murphy, and J. M. Sreenan. 2006. Embryo survival in dairy cows managed under pastoral conditions. Anim Reprod Sci 96(3-4):297-311.

Dorniak, P., F. W. Bazer, and T. E. Spencer. 2013. Physiology and Endocrinology Symposium: biological role of interferon tau in endometrial function and conceptus elongation. J Anim Sci 91(4):1627-1638.

Elenkov, I. J. 2004. Glucocorticoids and the Th1/Th2 balance. Ann N Y Acad Sci 1024:138-146.

England, P. C., L. G. Skinner, K. M. Cottrell, and R. A. Sellwood. 1974. Serum oestradiol-17 beta in normal women. Br J Cancer 29(6):462-469.

Fair, T. 2015. The contribution of the maternal immune system to the establishment of pregnancy in cattle. Front Immunol 6:7.

Farin, C. E., K. Imakawa, and R. M. Roberts. 1989. In situ localization of mRNA for the interferon, ovine trophoblast protein-1, during early embryonic development of the sheep. Molecular Endocrinology 3:1099-1107.

Ferguson, C. E., D. J. Kesler, and R. A. Godke. 2012. Progesterone enhances in vitro development of bovine embryos. Theriogenology 77(1):108-114.

Forde, N., M. E. Beltman, P. Lonergan, M. Diskin, J. F. Roche, and M. A. Crowe. 2011a. Oestrous cycles in Bos taurus cattle. Anim Reprod Sci 124(3-4):163-169.

Forde, N., F. Carter, T. E. Spencer, F. W. Bazer, O. Sandra, N. Mansouri-Attia, L. A. Okumu, P. A. McGettigan, J. P. Mehta, R. McBride, P. O'Gaora, J. F. Roche, and P. Lonergan. 2011b. Conceptus-induced changes in the endometrial transcriptome: how soon does the cow know she is pregnant? Biol Reprod 85(1):144-156.

Forde, N., G. B. Duffy, P. A. McGettigan, J. A. Browne, J. P. Mehta, A. K. Kelly, N. Mansouri-Attia, O. Sandra, B. J. Loftus, M. A. Crowe, T. Fair, J. F. Roche, P. Lonergan, and A. C. Evans. 2012a. Evidence for an early endometrial response to pregnancy in cattle: both dependent upon and independent of interferon tau. Physiol Genomics 44(16):799-810.

Forde, N. and P. Lonergan. 2017. Interferon-tau and fertility in ruminants. Reproduction 154(5):F33-F43.

Forde, N., J. P. Mehta, M. Minten, M. A. Crowe, J. F. Roche, T. E. Spencer, and P. Lonergan. 2012b. Effects of low progesterone on the endometrial transcriptome in cattle. Biol Reprod 87(5):124.

Friedman, S. L. 2015. Hepatic Fibrosis: Emerging Therapies. Digestive Diseases 33(4):504-507.

García-Ruiz, A., J. B. Cole, P. M. VanRaden, G. R. Wiggans, F. J. Ruiz-López, and C. P. V. Tassell. 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Proceedings of the National Academy of Sciences 113(28):E3995-E4004.

Garrett, J. E., R. D. Geisert, M. T. Zavy, L. K. Gries, R. P. Wettemann, and D. S. Buchanan. 1988. Effect of exogenous progesterone on prostaglandin F2 alpha release and the interestrous interval in the bovine. Prostaglandins 36(1):85-96.

Gifford, C. A., K. Racicot, D. S. Clark, K. J. Austin, T. R. Hansen, M. C. Lucy, C. J. Davies, and T. L. Ott. 2007. Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. J Dairy Sci 90(1):274-280.

Godkin, J. D., F. W. Bazer, W. W. Thatcher, and R. M. Roberts. 1984. Proteins released by cultured Day 15–16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. Reproduction 71(1):57-64.

Goff, A. K. and L. C. Smith. 1998. Effect of steroid treatment of endometrial cells on blastocyst development during co-culture. Theriogenology 49(5):1021-1030.

Green, J. C., C. S. Okamura, S. E. Poock, and M. C. Lucy. 2010. Measurement of interferon-tau (IFN-tau) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18-20d after insemination in dairy cattle. Anim Reprod Sci 121(1-2):24-33.

Griffin, P. and O. J. Ginther. 1991. Dynamics of uterine diameter and endometrial morphology during the estrous cycle and early pregnancy in mares. Animal Reproduction Science 25:133-142.

Guillomot, M., C. Michel, P. Gaye, N. Charlier, J. Trojan, and J. Martal. 1990. Cellular localization of an embryonic interferon, ovine trophoblastin and its mRNA in sheep embryos during early pregnancy. Biology of the Cell 68:205-211.

Han, H., K. J. Austin, L. A. Rempel, and T. R. Hansen. 2006a. Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. J Endocrinol 191(2):505-512.

Hansen, P. J. 2011. The immunology of early pregnancy in farm animals. Reprod Domest Anim 46 Suppl 3:18-30.

Hansen, T. R., L. K. Henkes, R. L. Ashley, R. C. Bott, A. Q. Antoniazzi, and H. Han. 2010. Endocrine actions of interferon-tau in ruminants. Soc Reprod Fertil Suppl 67:325-340.

Hansen, T. R., M. Kazemi, D. H. Keisler, P.-V. Malathy, K. Imakawa, and R. M. Roberts. 1989. Complex Binding of the Embryonic Interferon, Ovine Trophoblast Protein-1, to Endometrial Receptors. Journal of Interferon Research 9(2):215-225.

Hansen, T. R. and J. K. Pru. 2014. ISGylation: a conserved pathway in mammalian pregnancy. Adv Exp Med Biol 759:13-31.

Hansen, T. R., L. D. P. Sinedino, and T. E. Spencer. 2017. Paracrine and endocrine actions of interferon tau (IFNT). Reproduction 154(5):F45-f59.

Hochberg, Y. B. a. Y. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Royal Statistical Society 57(1):289-300.

Johnson, G. A., K. J. Austin, E. A. Van Kirk, and T. R. Hansen. 1998. Pregnancy and Interferon-Tau Induce Conjugation of Bovine Ubiquitin Cross-Reactive Protein to Cytosolic Uterine Proteins1. Biology of Reproduction 58(4):898-904.

Johnson, G. A., T. E. Spencer, T. R. Hansen, K. J. Austin, R. C. Burghardt, and F. W. Bazer. 1999. Expression of the Interferon Tau Inducible Ubiquitin Cross-Reactive Protein in the Ovine Uterus1. Biology of Reproduction 61(1):312-318.

Kerbrat, S. and C. Disenhaus. 2004. A proposition for an updated behavioural characterisation of the oestrus period in dairy cows. Applied Animal Behaviour Science 87(3):223-238.

Kim, H.-S., J.-S. Koh, Y.-B. Choi, J. Ro, H.-K. Kim, M.-K. Kim, B.-H. Nam, K.-T. Kim, V. Chandra, H.-S. Seol, W.-C. Noh, E.-K. Kim, J. Park, C.-D. Bae, and K.-M. Hong. 2014. Chromatin

CKAP2, a New Proliferation Marker, as Independent Prognostic Indicator in Breast Cancer. PLOS ONE 9(6):e98160.

Klein, C., S. Bauersachs, S. E. Ulbrich, R. Einspanier, H. H. Meyer, S. E. Schmidt, H. D. Reichenbach, M. Vermehren, F. Sinowatz, H. Blum, and E. Wolf. 2006. Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. Biol Reprod 74(2):253-264.

Konduri, S. D., R. Medisetty, W. Liu, B. A. Kaipparettu, P. Srivastava, H. Brauch, P. Fritz, W. M. Swetzig, A. E. Gardner, S. A. Khan, and G. M. Das. 2010. Mechanisms of estrogen receptor antagonism toward p53 and its implications in breast cancer therapeutic response and stem cell regulation. Proc Natl Acad Sci U S A 107(34):15081-15086.

Liebig, B. E., J. V. Bishop, K. D. McSweeney, H. Van Campen, C. L. Gonzalez-Berrios, T. R. Hansen, and M. G. Thomas. 2022. Direct genomic value daughter pregnancy rate and services per conception are associated with characteristics of day-16 conceptuses and hormone signaling for maternal recognition of pregnancy in lactating Holstein cows. Applied Animal Science 38(2):157-169.

Lin, H., T. R. Mosmann, L. Guilbert, S. Tuntipopipat, and T. G. Wegmann. 1993. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J Immunol 151(9):4562-4573.

Liu, Y., A. Beyer, and R. Aebersold. 2016. On the Dependency of Cellular Protein Levels on mRNA Abundance. Cell 165(3):535-550.

Lonergan, P., T. Fair, N. Forde, and D. Rizos. 2016a. Embryo development in dairy cattle. Theriogenology 86(1):270-277.

Lonergan, P., N. Forde, and T. Spencer. 2016b. Role of progesterone in embryo development in cattle. Reprod Fertil Dev 28(1-2):66-74.

Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 81(3-4):209-223.

Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15(12):550.

Maeda, Y., H. Ohtsuka, M. Tomioka, and M. Oikawa. 2013. Effect of progesterone on Th1/Th2/Th17 and regulatory T cell-related genes in peripheral blood mononuclear cells during pregnancy in cows. Vet Res Commun 37(1):43-49.

Mamo, S., J. P. Mehta, N. Forde, P. McGettigan, and P. Lonergan. 2012. Conceptus-endometrium crosstalk during maternal recognition of pregnancy in cattle. Biol Reprod 87(1):6, 1-9.

Mamo, S., J. P. Mehta, P. McGettigan, T. Fair, T. E. Spencer, F. W. Bazer, and P. Lonergan. 2011. RNA sequencing reveals novel gene clusters in bovine conceptuses associated with maternal recognition of pregnancy and implantation. Biol Reprod 85(6):1143-1151.

Mansouri-Attia, N., L. J. Oliveira, N. Forde, A. G. Fahey, J. A. Browne, J. F. Roche, O. Sandra, P. Reinaud, P. Lonergan, and T. Fair. 2012. Pivotal Role for Monocytes/Macrophages and Dendritic Cells in Maternal Immune Response to the Developing Embryo in Cattle1. Biology of Reproduction 87(5).

Martin, C., L. Pessemesse, M. P. De La Llosa-Hermier, J. Martal, J. Djiane, and M. Charlier. 2004. Interferon-tau upregulates prolactin receptor mRNA in the ovine endometrium during the peri-implantation period. Reproduction 128(1):99-105.

Matsuyama, S., T. Kojima, S. Kato, and K. Kimura. 2012. Relationship between quantity of IFNT estimated by IFN-stimulated gene expression in peripheral blood mononuclear cells and bovine embryonic mortality after AI or ET. Reprod Biol Endocrinol 10:21.

McCracken, J. A., E. E. Custer, and J. C. Lamsa. 1999. Luteolysis: a neuroendocrine-mediated event. Physiol Rev 79(2):263-323.

McCracken, J. A., M. E. Glew, and R. J. Scaramuzzi. 1970. Corpus luteum regression induced by prostagland in F2-alpha. J Clin Endocrinol Metab 30(4):544-546.

Meadows, C., P. J. Rajala-Schultz, and G. S. Frazer. 2005. A spreadsheet-based model demonstrating the nonuniform economic effects of varying reproductive performance in Ohio dairy herds. J Dairy Sci 88(3):1244-1254.

Merlo, B., E. Iacono, and G. Mari. 2007. EFFECT OF PROGESTERONE AND EPIDERMAL GROWTH FACTOR ON IN VITRO-PRODUCED EIGHT-CELL BOVINE EMBRYOS IN SERUM-FREE CULTURE MEDIUM. Reproduction, Fertility and Development 19:0-0.

Meyerholz, M. M., K. Mense, H. Knaack, O. Sandra, and M. Schmicke. 2016. Pregnancy-Induced ISG-15 and MX-1 Gene Expression is Detected in the Liver of Holstein-Friesian Heifers During Late Peri-Implantation Period. Reprod Domest Anim 51(1):175-177.

Monteiro, P. L. J., E. S. Ribeiro, R. P. Maciel, A. L. G. Dias, E. Solé, F. S. Lima, R. S. Bisinotto, W. W. Thatcher, R. Sartori, and J. E. P. Santos. 2014. Effects of supplemental progesterone after artificial insemination on expression of interferon-stimulated genes and fertility in dairy cows. Journal of Dairy Science 97(8):4907-4921.

Moraes, J. G. N., S. K. Behura, T. W. Geary, P. J. Hansen, H. L. Neibergs, and T. E. Spencer. 2018. Uterine influences on conceptus development in fertility-classified animals. Proc Natl Acad Sci U S A 115(8):E1749-e1758.

Moridi, M., S. H. Hosseini Moghaddam, S. Z. Mirhoseini, and M. Bionaz. 2019. Transcriptome analysis showed differences of two purebred cattle and their crossbreds. Italian Journal of Animal Science 18(1):70-79.

National Academies of Sciences, E. and Medicine. 2021. Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. The National Academies Press, Washington, DC.

Niswender, G. D. 1973. Influence of the site of conjugation on the specificity of antibodies to progesterone. Steroids 22(3):413-424.

Niswender, G. D., T. L. Davis, R. J. Griffith, R. L. Bogan, K. Monser, R. C. Bott, J. E. Bruemmer, and T. M. Nett. 2007. Judge, jury and executioner: the auto-regulation of luteal function. Soc Reprod Fertil Suppl 64:191-206.

Oliveira, J. F., L. E. Henkes, R. L. Ashley, S. H. Purcell, N. P. Smirnova, D. N. Veeramachaneni, R. V. Anthony, and T. R. Hansen. 2008. Expression of interferon (IFN)-stimulated genes in

extrauterine tissues during early pregnancy in sheep is the consequence of endocrine IFN-tau release from the uterine vein. Endocrinology 149(3):1252-1259.

Oliveira, L. J., N. Mansouri-Attia, A. G. Fahey, J. Browne, N. Forde, J. F. Roche, P. Lonergan, and T. Fair. 2013. Characterization of the Th profile of the bovine endometrium during the oestrous cycle and early pregnancy. PLoS One 8(10):e75571.

Ott, T. L. 2020. Immunological detection of pregnancy: Evidence for systemic immune modulation during early pregnancy in ruminants. Theriogenology 150:498-503.

P.M. VanRaden, A. H. S., M.E. Tooker, R.H. Miller and H.D. Norman. 2003. Daughter pregnancy rate evaluation of cow fertility. USDA Animal Improvement Programs Laboratory, Beltsville, MD.

Pedraza-Alva, G., J. M. Zingg, A. Donda, and L. Pérez-Martínez. 2009. Estrogen receptor regulates MyoD gene expression by preventing AP-1-mediated repression. Biochem Biophys Res Commun 389(2):360-365.

Perkel, K. J., A. K. Tscherner, C. Merrill, J. LaMarre, and P. Madan. 2015. The ART of selecting the best embryo: A review of early embryonic mortality and bovine embryo viability assessment methods. Molecular Reproduction and Development 82.

Piccinni, B., S. Massari, A. Caputi Jambrenghi, F. Giannico, M. P. Lefranc, S. Ciccarese, and R. Antonacci. 2015. Sheep (Ovis aries) T cell receptor alpha (TRA) and delta (TRD) genes and genomic organization of the TRA/TRD locus. BMC Genomics 16:709.

Pierson, R. A. and O. J. Ginther. 1987. Ultrasonographic appearance of the bovine uterus during the estrous cycle. J Am Vet Med Assoc 190(8):995-1001.

Raghupathy, R. 1997. Th1-type immunity is incompatible with successful pregnancy. Immunol Today 18(10):478-482.

Ribeiro, E., K. Galvão, W. Thatcher, and J. Santos. 2012. Economic aspects of applying reproductive technologies to dairy herds. Animal reproduction 9:370-387.

Ribeiro, E. S. 2018. Symposium review: Lipids as regulators of conceptus development: Implications for metabolic regulation of reproduction in dairy cattle. J Dairy Sci 101(4):3630-3641.

Ribeiro, E. S., R. G. Bruno, A. M. Farias, J. A. Hernandez-Rivera, G. C. Gomes, R. Surjus, L. F. Becker, A. Birt, T. L. Ott, J. R. Branen, R. G. Sasser, D. H. Keisler, W. W. Thatcher, T. R. Bilby, and J. E. Santos. 2014. Low doses of bovine somatotropin enhance conceptus development and fertility in lactating dairy cows. Biol Reprod 90(1):10.

Rizos, D., S. Scully, A. K. Kelly, A. D. Ealy, R. Moros, P. Duffy, A. Al Naib, N. Forde, and P. Lonergan. 2012. Effects of human chorionic gonadotrophin administration on day 5 after oestrus on corpus luteum characteristics, circulating progesterone and conceptus elongation in cattle. Reprod Fertil Dev 24(3):472-481.

Roberts, R. M. 2007. Interferon-tau, a Type 1 interferon involved in maternal recognition of pregnancy. Cytokine Growth Factor Rev 18(5-6):403-408.

Rocha, C. C., S. C. da Silva Andrade, G. D. de Melo, I. G. Motta, L. L. Coutinho, A. M. Gonella-Diaza, M. Binelli, and G. Pugliesi. 2020. Early pregnancy-induced transcripts in peripheral blood immune cells in Bos indicus heifers. Scientific Reports 10(1):13733.

Romero, J. J., A. Q. Antoniazzi, T. M. Nett, R. L. Ashley, B. T. Webb, N. P. Smirnova, R. C. Bott, J. E. Bruemmer, F. W. Bazer, R. V. Anthony, and T. R. Hansen. 2015. Temporal Release, Paracrine and Endocrine Actions of Ovine Conceptus-Derived Interferon-Tau During Early Pregnancy. Biol Reprod 93(6):146.

Romero, J. J., A. Q. Antoniazzi, N. P. Smirnova, B. T. Webb, F. Yu, J. S. Davis, and T. R. Hansen. 2013. Pregnancy-associated genes contribute to antiluteolytic mechanisms in ovine corpus luteum. Physiol Genomics 45(22):1095-1108.

Sandler, N. G., M. M. Mentink-Kane, A. W. Cheever, and T. A. Wynn. 2003. Global gene expression profiles during acute pathogen-induced pulmonary inflammation reveal divergent roles for Th1 and Th2 responses in tissue repair. J Immunol 171(7):3655-3667.

Sanger, P. L. 2012. Pathways to Pregnancy and Parturition. 3 ed. Current Conceptions, Redmon, OR.

Shah, V. S. S. a. R. G. 2009. Bovine In vitro Embryo Production: An Overview. Veterinary World 2(12):478-479.

Shaw, A. E., J. Hughes, Q. Gu, A. Behdenna, J. B. Singer, T. Dennis, R. J. Orton, M. Varela, R. J. Gifford, S. J. Wilson, and M. Palmarini. 2017. Fundamental properties of the mammalian innate immune system revealed by multispecies comparison of type I interferon responses. PLoS Biol 15(12):e2004086.

Shirasuna, K., H. Matsumoto, E. Kobayashi, A. Nitta, S. Haneda, M. Matsui, C. Kawashima, K. Kida, T. Shimizu, and A. Miyamoto. 2012. Upregulation of interferon-stimulated genes and interleukin-10 in peripheral blood immune cells during early pregnancy in dairy cows. J Reprod Dev 58(1):84-90.

Shirasuna, K., H. Matsumoto, S. Matsuyama, K. Kimura, H. Bollwein, and A. Miyamoto. 2015. Possible role of interferon tau on the bovine corpus luteum and neutrophils during the early pregnancy. Reproduction 150(3):217-225.

Sinedino, L. D., P. M. Honda, L. R. Souza, A. L. Lock, M. P. Boland, C. R. Staples, W. W. Thatcher, and J. E. Santos. 2017. Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. Reproduction 153(5):707-723.

Skopets, B., J. Li, W. W. Thatcher, R. M. Roberts, and P. J. Hansen. 1992. Inhibition of lymphocyte proliferation by bovine trophoblast protein-1 (type I trophoblast interferon) and bovine interferon-alpha I1. Vet Immunol Immunopathol 34(1-2):81-96.

Spencer, T. E. and F. W. Bazer. 1996. Ovine interferon tau suppresses transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. Endocrinology 137(3):1144-1147.

Spencer, T. E., N. Forde, and P. Lonergan. 2016. The role of progesterone and conceptus-derived factors in uterine biology during early pregnancy in ruminants. J Dairy Sci 99(7):5941-5950.

Spencer, T. E. and T. R. Hansen. 2015. Implantation and Establishment of Pregnancy in Ruminants. Adv Anat Embryol Cell Biol 216:105-135.

Spencer, T. E., T. L. Ott, and F. W. Bazer. 1996. tau-Interferon: pregnancy recognition signal in ruminants. Proc Soc Exp Biol Med 213(3):215-229.

Spencer, T. E., O. Sandra, and E. Wolf. 2008. Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. Reproduction 135(2):165-179.

Stark, G. R., I. M. Kerr, B. R. Williams, R. H. Silverman, and R. D. Schreiber. 1998. How cells respond to interferons. Annu Rev Biochem 67:227-264.

Stephenson, R. O., Y. Yamanaka, and J. Rossant. 2010. Disorganized epithelial polarity and excess trophectoderm cell fate in preimplantation embryos lacking E-cadherin. Development 137(20):3383-3391.

Sugiura, T., S. Akiyoshi, F. Inoue, Y. Yanagawa, M. Moriyoshi, M. Tajima, and S. Katagiri. 2018. Relationship between bovine endometrial thickness and plasma progesterone and estradiol concentrations in natural and induced estrus. The Journal of reproduction and development 64(2):135-143.

Syedbasha, M. and A. Egli. 2017. Interferon Lambda: Modulating Immunity in Infectious Diseases. Frontiers in Immunology 8.

Sykes, D. J., J. S. Couvillion, A. Cromiak, S. Bowers, E. Schenck, M. Crenshaw, and P. L. Ryan. 2012. The use of digital infrared thermal imaging to detect estrus in gilts. Theriogenology 78(1):147-152.

Talukder, A. K., M. S. Yousef, M. B. Rashid, K. Awai, T. J. Acosta, T. Shimizu, K. Okuda, M. Shimada, K. Imakawa, and A. Miyamoto. 2017. Bovine embryo induces an anti-inflammatory response in uterine epithelial cells and immune cells in vitro: possible involvement of interferon tau as an intermediator. J Reprod Dev 63(4):425-434.

Thatcher, W. W., P. J. Hansen, T. S. Gross, S. D. Helmer, C. Plante, and F. W. Bazer. 1989. Antiluteolytic effects of bovine trophoblast protein-1. J Reprod Fertil Suppl 37:91-99.

Van Eerdenburg, F. J. C. M., H. S. H. Loeffler, and J. H. van Vliet. 1996. Detection of oestrus in dairy cows: A new approach to an old problem. Veterinary Quarterly 18(2):52-54.

van Leeuwen, J. J., M. R. Martens, J. Jourquin, M. A. Driancourt, A. Wagner, B. Kemp, and N. M. Soede. 2015. Follicle size and reproductive hormone profiles during a post-weaning altrenogest treatment in primiparous sows. Reprod Fertil Dev 27(2):304-312.

Walsh, S. W., E. J. Williams, and A. C. O. Evans. 2011. A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science 123(3):127-138.

Wegmann, T. G., H. Lin, L. Guilbert, and T. R. Mosmann. 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 14(7):353-356.

Wiltbank, M. C., G. M. Baez, A. Garcia-Guerra, M. Z. Toledo, P. L. Monteiro, L. F. Melo, J. C. Ochoa, J. E. Santos, and R. Sartori. 2016. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. Theriogenology 86(1):239-253.

Wynn, T. A. 2008. Cellular and molecular mechanisms of fibrosis. J Pathol 214(2):199-210.

Yang, L., X. L. Wang, P. C. Wan, L. Y. Zhang, Y. Wu, D. W. Tang, and S. M. Zeng. 2010. Up-regulation of expression of interferon-stimulated gene 15 in the bovine corpus luteum during early pregnancy. J Dairy Sci 93(3):1000-1011.

Yang, L., X. Yao, S. Li, K. Chen, Y. Wang, L. Chen, and L. Zhang. 2016. Expression of genes associated with luteolysis in peripheral blood mononuclear cells during early pregnancy in cattle. Mol Reprod Dev 83(6):509-515.

Yang, L. M., L. Y. Zhang, H. Qiao, N. Liu, Y. X. Wang, and S. J. Li. 2014. Maternal Immune Regulation by Conceptus During Early Pregnancy in the Bovine. Asian Journal of Animal and Veterinary Advances 9:610-620.

Yankey, S. J., B. A. Hicks, K. G. Carnahan, A. M. Assiri, S. J. Sinor, K. Kodali, J. N. Stellflug, and T. L. Ott. 2001. Expression of the antiviral protein Mx in peripheral blood mononuclear cells of pregnant and bred, non-pregnant ewes. J Endocrinol 170(2):R7-11.

Zhang, L. Y., Y. Xia, F. Tang, S. J. Li, L. G. Yang, and B. Wang. 2015. The regulation of intrauterine immune cytokines and chemokines during early pregnancy in the bovine. Large Animal Review 21:23-31.

CHAPTER 3: IDENTIFICATION OF CANDIDATE SNPS ASSOCIATED WITH EMBRYO MORTALITY AND FERTILITY TRAITS OF LACTATING HOLSTEIN-FRIESIAN COWS[2]

Summary

For over 50 years, intensive genetic selection for milk yield was responsible for the steep decline in fertility trait levels in Holstein-Friesian cows. More recently, the identification of single nucleotide polymorphisms (SNP) have been used to improve genomic estimates of predicted transmitting abilities due to the fertility traits being of low heritability. The objective of this study was to identify and validate SNPs that were associated with fertility traits and cows with failing pregnancies because of early embryo mortality. The RNA sequencing of conceptuses (normal and embryo mortality) in Holstein-Friesian (n=15) cows from a previous study were used to conduct the SNP discovery phase. Selection for the specific SNPs within genes were first based on the following steps: (1) Related or associated to reproductive/pregnancy/fertility traits within previous studies, (2) Differentially expressed genes within previous RNA Seq transcriptome data that had $\log_2$ fold change significant (adjusted $p<0.05$), (3) Sorting identified SNPs as diagnostics or non-diagnostic, (4) Evaluating in which region and type of differentially expressed SNP, (5) Asses the prediction value of the sorting intolerant from tolerant tool (**SIFT**) analyses when an amino acid substitution occurred and if it could affect protein function , (6) Confirm if the loci of the SNP was in proximity to other associated SNP associated to reproductive/fertility traits in Cattle Quantitative Trait Loci (**Cattle QTL**) database. Validation of candidate SNPs and genotype to

[2] Written and formatted with the intention to be submitted to the Journal of Dairy Science in fall of 2022.

phenotype analysis were conducted in a different cohort of Holstein-Friesian cows (n=500) by collecting blood samples to be genotyped via a genotyping assay panel and collecting cow farms records. Further filtering of candidate SNPs involved removing those that were monomorphic and not in minor allele frequency and a quality control pipeline via pLink software. Reproductive and production traits were also removed from the study due to missing values. Continuous numeric trait models were analyzed using GLM-one way ANOVA in SAS. While binary models were evaluated using logistic regression in SAS. Statistically significant models were further analyzes using a means separation tests within LSMEANS from the mixed procedure and included the Bonferroni adjustment for p-values to minimize false discovery error. A total of sixty-nine candidate SNPs were initially discovered but only twenty-three passed the quality control pipeline in pLink software. Also, the n of observations used for the statistical models of the study were n=466 after having removed traits and animals due to missing values. All candidate SNPs were found explain a higher amount of the R^2 variation of each of the models and were in close proximity to SNP that were associated with quantitative trait loci of fertility traits. Out of the twenty-three candidate SNPs, seven (DSC2 [rs211151260]: age of cows at 1st calving were older with A allele; SREBF1 [rs41912290] and UBD [rs209518868]: cows took longer to conceive with T or G allele, respectively; UMPS [rs110953962] and SREBF1 [rs41912290]: required longer time to their 1st artificial insemination with C allele; DECR1 [rs41580472] and FASN [rs41919985]: cows were less likely to become pregnant at 1st artificial insemination with C allele; SREBF1 [rs41912290] and BOLA-DMB [rs109032590]: cows were less likely to become pregnant at 150 days in milk with T allele) were significantly associated to fertility traits. It was also found that two candidate SNPs (DSC2 gene [rs109278906: 4 SNPs and rs211151260: 2 SNPs] were considered as TAG SNPs. Only two of the seven candidate SNPs had significant allele substitution

effects where DSC2 [rs211151260] in cows with G allele decreased in the age at 1st calving by 10 days and SREBF1 [rs41912290] in cows with the C allele decreased days to 1st artificial insemination by 5 days and the probability of becoming pregnant at 150 days in milk by 6%. Thus, this study's candidate SNPs could be used to aid farmers in making decisions of culling reproductively inefficient heifers and cows within a herd.

Introduction

Until early 2005, Holstein-Friesian cows had a continuous decline in fertility trait levels that was attributed to the intensive genetic selection for milk yield (García-Ruiz et al., 2016b). An example of a declined fertility trait is conception rate, which has been reported at a level of 34% in primiparous Holstein-Friesian cows and plummets to 26% by their fifth breeding (Pryce et al., 2004, VanRaden et al., 2004, Kuhn et al., 2006). Moreover, the heritability of cow and heifer conception rates are also low (0.001 to 0.016) when compared to milk yield traits (~0.30) (Van Tassell et al., 1999).This was to be expected when selecting for fertility and production traits given their negative correlation estimated to range from 0.35 to 0.60 (Boichard and Manfredi, 1994, VanRaden et al., 2004, Pritchard et al., 2013). Current available technologies such as the genetic merit prediction tools have attempted to aid the dairy industry in selecting for traits such as conception rate that have low heritability (García-Ruiz et al., 2016b). Despite this, both Holstein-Friesian heifers (35-40%) and lactating cows (55-60%) are still considered suboptimal in their conception rates (Lonergan et al., 2016a).

