Nutritional and hormonal strategies to improve fertility in lactating dairy cows

by

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B.S., University of Missouri, 2014
M.S., Kansas State University, 2016

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

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Abstract

Four experiments were conducted to assess nutritional and hormonal strategies to improve fertility in lactating dairy cows. In experiment 1, metabolites (−4, −2, +1, +2, +5, and +7 wk) and steroid hormones in ovarian follicular fluid (FF) and blood serum (BS) were examined in Holstein cows (n = 59) individually fed *Saccharomyces cerevisiae* fermentation product from −4 through +7 wk (calving = wk 0). Neither metabolites nor hormonal concentrations in BS or FF differed between treatments. In experiment 2 a rumen-protected glucose (RPG) product was supplemented at varying doses in the diet of lactating dairy cows with the objective to increase concentrations of glucose and insulin resulting in decreased activity of liver cytochrome P450 2C and P450 3A, thus increasing blood progesterone concentration. Neither pre-feeding nor post-feeding concentrations of glucose differed among treatments. Pre-feeding insulin did not differ among treatments, but a difference in the concentration of insulin (postprandial minus pre-prandial) was detected ($P = 0.01$). The increase in insulin was greater in control cows relative to the mean of the 3 RPG doses. We conclude that the insulin response to the RPG diets was diminished relative to the control. Supplementation with RPG did not impact concentrations of progesterone. Experiment 3 tested: (1) a shortened version of Ovsynch (SS: GnRH-1 − 7 d − $PGF_{2\alpha}$ − 24 h − $PGF_{2\alpha}$ − 32 h − GnRH-2 − 16 h − AI) that excluded GnRH-1 to resynchronize ovulation in cows bearing a corpus luteum (CL) after a non-pregnancy diagnosis (NPD); (2) the value of including progesterone-releasing intravaginal insert + Ovsynch (OVS + CIDR) in absence of a CL compared with presence of a CL + OVS; and (3) the accuracy of detecting a functional CL by transrectal ultrasonography. Pregnancy per AI (P/AI) risk tended ($P = 0.09$)) to be greater for OVS than SS but did not differ from OVS + CIDR at 32 d (30.3% [n = 644], 25.7% [n = 678], and 25.9% [n = 270]), respectively. In SS cows, P/AI was greater ($P = 0.01$)

when cows had a functional CL (progesterone was ≥ 1 ng/mL) vs. a nonfunctional CL at d 0, but did not differ from OVS cows. Short synch is a viable alternative to an entire OVS treatment when CL status is accurately detected. Experiment 4 was performed in 2 herds to determine if administering $PGF_{2\alpha}$ concurrent with timed artificial insemination in lactating dairy cows would enhance P/AI. Pregnancy per AI at d 32 and 80 did not differ between treatments. Cows treated with $PGF_{2\alpha}$ in one herd produced more twins than control cows (11.7 vs. 3.2%), whereas no treatment difference was detected in the second herd (5.6 vs. 5.6%), respectively. We conclude that i.m. treatment of lactating dairy cows with 10 mg of $PGF_{2\alpha}$ concurrent with timed AI did not improve P/AI or embryo survival, but increased twinning in one herd. Further research is warranted to determine nutritional and hormonal strategies to improve fertility.

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Four experiments were conducted to assess nutritional and hormonal strategies to improve fertility in lactating dairy cows. In experiment 1, metabolites (−4, −2, +1, +2, +5, and +7 wk) and steroid hormones in ovarian follicular fluid (FF) and blood serum (BS) were examined in Holstein cows (n = 59) individually fed *Saccharomyces cerevisiae* fermentation product from −4 through +7 wk (calving = wk 0). Neither metabolites nor hormonal concentrations in BS or FF differed between treatments. In experiment 2 a rumen-protected glucose (RPG) product was supplemented at varying dose in the diet of lactating dairy cows with the objective to increase concentrations of glucose and insulin resulting in decreased activity of liver cytochrome P450 2C and P450 3A, thus increasing blood progesterone concentration. Neither pre-feeding nor post-feeding concentrations of glucose differed among treatments. Pre-feeding insulin did not differ among treatments, but a difference in the change of insulin (postprandial – pre-prandial) was detected ($P = 0.01$). The increase in insulin was greater in control cows relative to the mean of the 3 RPG doses. We conclude that the insulin response to the RPG diets was diminished relative to the control. Supplementation with RPG did not impact concentrations of progesterone. Experiment 3 tested: (1) a shortened version of Ovsynch (OVS: GnRH-1 − 7 d − $PGF_{2\alpha}$ − 24 h − $PGF_{2\alpha}$ − 32 h − GnRH-2 − 16 h − AI) that excluded GnRH-1 to resynchronize ovulation in cows bearing a corpus luteum (CL) after a non-pregnancy diagnosis (NPD); (2) the value of including progesterone-releasing intravaginal insert + OVS in absence of a CL compared with presence of a CL + OVS; and (3) the accuracy of detecting a functional CL by transrectal ultrasonography. Pregnancy risk tended to be greater for OVS than SS but did not differ from CIDR at 32 d (30.3% [n = 644], 25.7% [n = 678], and 25.9% [n = 270]), respectively. Pregnancy per AI (P/AI) was greater for the SS treatment when cows had a functional (progesterone was ≥ 1 ng/mL) CL

