THE IMPACT OF POSTPARTUM HORMONAL INTERVENTION ON UTERINE HEALTH AND FERTILITY OF DAIRY COWS

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Animal Science – Doctor of Philosophy

2019

ABSTRACT

THE IMPACT OF POSTPARTUM FERTILITY TREATMENT ON THE MANAGEMENT OF REPRODUCTION IN DAIRY COWS

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This dissertation explores the complex mechanisms involved in the ability of a dairy cow to undergo parturition and then become pregnant once again in less than 3 months. This critical period in a cow's life is when the herd veterinarian spends most of their time ensuring this period is not fraught with problems that may impact the ability for this cow to become pregnant again. The first chapter reviews the importance of both ovarian and uterine physiology during this important period. The physiology of follicular and luteal function changes rapidly from parturition until 1 to 2 months later. Cows will generally have an ovulation and begin estrous cycles from 15 to 45 DIM. But a substantial percentage of cows will not have an ovulation before this time. We refer to these cows as "anovular" because they are not ovulating. Anovulation can be detrimental to the chances of a pregnancy following first AI as pointed out in Chapter 1. In addition, what transpires with regard to ovarian function also can have a direct impact on uterine physiology. In Chapter 2, the literature regarding the problem of endometritis on the health, welfare and chances of a pregnancy is presented. It was clear from this literature review that endometritis increases the time to pregnancy thus increasing the chances of cows leaving the herd too soon. This costs dairy producers a significant amount of money. Chapter 3 proposes a systematic way to solve the two main issues discussed in Chapters 1 and 2 with a hypothesis that utilizing GnRH and $PGF_{2\alpha}$ in tandem could resolve both the anovulatory condition of cows in addition to endometritis. Outcomes were clear that this strategy actually decreased fertility of primiparous lactating cows. The final chapter of this dissertation, Chapter

4, summarized the impact of a fertility program initiated at Rolling Acres Farm in Michigan. The impact of the fertility program, G6G, on a number of outcomes is described.

ACKNOWLEDGMENTS

Reflection on 40 years of veterinary dairy practice and graduate school experiences has been a humbling exercise as I realize how my family and I have been blessed by it. Perhaps the most impactful thing to me has been the personal relationships that I have had the privilege to form. It was thrilling to have had the responsibility to work on exceptional dairy cattle and with truly exceptional dairy people. The registered Holstein dairy persons like Gordon Schreur; John Tolsma; Henry and Tim Baker; Dennis Raterink family, Alvern Poest family; Gerald Geurink family; and the Wendell VanGunst family, along with their amazing pedigreed cows, provided a daily stress-test for me. I hope that most of the time, I passed. The commercial dairy producers, especially Merle Coffey and family; John and Phil Kuyers and Roger Headley; 3-L Scholten and Walt Horinga made up my clinical experiment station for learning to manage large dairy farm reproduction a bit differently. They encouraged me to think innovatively. I am forever grateful for their patience and good humor and for lessons that they have taught me.

The relationships that my wife and I have forged with veterinary student interns over the years has been a particular highlight for us. Liz has nurtured while I have mentored upwards of 40 students that eventually became veterinarians! Many of them lived with us while they completed their internship in our practice. These were life-style relationships as much as they were professional relationships. That aspect of my practice career brought me the most enjoyment. The interaction with students has enhanced my knowledge and understanding of physiology and medicine. More importantly, the relationships formed have greatly enriched our lives. It has been a joy to mentor the next generation of cow-vets that are now responsible for providing exceptional care for dairy farmers and the cows that depend on them.

My Northern Uganda Veterinary Service practice partner Dr. Tonny Kidega, our ‘daughter’ Susan Anago, and ‘grandchildren’ Kayla Elizabeth & Kyle Robert deserve acknowledgment for their genuine interest in my work and its application at our dairy farm in Gulu, Uganda, Africa. They are a constant reminder for me to keep it practical!

Our son Andy, an academic by nature, is the epitome of a perfect student. I have tried to emulate that during my graduate study to the extent that I could. His love for learning was my inspiration. Our daughter Betsy and her husband, Scott, have been a constant source of encouragement to me by their fascination with my unique graduate school experience. They have checked in often to assure me of their love and support – and to provide timely “counseling.” I must also recognize Hollis and Gwen Vlietstra, my dad and mom, for their unwavering support of my academic adventures and for their understanding of my preoccupied schedule during this endeavor. I am thankful for our grandson Samuel Robert, the fantastic culmination of a fertility treatment regimen under the direction of fertility specialists. Sam has been a true blessing to our family and a reminder to me that life is really about “who you love and who loves you”!

I am thankful that my WMVS practice partners allowed me this and many other diversions over the years. Special recognition is due to Dr. Susan Myers for supporting my graduate school application. Dr. John Gunther, a veterinary school Theriogenology professor and a dear friend, provided timely encouragement. Dr. Dan Grooms understood my goal and helped push me down the academic path. Each continues to be a hero to me within our profession.

Within the Department of Animal Science, Drs. Steve Bursian, Cathy Ernst and Pam Ruegg were collaborators in the “Dr. Bob redesign” effort. The RDSP faculty made me feel welcome and a valued part of the ANS team. The office staff, especially Karla Macelli, Lauri

Felton, Robbyn Davenport, and Debbie Romain, kept me in my lane with a gentle yet decisive direction. I am deeply appreciative of their cheerful assistance.

My mates in the Pursley Laboratory, Emily Middleton, Megan Ahearne, Drs. Thainá Minela, Alisson Da Mota Santos, and Lilian Martins were a constant source of inspiration, intellectual stimulation, and joy. I will especially miss Emily's sweet spirit and Excel spreadsheet acumen and expertise with SAS, Alisson's friendly nature, and Thainá's creative genius. They have added depth and breadth to my life. In reality, we became a kind of family, and I value our friendship greatly.

Our lab's undergraduate research assistants have been an integral aspect of our on-farm research efforts. Emily Peacock, Shianne Berthume, and Mike Herman worked tirelessly to record ovarian ultrasound evaluations and Metricheck examinations while Emmy Schuurmans and Jenna Smigielski administered fertility treatments and took hormone blood samples. Their dedication to excellence in their work has been greatly appreciated. I was especially grateful for their mostly good-natured tolerance of early morning data collection work during the harsh winter months.

My Guidance Committee members took on this "project" with some trepidation, I'm sure. I hope that, for the most part, I have not disappointed them in this journey. For my CVM colleagues, Drs. Ron Erskine, Dalen Agnew, and Jennifer Roberts, I offer my most sincere thanks. Ron and Dalen encouraged me to attain the academic level the program demanded. Jen included me in teaching CVM clinical Theriogenology courses that have jump-started my career as a reproductive physiology teacher. Dr. Miriam Weber-Nielsen was the cornerstone of the committee, having served as its chair. Miriam was the calming influence on my road to becoming. Her gentle spirit and wise counsel were most welcome when my confidence needed a boost.

It is difficult to find the correct words to adequately express my gratitude to my friend and colleague of 25 years who became my advisor and major professor, Richard Pursley. He must have been aware of the mental-baggage, a 67-year-old cow vet would be taking along to his research laboratory because, in our first meeting, he explained that he would be training me to think differently! I now realize that the retraining process was a steep mountain to climb, but I thank him for pushing me up the slope and providing me the methods to achieve that goal.

Richard has been a huge proponent of my aspiration to become a college reproductive physiology teacher. He tolerated my fascination with individual subfertility cases while redirecting my wandering mind. He was a master at offering timely and encouraging advice in my research, writing, and teaching. Dr. Pursley has been admired by his graduate students and respected by scientists worldwide. As a veterinary practitioner, I was one of his most ardent disciples. I have had the privilege of studying under his tutelage, and I am proud to be a graft from his coaching tree! I take seriously the responsibility that comes with it.

Above all, my wife, Liz, has always been supportive of my back-to-school antics. Unsurprisingly, she was my most enthusiastic cheerleader in this make-over decision. Her patience and forbearance of my penchant for an unconventional approach to my profession has allowed me to flourish. Liz continues to be my inspiration as she motivates me to be better than I am. She has encouraged me to be content in all things, but never satisfied with the status quo! We are life-long learners - together.

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KEY TO ABBREVIATIONS

AI	artificial insemination
BCS	body condition score
BHBA	beta-hydroxybutyrate
BUN	blood urea nitrogen
CA	corpus albicans
CE	clinical endometritis
CH	corpus hemorragicum
CI	calving interval
CL	corpus luteum
CR	conception rate(s)
CYT	cytological endometritis
d	day(s)
DF	dominant follicle
DFS	day(s) to first service
DIM	days in milk
DNB	do not breed
DO	days open
E_2	estrogen
E. coli	Escherichia coli
ELISA	enzyme-linked immunosorbent assay
EnPEC	Endometrial Pathogenic E. coli
ER	estrogen receptor
F. necrophorum	Fusobacterium necrophorum
FP	follicular puncture
FSH	follicle-stimulating hormone
FW	follicular wave
GnRH	gonadotropin-releasing hormone

h	hour(s)
HDR	heat detection rate
HFC	high fertility cycle
IGF-1+	insulin-like growth factor
IL	interleukin
IR	insemination rate
LH	luteinizing hormone
LPS	lipopolysaccharide
M	Metricheck
NEB	negative energy balance
NEFA	nonesterified fatty acid(s)
OR	odds ratio
OXTR	oxytocin receptor
P_4	progesterone
PAGs	pregnancy-associated glycoproteins
PE	physical exam
PGE	prostaglandin E
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PMN	polymorphonuclear
PR	progesterone receptor
P/AI	pregnancy per artificial insemination
PSPB	pregnancy-specific protein B
PVD	purulent vaginal discharge
RFM	retained fetal membranes
SE	subclinical endometritis
T. pyogenes	Trueperella pyogenes
TAI	timed artificial insemination
TCI	Transition Cow Index
TLR	toll-like receptor
TMR	total mixed ration

TNF_{α}	tumor necrosis factor-alpha
VWP	voluntary waiting period

CHAPTER 1

OVARIAN AND UTERINE PHYSIOLOGY EARLY POSTPARTUM

GENERAL INTRODUCTION: REPRODUCTIVE PHYSIOLOGY FROM PARTURITION TO 1ST AI IN THE LACTATING DAIRY COW

Successful reproduction in the high producing dairy cow has been the focus of the dairy industry for as long as dairy producers have depended on milk sales for income. Metabolic demands on the cow have negatively impacted the reproductive function of postpartum cows (Beam and Butler, 1999; Chagas et al., 2007) as milk production per cow increased over the past 70 years due to rapid progress in genetics and management practices. However, recent research has increased knowledge and understanding of the bovine estrous cycle and its endocrinology, resulting in conception rate resurgence (Sakaguchi et al., 2004; Sartori et al., 2004; Lauderdale, 2009; Wiltbank et al., 2011; Wiltbank and Pursley, 2014). For example, the availability and use of trans-rectal high resolution and Doppler ultrasonography by competent researchers has allowed precise evaluation of the processes of follicular dynamics and CL function (Schams and Berisha, 2004; Wiltbank et al., 2011). These processes include follicular emergence, selection, growth, divergence, dominance, atresia, ovulation, and CL function. The use of serum testing for reproductive hormones has strengthened the understanding of the endocrine system's role surrounding fertility in the dairy cow (Stevenson and Britt, 2017).

The concept of induced ovulation and synchronized TAI to increase fertility in the reproduction programming of dairy cattle and the more in-depth understanding of ovarian physiology has revolutionized bovine reproduction efficiency over the past 24 years. Adaptations to Ovsynch are one of the most widely used and successful reproductive technologies in the dairy industry (Wiltbank and Pursley., 2014). During that time, the industry has experienced overall CR that has reached acceptable levels. Some farms are able to achieve an average of 70% 1st insemination P/AI utilizing the G-6-G protocol, an adaptation of the Ovsynch program. Fricke and Pursley (2019) refer to that success as a "reproduction revolution!" Many contributing factors are involved in the transformation in reproductive efficiencies such as the attention given to sire fertility, maternal fertility, and the intentional management of them.

On-farm sire fertility management is limited to the use of the USDA-ARS sire CR summaries in choosing bulls due to the almost exclusive use of AI (Stevenson and Britt, 2017). Semen quality is primarily dependent on proper semen tank maintenance, semen straw storage, and use technique.

Subfertility, due to the semen factor in the fertility equation, was found to be no less complex (Saacke et al., 2000). The seminal traits they discussed, included compensable (sperm transport, function in the female tract, initiation of the fertilization process, polyspermy blockage) and uncompensable (maintenance of the fertilization event and subsequent embryogenesis, errors in spermatozoa chromatin) traits. Saacke et al. (2000) concluded that a positive association existed between accessory sperm number and embryo quality. That indicated a morphologically more competent sperm was successful in the competition for fertilization at the zona pellucida.

When taking semen into account in the quest to evaluate reproductive strategies, the morula stage embryo served as a biomonitor since pregnancy rate was impacted by both fertilization rate and embryogenesis (Saacke et al., 2000). Accessory sperm reflected both fertilization rate and embryo quality; therefore, it was suggested to be evaluated as well. According to the research, optimum timing for artificial insemination in dairy cows was a compromise. Early insemination resulted in reduced fertilization rates with low numbers of accessory sperm but good quality embryos. The opposite was found to be the case in late inseminated cows (Saacke et al., 2000). Optimal timed TAI to achieve pregnancy with commercially available semen was performed at 16 h after the last GnRH treatment. It was the standard for the three suggested "fertility programs," Presynch-11, G-6-G, and Double Ovsynch (Bello et al., 2006; Sousa et al., 2008; Wiltbank et al., 2011; Wiltbank and Pursley, 2014; Martins and Pursley, 2016).

Maternal fertility has been defined as "the mother's ability to ovulate a competent oocyte and provide an oviductal and uterine environment capable of fertilization and complete embryonic and fetal development" (Pursley, personal communication). Pre-synchrony adaptations of the Ovsynch program have achieved the goal to routinely present an optimal fertile oocyte at ovulation (Bello et al., 2006; Sousa et al., 2008; Wiltbank and Pursley., 2014). However, far less attention has been given to the oviductal and uterine portion of the maternal fertility equation, and clinicians often ignore it. That neglect could lead to issues of reduced fertility when pathology is encountered, resulting in far-reaching medical problems in the cow and economic loss for the farm enterprise (De Vries, 2006; Ribeiro et al., 2016; Middleton et al., 2019). In short, the key limiting factors in attaining profitable reproductive performance in lactating dairy cattle are primarily issues of maternal sub-fertility and the multifaceted preventative management of it.

Increasing P/AI has the potential to maintain farm sustainability and to increase profit margins by employing the high fertility cycle (HFC) philosophy (Middleton et al., 2019). That philosophy is centered around attaining 80% P/AI by 130 DIM, coupled with the achievement and maintenance of a 13-month CI. That lofty standard cannot be reached with complacent farm reproduction program management. The transition, peripartum, and postpartum periods are essential times for intense gynecologic management of the high producing dairy cow. That collective period when neglected, has the potential to limit conception and negatively affect embryonic and fetal development.

In reality, the success of any on-farm reproductive program is largely dependent upon the management of it. One of the main determinates of dairy farm profitability and sustainability was reproductive performance (De Vries, 2006; Galvao et al., 2013). Farm personnel control programs to the extent that they can or will and to the extent that protocol complexities are understood. The development of a comprehensive reproductive program that addresses every accepted nuance of dairy cattle fertility and accounts for individual cases of sub-fertility is the easier variable in a successful program. That is because of the expansive information base available to farm consultants. The more difficult component in achieving reproductive success is obtaining and maintaining strict compliance to the program as it is written. Reducing program variation by rigid event observance is where science and art intersect in the practice of applied reproductive physiology and theriogenology, and it is there that failure frequently occurs!

TRANSITION / PERIPARTUM PERIOD

Parturition in a 13-month interval was a goal for successful dairy cow reproduction management (Middleton et al., 2019). Walsh et al., (2011) considered the points with the most significant impact on fertility to be little or no time spent in NEB, no infection of the postpartum uterus, good HDR and IR using high fertility semen and the ovulation of a high-quality oocyte. Additional points that impacted fertility were early and increasing P_4 secretion from CL, the uterine endometrium having created the appropriate environment to stimulate embryo development resulting in a healthy embryo that produced adequate interferon tau quantities, and the alteration of endometrial prostaglandin secretion signaling maternal pregnancy recognition (Walsh et al., (2011).

TCI had been developed as an objective evaluation of herd-level transition cow management (Nordlund, 2006). It utilized fourteen factors from a cow's historical DHIA record to project her first test date milk yield. Deviations from the expected milk yield were calculated and used to evaluate the effectiveness of cow management programs. Nordlund (2006, 2009) found five critical factors as major constituents associated with average TCI scores (Table 1.1. Nordlund, 2006).

TCI is an objective tool that dairy producers and their consultants could implement to benchmark, evaluate, and monitor the effectiveness of interventions during the transition period. Particular attention to detail in the five management areas listed are essential for optimal cow transitioning. A dairy cow's successful transition during the parity continuum was mostly dependent on a timely pregnancy (Middleton et al., 2019).

POSTPARTUM PERIOD

The healthy postpartum cow typically begins to develop a follicular wave in the first week postpartum, and the first DF was selected by d 10 (Leslie, 1983; Sakaguchi et al., 2004) or 15 d postpartum (Britt et al., 1974). A first ovulation occurred within two weeks after calving in dairy cows (Britt et al., 1974; Rajamahendran et al., 1990; Savio et al., 1990; McDougall et al., 1995). In one study, it was found that healthy ovarian follicular dynamics were regained after the second ovulation postpartum (Sakaguchi et al., 2004). Shrestha et al. (2004) found that 37% of cows experienced a normal resumption of ovarian cyclicity within 45 d postpartum and 46.3% of the cows did not resume ovarian cyclicity until greater than 65 d postpartum.