Another key contributor that causes 43% of all reproductive failures in Holstein-Friesian cows is embryo mortality (**EM**) (Diskin et al., 2006). Although the occurrence of pregnancies with

EM are associated with multiple factors, a study hypothesized that it may be due to the amount of interferon tau (**IFNT**) secreted by the trophectoderm of the conceptus (Evans et al., 2012). IFNT's main role is to establish and maintain pregnancy by preventing luteolysis of the corpus luteum (**CL**) (Rizos et al., 2012, Hansen et al., 2017, Moraes et al., 2018). IFNT protects the CL via paracrine actions on the endometrial luminal epithelium that inhibit estrogen receptor 1 upregulation from activating the oxytocin pathway via its receptors and thus, attenuating the release of prostaglandin $F_{2\alpha}$ that cause luteolysis (Spencer et al., 1996, McCracken et al., 1999). Furthermore, production of progesterone from the CL can continue which is essential for supporting conceptus growth and maintaining a competent uterine environment for the implanting conceptus (Bazer et al., 1975, Bazer et al., 1997). The problem with pregnancies with EM, is that they take place during the pre-implantation period, on days 7 to 16 of pregnancy, and it remains undetected until pregnancy status can be determined at day 32 via ultrasound (Lonergan and Forde, 2014). This then hinders resynchronizing a non-pregnant cow in a timely manner, limits management of a cow's reproductive performance and therefore, generates an dairy industry annual loss of $1.6 billion in the United States of America and $1.28 trillion worldwide (Shah, 2009, Perkel et al., 2015). Thus, providing rationale for selection of cows with superior fertility traits rather than milk yield and other production traits has only occurred in recent years.

Other types of technological advancements occurred when scientists shifted from traditional quantitative techniques to also include molecular genetics in animal breeding that resulted in genotyping for single nucleotide polymorphisms (**SNPs**) (Barillet, 2007). Hence, the identification of SNPs has allowed room for studies to corroborate their association to specific traits such as yield and reproduction in cattle (Amos et al., 2011, Khatkar et al., 2014). Such studies of SNP associations to traits were also essential for revolutionizing animal breeding programs

since they are responsible for 84% of the variation in gene expression (Spencer Thomas, 2014). A SNP's location relative to coding and regulatory regions of genes that could then affect protein structure, production, function and (or) become a phenotype (Ortega, 2018). Previous studies have also shown that variability in gene expression in reproductive tissues or cells could be due to the reproductive status of an animal (El-Sayed et al., 2006, Beltman et al., 2010, Kommadath et al., 2011). Thus, suggesting the potential use candidate SNPs associated to reproductive traits in genomic assay panels as a way to improve genomic estimates of predicted transmitting abilities due to the traits low heritability (Cochran et al., 2013). A common strategy performed to validate and ascertain candidate SNP's associations found in a particular cattle population are to genotype a different cohort of animals. According to Pryce et al. (Pryce et al., 2010), the probability of candidate SNPs being a false positive in another population are extremely low. Consequently, the use of SNP genotyping could further aid in the culling of cattle that are reproductively inferior (Singh et al., 2014). Our study hypothesized that pregnancies with EM are associated with intragenic SNPs that impair maternal-conceptus communication. Thus, we aimed to validate 69 candidate SNPs that were identified from pregnancies with conceptuses that were either normal (**N**) or EM from an initial fifteen purebred lactating multiparous Holstein-Friesian cows and then validated in a different cohort population of lactating primiparous Holstein-Friesian cows (n=500). We then propose that in order for the cattle industry to select for reproductively superior cows they might cull cows that are genotyped with the identified candidate SNPs that are associated to pregnancies with EM.

Materials and Methods

Figure 3.1 is a flow chart of the methodology from the initial purebred lactating multiparous Holstein-Friesian cow population (n=15) in which conceptus samples were collected for the identification of genes with potential SNPs (i.e., discovery) that may influence pregnancies to become EM. These potential SNPs were then validated in a different cohort of purebred lactating primiparous Holstein-Friesian cows (n=500) in Colorado, USA. Details on collection of conceptuses (N and EM), processing for RNA Sequencing (**RNA-Seq**) and analysis of raw sequence reads in RNA-Seq data were described in Gonzalez-Berrios et al. 2022.

Animal Care

An initial group of fifteen healthy purebred lactating multiparous lactating Holstein-Friesian from a previous study (Gonzalez-Berrios et al., 2022; protocol number 17-7539A) were located in Blue Sky Dairy, Meade, Colorado, USA to generate transcriptome data and identification of genes associated with fertility status. An additional five-hundred healthy purebred lactating primiparous Holstein-Friesian from the Aurora Organic Dairy herd at Gill, Colorado, USA had blood samples collected for DNA analysis as per approval of Colorado State University's Institutional Animal Care and Use Committee (protocol number 1036).

Holstein-Friesian Cows and Blood collections, Enrollment and Collection of Cow Records

Purebred lactating primiparous Holstein-Friesian cows (n=500) were randomly enrolled into study. Data of interest were divided into three categories. The first being the category of health traits which were separated into two sub-categories: reproductive disease affecting the reproductive system (mastitis, metritis, endometriosis and pyometra) or non-reproductive disease not affecting the reproductive system (lameness, respiratory, digestive and ketosis). All health

traits (non- or reproductive-diseases) were recorded for the date of incidence of a disease, the number of incidences that it occurred and if it occurred at or before 60 days of milk (**DIM**). Both non- or reproductive-diseases sub-categories were recorded to have either the number one for presence of disease or zero for disease not being present at 60 DIM. The second category was composed of reproductive traits after 1st calving which included: breeding and calving date of cow (warm [w] season from: June to September or cool [c] season from: October to May), sire ID used for artificial insemination (**AI**), AI tech ID, number of AI(s), pregnancy outcome (**p**: pregnant or **o**: non-pregnant [open]) and pregnancy loss (0: no pregnancy loss or 1: pregnancy loss; when explicitly recorded as an abortion after conception) up to 4 AI. The third and final category were on production traits: predicted 1st lactation milk production at 305 DIM, culling of a cow (0: no culling or 1: culling), reason for culling, date of culling, unit in which cow was housed (High Plains or High Meadows).

Blood Collections

Blood collections were performed using evacuated tubes containing K_2 EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) on tail vein while Holstein-Friesian cows restricted with individual headlocks. Blood samples were then placed on ice for transport to the Animal Reproduction and Biotechnology Laboratory facility. Upon arrival, blood samples were centrifuged for 30 minutes at 2,500 rpm to fractionate the blood components. The buffy coat was extracted from each sample and placed in a 1.5 mL microcentrifuge tube. Each tube with buffy coat was suspended up to a volume of 1 mL in 1x phosphate buffered saline (**PBS**) and stored at -20C.

DNA Isolation

Genomic DNA was extracted from the buffy coat of each animal using the Qiagen DNeasy Blood and Tissue Kit (Cat. No. 69504) according to the instructions provided for each sample type. Sample purity and quality was then determined by NanoDrop 2000 Spectrophotometer (Thermo Scientific, manufacture: ND2000USCAN) by diving the absorbance's (A) wavelength reading, A_{260} over A_{280}. Values of 2.0 were considered pure DNA samples.

Identification of Differentially Expressed Genes in RNA-Sequencing

For the RNA-Seq data, the DESeq2 package from R program was used to control for any false discovery by adjusting for the p-value and Benjamini-Hochberg method. Comparisons (1x1) for conceptuses (EM vs N) were performed using a negative binomial distribution model. As a result, all differentially expressed genes (**DEGs**) were identified by having adjusted *p*-values of <0.05.

Identification of Candidate SNPs within Genes

RNA-Seq single-end read sequences (75 base pairs [**bp**]), from the initial fifteen multiparous Holstein Friesian cows, were aligned to the bovine reference genome (ARS-UCD1.2) and SNPs were identified using the Qiagen CLC Genomics Workbench. This then led to the SNP Discovery phase and was made up of four steps. The first step consisted in selecting specific genes with SNPs which was based on: (a) genes with SNPs associated to reproductive/fertility traits in previously published genome wide association studies (**GWAS**), (b) DEGs in canonical pathways or lists from Ingenuity Pathway Analysis in our previous study (Gonzalez-Berrios et al. 2022), (c) genes that were pregnancy related. The second step was to filter the genes from step 1 by only selecting those that were significant (P<0.05) in our RNA Seq data of initial population of

Holstein-Friesian cows (n=15). The third step was to separate the SNPs into the groups of diagnostics (only individuals of a one type of pregnancy [N or EM] make up the complete frequency of a genotype) or non-diagnostic (individuals of both types of pregnancies [N or EM] were used to calculate the frequency of a genotype). The fourth step consisted of: (a) evaluating the function of each gene that had SNP(s), (b) verifying the region of SNP (i.e., non-synonymous), type of SNP (i.e., missense), (c) conduct Sorting Intolerant From Tolerant tool (**SIFT**) analyses to predict amino acid substitution could affect protein function by evaluating SNP values (0.0-1.0; closer to zero is predicted to have a significant effect in protein function), (d) verify if the SNP location within the gene had been previously associated or was nearby (5 centimorgan = 5 million nucleotides) to a reproductive or fertility trait in the Cattle Quantitative Trait Loci (**Cattle QTL**) database and (e) verify the expression in $\log_2$ fold change of genes with SNP(s) within the RNA-Seq data.

Design of Custom SNP Genotyping Assay Panel

A custom SNP genotyping assay panel for the candidate SNPs was designed using the Agena Plex panel (MassARRAY System with 90 well plate) from Neogen® and was divided into four major steps. The first step was to provide the list of candidate SNPs to Neogen® using both reference SNP identification (**rsID**) and the FASTA sequence with the location of the SNP (150 bp up-stream sequence, SNP and 150 bp down-stream sequence). The second step consisted of Neogen® employees conducting an *in-silico* assay design to verify for the efficiency and robustness of the assay through percentage of SNPs that will work in one panel without overlap between SNPs (SNPs that were less than 150 bp from each other). If any overlap was found between SNPs, then SNPs were separated into different panels. The third step was to test the custom assay with the primers that were created for candidate SNPs. This step verified once again

the robustness and efficiency of the assay by genotyping only a subset of DNA samples (n=24). The final step consisted in having an optimized assay that could be used to genotype the remaining samples of the data (n=476).

Quality Control Pipeline for Candidate SNPs

Filtering candidate SNPs, after having received all samples (n=500) genotyped, started by estimating genotypic and allelic frequencies to verify which SNP were monomorphic across all animals. We then verified which SNPs were in minor allele frequency (**MAF**; ≥10%). The candidate SNPs that were not in the MFA and/or were monomorphic were eliminated from the study. The remaining candidate SNPs were evaluated using pLink software using a quality control pipeline that consisted of five steps. The first step was to remove any SNP that 20% or higher genotypes missing in the data. The second step was to remove individual animals that were not genotyped for 10% or higher of the candidate SNPs. The third step was to remove any candidate SNPs that were not in Hardy-Weinberg Equilibrium by the metric of $1e^{-15}$ (i.e., SNP that were above $1e^{-15}$). The fourth was then to evaluate the remaining candidate SNPs (Table 3.1) for linkage disequilibrium via r^2 and d' (Table 2). The final step was to identify tag SNPs (Table 3.3, Fig. 3.2) within the candidate SNPs. If there were more than one tag SNP for a group of SNPs, the tag SNP was selected based on r^2 and d' having the highest values (i.e. strongest relationship to the group of SNPs). An additional number of animals were removed from the study due to missing AI information. Furthermore, some of the reproductive (breeding date, calving of cow and dystocia score) and production traits (predicted 1st lactation milk production at 305 DIM) were also removed from the study due to missing values. By having removed these animals and traits from the study, the n of observations used for the statistical models of the study were n=466.

Statistical Analysis

By using data from cow records and genotypes for each individual, the following models ($\hat{y}$) were for these traits: services per conception, age at first calving (days), days to conception, days to first artificial insemination, pregnant at first artificial insemination, pregnant at 150 days in milk and pregnancy loss (Table 3.4). All variables were evaluated for mean, standard deviation, minimum, maximum via the means procedure of SAS (Table 3.4) (SAS Institute Inc., 2014). Continuous numeric trait models (services per conception, age at first calving (days), days to conception and days to first artificial insemination) were evaluated for statistical significance with and without single genotype term from candidate SNPs using GLM one-way ANOVA. While binary models (i.e., 1: event occurred and 0: event did not occur; pregnant at first artificial insemination, pregnant at 150 days in milk and pregnancy loss) were evaluated for statistical significance with and without single genotype term from candidate SNPs using logistic regression. Statistically significant ($p \leq 0.05$) models with single genotype term from candidate SNPs used the means separation tests within LSMEANS from the mixed procedure and included the Bonferroni adjustment for p-values to minimize false discovery error (Weir, 2001). Interactions between genotype terms and/or other fixed effects that were significantly different within a same model were evaluated. The R^2 of each of the significant models were also evaluated with and without the genotype term in order to demonstrate how much of the observed variation can be explained by the model's inputs. Furthermore, we evaluated the effect of genotype additive and dominance allele effects on candidate SNPs that were significantly different ($p \leq 0.05$) via the statistical models. These procedures were described by Luna-Nevarez et al. (Luna-Nevarez et al., 2011). To confirm or reject such additive or dominant allele effects, there were linear and quadratic contrasts

conducted. All previously mentioned statistical procedures were conducted in SAS OnDemand for academic's software (SAS Institute Inc., 2014)

Results

Quality Control Pipeline for Candidate SNPs

Sixty-nine candidate SNPS were discovered within the RNA of EM vs N conceptuses and therefore, associated to pregnancies with EM in Holstein-Friesian cows. Only thirty out of the sixty-nine candidate SNPs were considered to be in >10% MFA and non-monomorphic. These remaining candidate SNPs (n=30) were evaluated in a five-step quality control pipeline using pLink software. In the first step, three candidate SNPs were eliminated since they had ≥20% of the genotypes missing in the total animal population (n=500). Twenty individual animals were then removed in the second step for not being genotyped for ≥10% of the candidate SNPs (n=27). The third step, eliminated four candidate SNPs that were not in Hardy-Weinberg Equilibrium ($>1e^{-15}$). The final amount of candidate SNPs for this study were twenty-three (Table 1). The fourth step found that eight out of the twenty-three candidate SNPs were linkage disequilibrium via r^2 and d' (Table 3.2). In the last step, it was identified that two out of the twenty-three candidate SNPs within the DSC2 gene (rs109278906: 4 SNPs and rs211151260: 2 SNPs) were considered tag SNPs (Table 3.3; See Appendix Fig. 10).

Potential Functional SNPs

All candidate SNPs were found in a location that were in close proximity (≤5 centimorgans) of at least one SNP that have been previously documented in the cattle QTL database and was associated with fertility traits of economic importance (Figure 3.2). Within the

models used to evaluate reproductive traits with single genotype terms, six instances (UBD [rs109295136] for services per conception; DSC2 [rs109300814] for age at 1st calving; HSD17B7 [rs110828053], FASN [rs41919985], BOLA-DMB [rs109032590], and BOLA-NC1 [rs109291107] for days to 1st AI; DECR1 [rs41580472] for pregnant at 150 DIM; and ACAT2 [rs109967779] for pregnancy loss) of having tendencies to be significantly different ($p \leq 0.10$; Table 3.6). While there were nine instances (DSC2 [rs211151260] for age at 1st calving; SREBF1 [rs41912290] and UBD [rs209518868] for days to conception; UMPS [rs110953962] and SREBF1 [rs41912290] for days to 1st AI; DECR1 [rs41580472] and FASN [rs41919985] for pregnant at 1st AI; SREBF1 [rs41912290] and BOLA-DMB [rs109032590] for pregnant at 150 DIM) were identified to be significantly different ($p \leq 0.05$; Tables 3.6-3.13; Figure 3.3). The R^2 of each model that had a significant instance with genotype term were demonstrated to explain a higher amount of the variance that was present (Table 3.7).

Each of the potential functional SNPs that had genotypes tested for the association with reproductive trait were conducted with the data from primiparous lactating Holstein-Friesian cows. For the DSC2 SNP [rs211151260], in age at 1st calving, cows with AA genotype vs AG and GG genotype were older (Table 3.8). For SREBF1 SNP [rs41912290], in days to conception, cows with TT genotype vs CT and CC genotypes took longer to become pregnant (Table 3.9). While UBD SNP [rs209518868], in days to conception, cows with GG genotype vs AG and AA genotypes also took longer to become pregnant (Table 3.10). For both models, reproductive diseases impacted health of the animal and the unit in which cows were housed were significantly different ($p<0.05$) as well as their interactions ($p<0.01$) but not their interactions with UBD SNP [rs209518868] or SREBF1 SNP [rs41912290] in days to conception. Also, no significant interactions were found between the SREBF1 SNP [rs41912290] and UBD SNP [rs209518868] in

days to conception. For UMPS SNP [rs110953962], in days to 1st AI, cows with TT genotype vs CT and CC genotypes waited longer to have their first AI (Table 3.11). While SREBF1 SNP [rs41912290], in days to 1st AI, cows with TT genotype vs CT and CC genotypes also waited longer to have their first AI (Table 3.9). For both models, age and reproductive diseases were significant (p<0.001) but no interactions were found between them nor with UMPS SNP [rs110953962] or SREBF1 SNP [rs41912290] in days to 1st AI. Furthermore, there was a tendency (p<0.10) for significant interactions between the UMPS SNP [rs110953962] and SREBF1 SNP [rs41912290] in days to 1st AI. For DECR1 SNP [rs41580472], in pregnant at 1st AI, cows with CC genotype vs CT and TT genotypes were less probable to become pregnant on their first AI (Table 3.12). While for FASN SNP [rs41919985], in pregnant at 1st AI, cows with AA genotype vs AG and GG genotypes also less probable to become pregnant on their first AI (Table 3.13). For both models, there were no significant variables apart from the genotype term in pregnant at 1st AI. There were also significant interactions (p<0.05) between DECR1 SNP [rs41580472] and FASN SNP [rs41919985] in pregnant at 1st AI. For SREBF1 SNP [rs41912290], in pregnant at 150 DIM, cows with TT genotype vs CC and CT genotypes were less probable to become pregnant at or before 150 DIM (Table 3.9). While BOLA-DMB SNP [rs109032590], in pregnant at 150 DIM, cows with TT genotype vs CC and CT genotypes were also less likely to become pregnant at or before 150 DIM (Table 3.14). For both models, there were no significant variables apart from the genotype term in pregnant at 150 DIM. There were significant interactions (p<0.01) between SREBF1 SNP [rs41912290] and BOLA-DMB SNP [rs109032590] in pregnant at 150 DIM.

The significant instances within reproductive traits were also verified for type of SNP. Meaning the prediction of the potential functional SNPs having an effect on the protein function of the gene (Tables 3.15-3.21), the allele substitution and additive/dominant effect (Tables 3.21-

3.27) were tested. Among the potential functional SNPs, all were found to be in non-synonymous coding regions and were classified as missense. The SIFT tool, from Ensembl, predicted that three (DSC2 [rs211151260]; SREBF1 [rs41912290]; and UMPS [rs110953962]) out of the seven potential functional SNPs would have an effect on the protein function of the gene ($p<0.05$; Tables 3.15, 3.16 and 3.18). It was also confirmed that all seven potential functional SNPs previously mentioned were found to have an additive effect ($p\leq0.05$) due to a linear trend observed when the genotype term was a fixed effect within the model (Tables 3.22-3.28). Conversely, only two of the potential functional SNPs (DSC2 [rs211151260] for age at 1st calving and SREBF1 [rs41912290] for days to 1st AI and pregnant at 150 DIM) had significant allele substitution effects ($p\leq0.05$; Tables 3.22 and 3.23) and only one potential functional SNP (DECR1 [rs41580472] for pregnant at 1st AI) tended to be significant ($p\leq0.10$; Table 3.26). For DSC2 [rs211151260], cows with the allele G improved the trait level decreasing the age at 1st calving by 10 days (Table 3.22). For SREBF1 [rs41912290], cows with the C allele improved the trait level by decreasing the days to 1st AI by 5 days and the probability of becoming pregnant at 150 DIM by 6% (Table 3.23).

Discussion

Potential Functional SNPs

Discovery of potential functional SNPs are important for genetic progress of fertility traits due to their low heritability within the Holstein-Friesian cow breed (García-Ruiz et al., 2016b). Moreover, improvement from these strategies likely have direct impact on the economic efficacy and culling practices of the dairy industry (Pryce et al., 1997, Inchaisri et al., 2010). Among the seven models that were evaluated in this study for reproductive traits, only five (age at 1st calving,

days to conception, days to 1st AI, pregnant at 1st AI and pregnant at 150 DIM) had potential functional SNPs that were predictive of these traits.

Identification of at least one QTL that has been associated to fertility traits was found to be in a location of close proximity to our candidate SNPs. Previous studies have shown that proximity to those QTL could be associated in having an effect over other nearby SNPs that control similar traits (Khatkar et al., 2004, Daetwyler et al., 2008). This of importance when conducting genotype to phenotype association studies because candidate SNPs that become associated with QTL(s) can be implemented into breeding programs to improve traits such as those within fertility that are of low heritability since traditional selection has been less effective (Spelman and Garrick, 1997, Van Tassell et al., 1999, Abdel-Azim and Freeman, 2002).

In all of the potential functional SNPs, that were significant, it was demonstrated that they had a larger R^2 number in each model when compared to a model without the genotype term (Table 7). This means that a higher amount of the variance found in the model was explained by the genotype term (SAS Institute Inc., 2014). Furthermore, it was observed that the R^2 for each of the model with or without genotype term were a low number. This was to be expected because fertility traits, as previously mentioned, are of low heritability (Van Tassell et al., 1999).

For age at 1st calving, cows with a DSC2 SNP [rs211151260] and A allele were older when compared to those with a G allele (Table 8). Various studies reported that the optimum age of cows at 1st calving is averaged between 23 to 24 months (700 to 730 days) (Heinrichs, 1993, Hoffman, 1997, Gabler et al., 2000, Boulton AC, 2015). This is of great importance because of the impact that this trait has on the cost of rearing replacements heifers in dairy herds (Tozer and Heinrichs, 2001, Ettema and Santos, 2004, Boulton AC, 2015). In context of the DSC2 gene, it is mainly known for its role in cell junction and adhesion (Lewin et al., 2022) and therefore, has been studied

for its potential role in the development and function of bovine embryos via compaction and cavitation (Wrenzycki et al., 2001). Currently, only one SNP [rs109503725] within the DSC2 gene has been previously associated fertility traits such as daughter pregnancy rate in Holstein-Friesian cows (Cochran et al., 2013). For this study's DSC2 SNP [rs211151260], it has only been submitted for a rsid within an Iranian *Bos taurus* breed and no previous associations report for age at 1st calving in Holstein-Friesian cows.

For the reproductive trait of days to conception, two sets of SNPs (SREBF1 [rs41912290] and UBD [rs209518868]) were found to take longer to become pregnant when cows had T (Table 3.9) or G allele (Table 3.10) when compared to C or A alleles, respectively. Furthermore, interactions were found between reproductive diseases (i.e., having more instances of endometritis or combination of metritis and endometritis) that impacted health of the animal and housing (i.e., High Meadows) of cows were associated to influence the cow's ability to become pregnant later rather than sooner. Interval of conception after calving is often used as a parameter to determine reproductive performance of dairy cows (Harman et al., 1996) and should ideally take place prior to 85 days post calving (Grimard et al., 2006, Tillard et al., 2008). However, the interval average of conception after calving is highly dependent on diverse factors such as season (i.e., summer), peripartum disorders (i.e., metritis and endometritis) and cow management (Grimard et al., 2006, Tillard et al., 2008, Siddiqui et al., 2013). Therefore, minimizing the number of days that a cow is not pregnant (open) is crucial to decreasing costs (i.e., food, labor, breeding) and culling rates but also increasing the number of calves and longevity of the animal (González-Recio et al., 2004, Inchaisri et al., 2010, Cabrera, 2014). In the SREBF1 gene, its function has been mainly documented for being a master regulator the transcription of genes that synthesize milk fat and its secretion in the mammary epithelial cells of dairy cows (Harvatine and Bauman, 2006, Ma and

Corl, 2012, Oppi-Williams et al., 2013, Li et al., 2014). As a result, the potential functional SNP [rs41912290] discussed in this study within SREBF1 has been previously submitted to ENSEMBL SNP database and associated with fatty acid indexes which had an effect on milk fat and protein percentage in Holstein-Friesian cows (Rincon et al., 2012, Cochran et al., 2013). Despite this, no associations for SREBF1 SNP [rs41912290] on its impact on days to conception have been made in Holstein-Friesian cows. In the case of the UMD gene, it has general roles in DNA-replication, -recombination and -repair, cell-development, -assembly, -maintenance, -survival and -death (Salehi et al., 2016). Previously discovered SNPs within UBD have mainly been associated or thought to regulate response from the immune system (Russell et al., 2012, Thompson-Crispi et al., 2014). The current study's UBD SNP [rs209518868] has been previously reported and submitted for a total of 14 different cow/bull populations but no association with days to conception in Holstein-Friesian cows.

In days to 1st AI, individuals with a T allele for UMPS SNP [rs110953962] and SREBF1 SNP [rs41912290] waited longer to have their first AI after first calving when compared to C allele, respectively (Tables 3.9 and 3.11). It was also found that age (i.e., average of 760 days or older) and reproductive diseases (i.e., endometritis or combination of metritis and endometritis) were associated with waiting longer to have their first AI after their first calving. Studies in the past have demonstrated that the average days to 1st AI ranged from 67 to 84 (Esslemont, 1992, Darwash et al., 1997, Royal et al., 2000, Waldmann et al., 2001, Yusuf et al., 2011) which demonstrated herd management favoring an extension of DIM before conducting their first AI after calving. In fact, it has been recently proven that higher conception rates are obtained in later rather than earlier stages of lactation (Dohoo, 1983, Royal et al., 2000, Quintela Arias et al., 2004, Bouchard E, 2008). For the UMPS gene, its role is essential in the synthesis of nucleotides used in

both DNA and RNA (Healey and Shanks, 1987). Deficiency (inactivation) of UMPs, also known as DUMPs, is hereditary recessive disorder that causes arrests growth and development of pregnancies which leads to EM taking place in Holstein-Friesian cows (Shanks and Robinson, 1990, Shanks and Greiner, 1992, Robinson et al., 1993, Kuhn and Shanks, 1994). Furthermore, carriers of DUMPs have been found to remain non-pregnant for longer periods (Čítek and Barbora, 2004). Prevention of this disease consists of early detection of defect carriers by screening herds routinely through haplotype tests (VĂTĂŞEscu-Balcan et al., 2006). For this study's UMPS SNP [rs110953962], it was only reported within an Iranian Bos taurus breed and no associations to days to 1st AI such as we made in Holstein-Friesian cows. As previously mentioned, the SREBF1 gene regulates synthesis of milk and fat (Harvatine and Bauman, 2006, Ma and Corl, 2012, Oppi-Williams et al., 2013, Li et al., 2014) and this study's SNP [rs41912290] have been previously associated with having an effect on milk fat and protein percentage in Holstein-Friesian cows (Rincon et al., 2012, Cochran et al., 2013). No associations of the SREBF1 SNP [rs41912290] have been made with days to 1st AI.

For pregnant at 1st AI, cows with the C allele (Table 3.12) from DECR1 SNP [rs41580472], A allele (Table 3.13) from FASN SNP [rs41919985] or carrier of both alleles were less probable to become pregnant on their first AI when compared to those with a T or G allele, respectively. Just like days to conception, pregnancy at 1st AI depends on the time of year, voluntary waiting period, the cow's state with reproductive/peripartum disorders and the reproductive management (Grimard et al., 2006, Tillard et al., 2008, Siddiqui et al., 2013). Furthermore, it has been proven that dairy cows that are serviced more than one AI have less pregnancy rates at first AI (77.3 vs 35.7%) and a reduced probability to become pregnant by 4.5% for each previously unsuccessful service (Barrett, 1948, Sprott et al., 1998). The DECR1 gene function is to act as a mitochondrial

enzyme involved in beta-oxidation that regulates the rate of fatty acid metabolism which contributes to energy production (Kunau and Dommes, 1978, Wathes et al., 2012). Potential functional SNPs within the DECR1 gene have been found to be associated with an effect on lipid metabolism, milk production, back fat thickness, days to first service and calving interval in beef cattle (Marques et al., 2009, Clempson et al., 2012). Boussaha et al. (Boussaha et al., 2015) has previously submitted our study's DECR1 SNP [rs41580472] within a GWAS data set but no publications have been made to associated with pregnancy at 1st AI in Holstein-Friesian cows. In the case of the FASN gene, its role is to catalyze the production of long chain fatty acids and therefore, has been evaluated as a candidate for improving fat levels observed in both milk and muscle for dairy and beef cattle (Roy et al., 2006, Schennink et al., 2009, Matsumoto et al., 2012, Li et al., 2016). SNPs within the FASN gene have been associated with lactation traits, composition of fatty acids within milk, and reconstitution of body reserves during gestation (Matsumoto et al., 2012, Elis et al., 2013, Mauric, 2019) but not this study's FASN SNP [rs41919985] to pregnancy at 1st AI.