at d 0. Short synch is a viable alternative to an entire OVS treatment when CL status is accurately detected. Experiment 4 was performed in 2 herds to determine if administering $PGF_{2\alpha}$ concurrent with timed artificial insemination in lactating dairy cows would enhance P/AI. Pregnancy per AI at d 32 ($P = 0.50$) and 80 ($P = 0.33$) did not differ between treatments. Cows treated with $PGF_{2\alpha}$ in one herd produced more twins than control cows (11.7 vs. 3.2%), whereas no treatment difference was detected in the second herd (5.6 vs. 5.6%), respectively. We conclude that i.m. treatment of lactating dairy cows with 10 mg of $PGF_{2\alpha}$ concurrent with timed AI did not improve P/AI or embryo survival, but increased twinning in one herd. Further research is warranted to determine nutritional and hormonal strategies to improve fertility.

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Dedication

The research summarized herein is dedicated to the pillars of my support system, Michael and Carole Sauls, and Randy Hiesterman. Without their unconditional love and support I would not be the person I am today.

Preface

The chapters in this dissertation have inconsistent formatting because they will be submitted to different journals for publication. The manuscript entitled "Relationships of metabolites and hormones in follicular fluid and blood serum in transition dairy cows supplemented with a *Saccharomyces cerevisiae* fermentation product" (chapter 2) will be submitted to Animal Reproduction Science. The manuscripts entitled "Physiologic responses to feeding rumen-protected glucose to lactating dairy cows" (chapter 4), and "A shortened resynchronization treatment for dairy cows after a non-pregnancy diagnosis based on corpus luteum status" (chapter 6) will be submitted to the Journal of Dairy Science for publication. The manuscript entitled "Additional Small Dose of Prostaglandin $F_{2\alpha}$ at Timed Artificial Insemination Failed to Improve Pregnancy Risk of Lactating Dairy Cows" is published in the Journal of Theriogenology (https://doi.org/10.1016/j.theriogenology.2017.12.051).

Chapter 1 - REVIEW OF LITERATURE- *SACCHAROMYCES CEREVISIAE* FERMENTATION PRODUCTS

Introduction

Reproductive success is critical to the survival of a dairy herd. Failure to produce a calf at regular intervals negatively impacts profitability (Royal et al., 2000). Milk production combined with reduced appetite and dry matter intake (DMI) in early lactation dairy cows causes a period of negative energy balance (NEB) and increased metabolic stress that is associated with decreased fertility (Leroy et al., 2012). In fact, energy balance was found to be the most influential factor in a model that predicted days to first ovulation (Francisco et al., 2003). Physiological adaptations that occur in lactating dairy cows to cope with NEB include increased concentrations of free fatty acids (FFA), hypoglycemia, or ketonemia (Beam and Butler, 1997). Yeast products are commonly used around the world for inclusion in diets of production animals (Poppy et al., 2012). Research is currently ongoing to determine how yeast products affect the rumen ecosystem and the impact yeast has on the transition period in dairy cattle.