Of particular importance was the role of P_4 as it related to the postpartum high production dairy cow (Wiltbank et al., 2014). Approximately 20% of cows remained in an anovulatory state for an extended period postpartum even though early post-partum cows typically resumed FW within the second week of lactation (Wiltbank et al., 2011; Vercouteren et al., 2015). Santos et al. (2016) reviewed the ovarian related mechanisms underlying reduced fertility in anovulatory dairy cows. It was reported that resumption of ovulation in the dairy cow after parturition was a coordinated process between many organs, biochemical/endocrine pathways, genetic factors, disease processes and management practices (Shrestha et al., 2004; Opsomer et al., 2000; Santos et al., 2016). Insufficient P_4 concentrations during follicle development through the early postpartum period affected ovarian dynamics and had direct effects on fertility in general and on embryo development and uterine receptivity to pregnancy (Santos et al., 2009; Cerri et al., 2011).

Uterine bacterial infections in dairy cows caused slower dominant follicle growth, lower peripheral plasma E_2, and suppression of GnRH and LH release that resulted in fewer ovulations (Sheldon et al., 2009). Vercouteren et al. (2015) concluded that metritis was negatively associated with early cyclicity in dairy cows. Ovulation of a DF from the first follicular wave ranged between 74% (Savio et al., 1990) and 31% (Butler et al., 2006), which resulted in a functional CL producing P_4. The circulating P_4 had beneficial effects on future ovarian function as it related to the production of fertile oocytes that resulted in healthy embryos (Pursley and Martins, 2011; Wiltbank et al., 2011). However, high levels of P_4 could have deleterious effects on the uterine immune function of a cow with metritis (Lewis, 2004; Sheldon and Dobson, 2004). The regression of a functional CL was imperative in those cases to ultimately provide for a healthy uterine lumen to allow for transport of sperm to the oviduct, fertilize the oocyte and result in a pregnancy that completed gestation.

It was reported that the resulting multiple estrus events could have resulted in a healthier uterine environment that may have enhanced fertility (Thacker and Wilcox., 1973; Britt et al., 1974; Foote and Reik, 1999). However, a few investigators have found that early administration of GnRH might not affect (Britt et al., 1974) or have a negative effect (Etherington et al., 1984) on P/AI. Studies have shown that $PGF_{2\alpha}$ had positive effects on reproductive performance when administered postpartum, even without luteolytic action (McClary et al., 1989; Lewis, 2004). In contrast, some researchers have found no beneficial effects of early $PGF_{2\alpha}$ use (Haimerl et al., 2012), while its positive effects on fertility could be seen when used in the later postpartum period and during the synchronization protocol (Gumen et al., 2011).

Anestrous was negatively associated with postpartum fertility (Peter et al., 2009). It is characterized clinically by the lack of estrus behavior within the context of the estrous cycle. Peter et al. (2009) classified anestrous based on follicular development function, that is the emergence of a new FW, deviation, and ovulation. Emergence is usually not affected by postpartum. However, growth, deviation, and ovulation can be perturbed.

PRE-BREEDING PERIOD

The Estrous Cycle and Endocrine System

A general knowledge of the bovine estrous cycle and the controlling hormones was the foundation for the successful application of programmed breeding strategies (Thatcher, 2017). The bovine reproductive cycle could be divided didactically into four distinct phases beginning with puberty. The phases in order are estrus, metestrus, diestrus, and proestrus.

Estrus is the period of sexual receptivity with standing to be mounted as the primary sign. All other signs, including clear mucous discharge, hyperemia, and vulvar edema, are examples of secondary signs. Ovulation occurs at the end of estrus and is hormone dependent.

Metestrus directly follows ovulation and is the period in which fertilization occurs. A bloody discharge is a physiologic marker for ovulation occurring during metestrus. It usually occurs within 48 h of ovulation. Wiltbank et al. (2011) reported that the second of two FSH surges peri-ovular starts the next FW. Emergence and development of follicles occur in a wave-like pattern with deviation and then dominance of the largest cohort follicle resulting in ovulation or atresia. It is more typical for a multiparous cow to have a two FW cycle, while primiparous heifers can have three or more FW in each estrous cycle (Sakaguchi et al., 2004).

Follicular development and maturation of CL occur during diestrus. It is characterized by FW dynamics and increased P_4 from the CL derived from the past ovulated follicle.

Proestrus is the period when the final development of a pre-ovulatory follicle occurs. It is marked by decreasing or basal levels of P_4, high levels of E_2, and inhibin from the pre-ovulatory follicle and $PGF_{2\alpha}$ from the uterine endometrium.

Reproductive hormones interconnected to important reproductive events were described by Leslie (1983b), Wiltbank et al. (2011) and Thatcher (2017). Reproductive hormones are divided into the hypothalamic-pituitary axis, gonadal, and uterine hormones. Hypothalamic neurosecretory neurons secrete a decapeptide, releasing hormone GnRH into the vasculature of the hypophyseal portal system arriving at the anterior pituitary gland. GnRH stimulates the release of FSH and LH from the pituitary. Low-frequency GnRH pulses are responsible for FSH secretion, whereas high-frequency pulses caused LH secretion (Wiltbank et al., 2011). Gonadotropin hormones had a trophic effect in the target gonadal tissue – the ovary.

The glycoprotein hormone FSH stimulates recruitment, growth, and maturation of primordial follicles into tertiary follicles, commonly called antral follicles. FSH growth-dependent follicles are pre deviation follicles. The primary driver of follicular growth post-deviation was found to be low-level pluses of LH (Wiltbank et al., 2011). The glycoprotein LH was found to be synthesized by the anterior pituitary and secreted in response to GnRH. LH concentrations were positively associated with FSH and E_2 and negatively associated with P_4 (Ireland and Roche, 1982; Gobikrushanth et al., 2017). That finding agrees with Roberson et al. (1989) who reported that an increase in LH pulse frequency was associated with low levels of P_4.

Wiltbank et al. (2011) found that LH had an essential responsibility in the final maturation of a pre-ovulatory DF due to the acquisition of LH receptors in granulosa cells. Xu et al. (1995) found that early-stage FW follicles do not ovulate due to a lack of LH receptors. Pulsatile LH release during the follicle's dominance phase of the FW was most likely the major driver of growth of the DF and its production of E_2 (Wiltbank et al., 2011). Ovulation occurred due to a high-amplitude LH surge from the anterior pituitary. Following ovulation, LH induced follicular cells morph into luteal cells. Stimulated luteal cells maintained luteal function by releasing periodic LH pulses (McCracken et al., 1999).

The gonadal hormones P_4, E_2, and inhibin are produced in the ovaries. The developing follicular structure produces the steroid hormone E_2. It acts in tissue that expresses ER. E_2 is responsible for increasing blood supply, cellular proliferation, and growth of reproductive tissues. It increases the expression of OXTR in the uterus, thereby stimulating uterine myometrial contractility. High levels of E_2 and basal levels of P_4 promote sexual receptivity behavior. E_2 also regulates the production and release of hypothalamic-pituitary hormones.

Inhibin is a glycoprotein hormone produced by granulosa cells in a DF. It is the primary source of FSH inhibition, suppressing its release from the anterior pituitary gland.

P_4 is a steroid hormone produced by CL. It acts in tissue that expresses its PR, primarily the uterus. P_4 bound to PR in the uterus caused smooth muscle relaxation, decreased OXTR, and increased uterine gland growth and secretory function. It is preparatory for pregnancy, and if fertilization occurred, its role becomes one of pregnancy maintenance. P_4 also had an essential role in the regulation of hypothalamic-pituitary hormone production and release (Carvalho et al., 2015).

$PGF_{2\alpha}$ is a hormone produced in the uterine endometrium that is vitally important for reproductive function. $PGF_{2\alpha}$ is derived from arachidonic acid and is produced in response to the binding of oxytocin to OXTR. When $PGF_{2\alpha}$ was released into the venoarterial pathway (Mapletoft et al., 1976; Ginther, 1981), it acted on CL to cause an initial transient increase in blood flow before a decrease in plasma P_4 (Ginther et al., 2009) followed by dramatically reducing its vascularization in association with luteolysis (Niswender et al., 1976).

Reproductive hormonal interactions are dependent upon how their properties relate to performance. Protein hormones, for example, glycoproteins and peptides, act on cell surface receptors. Binding activates other molecules inside the cell resulting in an immediate hormonal response. The response is not a time-dependent function since the response occurred as long as receptors were present. Contrary, steroid hormones act on intracellular receptors that result in a less-immediate effect of hormone and receptor binding. As levels of steroid hormone rise, increased expression of their receptors takes place. Prolonged and continuous hormone stimulus results in decreased hormone binding with its receptor and, consequently, a decreased expected hormonal response.

Stevenson and Pulley (2016) agreed that three main negative feedback loops are involved in the estrous cycle's hormonal pattern. First, there was a direct relationship between P_4 and E_2 levels and the expression of their receptors. Greater PR expression resulted from high or increasing levels of P_4. Low or decreasing levels of E_2 resulted in lower ER expression. Also, the binding of P_4 with PRs decreased expression of OXTR in the uterus. With the decreased uterine OXTR expression, the binding of oxytocin to OXTR did not occur. As described above, $PGF_{2\alpha}$ release is blocked.

Second, high levels of E_2 and inhibin produced by the DF theca and granulosa cells suppressed the pituitary FSH release. E_2 exerted a negative feedback effect on GnRH release from the hypothalamus; therefore, FSH release did not occur. Inhibin suppressed production and release of FSH at the level of the anterior pituitary gland. If P_4 levels are high after follicle deviation, the FSH surge is blocked.

Third, high levels of P_4 exerted a hypothalamic level negative feedback blocking GnRH release frequency and pulsatility. Consequently, a pituitary LH surge and ovulation cannot manifest. However, a low-amplitude LH pulse is expected every six h while P_4 levels are high. That was important for the DF development where LH receptors are located.

Stevenson and Pulley (2016) agreed that three positive feedback systems controlled hormonal influence over the estrous cycle. First, as described previously, stimulation of steroid hormone receptors over time resulted in decreased hormone binding with its receptor. When P_4 was elevated into late diestrus (d 15 – 18), its receptor became less sensitive to P_4 binding. Low or decreased P_4 levels resulted in lower PR expression, and blocking OXTR expression was not as efficient. Concomitant, the DF produced increased levels of E_2, resulting in greater ER expression elevating expression of OXTR in the uterus. The binding of oxytocin with its receptor OXTR occurred, and as a result, $PGF_{2\alpha}$ was released by the endometrium (Wolfenson et al., 1985; McCracken et al., 1999; Schams and Berisha, 2004). Regression of CL lowering P_4 resulted during proestrus ushering in the proper hormonal environment for the developing DF and impending estrus event.

The second positive feedback system involved a lack of inhibition through decreased inhibin. High levels of E_2 and inhibin negatively affected the production and release of FSH from the pituitary. Therefore, the absence of E_2 and inhibin and their inhibition allowed for FSH to return to basal levels, including the FSH surge. The FSH surge occurred while P_4 levels were elevated.

Finally, while P_4 was low and E_2 increased during proestrus, a hypothalamic level positive feedback was exerted, having increased the pulsatility of GnRH release. That resulted in a high-amplitude LH and FSH surge culminating in ovulation after estrus.

Physiological and, therefore, endocrine events are predictable during the bovine estrous cycle (Stevenson and Pulley, 2016). The second peri-ovular FSH surge caused a new FW (Wiltbank et al., 2011). The emerging cohort of follicles grew about 1.5 mm per d under FSH stimulation. During that early period. follicle size averaged 4 - 5 mm. At the same time, FSH concentrations were decreasing on d 5 and 15 of the estrous cycle due to increased E_2 and inhibin production by the largest emerging follicles in the wave. There was a positive correlation between the production of E_2 and inhibin and follicle size (Wiltbank et al., 2011). In each wave, one or two follicles were more efficient at secreting E_2 and inhibin that adversely affected subordinate follicle growth and development.

Deviation of one or two follicles from others in the FW cohort occurred 4 - 5 d after FW emergence. It was characterized by the deviated follicle(s) at ~8 mm, acquiring LH receptors (Wiltbank et al., 2011). Continued proper follicle growth and development after deviation are LH pulse dependent. The follicle that acquires LH receptors could become the DF secreting copious amounts of E_2 and inhibin suppressing basal levels of FSH (Ireland and Roche, 1982; Roberson et al., 1989; Butler et al., 2008).

The DF had three usual outcomes; ovulation, atresia, or an anovulatory condition such as an enlarged and aged follicular cyst (Wiltbank et al., 2011). Increasingly more common in high producing dairy cows was the incidence of double ovulation (Wiltbank et al., 2006; Wiltbank et al., 2011).

The anovulatory conditions described by Wiltbank et al. (2011), include a cystic follicle condition associated with low E_2. Insufficient LH pulses during the dominance phase of the follicular wave caused an insufficient production of E_2. Cows with that scenario were likely to present in a state of anestrous (Peter et al., 2009). Contrary, Bergfeld et al. (1996) reported that the reduced P_4 found in cows versus heifers (Sartori et al., 2001, 2004; Wolfenson et al., 2004) might result in larger DF diameters due to more luteal phase LH pulses. Secondly, a large anovulatory follicle producing large amounts of E_2, an elevated LH basal level but lacking an LH surge (Leslie, 1983), could present as a cow experiencing varying degrees of nymphomania. Wiltbank et al., (2011), suggests that that physiological change may be governed by the lack of a GnRH / LH surge in response to elevated E_2. E_2 positive feedback block on the hypothalamus could be driven by low serum P_4 interference. Cows that lose body condition early postpartum could have an association with sub-luteal release of P_4 from fat. Finally, there may be a disruption in the development of LH responsiveness in granulosa cells that are required for ovulatory capacity (Wiltbank et al., 2011). The acquisition of LH receptors near follicle deviation depended on LH activity. Therefore, after the DF deviation period, the presence of granulosa cell LH receptors suggested a significant role for LH in follicle growth (Wiltbank et al., 2011).

Anovulation occurs in about 20% of high producing dairy cows. In addition to infectious and inflammatory disease leading to follicular perturbation (Sheldon et al., 2009; Careiro et al., 2016; Cheong et al., 2017), nutrition management of the cow and her metabolic system had an effect on ovulation and fertility (Beam and Butler, 1999; Butler et al., 2006; Gumen et al., 2011; Kawashima, et al., 2011; Wiltbank et al., 2015). The failure to ovulate a DF may lead to a low P_4 environment that could have a profound effect on ovarian dynamics and subsequent fertility in lactating dairy cows. As discussed in a prior section, the early postpartum cow with a depressed DMI resulting in increased NEB, lost body condition (Beam and Butler, 1999; Butler et al., 2006; Gumen et al., 2011; Drackley and Cardoso, 2014). Anestrus and anovulatory cows are an important subset of any dairy's herd eligible for 1st AI.

Anovulation has more recently become a less-practical issue in large cohort dairy farms. The extensive use of fertility treatments (GnRH) to ovulate any DF with LH receptors in presynch / Ovsynch protocols and high energy balanced TMR rations has alleviated much of the anovulatory condition. However, Vasconcelos et al. (2001) reported that some of the induced small follicle ovulation resulted in smaller volume CL secreting less P_4 resulting in reduced fertility.

Atresia refers to the regression or death of a follicular structure. Subordinate follicles that lacked LH receptors post-deviation or a 10 d first wave DF with insufficient LH resulted in insufficient E_2 production ceasing its growth and becoming atretic (Wiltbank et al., 2011). Atresia was a response to the negative feedback of high P_4 concentration interfering with hypothalamic GnRH pulses eliminating an LH surge. When the DF becomes atretic, decreased E_2 and inhibin removed the negative feedback on FSH secretion. A new FW was initiated.

Ovulation was the outcome for a DF with sufficient expression of its LH receptors after an LH surge (Wiltbank et al., 2011). It was the physical rupture of the pre-ovulatory DF and the release of the oocyte. The average ovulatory follicle is ~17.5 mm in cows and ~15 mm in heifers

(Vasconcelos et al., 2001). The final maturation of the DF was due to increased LH pulses in response to decreasing levels of P_4 from the regressed CL around d 19. A concomitant increase in E_2 with decreased P_4 provided for positive feedback at the hypothalamus allowed for a GnRH surge, followed by an LH surge resulting in ovulation. With exogenous GnRH (Britt et al., 1974; Britt et al., 1981), ovulation can be induced in a high P_4 environment when the DF is on d 6 - 7 especially. Physiological manipulation induced an auxiliary CL, thereby increasing diestrus P_4 concentrations (Bello et al., 2006). That was shown to positively affect fertility in high producing dairy cows experiencing elevated levels of steroid hormone metabolism (Bello et al., 2006, 2007; Wiltbank et al., 2006).

The granulosa and internal theca cells from the ovulated follicle morphologically become luteal cells forming the CH from d 0 - 2. The CL develops rapidly, and a cavity may be present through d 10 - 11. Veterinarians use that anatomic finding as information when staging a cow within her estrous cycle. Low-amplitude LH pulses stimulated large and small luteal cells that produced and released P_4. Maintenance of the CL during diestrus until day 18 produced high levels of P_4. The endometrial release of $PGF_{2\alpha}$ determines the functional lifespan of the CL. The regressed CL becomes a CA. The regression process is called luteolysis.

Luteolysis was defined as the functional and morphological regression of CL to become CA (Pharriss et al., 1971; McCracken et al., 1971, 1999; Nett et al., 1976; Knickerbocker et al., 1988; Schams and Berisha, 2004; Pate et al., 2012). The lysis of luteal tissue was primarily regulated by the release of $PGF_{2\alpha}$ when oxytocin binds to OXTR in the uterus. The P_4 and E_2 serum levels regulated the expression of OXTR. During the long exposure of PR to P_4 during diestrus (d 3 - 18), PR expression in the uterus was downregulated. E_2 levels and ER expression increase around d 16, resulting in the induction of endometrial OXTR expression activated by regular pulses of oxytocin. Wiltbank et al., (2016) observed that the primary mechanisms were activated due to the venoarterial pathway's ipsilateral transfer of $PGF_{2\alpha}$ (Ginther, 1974; Wolfenson et al., 1985; Knickerbocker et al., 1988; Ginther et al., 2009). Around d 18, 4 - 5 endometrial $PGF_{2\alpha}$ peaks occurred initiating the luteolytic cascade. P_4 decreased dramatically, and when serum concentrations were below one ng/mL, a CL was considered completely regressed and non-functional (Schams and Berisha, 2004; Wiltbank et al., 2016).

Martins et al., (2011, 2016) reported that delayed or incomplete luteal regression occurred in 20% of cows treated with dinoprost tromethamine during fertility program enrollment. Other pathophysiologic events that may result in reduced CL regression will be presented in chapter 2.