In the trait of pregnant at 150 DIM, cows with the T allele (Tables 9 and 14) from SREBF1 SNP [rs41912290], BOLA-DMB SNP [rs109032590] or carrier of both alleles were less probable to become pregnant at or before 150 DIM when compared to C alleles. For pregnancy to take place before or at 150 DIM it is contingent of reproductive diseases, detection of estrus, voluntary waiting period and season (Grimard et al., 2006, Tillard et al., 2008, Siddiqui et al., 2013). The importance of timing when this pregnancy occurs in dairy cows is based on sustaining profitability within the industry (Giordano et al., 2011). In the case of the SREBF1 SNP [rs41912290], no associations with pregnant at 150 DIM have been made previously. For the BOLA-DMB gene, it is part of the bovine immune system's major histocompatibility complex in the class IIb region

and it is responsible for aiding with the loading of peptides in antigen presenting cells (Pathak et al., 2001, La Rocca et al., 2014). Interestingly, regulation of BOLA-DMB is different in the endometrium of cows who are pregnant vs non-pregnant cows (Cerri et al., 2012). Associations for this study's BOLA-DMB SNP has been previously made for daughter pregnancy rate, cow conception rate, heifer pregnancy rate and milk yield (Cochran et al., 2013, Ortega et al., 2016, Ortega et al., 2017, Ortega, 2018) but not specifically to pregnancy at 150 DIM.

For allele substitution and additive effects, cows with a G allele that had the DSC2 [rs211151260] SNP for age at 1st calving improved the trait level decreasing the age at 1st calving by 10 days (Table 22). Eastham et al. (Eastham et al., 2018) previously found that cows that were younger during their first calving has an association with udder health, increased longevity, milk yield, improved reproductive performance and increased probability to of calve for a second time. While the SREBF1 [rs41912290], had cows with the C allele improved the trait level by decreasing the days to 1st AI by 5 days and the probability of becoming pregnant at 150 DIM by 6% (Table 23). For cows that are having their first AI sooner it has been previously demonstrated to be dependent on the management of herd and is unique to each individual dairy (Esslemont, 1992, Darwash et al., 1997, Royal et al., 2000, Waldmann et al., 2001, Yusuf et al., 2011). Moreover, it has been shown that cows had higher conception rates when AI occurs at later stages of lactation (Dohoo, 1983, Royal et al., 2000, Quintela Arias et al., 2004, Bouchard E, 2008). Conversely, pregnancy occurring before or at 150 DIM is of high importance to be able to sustain profitability within the industry by pregnancy occurring between 90 and 130 DIM (Giordano et al., 2011). Regardless of our study's findings, the potential candidate SNPs being validated within the models of days to 1st AI, pregnant at 1st AI and 150 DIM may be somewhat unique to the management

practices of an organic farm (i.e., Aurora Organic Dairy), and therefore, reproductive management taking longer for each lactation cycle (Sorge et al., 2016).

Another aspect of this study, was being able to demonstrate the true additive effects of alleles by having genotyped and validated these effects in a different Holstein-Friesian cow population. This is of importance because genetic panels/evaluations performed in cattle such as expected progeny differences (EPDs) in bulls, predictive transmissibility abilities (PTA) or those commercially available (i.e., Clarifide from Zoetis) are based on these values that are used when breeding. Therefore, aiding in the development of breeding values that encompass fertility traits are of great value when determining the expected fertility of replacement heifers and cows that remain in the herd for the Holstein-Friesian breed (Weigel, 2006, García-Ruiz et al., 2016b).

Conclusions

Having first conducted a gene expression study (RNA-Seq) on day 16 conceptus tissues (EM vs N) from multiparous lactating Holstein-Friesian cows (n=15) in a traditional dairy farm (Blue Sky Dairy) revealed an initial 17,156 candidate SNPs. All of the candidate SNPs were filtered to 69 in 18 genes. The remaining candidate SNPs were found to have been previously reported QTL associated to fertility traits within beef and dairy cattle. Validation of such candidate SNPs (n=69) entailed on conducting a genotype to phenotype analyses on a second dairy (Aurora Organic Dairy) using primiparous lactating Holstein-Friesian cows (n=500) and having their records collected. Furthermore, a quality control pipeline aided in reducing the number of candidate SNPs to 23 in 14 genes. Genotype to phenotype analysis suggested that 7 SNP are predictive of causing fertility traits that are of economic importance to be inferior in Holstein-Friesian cows. Therefore, we

propose that the candidate SNPs that are associated to pregnancies with EM might be considered for sorting and culling heifer and cows that are reproductively inefficient. However, additional research should be conducted to determine the utility of these candidate SNPs in genome-enhanced PTA estimations and (or) commercial genotyping tools for early life sorting of heifers.

Table 3.1: Single nucleotide polymorphisms (SNP) candidates (n=23) that were non-monomorphic and passed quality control pipeline using PLINK software in primiparous lactating Holstein-Friesian cows (n=466). chrm=chromosome.

Gene	rsid	chrm	SNP location	genotype	genotyping	maf	hwe
UMPS	rs110953962	1	69148086	C/T	463	0.29	0.8
HSD17B7	rs110828053	3	6635945	C/T	464	0.19	0.5
CAST	rs110914810	7	96152634	C/G	466	0.38	1
IFNGR1	rs109049057	9	75092093	C/T	466	0.29	0.3
ACAT2	rs109967779	9	96041211	A/G	464	0.40	0.6
DECR1	rs41580472	14	73708561	C/T	465	0.27	0.9
MRPL48	.	15	53332881	A/G	466	0.48	0.5
SREBF1	rs41912290	19	34646676	C/T	465	0.40	0.6
FASN	rs41919985	19	50793357	A/G	433	0.29	$1.2e^{-05}$
BOLA-DMB	rs109032590	23	7249490	C/T	464	0.30	0.7
BLA-DQB	rs109291107	23	25674287	A/G	409	0.20	$2e^{-13}$
BOLA-NC1	rs382125666	23	28551269	A/C	401	0.22	$2.68e^{-06}$
UBD	rs209518868	23	29119086	A/G	465	0.11	0.007
UBD	rs109295136	23	29119334	A/G	400	0.42	$3e^{-06}$
DSC2	rs109300814	24	26043125	A/C	463	0.45	$3.4e^{-12}$
DSC2	rs210995078	24	26048022	A/G	466	0.45	1
DSC2	rs211151260	24	26050992	A/G	466	0.36	0.3
DSC2	rs385100256	24	26057277	C/G	466	0.36	0.3
DSC2	rs109503725	24	26057282	C/T	466	0.45	1
DSC2	.	24	26060104-5	AA/GT	459	0.44	0.8
DSC2	rs109278906	24	26060155	A/T	463	0.44	1
DSC2	rs110651429	24	26060157	C/T	464	0.44	0.9
DSC2	rs210416248	24	26063437	A/G	466	0.36	0.3

Table 3.2: Candidate single nucleotide polymorphisms (SNPs) (n= 12 out of 23) that were in linkage disequilibrium (r2 and d') using PLINK software in primiparous lactating Holstein-Friesian cows (n=428). chrm=chromosome.

Gene	SNP A rsid	chrm	SNP A location	SNP B	SNP B rsid	SNP B location	r^2	d'
BOLA-NC1	rs382125666	23	69148086	UBD	rs209518868	29119086	0.00165162	0.0737591
BOLA-NC1	rs382125666	23	69148086	UBD	rs109295136	29119334	0.0943097	0.477845
UBD	rs209518868	23	29119086	UBD	rs109295136	29119334	0.104576	0.908803
DSC2	rs109300814	24	26043125	DSC2	rs210995078	26048022	0.705424	1
DSC2	rs109300814	24	26043125	DSC2	rs211151260	26050992	0.665203	1
DSC2	rs109300814	24	26043125	DSC2	rs385100256	26057277	0.665203	1
DSC2	rs109300814	24	26043125	DSC2	rs109503725	26057282	0.693562	1
DSC2	rs109300814	24	26043125	DSC2	.	26060104-5	0.677046	0.97805
DSC2	rs109300814	24	26043125	DSC2	rs109278906	26060155	0.687309	0.994494
DSC2	rs109300814	24	26043125	DSC2	rs110651429	26060157	0.695425	1
DSC2	rs109300814	24	26043125	DSC2	rs210416248	26063437	0.653245	1
DSC2	rs210995078	24	26048022	DSC2	rs211151260	26050992	0.4594	1
DSC2	rs210995078	24	26048022	DSC2	rs385100256	26057277	0.4594	1
DSC2	rs210995078	24	26048022	DSC2	rs109503725	26057282	0.983269	1
DSC2	rs210995078	24	26048022	DSC2	.	26060104-5	0.965927	0.982816
DSC2	rs210995078	24	26048022	DSC2	rs109278906	26060155	0.978915	0.995715
DSC2	rs210995078	24	26048022	DSC2	rs110651429	26060157	0.983198	1
DSC2	rs210995078	24	26048022	DSC2	rs210416248	26063437	0.45117	1
DSC2	rs211151260	24	26050992	DSC2	rs385100256	26057277	1	1
DSC2	rs211151260	24	26050992	DSC2	rs109503725	26057282	0.451713	1
DSC2	rs211151260	24	26050992	DSC2	.	26060104-5	0.436002	0.975585
DSC2	rs211151260	24	26050992	DSC2	rs109278906	26060155	0.44366	0.991819
DSC2	rs211151260	24	26050992	DSC2	rs110651429	26060157	0.452195	1
DSC2	rs211151260	24	26050992	DSC2	rs210416248	26063437	0.982087	1
DSC2	rs385100256	24	26057277	DSC2	rs109503725	26057282	0.451713	1
DSC2	rs385100256	24	26057277	DSC2	.	26060104-5	0.436002	0.975585
DSC2	rs385100256	24	26057277	DSC2	rs109278906	26060155	0.44366	0.991819
DSC2	rs385100256	24	26057277	DSC2	rs110651429	26060157	0.452195	1
DSC2	rs385100256	24	26057277	DSC2	rs210416248	26063437	0.982087	1
DSC2	rs109503725	24	26057282	DSC2	.	26060104-5	0.983017	1
DSC2	rs109503725	24	26057282	DSC2	rs109278906	26060155	0.995765	1
DSC2	rs109503725	24	26057282	DSC2	rs110651429	26060157	1	1
DSC2	rs109503725	24	26057282	DSC2	rs210416248	26063437	0.443622	1
DSC2	.	24	26060104-5	DSC2	rs109278906	26060155	0.995702	1
DSC2	.	24	26060104-5	DSC2	rs110651429	26060157	0.987178	1
DSC2	.	24	26060104-5	DSC2	rs210416248	26063437	0.449772	1
DSC2	rs109278906	24	26060155	DSC2	rs110651429	26060157	1	1
DSC2	rs109278906	24	26060155	DSC2	rs210416248	26063437	0.446917	1
DSC2	rs110651429	24	26060157	DSC2	rs210416248	26063437	0.446088	1

Table 3.3: Candidate single nucleotide polymorphisms (SNPs) (n=8 out of 23) that have a tag SNP using PLINK software in primiparous lactating Holstein-Friesian cows.

Gene	rsid	chrm	SNP location	Tag SNP	Tag SNP rsid	Tag SNP location
DSC2	rs210995078	24	26048022	DSC2	rs109278906	26060155
DSC2	rs109503725	24	26057282	DSC2	rs109278906	26060155
DSC2	rs110651429	24	26060157	DSC2	rs109278906	26060155
DSC2	.	24	26060104-5	DSC2	rs109278906	26060155
DSC2	rs385100256	24	26057277	DSC2	rs211151260	26050992
DSC2	rs210416248	24	26063437	DSC2	rs211151260	26050992

Table 3.4: Basic model and qualitative and quantitative explanatory variables used to predict the categorical traits.

Statistical model
$Y_{ijklm} = \mu + A_i + B_j + C_k + D_l + E_m + e_{ijklm}$

$^{a}Y_{ijkmqr}$ = qualitative trait for the rth cow in the (i, j, k, l, m)th cell, μ = general mean for the reproductive trait, A_i = fixed effect of genotypes, B_j =co-variate effect of the age in days at first calving, C_k = fixed effect of the health reproductive disease, D_l = fixed effect of the health non-reproductive disease, E_m = fixed effect of the housing unit and $e_{ijklmnopq}$ = random error term.

Table 3.5: MEANS, GLMa and logisticb for conception, age at calving and pregnancy in primiparous lactating Holstein-Friesian cows.

Trait	n	Mean + SD	Min	Max	p-value	X^2 test *p*-value
Services per conception[a]	466	3.8±2.8	1	13	0.001*	.
Age at 1st calving, days[a]	466	751.2±62.1	560	993	0.1	.
Days to conception[a]	417	159.2±102.5	45	579	0.0105*	.
Days to 1st AI[a]	466	75.7±32.4	37	266	<0.0001*	.
Pregnant at 1st AI[b]	466	0.2±0.4	0	1	.	0.1
Pregnant at 150 DIM[b]	466	0.5±0.5	0	1	.	0.008*
Pregnancy loss[b]	466	0.07±0.2	0	2	.	0.6

[a]Non-binary models that were evaluated with GLM-one way ANOVA. [b]Binary models that were evaluated with logistic regression. *Models that were statistically significant ($p \leq 0.05$) without genotype term.

Table 3.6: Summary of conception, age at calving and pregnancy with candidate single nucleotide polymorphisms (SNPs) (n=17) in primiparous lactating Holstein-Friesian cows.

Trait	Gene	rsid	n	Model *p*-value	Model X^2 Test *p*-value	SNP *p*-value	SNP X^2 Test *p*-value
Services per conception[a]							
	UMPS	rs110953962	463	0.002	.	0.2	.
	HSD17B7	rs110828053	464	0.001	.	0.1	.
	CAST	rs110914810	466	0.006	.	0.9	.
	ACAT2	rs109967779	464	0.005	.	0.6	.
	IFNGR1	rs109049057	466	0.004	.	0.6	.
	DECR1	rs41580472	465	0.003	.	0.3	.
	MRPL48	.	466	0.002	.	0.2	.
	FASN	rs41919985	433	0.005	.	0.3	.
	SREBF1	rs41912290	465	0.001	.	0.1	.
	BOLA-DMB	rs109032590	464	0.004	.	0.5	.
	BLA-DQB	rs109291107	409	0.004	.	0.5	.
	BOLA-NC1	rs382125666	401	0.006	.	0.2	.
	UBD	rs209518868	465	0.006	.	0.8	.
	UBD	rs109295136	400	0.002	.	0.09†	.
	DSC2	rs109300814	463	0.005	.	0.4	.
	DSC2	rs211151260	466	0.007	.	0.9	.
	DSC2	rs109278906	463	0.005	.	0.6	.
Age at 1st calving[a]							
	UMPS	rs110953962	463	0.2	.	0.4	.
	HSD17B7	rs110828053	464	0.4	.	0.8	.
	CAST	rs110914810	466	0.4	.	0.9	.
	ACAT2	rs109967779	464	0.2	.	0.5	.
	IFNGR1	rs109049057	466	0.2	.	0.4	.
	DECR1	rs41580472	465	0.2	.	0.3	.
	MRPL48	.	466	0.2	.	0.4	.
	FASN	rs41919985	433	0.1	.	0.07	.
	SREBF1	rs41912290	465	0.2	.	0.4	.
	BOLA-DMB	rs109032590	464	0.1	.	0.1	.
	BLA-DQB	rs109291107	409	0.3	.	0.9	.
	BOLA-NC1	rs382125666	401	0.2	.	0.4	.
	UBD	rs209518868	465	0.3	.	0.8	.
	UBD	rs109295136	400	0.3	.	0.8	.
	DSC2	rs109300814	463	0.05	.	0.04†	.
	DSC2	rs211151260	466	0.04	.	0.03*	.
	DSC2	rs109278906	463	0.3	.	0.7	.
Days to conception[a]							
	UMPS	rs110953962	416	0.01	.	0.3	.
	HSD17B7	rs110828053	415	0.01	.	0.2	.
	CAST	rs110914810	417	0.03	.	0.9	.
	ACAT2	rs109967779	415	0.02	.	0.5	.
	IFNGR1	rs109049057	417	0.01	.	0.3	.
	DECR1	rs41580472	416	0.02	.	0.6	.
	MRPL48	.	417	0.04	.	0.9	.
	FASN	rs41919985	388	0.04	.	0.5	.
	SREBF1	rs41912290	416	0.003	.	0.04*	.
	BOLA-DMB	rs109032590	415	0.007	.	0.1	.
	BLA-DQB	rs109291107	367	0.03	.	0.3	.
	BOLA-NC1	rs382125666	358	0.01	.	0.5	.
	UBD	rs209518868	416	0.001	.	0.009*	.
	UBD	rs109295136	361	0.05	.	0.1	.

	DSC2	rs109300814	414	0.02	.	0.2	.
	DSC2	rs211151260	417	0.03	.	0.9	.
	DSC2	rs109278906	414	0.01	.	0.1	.
Days to 1st AI[a]							
	UMPS	rs110953962	463	<.0001	.	0.04*	.
	HSD17B7	rs110828053	464	<.0001	.	0.06†	.
	CAST	rs110914810	466	<.0001	.	0.6	.
	ACAT2	rs109967779	464	<.0001	.	0.1	.
	IFNGR1	rs109049057	466	<.0001	.	0.7	.
	DECR1	rs41580472	465	<.0001	.	0.3	.
	MRPL48	.	466	<.0001	.	0.2	.
	FASN	rs41919985	433	<.0001	.	0.07†	.
	SREBF1	rs41912290	465	<.0001	.	0.01*	.
	BOLA-DMB	rs109032590	464	<.0001	.	0.08†	.
	BLA-DQB	rs109291107	409	<.0001	.	0.06†	.
	BOLA-NC1	rs382125666	401	<.0001	.	0.7	.
	UBD	rs209518868	465	<.0001	.	0.4	.
	UBD	rs109295136	400	0.002	.	0.7	.
	DSC2	rs109300814	463	<.0001	.	0.1	.
	DSC2	rs211151260	466	<.0001	.	0.5	.
	DSC2	rs109278906	463	<.0001	.	0.5	.
Pregnant at 1st AI[b]							
	UMPS	rs110953962	463	.	0.1	.	0.3
	HSD17B7	rs110828053	464	.	0.1	.	0.6
	CAST	rs110914810	466	.	0.2	.	0.8
	ACAT2	rs109967779	464	.	0.1	.	0.6
	IFNGR1	rs109049057	466	.	0.2	.	0.7
	DECR1	rs41580472	465	.	0.02	.	0.02*
	MRPL48	.	466	.	0.1	.	0.4
	FASN	rs41919985	433	.	0.01	.	0.02*
	SREBF1	rs41912290	465	.	0.1	.	0.4
	BOLA-DMB	rs109032590	464	.	0.09	.	0.1
	BLA-DQB	rs109291107	409	.	0.08	.	0.9
	BOLA-NC1	rs382125666	401	.	0.2	.	0.5
	UBD	rs209518868	465	.	0.1	.	0.8
	UBD	rs109295136	400	.	0.04	.	0.03
	DSC2	rs109300814	463	.	0.1	.	0.3
	DSC2	rs211151260	466	.	0.1	.	0.2
	DSC2	rs109278906	463	.	0.1	.	0.3
Pregnant at 150 DIM[b]							
	UMPS	rs110953962	463	.	0.006	.	0.1
	HSD17B7	rs110828053	464	.	0.01	.	0.3
	CAST	rs110914810	466	.	0.02	.	0.8
	ACAT2	rs109967779	464	.	0.01	.	0.3
	IFNGR1	rs109049057	466	.	0.01	.	0.2
	DECR1	rs41580472	465	.	0.003	.	0.06†
	MRPL48	.	466	.	0.01	.	0.4
	FASN	rs41919985	433	.	0.02	.	0.3
	SREBF1	rs41912290	465	.	0.001	.	0.01*
	BOLA-DMB	rs109032590	464	.	0.002	.	0.04*
	BLA-DQB	rs109291107	409	.	0.02	.	0.9
	BOLA-NC1	rs382125666	401	.	0.01	.	0.8
	UBD	rs209518868	465	.	0.01	.	0.6
	UBD	rs109295136	400	.	0.07	.	0.1
	DSC2	rs109300814	463	.	0.009	.	0.1
	DSC2	rs211151260	466	.	0.03	.	0.9
	DSC2	rs109278906	463	.	0.008	.	0.1
Pregnancy loss[b]							
	UMPS	rs110953962	463	.	0.7	.	0.7
	HSD17B7	rs110828053	464	.	0.3	.	0.1
	CAST	rs110914810	466	.	0.7	.	0.6
	ACAT2	rs109967779	464	.	0.07	.	0.05†

IFNGR1	rs109049057	466	.	0.1	.	0.01
DECR1	rs41580472	466	.	0.5	.	0.4
MRPL48	.	466	.	0.7	.	0.8
FASN	rs41919985	433	.	0.4	.	0.3
SREBF1	rs41912290	465	.	0.4	.	0.1
BOLA-DMB	rs109032590	464	.	0.6	.	0.4
BLA-DQB	rs109291107	409	.	0.5	.	0.3
BOLA-NC1	rs382125666	401	.	0.7	.	0.4
UBD	rs209518868	465	.	0.7	.	0.7
UBD	rs109295136	400	.	0.7	.	0.8
DSC2	rs109300814	463	.	0.7	.	0.7
DSC2	rs211151260	466	.	0.7	.	0.7
DSC2	rs109278906	463	.	0.7	.	0.7

[a]Non-binary models that were evaluated with GLM-one way ANOVA. [b]Binary models that were evaluated with logistic regression. †Candidate single nucleotide polymorphism that were a tendency to be significant ($p>0.05$) within models. *Candidate single nucleotide polymorphism that were statistically significant ($p\leq0.05$) within models. AI=artificial insemination, DIM= days in milk.

Table 3.7: Summary of R2 of model without and with potential functional candidate single nucleotide polymorphisms (SNPs) (n=7) in primiparous lactating Holstein-Friesian cows.

Trait	SNP	rsid	n	R^2 without SNP	*p*-value	n	R^2 with SNP	*p*-value
Age at 1st calving, days	DSC2	rs211151260	466	0.01	0.1	466	0.02	0.04
Days to conception	SREBF1	rs41912290	417	0.03	0.01	416	0.04	0.003
Days to conception	UBD	rs209518868	417	0.03	0.01	416	0.05	0.001
Days to 1st AI	SREBF1	rs41912290	466	0.06	<0.0001	465	0.08	<0.0001
Days to 1st AI	UMPS	rs110953962	466	0.06	<0.0001	463	0.07	<0.0001
Pregnant at 1st AI	DECR1	rs41580472	466	0.01	0.1042	465	0.03	0.02
Pregnant at 1st AI	FASN	rs41919985	466	0.01	0.1042	433	0.03	0.01
Pregnant at 150 DIM	SREBF1	rs41912290	466	0.02	0.008	465	0.04	0.001
Pregnant at 150 DIM	BOLA-DMB	rs109032590	466	0.02	0.008	464	0.04	0.002

Table 3.8: Least square means ± standard error for age at 1st calving among genotypes of the SNP within DSC2 in primiparous lactating Holstein-Friesian cows.

		SNP (rs211151260) in DSC2			
Trait	n	AA	AG	GG	*p*-value
Age at 1st calving, days	466	761.9±8.7	757.8±5.7	744.1±5.7	0.03*

*Candidate single nucleotide polymorphisms (SNPs) that were statistically significant ($p<0.05$) within models.

Table 3.9: Least square means ± standard error for fertility traits among genotypes of the SNP within SREBF1 in primiparous lactating Holstein-Friesian cows.

		SNP (rs41912290) in SREBF1			
Trait	n	CC	CT	TT	*p*-value
Days to conception	416	176.3±10.9	162.5±10.09	197.7±14.04	0.04*
Days to 1st AI	465	80.08±3.1	80.5±2.8	91.4±3.9	0.01*
Pregnant at 150 DIM	465	0.4±0.04	0.4±0.04	0.3±0.06	0.01*

*Candidate single nucleotide polymorphisms (SNPs) that were statistically significant ($p \leq 0.05$) within models.

Table 3.10: Least square means ± standard error for days to conception among genotypes of the SNP within UBD in primiparous lactating Holstein-Friesian cows.

		SNP (rs209518868) in UBD			
Trait	n	AA	AG	GG	*p*-value
Days to conception	416	173.1±9.008	175.7±12.5	481.83±101.2	0.009*

*Candidate single nucleotide polymorphisms (SNPs) that were statistically significant ($p \leq 0.05$) within models.

Table 3.11: Least square means ± standard error for days to 1st artificial insemination among genotypes of the SNP within UMPS in primiparous lactating Holstein-Friesian cows.

		SNP (rs110953962) in UMPS			
Trait	n	CC	CT	TT	*p*-value
Days to 1st AI	463	83.01±2.7	79.2±3.04	93.03±5.3	0.04*

*Candidate single nucleotide polymorphisms (SNPs) that were statistically significant ($p \leq 0.05$) within models.

Table 3.12: Least square means ± standard error for pregnant at 1st artificial insemination among genotypes of the SNP within DECR1 in primiparous lactating Holstein-Friesian cows.

		SNP (rs41580472) in DECR1			
Trait	n	CC	CT	TT	*p*-value
Pregnant at 1st AI	465	0.2±0.03	0.2±0.04	0.4±0.07	0.02*

*Candidate single nucleotide polymorphisms (SNPs) that were statistically significant ($p \leq 0.05$) within models. AI=artificial insemination.

Table 3.13: Least square means ± standard error for pregnant at 1st artificial insemination among genotypes of the SNP within FASN in primiparous lactating Holstein-Friesian cows.

		SNP (rs41919985) in FASN			
Trait	n	AA	AG	GG	*p*-value
Pregnant at 1st AI	433	0.01±0.09	0.2±0.03	0.1±0.04	0.0240*

*Candidate single nucleotide polymorphism (SNPs) that were statistically significant ($p \leq 0.05$) within models. AI=artificial insemination.

Table 3.14: Least square means ± standard error for pregnant at 150 days in milk among genotypes of the SNP within BOLA-DMB in primiparous lactating Holstein-Friesian cows.

		SNP (rs109032590) in BOLA-DMB			
Trait	n	CC	CT	TT	*p*-value
Pregnant at 150 DIM	464	0.4±0.04	0.5±0.04	0.3±0.08	0.04*

*Candidate single nucleotide polymorphism candidates (SNPs) that were statistically significant ($p \leq 0.05$) within models. DIM=days in milk.

Table 3.15: Variant effect predictor analysis for SNP within DSC2 in primiparous lactating Holstein-Friesian cows.

Coding Region	Type of SNP	rsid	Exon	Codon Change	Amino Acid Change	SIFT
Non-Synonymous	Missense	rs211151260	11	**G**AA/**A**AA	**E/K**	0.01

Table 3.16: Variant effect predictor analysis for SNP within SREBF1 in primiparous lactating Holstein-Friesian cows.

Coding Region	Type of SNP	rsid	Exon	Codon Change	Amino Acid Change	SIFT
Non-Synonymous	Missense	rs41912290	2	C**C**T/C**T**T	**P**/**L**	0.03

Table 3.17: Variant effect predictor analysis for SNP within UBD in primiparous lactating Holstein-Friesian cows.

Coding Region	Type of SNP	rsid	Exon	Codon Change	Amino Acid Change	SIFT
Non-Synonymous	Missense	rs209518868	2	A**A**G/A**G**G	**K**/**R**	0.4

Table 3.18: Variant effect predictor analysis for SNP within UMPS in primiparous lactating Holstein-Friesian cows.

Coding Region	Type of SNP	rsid	Exon	Codon Change	Amino Acid Change	SIFT
Non-Synonymous	Missense	rs211151260	3	**C**GT/**T**GT	**R**/**C**	0.01

Table 3.19: Variant effect predictor analysis for SNP within DECR1 in primiparous lactating Holstein-Friesian cows.

Coding Region	Type of SNP	rsid	Exon	Codon Change	Amino Acid Change	SIFT
Non-Synonymous	Missense	rs41580472	5	**A**TA/**G**TA	**I/V**	0.06

Table 3.20: Variant effect predictor analysis for SNP within FASN in primiparous lactating Holstein-Friesian cows.

Coding Region	Type of SNP	rsid	Exon	Codon Change	Amino Acid Change	SIFT
Non-Synonymous	Missense	rs41919985	18	**A**CC/**G**CC	**T**/**A**	0.7

Table 3.21: Variant effect predictor analysis for SNP within BOLA-DMB in primiparous lactating Holstein-Friesian cows.

Coding Region	Type of SNP	rsid	Exon	Codon Change	Amino Acid Change	SIFT
Non-Synonymous	Missense	rs109032590	3	**A**TC/**G**TC	**I/V**	0.5

Table 3.22: Allele substitution estimates and fixed effect estimates of additive and dominance of the single nucleotide polymorphism within DSC2 in primiparous lactating Holstein-Friesian cows.

	Allele Substitution Effects				Fixed Effects		
Trait	n	*p*-value[b]	Estimate[c]	SE	*p*-value[d]	Additive Effect [e]	Dominant Effect [f]
A1C[a], days	466	0.01*	-10.09	4.1	0.03*	8.9	748.9

*Candidate single nucleotide polymorphism candidates (SNPs) that were statistically significant ($p \leq 0.05$) within models. [a]AIC=age at 1st calving. [b]*p*-values obtained from allele substitution analysis in SAS which included the term genotype as a covariate. [c]Estimates of the effect expressed in units of the traits. [d]*p*-values for fixed effects were obtained from the substitution of favorable allele analysis which included the genotype term as a fixed effect. [e]Additive effect was estimated as the difference between the 2 homozygous means divided by 2. [f]Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous. SNP=single nucleotide polymorphism.