Yeast Product Supplementation

Supplementation of direct-fed microbials to high-yielding dairy cattle has been in practice for more than 60 years with varied responses (Schingoethe et al., 2004). Two types of yeast products are commercially available (active dry yeast and yeast cultures) as commonly used direct-fed microbials (AAFCO, 2011). Active dry yeast products are products that must contain > 15 live yeast cells/g. The effect seen from active dry yeast is assumed to be depended on the yeast cell being alive in the rumen to influence production (Newbold et al., 1996). Yeast cultures made through yeast fermentation are not dependent on the live yeast for their physiological effect, rather the yeast culture contains fermentation by-products that impact growth of various types of rumen bacteria and protozoa (Harrison et al., 1998). The yeast *Saccharomyces cerevisiae* is made through yeast fermentation and is a commonly used direct-fed microbial (Dann et al., 2000).

Mode of Action

The rumen is relatively anaerobic; however, rumen gas has been found to contain between 5 and 10 mL of oxygen per liter with a significant concentration of oxygen detected in the liquid phase (McArthur and Multimore, 1962). Many rumen microorganisms are incredibly sensitive to oxygen (Loesche, 1969). Oxygen is toxic to anaerobic bacteria and inhibits growth of rumen bacteria in pure culture (Marouenk and Wallace, 1984) as well as the adhesion of cellulolytic rumen bacteria to cellulose (Roger et al., 1990). *Saccharomyces cerevisiae* does not replicate in rumen fluid (Arambel and Tung, 1987); however, it retains its metabolic activity (Ingledew and Jones, 1982) and viability (El Hassan, 1993). It was speculated that *S. cerevisiae* might protect the anaerobic bacteria by scavenging oxygen because of its high respiratory activity (Rose, 1987). Ability of varying yeast preparations to stimulate bacterial growth in the rumen of sheep corresponds with the yeast ability to remove oxygen from rumen fluid (Newbold, 1996).

Because yeast supplementation stimulates cellulolytic bacteria it enhances potential fiber digestion in the rumen (Newbold, 1996). An enhancement in fiber digestion prevents a drop of rumen pH which increases utilization of lactic acid by bacteria, decreases lactic acid production, or both (Chaucheyras et al., 1996; Callaway and Martin, 1997). Yeast supplementation also influences the profile of volatile fatty acid production because it influences feed degradability (Carro et al., 1992; Zelenak et al., 1994; Guedes et al., 2008).

Previous researchers reported a rumen buffering effect of yeast supplemented to goats (Desnoyers et al., 2009). A meta-analysis was performed using 157 studies and found that rumen pH and VFA concentration were increased by yeast supplementation (Desnoyers et al., 2009). In addition, a tendency was detected for a reduction in lactic acid production (Desnoyers et al., 2009). Therefore, it is thought that yeast supplementation acts by influencing the microbial population to alter volatile fatty acid production and stabilize rumen pH.

Production/Performance Impacts of Yeast Supplementation

Results from feeding *S. cerevisiae* fermentation product to dairy calves, pigs, and chickens indicate improved immune function (Magãlhaes et al., 2008; Gao et al., 2009; Shen et al., 2009) by activation of the innate and adaptive immune response (Jensen et al., 2008). A decrease in morbidity, recovery time from bovine respiratory disease (BRD), and negative effects of antibiotic treatment on feed intake in heifers have been reported after supplementation of *S. cerevisiae* (Keyser et al., 2007). A component of yeast products, β-glucan, has been shown to improve immune function against invading pathogens by enhancing the ability of macrophages, neutrophils, and eosinophils to clear pathogens (Volman et al., 2008). Receiving steer calves supplemented with yeast had reduced neutrophil to lymphocyte ratios and lower mortality risk compared with steers not supplemented (Finck et al., 2014).

Supplementation of yeast products has most commonly been studied in dairy cattle. Because it is thought that yeast products affect rumen microbial populations and alter VFA production, supplementation of yeast could result in increased milk production as well as increases in milk fat and protein (Erasmus et al., 1992). Several studies have reported a significant increase in milk production after supplementing yeast products (Harrison et al., 1988; Hippen et al., 2007; Lehloenya et al., 2007; Ramsing et al., 2009). Others have reported only a trend for increased production (Dan et al., 2000; Wang et al., 2001), whereas others report no differences when supplementing yeast products (Robinson, 1997; Schingoethe et al., 2004). Piva et al. (1993)

described improved milk composition when supplementing with *S. cerevisiae*, whereas other studies found no difference in milk composition (Swartz et al., 1994; Robinson et al., 1999). Milk yield and milk components are influenced by dry matter intake. In a meta-analysis of studies employing supplemental yeast products, milk yield was significantly improved with an increase in dry matter intake; however, an increase in dry matter intake also resulted in more intake of the yeast (Desnoyers et al., 2009). Thus, a conclusion that yeast supplementation increases milk yield is confounded because it is not possible to determine if the increase in milk yield was a result of increased dry matter intake, a physiological response to yeast supplementation, or a combination of both (Desnoyers et al., 2009).