Follicular Wave Physiology

A follicle is a structure on the ovary containing the developing oocyte. It is classified according to histological and morphological characteristics. Primordial or primary follicles include an oocyte surrounded by a single layer of granulosa cells. They are quiescent structures present in the ovarian cortex. They rapidly decrease in number with age. Some primary follicles are recruited into the ovulatory pool, becoming secondary. A secondary follicle will typically have 2 - 4 layers of granulosa cells and zona pellucida formation, without theca cells or antrum present. When an antrum is formed, the follicle is classified as tertiary or Graafian. Internal and external theca cells surround the follicle. In the Graafian follicle, theca cells produce steroid precursors (androstenedione) that the granulosa cells convert to E_2 via the enzyme androgenase. The granulosa cells are responsible for E_2 production and release (Shirasuna et al., 2013).

Upon ovulation, the CL is formed by large and small luteal cells derived from follicular granulosa and internal theca cells, respectively (Milvae and Hansel, 1983; Diaz et al., 2002;

Wiltbank et al., 2016). The cellular morphologic change to theca and granulosa cells in response to the luteinization process caused by LH (McCracken et al., 1999). The CL is a transitory secretory glandular structure with the capacity for the great production of P_4. LH stimulates P_4 production by the small luteal cells where most LH receptors are found (Schams and Berisha, 2004). Pate et al. (2012) speaks to the increased secretion of P_4 mirroring the CL tissue's rapid growth rate that is related to the intense angiogenesis process. Blood capillaries have an intimate anatomic relationship with luteal cells accounting for the accelerated functional change (Shirasuna et al., 2013). The other key feature of CL is its capacity to undergo cell death or regression. Histological characteristics can classify CL in three distinct stages of development from youngest to oldest; CH, CL, or CA.

As described, the bovine estrous cycle was characterized by usually two to three FW, with a range of one to five FW in a 21 – 23 d estrous cycle period (Sakaguchi et al., 2004). The periovulatory phase was characterized by two distinct surges of FSH (Wiltbank et al., 2011). The DF production of E_2 induced a hypothalamic preovulatory GnRH surge, most likely regulated by the kisspeptin neuronal system (Okamura et al., 2013). GnRH was released into the portal vessels and acted on the anterior pituitary gland stimulating secretion of LH and FSH. The first distinct FSH surge stimulates the anterior pituitary to release LH and FSH (Kulick et al., 1999), causing an LH surge-induced ovulation and the FSH-stimulated new FW. The second FSH surge is linked to the emergence of the next wave of follicles (Ginther et al., 1996; Wiltbank et al., 2011).

The post-ovulatory phase is characterized by the appearance of a CL producing P_4 at the site of the just ovulated follicle. During that early diestrus phase, the lack of inhibition by ovarian factors E_2 and inhibin A allowed FSH to remain above basal levels promoting the growth of the next wave of follicles (Wiltbank et al., 2011). The largest follicle growing under the influence of FSH and LH pulses, became the DF after deviation, at around 8.5 - 10 mm on about d 4. As the follicle reaches the point of deviation, FSH secretion decreases from the anterior pituitary due to negative feedback from ovarian hormonal influences, E_2, and inhibin A. Wiltbank et al. (2011) described that the selected DF continued to grow. The granulosa cells in the DF had an increased expression of LH receptors. Ovulatory capacity had been associated with the development of LH responsiveness, which occurs in follicles that have grown past the point of deviation (Sartori et al., 2001). Wiltbank et al. (2011) found that most data consistently described an increasing role for LH in the DF growth after deviation. It was concluded that this is most likely due to granulosa cell LH receptors present in the DF.

Subordinate follicle growth slows and decreases in volume due to the negative feedback pathway exerted by increased E_2 and inhibin A secretion from the growing follicle (Wiltbank et al., 2011). Meanwhile, during the first FW, the expanding CL was increasing its production of P_4, and the first wave DF would undergo atresia by usually d 10 of the estrous cycle in the 2 FW cow. With ovarian hormone negative feedback on the pituitary removed, another FSH surge occurs, and a second FW begins (Wiltbank et al., 2011). Wiltbank et al. (2016) reported that in the two FW cow, the second wave begins around d 7 - 11. The ovarian activity pattern is repeated in the 2nd wave except the endogenous $PGF_{2\alpha}$ released by the endometrium begins the CL lysis process at about d 18 - 25 (Wiltbank et al., 2016). With complete CL regression, P_4 levels returned to below basal levels causing a hypothalamic surge of GnRH to be released, resulting in an LH surge from the anterior pituitary. That LH surge caused the DF to ovulate on d 21 – 23, and the cyclic nature of the process is replicated until pregnancy is obtained.

BREEDING PERIOD

Estrous Cycle Regulation and its Effect to Increase Synchrony

Precise regulation of the estrous cycle to obtain pregnancy in the high producing dairy cow has become the standard adopted tool to manage modern dairy cow reproduction (Pursley et al., 1995; Stevenson and Britt, 2017; Thatcher, 2017). Generally, the management programs are focused on either estrus synchrony or ovulation synchrony. Estrus Synchrony in virgin heifers remains popular and can be very successful due to the typical high fertility of nulliparous bovines and their propensity to exhibit estrus (Martins and Pursley, 2016). In postpartum primiparous and multiparous cows with high production, ovulation synchrony protocols were the preferred choice to obtain high P/AI (Wiltbank et al., 2006; Martins and Pursley, 2016). With the ovulation pre-synchrony programs, ovulatory follicle size and age were controlled (Vasconcelos et al., 1999; Santos et al., 2010; Wiltbank et al., 2011; Martins and Pursley, 2016). P/AI was improved when the insemination rate was increased because the heat detection variable was excluded (Lucy, 2001) and AI timing variables were eliminated from the process. The ovulation synchrony protocols utilized GnRH and $PGF_{2\alpha}$ treatments and TAI in a precise timing sequence to achieve a higher rate of pregnancies in a timely manner (Pursley et al., 1995; Peters and Pursley, 2003; Middleton et al., 2019).

An exogenous injection of GnRH will result in ovulation of a responsive DF about 28 h post-injection. If it is a first wave DF at d 6 - 8 of the estrous cycle primarily, the follicle has LH receptors, will ovulate and become an accessory CL (Bello et al., 2006; Sousa et al., 2008). The additional P_4 produced by the accessory CL can be beneficial for fertility in the next follicular phase of the estrous cycle (Wiltbank et al., 2012). The increased circulating P_4 has a positive influence on the developing DF with respect to the time it spends in dominance and its timing of ovulation (Vasconcelos et al., 1999; Santos et al., 2010; Wiltbank et al., 2011, 2014). A successful ovulation to the first FW GnRH injection helps to ensure that a second FW ovulation will occur due to successful synchrony for the final GnRH treatment of Ovsynch (Bello et al., 2006; Pursley and Martins, 2011).

Impacts of Increased P_4 on Ovarian Dynamics and Fertility

The concentration of circulating P_4, therefore, had a substantial effect on fertility and can be used as a predictor of it (Wiltbank et al., 2011, 2014). Fonseca et al. (1983) reported a relationship between average P_4 concentrations during the 12 d before AI and subsequent first service P/AI. They found an approximately 10% increase in P/AI for each one ng/mL increase in average P_4 concentration. Fertility may also be improved by use of the presynch / Ovsynch programs (Bello and Pursley, 2007; Souza et al., 2008) due to the increased P_4 produced by the additional CL present during the early and middle luteal phase (d 5 - 13) of the estrous cycle (Xu et al., 1997). Attenuated conception rates of dairy cattle were attributed to reduced P_4 concentrations during the growth of the ovulatory follicle (Pursley and Martins, 2011).

Wiltbank et al. (2014) found that elevating P_4 concentration before AI generally decreased double ovulation while increasing fertility to TAI. Cerri et al. (2011) reported that double ovulation was increased in cows with low P_4 concentration. In addition, E_2 concentrations were altered, but the study results determined similar fertilization rates and only minor changes in embryo quality. Martins et al. (2018) described an increased double ovulation rate in cows with low P_4 during the pre-dominance through dominance phase of follicle development. It was discovered that cows with double ovulations had a greater P/AI on d 23 after AI compared to cows with single ovulations (Martins et al., 2018). The increased pregnancy rate was attributed to the increased ovulatory rate in the double ovulation cows. Pregnancy loss was greater in cows with unilateral twins versus bilateral or a single fetus. Martins et al. (2018) concluded that P_4 concentrations during ovulatory follicle development affect the number of follicles ovulated and the subsequent timing of pregnancy loss. In a study designed to study risk prevention of twin pregnancy via subordinate follicle puncture and drainage, Lopez-Gatius and Hunter, (2018) found that the procedure may eliminate the risk of twin pregnancy and reduce pregnancy loss by increased auxiliary CL formation.

Importance of Prostaglandin $F_{2\alpha}$ in Fertility Programs for Lactating Cows

$PGF_{2\alpha}$ is integral in the regression of luteal tissue; therefore, complete CL regression has been the focus of on-going research studies. Recently, the Pursley laboratory reported that in order to obtain an optimal fertile oocyte at ovulation, complete luteal regression must occur before an endogenous or exogenous GnRH / LH surge culminating in ovulation (Martins et al., 2011). It was discovered that up to 20% of cows treated with a single dose (25 mg) dinoprost tromethamine (Lutalyse®, Zoetis Animal Health) had delayed or incomplete luteolysis resulting in no chance for pregnancy at AI. Martins et al., (2018) reported that cows had lower P_4 and higher E_2 serum concentrations in the first 12 h following a single treatment with cloprostenol sodium, a synthetic alternative $PGF_{2\alpha}$ (Estrumate®, Schering Plough Animal Health Inc. Union, NJ). Also, the study found no difference in time to complete luteolysis or time to ovulation for the two $PGF_{2\alpha}$ analogs. Assurance of complete luteal regression is vital to increasing P/AI in ovulation synchrony TAI programs (Brusveen et al., 2009; Carvalho et al., 2015; Wiltbank et al., 2015). In the Carvalho et al. (2015) study, a GnRH treatment was administered 6 d before the Ovsynch protocol initiation and a supplemental $PGF_{2\alpha}$ therapeutic dose 24 h after the first $PGF_{2\alpha}$ was administered within the Ovsynch protocol. This modification resulted in optimized P_4 serum concentrations at each treatment. P/AI was also increased for cows resynchronized when treated with a supplemental $PGF_{2\alpha}$ injection. Wiltbank et al. (2015) discovered that complete regression of CL with a supplemental treatment of $PGF_{2\alpha}$ in an Ovsynch protocol could result in about 10% increased P/AI in multiparous cows. Borchardt et al. (2018) published a literature review and meta-analytical assessment describing a benefit to the supplemental $PGF_{2\alpha}$ treatment in complete luteal regression resulting in a 4.6 percentage unit increase in P/AI. Carvalho et al. (2018) concluded that in developing controlled ovulation fertility programs using GnRH and $PGF_{2\alpha}$, one must attain medium P_4 at G3, high P_4 at PGF4, and low P_4 concentrations at G4 to achieve increased P/AI. It is best accomplished by the institution of a pre-synch / Ovsynch program, including the supplemental $PGF_{2\alpha}$ treatment, particularly in multiparous cows before TAI.

Published papers have shown lower luteal phase P_4 and follicular phase E_2 serum levels in healthy high producing dairy cows (Sartori et al., 2004; Santos et al., 2016). The high DMI and increased portal blood volume in multiparous cows versus nulliparous heifers results in increased steroid hormone metabolism in the liver (Sangsritavong et al., 2002). P/AI in multiparous dairy cows decreased as DMI and milk production per cow escalated until the mid-1990s when ovulation synchrony programming (Ovsynch) was introduced (Pursley et al., 1995, 1997). Another increase in dairy cattle reproductive efficiency was obtained after pre-synchrony protocols were published (Bello et al., 2006; Souza et al., 2008) that physiologically targeted the ovary to produce an auxiliary CL before Ovsynch resulting in increased P_4 levels during the pre-ovulatory follicular growth phase.

SUMMARY

Reproductive competence in the female bovine is the goal for normal hypothalamic and pituitary control of reproductive and metabolic hormones and its integration with pituitary ovarian and ovarian uterine physiology. The function of the healthy bovine reproductive system is to achieve pregnancy while performing as a profitable high milk production animal. William Hansel (Cornell University) famously proclaimed to his students, “it is not a wonder that reproduction sometimes fails, but rather a miracle that so many pregnancies terminate successfully” (Stevenson and Britt, 2017). The human component in achieving pregnancy may be the most crucial variable in cows suffering from reduced fertility! Successful management of dairy cow reproduction requires a mixture of scientific knowledge and technology with an artful understanding of cattle husbandry.

APPENDIX

PRIMARY TCI FACTORS	RECOMMENDED ACTIONS
1. Feed bunk space	30 inches in transition, peri and postpartum
2. Minimize pen moves and social stress peripartum	10 d prior calving
3. Increased cow comfort through the period	Amply sized free stalls
4. Sand bedding	Adequate type and amount on which to lie and rise
5. Identify sick cows	Efficient and effective screening process to identify cows requiring medical attention or nursing care

Table 1.1 5 Primary factors and recommended actions associated with herd average TCI scores (Nordlund, 2006). The recommended actions can control issues of transition cow mismanagement that may affect herd fertility.

CHAPTER 2

REVIEW OF POSTPARTUM UTERINE INVOLUTION IN THE LACTATING DAIRY COW

GENERAL INTRODUCTION

Although governed by the homeorhesis mechanism, early lactation cows exist in an intense changing biological ecosystem that can greatly impact performance. A significant part of that dynamic process is the monumental physiologic and metabolic transformation that occurs periparturient. That transition culminates in either the natural or a traumatic process of parturition.

The proportion of fat carried by a cow determines her body condition. Cows were evaluated within the parameters of an arbitrary scale of 1 signifying emaciation to 4 (Ferguson et al., 1994) or 5 (Wildman et al., 1982) indicating obesity and that defined her BCS. Management of the dairy cow utilizing her BCS is useful when done in a comparative manner in addition to the score given at any given point in time. The usual time in a cow's parity receiving a score are at dry off, at parturition and at 30 DIM. Targeted management decisions can be made for specific cow groups when a comparison is made between the scores noting positive or no change (gained or maintained) versus negative change (loss). It is a monitoring tool and the reflective BCS data in addition to TCI scores (Nordlund, 2006) may benefit management of future animal cohorts.

The lactation curve and intercalving profile of BCS were mirror images of each other in most high production dairy cows (Roche et al., 2009). BCS decreased for up to 100 DIM in many cows and feeding management had little effect on the loss until the somatotrophic axis had recoupled and the natural insulin resistance period had passed. Roche et al., (2009) determined that BCS loss early postpartum was a natural homeostatic controlled event. Upregulation of lipolytic pathways in adipose tissue were expressed during the postpartum period. Roche et al., (2009) reported that there were clear associations between BCS loss on milk production, metritis, metabolic disease risk, postpartum anestrus, DO, and pregnancy probability. In the study, it was determined that 3.0 – 3.25 (1 - 5 scale) was optimum for function as a healthy high production dairy cow (Roche et al., 2009). Lower scores were associated with decreased production and reproduction while higher scores resulted in depressed DMI and production resulting in a greater risk for metabolic disease during the early lactation period. Middleton et al., (2019) found 2.7 BCS to be optimum in maintaining a 13-month CI. Middleton et al., (2019) reported that a postpartum BCS loss of no more than 0.1, may maintain cows in a cycle of high fertility.

Berry et al., (2007) reported that BCS did not impact dystocia; however, dystocia did impact BCS. Dystocia is a risk factor along with metabolic stress associated with milk production, for subsequent infectious disease by impairment of the inflammatory response to pathogens (Sheldon et al., 2018). Pantaleo et al., (2014) found the complex interaction between endocrine, immune, and infectious factors and the dysregulation of uterine defenses often resulted in metritis or endometritis in the early postpartum cow. Aleri et al., (2016) reported that contributing factors implicated in decreased periparturient immunity were the parturition process, leukocyte activity dysfunction, colostrogenesis and lactogenesis, and metabolic associated maladies like hypocalcemia and NEB.

Two primary postpartum challenges the dairy cow must overcome to achieve good fertility were reported by Cheong et al., (2017); restoration of uterine function and health, and resumption of normal ovarian follicular and luteal function. Interactions between the two distinct processes leading to higher fertility at first service TAI were discovered (Cheong et al., 2017).

In Chapter 1, normal reproductive physiology was reviewed. In this chapter, the pathophysiology of impaired reproductive function in the dairy cow and its effect on fertility will be reviewed.

TRANSITION / PERIPARTUM PERIOD

A successful productive lifetime for the high production dairy cow is equal to the sum of its parities. One parity does not tell the complete story nor is it a predictor of overall success. A definitive predictor of future success is in many ways the illusive 'holy grail' of dairy cattle management research. In that spirit, Nordlund (2006) developed the TCI management tool, a practical approach to objectively evaluate the effects of transition cow management at the herd level. Recently, Middleton et al., (2019) described a novel approach to dairy cow management addressing the concept of an HFC for the high producing dairy cow (Figure 2.1). It was reported that an association was found between a cow's body condition loss and future health and reproductive outcomes. Healthy cows that attain pregnancy in early lactation soon after the VWP, have more profitable lifetime records. Fricke and Pursley (2019) confirmed that timely pregnancy in a current lactation leads to reduced BCS loss, fewer health-related events, increased fertility and reduced pregnancy loss in the following lactation. Middleton et al., (2019) defined a relationship between increased conception rates at first TAI and cows with less BCS at parturition. Pregnancy by 130 DIM drives the metabolic mechanisms and DMI necessary for maintaining or gaining BCS. Cows that maintain or gain BCS reside in the HFC and have greater fertility than those experiencing BCS loss (Middleton et al., 2019).

Managing BCS has its roots in ration and DMI management. Typically, metritis and endometritis were preceded by decreased DMI and gene expression for pro-inflammatory cytokines, including IL-1, IL-6 and IL-8, increased circulating levels of BHBA or NEFA, and innate immune function (Hammond et al., 2006; LeBlanc et al., 2011). Hammond et al., (2006) and LeBlanc et al., (2011) suggested that it occurred several weeks prior to clinical disease symptoms and that it was characterized by a loss of BCS. The risk of reproductive disease is thought to be best mitigated by employment of commonly recommended best practice management during the transition period (LeBlanc et al., 2011).