Table 3.23: Allele substitution estimates and fixed effect estimates of additive and dominance of the single nucleotide polymorphism within SREBF1 in primiparous lactating Holstein-Friesian cows.

Trait	n	Allele Substitution Effects			Fixed Effects		
		p-value[b]	Estimate[c]	SE	*p*-value[d]	Additive Effect [e]	Dominant Effect [f]
D2C[a]	416	0.4	5.8	7.2	0.04	10.7	151.8
D21AI[a]	465	0.02*	4.7	2.08	0.01*	5.6	74.8
P150DIM[a]	465	0.04*	-0.06	0.03	0.01*	0.08	0.1

*Candidate single nucleotide polymorphism candidates (SNPs) that were statistically significant ($p \leq 0.05$) within models. [a]D2C=days to conception, D21AI=days to 1st artificial insemination and P150DIM=pregnant at 150 days in milk. [b]*p*-values obtained from allele substitution analysis in SAS which included the term genotype as a covariate. [c]Estimates of the effect expressed in units of the traits. [d]*p*-values for fixed effects were obtained from the substitution of favorable allele analysis which included the genotype term as a fixed effect. [e]Additive effect was estimated as the difference between the 2 homozygous means divided by 2. [f]Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous. SNP=single nucleotide polymorphism.

Table 3.24: Allele substitution estimates and fixed effect estimates of additive and dominance of the single nucleotide polymorphism within UBD in primiparous lactating Holstein-Friesian cows.

Trait	n	Allele Substitution Effects			Fixed Effects		
		p-value[b]	Estimate[c]	SE	*p*-value[d]	Additive Effect [e]	Dominant Effect [f]
D2C[a]	416	0.4	9.7	11.7	0.009	308.7	132.9

[a]D2C=days to conception. [b]*p*-values obtained from allele substitution analysis in SAS which included the term genotype as a covariate. [c]Estimates of the effect expressed in units of the traits. [d]*p*-values for fixed effects were obtained from the substitution of favorable allele analysis which included the genotype term as a fixed effect. [e]Additive effect was estimated as the difference between the 2 homozygous means divided by 2. [f]Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous. SNP=single nucleotide polymorphism.

Table 3.25: Allele substitution estimates and fixed effect estimates of additive and dominance of the single nucleotide polymorphism within UMPS in primiparous lactating Holstein-Friesian cows.

		Allele Substitution Effects			Fixed Effects		
Trait	n	*p*-value[b]	Estimate[c]	SE	*p*-value[d]	Additive Effect [e]	Dominant Effect [f]
D21AI[a]	463	0.5	1.2	2.2	0.04	5.01	74.2

[a]D21AI=days to 1st artificial insemination. [b]*p*-values obtained from allele substitution analysis in SAS which included the term genotype as a covariate. [c]Estimates of the effect expressed in units of the traits. [d]*p*-values for fixed effects were obtained from the substitution of favorable allele analysis which included the genotype term as a fixed effect. [e]Additive effect was estimated as the difference between the 2 homozygous means divided by 2. [f]Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous. SNP=single nucleotide polymorphism.

Table 3.26: Allele substitution estimates and fixed effect estimates of additive and dominance of the single nucleotide polymorphism within DECR1 in primiparous lactating Holstein-Friesian cows.

Trait	n	Allele Substitution Effects			Fixed Effects		
		p-value[b]	Estimate[c]	SE	*p*-value[d]	Additive Effect [e]	Dominant Effect [f]
P1AI[a]	465	0.05	0.06	0.03	0.01	0.1	0.1

[a]P1AI=pregnant at first artificial insemination. [b]*p*-values obtained from allele substitution analysis in SAS which included the term genotype as a covariate. [c]Estimates of the effect expressed in units of the traits. [d]*p*-values for fixed effects were obtained from the substitution of favorable allele analysis which included the genotype term as a fixed effect. [e]Additive effect was estimated as the difference between the 2 homozygous means divided by 2. [f]Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous. SNP=single nucleotide polymorphism.

Table 3.27: Allele substitution estimates and fixed effect estimates of additive and dominance of the single nucleotide polymorphism within FASN in primiparous lactating Holstein-Friesian cows.

		Allele Substitution Effects			Fixed Effects		
Trait	n	*p*-value[b]	Estimate[c]	SE	*p*-value[d]	Additive Effect [e]	Dominant Effect [f]
P1AI[a]	433	0.7	-0.01	0.03	0.01	0.08	0.1

[a]P1AI=pregnant at first artificial insemination. [b]*p*-values obtained from allele substitution analysis in SAS which included the term genotype as a covariate. [c]Estimates of the effect expressed in units of the traits. [d]*p*-values for fixed effects were obtained from the substitution of favorable allele analysis which included the genotype term as a fixed effect. [e]Additive effect was estimated as the difference between the 2 homozygous means divided by 2. [f]Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous. SNP=single nucleotide polymorphism.

Table 3.28: Allele substitution estimates and fixed effect estimates of additive and dominance of the single nucleotide polymorphism within BOLA-DMB in primiparous lactating Holstein-Friesian cows.

Trait	n	Allele Substitution Effects			Fixed Effects		
		p-value[b]	Estimate[c]	SE	*p*-value[d]	Additive Effect [e]	Dominant Effect [f]
P150DIM[a]	464	0.3	0.03	0.03	0.04	-0.07	0.07

[a]P150DIM=pregnant at 150 days in milk. [b]*p*-values obtained from allele substitution analysis in SAS which included the term genotype as a covariate. [c]Estimates of the effect expressed in units of the traits. [d]*p*-values for fixed effects were obtained from the substitution of favorable allele analysis which included the genotype term as a fixed effect. [e]Additive effect was estimated as the difference between the 2 homozygous means divided by 2. [f]Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous. SNP=single nucleotide polymorphism.

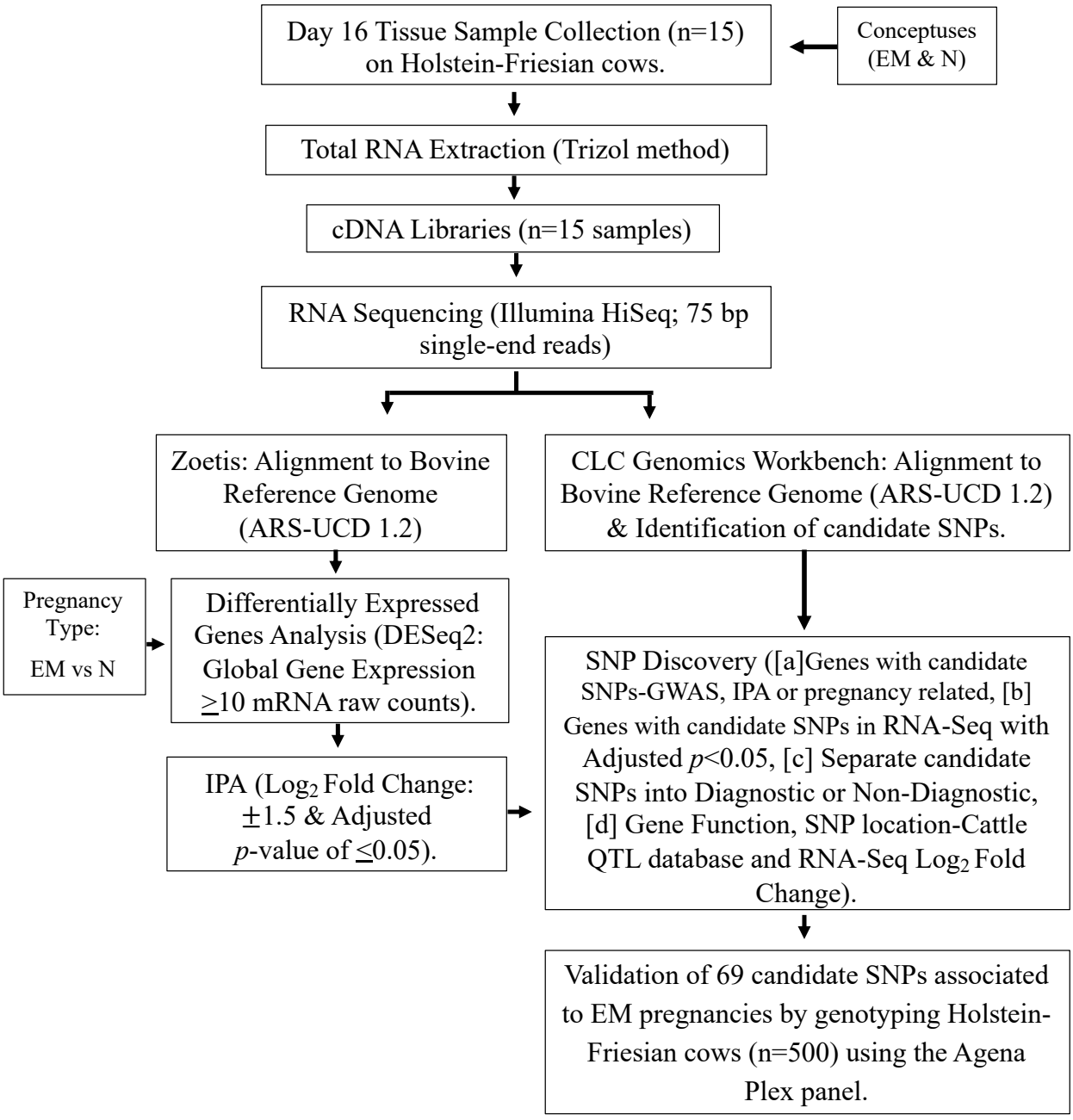


Figure 3.1: Flowchart of analytical steps from conceptus tissue collection to selection of genes with candidate single nucleotide polymorphism candidates.

SNPs=Single Nucleotide Polymorphisms; EM=Embryo Mortality; N=Normal pregnancy are the cow's pregnancy status.

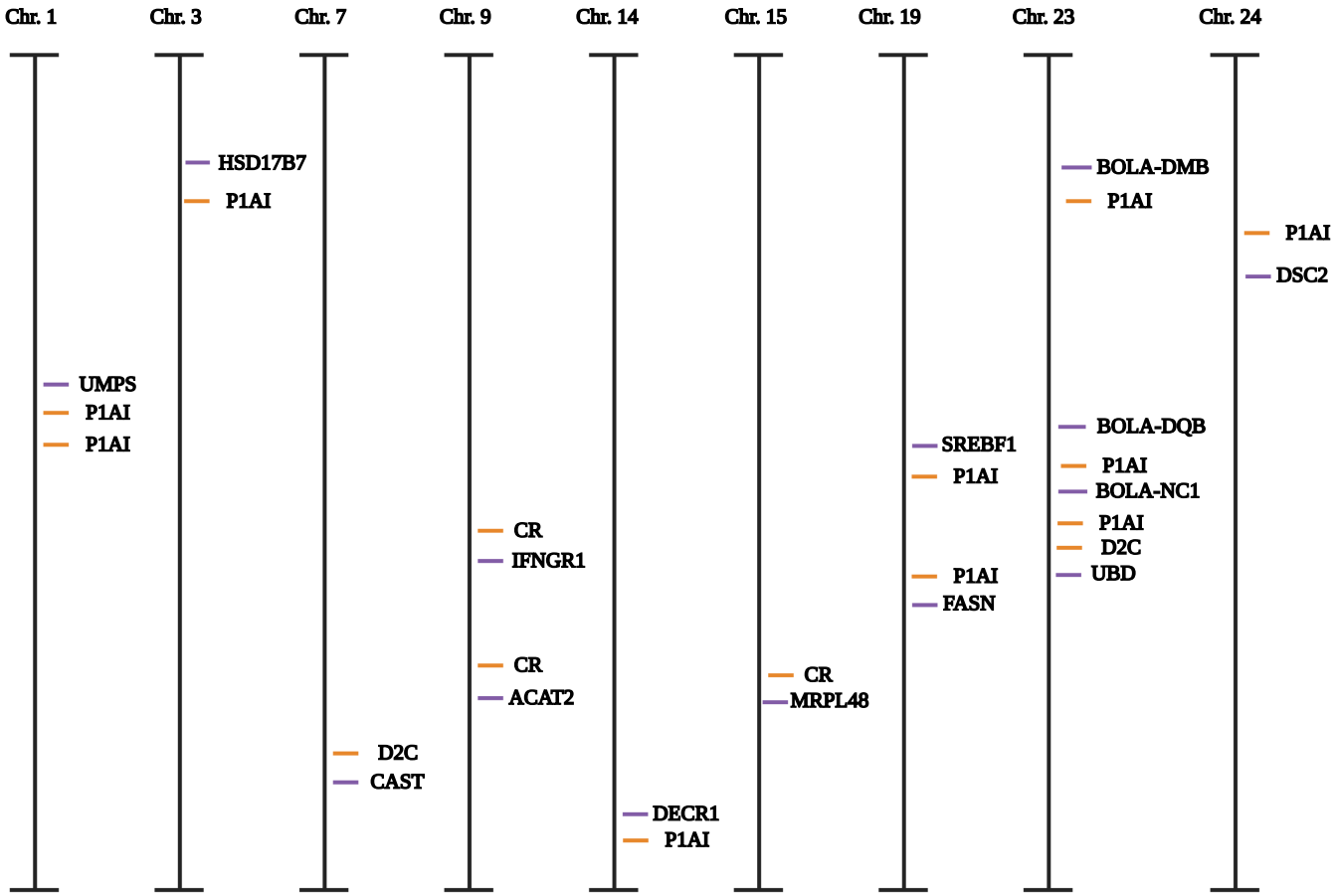


Figure 3.2: Candidate single nucleotide polymorphisms and published single nucleotide polymorphisms associated with quantitative trait loci within fertility for each chromosome.
Chr=chromosome, CR=conception rate, D2C=days to conception, and pregnant at 1st artificial insemination=P1AI. Orange lines= published SNPs associated with quantitative trait loci; purple lines= candidate SNPs. Created with Biorender.com.

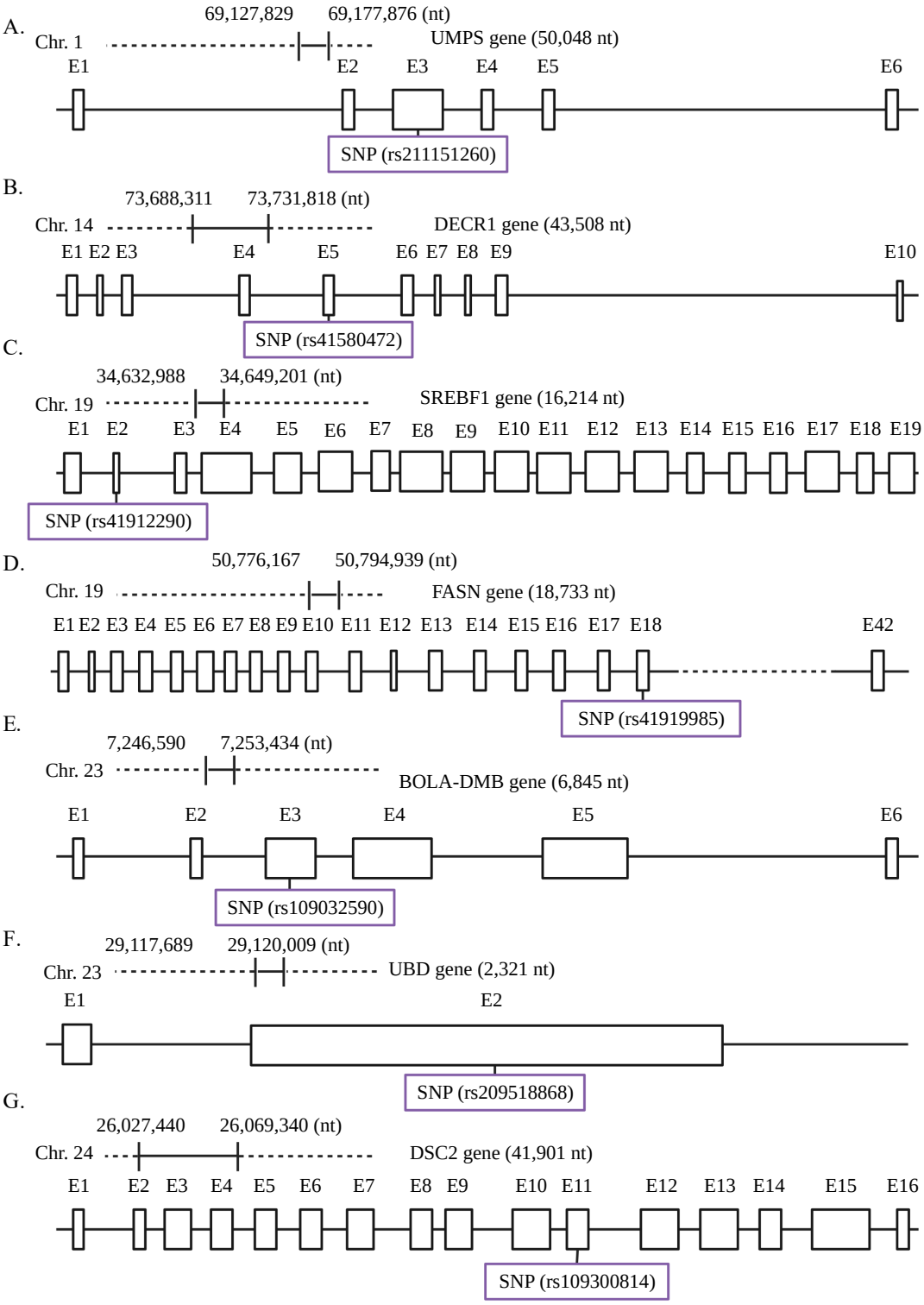


Figure 3.3: Seven potential functional single nucleotide polymorphisms (SNPs) within exons (E) of each gene: (A) UMPS, (B) DECR1, (C) SREBF1, (D) FASN, (E) BOLA-DMB, (F) UBD and (G) DSC2 in their designated chromosome.
Chr=Chromosome. Created with Biorender.com.

REFERENCES

Abdel-Azim, G. and A. E. Freeman. 2002. Superiority of QTL-Assisted Selection in Dairy Cattle Breeding Schemes. Journal of Dairy Science 85(7):1869-1880.

Agriculture, N. A. S. S.-U. S. D. o. 2022. Production per Cow, 2012-2021. National Agricultural Statistics Service-United States Department of Agriculture.

Alila, H. W. and W. Hansel. 1984. Origin of different cell types in the bovine corpus luteum as characterized by specific monoclonal antibodies. Biol Reprod 31(5):1015-1025.

Ambrose, D. J., J. P. Kastelic, R. Corbett, P. A. Pitney, H. V. Petit, J. A. Small, and P. Zalkovic. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in alpha-linolenic acid. J Dairy Sci 89(8):3066-3074.

Amos, W., E. Driscoll, and J. I. Hoffman. 2011. Candidate genes versus genome-wide associations: which are better for detecting genetic susceptibility to infectious disease? Proc Biol Sci 278(1709):1183-1188.

Antoniazzi, A. Q., B. T. Webb, J. J. Romero, R. L. Ashley, N. P. Smirnova, L. E. Henkes, R. C. Bott, J. F. Oliveira, G. D. Niswender, F. W. Bazer, and T. R. Hansen. 2013. Endocrine delivery of interferon tau protects the corpus luteum from prostaglandin F2 alpha-induced luteolysis in ewes. Biol Reprod 88(6):144.

Austin, K. J., A. L. Carr, J. K. Pru, C. E. Hearne, E. L. George, E. L. Belden, and T. R. Hansen. 2004. Localization of ISG15 and Conjugated Proteins in Bovine Endometrium Using Immunohistochemistry and Electron Microscopy. Endocrinology 145(2):967-975.

Austin, K. J., S. K. Ward, M. G. Teixeira, V. C. Dean, D. W. Moore, and T. R. Hansen. 1996. Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. Biol Reprod 54(3):600-606.

Barillet, F. 2007. Genetic improvement for dairy production in sheep and goats. Small Ruminant Research 70(1):60-75.

Barnwell, C. V., P. W. Farin, C. M. Ashwell, W. T. Farmer, S. P. Galphin, Jr., and C. E. Farin. 2016. Differences in mRNA populations of short and long bovine conceptuses on Day 15 of gestation. Mol Reprod Dev 83(5):424-441.

Barrett, J. W., C. A. Lloyd, and R. A. Carpenter. 1948. Order number of insemination and conception rate. J. Dairy Sci. 31:683.

Bartolome, J. A., J. J. van Leeuwen, M. Thieme, O. G. Sa'filho, P. Melendez, L. F. Archbald, and W. W. Thatcher. 2009. Synchronization and resynchronization of inseminations in lactating dairy cows with the CIDR insert and the Ovsynch protocol. Theriogenology 72(6):869-878.

Baruselli, P. S., M. F. de Sá Filho, C. M. Martins, L. F. Nasser, M. F. Nogueira, C. M. Barros, and G. A. Bó. 2006. Superovulation and embryo transfer in Bos indicus cattle. Theriogenology 65(1):77-88.

Bauersachs, S., S. E. Ulbrich, H. D. Reichenbach, M. Reichenbach, M. Buttner, H. H. Meyer, T. E. Spencer, M. Minten, G. Sax, G. Winter, and E. Wolf. 2012. Comparison of the effects of early

pregnancy with human interferon, alpha 2 (IFNA2), on gene expression in bovine endometrium. Biol Reprod 86(2):46.
Bauersachs, S., E. Ulbrich Susanne, V. Zakhartchenko, M. Minten, M. Reichenbach, H.-D. Reichenbach, H. Blum, E. Spencer Thomas, and E. Wolf. 2009. The endometrium responds differently to cloned versus fertilized embryos. Proceedings of the National Academy of Sciences 106(14):5681-5686.

Bauersachs, S. and E. Wolf. 2012. Transcriptome analyses of bovine, porcine and equine endometrium during the pre-implantation phase. Anim Reprod Sci 134(1-2):84-94.

Bazer, F. W., T. T. Chen, J. W. Knight, D. Schlosnagle, N. J. Baldwin, and R. M. Roberts. 1975. Presence of a progesterone-induced, uterine specific, acid phosphatase in allantoic fluid of gilts. J Anim Sci 41(4):1112-1119.

Bazer, F. W., M. A. Mirando, T. L. Ott, J. P. Harney, D. H. Dubois, T. K. Schalue, C. H. Pontzer, C. Hostetler, H. M. Johnson, and T. Ogle. 1992. Roles of ovine trophoblast protein-1 and oestradiol/prolactin in the establishment of pregnancy in sheep and pigs. Reprod Fertil Dev 4(3):335-340.

Bazer, F. W., T. E. Spencer, G. A. Johnson, and R. C. Burghardt. 2011. Uterine receptivity to implantation of blastocysts in mammals. Front Biosci (Schol Ed) 3(2):745-767.

Bazer, F. W., T. E. Spencer, and T. L. Ott. 1997. Interferon tau: a novel pregnancy recognition signal. Am J Reprod Immunol 37(6):412-420.

Bazer, F. W., W. W. Thatcher, P. J. Hansen, M. A. Mirando, T. L. Ott, and C. Plante. 1991. Physiological mechanisms of pregnancy recognition in ruminants. J Reprod Fertil Suppl 43:39-47.

Bazer, F. W., G. Wu, T. E. Spencer, G. A. Johnson, R. C. Burghardt, and K. Bayless. 2010. Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. Mol Hum Reprod 16(3):135-152.

Bellows, D. S., S. L. Ott, and R. A. Bellows. 2002. Review: Cost of Reproductive Diseases and Conditions in Cattle11Portions of this research were conducted under a cooperative agreement between USDA, ARS and the Montana Agric. Exp. Stn. Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, Montana Agric. Exp. Stn., or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA, ARS, Northern Plains Area, is an equal opportunity/affirmative action employer, and all agency services are available without discrimination. The Professional Animal Scientist 18(1):26-32.

Beltman, M. E., N. Forde, P. Furney, F. Carter, J. F. Roche, P. Lonergan, and M. A. Crowe. 2010. Characterisation of endometrial gene expression and metabolic parameters in beef heifers yielding viable or non-viable embryos on Day 7 after insemination. Reprod Fertil Dev 22(6):987-999.

Berg, D. K., J. van Leeuwen, S. Beaumont, M. Berg, and P. L. Pfeffer. 2010. Embryo loss in cattle between Days 7 and 16 of pregnancy. Theriogenology 73(2):250-260.

Bernard, C., J. P. Valet, R. Béland, and R. D. Lambert. 1983. Prediction of bovine ovulation by a rapid radioimmunoassay for plasma LH. J Reprod Fertil 68(2):425-430.

Berry, D. P., F. Buckley, P. Dillon, R. D. Evans, M. Rath, and R. F. Veerkamp. 2003. Genetic parameters for body condition score, body weight, milk yield, and fertility estimated using random regression models. J Dairy Sci 86(11):3704-3717.
Berry, D. P., B. Coughlan, B. Enright, S. Coughlan, and M. Burke. 2013. Factors associated with milking characteristics in dairy cows. J Dairy Sci 96(9):5943-5953.

Berry, D. P., P. Lonergan, S. T. Butler, A. R. Cromie, T. Fair, F. Mossa, and A. C. Evans. 2008. Negative influence of high maternal milk production before and after conception on offspring survival and milk production in dairy cattle. J Dairy Sci 91(1):329-337.

Berry, D. P., E. Wall, and J. E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8 Suppl 1:105-121.

Betteridge, K. J., M. D. Eaglesome, G. C. Randall, and D. Mitchell. 1980. Collection, description and transfer of embryos from cattle 10--16 days after oestrus. J Reprod Fertil 59(1):205-216.

Bilodeau-Goeseels, S. 2003. Effect of oocyte quality on the relative abundance of specific gene transcripts in bovine mature oocytes and 16-cell embryos. Can J Vet Res 67(2):151-156.

Bilodeau-Goeseels, S. and J. P. Kastelic. 2003. Factors affecting embryo survival and strategies to reduce embryonic mortality in cattle. Canadian Journal of Animal Science 83(4):659-671.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J Dairy Sci 87(10):3105-3124.

Boichard, D. and E. Manfredi. 1994. Genetic Analysis of Conception Rate in French Holstein Cattle. Acta Agriculturae Scandinavica, Section A — Animal Science 44(3):138-145.

Bols, P. E. J. and T. A. E. Stout. 2018. Transvaginal Ultrasound-Guided Oocyte Retrieval (OPU: Ovum Pick-Up) in Cows and Mares.

Boni, R., Tosti, E., Roviello, S. and Dale, B. 1999. Intercellular communication in in vivo- and in vitro-produced bovine embryos. Biology of Reproduction 61(4):1050-1055.

Bott, R. C., R. L. Ashley, L. E. Henkes, A. Q. Antoniazzi, J. E. Bruemmer, G. D. Niswender, F. W. Bazer, T. E. Spencer, N. P. Smirnova, R. V. Anthony, and T. R. Hansen. 2010. Uterine vein infusion of interferon tau (IFNT) extends luteal life span in ewes. Biol Reprod 82(4):725-735.

Bouchard E, T. D. 2008. Dairy herd production and reproduction in Quebec and Canada. in Proc. World Buiatrics Congress, Budapest, Hungary.

Boulton AC, R. J., Wathes DC. 2015. A study of dairy heifer rearing practices from birth to weaning and their associated costs on uk dairy farms. Open Journal of Animal Sciences (5):185-197.

Boussaha, M., D. Esquerré, J. Barbieri, A. Djari, A. Pinton, R. Letaief, G. Salin, F. Escudié, A. Roulet, S. Fritz, F. Samson, C. Grohs, M. Bernard, C. Klopp, D. Boichard, and D. Rocha. 2015. Genome-Wide Study of Structural Variants in Bovine Holstein, Montbéliarde and Normande Dairy Breeds. PLoS One 10(8):e0135931.

Bovine HapMap, C., R. A. Gibbs, J. F. Taylor, C. P. Van Tassell, W. Barendse, K. A. Eversole, C. A. Gill, R. D. Green, D. L. Hamernik, S. M. Kappes, S. Lien, L. K. Matukumalli, J. C. McEwan, L. V. Nazareth, R. D. Schnabel, G. M. Weinstock, D. A. Wheeler, P. Ajmone-Marsan, P. J. Boettcher, A. R. Caetano, J. F. Garcia, O. Hanotte, P. Mariani, L. C. Skow, T. S. Sonstegard, J. L.

Williams, B. Diallo, L. Hailemariam, M. L. Martinez, C. A. Morris, L. O. C. Silva, R. J. Spelman, W. Mulatu, K. Zhao, C. A. Abbey, M. Agaba, F. R. Araujo, R. J. Bunch, J. Burton, C. Gorni, H. Olivier, B. E. Harrison, B. Luff, M. A. Machado, J. Mwakaya, G. Plastow, W. Sim, T. Smith, M. B. Thomas, A. Valentini, P. Williams, J. Womack, J. A. Woolliams, Y. Liu, X. Qin, K. C. Worley, C. Gao, H. Jiang, S. S. Moore, Y. Ren, X.-Z. Song, C. D. Bustamante, R. D. Hernandez, D. M. Muzny, S. Patil, A. San Lucas, Q. Fu, M. P. Kent, R. Vega, A. Matukumalli, S. McWilliam, G. Sclep, K. Bryc, J. Choi, H. Gao, J. J. Grefenstette, B. Murdoch, A. Stella, R. Villa-Angulo, M. Wright, J. Aerts, O. Jann, R. Negrini, M. E. Goddard, B. J. Hayes, D. G. Bradley, M. Barbosa da Silva, L. P. L. Lau, G. E. Liu, D. J. Lynn, F. Panzitta, and K. G. Dodds. 2009. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. Science 324(5926):528-532.