Improved dry matter intake has been reported in previous studies when dairy cattle were supplemented with yeast culture (Wohlt et al., 1991; Dan et al., 2000); yet other studies found no effect on dry matter intake (Robinson et al., 1997, 1999). Researchers reported from a meta-analysis that no difference in dry matter intake was observed; however, organic matter digestibility was improved with yeast supplementation (Desnoyers et al., 2009). Another meta-analysis using 61 research papers reported an increase in dry matter intake (0.62 kg/d) when cows were supplemented with yeast culture during early lactation (Poppy et al., 2012).

Negative Energy Balance in Dairy Cattle

Dry matter intake is critical during the transition period in dairy cattle. The transition from late gestation to early lactation is a time of drastic physiological and metabolic adaptations for dairy cows (Reynolds et al., 2003). The nutritional demands of the growing fetus and the placenta increase during late gestation while dry matter intake is reduced and is associated with endocrine changes that result in parturition and other factors influencing intake (Ingvarsten and Andersen, 2000). After parturition, increased nutrient demand for synthesis of milk and hypertrophy of visceral tissues outpaces an increase in dry matter intake (Bell, 1995). The most severe nutritional imbalances typically occur during the transition period of dairy cows (Grummer, 1995). Therefore, during early lactation, cows are unable to compensate for the increased energy demands by consuming adequate feed and thus enter a NEB (Leroy et al., 2012).

To compensate for the energy deficit, cows mobilize body fat and to a lesser extent muscle protein (Van Knegsel et al., 2005). In a ruminant animal that is not in a state of NEB, acetate and butyrate are oxidized to acetyl-CoA and propionate is oxidized to oxaloacetate (Van Knegsel et al., 2005). Oxaloacetate and acetyl-CoA form citrate in a molecular ratio of 1:1; citrate proceeds through the Krebs cycle to generate ATP, NADH, and $FADH_2$ (Van Knegsel et al., 2005). Energy is then made for the body, in the form of ATP, through NADH and $FADH_2$ reacting with oxygen in the respiratory chain reaction (Van Knegsel et al., 2005).

Body fat mobilization results in elevated free fatty acid (FFA) concentrations in the blood, which can be oxidized to acetyl-CoA or stored in the liver as tri-acyl glycerol (Van Knegsel et al., 2005). Demand for milk synthesis in early lactation requires increased lactose production resulting in decreased glucose and insulin concentrations (Bell, 1995). Therefore, during early lactation, production of acetyl-CoA from acetate, butyrate, and body fat reserves is increased while propionate and glucogenic precursors are partitioned toward lactose production causing the ratio of oxaloacetate to acetyl-CoA to be out of balance (Van Knegsel et al., 2005). Because the ratio of oxaloacetate to acetyl-CoA is out of balance, the availability of citrate to form ATP in the Krebs cycle is decreased, and acetyl-CoA is diverted into making ketone bodies such as acetone, acetoacetate and β-hydroxybutyrate (BHB; Van Knegsel et al., 2005). Although tissue mobilization is crucial to maintain lactogenesis during the energy deficit, excessive loss of body condition during the transition period increases the risk of health and reproductive disorders (Roche et al., 2007).

Because major changes occur in energy partitioning in different body tissues during the transition period, a conflict of energy partitioning occurs between the uterus, mammary gland, and other organs (Leroy et al., 2012). Britt (1992) first hypothesized that the developmental competence of oocytes of the follicle is impacted by their environment during the long period of follicular growth before eventual fates of ovulation or atresia. Primary follicles developing during negative energy balance may be less capable of steroidogenesis once they become selected as the dominant follicle (Britt, 1992; Roth et al., 2001). Other researchers reported that morphological oocyte quality in dairy cows was greater before 30 days in milk (DIM) compared with three to four months later supporting Britt's hypothesis (Kendrick et al., 1999; Gwazdauskas et al., 2000). Unfortunately, follicles bearing potentially less viable oocytes that develop during negative energy balance also ovulate at 50 to 80 DIM coinciding with the time of first insemination (Britt, 1992; Roth et al., 2001).