POSTPARTUM PERIOD

Resumption of normal estrous cycles culminating in ovulation are an expectation for each individual cow soon after parturition. Ovarian cyclicity prior to 1st TAI (Galvao et al., 2004, 2010; Chebel et al., 2007; Santos et al., 2009) but especially resumption in early lactation (Darwash et al., 1997; McCoy, 2006) was associated with improved reproductive performance. That conclusion was shared by Galvao et al., (2010) and Dubuc et al., (2012) when they reported that Holstein cows that were cyclic by 21 DIM had improved reproductive performance compared to cows not cyclic by 60 DIM.

The early postpartum cow that had a loss in BCS was often suffering from a depressed DMI that resulted in a state of NEB as indicated by elevated BHBA and NEFA levels and by elevated indicators of inflammation (i.e., haptoglobin) (Beam and Butler, 1999; Butler et al., 2006; Gumen et al., 2011; Drackley and Cardoso, 2014). The elevated indicators were found to negatively affect cyclicity (Dubuc et al., 2012) and fertility (Gumen et al., 2011). LeBlanc (2012) contended that NEB contributed to immune dysfunction peri and postpartum and was a major element in reproductive tract inflammatory disease. Kawashima et al., (2018) found that hepatic dysfunction from peripartum diseases caused delayed uterine involution and endometritis. Cows suffering from elevated BUN levels may have depressed DMI (Kawashima et al., 2018).

Cows in NEB experienced greater incidences of metabolic maladies that resulted in RFM, metritis and delayed cyclicity by 21 DIM (Vercouteren et al., 2015). Heppelmann et al., (2015), described the effect metritis and hypocalcemia had on uterine involution as evaluated by sonomicrometry. It was demonstrated that both diseases affected uterine size reduction (involution) until d 28. Metritis positive cows had a larger uterine diameter and those with subclinical hypocalcemia had delayed uterine length reduction which may be due to decreased myometrial contractility (Heppelmann et al., 2015). If the NEB was significant, it adversely affected the first dominant follicle postpartum, as described by Cheong et al., (2016). The NEB resulted in reduced LH pulses and increased hypothalamic negative feedback sensitivity to E_2 that resulted in ovulation failure and a large incidence of anovulation in lactating dairy cows (Wiltbank et al., 2011). E_2 production impairment was also associated with depressed circulating insulin, IGF-1+, and glucose (Canfield and Butler, 1991; Beam and Butler, 1999; Butler et al., 2004).

Dubuc et al., (2012) demonstrated that metritis positive cows had increased levels of haptoglobin and decreased cyclicity by 3 weeks postpartum (Cheong et al., 2017). In addition, Cheong et al., (2017) reported increased serum concentrations of BHBA and NEFA resulting from a postpartum NEB status. NEB adversely impacts the developing follicle resulting in E_2 production impairment and failure of first ovulation. Failure to ovulate a DF led to a low P_4 environment that had a profound effect on ovarian dynamics and subsequent fertility in lactating dairy cows (Wiltbank et al., 2011; Cheong et al., 2017). Contrary, the lower P_4 serum concentrations were found to be beneficial to early and complete uterine involution and resolution of any endometritis present (Heppelmann et al., 2015). Lewis, (2004) found P_4 to be "permissive" to postpartum disease of the uterus. Sheldon and Dobson, (2004) reported that the uterus was more susceptible to infection during the luteal phase of the estrous cycle. The local uterine immune response was suppressed by P_4.

Heppelmann et al., (2015) also investigated the effect of time for first postpartum ovulation on endometrial inflammation in cows. The study included cows with and without uterine disease during the early puerperal period. The transvaginal FP procedure was used to suppress postpartum ovulation and formation of CL until 42 DIM. Heppelmann et al., (2015) concluded that suppression of early ovulation by transvaginal FP enhances uterine inflammation healing in postpartum cows. The low P_4 environment produced by transvaginal FP provides additional evidence that serial regression of CL in cows ovulating normally, thereby periodically decreasing P_4 could be beneficial in the resolution of metritis cases leading to increased pregnancy at first TAI.

Impact of Uterine Disease on Ovarian Function and Fertility

The ubiquitous nature of postpartum uterine bacterial contamination and the persistence of pathologic bacteria were frequently a cause of reduced fertility in high production dairy cows (Sheldon and Dobson, 2004; Sheldon et al., 2006). Subfertility in cattle was associated with uterine infection by hypothalamic, pituitary and ovarian function perturbation (Sheldon et al., 2002; Carneiro et al., 2016).

Multiorgan dysfunction occurrences are often related; they are not typically independent events. Designing effective and relevant prevention and control programs to adequately address the management of uterine disease and its relationship to other organ function are necessary yet challenging in high production dairy cattle. Bovine uterine disease has achieved the notice of researchers as more than 500 papers in the past 50 years have been published on the subject. Paradoxically, in spite of the additional available information and the extra attention given to cow management the incidence of uterine disease in dairy cows had not significantly changed in that time (Sheldon et al., 2002; Sheldon and Dobson, 2004). If fertility in the high production dairy cow was to be positively impacted, it was found to be imperative that veterinarians properly diagnose conditions implicated in lowered fertility causation (Sheldon and Dobson,

2004) and that herdsmen utilize effective treatment protocols that are efficacious (Gilbert et al., 2005) maintaining the cow in the HFC (Middleton et al., 2019).

Uterine Disease

The lack of validated and consistent diagnostics and definitions for reproductive diseases such as vaginitis, cervicitis, metritis, endometritis, subclinical endometritis, salpingitis, and oophoritis complicated the discussion. To decrease confusion, practical definitions that were descriptive, based on key clinical features, were necessary to properly diagnose and successfully treat uterine disease (LeBlanc et al., 2002; Sheldon et al., 2006). In general, infection occurred when there was adherence of pathologic organisms to mucosa, colonization or penetration of epithelium and/or release of bacterial toxins (Sheldon et al., 2002; Carneiro et al., 2016). It was reported that the severity of infection depended on the immune response of the host, the bacteria species and the bacterial challenge involved.

The following five working disease classifications will be addressed by reflecting on unique characteristics thereby making it a useful cow-side tool for use by veterinarians and dairy cow managers:

1.) ***Puerperal Metritis***. High production dairy cows were prone to uterine contamination from numerous sources and by various different bacterial strains (Sheldon and Dobson, 2004). The most common bacteria were E. coli (gram negative. Find a description of EnPEC in the bacteriology section (Sheldon et al., 2010)), T. pyogenes (gram positive Group A strep), F. necrophorum (gram negative anaerobe), and Prevotella species (gram negative anaerobe) (Sheldon and Dobson, 2004). Approximately 95% of dairy cow uteruses were initially contaminated by a non-specific range of bacterial species (Sheldon and Dobson, 2004), then became infected in the first 14 d postpartum, with the peak incidence between 5 –7 DIM (Sheldon et al., 2006). In that report, Sheldon et al. (2006) defined puerperal metritis as a more acute infection of the uterus. It was characterized in the study by an abnormally enlarged uterus indicating delayed involution. A fetid watery, red-brown uterine discharge was present. Pyrexia should be common (>103° F), but 60% (Galvao et al., 2010) or 50% (Hammond et al., 2006) of cows with puerperal metritis did not exhibit a fever. [editorial: The complication encountered when using pyrexia as a key component in treatment decisions for uterus infection therapy, is that the presence of fever is largely dependent upon timing of the PE relative to uterine disease progression. Bacteremia (bacterial growth phase) occurs early-on in the disease progression, resulting in pyrexia. Septicemia / Toxemia occurs later, during the bacterial products phase (for example: endotoxin increase from the gram-negative bacteria cell wall). It can cause severe clinical illness resulting in a normal to subnormal basal body temperature. Systemic, multisystem dysfunction (septicemia and/or toxemia), is often present causing an elevated heart and respiratory rate, dehydration, low blood pressure, an already low blood glucose from depressed DMI, depression – varied responsiveness and an ineffective immune response or an overwhelmed immune system. (R.J. Vlietstra, WMVS).]

2.) Metritis. Metritis was defined by Sheldon and Dobson, (2004) as a severe inflammatory reaction involving all layers of the uterus within 21 d and most commonly, within 10 d of parturition (Sheldon et al., 2009a). It included the endometrium, submucosa, muscularis and the serosa. Histology examination revealed inflammation characterized by edema, leukocyte infiltration, myometrial degeneration, and congested mucosa (BonDurant, 1999; Sheldon et al., 2009a). The clinical manifestation of metritis was characterized by an abnormally enlarged uterus indicating delayed involution. There was a fetid uterine discharge without systemic signs of illness (Sheldon and Dobson, 2004). The incidence rate ranges from 18% (Drillich et al., 2001) to 40% (Markusfeld, 1987).

3.) ***Endometritis***. Sheldon and Dobson (2004) define endometritis as a superficial inflammation of the endometrium after 21 d postpartum. Endometritis extends no deeper than the stratum spongiosum (BonDurant, 1999; Sheldon et al., 2006). BonDurant (1999) reported histologic evidence of inflammation, as evidenced by fibrosis and leukocyte infiltration. Sheldon et al., (2006) reported that histological examination of endometritis biopsy samples exhibited some disruption of the surface epithelium. Infiltration of the endometrial layer with inflammatory cells (neutrophils and macrophages) and vascular congestion was prominent. Endometritis was also characterized by depletion of endometrial glands and atrophy of remaining glands with congested mucosa (Sheldon et al., 2006).

4.) ***Clinical Endometritis***. was diagnosed by the presentation of a cervical measurement of ≥7.5 cm diameter at ≥20 d postpartum (LeBlanc et al., 2002) or the presence of purulent uterine discharge or both after 21 d or mucopurulent vaginal discharge after 26 d postpartum (Sheldon et al., 2006) as determined by a vaginal speculum (LeBlanc et al., 2002), a Metricheck devise (McDougall et al., 2007; Kawashima et al., 2018) or a digital exam (Williams et al., 2005). Williams et al., (2005) found that an evaluation of vaginal mucus character and odor reflected the number of uterine bacteria and the acute phase protein response. Kawashima et al., (2016, 2018) described delayed uterine involution associated with elevated BUN levels indicating hepatic dysfunction (often a peripartum disease issue) as indicative of endometritis. The incidence rate range was 10 – 20% (Borsberry & Dobson, 1989; LeBlanc et al., 2002).

Cytological endometritis was defined by Dubuc et al., (2010) as an endometrial cytology sample (cytobrush, uterine low volume lavage, biopsy) that had an increased percent PMN leukocytes or neutrophils. They described 3 different uterine health status classifications in the following way:

1. Purulent vaginal discharge only
2. Cytological endometritis only
3. Both purulent vaginal discharge and cytological endometritis

Barlund (2008) stated that in diagnosing endometritis, cytology was more sensitive than an ultrasound examination. Savc et al., (2016) suggested that a postpartum examination of the reproductive tract should include both an ultrasound and Metricheck examination (vaginal mucus scoring). Jennifer Roberts (personal communication) asserted that a diagnosis of metritis based solely on the presence of purulent or mucopurulent vaginal discharge could be misleading. Examination of vaginal contents do not necessarily provide an accurate diagnosis of uterine disease, i.e. vaginitis and/or cervicitis could be causative of an abnormal vaginal discharge. Wagener et al., (2017) agree, "Because a poor agreement between vaginal and cytological findings has been found, the terms PVD and CYT have been suggested instead of CE and SE. Whereas the terms CE and SE follow the classical rules of terminology for a disease, the terms PVD and CYT represent a mixture of clinical symptoms (purulent discharge), diagnostic technique (cytological), and diagnosis (endometritis)."

5.) ***Subclinical Endometritis*.** Dubuc et al., (2010) described cows with no clinical signs of endometritis but an increased cytological percent PMN, associated with reduced reproductive performance, as having SE. Kasimanickam (2004), Galvao et al., (2011) and Savc et al., (2016) agreed that the following cytology sample parameters were diagnostic for SE with a decreased risk for pregnancy if found above the listed threshold:

1. 21 – 33 d postpartum: >18% neutrophils (PMN)
2. 34 – 47 d postpartum: >10% neutrophils (PMN)

Wagener et al., (2017) reported in their review that at 21 – 62 d postpartum, a general threshold of 5% PMN (range: 5 – 18% PMN) had a negative impact on fertility. In that study, Wagner et al., (2017) determined that anything above the threshold had a decreased P/AI, increased DFS and DO., Embryo quality and survival were decreased in the event of pregnancy. McDougall et al., (2011) concluded that with respect to a predictor of reproductive performance, the percent polymorphonuclear leukocytes present in the uterus was better than vaginal inflammation scoring or bacteriology.

Endometrial damage causing an increased inflammatory reaction in the stratum compactum had a strong association with poor reproductive performance especially in the presence of T. pyogenes or other anaerobic bacteria (Bonnett et al., 1991). Endometritis causes reduced fertility while bacterial infection is present.

Bacteriology, Immunology, Endocrinology and Fertility

As described by Sheldon et al., (2002) the four predominant intrauterine pathogens are E. coli, T. pyogenes, F. necrophrum and Prevotella species. E. coli bacteria and its products caused immune suppression by inhibiting the phagocytic ability of neutrophils (Sheldon et al., 2002, 2009; Sheldon and Dobson, 2004). E. coli infection was most common in the first week postpartum (see clinical metritis). Infection resulted in endometrial inflammation and ovarian dysfunction since LPS produced had an affinity for ("sink") follicular fluid (Sheldon et al., 2009; Carneiro et al., 2016; Cheong et al., 2017). It was reported that LPS binds to TLR_4 on granulosa and endometrial cells. Down-regulated Aromatase leads to decreased E_2 from circulating androgens and an increased PGE to $PGF_{2\alpha}$ ratio from PGF: PGE at 2: 1 to PGE: PGF at 5: 1 (Sheldon and Dobson, 2004).

The immune response may have a localized effect on ovarian function. Sheldon and Dobson (2004) reported that perhaps the previously gravid horn that is larger can contain greater numbers of bacteria where more bacterial products are delivered to the ipsilateral ovary through the larger volume uterine venous vasculature (venoarterial pathway). Proinflammatory molecules (cytokines), a bacterial product, could be an intermediary affecting ovarian function and subsequent fertility (Spicer, 1998). For example, he found TNF_α suppressed theca and granulosa cell E_2 secretion in addition to the effects of down-regulated Aromatase. Sheldon et al., (2009) and Cerneiro et al., (2016) report that bovine luteal cells were highly responsive to a number of cytokines explaining the reduced P_4 secretion by CL in cows with metritis. Cytokines influenced proper luteolysis, a process considered important in resolving metritis. It was reported that in some cows, extended luteal phases were associated with effects on luteolysis or on the function of luteal cells (Sheldon et al., 2009). The luteolytic mechanism can be disrupted because of the switch in endometrial prostaglandin from PGF to PGE (Sheldon and Dobson, 2004). PGF is luteolytic while PGE is luteotrophic in cattle. Sheldon and Dobson, (2004) suggest that shift may prolong the luteal phase P_4 influence, exacerbating metritis.

E. coli was found to be associated with the hepatic secretion acute phase protein response, severity of metritis and the extent of the resultant reduced fertility (Sheldon and

Dobson, 2004). EnPEC, introduced before, was described by Sheldon et al., (2010) as a strain of E. coli more adherent and invasive for endometrial epithelial and stromal cells. These cells produced more PGE and IL-8 in response to LPS. EnPEC is associated with bacterial invasion of the endometrium and myometrium. Clinically, it was causative of severe puerperal metritis usually within 5 d postpartum (Hammond et al., 2006).

T. pyogenes, F. necrophorum and Prevotella species bacteria were found to act synergistically causing or prolonging uterine disease (Sheldon and Dobson, 2004). T. pyogenes produced a growth factor for F. necrophorum. Strains of T. pyogenes expressed the virulence gene, 'plo', that encoded for a cholesterol dependent cytotoxin, pyolysin, that led to cell death. F. necrophorum caused immune suppression and it produced a leukotoxin while Prevotella produced a substance that inhibited phagocytosis (Sheldon and Dobson, 2004).

Innate immunity was reported to be responsible for bacterial contamination defense via anatomic, physiologic, phagocytic and inflammatory systems (Sheldon et al., 2018). The cow's anatomy; the vulva, vestibule, vagina and cervix are her first defense against ascending bacterial infection. The vaginal and cervical mucus produced particularly during estrus, were found to be a physiological barrier to bacteria ascending the genital tract (Gunther, 1982). Bacterial challenge caused a phagocytic barrier by the invasion of neutrophils first, then macrophages become important (Sheldon et al., 2018). In that study, inflammatory barriers to uterine disease were revealed to include the non-specific defense molecules like lactoferrin, defensins (host defense peptides) and acute phase proteins. Beutler et al., (2003) reported that bacterial components such as endotoxin and peptidoglycan via TLR activating down-stream signaling releasing cytokines were detected by immune cells. The cytokines, TNFα and IL-1, IL-6, and IL-8 caused pyrexia resulting in increased immune cell mobilization via a positive feedback loop (Zerbe et al., 2003).

The pro-inflammatory cytokines also stimulated hepatic secretion of acute phase proteins (i.e., haptoglobin) that are already typically elevated at parturition.

The first postpartum transient FSH increase and therefore, follicle wave emergence was not altered in cows with metritis (Sheldon and Dobson, 2004). However, Opsomer et al., (2000) reported in an epidemiological study that uterine infection was a risk factor for delayed ovulation (OR 4.5). Furthermore, greater uterine bacterial contamination perturbs ovarian function in that the first postpartum DF had a slower growth rate producing less E_2 at the end of its growth phase. That effect was caused by multi-level mechanisms affecting the hypothalamus, pituitary and ovary (Sheldon and Dobson, 2004; Sheldon et al., 2009).