Brandão, D. O., P. Maddox-Hyttel, P. Løvendahl, R. Rumpf, D. Stringfellow, and H. Callesen. 2004. Post hatching development: a novel system for extended in vitro culture of bovine embryos. Biol Reprod 71(6):2048-2055.

C. Bartlett, P., J. H. Kirk, M. A. Wilke, J. B. Kaneene, and E. C. Mather. 1986. Metritis complex in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. Preventive Veterinary Medicine 4(3):235-248.

Cabrera, V. E. 2014. Economics of fertility in high-yielding dairy cows on confined TMR systems. Animal 8 Suppl 1:211-221.

Carter, F., N. Forde, P. Duffy, M. Wade, T. Fair, M. A. Crowe, A. C. Evans, D. A. Kenny, J. F. Roche, and P. Lonergan. 2008. Effect of increasing progesterone concentration from Day 3 of pregnancy on subsequent embryo survival and development in beef heifers. Reprod Fertil Dev 20(3):368-375.

Cavestany, D. and R. H. Foote. 1985. Reproductive performance of Holstein cows administered GnRH analog HOE 766 (Buserelin) 26 to 34 days postpartum. J Anim Sci 61(1):224-233.

Ceciliani, F., C. Lecchi, C. Urh, and H. Sauerwein. 2018. Proteomics and metabolomics characterizing the pathophysiology of adaptive reactions to the metabolic challenges during the transition from late pregnancy to early lactation in dairy cows. J Proteomics 178:92-106.

Cerri, R. L., I. M. Thompson, I. H. Kim, A. D. Ealy, P. J. Hansen, C. R. Staples, J. L. Li, J. E. Santos, and W. W. Thatcher. 2012. Effects of lactation and pregnancy on gene expression of endometrium of Holstein cows at day 17 of the estrous cycle or pregnancy. J Dairy Sci 95(10):5657-5675.

Chaouat, G., N. Ledée-Bataille, K. B. Chea, and S. Dubanchet. 2005. Cytokines and implantation. Chem Immunol Allergy 88:34-63.

Chaouat, G., N. Ledée-Bataille, S. Dubanchet, S. Zourbas, O. Sandra, and J. Martal. 2004. Th1/Th2 Paradigm in Pregnancy: Paradigm Lost? International Archives of Allergy and Immunology 134(2):93-119.

Chebel, R. C., J. E. Santos, J. P. Reynolds, R. L. Cerri, S. O. Juchem, and M. Overton. 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim Reprod Sci 84(3-4):239-255.

Chebel, R. C., P. R. B. Silva, M. I. Endres, M. A. Ballou, and K. L. Luchterhand. 2016. Social stressors and their effects on immunity and health of periparturient dairy cows1. Journal of Dairy Science 99(4):3217-3228.

Clemente, M., J. de La Fuente, T. Fair, A. Al Naib, A. Gutierrez-Adan, J. F. Roche, D. Rizos, and P. Lonergan. 2009. Progesterone and conceptus elongation in cattle: a direct effect on the embryo or an indirect effect via the endometrium? Reproduction 138(3):507-517.

Clempson, A. M., G. E. Pollott, J. S. Brickell, and D. C. Wathes. 2012. Associations between bovine IGFBP2 polymorphisms with fertility, milk production, and metabolic status in UK dairy cows. Anim Biotechnol 23(2):101-113.

Cochran, S. D., J. B. Cole, D. J. Null, and P. J. Hansen. 2013. Discovery of single nucleotide polymorphisms in candidate genes associated with fertility and production traits in Holstein cattle. BMC Genet 14:49.

Cole, J. B. and P. M. VanRaden. 2018. Symposium review: Possibilities in an age of genomics: The future of selection indices. J Dairy Sci 101(4):3686-3701.

Consortium, T. U. 2021. UniProt: the universal protein knowledgebase in 2021. 2021 ed, Uniprot.

Corcoran, D., T. Fair, S. Park, D. Rizos, O. V. Patel, G. W. Smith, P. M. Coussens, J. J. Ireland, M. P. Boland, A. C. Evans, and P. Lonergan. 2006. Suppressed expression of genes involved in transcription and translation in in vitro compared with in vivo cultured bovine embryos. Reproduction 131(4):651-660.

Correa, M. T., H. Erb, and J. Scarlett. 1993. Path analysis for seven postpartum disorders of Holstein cows. J Dairy Sci 76(5):1305-1312.

Crosier, A. E., P. W. Farin, M. J. Dykstra, J. E. Alexander, and C. E. Farin. 2000. Ultrastructural morphometry of bovine compact morulae produced in vivo or in vitro. Biol Reprod 62(5):1459-1465.

Crowe, M. A. 1999. Gonadotropic control of terminal follicular growth in cattle. Reproduction of Domestic Animals 34:157-166.

Crowe, M. A. 2008. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Reprod Domest Anim 43 Suppl 5:20-28.

Daetwyler, H. D., F. S. Schenkel, M. Sargolzaei, and J. A. Robinson. 2008. A genome scan to detect quantitative trait loci for economically important traits in Holstein cattle using two methods and a dense single nucleotide polymorphism map. J Dairy Sci 91(8):3225-3236.

Darwash, A. O., G. E. Lamming, and J. A. Wooliams. 1997. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. Animal Science 65(1):9-16.

De Vries, A. 2006. Economic value of pregnancy in dairy cattle. J Dairy Sci 89(10):3876-3885.
Dickson, M. J., J. V. Bishop, T. R. Hansen, I. M. Sheldon, and J. J. Bromfield. 2022. The endometrial transcriptomic response to pregnancy is altered in cows after uterine infection. PLoS One 17(3):e0265062.

Dickson, M. J., R. L. Piersanti, R. Ramirez-Hernandez, E. B. de Oliveira, J. V. Bishop, T. R. Hansen, Z. Ma, K. C. C. Jeong, J. E. P. Santos, M. I. Sheldon, J. Block, and J. J. Bromfield. 2020. Experimentally Induced Endometritis Impairs the Developmental Capacity of Bovine Oocytes†. Biology of reproduction 103(3):508-520.

Diskin, M. G. and D. G. Morris. 2008. Embryonic and early foetal losses in cattle and other ruminants. Reprod Domest Anim 43 Suppl 2:260-267.
Diskin, M. G., J. J. Murphy, and J. M. Sreenan. 2006. Embryo survival in dairy cows managed under pastoral conditions. Anim Reprod Sci 96(3-4):297-311.

Diskin, M. G., M. H. Parr, and D. G. Morris. 2011. Embryo death in cattle: an update. Reprod Fertil Dev 24(1):244-251.

Dohoo, I. R. 1983. The effects of calving to first service interval on reproductive performance in normal cows and cows with postpartal disease. Can Vet J 24(11):343-346.

Dohoo, I. R. and S. W. Martin. 1984. Subclinical ketosis: prevalence and associations with production and disease. Can J Comp Med 48(1):1-5.

Dorniak, P., F. W. Bazer, and T. E. Spencer. 2013. Physiology and Endocrinology Symposium: biological role of interferon tau in endometrial function and conceptus elongation. J Anim Sci 91(4):1627-1638.

Driancourt, M. A., Gougeon, A., Royere, A. and Thibault, C. 1993. Ovarian Function. Reproduction in Mammals and Man. Ellipses, Paris, France.

Ealy, A. D., M. Drost, and P. J. Hansen. 1993. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. J Dairy Sci 76(10):2899-2905.

Eastham, N. T., A. Coates, P. Cripps, H. Richardson, R. Smith, and G. Oikonomou. 2018. Associations between age at first calving and subsequent lactation performance in UK Holstein and Holstein-Friesian dairy cows. PLoS One 13(6):e0197764.

Edwards, J. L., W. A. King, S. J. Kawarsky, and A. D. Ealy. 2001. Responsiveness of early embryos to environmental insults: potential protective roles of HSP70 and glutathione. Theriogenology 55(1):209-223.

El-Sayed, A., M. Hoelker, F. Rings, D. Salilew, D. Jennen, E. Tholen, M. A. Sirard, K. Schellander, and D. Tesfaye. 2006. Large-scale transcriptional analysis of bovine embryo biopsies in relation to pregnancy success after transfer to recipients. Physiol Genomics 28(1):84-96.

Elenkov, I. J. 2004. Glucocorticoids and the Th1/Th2 balance. Ann N Y Acad Sci 1024:138-146.

Elis, S., S. Coyral-Castel, S. Freret, J. Cognié, A. Desmarchais, A. Fatet, C. Rame, E. Briant, V. Maillard, and J. Dupont. 2013. Expression of adipokine and lipid metabolism genes in adipose tissue of dairy cows differing in a female fertility quantitative trait locus. J Dairy Sci 96(12):7591-7602.

England, P. C., L. G. Skinner, K. M. Cottrell, and R. A. Sellwood. 1974. Serum oestradiol-17 beta in normal women. Br J Cancer 29(6):462-469.

Esslemont, R. J. 1992. Measuring dairy herd fertility. Vet Rec 131(10):209-212.

Ettema, J. F. and J. E. Santos. 2004. Impact of age at calving on lactation, reproduction, health, and income in first-parity Holsteins on commercial farms. J Dairy Sci 87(8):2730-2742.

Evans, A. C., F. Mossa, S. W. Walsh, D. Scheetz, F. Jimenez-Krassel, J. L. Ireland, G. W. Smith, and J. J. Ireland. 2012. Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. Reprod Domest Anim 47 Suppl 4:31-37.

Fair, T. 2015. The contribution of the maternal immune system to the establishment of pregnancy in cattle. Front Immunol 6:7.

Farin, C. E., K. Imakawa, and R. M. Roberts. 1989. In situ localization of mRNA for the interferon, ovine trophoblast protein-1, during early embryonic development of the sheep. Molecular Endocrinology 3:1099-1107.

Farin, C. E., T. M. Nett, and G. D. Niswender. 1990. Effects of luteinizing hormone on luteal cell populations in hypophysectomized ewes. J Reprod Fertil 88(1):61-70.

Farin, P. W., A. E. Crosier, and C. E. Farin. 2001. Influence of in vitro systems on embryo survival and fetal development in cattle. Theriogenology 55(1):151-170.

Ferguson, C. E. 2004. The use of altrenogest to control reproductive function in beef cattle. Louisiana State University and Agricultural & Mechanical College.

Ferguson, C. E., D. J. Kesler, and R. A. Godke. 2012. Progesterone enhances in vitro development of bovine embryos. Theriogenology 77(1):108-114.

Fessenden, B., D. J. Weigel, J. Osterstock, D. T. Galligan, and F. Di Croce. 2020. Validation of genomic predictions for a lifetime merit selection index for the US dairy industry. Journal of Dairy Science 103(11):10414-10428.

Firk, R., E. Stamer, W. Junge, and J. Krieter. 2002. Automation of oestrus detection in dairy cows: a review. Livestock Production Science 75(3):219-232.

Forde, N., M. E. Beltman, P. Lonergan, M. Diskin, J. F. Roche, and M. A. Crowe. 2011a. Oestrous cycles in Bos taurus cattle. Anim Reprod Sci 124(3-4):163-169.

Forde, N., F. Carter, T. Fair, M. A. Crowe, A. C. Evans, T. E. Spencer, F. W. Bazer, R. McBride, M. P. Boland, P. O'Gaora, P. Lonergan, and J. F. Roche. 2009. Progesterone-regulated changes in endometrial gene expression contribute to advanced conceptus development in cattle. Biol Reprod 81(4):784-794.

Forde, N., F. Carter, T. E. Spencer, F. W. Bazer, O. Sandra, N. Mansouri-Attia, L. A. Okumu, P. A. McGettigan, J. P. Mehta, R. McBride, P. O'Gaora, J. F. Roche, and P. Lonergan. 2011b. Conceptus-induced changes in the endometrial transcriptome: how soon does the cow know she is pregnant? Biol Reprod 85(1):144-156.

Forde, N., G. B. Duffy, P. A. McGettigan, J. A. Browne, J. P. Mehta, A. K. Kelly, N. Mansouri-Attia, O. Sandra, B. J. Loftus, M. A. Crowe, T. Fair, J. F. Roche, P. Lonergan, and A. C. Evans. 2012a. Evidence for an early endometrial response to pregnancy in cattle: both dependent upon and independent of interferon tau. Physiol Genomics 44(16):799-810.

Forde, N. and P. Lonergan. 2017. Interferon-tau and fertility in ruminants. Reproduction 154(5):F33-F43.

Forde, N., P. A. McGettigan, J. P. Mehta, L. O'Hara, S. Mamo, F. W. Bazer, T. E. Spencer, and P. Lonergan. 2014. Proteomic analysis of uterine fluid during the pre-implantation period of pregnancy in cattle. Reproduction 147(5):575-587.

Forde, N., J. P. Mehta, M. Minten, M. A. Crowe, J. F. Roche, T. E. Spencer, and P. Lonergan. 2012b. Effects of low progesterone on the endometrial transcriptome in cattle. Biol Reprod 87(5):124.

Forde, N., C. A. Simintiras, R. G. Sturmey, A. Graf, E. Wolf, H. Blum, and P. Lonergan. 2017. Effect of lactation on conceptus-maternal interactions at the initiation of implantation in cattle: I. Effects on the conceptus transcriptome and amino acid composition of the uterine luminal fluid. Biol Reprod 97(6):798-809.

Friedman, S. L. 2015. Hepatic Fibrosis: Emerging Therapies. Digestive Diseases 33(4):504-507.

Friggens, N. C., C. Disenhaus, and H. V. Petit. 2010. Nutritional sub-fertility in the dairy cow: towards improved reproductive management through a better biological understanding. Animal 4(7):1197-1213.

Gabler, M. T., P. R. Tozer, and A. J. Heinrichs. 2000. Development of a cost analysis spreadsheet for calculating the costs to raise a replacement dairy heifer. J Dairy Sci 83(5):1104-1109.

Gad, A., M. Hoelker, U. Besenfelder, V. Havlicek, U. Cinar, F. Rings, E. Held, I. Dufort, M. A. Sirard, K. Schellander, and D. Tesfaye. 2012. Molecular mechanisms and pathways involved in bovine embryonic genome activation and their regulation by alternative in vivo and in vitro culture conditions. Biol Reprod 87(4):100.

Garbarino, E. J., J. A. Hernandez, J. K. Shearer, C. A. Risco, and W. W. Thatcher. 2004. Effect of lameness on ovarian activity in postpartum holstein cows. J Dairy Sci 87(12):4123-4131.

García-Ruiz, A., J. B. Cole, P. M. VanRaden, G. R. Wiggans, F. J. Ruiz-López, and C. P. V. Tassell. 2016a. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Proceedings of the National Academy of Sciences 113(28):E3995-E4004.

García-Ruiz, A., J. B. Cole, P. M. VanRaden, G. R. Wiggans, F. J. Ruiz-López, and C. P. Van Tassell. 2016b. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Proc Natl Acad Sci U S A 113(28):E3995-4004.

Garrett, J. E., R. D. Geisert, M. T. Zavy, L. K. Gries, R. P. Wettemann, and D. S. Buchanan. 1988. Effect of exogenous progesterone on prostaglandin F2 alpha release and the interestrous interval in the bovine. Prostaglandins 36(1):85-96.

Gifford, C. A., K. Racicot, D. S. Clark, K. J. Austin, T. R. Hansen, M. C. Lucy, C. J. Davies, and T. L. Ott. 2007. Regulation of interferon-stimulated genes in peripheral blood leukocytes in pregnant and bred, nonpregnant dairy cows. J Dairy Sci 90(1):274-280.

Giordano, J. O., P. M. Fricke, M. C. Wiltbank, and V. E. Cabrera. 2011. An economic decision-making support system for selection of reproductive management programs on dairy farms. J Dairy Sci 94(12):6216-6232.

Godkin, J. D., F. W. Bazer, W. W. Thatcher, and R. M. Roberts. 1984. Proteins released by cultured Day 15–16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. Reproduction 71(1):57-64.

Goff, A. K. and L. C. Smith. 1998. Effect of steroid treatment of endometrial cells on blastocyst development during co-culture. Theriogenology 49(5):1021-1030.

González-Recio, O., M. A. Pérez-Cabal, and R. Alenda. 2004. Economic value of female fertility and its relationship with profit in Spanish dairy cattle. J Dairy Sci 87(9):3053-3061.

Gordon, R. and C. A. Melvin. 2003. Reverse engineering the embryo: a graduate course in developmental biology for engineering students at the University of Manitoba, Canada. Int J Dev Biol 47(2-3):183-187.
Goshen, T., K. Tsitrin, and M. van Straten. 2015. Reproductive Performance in Dairy Cows Synchronized with the Ovsynch Protocol at Different Stages of the Estrus Cycle. Israel Journal of Veterinary Medicine 70:22-27.

Graf, A., S. Krebs, M. Heininen-Brown, V. Zakhartchenko, H. Blum, and E. Wolf. 2014a. Genome activation in bovine embryos: review of the literature and new insights from RNA sequencing experiments. Anim Reprod Sci 149(1-2):46-58.

Graf, A., S. Krebs, V. Zakhartchenko, B. Schwalb, H. Blum, and E. Wolf. 2014b. Fine mapping of genome activation in bovine embryos by RNA sequencing. Proc Natl Acad Sci U S A 111(11):4139-4144.

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. 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):1624-1631.

Green, J. C., C. S. Okamura, S. E. Poock, and M. C. Lucy. 2010. Measurement of interferon-tau (IFN-tau) stimulated gene expression in blood leukocytes for pregnancy diagnosis within 18-20d after insemination in dairy cattle. Anim Reprod Sci 121(1-2):24-33.

Griffin, J. F., P. J. Hartigan, and W. R. Nunn. 1974. Non-specific uterine infection and bovine fertility. I. Infection patterns and endometritis during the first seven weeks post-partum. Theriogenology 1(3):91-106.

Griffin, P. and O. J. Ginther. 1991. Dynamics of uterine diameter and endometrial morphology during the estrous cycle and early pregnancy in mares. Animal Reproduction Science 25:133-142.

Grimard, B., S. Freret, A. Chevallier, A. Pinto, C. Ponsart, and P. Humblot. 2006. Genetic and environmental factors influencing first service conception rate and late embryonic/foetal mortality in low fertility dairy herds. Anim Reprod Sci 91(1-2):31-44.

Gröhn, Y. T. and P. J. Rajala-Schultz. 2000. Epidemiology of reproductive performance in dairy cows. Anim Reprod Sci 60-61:605-614.

Guillomot, M., C. Michel, P. Gaye, N. Charlier, J. Trojan, and J. Martal. 1990. Cellular localization of an embryonic interferon, ovine trophoblastin and its mRNA in sheep embryos during early pregnancy. Biology of the Cell 68:205-211.

Gustavsson, I. 1979. Distribution and effects of the 1/29 Robertsonian translocation in cattle. J Dairy Sci 62(5):825-835.

Han, H., K. J. Austin, L. A. Rempel, and T. R. Hansen. 2006a. Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. Journal of Endocrinology 191(2):505-512.

Han, H., K. J. Austin, L. A. Rempel, and T. R. Hansen. 2006b. Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. J Endocrinol 191(2):505-512.

Hansen, P. J. 2006. Realizing the promise of IVF in cattle--an overview. Theriogenology 65(1):119-125.

Hansen, P. J. 2009. Effects of heat stress on mammalian reproduction. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 364(1534):3341-3350.

Hansen, P. J. 2011. The immunology of early pregnancy in farm animals. Reprod Domest Anim 46 Suppl 3:18-30.

Hansen, P. J. and P. Tríbulo. 2019. Regulation of present and future development by maternal regulatory signals acting on the embryo during the morula to blastocyst transition - insights from the cow. Biol Reprod 101(3):526-537.

Hansen, T. R., L. K. Henkes, R. L. Ashley, R. C. Bott, A. Q. Antoniazzi, and H. Han. 2010. Endocrine actions of interferon-tau in ruminants. Soc Reprod Fertil Suppl 67:325-340.

Hansen, T. R., M. Kazemi, D. H. Keisler, P.-V. Malathy, K. Imakawa, and R. M. Roberts. 1989. Complex Binding of the Embryonic Interferon, Ovine Trophoblast Protein-1, to Endometrial Receptors. Journal of Interferon Research 9(2):215-225.

Hansen, T. R. and J. K. Pru. 2014. ISGylation: a conserved pathway in mammalian pregnancy. Adv Exp Med Biol 759:13-31.

Hansen, T. R., L. D. P. Sinedino, and T. E. Spencer. 2017. Paracrine and endocrine actions of interferon tau (IFNT). Reproduction 154(5):F45-f59.

Harman, J. L., G. Casella, and Y. T. Gröhn. 1996. The application of event-time regression techniques to the study of dairy cow interval-to-conception. Preventive Veterinary Medicine 26(3):263-274.

Harvatine, K. J. and D. E. Bauman. 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. J Nutr 136(10):2468-2474.

Havlicek, V., F. Wetscher, T. Huber, G. Brem, M. Mueller, and U. Besenfelder. 2005. In vivo culture of IVM/IVF embryos in bovine oviducts by transvaginal endoscopy. J Vet Med A Physiol Pathol Clin Med 52(2):94-98.

Healey, M. H. and R. D. Shanks. 1987. Performance of females heterozygous for deficiency of uridine monophosphate synthase. J Dairy Sci 70(5):945-951.

Heinrichs, A. J. 1993. Raising dairy replacements to meet the needs of the 21st century. J Dairy Sci 76(10):3179-3187.

Hochberg, Y. B. a. Y. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Royal Statistical Society 57(1):289-300.

Hoffman, P. C. 1997. Optimum body size of Holstein replacement heifers. J Anim Sci 75(3):836-845.

Horan, B., P. Dillon, P. Faverdin, L. Delaby, F. Buckley, and M. Rath. 2005. The interaction of strain of Holstein-Friesian cows and pasture-based feed systems on milk yield, body weight, and body condition score. J Dairy Sci 88(3):1231-1243.

Howell, J. L., J. W. Fuquay, and A. E. Smith. 1994. Corpus luteum growth and function in lactating Holstein cows during spring and summer. J Dairy Sci 77(3):735-739.
Huang, W., B. W. Kirkpatrick, G. J. Rosa, and H. Khatib. 2010. A genome-wide association study using selective DNA pooling identifies candidate markers for fertility in Holstein cattle. Anim Genet 41(6):570-578.

Hue, I., S. A. Degrelle, E. Campion, and J. P. Renard. 2007. Gene expression in elongating and gastrulating embryos from ruminants. Soc Reprod Fertil Suppl 64:365-377.

Hue, I., S. A. Degrelle, and N. Turenne. 2012. Conceptus elongation in cattle: genes, models and questions. Anim Reprod Sci 134(1-2):19-28.

Hussein, H. A., K. A. E.-H. A. El-Razik, A. M. Gomaa, M. K. Elbayoumy, K. A. Abdelrahman, and H. I. Hosein. 2018. Milk amyloid A as a biomarker for diagnosis of subclinical mastitis in cattle. Veterinary world 11(1):34-41.

Huzzey, J. M., D. M. Veira, D. M. Weary, and M. A. von Keyserlingk. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J Dairy Sci 90(7):3220-3233.

Inchaisri, C., R. Jorritsma, P. L. Vos, G. C. van der Weijden, and H. Hogeveen. 2010. Economic consequences of reproductive performance in dairy cattle. Theriogenology 74(5):835-846.

Ingvartsen, K. L., R. J. Dewhurst, and N. C. Friggens. 2003. On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livestock Production Science 83(2):277-308.

Ingvartsen, K. L. and N. C. Friggens. 2005. To what extent do variabilities in hormones, metabolites and energy intake explain variability in milk yield? Domest Anim Endocrinol 29(2):294-304.

Johnson, G. A., K. J. Austin, E. A. Van Kirk, and T. R. Hansen. 1998. Pregnancy and Interferon-Tau Induce Conjugation of Bovine Ubiquitin Cross-Reactive Protein to Cytosolic Uterine Proteins1. Biology of Reproduction 58(4):898-904.

Johnson, G. A., T. E. Spencer, T. R. Hansen, K. J. Austin, R. C. Burghardt, and F. W. Bazer. 1999. Expression of the Interferon Tau Inducible Ubiquitin Cross-Reactive Protein in the Ovine Uterus1. Biology of Reproduction 61(1):312-318.

Kadarmideen, H. N. 2014. Genomics to systems biology in animal and veterinary sciences: Progress, lessons and opportunities. Livestock Science 166:232-248.

Kerbrat, S. and C. Disenhaus. 2004. A proposition for an updated behavioural characterisation of the oestrus period in dairy cows. Applied Animal Behaviour Science 87(3):223-238.

Khan, D. R., D. Dubé, L. Gall, N. Peynot, S. Ruffini, L. Laffont, D. Le Bourhis, S. Degrelle, A. Jouneau, and V. Duranthon. 2012. Expression of pluripotency master regulators during two key developmental transitions: EGA and early lineage specification in the bovine embryo. PLoS One 7(3):e34110.

Khatib, H., C. Maltecca, R. L. Monson, V. Schutzkus, and J. J. Rutledge. 2009. Monoallelic maternal expression of STAT5A affects embryonic survival in cattle. BMC Genet 10:13.

Khatkar, M. S., I. A. S. Randhawa, and H. W. Raadsma. 2014. Meta-assembly of genomic regions and variants associated with female reproductive efficiency in cattle. Livestock Science 166:144-157.

Khatkar, M. S., P. C. Thomson, I. Tammen, and H. W. Raadsma. 2004. Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genet Sel Evol 36(2):163-190.

Kim, H.-S., J.-S. Koh, Y.-B. Choi, J. Ro, H.-K. Kim, M.-K. Kim, B.-H. Nam, K.-T. Kim, V. Chandra, H.-S. Seol, W.-C. Noh, E.-K. Kim, J. Park, C.-D. Bae, and K.-M. Hong. 2014. Chromatin CKAP2, a New Proliferation Marker, as Independent Prognostic Indicator in Breast Cancer. PLOS ONE 9(6):e98160.

Klein, C., S. Bauersachs, S. E. Ulbrich, R. Einspanier, H. H. Meyer, S. E. Schmidt, H. D. Reichenbach, M. Vermehren, F. Sinowatz, H. Blum, and E. Wolf. 2006. Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. Biol Reprod 74(2):253-264.

Kojima, F. N., E. G. Bergfeld, M. E. Wehrman, A. S. Cupp, K. E. Fike, D. V. Mariscal-Aguayo, T. Sanchez-Torres, M. Garcia-Winder, D. T. Clopton, A. J. Roberts, and J. E. Kinder. 2003. Frequency of luteinizing hormone pulses in cattle influences duration of persistence of dominant ovarian follicles, follicular fluid concentrations of steroids, and activity of insulin-like growth factor binding proteins. Anim Reprod Sci 77(3-4):187-211.

Kommadath, A., H. Woelders, B. Beerda, H. A. Mulder, A. A. de Wit, R. F. Veerkamp, M. F. te Pas, and M. A. Smits. 2011. Gene expression patterns in four brain areas associate with quantitative measure of estrous behavior in dairy cows. BMC Genomics 12:200.

Konduri, S. D., R. Medisetty, W. Liu, B. A. Kaipparettu, P. Srivastava, H. Brauch, P. Fritz, W. M. Swetzig, A. E. Gardner, S. A. Khan, and G. M. Das. 2010. Mechanisms of estrogen receptor antagonism toward p53 and its implications in breast cancer therapeutic response and stem cell regulation. Proc Natl Acad Sci U S A 107(34):15081-15086.

Kuhn, M. T., J. L. Hutchison, and G. R. Wiggans. 2006. Characterization of Holstein heifer fertility in the United States. J Dairy Sci 89(12):4907-4920.

Kuhn, M. T. and R. D. Shanks. 1994. Association of deficiency of uridine monophosphate synthase with production and reproduction. J Dairy Sci 77(2):589-597.

Kunau, W. H. and P. Dommes. 1978. Degradation of unsaturated fatty acids. Identification of intermediates in the degradation of cis-4-decenoly-CoA by extracts of beef-liver mitochondria. Eur J Biochem 91(2):533-544.

La Rocca, C., F. Carbone, S. Longobardi, and G. Matarese. 2014. The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. Immunol Lett 162(1 Pt A):41-48.

Ledgard, A. M., M. C. Berg, W. H. McMillan, G. Smolenski, and A. J. Peterson. 2012. Effect of asynchronous transfer on bovine embryonic development and relationship with early cycle uterine proteome profiles. Reprod Fertil Dev 24(7):962-972.

Lefebvre, R., Fritz, S,Ledoux, D, Gatien, J, Genestout, L, Rossignol, M-N, Grimard, B, Boichard, D, Humblot, P and Ponsart, C. 2011. GENIFER: fine mapping and fertility QTL effects in the Holstein cattle breed. Rencontres Recherche Ruminants 18:415.

Lewin, T. D., A. A. Fouladi-Nashta, and P. W. H. Holland. 2022. PRD-Class Homeobox Genes in Bovine Early Embryos: Function, Evolution, and Overlapping Roles. Molecular Biology and Evolution 39(5):msac098.