Negative energy balance can impede the process of follicular growth at different points in the hypothalamic-pituitary-ovarian axis (Webb et al., 1999; Wathes et al., 2007). An increase in growth hormone concentrations, a decrease in concentrations of insulin and insulin-like growth factor (IGF-1), and a reduction in leptin concentrations are associated with final follicular growth and ovulation (Leroy et al., 2012). A possible direct interaction between energy status and follicular growth is implicated by research that demonstrated the presence of insulin receptor protein in granulosa cells of bovine follicles (Bossaert et al., 2010). Associations between concentrations of insulin and LH pulsatility have been reported (Webb et al., 1999; Lucy, 2003). Hypoglycemia is associated with reduced GnRH secretion by the hypothalamus, and consequently, reduced LH pulsatility (Leroy et al., 2012). A reduction in steroidogenesis in response to a lack of LH pulse prolonged anestrus in suckled beef cows (Stagg et al., 1998). In addition, energy balance impacts the intrafollicular bioavailability of leptin, growth hormone, IGF-1, and insulin (Comin et al., 2002). These hormones are important in the role of oocyte maturation (Leroy et al., 2008).

Few studies have examined the effects of NEB associated with hypoglycemia, BHB, or FFA concentrations in dairy cows on the composition of follicular fluid and oocyte quality (Leroy et al., 2012). Hypoglycemia is reflected in the micro-environment of the preovulatory oocyte and can compromise the oocyte's developmental capacity (Leroy et al., 2006). Glucose is essential for expansion of the cumulus cells as well as normal oocyte maturation (Bilodeau-Goeseels, 2006; Sutton-McDowall et al., 2010). Kruip and Kemp (1999) were the first to suggest that FFA had a direct toxic effect at the ovarian level. Granulosa cell steroidogenic capacity and viability are reduced when incubated with elevated concentrations of FFA (Vanholder et al., 2005). Incubation of oocytes with FFA in vitro reduced risks of maturation, fertilization, cleavage, blastocyst formation, as well as increased apoptosis and cumulus cell necrosis (Leroy et al., 2005; Aardema et al., 2011). Oocyte maturation in the presence of increased FFA concentrations had adverse carryover effects on blastocyst quality in terms of embryo energy metabolism, amino acid turnover, and gene expression patterns without altering any embryo culture conditions (Shehab-El-Deen et al., 2009; Van Hoeck et al., 2011). Optimal mitochondrial function is essential for embryo development and implantation (Wakefield et al., 2011) and an increase in FFA concentration during final bovine oocyte maturation compromised mitochondrial function (Van Hoeck et al., 2011). A disruption in mitochondrial activity has been associated with suboptimal fetal and placental development (Wakefield et al., 2011).

Negative energy balance is detrimental to reproductive success (Wathes et al., 2007). Decreased reproductive performance related to delayed luteal activity is associated with increased early postpartum BHB concentrations (Wathes et al., 2007). Cows with excessive NEB had decreased pregnancy risk during 70 days after the voluntary waiting period (Ospina et al., 2010). An increase in both concentrations of FFA and BHB reduced the risk of conception; however, an increase in FFA concentrations has a stronger association with reduced reproductive performance compared with BHB (Ospina et al., 2010).

Summary

The current literature on supplementing yeast products to dairy cattle are conflicting. Some researchers report an increase in dry matter intake (Dan et al., 2000), milk production (Ramsing et al., 2009), milk components (Piva et al., 1993), and improved immune function (Shen et al., 2009), whereas others report no positive effects (Schingoethe et al., 2004; Robinson et al., 1997). The transition period is a critical time for dairy cattle. The transition from gestation to lactation induces many physiological and metabolic adaptations in dairy cattle (Reynolds et al., 2003). This period is marked by decreased dry matter intake resulting in a NEB (Grummer, 1995). Negative energy balance decreases the odds for reproductive success because of delayed resumption of estrous cycles, increased metabolic stress resulting in less viable and healthy oocytes, and decreased pregnancy risk (Leroy et al., 2012). Few studies have examined the effects of *S. cerevisiae* yeast culture products on reproductive functions. A previous study in Ghezel ewes demonstrated that diets supplemented with *S. cerevisiae* yeast during the breeding season improved pregnancy risk (Ahmadzadeh et al., 2018). If *S. cerevisiae* yeast increases dry matter intake in dairy cattle, it could decrease the severity of NEB and therefore increase reproductive success. Research is needed to determine if *S. cerevisiae* can improve measures of reproductive function in lactating dairy cattle.