The effect of uterine bacterial infection on hypothalamus and pituitary function centered on the role of endotoxin originating from gram negative bacterial cell walls. Endotoxin was absorbed from the uterine lumen into the peripheral circulation that resulted in elevated plasma endotoxin concentrations (Peter et al., 1990; Mateus et al., 2003). Peter et al., (1990) found that E. coli endotoxin injected into the uterine lumen of heifers prevented the expected pre-ovulatory LH surge and ovulation resulting in persistent follicles or follicular cysts. Endotoxin had also been reported to perturb the E_2 rise during the follicular phase (Karsch et al., 2003). Cheong et al., (2017) described how LPS from endotoxin inhibited pulsatile LH secretion from the pituitary by the suppression of hypothalamic GnRH release resulting in reduced pituitary responsiveness to endogenous or exogenous GnRH pulses. The consequence of this was a decreased likelihood of ovulation (Cheong et al., 2017). There may also be a direct effect of endotoxin on the ovary because it was reported that in some animals LPS from endotoxin blocked the pre-ovulatory increase in peripheral plasma E_2 (Battaglia et al., 2000; Sheldon et al., 2009).

As described by Sheldon et al., (2009), Carneiro et al., (2016), and Cheong et al., (2017) follicular fluid was found to be a "sink" for LPS and LPS reduced E_2 secretion. Theca cells convert cholesterol to androstenedione passing across the ovarian follicle basement membrane where it was reported to be converted to E_2 by the granulosa cells (Sheldon et al., 2018). Aromatase enzyme important in the conversion of androgens to E_2, was reduced by LPS that resulted in lower E_2 levels. Steroidogenesis was impaired by pro-inflammatory cytokines and stress-related mechanisms (Sheldon et al., 2018).

The unequal distribution of postpartum follicular growth in cattle may also indicate a direct effect of endotoxin on the ovary. Sheldon et al., (2000) reported fewer follicles >8 mm diameter in the ovary ipsilateral to the previous gravid horn than in the ovary contralateral. That ovarian difference was found to decline over time postpartum due to uterine involution and elimination of bacterial contamination (Sheldon et al., 2000). Practitioners find that observation interesting because Bonnett et al., (1991) and Sheldon et al., (2000) reported that the presence of a DF in the ipsilateral ovary to the previously gravid horn is a marker of subsequent fertility. The presence of an ipsilateral DF may reflect uterine health.

PRE-BREEDING PERIOD

Uterine infection involves perturbation of the hypothalamus, pituitary and ovary. Sub-fertility persisted even after successful infection resolution due to disturbance of ovarian function and the effects of uterine damage (Sheldon and Dobson, 2004). One of the effects of uterine damage was disruption to the luteolytic mechanism that caused prolonged postpartum luteal phases (Opsomer et al., 2000). As described, luteal phase concentrations of P_4 could enhance the likelihood and severity of bacterial uterine disease due to suppression of the immune response (Lewis, 2004). Elevated P_4 during the follicular phase of follicle development may result in older and larger DF that have reduced oocyte fertility upon ovulation or could result in an anovular condition (Wiltbank et al., 2011).

The prompt and effective management of postpartum health were found to be imperative if the goal was to achieve a timely pregnancy. McCoy et al., (2006) asserted that best management practices in prevention and treatment of postpartum associated diseases may be more rewarding than hormonal therapy. Galvao et al., (2010) and Dubuc et al., (2012) supported the conventional wisdom accepting the premise that cows in excellent health experiencing regular normal estrous cycles have greater fertility resulting in increased P/AI.

Anestrus or anovulation had a negative influence on fertility after the postpartum period (Peter et al., 2009). That could be the result of numerous suboptimal conditions. For example, inadequate nutrition peripartum (Canfield and Butler, 1991; Butler et al., 2006), excessive body condition loss (Gumen et al., 2011), metabolic disease (Cheong et al., 2016) and acute and chronic uterine (Sheldon and Dobson, 2004; Sheldon et al., 2006; Cheong et al., 2017) and systemic infectious disease were correlated with anestrus (Peter et al., 2009). Cows in a state of NEB postpartum were found to have fewer LH pulses and increased hypothalamic negative feedback sensitivity to E_2 resulting in an anovulation condition (Wiltbank et al., 2011). Other studies concluded that improvements in fertility should be achieved after peripartum health and nutrition management elevate postpartum energy status for the high production cow that could result in less body condition loss and earlier resumption of normal estrous cyclicity (Santos et al., 2009; Kawashima et al., 2012; Drackley and Cardoso, 2014; Wiltbank et al., 2015).

With respect to fertility, endometrial morphology was influenced by ovarian steroid concentrations in cows with uninfected and normally involuted uteri. Wiltbank et al., (2011) observed an increase in endometrial thickness with complete luteal regression at estrus. Delayed or incomplete CL regression resulted in a thinner endometrium. A relationship between fertility and endometrial thickness was determined in lactating dairy cows after a TAI protocol (Wiltbank et al., 2011). The study reported an endometrial thickness of 6 mm resulted in a P/AI of less than 20%, whereas an endometrial thickness of 10 mm or greater, produced a P/AI of 40%. Moreover, cows with the thinnest endometrium and lowest fertility also ovulated the smallest follicles with lower E_2 serum concentrations prior to AI. Smaller ovulatory follicles producing insufficient E_2 at AI resulted in lower fertility (Vasconcelos et al., 2001).

BREEDING PERIOD

The dairy industry's "reproduction revolution" (Fricke and Pursley, 2019) has not come about by coincidence. Revolution happens when intentionally "a sudden, radical, or complete change" (Merriam-Webster.com) is made. For dairy cattle reproduction, change began with the advent of ovulation synchrony (Pursley et al., 1995; Bello et al., 2006; Sousa et al., 2008) programs and estrous and pregnancy detection technology (Bio-Tracking Inc Moscow, ID.). However, a revolution cannot be sustained over time without focused relevant leadership to maintain its momentum. Modern dairy farms have adopted published fertility programs to the extent that the management team demands and most barn crews comply with the listed protocols equal to the extent that their understanding of them allows.

Strict and repeatable protocol compliance is the intangible component – the human behavior aspect of reproduction management - that is equal to the science. Fricke and Pursley, (2019) contend that there was more to the reproduction revolution than the combination of science and art. As a basis the accurate diagnosis and proper treatment of uterine disease were key factors in fertility program management (Sheldon and Dobson, 2004). However, if the primary goal in the overall successful management of dairy cattle was to control the rates and reasons for culling cows, i.e., to attain voluntary culling status (DeVries, 2006) there must be more to the subfertility story.

In pondering why equally programmed herds with similar management practices have large variation in conception rates, Middleton et al., (2019) conducted a study in a single herd to examine the Britt hypothesis (Britt, 1992). It was reported in agreement with Britt that cows in early lactation with body condition loss had poorer fertility than cows that maintained or gained body condition. Middleton et al., (2019) reported cows that became pregnant by 130 DIM had a greater potential for maintaining or gaining body condition postpartum in the following lactation. That translated into higher pregnancy rates at first service AI and decreased incidence of adverse health events that included early pregnancy loss. Middleton et al., (2019) referred to the scenario as the HFC.

SUMMARY

It is evident from this review, that uterine disease complications in dairy cows can have a profound negative effect on fertility resulting in decreased conception rates. Practical dairy cow fertility management places emphasis on the adaptation of fertility programs and its proper administration. However, to optimize P/AI, one must integrate protocols for the pre, peri and postpartum phases allowing managers to enroll and maintain cows in the HFC. The dry cow especially transition through the early postpartum phase, must be controlled by the dairy management team with precise attention given to program compliance. Elite conception rate attainment demands protocol superintendence. A quality reproduction management program scheme includes attentiveness given to the uterus in equal measure to the ovary. They are in reality, biologically interdependent.

APPENDIX

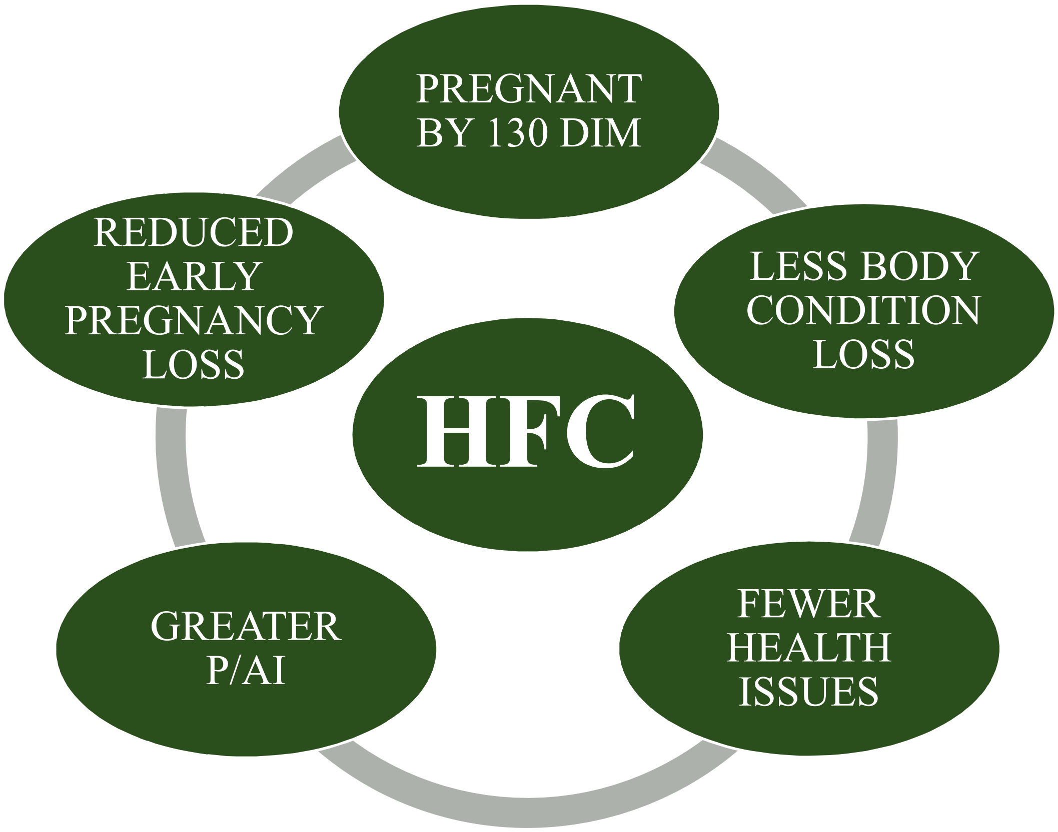

Figure 2.1 A pictorial depiction of the "high fertility cycle".

CHAPTER 3

THE EFFECT OF A POSTPARTUM TREATMENT ON THE INTERACTION OF BODY CONDITION, MUCOPURULENT DISCHARGE, PREGNANCY RATE AND PREGNANCY LOSS IN LACTATING DAIRY COWS

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ABSTRACT

A goal of the successful modern dairy enterprise has become the achievement of timely pregnancy for each cow. The primary objective of this chapter was to evaluate the effect of postpartum treatment on fertility of lactating dairy cows. The strategy tested in the treatment group sought to: 1) induce an early ovulation by intramuscular (IM) administration of a gonadal-releasing hormone (GnRH) treatment (GONAbreed® (gonadorelin acetate, 100 mcg), Parnell U.S. Overland Park, KS), and 2) to decrease subsequent P4 by the regression of corpus luteum (CL) in a serial fashion by the IM administration of prostaglandin F2α (PGF2α) (estroPLAN® (cloprostenol sodium, 250 mcg), Parnell U.S. Overland Park, KS). The control group did not receive any GnRH or PGF2α prior to the G-6-G protocol of first timed artificial insemination (TAI) for the control and treatment groups. A secondary objective of this study was to determine if this treatment could reduce the incidence of endometritis. Treatment reduced pregnancy per AI (P/AI) for first AI at 35 d post-AI in primiparous cows (40.9% versus 58.7% respectively; P = 0.01) but not in multiparous cows (40.8 % versus 43.2 %, respectively; P = 0.65). That difference in primiparous cows was maintained throughout each period of pregnancy beginning at 35 d, 60 d (57.8% vs. 40.0%; P = 0.01), 119 d (54.2% vs. 39.5%; P = 0.03), and 188 d (52.8% vs. 38.9%; P = 0.04) post TAI. There were no differences in treatments for pregnancy loss between each pregnancy determination period. Total pregnancy losses after first TAI were 14.3% and 18% for control versus treatment respectively (P = 0.87; n = 252). A Metricheck (M) device was used on the control group (n = 161) and the treatment group (n = 163) as an indicator of endometritis. Five vaginal discharge examinations were performed at M1 (21 ± 3 days in milk (DIM)), M2 (28 ± 3 DIM), M3 (42 ± 3 DIM), M4 (56 ± 3 DIM), and M5 (77 ± 3 DIM). No significant differences were found between treatments and treatments x time (P > 0.05). There was no effect

of treatment (11.5%) on cows that left the herd from time of treatment to 188 DIM compared to controls (8.9 %; n = 614). In summary, treatment did not improve fertility or % of cows with endometritis throughout the treatment period in lactating dairy cows. Treatment had an adverse effect on primiparous cows. It was not clear why treatment did not improve fertility and actually had a negative impact on fertility of primiparous cows.

INTRODUCTION

High producing dairy cows depends on consistent management. A comprehensive management guidance program is necessary because of the dynamic nature of biological systems and the inherent variables involved in its control. The program is based on cohorts where each cow is enrolled and treated individually. System analytics including data collection and measurement and monitoring are important aspects of the program. Timely review and disciplined reaction to the data is imperative if the exercise is to be successful.

Individual cows must be managed in such a way as to attain a profitable lifetime. A cow's circular parity continuum demands a calving interval (**CI**) of 12 to 13-months for optimum function as a high producing dairy cow (Dijkhuisen et al., 1984; Meadows, 2005). That objective lies at the heart of the voluntary cull goal. Preparation for a successful outcome to the first insemination is a part of that continuum. It demands intentional management by planned programming during each parity phase beginning with heifer development. Managing dairy cow reproduction to attain that objective requires specific attention be given to first service pregnancy rate per each artificial insemination (**P/AI**).

Optimal production begins with reproduction. Since Pursley et al. (1995) unraveled fertility program biology, the physiology of fertility and its proper understanding and implementation has largely been elucidated (Stevenson and Britt, 2017; Thatcher, 2017). Pregnancy has become more common outcome for the high producing dairy cow.

The incidence of uterine and ovarian dysfunction in dairy cows has not changed over the past 50 years even though management skill and practice has greatly improved the health of peri and postpartum cows (Sheldon and Dobson, 2004). Infectious and metabolic disease risk is typically at its peak during the transition phase of parity, especially if excessive loss of body condition occurs (Ruegg and Milton, 1995; Middleton et al., 2019).

The dairy cow is dependent upon the natural uterine involution process postpartum without exogenous hormone or antibiotic intervention. Endometritis was associated with profoundly impaired reproductive performance (Gilbert et al., 2005) and that uterine bacterial contamination after parturition inhibits follicle growth and function (Sheldon et al., 2002). That may have a negative impact on first service P/AI and subsequent services (Gobikrushanth et al., 2016). The involution mechanism can be disrupted in dairy cows diagnosed with metritis (Leslie, 1983; Cheong et al., 2017).

The most common intervention for clinical metritis and endometritis have included timely $PGF_{2\alpha}$ treatments (McClary et al., 2011) or systemic antibiotic therapy or both (Kaufmann et al., 2010). Hormonal manipulation of responsive ovaries may help to resolve uterine infection effectively resulting in a best-case scenario for a conception at first service (Gumen et al., 2011). Contrary, Haimerl et al., (2012) and Lefebvre and Stock (2012) were unable to find enough clinical evidence that using $PGF_{2\alpha}$ provided a significant benefit for endometritis cases. The limited number of treatment-oriented studies and the differing results leave us with little confidence in the use of $PGF_2\alpha$ or antibiotics (cephalosporins) or both for a definitive treatment of subclinical endometritis (Aris, et al., 2018).

The healthy postpartum cow typically begins to develop a follicular wave (**FW**) in the first week postpartum and the first dominant follicle (**DF**) is selected by day (**d**) 10 (Leslie, 1983; Sakaguchi et al., 2004) or 15 d postpartum (Britt et al., 1974). A first ovulation can occur within 2 weeks after calving in dairy cows (Britt et al., 1974; Rajamahendran et al., 1990; Savio et al., 1990; McDougall et al., 1995). In one study it was found that normal ovarian follicular dynamics were regained after the second ovulation postpartum (Sakaguchi et al., 2004). Shrestha et al. (2004) found that 37% of cows experienced normal resumption of ovarian cyclicity within 45 d postpartum and 46.3% of the cows did not resume ovarian cyclicity until greater than 65 d postpartum.

Anestrus had a negative influence on fertility during the postpartum period (Peter et al., 2009). Anestrus can be the result of numerous suboptimal conditions. For example, inadequate nutrition peripartum (Canfield and Butler, 1991; Butler et al., 2006), excessive body condition loss (Gumen et al., 2011), metabolic disease (Cheong et al., 2016), and acute and chronic uterine (Sheldon and Dobson, 2004; Sheldon et al., 2006; Cheong et al., 2017) and systemic infectious disease are correlated with anestrus (Peter et al., 2009; Figure 4. Middleton et al., 2019).

Cows with negative energy balance (**NEB**) postpartum were found to have fewer LH pulses and increased hypothalamic negative feedback sensitivity to estrogen (**E_2**) resulting in an anovulation condition (Wiltbank et al., 2011). Approximately 20% of any postpartum population of cows are anovulatory (Shrestha et al., 2003; Butler et al., 2006; Martins et al., 2011; Wiltbank et al., 2011). Dubuc et al. (2012) reported that postpartum anovulation was associated with indicators of energy balance and uterine inflammation resulting in a detrimental effect on reproductive performance. Although the experimental treatment protocol may prove to be a therapy for anovulation (Bittar et al., 2014), a subset of treatment group cows may not respond to the initial GnRH injection if the follicle present has not deviated to achieve dominance lacking LH receptors in the granulosa cells (Wiltbank et al., 2011).

Anestrous and anovulation are distinct in definition yet may be present concurrently. Ovulation results in CL producing progesterone (**P_4**). Endometritis in the presence of high or persistent P_4 might become a chronic condition promoting infertility (Etherington et al., 1984; Sheldon et al., 2006).