Li, C., D. Sun, S. Zhang, S. Yang, M. A. Alim, Q. Zhang, Y. Li, and L. Liu. 2016. Genetic effects of FASN, PPARGC1A, ABCG2 and IGF1 revealing the association with milk fatty acids in a Chinese Holstein cattle population based on a post genome-wide association study. BMC Genet 17:110.

Li, J., Y. Zhu, B. Shoemake, B. Liu, P. Adkins, and L. Wallace. 2022. A systematic review of the utility of biomarkers as aids in the early diagnosis and outcome prediction of bovine respiratory disease complex in feedlot cattle. J Vet Diagn Invest:10406387221081232.

Li, N., F. Zhao, C. Wei, M. Liang, N. Zhang, C. Wang, Q. Z. Li, and X. J. Gao. 2014. Function of SREBP1 in the milk fat synthesis of dairy cow mammary epithelial cells. Int J Mol Sci 15(9):16998-17013.

Liebig, B. E., J. V. Bishop, K. D. McSweeney, H. Van Campen, C. L. Gonzalez-Berrios, T. R. Hansen, and M. G. Thomas. 2022. Direct genomic value daughter pregnancy rate and services per conception are associated with characteristics of day-16 conceptuses and hormone signaling for maternal recognition of pregnancy in lactating Holstein cows. Applied Animal Science 38(2):157-169.

Lin, H., T. R. Mosmann, L. Guilbert, S. Tuntipopipat, and T. G. Wegmann. 1993. Synthesis of T helper 2-type cytokines at the maternal-fetal interface. J Immunol 151(9):4562-4573.

Liu, Y., A. Beyer, and R. Aebersold. 2016. On the Dependency of Cellular Protein Levels on mRNA Abundance. Cell 165(3):535-550.

Lonergan, P., T. Fair, D. Corcoran, and A. C. Evans. 2006. Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. Theriogenology 65(1):137-152.

Lonergan, P., T. Fair, N. Forde, and D. Rizos. 2016a. Embryo development in dairy cattle. Theriogenology 86(1):270-277.

Lonergan, P. and N. Forde. 2014. Maternal-embryo interaction leading up to the initiation of implantation of pregnancy in cattle. Animal 8 Suppl 1:64-69.

Lonergan, P., N. Forde, and T. Spencer. 2016b. Role of progesterone in embryo development in cattle. Reprod Fertil Dev 28(1-2):66-74.

Lonergan, P., D. Rizos, A. Gutierrez-Adan, T. Fair, and M. P. Boland. 2003. Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. Reprod Domest Anim 38(4):259-267.

Lonergan, P., A. Woods, T. Fair, F. Carter, D. Rizos, F. Ward, K. Quinn, and A. Evans. 2007. Effect of embryo source and recipient progesterone environment on embryo development in cattle. Reprod Fertil Dev 19(7):861-868.

Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 81(3-4):209-223.

Love, M. I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15(12):550.

Lucy, M. C. 2007. Fertility in high-producing dairy cows: reasons for decline and corrective strategies for sustainable improvement. Soc Reprod Fertil Suppl 64:237-254.

Lucy, M. C., S. McDougall, and D. P. Nation. 2004. The use of hormonal treatments to improve the reproductive performance of lactating dairy cows in feedlot or pasture-based management systems. Animal Reproduction Science 82-83:495-512.

Lucy, M. C., Savio, J.D., Badiga, L., De La Sota, R.L. and Thatcher, W.W. 1992. Factors that affect ovarian follicular dynamics in cattle. Journal of Animal Science 70:3615-3626.

Luna-Nevarez, P., G. Rincon, J. F. Medrano, D. G. Riley, C. C. Chase, Jr., S. W. Coleman, D. M. Vanleeuwen, K. L. DeAtley, A. Islas-Trejo, G. A. Silver, and M. G. Thomas. 2011. Single nucleotide polymorphisms in the growth hormone-insulin-like growth factor axis in straightbred and crossbred Angus, Brahman, and Romosinuano heifers: population genetic analyses and association of genotypes with reproductive phenotypes. J Anim Sci 89(4):926-934.

López-Gatius, F., P. Santolaria, I. Mundet, and J. L. Yániz. 2005. Walking activity at estrus and subsequent fertility in dairy cows. Theriogenology 63(5):1419-1429.

Ma, L. and B. A. Corl. 2012. Transcriptional regulation of lipid synthesis in bovine mammary epithelial cells by sterol regulatory element binding protein-1. J Dairy Sci 95(7):3743-3755.

Macdonald, K. A., J. W. Penno, J. A. Lancaster, and J. R. Roche. 2008. Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. J Dairy Sci 91(5):2151-2163.

Maeda, Y., H. Ohtsuka, M. Tomioka, and M. Oikawa. 2013. Effect of progesterone on Th1/Th2/Th17 and regulatory T cell-related genes in peripheral blood mononuclear cells during pregnancy in cows. Vet Res Commun 37(1):43-49.

Mamo, S., J. P. Mehta, N. Forde, P. McGettigan, and P. Lonergan. 2012. Conceptus-endometrium crosstalk during maternal recognition of pregnancy in cattle. Biol Reprod 87(1):6, 1-9.

Mamo, S., J. P. Mehta, P. McGettigan, T. Fair, T. E. Spencer, F. W. Bazer, and P. Lonergan. 2011. RNA sequencing reveals novel gene clusters in bovine conceptuses associated with maternal recognition of pregnancy and implantation. Biol Reprod 85(6):1143-1151.

Mansouri-Attia, N., L. J. Oliveira, N. Forde, A. G. Fahey, J. A. Browne, J. F. Roche, O. Sandra, P. Reinaud, P. Lonergan, and T. Fair. 2012. Pivotal Role for Monocytes/Macrophages and Dendritic Cells in Maternal Immune Response to the Developing Embryo in Cattle1. Biology of Reproduction 87(5).

Marques, E., J. D. Nkrumah, E. L. Sherman, and S. S. Moore. 2009. Polymorphisms in positional candidate genes on BTA14 and BTA26 affect carcass quality in beef cattle. J Anim Sci 87(8):2475-2484.

Martin, C., L. Pessemesse, M. P. De La Llosa-Hermier, J. Martal, J. Djiane, and M. Charlier. 2004. Interferon-tau upregulates prolactin receptor mRNA in the ovine endometrium during the peri-implantation period. Reproduction 128(1):99-105.

Matsumoto, H., S. Inada, E. Kobayashi, T. Abe, H. Hasebe, S. Sasazaki, K. Oyama, and H. Mannen. 2012. Identification of SNPs in the FASN gene and their effect on fatty acid milk composition in Holstein cattle. Livestock Science 144(3):281-284.

Matsuyama, S., T. Kojima, S. Kato, and K. Kimura. 2012. Relationship between quantity of IFNT estimated by IFN-stimulated gene expression in peripheral blood mononuclear cells and bovine embryonic mortality after AI or ET. Reprod Biol Endocrinol 10:21.

Mauric, M. 2019. Effects of different variants of the FASN gene on production performance and milk fatty acid composition in Holstein × Simmental dairy cows. Veterinární medicína v. 64(no. 03):pp. 101-108-v.164 no.103.

Mazzoni, G., S. M. Salleh, K. Freude, H. S. Pedersen, L. Stroebech, H. Callesen, P. Hyttel, and H. N. Kadarmideen. 2017. Identification of potential biomarkers in donor cows for in vitro embryo production by granulosa cell transcriptomics. PLoS One 12(4):e0175464.

McCracken, J. A., E. E. Custer, and J. C. Lamsa. 1999. Luteolysis: a neuroendocrine-mediated event. Physiol Rev 79(2):263-323.

McCracken, J. A., M. E. Glew, and R. J. Scaramuzzi. 1970. Corpus luteum regression induced by prostagland in F2-alpha. J Clin Endocrinol Metab 30(4):544-546.

McGee, E. A. a. A. J. W. H. 2000. Initial and cyclic recruitment of ovarian follicles. Endocrine Reviews 21(2):200-214.

McHughes, C. E., G. K. Springer, L. D. Spate, R. Li, R. Woods, M. P. Green, S. W. Korte, C. N. Murphy, J. A. Green, and R. S. Prather. 2009. Identification and quantification of differentially represented transcripts in in vitro and in vivo derived preimplantation bovine embryos. Mol Reprod Dev 76(1):48-60.

Meadows, C., P. J. Rajala-Schultz, and G. S. Frazer. 2005. A spreadsheet-based model demonstrating the nonuniform economic effects of varying reproductive performance in Ohio dairy herds. J Dairy Sci 88(3):1244-1254.

Merlo, B., E. Iacono, and G. Mari. 2007. EFFECT OF PROGESTERONE AND EPIDERMAL GROWTH FACTOR ON IN VITRO-PRODUCED EIGHT-CELL BOVINE EMBRYOS IN SERUM-FREE CULTURE MEDIUM. Reproduction, Fertility and Development 19:0-0.

Mermillod, P., P. Lonergan, C. Carolan, H. Khatir, N. Poulin, and Y. Cognié. 1996. [In vitro oocyte maturation in domestic ruminants]. Contracept Fertil Sex 24(7-8):552-558.

Mermillod, P., A. Massip, and F. Dessy. 1992. In vitro production of cattle embryos: review and Belgian results. Int J Dev Biol 36(1):185-195.

Meuwissen, T. H., B. J. Hayes, and M. E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157(4):1819-1829.

Meyerholz, M. M., K. Mense, H. Knaack, O. Sandra, and M. Schmicke. 2016. Pregnancy-Induced ISG-15 and MX-1 Gene Expression is Detected in the Liver of Holstein-Friesian Heifers During Late Peri-Implantation Period. Reprod Domest Anim 51(1):175-177.

Miglior, F., A. Fleming, F. Malchiodi, L. F. Brito, P. Martin, and C. F. Baes. 2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. J Dairy Sci 100(12):10251-10271.

Miretti, S., C. Lecchi, F. Ceciliani, and M. Baratta. 2020. MicroRNAs as Biomarkers for Animal Health and Welfare in Livestock. Frontiers in veterinary science 7:578193-578193.

Momont, H. 1990. Rectal palpation. The Bovine Practitioner:122-123.

Mondou, E., I. Dufort, M. Gohin, E. Fournier, and M. A. Sirard. 2012. Analysis of microRNAs and their precursors in bovine early embryonic development. Mol Hum Reprod 18(9):425-434.

Monteiro, P. L. J., E. S. Ribeiro, R. P. Maciel, A. L. G. Dias, E. Solé, F. S. Lima, R. S. Bisinotto, W. W. Thatcher, R. Sartori, and J. E. P. Santos. 2014. Effects of supplemental progesterone after artificial insemination on expression of interferon-stimulated genes and fertility in dairy cows. Journal of Dairy Science 97(8):4907-4921.

Moore, N. W. and J. N. Shelton. 1964. EGG TRANSFER IN SHEEP. EFFECT OF DEGREE OF SYNCHRONIZATION BETWEEN DONOR AND RECIPIENT, AGE OF EGG, AND SITE OF TRANSFER ON THE SURVIVAL OF TRANSFERRED EGGS. J Reprod Fertil 7:145-152.

Moore, R. E., J. Kirwan, M. K. Doherty, and P. D. Whitfield. 2007. Biomarker discovery in animal health and disease: the application of post-genomic technologies. Biomarker insights 2:185-196.

Moraes, J. G. N., S. K. Behura, T. W. Geary, P. J. Hansen, H. L. Neibergs, and T. E. Spencer. 2018. Uterine influences on conceptus development in fertility-classified animals. Proc Natl Acad Sci U S A 115(8):E1749-e1758.

Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 84(7):1646-1659.

Moridi, M., S. H. Hosseini Moghaddam, S. Z. Mirhoseini, and M. Bionaz. 2019. Transcriptome analysis showed differences of two purebred cattle and their crossbreds. Italian Journal of Animal Science 18(1):70-79.

Márquez, C., M. Sandalinas, M. Bahçe, M. Alikani, and S. Munné. 2000. Chromosome abnormalities in 1255 cleavage-stage human embryos. Reprod Biomed Online 1(1):17-26.

National Academies of Sciences, E. and Medicine. 2021. Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. The National Academies Press, Washington, DC.

Nel-Themaat, L. and Z. P. Nagy. 2011. A review of the promises and pitfalls of oocyte and embryo metabolomics. Placenta 32 Suppl 3:S257-263.

Niemann, H., J. W. Carnwath, D. Herrmann, G. Wieczorek, E. Lemme, A. Lucas-Hahn, and S. Olek. 2010. DNA methylation patterns reflect epigenetic reprogramming in bovine embryos. Cell Reprogram 12(1):33-42.

Niswender, G. D. 1973. Influence of the site of conjugation on the specificity of antibodies to progesterone. Steroids 22(3):413-424.

Niswender, G. D., T. L. Davis, R. J. Griffith, R. L. Bogan, K. Monser, R. C. Bott, J. E. Bruemmer, and T. M. Nett. 2007. Judge, jury and executioner: the auto-regulation of luteal function. Soc Reprod Fertil Suppl 64:191-206.

Norman, H. D., J. R. Wright, R. L. Powell, P. M. VanRaden, F. Miglior, and G. de Jong. 2007. Consistency of maturity rate for milk yield across countries and generations. J Dairy Sci 90(8):3937-3944.

Northrop, E. J., J. J. J. Rich, J. R. Rhoades, and G. A. Perry. 2019. Comparison of two bovine serum pregnancy tests in detection of artificial insemination pregnancies and pregnancy loss in beef cattle. PloS one 14(1):e0211179-e0211179.

Nowicki, A., W. Barański, A. Baryczka, and T. Janowski. 2017. OvSynch Protocol and its Modifications in the Reproduction Management of Dairy Cattle Herds - an Update. J Vet Res 61(3):329-336.

Oliveira, J. F., L. E. Henkes, R. L. Ashley, S. H. Purcell, N. P. Smirnova, D. N. Veeramachaneni, R. V. Anthony, and T. R. Hansen. 2008. Expression of interferon (IFN)-stimulated genes in extrauterine tissues during early pregnancy in sheep is the consequence of endocrine IFN-tau release from the uterine vein. Endocrinology 149(3):1252-1259.

Oliveira, L. J., N. Mansouri-Attia, A. G. Fahey, J. Browne, N. Forde, J. F. Roche, P. Lonergan, and T. Fair. 2013. Characterization of the Th profile of the bovine endometrium during the oestrous cycle and early pregnancy. PLoS One 8(10):e75571.

Oppi-Williams, C., J. K. Suagee, and B. A. Corl. 2013. Regulation of lipid synthesis by liver X receptor α and sterol regulatory element-binding protein 1 in mammary epithelial cells. J Dairy Sci 96(1):112-121.

Opsomer, G., Y. T. Gröhn, J. Hertl, M. Coryn, H. Deluyker, and A. de Kruif. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology 53(4):841-857.

Ortega, M. S., A. C. Denicol, J. B. Cole, D. J. Null, and P. J. Hansen. 2016. Use of single nucleotide polymorphisms in candidate genes associated with daughter pregnancy rate for prediction of genetic merit for reproduction in Holstein cows. Anim Genet 47(3):288-297.

Ortega, M. S., A. C. Denicol, J. B. Cole, D. J. Null, J. F. Taylor, R. D. Schnabel, and P. J. Hansen. 2017. Association of single nucleotide polymorphisms in candidate genes previously related to genetic variation in fertility with phenotypic measurements of reproductive function in Holstein cows. Journal of Dairy Science 100(5):3725-3734.

Ortega, S. 2018. Identification of genes associated with reproductive function in dairy cattle. Animal Reproduction 15:923-932.

Ott, T. L. 2020. Immunological detection of pregnancy: Evidence for systemic immune modulation during early pregnancy in ruminants. Theriogenology 150:498-503.

P.M. VanRaden, A. H. S., M.E. Tooker, R.H. Miller and H.D. Norman. 2003. Daughter pregnancy rate evaluation of cow fertility. USDA Animal Improvement Programs Laboratory, Beltsville, MD.

Paisley, L. G., W. D. Mickelsen, and P. B. Anderson. 1986. Mechanisms and therapy for retained fetal membranes and uterine infections of cows: A review. Theriogenology 25(3):353-381.

Parmar, S., A. Dhami, K. Hadiya, and C. Parmar. 2016. Early Embryonic Death in Bovines: An Overview. Raksha Technical Review 6:6-12.

Pathak, S. S., J. D. Lich, and J. S. Blum. 2001. Cutting edge: editing of recycling class II:peptide complexes by HLA-DM. J Immunol 167(2):632-635.

Pedraza-Alva, G., J. M. Zingg, A. Donda, and L. Pérez-Martínez. 2009. Estrogen receptor regulates MyoD gene expression by preventing AP-1-mediated repression. Biochem Biophys Res Commun 389(2):360-365.

Pereira, G., R. Bexiga, J. Chagas e Silva, E. Silva, C. Ramé, J. Dupont, Y. Guo, P. Humblot, and L. Lopes-da-Costa. 2020. Adipokines as biomarkers of postpartum subclinical endometritis in dairy cows. Reproduction 160(3):417-430.

Perkel, K. J., A. K. Tscherner, C. Merrill, J. LaMarre, and P. Madan. 2015. The ART of selecting the best embryo: A review of early embryonic mortality and bovine embryo viability assessment methods. Molecular Reproduction and Development 82.

Perry, G. A. 2012. Physiology and Endocrinology Symposium: Harnessing basic knowledge of factors controlling puberty to improve synchronization of estrus and fertility in heifers. J Anim Sci 90(4):1172-1182.

Perry, G. A., M. F. Smith, and T. W. Geary. 2004. Ability of intravaginal progesterone inserts and melengestrol acetate to induce estrous cycles in postpartum beef cows. J Anim Sci 82(3):695-704.

Petit, H. V. and H. Twagiramungu. 2006. Conception rate and reproductive function of dairy cows fed different fat sources. Theriogenology 66(5):1316-1324.

Piccinni, B., S. Massari, A. Caputi Jambrenghi, F. Giannico, M. P. Lefranc, S. Ciccarese, and R. Antonacci. 2015. Sheep (Ovis aries) T cell receptor alpha (TRA) and delta (TRD) genes and genomic organization of the TRA/TRD locus. BMC Genomics 16:709.

Pierson, R. A. and O. J. Ginther. 1987. Ultrasonographic appearance of the bovine uterus during the estrous cycle. J Am Vet Med Assoc 190(8):995-1001.

Pontes, J. H., I. Nonato-Junior, B. V. Sanches, J. C. Ereno-Junior, S. Uvo, T. R. Barreiros, J. A. Oliveira, J. F. Hasler, and M. M. Seneda. 2009. Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore (Bos indicus) donor cows. Theriogenology 71(4):690-697.

Poock, S., Horner, J and Milhollin, R. 2009. Missouri Dairy Growth Council: Dairy cattle reproductive manual. University of Missouri: University of Missouri Extension Commercial Agriculture Program, Missouri, USA.

Pope, W. F. 1988. Uterine asynchrony: a cause of embryonic loss. Biol Reprod 39(5):999-1003.

Pritchard, T., M. Coffey, R. Mrode, and E. Wall. 2013. Genetic parameters for production, health, fertility and longevity traits in dairy cows. Animal 7(1):34-46.

Pritchard, T. J. and C. W. Donnelly. 1999. Combined secondary enrichment of primary enrichment broths increases Listeria detection. J Food Prot 62(5):532-535.

Pryce, J. E., S. Bolormaa, A. J. Chamberlain, P. J. Bowman, K. Savin, M. E. Goddard, and B. J. Hayes. 2010. A validated genome-wide association study in 2 dairy cattle breeds for milk production and fertility traits using variable length haplotypes. J Dairy Sci 93(7):3331-3345.

Pryce, J. E., M. P. Coffey, and G. Simm. 2001. The Relationship Between Body Condition Score and Reproductive Performance. Journal of Dairy Science 84(6):1508-1515.

Pryce, J. E., T. T. T. Nguyen, M. Axford, G. Nieuwhof, and M. Shaffer. 2018. Symposium review: Building a better cow-The Australian experience and future perspectives. J Dairy Sci 101(4):3702-3713.

Pryce, J. E., M. D. Royal, P. C. Garnsworthy, and I. L. Mao. 2004. Fertility in the high-producing dairy cow. Livestock Production Science 86(1):125-135.

Pryce, J. E., R. F. Veerkamp, R. Thompson, W. G. Hill, and G. Simm. 1997. Genetic aspects of common health disorders and measures of fertility in Holstein Friesian dairy cattle. Animal Science 65(3):353-360.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 44(7):915-923.

Quintela Arias, L., A. Peña, M. J. Taboada, G. Alonso, B. Portas, C. Diaz, M. Barrio, M. E. García, J. J. Becerra, and P. García Herradón. 2004. Risk factors for low pregnancy rate in dairy cattle: a retrospective study in the north west of Spain. Archivos de zootecnia, ISSN 0004-0592, Vol. 53, Nº 201, 2004, pags. 69-76 53.

Raghupathy, R. 1997. Th1-type immunity is incompatible with successful pregnancy. Immunol Today 18(10):478-482.

Reggio, B., J. Lynn, and R. Godke. 1997. The effect of progesterone on the development of IVF-derived bovine embryos cultured in a semi-defined culture medium. Theriogenology 47:284.

Ribeiro, E., K. Galvão, W. Thatcher, and J. Santos. 2012. Economic aspects of applying reproductive technologies to dairy herds. Animal reproduction 9:370-387.

Ribeiro, E. S. 2018. Symposium review: Lipids as regulators of conceptus development: Implications for metabolic regulation of reproduction in dairy cattle. J Dairy Sci 101(4):3630-3641.

Ribeiro, E. S., R. G. Bruno, A. M. Farias, J. A. Hernandez-Rivera, G. C. Gomes, R. Surjus, L. F. Becker, A. Birt, T. L. Ott, J. R. Branen, R. G. Sasser, D. H. Keisler, W. W. Thatcher, T. R. Bilby, and J. E. Santos. 2014. Low doses of bovine somatotropin enhance conceptus development and fertility in lactating dairy cows. Biol Reprod 90(1):10.

Rincon, G., A. Islas-Trejo, A. R. Castillo, D. E. Bauman, B. J. German, and J. F. Medrano. 2012. Polymorphisms in genes in the SREBP1 signalling pathway and SCD are associated with milk fatty acid composition in Holstein cattle. J Dairy Res 79(1):66-75.

Rizos, D., S. Scully, A. K. Kelly, A. D. Ealy, R. Moros, P. Duffy, A. Al Naib, N. Forde, and P. Lonergan. 2012. Effects of human chorionic gonadotrophin administration on day 5 after oestrus on corpus luteum characteristics, circulating progesterone and conceptus elongation in cattle. Reprod Fertil Dev 24(3):472-481.

Rizos, D., F. Ward, P. Duffy, M. P. Boland, and P. Lonergan. 2002. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. Mol Reprod Dev 61(2):234-248.

Roberts, R. M. 2007. Interferon-tau, a Type 1 interferon involved in maternal recognition of pregnancy. Cytokine Growth Factor Rev 18(5-6):403-408.

Roberts, S. J. 1971. Veterinary obstetrics and genital diseases (Theriogenology).

Robinson, J. L., R. G. Popp, R. D. Shanks, A. Oosterhof, and J. H. Veerkamp. 1993. Testing for deficiency of uridine monophosphate synthase among holstein-friesian cattle of North America and Europe. Livestock Production Science 36(4):287-298.

Rocha, C. C., S. C. da Silva Andrade, G. D. de Melo, I. G. Motta, L. L. Coutinho, A. M. Gonella-Diaza, M. Binelli, and G. Pugliesi. 2020. Early pregnancy-induced transcripts in peripheral blood immune cells in Bos indicus heifers. Scientific Reports 10(1):13733.

Romano, J. and D. Magee. 2001. Applications of trans-rectal ultrasonography in cow/heifer reproduction. Pages 99-104 in Proc. Annual food conference. Conception to parturition: fertility in Texas beef cattle.

Romano, J. E., J. A. Thompson, D. C. Kraemer, M. E. Westhusin, D. W. Forrest, and M. A. Tomaszweski. 2007. Early pregnancy diagnosis by palpation per rectum: Influence on embryo/fetal viability in dairy cattle. Theriogenology 67(3):486-493.

Romero, J. J., A. Q. Antoniazzi, T. M. Nett, R. L. Ashley, B. T. Webb, N. P. Smirnova, R. C. Bott, J. E. Bruemmer, F. W. Bazer, R. V. Anthony, and T. R. Hansen. 2015. Temporal Release, Paracrine and Endocrine Actions of Ovine Conceptus-Derived Interferon-Tau During Early Pregnancy. Biol Reprod 93(6):146.

Romero, J. J., A. Q. Antoniazzi, N. P. Smirnova, B. T. Webb, F. Yu, J. S. Davis, and T. R. Hansen. 2013. Pregnancy-associated genes contribute to antiluteolytic mechanisms in ovine corpus luteum. Physiol Genomics 45(22):1095-1108.

Rowson, L. E. and R. M. Moor. 1966. Non-surgical transfer of cow eggs. J Reprod Fertil 11(2):311-312.

Rowson, L. E., R. Tervit, and A. Brand. 1972. The use of prostaglandins for synchronization of oestrus in cattle. J Reprod Fertil 29(1):145.

Roy, R., L. Ordovas, P. Zaragoza, A. Romero, C. Moreno, J. Altarriba, and C. Rodellar. 2006. Association of polymorphisms in the bovine FASN gene with milk-fat content. Anim Genet 37(3):215-218.

Royal, M. D., A. O. Darwash, A. P. F. Flint, R. Webb, J. A. Woolliams, and G. E. Lamming. 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Animal Science 70(3):487-501.

Russell, G. C., J. Benavides, D. M. Grant, H. Todd, J. Thomson, V. Puri, M. Nath, and D. M. Haig. 2012. Host gene expression changes in cattle infected with Alcelaphine herpesvirus 1. Virus Res 169(1):246-254.

Salehi, R., M. G. Colazo, S. Tsoi, A. Behrouzi, B. K. Tsang, M. K. Dyck, M. Oba, and D. J. Ambrose. 2016. Morphologic and transcriptomic assessment of bovine embryos exposed to dietary long-chain fatty acids. Reproduction 152(6):715-726.

Salumets, A., C. Hydén-Granskog, A. M. Suikkari, A. Tiitinen, and T. Tuuri. 2001. The predictive value of pronuclear morphology of zygotes in the assessment of human embryo quality. Hum Reprod 16(10):2177-2181.

Salumets, A., T. Tuuri, S. Mäkinen, S. Vilska, L. Husu, R. Tainio, and A. M. Suikkari. 2003. Effect of developmental stage of embryo at freezing on pregnancy outcome of frozen-thawed embryo transfer. Hum Reprod 18(9):1890-1895.

Sandals, W. C., R. A. Curtis, J. F. Cote, and S. W. Martin. 1979. The effect of retained placenta and metritis complex on reproductive performance in dairy cattle -- a case control study. The Canadian veterinary journal = La revue veterinaire canadienne 20(5):131-135.

Sandler, N. G., M. M. Mentink-Kane, A. W. Cheever, and T. A. Wynn. 2003. Global gene expression profiles during acute pathogen-induced pulmonary inflammation reveal divergent roles for Th1 and Th2 responses in tissue repair. J Immunol 171(7):3655-3667.

Sandra, O., N. Mansouri-Attia, and R. G. Lea. 2011. Novel aspects of endometrial function: a biological sensor of embryo quality and driver of pregnancy success. Reprod Fertil Dev 24(1):68-79.

Sanger, P. L. 2012. Pathways to Pregnancy and Parturition. 3 ed. Current Conceptions, Redmon, OR.

Santos, J. E., W. W. Thatcher, R. C. Chebel, R. L. 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., M. R. Bastos, and M. C. Wiltbank. 2010. Factors affecting fertilisation and early embryo quality in single- and superovulated dairy cattle. Reprod Fertil Dev 22(1):151-158.

SAS Institute Inc. 2014. User's Guide: Statistic 9.4 ed.

Sasser, R. G., C. A. Ruder, K. A. Ivani, J. E. Butler, and W. C. Hamilton. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. Biol Reprod 35(4):936-942.

Satterfield, M. C., F. W. Bazer, and T. E. Spencer. 2006. Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. Biol Reprod 75(2):289-296.

Schennink, A., H. Bovenhuis, K. M. Léon-Kloosterziel, J. A. van Arendonk, and M. H. Visker. 2009. Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. Anim Genet 40(6):909-916.

Schwenger, B., S. Schöber, and D. Simon. 1993. DUMPS cattle carry a point mutation in the uridine monophosphate synthase gene. Genomics 16(1):241-244.

Shah, V. S. S. a. R. G. 2009. Bovine In vitro Embryo Production: An Overview. Veterinary World 2(12):478-479.

Shanks, R. D. and M. M. Greiner. 1992. Relationship between genetic merit of Holstein bulls and deficiency of uridine-5'-monophosphate synthase. J Dairy Sci 75(7):2023-2029.

Shanks, R. D. and J. L. Robinson. 1990. Deficiency of uridine monophosphate synthase among Holstein cattle. Cornell Vet 80(2):119-122.

Shaw, A. E., J. Hughes, Q. Gu, A. Behdenna, J. B. Singer, T. Dennis, R. J. Orton, M. Varela, R. J. Gifford, S. J. Wilson, and M. Palmarini. 2017. Fundamental properties of the mammalian innate immune system revealed by multispecies comparison of type I interferon responses. PLoS Biol 15(12):e2004086.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65(8):1516-1530.