References

AAFCO. 2011. Official Publication. Association of the American Feed Control Officials, Oxford, IN.

Aardema, H., P. L. A. M. Vos, F. Lolicato, B. A. J. Roelen, H. M. Knijn, A. B. Vaandrager, J. B. Helms, and B. M. Gadella. 2011. Oleic acid prevents detrimental effects of saturated fatty acids on bovine oocyte developmental competence. Biol. Reprod. 85:62-69.

Ahmadzadeh, L., A. Hosseninkhani, and H. Daghigh Kia. 2018. Effect of supplementing a diet with monensin sodium and *Saccharomyces cerevisiae* on reproductive performance of Ghezel ewes. Anim. Reprod. Sci. 188:93-100.

Arambel, M. J., and R. S. Tung. 1987. Evaluation of *Saccharomyces cerevisiae* growth in the rumen ecosystem. Proc. 19th Bienn. Conf. Rumen Function, Chicago, p. 29 (Abstr.).

Beam, S. W., and W. R. Butler. 1997. Effects of energy balance on follicular development and first ovulation postpartum dairy cows. J. Reprod. Fertil. 54:411-424.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bilodeau-Goeseels, S. 2006. Effect of culture media and energy source on the inhibition of nuclear maturation in bovine oocytes. Theriogenology 66:297-306.

Bossaert, P., H. De Cock, J. L. M. R. Leroy, S. De Campeneere, P. E. J. Bols, M. Filliers, and G. Opsomer. 2010. Immunohistochemical visualization of insulin receptors in formalin-fixed bovine ovaries post mortem and in granulosa cells collected in vivo. Theriogenology 73:1210-1219.

Britt, J. H. 1992. Impacts of early postpartum metabolism on follicular development and fertility. Pp 39-43 in Proc. Annu. Convention Am. Assoc. Bovine Pract., pp 39-43.

Callaway, E. S., and S. A. Martin. 1997. Effects of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

Carro, M. D., P. Lebzien, and K. Rohr. 1992. Influence of yeast culture on the in vitro fermentation (Rusitec) of diets containing variable portions of concentrates. Anim. Feed Sci. Tech. 37:209-220.

Chaucheyras, F., G. Fonty, P. Gouet, G. Bertin, and J. M. Salmon. 1996. Effects of a strain of *Saccharomyces cerevisiae* (Levucell SC), a microbial additive for ruminants, on lactate metabolism in vitro. Can. J. Microbiol. 42:927-933.

Comin, A., D. Gerin, A. Cappa, V. Marchi, R. Renaville, M. Motta, U. Fazzini, and A. Prandi. 2002. The effect of an acute energy deficit on the hormone profile of dominant follicles in dairy cows. Theriogenology 58:899-910.

Dann, H. M., J. K. Drackley, G. C. McCoy, M. F. Hutjens, and J. E. Garrett. 2000. Effects of yeast culture (*Saccharomyces cerevisiae* on prepartum intake and postpartum intake and milk production of Jersey cows. J. Dairy Sci. 83:123-127.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Ponter, and D. Sauvant. 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 92:1620-1632.

El Hassan, S. M., C. J. Newbold, and R. J. Wallace. 1993. The effect of yeast in the rumen and the requirement for viable yeast cells. Anim. Prod. 54:504 (Abstr.).

Erasmus, L. J., P. M. Botha, and A. Kistner. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056-3065.

Finck, D. N., F. R. B. Ribeiro, PAS, N. C. Burdick, S. L. Parr, J. A. Carroll, T. R. Young, B. C. Berhard, J. R. Corley, A. G. Estefan, R. J. Rathmann, and B. J. Johnson. 2014. Yeast supplementation alters the performance and health status of receiving cattle. Prof. Anim. Sci. 30:333-341.

Francisco, C. C., L. J. Spicer, and M. E. Payton. 2003. Predicting cholesterol, progesterone, and days to ovulation using postpartum metabolic and endocrine measures. J. Dairy Sci. 86:2852-2863.