Anovulation and/or anestrous can be the result of peri and postpartum disease processes or BCS loss that adversely affect maintaining a cow within the HFC (Middleton et al., 2019). Other studies concluded that improvements in fertility could be achieved after peripartum health and nutrition management improve postpartum energy status for the high production cow resulting in less body condition loss and earlier resumption of normal estrous cyclicity resulting in ovulation (Santos et al., 2009; Kawashima et al., 2012; Drackley and Cardoso, 2014; Wiltbank et al., 2015).

Normal estrous cycles are achieved in most dairy cows in the first 30 d postpartum (Vercouteren et al., 2015). This is usual for metritis negative or metritis positive cows. In the metritis positive cows classified as a pyometra case, a persistent CL can lock them into a perpetual diestrus phase of the estrous cycle. This condition does not allow for the expulsion of the uterine lumen contents and the elevated P_4 levels may adversely affect the uterine immune function (Lewis, 2004). Lewis (2004) also reported that P_4 can suppress uterine eicosanoid synthesis and that seems to be a factor in the onset of uterine infections. This is due to eicosanoids function in enhancement of uterine immune defenses. Lewis (2004) demonstrated that exogenous $PGF_{2\alpha}$ can promote the resolution of uterine infections even while P_4 levels are maintained at luteal phase levels. $PGF_{2\alpha}$ is a proinflammatory molecule that stimulates proinflammatory cytokine production that is implicated in uterine production of leukotriene B4 (**LTB_4**) (Lewis, 2004; Sheldon et al., 2009; Carneiro et al., 2016). LTB_4 stimulates various neutrophil functions of which one is to enhance the neutrophil's phagocytic activity (Lewis, 2004). Failure of this local immune cascade could be a significant contributor to reduced fertility in dairy cattle.

The objective of the chapter was to determine if ovarian manipulation using GnRH and $PGF_{2\alpha}$ prior to the presynch / Ovsynch (G-6-G) protocol of first service AI could result in greater fertility in lactating dairy cows at first service AI. We hypothesize that treatment will have a positive effect on resumption of cyclicity and uterine involution. We further hypothesize that cows with a positive Metrichek examination will have reduced fertility following a timed-AI protocol.

EXPERIMENTAL DESIGN AND METHODOLOGIES

Cows, Housing, and Materials

All animal handling and experimental procedures were approved by the Michigan State University Institutional Animal Care and Use Committee according to application number 11/11-254-00, dated 11/16/2011.

The trial required 7 months for data collection. The experiment was conducted on a commercial Holstein dairy farm in Michigan. The farm milked about 1,000 dairy cows 3 times daily at approximately 8-hour intervals in a modified herringbone parlor. Cows were fed a TMR balanced for optimum milk production and health. The ration was fed once daily and consisted of corn, wheat and alfalfa silage as forage with corn and a soybean meal-based concentrate formulated to meet or exceed the minimum nutritional requirements (NRC, 2001). There were three basic lactating cow diets fed to: 1) early lactation, 2) 1st parity, and 3) multiparous cows.

Two distinct rations were fed to dry cows: 1) cows entering the dry period, and 2) cows within 3 weeks of their calving date. Both dry cow diets consisted of corn silage as the primary forage but with grass hay when entering the dry period and straw plus concentrates in the close-up ration. Cows entering ≥ third lactation and any cows with twins (diagnosed by veterinarian ultrasound examination) received calcium supplementation at calving. They had ad libitum access to feed and water. All postpartum primiparous and multiparous cows were housed in 4 row free-stall sand bedded barns with lock-ups at the feed bunk. The sidewall curtained barns were equipped with fans but not sprinklers that were thermostatically controlled. The average daily milk production was 42 kg / cow with 3.4% fat and 2.9% protein. All trial enrollees were divided into 2 separate groups within one free-stall barn and moved into other barns as subsequent cows entered the lactation herd. Animals that required special needs care were located in a separate hospital pen group near the treatment area and milking parlor.

Experimental Design

All cows were given a score for body condition (**BCS**) and all disease incidences were recorded. BCS was assessed on a 1 to 5 scale with 0.1 increments within 1 week of parturition then again 27 to 33 DIM (Middleton et al. 2019). Weekly cow cohorts in parity 1, 2 and ≥3 at 18 to 24 days in milk (**DIM**) were divided equally into a treatment and control group for each of the 3 parities. Cows were randomly assigned by picking numbers from a selection box. Half of each group (subset) within every cohort was randomly assigned to a Scan (Ibex Pro®, E.I. Medical Imaging, Loveland, CO. 7.5-MHz linear-array transducer) and Metricheck examination group versus a no ultrasound / no Metricheck group. The ovaries of the ultrasound cohort were examined via transrectal palpation with ultrasound and the structures recorded. A functional corpus luteum (**CL**) present on an ovary was the determinate of cyclicity. The cervical mucus /

vaginal discharge for visual determination of mucopurulent discharge status, was obtained via the Metricheck device in accordance with its recommended use. Collected material was examined and recorded as a positive or negative mucopurulent discharge finding. A discharge other than clear mucus was considered positive. The Metricheck positive cows did not receive any treatment other than the prescribed treatment protocol prior to G-6-G, if they were randomly selected for the experimental treatment cohort.

The treatment cohort received fertility injections as described in Figure 3.1; **1)** 1 ml injection of GONAbreed® (gonadorelin acetate, 100 mcg / ml. Parnell Technologies Pty. Ltd.) (G1) at 21 (±3) DIM. G1 was followed by **2)** 2 ml injection of estroPLAN® (cloprostenol sodium, 250 mcg / ml. Parnell Technologies Pty. Ltd) (PGF1). PGF1 was administered 11 days after the initial GnRH injection (G1). **3)** PGF2 injection was administered at 46 (±3) DIM 14 days after PGF1. Cows designated for ovarian ultrasound and Metricheck® examination were completed and recorded on the appointed days. All prescribed treatments were administered using 3cc Monoject® syringes with 20-gauge, 1.5-inch needles attached. Treatments were therapeutic doses and were administered by Pursley laboratory personnel centered around 9:00 am on each treatment day. The exception was the G4 injection that was done at or around 3:00 pm at 84.5 (±3) DIM.

The treatment and control cohort cows were enrolled in the G-6-G / Ovsynch protocol beginning with PGF3 at 67 (±3) DIM 21 d after the final treatment prostaglandin (**$PGF_{2\alpha}$**) injection. G-6-G / Ovsynch protocol was followed until the cow received the 1st timed artificial insemination (**TAI**) insemination at 85 (±3) DIM as described in Figure 3.1. TAI was done by the farm owner and herdsman as was usual and customary farm practice.

The purpose of the presynchrony aspect of G-6-G was two-fold. First, it aimed to regress a responsive CL with an injection of $PGF_{2\alpha}$ (PGF3). Secondly, the GnRH (G2) injection will ovulate a dominant follicle if present resulting in a CL while a new follicular wave emerges. Ovsynch is started 6 d later by administering another GnRH (G3) injection. The stimulated LH surge induces an ovulation of the next dominant follicle creating an auxiliary CL resulting in increased P_4 serum levels. That is an essential step in enhancing fertility through the ovulation of an optimal oocyte. The remainder of the protocol is followed as described. Cows removed from the study were done so because of farm management culling decisions, death loss or protocol mistakes by farm personnel or research team members.

Ultrasound Evaluation of the Ovaries

The ultrasound / Metricheck cohort ovarian structures were examined with a portable Ibex Pro as commonly used in veterinary practice. Ultrasound evaluations were completed as per protocol (Table 3.1).

Images were "fixed" in place when the ovarian structures were visualized to be at their maximal size. Structure size was determined by taking a measurement at the structures greatest diameter using the screen grid as the guide. All visualized structures were mapped and recorded.

Ovulation in the treatment and control groups were determined by the disappearance of one or more ovulatory-sized follicles at ≥10 mm in diameter (Sartori et al., 2001) and the appearance of a functional CL at that same site. The size and age of the CL if present was dependent on the ultrasound examination date relative to the prior $PGF_{2\alpha}$ injection for the treatment cohort. CL age was further determined by the presence of an appropriately sized structure for the ultrasound examination date and the presence of a CL cavity indicating an earlier aged CL (d 1 – 10).

Pregnancy Diagnosis

All eligible inseminated cows were pregnancy examined weekly by the herd veterinarian via transrectal palpation with ultrasound at d 34 ± 3 after AI. Cows found pregnant were re-examined for pregnancy after d 60 post-AI. Pregnancy was re-confirmed using a pregnancy associated glycoprotein (PAG) ELISA assay 120 d post-AI and if pregnant, the PAG test was repeated at 188 d post-AI (AntelBio, NorthStar Cooperative DHI Services, Grand Ledge, MI). Pregnancy results were recorded in PCDART for P/AI determination. Pregnancy loss during that time was determined, recorded and analyzed.

Statistical Analyses

All information was recorded in a Microsoft Excel (Microsoft Corp., Redmond, WA) spreadsheet for organization before statistical analysis. All ovarian structures found on ultrasound examination were recorded by the standard Ovary Mapping technique. For selected analysis of Ovulation, treatment versus control and parity effect, Cochran-Mantel-Haenszel chi-squared analyses using the FREQ procedure of SAS was performed. For the interaction of parity effect within treatment, generalized linear mixed model fitted with the GLIMMIX procedure of the statistical software SAS (version 9.4, SAS Institute Inc., Cary, NC) was used. The model considered treatment, parity, and their interactions as fixed effects and weekly groups as a random effect.

In the analysis of Metricheck positive incidence, the Cochran-Mantel-Haenszel Chi-squared analyses using the FREQ procedure of SAS was implemented. It was also used in the analysis of Metricheck positive incidence between treatment and control groups, parity, conception rate, and the interaction between conception rate and parity. P/AI for the Metricheck positive / Metricheck negative cohorts by parity versus control / treatment interaction was analyzed using a generalized linear mixed model fitted with the GLIMMIX procedure of the statistical software SAS. The model considered treatment, parity, and the interactions as fixed effects and weekly groups as a random effect.

Binomial variables were analyzed using a generalized linear mixed model fitted with the GLIMMIX procedure of the statistical software SAS. The model considered Metricheck positive, treatment, ovulation, parity, and their interactions as fixed effects and weekly cohorts as a random effect. Predicted probabilities of pregnancy were computed using the LOGISTIC procedure of SAS. Pregnancy loss (differences in P/AI between P1 and P2) were analyzed using a generalized linear mixed model fitted with the GLIMMIX procedure of the statistical software SAS.

RESULTS

Treatment Effects on Metricheck Examinations

The Metricheck examination for the presence of mucopurulent vaginal contents was conducted at five specific times early postpartum (Table 3.2). Metricheck positive cows in the primiparous and multiparous combined treatment and control groups decreased in frequency in a continuous decreasing order from M1 through M5 as described in Table 3.4. The rate at which both groups decreased was virtually identical except for treatment versus control at M2. The d 28 - M2 treatment cows had a tendency for a faster rate of decline than did the control group. However, by the time the cows reached d 77 – M5 there was no difference in Metricheck positive cows for the two groups.

A difference was found in the primiparous versus multiparous M1 ($p = 0.01$; $n = 381$) and M3 ($p = 0.05$; $n = 304$) sampled cows. The primiparous animals had a greater propensity for a positive Metricheck exam at d 21 and d 42. The differences were not found at d 56 and at the termination of the experimental treatment time at d 77.

Treatment Effects on Fertility

There was a greater P/AI for 1^{st} AI at 35 d post-AI in the control versus the treatment group (49.1% versus 40.8%; $p = 0.05$). That difference was maintained throughout each period of pregnancy at 60 d post-AI, 119 d post-AI and 188 d post-AI as shown in Table 3.3. The difference in P/AI was found in the primiparous cows where the control outpaced the treatment group 35 d post-AI (58.7% versus 40.9%; $p = 0.01$).

Treatment Effects on Pregnancy Loss

There were no differences found within each period of pregnancy loss between treatments. Total pregnancy losses after 1^{st} AI were 7.3% and 7.8% for control versus treatment respectively ($p = 0.87$; $n = 252$).

There were no differences in the percent of cows that left the herd between the treatment and control (11.5% versus 8.9%; $p = 0.29$; $n = 614$). The treatment had n = 23 cows that were culled or died before 35 d post-AI, an additional n = 3 before 60 d post-AI, an additional n = 6 before 119 d post-AI, and an additional n = 4 before 188 d post-AI. The control had n = 17 cows that were culled or died before 35 d post-AI, an additional n = 2 before 60 d post-AI, an additional n = 4 before 119 d post-AI, and an additional n = 4 before 188 d post-AI.

DISCUSSION

The experiment tested the hypothesis that the treatment regime prior to presynch / Ovsynch would result in greater fertility at first TAI. Outcomes from the study did not support that hypothesis because a greater proportion of control versus treatment cows became pregnant after first TAI. A greater difference was apparent in the primiparous cows versus multiparous cows. A relationship between fertility and Metricheck positive examination could not be found. Paradoxically, the difference in the P/AI at first TAI for control versus treatment (58.7% vs. 40.9%; p = 0.01) groups was found in the primiparous cows. That effect was not determined to be true for multiparous control versus treatment cows (43.2% vs. 40.8%; p = 0.65).

Total pregnancy losses after first AI for control (7.3%) versus treatment (7.8%) were unremarkable. The expected pregnancy loss results could indicate a disassociation of Metricheck positive cows' influence on fertility / fetal attachment.

With respect to the Metricheck data, the treatment had no effect on primiparous versus multiparous or control versus treatment groups. Time postpartum had the same apparent effect in all categories with about 20% of cows in any category positive on the final Metricheck exam at 77 d postpartum.

Savc et al., (2016) suggested that a postpartum examination of the reproductive tract should include both an ultrasound and Metricheck examination (vaginal mucus scoring). Theriogenologists have asserted that a diagnosis of metritis based solely on the presence of purulent or mucopurulent vaginal discharge could be misleading. Examination of vaginal contents do not necessarily provide an accurate diagnosis of uterine disease, i.e. vaginitis and/or cervicitis could be causative of an abnormal vaginal discharge.

Wagener et al., (2017) agree, "Because a poor agreement between vaginal and cytological findings has been found, the terms PVD and CYT have been suggested instead of CE and SE. Whereas the terms CE and SE follow the classical rules of terminology for a disease, the terms PVD and CYT represent a mixture of clinical symptoms (purulent discharge), diagnostic technique (cytological), and diagnosis (endometritis)."

The association between the ubiquitous nature of uterine contamination (Sheldon and Dobson, 2004; Sheldon et al., 2006) and Metricheck positive cows is unknown. The effect of Metricheck positive on fertility was unremarkable in the study.

Resumption of normal estrous cycles culminating in ovulation are an expectation for each individual cow soon after parturition according to traditional thought and practice. Ovarian cyclicity prior to 1st TAI (Galvao et al., 2004, 2010; Chebel et al., 2007; Santos et al., 2009) but especially resumption in early lactation (Darwash et al., 1997; McCoy, 2006) was associated with improved reproductive performance. That conclusion was shared by Galvao et al., (2010) and Dubuc et al., (2012) when they reported that Holstein cows that were cyclic by 21 DIM had improved reproductive performance compared to cows not cyclic by 60 DIM.

The early postpartum cow that had a loss in BCS was often suffering from a depressed DMI that resulted in a state of NEB as indicated by elevated BHBA and NEFA levels and by elevated indicators of inflammation (i.e., haptoglobin) (Beam and Butler, 1999; Butler et al., 2006; Gumen et al., 2011; Drackley and Cardoso, 2014). The elevated indicators were found to negatively affect cyclicity (Dubuc et al., 2012) and fertility (Gumen et al., 2011). LeBlanc (2012) contended that NEB contributed to immune dysfunction peri and postpartum and was a major element in reproductive tract inflammatory disease. Kawashima et al., (2018) found that hepatic dysfunction from peripartum diseases caused delayed uterine involution and endometritis. Cows suffering from elevated BUN levels may have depressed DMI (Kawashima et al., 2018).

Cows in NEB experienced greater incidences of metabolic maladies that resulted in retained fetal membranes (**RFM**), metritis and delayed cyclicity by 21 DIM (Vercouteren et al., 2015). Heppelmann et al., (2015), described the effect metritis and hypocalcemia had on uterine involution as evaluated by sonomicrometry. It was demonstrated that both diseases affected uterine size reduction (involution) until d 28. Metritis positive cows had a larger uterine diameter and those with subclinical hypocalcemia had delayed uterine length reduction which may be due to decreased myometrial contractility (Heppelmann et al., 2015). If the NEB was significant, it adversely affected the first dominant follicle postpartum, as described by Cheong et al., (2016). The NEB resulted in reduced LH pulses and increased hypothalamic negative feedback sensitivity to E_2 that resulted in ovulation failure and a large incidence of anovulation in lactating dairy cows (Wiltbank et al., 2011). E_2 production impairment was also associated with depressed circulating insulin, IGF-1+, and glucose (Canfield and Butler, 1991; Beam and Butler, 1999; Butler et al., 2004).

Dubuc et al., (2012) demonstrated that metritis positive cows had increased levels of haptoglobin and decreased cyclicity by three weeks postpartum (Cheong et al., 2017). In addition, Cheong et al., (2017) reported increased serum concentrations of BHBA and NEFA resulting from a postpartum NEB status. NEB adversely impacts the developing follicle resulting in E_2 production impairment and failure of first ovulation. Failure to ovulate a DF led to a low P_4 environment that had a profound effect on ovarian dynamics and subsequent fertility in lactating dairy cows (Wiltbank et al., 2011; Cheong et al., 2017). Contrary, the lower P_4 serum concentrations were found to be beneficial to early and complete uterine involution and resolution of any endometritis present (Heppelmann et al., 2015). Lewis, (2004) found P_4 to be "permissive" to postpartum disease of the uterus. Sheldon and Dobson, (2004) reported that the uterus was more susceptible to infection during the luteal phase of the estrous cycle. The local uterine immune response was suppressed by P_4.

Heppelmann et al., (2015) also investigated the effect of time for first postpartum ovulation on endometrial inflammation in cows. The study included cows with and without uterine disease during the early puerperal period. The transvaginal FP procedure was used to suppress postpartum ovulation and formation of CL until 42 DIM. Heppelmann et al., (2015) concluded that suppression of early ovulation by transvaginal FP enhances uterine inflammation healing in postpartum cows. The low P_4 environment produced by transvaginal FP could be beneficial in the resolution of metritis.