Shirasuna, K., H. Matsumoto, E. Kobayashi, A. Nitta, S. Haneda, M. Matsui, C. Kawashima, K. Kida, T. Shimizu, and A. Miyamoto. 2012. Upregulation of interferon-stimulated genes and interleukin-10 in peripheral blood immune cells during early pregnancy in dairy cows. J Reprod Dev 58(1):84-90.

Shirasuna, K., H. Matsumoto, S. Matsuyama, K. Kimura, H. Bollwein, and A. Miyamoto. 2015. Possible role of interferon tau on the bovine corpus luteum and neutrophils during the early pregnancy. Reproduction 150(3):217-225.

Siddiqui, M. A., Z. C. Das, J. Bhattacharjee, M. M. Rahman, M. M. Islam, M. A. Haque, J. J. Parrish, and M. Shamsuddin. 2013. Factors affecting the first service conception rate of cows in smallholder dairy farms in Bangladesh. Reprod Domest Anim 48(3):500-505.

Sinedino, L. D., P. M. Honda, L. R. Souza, A. L. Lock, M. P. Boland, C. R. Staples, W. W. Thatcher, and J. E. Santos. 2017. Effects of supplementation with docosahexaenoic acid on reproduction of dairy cows. Reproduction 153(5):707-723.

Singh, U., R. Deb, R. R. Alyethodi, R. Alex, S. Kumar, S. Chakraborty, K. Dhama, and A. Sharma. 2014. Molecular markers and their applications in cattle genetic research: A review. Biomarkers and Genomic Medicine 6(2):49-58.

Skopets, B., J. Li, W. W. Thatcher, R. M. Roberts, and P. J. Hansen. 1992. Inhibition of lymphocyte proliferation by bovine trophoblast protein-1 (type I trophoblast interferon) and bovine interferon-alpha I1. Vet Immunol Immunopathol 34(1-2):81-96.

Smith, B. I. and C. A. Risco. 2005. Management of periparturient disorders in dairy cattle. Vet Clin North Am Food Anim Pract 21(2):503-521.

Soler, L., S. Uzbekova, E. Blesbois, X. Druart, and V. Labas. 2020. Intact cell MALDI-TOF mass spectrometry, a promising proteomic profiling method in farm animal clinical and reproduction research. Theriogenology 150:113-121.

Sorge, U. S., R. Moon, L. J. Wolff, L. Michels, S. Schroth, D. F. Kelton, and B. Heins. 2016. Management practices on organic and conventional dairy herds in Minnesota. J Dairy Sci 99(4):3183-3192.

Spelman, R. and D. Garrick. 1997. Utilisation of marker assisted selection in a commercial dairy cow population. Livestock Production Science 47(2):139-147.

Spencer, T. E. and F. W. Bazer. 1996. Ovine interferon tau suppresses transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. Endocrinology 137(3):1144-1147.

Spencer, T. E., N. Forde, and P. Lonergan. 2016. The role of progesterone and conceptus-derived factors in uterine biology during early pregnancy in ruminants. J Dairy Sci 99(7):5941-5950.

Spencer, T. E. and T. R. Hansen. 2015. Implantation and Establishment of Pregnancy in Ruminants. Adv Anat Embryol Cell Biol 216:105-135.

Spencer, T. E., G. A. Johnson, F. W. Bazer, R. C. Burghardt, and M. Palmarini. 2007. Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. Reprod Fertil Dev 19(1):65-78.

Spencer, T. E., T. L. Ott, and F. W. Bazer. 1996. tau-Interferon: pregnancy recognition signal in ruminants. Proc Soc Exp Biol Med 213(3):215-229.

Spencer, T. E., O. Sandra, and E. Wolf. 2008. Genes involved in conceptus-endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. Reproduction 135(2):165-179.

Spencer Thomas, E., Hansen Peter, J., Cole John, B., Dalton Joseph, Neibergs Holly. 2014. Genomic Selection and Reproductive Efficiency in Dairy Cattle Pages 16-31 in Proc. Dairy Cattle Reproduction The Dairy Cattle Reproduction Council, Salt Lake City, Utah.

Sprott, L. R., M. D. Harris, J. W. Richardson, A. W. Gray, D. W. Forrest, S. Northcutt-Dolezal, and L. V. Cundiff. 1998. Pregnancy to Artificial Insemination in Beef Cows as Affected by Body Condition and Number of Services1, 21This research was supported by Select Sires, Inc., Plain City, OH.2Authors gratefully acknowledge J. O. Sanders and D. K. Lunt for supplying animals and facilities. The Professional Animal Scientist 14(4):231-235.

Starbuck, G. R., A. O. Darwash, G. E. Mann, and G. E. Lamming. 2001. The detection and treatment of post insemination progesterone insufficiency in dairy cows. BSAP Occasional Publication 26(2):447-450.

Stark, G. R., I. M. Kerr, B. R. Williams, R. H. Silverman, and R. D. Schreiber. 1998. How cells respond to interferons. Annu Rev Biochem 67:227-264.

Stephenson, R. O., Y. Yamanaka, and J. Rossant. 2010. Disorganized epithelial polarity and excess trophectoderm cell fate in preimplantation embryos lacking E-cadherin. Development 137(20):3383-3391.

Strimbu, K. and J. A. Tavel. 2010. What are biomarkers? Curr Opin HIV AIDS 5(6):463-466.

Stringfellow, D., Seidel,SM. 1998. Manual of the International Embryo Transfer Society: A Procedural Guide and General Information for the Use of Embryo Transfer Technology Emphasizing Sanitary Procedures. 3rd ed. International Embryo Transfer Society, Savory, IL.

Sudano, M. J., D. M. Paschoal, T. da Silva Rascado, L. F. Crocomo, L. C. Magalhães, A. M. Junior, R. Machado, and F. da Cruz Landim-Alvarenga. 2014. Crucial surviving aspects for vitrified in vitro-produced bovine embryos. Zygote 22(2):124-131.

Sugiura, T., S. Akiyoshi, F. Inoue, Y. Yanagawa, M. Moriyoshi, M. Tajima, and S. Katagiri. 2018. Relationship between bovine endometrial thickness and plasma progesterone and estradiol concentrations in natural and induced estrus. The Journal of reproduction and development 64(2):135-143.

Suravajhala, P., L. J. A. Kogelman, and H. N. Kadarmideen. 2016. Multi-omic data integration and analysis using systems genomics approaches: methods and applications in animal production, health and welfare. Genetics, selection, evolution : GSE 48(1):38-38.

Syedbasha, M. and A. Egli. 2017. Interferon Lambda: Modulating Immunity in Infectious Diseases. Frontiers in Immunology 8.

Sykes, D. J., J. S. Couvillion, A. Cromiak, S. Bowers, E. Schenck, M. Crenshaw, and P. L. Ryan. 2012. The use of digital infrared thermal imaging to detect estrus in gilts. Theriogenology 78(1):147-152.

Sánchez, J. M., D. J. Mathew, S. K. Behura, C. Passaro, G. Charpigny, S. T. Butler, T. E. Spencer, and P. Lonergan. 2019. Bovine endometrium responds differentially to age-matched short and long conceptuses†. Biology of Reproduction 101(1):26-39.

Talukder, A. K., M. S. Yousef, M. B. Rashid, K. Awai, T. J. Acosta, T. Shimizu, K. Okuda, M. Shimada, K. Imakawa, and A. Miyamoto. 2017. Bovine embryo induces an anti-inflammatory response in uterine epithelial cells and immune cells in vitro: possible involvement of interferon tau as an intermediator. J Reprod Dev 63(4):425-434.

Thatcher, W. W., A. Guzeloglu, A. Meikle, S. Kamimura, T. Bilby, A. A. Kowalski, L. Badinga, R. Pershing, J. Bartolome, and J. E. Santos. 2003. Regulation of embryo survival in cattle. Reprod Suppl 61:253-266.

Thatcher, W. W., P. J. Hansen, T. S. Gross, S. D. Helmer, C. Plante, and F. W. Bazer. 1989. Antiluteolytic effects of bovine trophoblast protein-1. J Reprod Fertil Suppl 37:91-99.

Thatcher, W. W., F. Moreira, S. M. Pancarci, J. A. Bartolome, and J. E. Santos. 2002. Strategies to optimize reproductive efficiency by regulation of ovarian function. Domest Anim Endocrinol 23(1-2):243-254.

Thompson-Crispi, K. A., M. Sargolzaei, R. Ventura, M. Abo-Ismail, F. Miglior, F. Schenkel, and B. A. Mallard. 2014. A genome-wide association study of immune response traits in Canadian Holstein cattle. BMC Genomics 15(1):559.

Tillard, E., P. Humblot, B. Faye, P. Lecomte, I. Dohoo, and F. Bocquier. 2008. Postcalving factors affecting conception risk in Holstein dairy cows in tropical and sub-tropical conditions. Theriogenology 69(4):443-457.

Tozer, P. R. and A. J. Heinrichs. 2001. What affects the costs of raising replacement dairy heifers: a multiple-component analysis. J Dairy Sci 84(8):1836-1844.

Tríbulo, P., L. G. B. Siqueira, L. J. Oliveira, T. Scheffler, and P. J. Hansen. 2018. Identification of potential embryokines in the bovine reproductive tract. J Dairy Sci 101(1):690-704.

Urton, G., M. A. von Keyserlingk, and D. M. Weary. 2005. Feeding behavior identifies dairy cows at risk for metritis. J Dairy Sci 88(8):2843-2849.

Ushizawa, K., C. B. Herath, K. Kaneyama, S. Shiojima, A. Hirasawa, T. Takahashi, K. Imai, K. Ochiai, T. Tokunaga, Y. Tsunoda, G. Tsujimoto, and K. Hashizume. 2004. cDNA microarray analysis of bovine embryo gene expression profiles during the pre-implantation period. Reprod Biol Endocrinol 2:77.

Valour, D., P. Michot, C. Eozenou, R. Lefebvre, A. Bonnet, A. Capitan, S. Uzbekova, E. Sellem, C. Ponsart, and L. Schibler. 2015. Dairy cattle reproduction is a tightly regulated genetic process: Highlights on genes, pathways, and biological processes. Animal Frontiers 5(1):32-41.

Van Eerdenburg, F. J. C. M., H. S. H. Loeffler, and J. H. van Vliet. 1996. Detection of oestrus in dairy cows: A new approach to an old problem. Veterinary Quarterly 18(2):52-54.

van Leeuwen, J. J., M. R. Martens, J. Jourquin, M. A. Driancourt, A. Wagner, B. Kemp, and N. M. Soede. 2015. Follicle size and reproductive hormone profiles during a post-weaning altrenogest treatment in primiparous sows. Reprod Fertil Dev 27(2):304-312.

Van Soom, A., P. Mijten, I. Van Vlaenderen, J. Van den Branden, A. R. Mahmoudzadeh, and A. de Kruif. 1994. Birth of double-muscled Belgian Blue calves after transfer of in vitro produced embryos into dairy cattle. Theriogenology 41(4):855-867.

Van Tassell, C. P., G. R. Wiggans, and H. D. Norman. 1999. Method R estimates of heritability for milk, fat, and protein yields of United States dairy cattle. J Dairy Sci 82(10):2231-2237.

VanRaden, P. M. and R. H. Miller. 2006. Effects of nonadditive genetic interactions, inbreeding, and recessive defects on embryo and fetal loss by seventy days. J Dairy Sci 89(7):2716-2721.

VanRaden, P. M., A. H. Sanders, M. E. Tooker, R. H. Miller, H. D. Norman, M. T. Kuhn, and G. R. Wiggans. 2004. Development of a national genetic evaluation for cow fertility. J Dairy Sci 87(7):2285-2292.

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):153-165.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, E. P. Stanisiewski, and R. L. Fogwell. 1990. Influence of Energy Balance and Body Condition on Estrus and Estrous Cycles in Holstein Heifers1. Journal of Dairy Science 73(10):2759-2765.

VĂTĂŞEscu-Balcan, R.-A., S. Georgescu, M.-A. Manea, A. Dinischiotu, C.-D. Tesio, and M. Costache. 2006. A METHOD FOR DIAGNOSIS OF DEFICIENCY OF URIDINE MONOPHOSPHATE SYNTHASE IN CATTLE. Romanian Journal of Biochemistry 43-44:81-85.

Waldmann, A., O. Reksen, K. Landsverk, E. Kommisrud, E. Dahl, A. O. Refsdal, and E. Ropstad. 2001. Progesterone concentrations in milk fat at first insemination--effects on non-return and repeat-breeding. Anim Reprod Sci 65(1-2):33-41.

Walsh, S. W., E. J. Williams, and A. C. O. Evans. 2011. A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science 123(3):127-138.

Wan, D. C., D. Scanlon, B. Power, C. L. Choi, P. Hudson, and B. G. Livett. 1987. Synthesis of a specific oligodeoxyribonucleotide probe and its use for studying proenkephalin A gene expression. Neurosci Lett 76(1):74-80.

Wathes, D. C., A. M. Clempson, and G. E. Pollott. 2012. Associations between lipid metabolism and fertility in the dairy cow. Reprod Fertil Dev 25(1):48-61.

Wegmann, T. G., H. Lin, L. Guilbert, and T. R. Mosmann. 1993. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 14(7):353-356.

Weigel, K. A. 2006. Prospects for improving reproductive performance through genetic selection. Anim Reprod Sci 96(3-4):323-330.

Weir, B. 2001. Forensics. Statistical Genetics. John Wiley and Sons, New York.

Williams, E. J., D. P. Fischer, D. E. Noakes, G. C. England, A. Rycroft, H. Dobson, and I. M. Sheldon. 2007. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68(4):549-559.

Wiltbank, J. N., R. P. Shumway, W. R. Parker, and D. R. Zimmerman. 1967. Duration of estrus, time of ovulation and fertilization rate in beef heifers synchronized with dihydroxyprogesterone acetophenide. J Anim Sci 26(4):764-767.

Wiltbank, M. C., G. M. Baez, A. Garcia-Guerra, M. Z. Toledo, P. L. Monteiro, L. F. Melo, J. C. Ochoa, J. E. Santos, and R. Sartori. 2016. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. Theriogenology 86(1):239-253.

Wiltbank, M. C. and J. R. Pursley. 2014. The cow as an induced ovulator: timed AI after synchronization of ovulation. Theriogenology 81(1):170-185.

Witkamp, R. F. 2005. Genomics and systems biology--how relevant are the developments to veterinary pharmacology, toxicology and therapeutics? J Vet Pharmacol Ther 28(3):235-245.

Wolfenson, D., F. F. Bartol, L. Badinga, C. M. Barros, D. N. Marple, K. Cummins, D. Wolfe, M. C. Lucy, T. E. Spencer, and W. W. Thatcher. 1993. Secretion of PGF2alpha and oxytocin during hyperthermia in cyclic and pregnant heifers. Theriogenology 39(5):1129-1141.

Wolfenson, D., B. J. Lew, W. W. Thatcher, Y. Graber, and R. Meidan. 1997. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. Anim Reprod Sci 47(1-2):9-19.

Wolfenson, D., Z. Roth, and R. Meidan. 2000. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Anim Reprod Sci 60-61:535-547.

Wrenzycki, C., D. Wells, D. Herrmann, A. Miller, J. Oliver, R. Tervit, and H. Niemann. 2001. Nuclear transfer protocol affects messenger RNA expression patterns in cloned bovine blastocysts. Biol Reprod 65(1):309-317.

Wright, I. A., S. M. Rhind, T. K. Whyte, and A. J. Smith. 1992. Effects of body condition at calving and feeding level after calving on LH profiles and the duration of the post-partum anoestrous period in beef cows. Animal Science 55(1):41-46.

Wynn, T. A. 2008. Cellular and molecular mechanisms of fibrosis. J Pathol 214(2):199-210.

Xu, K. P., B. R. Yadav, W. A. King, and K. J. Betteridge. 1992. Sex-related differences in developmental rates of bovine embryos produced and cultured in vitro. Mol Reprod Dev 31(4):249-252.

Yang, L., X. L. Wang, P. C. Wan, L. Y. Zhang, Y. Wu, D. W. Tang, and S. M. Zeng. 2010. Up-regulation of expression of interferon-stimulated gene 15 in the bovine corpus luteum during early pregnancy. J Dairy Sci 93(3):1000-1011.

Yang, L., X. Yao, S. Li, K. Chen, Y. Wang, L. Chen, and L. Zhang. 2016. Expression of genes associated with luteolysis in peripheral blood mononuclear cells during early pregnancy in cattle. Mol Reprod Dev 83(6):509-515.

Yang, L. M., L. Y. Zhang, H. Qiao, N. Liu, Y. X. Wang, and S. J. Li. 2014. Maternal Immune Regulation by Conceptus During Early Pregnancy in the Bovine. Asian Journal of Animal and Veterinary Advances 9:610-620.

Yankey, S. J., B. A. Hicks, K. G. Carnahan, A. M. Assiri, S. J. Sinor, K. Kodali, J. N. Stellflug, and T. L. Ott. 2001. Expression of the antiviral protein Mx in peripheral blood mononuclear cells of pregnant and bred, non-pregnant ewes. J Endocrinol 170(2):R7-11.

Youngquist. 2007. Pregnancy diagnosis. Pages 294-303 in Current therapy in large animal theriogenology. Elsevier.

Yusuf, M., T. Nakao, C. Yoshida, S. T. Long, G. Gautam, R. M. Ranasinghe, K. Koike, and A. Hayashi. 2011. Days in milk at first AI in dairy cows; its effect on subsequent reproductive performance and some factors influencing it. J Reprod Dev 57(5):643-649.

Zemjanis, R. 1970. Diagnostic and therapeutic techniques in animal reproduction. Diagnostic and therapeutic techniques in animal reproduction. (2nd edn).

Zeron, Y., A. Ocheretny, O. Kedar, A. Borochov, D. Sklan, and A. Arav. 2001. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. Reproduction 121(3):447-454.

Zhang, L. Y., Y. Xia, F. Tang, S. J. Li, L. G. Yang, and B. Wang. 2015. The regulation of intrauterine immune cytokines and chemokines during early pregnancy in the bovine. Large Animal Review 21:23-31.

Ziebe, S., K. Petersen, S. Lindenberg, A. G. Andersen, A. Gabrielsen, and A. N. Andersen. 1997. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. Hum Reprod 12(7):1545-1549.

Čítek, J. and B. Barbora. 2004. Recessive disorders - a serious health hazard? Journal of Applied Biomedicine 2.

CHAPTER 4: SUMMARY

The objectives of this thesis were to elucidate mechanisms that are involved in pregnancies with EM by using transcriptomics (chapter 2) and identify candidate SNPs within EM conceptuses that were associated with inferior fertility traits (chapter 3). Interest to further understand how pregnancies with EM occur were based on their contribution to 43% of all failed pregnancies in high producing USA dairy cows. Due to pregnancies with EM occurring between days 7 to 19 after AI, current technology can only detect them at either day 28 (i.e., Biopryn and Alertys) or at day 32 (i.e., ultrasound). On the other hand, genetic panels have been found to aid in selecting traits such as fertility that have low heritability and identifying candidate SNPs add accuracy to breeding values. Currently, pregnancies with EM are poorly understood. Research is then needed to further explore its mechanisms and identify candidate SNPs within EM conceptuses which are associated with inferior fertility traits and could be used to sort and cull heifers.

In chapter 2, two experiments were conducted to reveal the transcriptome response of reproductive tissues (endometrium, PBMC and CL) to pregnancies with EM conceptuses via RNA-Sequencing. Transcriptomics revealed that EM pregnancies were associated with the failure of IFNT from the conceptus to achieve maternal recognition. Disruption of maternal-fetal crosstalk could then occur due to the activation of ESR1 up-regulating OXTR signaling pathway and pulsatile release of $PGF_{2\alpha}$. This was confirmed by EM conceptuses exhibiting a Th1 adaptive immune response that up-regulates pro-inflammatory cytokines through the potential stimulation of $PGF_{2\alpha}$. Furthermore, peripheral responses from reproductive tissues (endometrium, peripheral blood mononuclear cells and corpus luteum) were corresponding to that of an impaired IFNT action (paracrine and endocrine). In the endometrium, from pregnancies with EM, up-regulated

DEGs that were not only targeted but also estradiol-mediated luteolytic action that could indicate remodeling of the endometrium (thickness), uterine lumen (stops producing histotroph) and reset of the estrous cycle. These endometrial changes have been previously associated with being unfavorable for conceptus survival. For PBMC, within pregnancies with EM, DEGs that associated with tissue growth, remodeling and/or development, innate immune system, conceptus implantation and essential mineral transporters were down-regulated. This could be evidence of either an intermediate or failed attempt of a pregnancy taking place. Within the CL, of an EM pregnancy, the up-regulated DEGs were associated with the activation of the luteolysis cascade through increase in calcium, inflammation and estradiol steroidogenesis. To our knowledge, the transcriptomic responses of day 16 *in vivo* reproductive tissues (endometrium, PBMC and CL) to pregnancies with EM conceptuses in this study is the first of its kind conducted in purebred Holstein-Friesian cows. The results from this study could then be a crucial contributor to further understanding pregnancies with EM and what reproductive practices may be used to mitigate them.

For chapter 3, discovery of candidate SNPs were based upon chapter 2's RNA-Seq single end read sequences from day 16 conceptuses (EM and N) within multiparous lactating Holstein-Friesian cows (n=15). The initial 17,156 candidate SNPs were filtered into 69 in 18 genes and were previously reported to have QTL associated to fertility traits within beef and dairy cattle. Validation and genotype to phenotype analysis of such candidate SNPs were conducted in a second dairy using primiparous lactating Holstein-Friesian cows (n=500). Quality control pipeline further sorted and reduced candidate SNPs to 23 in 14 genes. The genotype to phenotype analysis suggested that 7 of the candidate SNPs were predictive of inferior fertility traits that are of economic importance in Holstein-Friesian cows. It is then proposed that the 7 candidate SNPs could be used for culling and sorting heifer and cows that are reproductively inefficient.

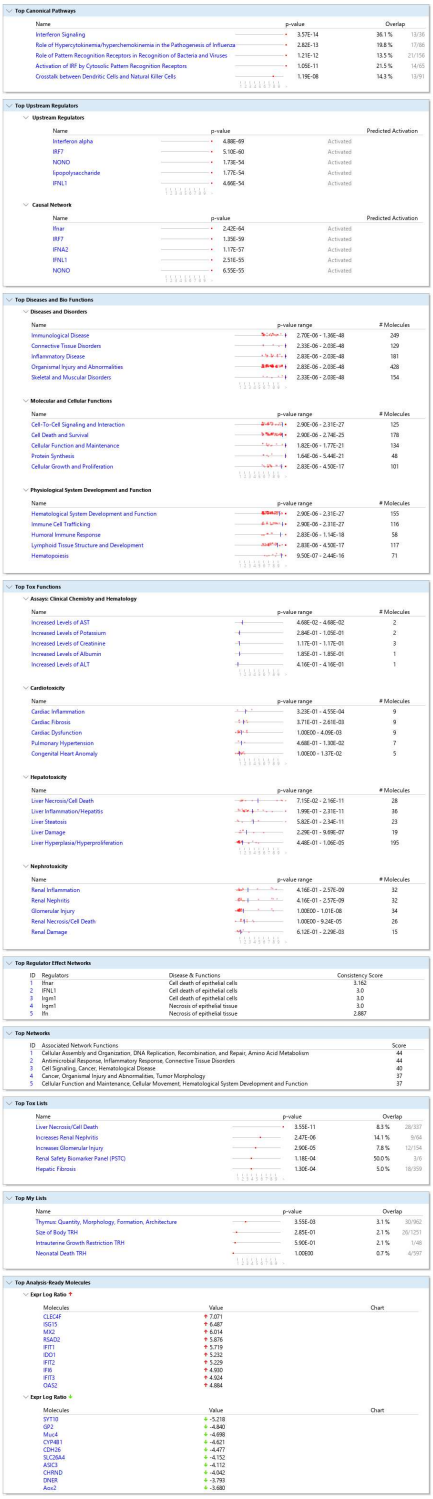
Recommendations moving forward from this theses research are three main things. The first is that more transcriptomic studies need to be conducted on *in vivo* EM conceptuses with reproductive tissues (endometrium, PBMC and CL) across different days of pregnancy with early EM (i.e., 7-15 and 17-24). The second is that additional research needs to be performed to not only determine the utility of the candidate SNPs in EM conceptuses that were associated with inferior fertility traits in genome-enhanced PTA estimations and (or) commercial genotyping tools for early life sorting and culling of heifers. The third is identifying candidate SNPs in reproductive tissues (endometrium, PBMC and CL) from pregnancies with EM and performing genotype to phenotype association studies to determine any associations to traits of economic importance (i.e., production, health, fertility).

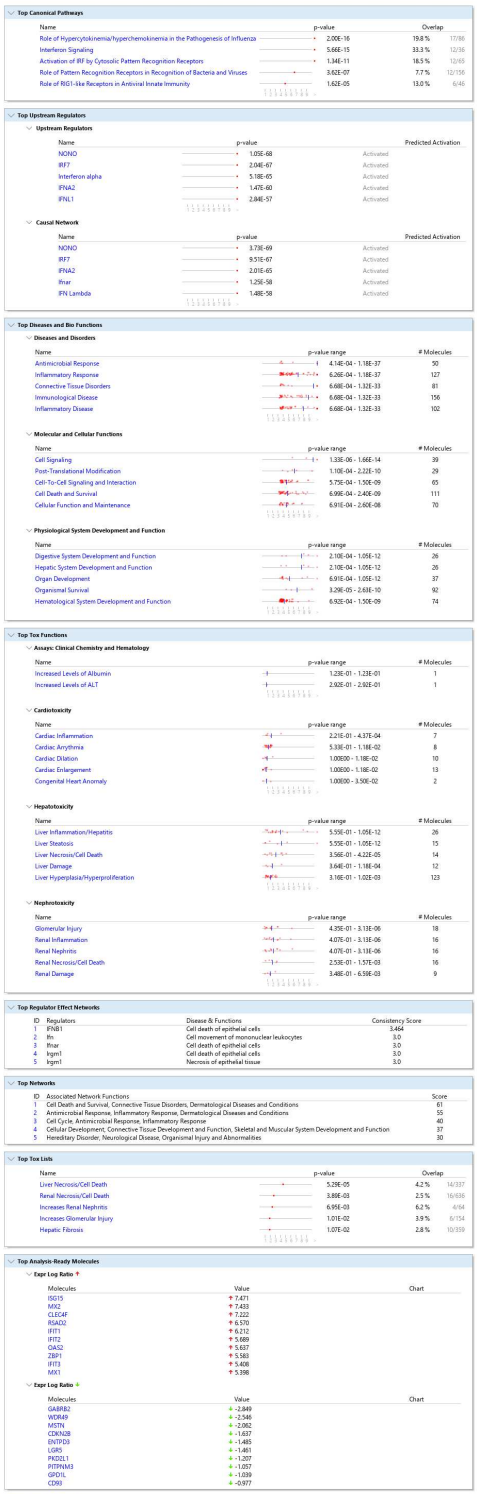
APPENDIX

E1 E2

Appendix Figure 1: Embryo Mortality vs Normal Conceptus.