Gao, J., H. J. Zhang, S. H. Yu, S. G. Wu, I. Yoon, J. Quigley, Y. P. Gao, and G. H. Qi. 2008. Effects of yeast culture in broiler diets on performance and immunomodulation functions. Poult. Sci. 87:1377-1384.

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

Guedes, C. M., D. Gonçalves, M. A. M. Rodrigues, and A. Dias-da-Silva. 2008. Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fiber degradation of maize silages in cows. Anim. Feed Sci. Tech. 145:27-40.

Gwazdauskas, F. C., K. W. Kendrick, A. W. Pryor, and T. L. Bailey. 2000. Impact of follicular aspiration on folliculogenesis as influenced by dietary energy and stage of lactation. J. Dairy Sci. 83:1625-1634.

Harrison, G. A., R. W. Hemken, K. A. Dawson, R. J. Harmon, and K. B. Barker. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci. 71:2967-2975.

Hippen, A. R., D. J. Schingoethe, K. F. Kalscheur, P. Linke, K. Gross, D. Rennich, and I. Yoon. 2007. Interactions of yeast culture and dried distillers grains plus solubles in diets of lactating dairy cows. J. Dairy Sci. 90(Suppl. 1):452 (Abstr.).

Ingledew, W. M., and G. A. Jones. 1982. The fate of live brewers yeast slurry in bovine rumen fluid. J. Inst. Brewing 88:18-20.

Ingvartsen, K. L., and J. B. Andersen. 2000. Integration of metabolism and intake regulation: A review focusing on periparturient animals. J. Dairy Sci. 83:1573-1597.

Jensen, G. S., K. M. Patterson, and I. Yoon. 2008. Yeast culture has anti-inflammatory effects and specifically activates NK cells. Comp. Immunol. Microbiol. Infect. Dis. 31:487-500.

Kendrick, K. S., T. L. Bailey, A. S. Garst, A. W. Pryor, A. Ahmadzadeh, R. M. Akers, W. E. Eyestone, R. E. Pearson, and F. C. Gwazdauskas. 1999. Effects of energy balance on hormones, ovarian activity, and recovered oocytes in lactating Holstein cows using transvaginal follicular aspiration. J. Dairy Sci. 82:1731-1741.

Keyser, S. A., J. P. McMeniman, D. R. Smith, J. C. MacDonald, and M. L. Galyean. 2007. Effects of *Saccharomyces cerevisiae* subspecies boulardii CNCM I-1079 on feed intake by healthy beef cattle treated with florfenicol and on health and performance of newly received beef heifers. J. Anim. Sci. 85:1264-1273.

Kruip, T. A. M., and B Kemp. 1999. Voeding en vruchtbaarheid bij landbouwhuisdieren. Tijdschr. Diergeneeskd. 124:462–464.

Lehloenya, K. V., D. R. Stein, D. T. Allen, G. E. Selk, D. A. Jones, M. M. Aleman, T. G. Rehberger, K. J. Mertz, and L. J. Spicer. 2007. Effects of feeding yeast and propionibacteria to dairy cows on milk yield and components, and reproduction. J. Anim. Physiol. Anim. Nutr. (Berl.) 92:190-202.

Leroy, J. L. M. R., D. Rizos, R. Sturmey, P. Bossaert, A. Gutierrez-Adan, V. Van Hoeck, S. Valckx, and P. E. J. Bols. 2012. Intrafollicular conditions as a major link between maternal metabolism and oocyte quality: a focus on dairy cow fertility. Reprod. Fertil. Dev. 24:1-12.

Leroy, J. L. M. R., T. Vanholder, B. Mateusen, A. Christophe, G. Opsomer, A. de Kruif, G. Genicot, and A. Van Soom. 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. Reproduction 130:485-495.

Leroy, J. L. M. R., T. Vanholder, G. Opsomer, A. Van Soom, and A. de Kruif. 2006. The in vitro development of bovine oocytes after maturation in glucose and beta-hydroxybutyrate concentrations associated with negative energy balance in dairy cows. Reprod. Domest. Anim. 41:119-123.

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

Loesche, W. J. 1969. Oxygen sensitivity of various anaerobic bacteria. Appl. Microbiol. 18:723-727.

Lucy, M. C. 2003. Mechanisms linking nutrition and reproduction in postpartum cows. Reprod. Suppl. 61:415-427.