The ubiquitous nature of postpartum uterine bacterial contamination and the persistence of pathologic bacteria were frequently a cause of reduced fertility in high production dairy cows (Sheldon and Dobson, 2004; Sheldon et al., 2006). Subfertility in cattle was associated with uterine infection by hypothalamic, pituitary and ovarian function perturbation (Sheldon et al., 2002; Carneiro et al., 2016).

SUMMARY

Postpartum treatment of cows with a mucopurulent vaginal discharge following administration of GnRH and $PGF_{2\alpha}$ had no overall benefit in fertility among multiparous cows. The apparent adverse effect of treatment on primiparous cows may suggest that decreasing P_4 concentration by the cessation of ovarian cyclicity may be beneficial in cows with a mucopurulent discharge (Hepplemann et al., 2015). It is unknown why there is a negative impact to treatment in primiparous cows by the data collected. It is unclear if there is a direct relationship between mucopurulent vaginal discharge found by Metricheck examination and metritis disease incidence (Wagner et al., 2017). It is also not clear that the incidence of mucopurulent discharge decreased fertility. An area of further research may be the question surrounding the treatment's negative impact on primiparous cows. Elucidation of the interactions between early ovarian cyclicity and fertility, and its effect on uterine health could help to make the association clearer.

The study did not elucidate the incidence of uterine bacterial disease (metritis or endometritis) from Metricheck positive cows and its subsequent effect on fertility. Further research into the question of estrous cycle perturbation and its relationship to resolution of mucopurulent vaginal discharge should be considered.

ACKNOWLEDGEMENTS

Funding for R.J. Vlietstra's research project was made possible from the Michigan Animal Agriculture Alliance. We especially thank Ken and Kerry Nobis and the employees at Nobis Dairy Farm (St. Johns, MI) for their assistance and use of their cows. We acknowledge Dr. Jerry Kerr (Countyline Veterinary Service. Pewamo, MI) and appreciate his long-suffering acceptance of and patience with our research efforts at the Nobis dairy. We specifically recognize Emily Peacock, Shianne Berthume and Michael Herman, undergraduate research assistants (Michigan State University, East Lansing), for their roles in this project. We are always grateful for the interest Parnell Animal Health (Overland Park, KS) displays in our research by their donation of GONAbreed and estroPLAN used for the G-P-P and G6G / Ovsynch at Nobis Dairy Farm.

APPENDIX

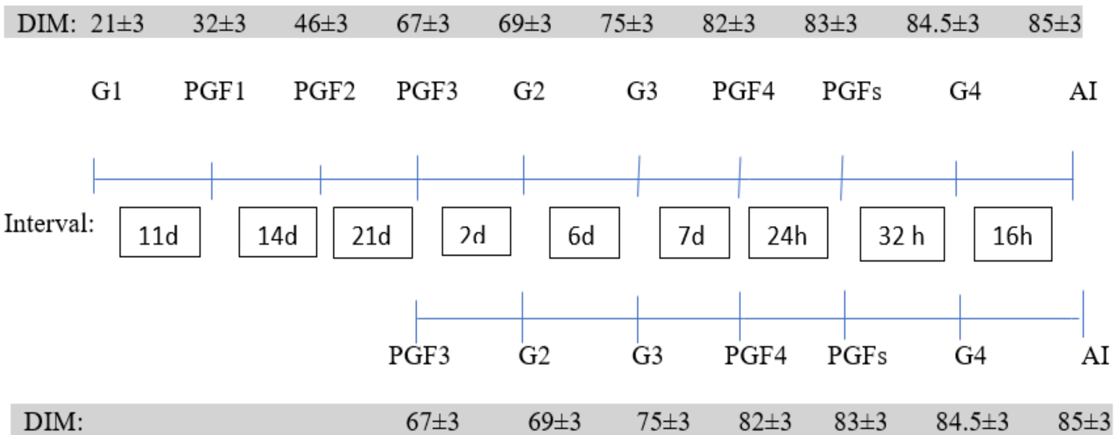


Figure 3.1 Schematic diagram of the experimental protocol for the Treatment and Control groups. In the treatment group, cows were enrolled in the G-P-P (Treatment) protocol at 21 ± 3 DIM with TAI at 85 ± 3 DIM. In the Control group, cows began the G-6-G / Ovsynch protocol at 67 ± 3 d and were TAI at 85 ± 3 DIM.

Metricheck No.	DIM (± 3 d)
1	21
2	28
3	42
4	56
5	77

Table 3.1 A description of the corresponding DIM for the n=5 postpartum examinations utilizing the Metricheck device.

Pregnancies per 1st AI	Control	Treatment	P - value
35 d post-AI			
Primiparous, % (n / n)	58.7 (64 / 109)	40.9 (47 / 115)	0.01
Multiparous, % (n / n)	43.2 (76 / 176)	40.8 (71 / 174)	0.65
Total, % (n / n)	49.1 (140 / 285)	40.8 (118 / 289)	0.05
60 d post-AI			
Primiparous, % (n / n)	57.8 (63 / 109)	40.0 (46 / 115)	0.01
Multiparous, % (n / n)	41.2 (72 / 174)	36.8 (63 / 171)	0.39
Total, % (n / n)	47.7 (135 / 283)	38.1 (109 / 286)	0.02
119 d post-AI			
Primiparous, % (n / n)	54.2 (58 / 107)	39.5 (45 / 114)	0.03
Multiparous, % (n / n)	40.7 (70 / 172)	33.7 (56 / 166)	0.19
Total, % (n / n)	45.9 (128 / 279)	36.1 (101 / 280)	0.02
188 d post-AI			
Primiparous, % (n / n)	52.8 (56 / 106)	38.9 (44 / 113)	0.04
Multiparous, % (n / n)	37.9 (64 / 169)	32.5 (53 / 163)	0.31
Total, % (n / n)	43.6 (120 / 275)	35.1 (97 / 276)	0.04

Table 3.2 The effect of treatment on pregnancies per AI for 1st AI in primiparous and multiparous lactating dairy cows.
*There were no differences within each period of pregnancy loss between treatments. Total pregnancy losses after 1st AI were 7.3% and 7.8% for control vs. treatment respectively (P = 0.87; n = 252).
*The treatment had n = 23 cows that were culled or died before 35 d post-AI, an additional n = 3 before 60 d post-AI, an additional n = 6 before 119 d post-AI, and an additional n = 4 before 188 d post-AI. The control had n = 17 cows that were culled or died before 35 d post-AI, an additional n = 2 before 60 d post-AI, an additional n = 4 before 119 d post-AI, and an additional n = 4 before 188 d post-AI. There were no differences in the percent of cows that left the herd between the treatment and control (11.5% vs. 8.9%; P = 0.29; n = 614).

Metricheck	Control	Treatment	P - value
M1			
Primiparous, % (n / n)	77.1 (54 / 70)	77.5 (55 / 71)	0.96
Multiparous, % (n / n)	63.1 (77 / 122)	64.4 (76 / 118)	0.84
Total, % (n / n)	68.2 (131 / 192)	69.3 (131 / 189)	0.82
M2			
Primiparous, % (n / n)	60.8 (31 / 51)	50.0 (34 / 68)	0.24
Multiparous, % (n / n)	51.2 (44 / 86)	41.7 (48 / 115)	0.19
Total, % (n / n)	54.7 (75 / 137)	44.8 (82 / 183)	0.08
M3			
Primiparous, % (n / n)	57.7 (30 / 52)	46.9 (30 / 64)	0.25
Multiparous, % (n / n)	43.6 (34 / 78)	38.2 (42 / 110)	0.46
Total, % (n / n)	49.2 (64 / 130)	41.2 (72 / 174)	0.17
M4			
Primiparous, % (n / n)	41.8 (23 / 55)	29.5 (18 / 61)	0.17
Multiparous, % (n / n)	24.2 (21 / 86)	34.3 (34 / 99)	0.14
Total, % (n / n)	31.2 (44 / 141)	32.5 (52 / 160)	0.81
M5			
Primiparous, % (n / n)	24.1 (13 / 54)	17.5 (10 / 57)	0.40
Multiparous, % (n / n)	18.6 (16 / 86)	25.0 (21 / 84)	0.31
Total, % (n / n)	20.7 (29 / 140)	22.0 (31 / 141)	0.80

Table 3.3. The effect of treatment on the percentage of primiparous and multiparous lactating dairy cows that had a positive Metricheck exam.

CHAPTER 4

ROLLING ACRES DAIRY FARM - CASE STUDY

LACTATING COW HERD REPRODUCTION PROGRAM

CHAPTER SUMMARY

The objective of the chapter was to include in this dissertation, a practical tool for teaching dairy cow reproductive management. The chapter describes a retrospective case study of how the owners of Rolling Acres Dairy Farm (Merle Coffey family, Allegan, MI) and their veterinarian (R.J. Vlietstra, West Michigan Veterinary Service. Coopersville, MI) developed a reproduction management program over a 13-year period (2006 – 2019) to address farm-level subfertility. Long-standing poor P/AI were the initial impetuous for what became a paradigm shift in reproduction management.

The introduction of PAG laboratory testing for individual cow pregnancy status information shifted the farm's budget for veterinary time utilization. That provided the farm unique opportunities that were not available before. Time spent by Dr. Vlietstra on this farm examining cows for pregnancy changed significantly during this 13-y period. Less time is now devoted to manual palpation and more time is invested in targeting the wellbeing of the transition and postpartum cow. Intentional attention given that critical parity phase resulted in optimizing fertility at 1st TAI. The change culminated in an eventual average 70% 1st insemination P/AI yielding 85% P/AI by 130 DIM. That point of convergence allowed for voluntary culling of less productive animals because of an adequate supply of replacement heifers. It turned out that achieving those indices increased reproduction efficiency and placed each individual cow squarely within what has been defined as the HFC parameters (Middleton, et al., 2019).

It was clear after this 13-year period that achieving reproductive programming success was a multifaceted management issue. It was as much about ensuring compliance of standard operating procedures as it was about manipulating reproductive physiology in healthy cows.

KEYWORDS

Conception rate, Days in Milk, High Fertility Cycle, PAG test, Transition, Pregnancy rate per artificial insemination

BACKGROUND

This reports a case study of a currently 1000 lactating cow commercial dairy farm located in Allegan, County, Michigan. Dr. Robert Vlietstra served as the clinical veterinary practitioner (VCPR) for Rolling Acres Dairy since the early 1980's.

Until the mid-1990's, Rolling Acres Dairy Farm struggled to maintain a consistent bi-weekly P/AI of 20% (personal experience). Pursley et al., (1995) introduced Ovsynch to the dairy industry in 1995 (Figure 1b). That was quickly adapted into this farm's reproduction program that until then, relied heavily upon artificial inseminating cows to perceived estrus. The "Ovsynch 48" target P/AI was originally set at 34%.

The use of $PGF_{2\alpha}$ for TAI synchrony was limited because of the problems associated with clean-up bulls within the lactation cow groups. The accompanying early pregnancy status uncertainty innate in bull exposed herds discouraged the use of $PGF_{2\alpha}$ for estrus synchrony. Rolling Acres experienced an inconsistent increase in P/AI from mid-twenty to low thirty percent using the Ovsynch 48 protocol. Numerous on-farm adaptations were made to the published Ovsynch protocol based on empirical impression. The inconsistent adjustments were done in a futile effort to increase P/AI.

Bello et al., (2006) published the G-6-G / Ovsynch protocol that more precisely synchronized ovulation and enhanced fertility by the formation of an auxiliary CL within the scheme. The additional CL created with G6G increased P_4 during the critical growth phase of the next DF.

At that point, a decision was made to trust the published science and strictly comply to the G-6-G protocol for 1^{st} TAI for each cow. The farm's reproduction program focal point became optimization of individual cow fertility at 1^{st} TAI by 90 DIM that resulted in maximizing IR at 100% for each weekly cohort of eligible cows.

During that same time, Dr. Vlietstra identified and implemented four other factors implicated in increasing fertility. 1) Attention to dry and transition cow diets and management 2) Implementation of a comprehensive postpartum cow health monitoring program 3) use of high-ranking sire conception rate (SCR) bulls; and 4) deep horn breeding (Pursley, J.R., Deep horn AI training. MSU Extension, 2002). These additions were introduced into the reproduction program routine.

Changes in farm ownership and management were consolidated into the Merle Coffey family ownership structure in 2010. Merle Coffey (Rolling Acres Farm primary owner) made the decision to personally administer all scheduled daily fertility treatments and any additional actions required for the reproduction program. The intent of management consolidation was to decrease treatment event and time variability. The goal was to optimize reproductive protocol control and compliance. Deficient or incompetent program compliance was considered to be a significant contributor to program dysfunction resulting in lower than expected P/AI.

All work was performed in the group pens away from the milking parlor. Cow comfort issues were addressed in the dry and transition groups using TCI as a guide (Nordlund, 2006, 2009). Because of a negative TCI assessment, a new transition barn was built. Soon after, a rotary parlor was installed complete with a post-partum cow pen in close proximity to the parlor. Transition and postpartum groups were designed with a target stocking density of 80%. Head-lockup space was set at 30 inches and sand bedding was used in the fan ventilated free stall barn.

The dry, transition and lactation rations were presented as a balanced TMR diet. DMI was especially targeted and encouraged by 6 daily feed push-up events.

On-farm veterinary consulting and MSU extension education were utilized to set the framework and monitor the prescribed program. An aggressive 1st TAI G-6-G protocol P/AI target was set at 60%, 10% higher than the P/AI published in the original limited "n" data (Bello et al., 2006; 2007). The expectation for subsequent TAI (Ovsynch 56) in cows diagnosed not pregnant was set at 50%. This would attain the goal of achieving 80% P/AI before 130 DIM.

The objective going into the paradigm shift in lactation dairy cow reproduction programming was to maximize 1st insemination P/AI. First insemination P/AI was the target because it was considered to be a marker for postpartum health event monitored outcomes. It was used as a general assessment of the herd's health and fertility.

Reproductive success hinged on the idea that culling reasons and rates were the ultimate key indicators of that accomplishment. For example; the goal of 80% pregnant by 130 DIM can be achieved in the following scenario: 60% P/AI at 1st TAI and 50% P/AI (Ovsynch 56). All remaining non-pregnant cows could be milked and sold as reproductive cull cows by today's commercial herd standard or at the dairy sale if at high production. If made, that herd decision would drastically reduce costs associated with TAI, DIM, DO, and infectious and metabolic disease incidence in subsequent lactations.

Postpartum Cow Examination Program

Rolling Acres' veterinary budget was scrutinized during the audit of the new-look herd visit day. Since a substantial savings on pregnancy examination service expense was realized by using the PAG test, it was decided to invest that same time into the largely neglected transition, peri and post-partum cow phases.

The program was simple, yet specific. Farm personnel identified cows prior to 21 DIM with adverse health events using a 'feed-bunk management' scheme. The scheme relied on observation of cattle behavior as a means to identify cows in need of attention. Cows with adequate rumen and udder fill and that eagerly came to the feed bunk when the mixer wagon approached, were considered to be healthy. Conversely, those that were reluctant to rise from free stalls or standing away from the feed bunk, most likely required attention.

Compromised cows had depressed appetites and reduced rumen fill with little vigor so were reluctant to walk to the feed bunk and eat. Those cows were overlooked in the past. That omission was often due to pen time constraints surrounding the milking parlor traffic flow and diminished employee motivation and understanding. Poor outcomes were a typical result of that oversight and neglect.

Adverse health events that were ruled out upon examination included fever, decreased rumen and udder fill, ketosis and dehydration (sunken eyes), metritis early postpartum and endometritis after 21 DIM and mastitis (Focus on Fresh Cows, Dairy Wellness. Zoetis, USA).

Treatment protocols were presented, discussed, agreed upon and written by the VCPR veterinarian. Diagnostic education and implementing treatment protocols were undertaken as part of employee training. Comprehensive programmed protocols were put in place for calf / heifer health and development, reproduction and cow production and health aspects of the farming enterprise.

It was considered ideal to achieve early postpartum resumption of continuous ovarian estrous cyclicity to enhance reproductive efficiency and fertility. Many factors peripartum influence that model, especially nutrition and specifically DMI. Healthy cows sporting healthy organ function had a propensity to consume large quantities of TMR, produce milk, and achieve a successful pregnancy. Conversely, anything that perturbs healthy organ function, including inhibited ovarian or uterine purpose furthers reproductive inefficiency and unproductivity.

A treatment protocol was devised and administered to uterine lumen positive or vaginal discharge positive cows beginning at 21 (±3 d) DIM. It was typical for the majority of first week cohort cows to be diagnosed with a cloudy vaginal discharge requiring treatment. A vaginal discharge positive cow was defined as one with a purulent or mucopurulent discharge.

Treatment was an antiseptic 60 cc intrauterine infusion that consisted of 20 cc dilute iodine solution plus 40 cc propylene glycol mixture. All vaginal discharge positive animals were administered a therapeutic dose of $PGF_{2\alpha}$ (25 mg dinoprost, Lutalyse®, Zoetis) beginning at 28 (±3) d postpartum if they remained on the treatment list after two weeks. $PGF_{2\alpha}$ was repeated every two weeks until the uterus was pronounced healthy. Persistent infection could delay enrollment in the G-6-G protocol beginning at 60 DIM but that was a rare occurrence.

The intensive post-partum intervention program included a veterinarian performed reproductive tract examination of every post-partum cow., A cohort of eligible cows was examined each week via trans-rectal palpation for manure quantity, consistency and temperature; for vaginal discharge and uterine involution, and ovarian activity. Body condition was noted but not recorded. Cows that had received treatment from prior weeks were reexamined as required by protocol. An additional program goal was to reduce the risk of antibiotic residue by using an antiseptic intrauterine infusion solution rather than an intrauterine antibiotic that would be an extra label use issue. Our challenge was to accomplish the lofty goal of 80% P/AI by 130 DIM.

Details of Fertility and Synchronization Program Logistics

All cows entering the lactating herd made their way through the intentional post-partum program prior to enrollment in the breeding phase protocol. As stated, the farm goal was to achieve optimal fertility at 1st TAI for each individual cow (Figure 4.1). Cows successfully graduating from the postpartum program were enrolled at 60 DIM into the G-6-G TAI protocol (Figure 4.2 A). The protocol was cohort specific for each cow's first chance for pregnancy at 80 DIM. After installation of the postpartum cow monitoring program into the cow management scheme, the 1st insemination P/AI goal was re-set to 70%.