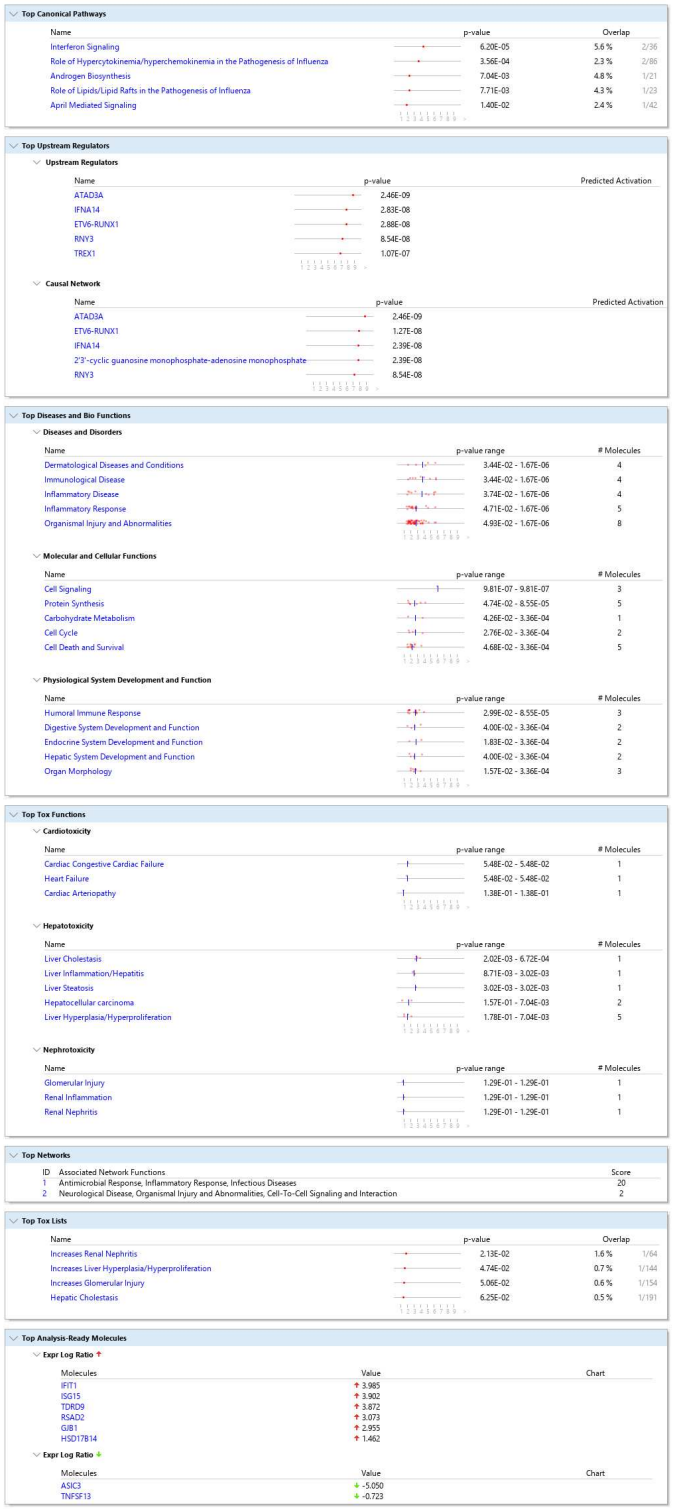
E1 E2



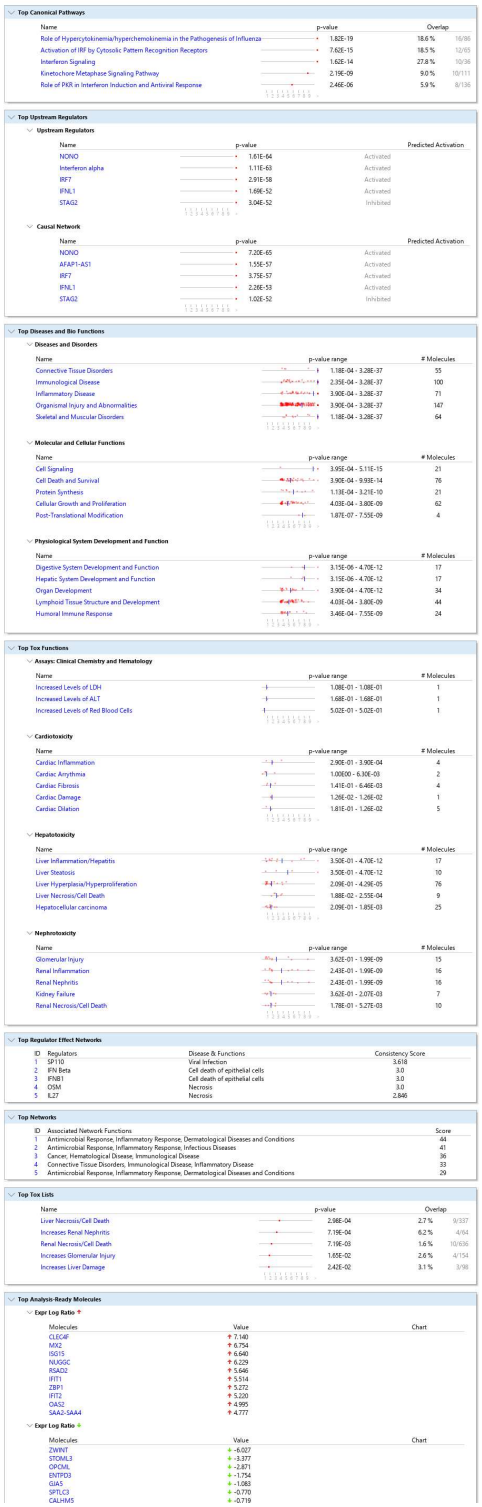


Appendix Figure 2: Normal vs Non-Pregnant Endometrium.

E1

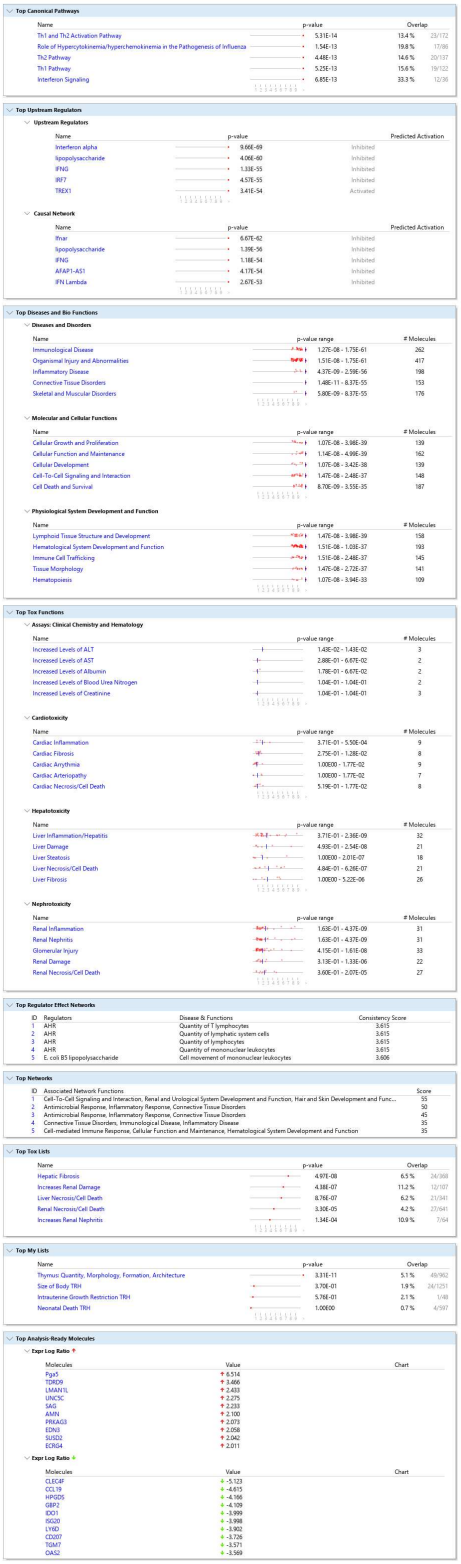


E2

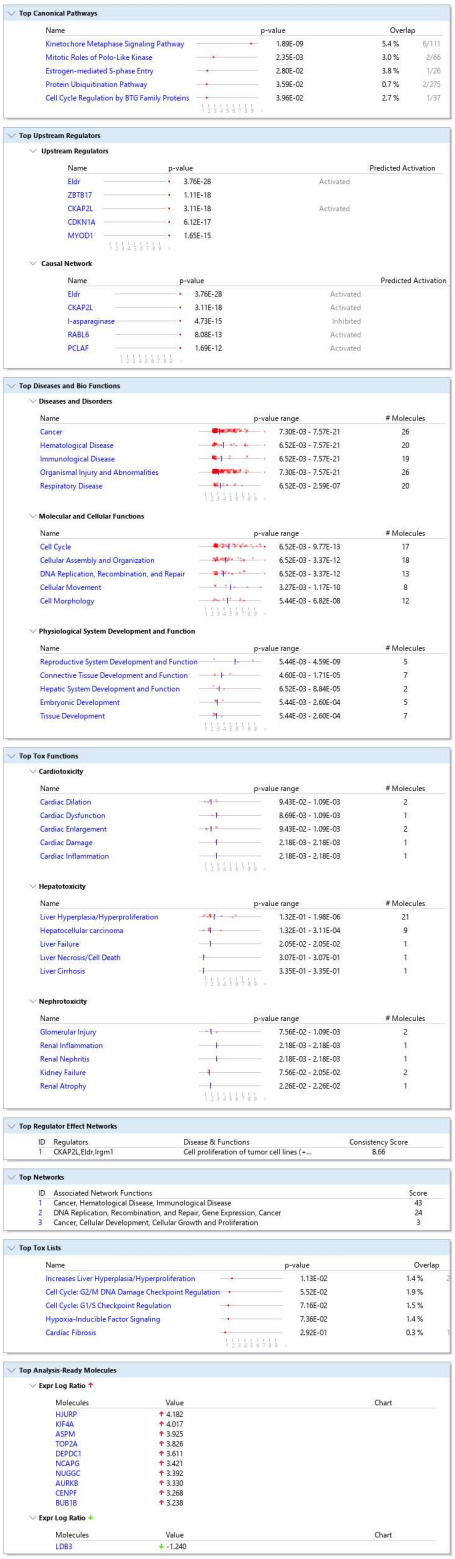


Appendix Figure 3: Embryo Mortality vs Non-Pregnant Endometrium.

E1

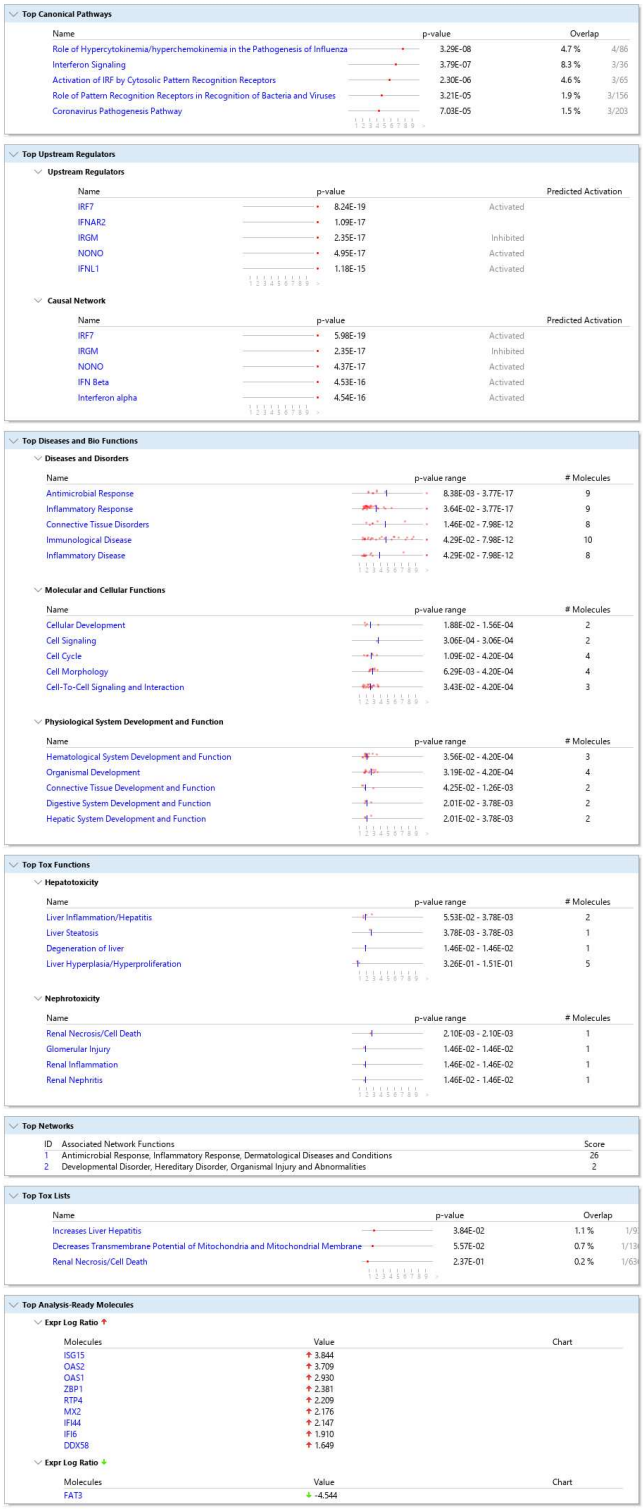


E2



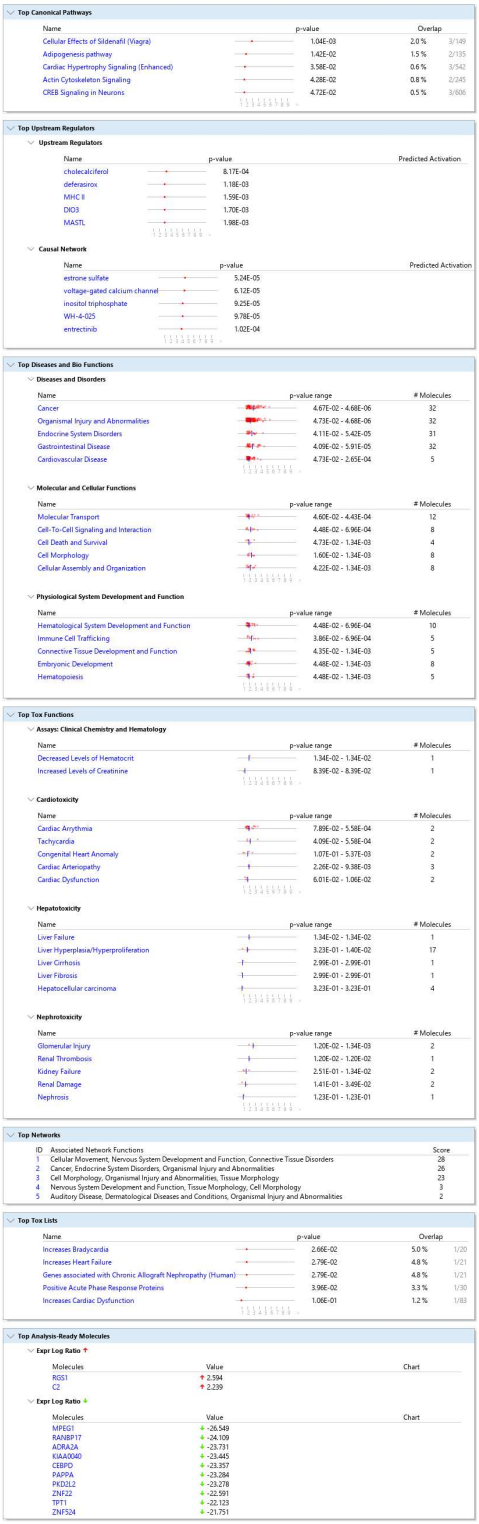
Appendix Figure 4: Embryo Mortality vs Normal Endometrium.

E2



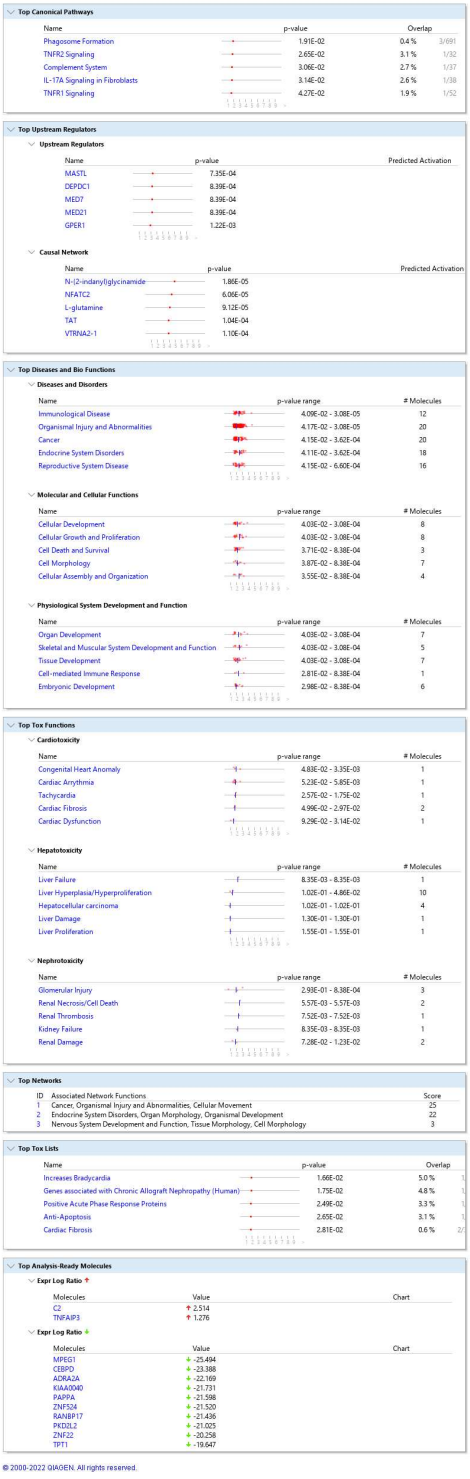
Appendix Figure 5: Normal vs Non-Pregnant Peripheral Blood Mononuclear Cells.

E1



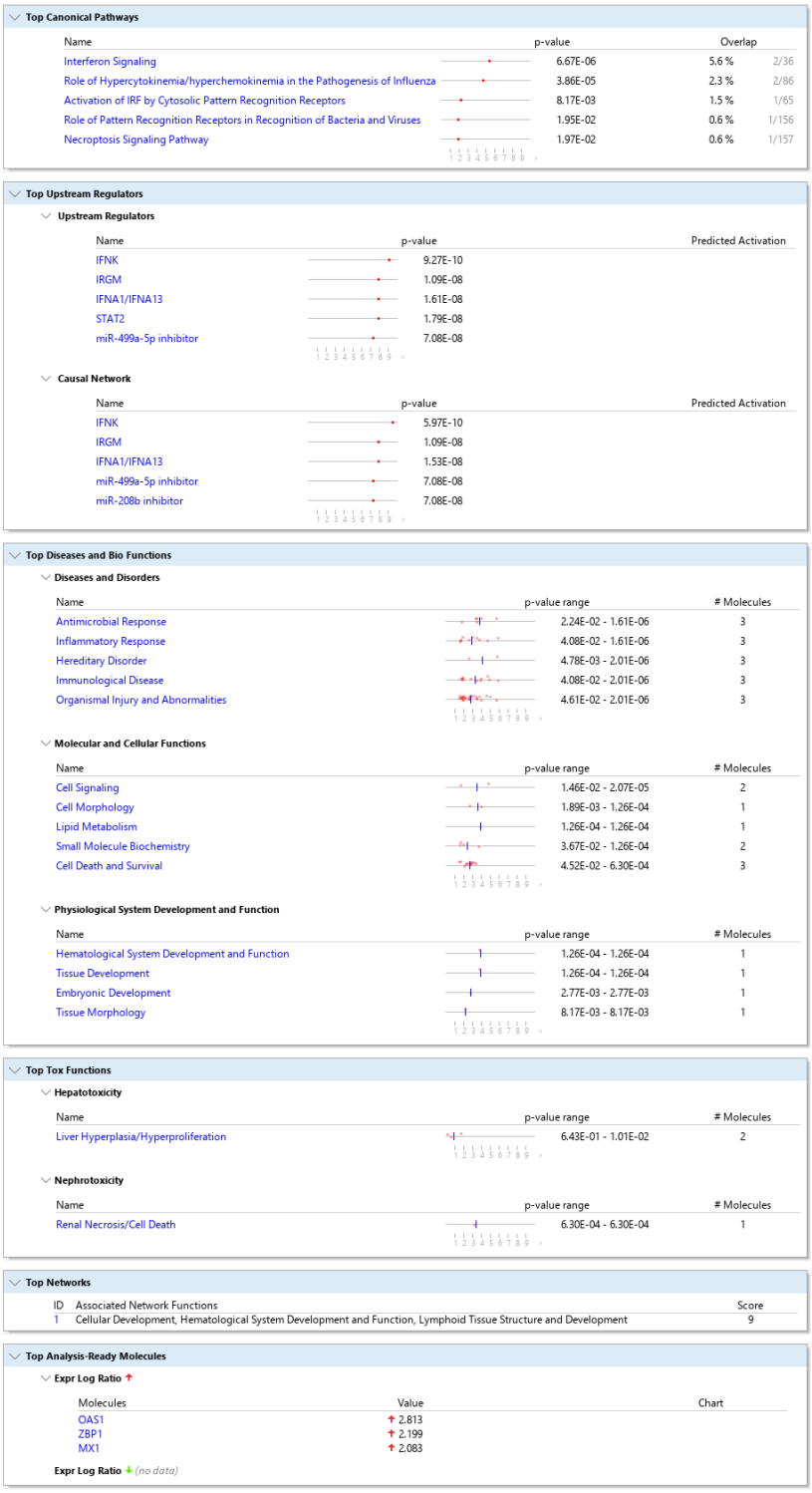
Appendix Figure 6: Embryo Mortality vs Non-Pregnant Peripheral Blood.

E1



Appendix Figure 7: Embryo Mortality vs Normal Peripheral Blood Mononuclear Cells.

E2



Top Canonical Pathways

Name	p-value	Overlap	
Interferon Signaling	6.67E-06	5.6 %	2/36
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	3.86E-05	2.3 %	2/86
Activation of IRF by Cytosolic Pattern Recognition Receptors	8.17E-03	1.5 %	1/65
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	1.95E-02	0.6 %	1/156
Necroptosis Signaling Pathway	1.97E-02	0.6 %	1/157

Top Upstream Regulators

Upstream Regulators

Name	p-value	Predicted Activation
IFNK	9.27E-10	
IRGM	1.09E-08	
IFNA1/IFNA13	1.61E-08	
STAT2	1.79E-08	
miR-499a-5p inhibitor	7.08E-08	

Causal Network

Name	p-value	Predicted Activation
IFNK	5.97E-10	
IRGM	1.09E-08	
IFNA1/IFNA13	1.53E-08	
miR-499a-5p inhibitor	7.08E-08	
miR-208b inhibitor	7.08E-08	

Top Diseases and Bio Functions

Diseases and Disorders

Name	p-value range	# Molecules
Antimicrobial Response	2.24E-02 - 1.61E-06	3
Inflammatory Response	4.08E-02 - 1.61E-06	3
Hereditary Disorder	4.78E-03 - 2.01E-06	3
Immunological Disease	4.08E-02 - 2.01E-06	3
Organismal Injury and Abnormalities	4.61E-02 - 2.01E-06	3

Molecular and Cellular Functions

Name	p-value range	# Molecules
Cell Signaling	1.46E-02 - 2.07E-05	2
Cell Morphology	1.89E-03 - 1.26E-04	1
Lipid Metabolism	1.26E-04 - 1.26E-04	1
Small Molecule Biochemistry	3.67E-02 - 1.26E-04	2
Cell Death and Survival	4.52E-02 - 6.30E-04	3

Physiological System Development and Function

Name	p-value range	# Molecules
Hematological System Development and Function	1.26E-04 - 1.26E-04	1
Tissue Development	1.26E-04 - 1.26E-04	1
Embryonic Development	2.77E-03 - 2.77E-03	1
Tissue Morphology	8.17E-03 - 8.17E-03	1

Top Tox Functions

Hepatotoxicity

Name	p-value range	# Molecules
Liver Hyperplasia/Hyperproliferation	6.43E-01 - 1.01E-02	2

Nephrotoxicity

Name	p-value range	# Molecules
Renal Necrosis/Cell Death	6.30E-04 - 6.30E-04	1

Top Networks

ID	Associated Network Functions	Score
1	Cellular Development, Hematological System Development and Function, Lymphoid Tissue Structure and Development	9

Top Analysis-Ready Molecules

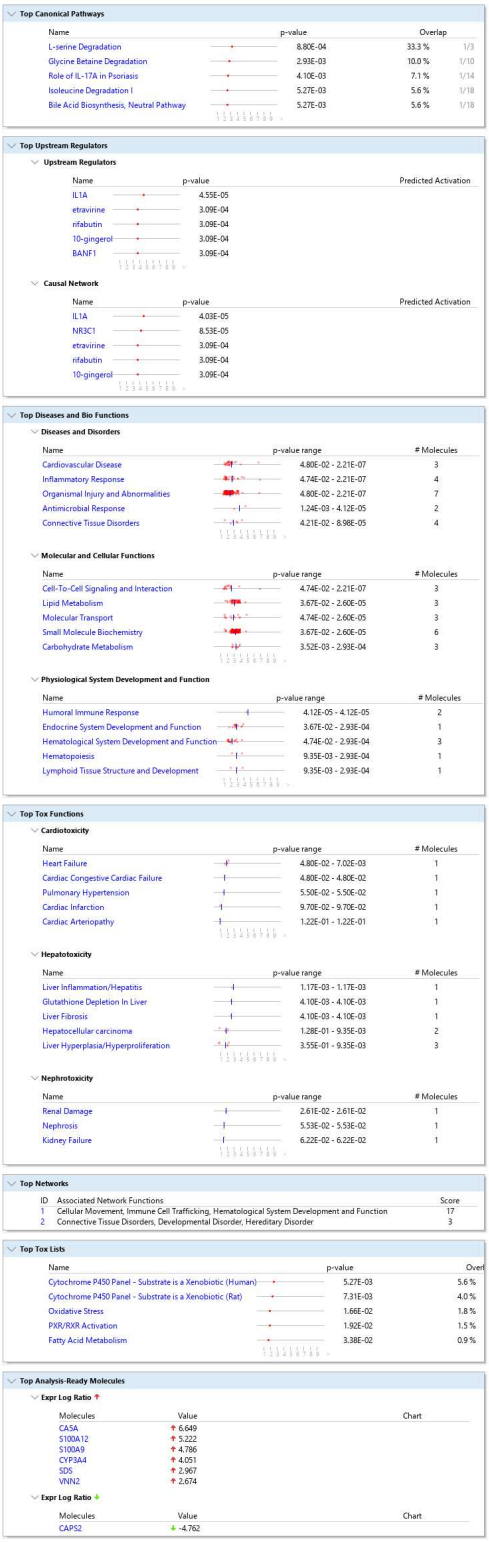
Expr Log Ratio ↑

Molecules	Value	Chart
OAS1	↑ 2.813	
ZBP1	↑ 2.199	
MX1	↑ 2.083	

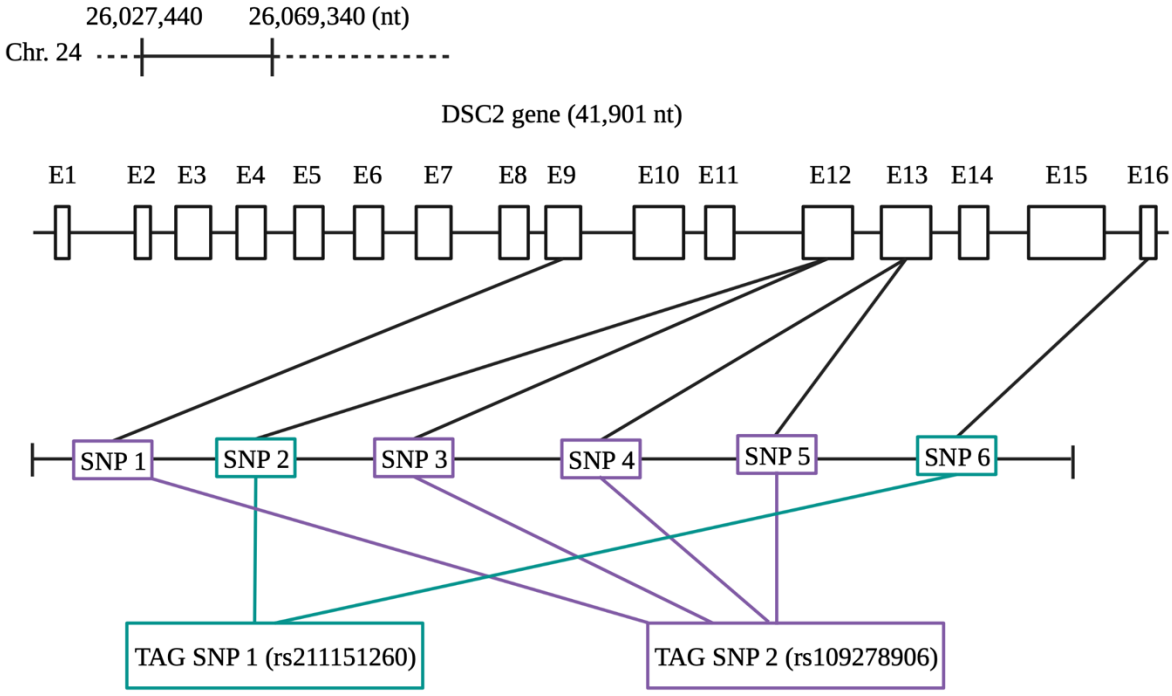
Expr Log Ratio ↓ *(no data)*

Appendix Figure 8: Normal vs Non-Pregnant Corpus Luteum.

E2



Appendix Figure 9: Embryo Mortality vs Normal Corpus Luteum.



Appendix Figure 10: Tag SNPs within exons of DSC2 gene in chromosome 24.
Chr=chromosome; E=exon.

LIST OF ABBREVIATIONS

AI	Artificial Insemination
BCS	Body Condition Score
CL	Corpus Luteum
Ct	Cycle Threshold
DEG	Differentially Expressed Genes
DIM	Days in Milk
DPR	Daughter Pregnancy Rate
DUMPS	Deficiency of Uridine Monophosphate Synthase
E1	Experiment 1
E2	Experiment 2
EM	Embryo Mortality
ESR1	Estrogen Receptor Alpha
ERT	Economic Relevant Traits
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing hormone
GWAS	Genome Wide Association Studies
HP	High Fertility
IFNT	Interferon Tau
IM	Intra-Muscular
IPA	Ingenuity Pathway Analysis
ISG	Interferon Stimulated Gene
LH	Luteinizing Hormone

LP	Low Fertility
MAF	Minor Allele Frequency
MHC	Major Histocompatibility Complex
N	Normal
NP	Non-Pregnant
O	Open
OXTR	Oxytocin Receptor
P	Pregnant
PAG	Pregnancy-Associated Glycoprotein
PCA	Principal Component Analysis
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline Solution
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PK-C	Protein Kinase-C
PSB	Pregnancy-Specific Protein B
PGR	Progesterone Receptor
QTL	Quantitative Trait Loci
RNA-Seq	RNA-Sequencing
RSID	Reference SNP Identification
RTqPCR	Reverse-Transcription Quantitative Polymerase Chain Reaction
SIFT	Sorting Intolerant From Tolerant
SNP	Single Nucleotide Polymorphism
STRING	Search Tool for the Retrieval of Interacting Genes/Protein

TAI	Timed Artificial Insemination
Th	T Helper
UF	Uterine Flushing