After programmed TAI, cows were monitored for activity as routine chores allowed for proximity observation of estrus. Cows that exhibited the primary sign of estrus ('stand to be ridden') at 21 - 23 d post-TAI, were re-inseminated and the pregnancy exam date was updated in the farm's Westphalia Dairy Plan 5 computerized record system. Cows that were diagnosed not pregnant via PAG test after TAI at 31 d (±3 d) were enrolled in the Ovsynch-56 program and inseminated 10 d later (Figure 4.2 B). This continued until the cow became pregnant or earned the status DNB and remained in the herd until culled.

Determination of Pregnancy

The method of pregnancy diagnosis was changed from trans-rectal palpation of the uterus to the Bio-PRYN ELISA laboratory test (Bio-Tracking Inc., Moscow, Idaho) performed at the Reproduction Laboratory, WMVS, Coopersville, Michigan. P1 was performed at 31 (±3) d after TAI. Blood samples were set-up in the laboratory on Monday morning. Client laboratory results were sent electronically to the farm on later Tuesday morning and the information was entered into the farm's computer data base. Cows diagnosed pregnant received a second PSPB test at 75 (±3) d post-TAI. Cows that exhibited signs of estrus 21 - 23 d post-TAI were inseminated according to the am / pm rule. Individual animals listed on the laboratory result sheet as not pregnant were enrolled in the Ovsynch - 56 protocol on d 31 - 37.

Logistics for the efficient use of a laboratory PAG test for pregnancy was carefully considered. Attention was given to the amount of time spent collecting samples, ease of locating cows and with personnel phlebotomizing cows. Sample collection had to coincide with laboratory testing hours. The farm owner collected blood samples via puncture of the median caudal vein or artery into a 6 ml evacuated plain serum collection tube (Atlantic Medical Supply, Inc. Deer Park, NY). Samples were assembled Friday afternoon through Sunday morning. They were labeled, refrigerated and stored prior to being transported to the lab on Monday morning. The WMVS Bio-PRYN laboratory technician began the testing set-up process for the samples on Monday afternoon and the results were reported by 11 am Tuesday. The routine of sampling cows by group each week was strictly maintained. It coincided with the TAI routine per group. Regimentation was an important aspect of compliance to protocol.

Record Keeping

A cow's examination history status and the action taken in the postpartum program group was recorded on a barn work sheet at herd visit day. The cow remained on the active treatment list until the attending veterinarian changed the cow's status to inactive. That was the indication that she was ready for presynch / Ovsynch program enrollment at the appropriate DIM (60 DIM).

Weekly cohort P/AI data was recorded in an excel spreadsheet with the following headings: Total cohort, TTL % pregnant; G-6-G (1st TAI) cohort, G-6-G % pregnant; Ovsynch (OVS) cohort, OVS % pregnant; Estrus cohort, E % pregnant. These data were transferred into a monthly and yearly graph format (Figure 4.3). Monitoring occurred weekly, monthly and yearly and its review was an agenda item at each management team meeting.

The idea of a controlled study was introduced that would help us understand the effects of each introduced variable on the improved P/AI results. However, there was no interest on the part of the dairy ownership to do so. In their view, there was little practical reason to return a control group to the possible poor reproductive performance of the past. In addition, the number of variables introduced (facility updates, nutrition/DMI focus, postpartum cow program, TAI breeding program, TAI program compliance, technician skill) would make a multivariable statistical analysis very complex. Ultimately, there was no support from ownership for such an undertaking.

Reproductive Performance During the 13-Year Period 2006 to 2018

At the onset of ovulation synchrony programming, the set targets were as follows: 60 % 1^{st} service P/AI goal was reached by 2008 and a spreadsheet record began to track progress. The first full year of P/AI data was 2009. A steady climb in 1^{st} service P/AI to 70 % by 2013 was realized (Table 4.1).

The story for the OVS cohort is similar to that of G-6-G. The goal of 40 % was reached by the end of 2008 and oscillated in the mid to low 40% range through 2017. With the Pursley laboratory research work on the HFC in 2018 considered, the OVS target was updated to 50% (Middleton et al., 2019). At that same time, the OVS protocol was reviewed and changed from a 5 d Ovsynch to the more traditional 7 d Ovsynch using a single $PGF_{2\alpha}$ injection prior to the GnRH of ovulation. That change resulted in attaining the 50% goal in 2018.

Conversely, cows inseminated to the estrus event had reduced fertility. Cows inseminated to estrus possess the inherent major variable of heat detection and the question of ovulation timing. Therefore, estrus inseminations were limited to the primary sign of estrus expression; “stand-to-be-ridden” indicator. Even then, pregnancy to estrus was a “bonus” for the dairy producer (increased % herd pregnant).

Total cohort P/AI was targeted at 50% because of the accepted traditional standard. Realistically, this index is only a result of the prior TAI actions.

The goal of the management program in its entirety at Rolling Acres dairy is selection for pregnancy in the most fertile cows at 1st TAI. To optimize fertility at 1st TAI, ovarian and uterine function were considered. Reduced fertility could be adversely affected by failure in any one or combination of the interdependent 5 cornerstones upon which the HFC is based. That failure may result in reduced fertility by ovarian or uterine perturbation or both.

The slight variation in P/AI in any category can be attributed to strict compliance to every aspect of the reproductive program. At Rolling Acres dairy, the owner manages the entire reproductive program. That seems to have an added benefit in quality control which is reflected in the lack of variation. Total management control is not usual and is not advised for the average commercial dairy.

BRIEF DISCUSSION

The implementation of a presynch / Ovsynch protocol for first TAI is advantageous for use at 1st insemination (Moreira et al., 2001; Bello et al., 2006; Sousa et al., 2008). Conversely, presynch programs are not ideal for subsequent inseminations (Wiltbank and Pursley, 2014). Pursley et al., (1997) reported that for 2nd and 3rd AI a reduction in median days to pregnancy by 19 d was found when a synchronization program was used versus estrus detection. Giordano et al., (2012) determined that a need to control days to insemination was important because estrus detection is not efficient enough to be used as the sole re-insemination strategy.

Another component of timely re-insemination is early and accurate non-pregnant diagnoses. Fricke (2002) and Giordano et al. (2013) found that to be critical to the establishment of timely pregnancies. The discovery of PSPB by Butler et al. (1982) localized to the binucleated cells of the placental trophoblastic ectoderm (Sasser et al., 1989) combined with the eventual readily accepted ovulation synchrony programs put reproductive management of the dairy industry on the verge of new biotechnological approaches. Romano and Larson, (2010) reported that relative to transrectal ultrasound examination for pregnancy at d 28, 30, and 35 after insemination, development of the ELISA test addressed the issue of cost, accuracy and simplicity for field use. The introduction of the enzyme-linked immunosorbent assay (**ELISA**) in 2002 for bovine pregnancy determination (BioTracking, Moscow, ID) was adopted by Rolling Acres as their choice for determining non-pregnant cows.

There were three suggested fertility programs for lactating dairy cows considered at 1st insemination TAI when the project started. These include A) "Presynch - 11"; B) "Double Ovsynch"; and C) "G-6-G" (Wiltbank and Pursley, 2014). The G-6-G protocol was chosen and implemented at Rolling Acres in 2006 as the best method of choice to improve fertility. Cows found not pregnant were immediately enrolled in Ovsynch - 48 initially (circa, 2006) followed by Ovsynch – 56 (Brusveen et al., 2008).

A discussion centered around whether estrus breedings should be excused from the reproduction program altogether. The reality was that if target P/AI could be attained and maintained by 130 DIM cows found not pregnant after the 2nd insemination could be considered a DNB animal. As a point of management, cows that do not achieve pregnancy at 1st TAI and show signs of estrus may be better served by enrollment in the Ovsynch protocol resulting in a 50% P/AI rather than saving ~20 d by inseminating to the 30% P/AI estrus breeding. That paradigm shift was an obstacle too large for the Rolling Acres ownership so it was decided to inseminate on primary estrus signs and accept the poorer pregnancy rate.

The typical expected average for estrus breedings in primiparous and multiparous cows in the US is 39 % (Pursley et al., 1997). Rolling Acres' low P/AI to estrus may be due to a culmination of an exceptionally high first service rate (fertile cows get pregnant) or lack of ovulation synchrony with AI.

Attaining program success had it's obvious up-side but in reality, it presented challenges for which one must plan. One issue to be aware of in HFC farms is the potential problem of drying off cows at high milk production. At Rolling Acres, primiparous heifers encountered high intramammary pressure that was a serious stress risk for clostridium mastitis. Monitoring this was imperative to maintain individual cow health and decrease involuntary culling or death. For example, high intramammary pressure at dry off could have been alleviated via greater control of the CI. As a consequence, DFS was increased to 120 DIM for the primiparous heifers. Incrementally, DFS was returned to 80 DIM with the appropriate management changes.

Another challenge was managing the increased population of heifer calves born from a larger cohort of pregnant cows giving birth. To accommodate health and wellbeing, Rolling Acres constructed 4 group pens of 25 calves with a Lely robotic calf feed station for each pen. One man was dedicated to daily calf management from birth to weaning. Calves were monitored for proper health, growth and development.

Choosing the correct TAI program at Rolling Acres resulted in excellent 1st insemination P/AI and helped to attain 80% P/AI at 130 DIM. This success can be attributed to a general philosophy of team member behavior especially in maintaining proper compliance of treatments and pregnancy diagnoses.

ACKNOWLEDGMENTS

I would like to thank Merle, Darren, Al and Adam Coffey for following me “off the cliff” with a different approach to the farm’s weekly herd visit. Merle’s wife, Elaine especially deserves recognition for tolerating the extra effort Merle invested in the strict rules about details. For the success achieved, there was a cost in time and dedication. I am deeply grateful to them for that commitment. Dr. Richard Pursley’s guidance in the process was also greatly appreciated.

APPENDIX

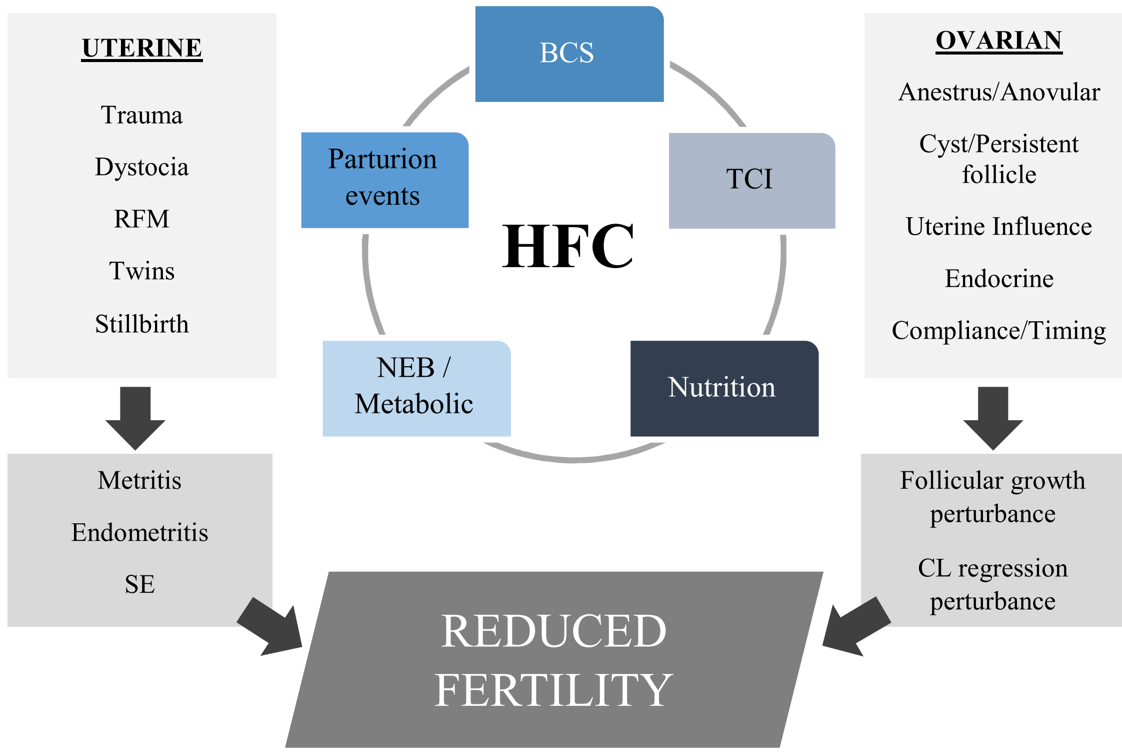


Figure 4.1 A clinical depiction of the interdependent nature for the 5 cornerstones upon which the "high fertility cycle" is based. Mismanagement of one or any combination of the 5 cornerstones can result in reduced fertility by ovarian and/or uterine perturbation. That dysfunction is described in Chapter 1 and 2 of this Dissertation.

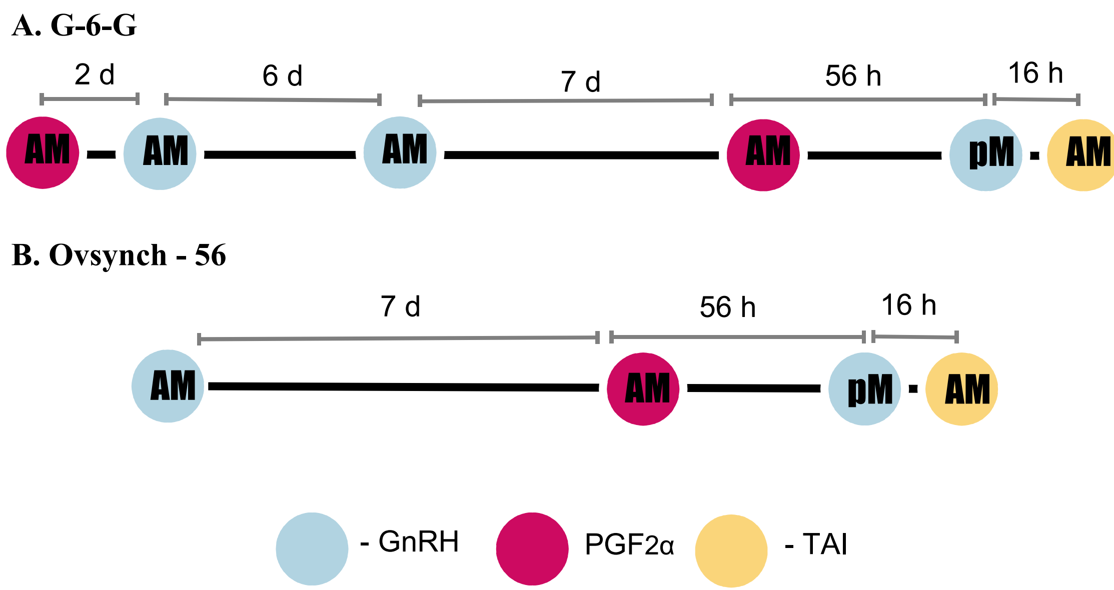
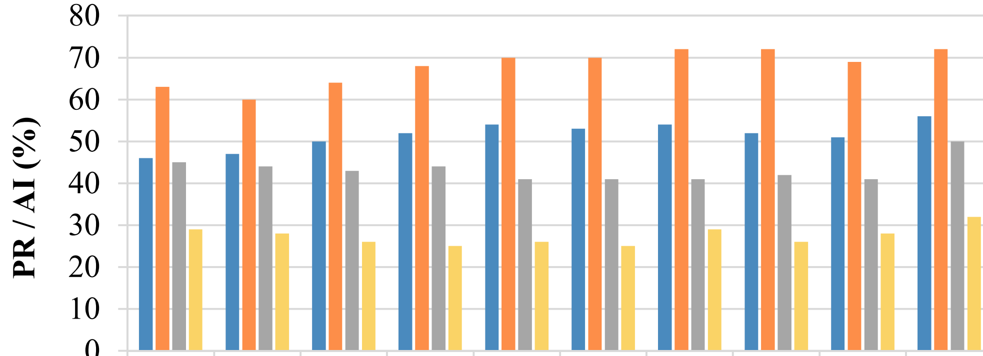


Figure 4.2 A. G-6-G, a presynch / Ovsynch adaptation of the Ovsynch – 56 protocol utilizing GnRH and PGF2α to increase P4 and synchronize ovulation (Bello et al., 2006). **4.2 B.** 7 d Ovsynch – 56; Rolling Acres dairy resynch program (Sousa et al., 2008).



	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
TTL	46	47	50	52	54	53	54	52	51	56
G-6-G	63	60	64	68	70	70	72	72	69	72
OVS	45	44	43	44	41	41	41	42	41	50
ESTRUS	29	28	26	25	26	25	29	26	28	32

Figure 4.3 All cows receive 1st AI with the G-6-G presynch / Ovsynch program at an average of 80 DIM. Following the PAG test for pregnancy at 28 – 34 d, all non-pregnant cows are enrolled in an Ovsynch-56 and receive a 2nd TAI. After 2nd AI, cows observed in standing estrus are inseminated on the am / pm rule. All other non-pregnant cows not observed in standing estrus are reenrolled in Ovsynch-56. The total is the average of each weekly cohort.

PROTOCOL	GOAL	SET	ACHIEVED
G-6-G	60%	2006	2008
G-6-G	70%	2011	2013
OVS – 48 (7 d)	34%	1995	2006
OVS – 56 (7 d)	40%	2006	2008
OVS – 56 (5 d)	40%	2013	2017
OVS – 56 (7 d)	50%	2018	2019
Estrus	20%	1982	1983
Estrus	30%	2006	2018
Total	50%	2006	2012

Table 4.1 Protocol PR / AI goal achievement by period. G-6-G cohort are cows inseminated for 1st TAI. OVS (Ovsynch 48 and 56; 5 and 7 d cohorts) includes all cows inseminated on the OVS protocol after 1st TAI. Estrus are AI inseminations in cows found in-heat (after 1st TAI diagnosed non-pregnant). The Total goal set was done as a matter of convention. The Total goal was achieved as a result of the cumulative effect of the individual protocols. The set date is the year in which the goal percentage was begun. The achieved date was the year in which the goal was reached.

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