Magalhāes, V. J. A., F. Susca, F. S. Lima, A. F. Branco, I. Yoon, and J. E. P. Santos. 2008. Effect of feeding yeast culture on performance, health, and immunocompetence of dairy calves. J. Dairy Sci. 91:1497-1509.

Marounek, M., and R. J. Wallace. 1984. Influence of culture Eh on the growth and metabolism of the rumen bacteria Selenomonas ruminantium, Bacteroides amylophilus, Bacteroides succinogenes, and Streptococcus bovis in batch culture. Microbiol. 130:223-229.

McArthur, J. M., and J. E. Miltimore. 1961. Rumen gas analysis by gas solid chromatography. Canadian J. Anim. Sci. 41:187-192.

Newbold, C. J., R. J. Wallace, and F. M. Mcintosh. 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. Brit. J. Nutr. 76:249-261.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93:1596-1603.

Piva, G., S. Belladonna, G. Fusconi, and F. Sicbaldi. 1993. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. J. Dairy Sci. 76:2717-2722.

Poppy, G. D., A. R. Rabiee, I. J. Lean, W. K. Sanchez, K. L. Dorton, and P. S. Morley. 2012. A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of *Saccharomyces cerevisiae* on milk production of lactating dairy cows. J. Dairy Sci. 95:6027-6041.

Ramsing, E. M., J. A. Davidson, P. D. French, I. Yoon, M. Keller, and H. Peters-Fleckenstein. 2009. Effects of yeast culture on peripartum intake and milk production of primiparous and multiparous Holstein cows. Prof. Anim. Sci. 25:487-495.

Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86:1201-1217.

Robinson, P. H. 1997. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to diets postpartum. J. Dairy Sci. 80:1119-1125.

Robinson, P. H., and J. E. Garrett. 1999. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to postpartum diets and on lactational performance. J. Anim. Sci. 77:988-999.

Roche, J. R., K. A. Macdonald, C. R. Burke, J. M Lee, and D. P. Berry. 2007. Associations among body condition score, body weight and reproductive performance in seasonal-calving dairy cattle. J. Dairy Sci. 90:376-391.

Roger, V., G. Fonty, S. Komisarczuk-Bony, and P. Gouet. 1990. Effects of physiochemical factors on the adhesion to cellulose Avicel of the rumen bacteria Ruminicoccus flavefaciens and Fibrobacter succinogenes subsp. Succinogenes. Appl. Environ. Microbiol. 56:3081-3087.

Rose, A. H. 1987. Yeast culture, a micro-organism for all species: a theoretical look at its mode of action. Biotechnology in the Feed industry, pp 113-118. [T. P. Lyons, editor]. Nicholasville, Kentucky: Alltech Tech. Pub.

Roth, A., A. Arav, A. Zeron, Y. Bra-Tal, and D. Wolfensohn. 2001. Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. Reproduction 122:737-744.

Royal, M., G. E. Mann, A. P. F. Flint. 2000. Strategies for reversing the trends toward subfertility in dairy cattle. Vet. J. 160:53-60.

Schingoethe, D. J., K. N. Linke, K. F. Kalscheur, A. R. Hippen, D. R. Rennich, and I. Yoon. 2004. Feed efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci. 87:4178-4181.

Shehab-El-Deen, M. A., J. L. M. R. Leroy, D. Maes, and A. Van Soom. 2009. Cryotolerance of bovine blastocyst is affected by oocyte maturation in media containing palmitic or stearic acid. Reprod. Domest. Anim. 44:140-142.

Shen, Y. B., X. S. Piao, S. W. Kim, L Wang, P. Liu, I. Yoon, and Y. G. Zhen. 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J. Anim. Sci. 87:2614-2624.

Stagg, K., L. J. Spicer, J. M. Sreenan, J. F. Roche, and M. G. Diskin. 1998. Effect of calf isolation on follicular wave dynamics, gonadotrophin and metabolic hormone changes, and interval to first ovulation in beef cows fed either of two energy levels postpartum. Biol. Reprod. 59:777-783.

Sutton-McDowall, M., R. Gilchrist, and J. G. Thompson. 2010. The pivotal role of glucose metabolism in determining oocyte developmental competence. Reprod. 139:685-695.

Swartz, D. L., L. D. Muller, G. W. Rogers, and G. A. Varga. 1994. Effect of yeast cultures on performance of lactating dairy cows: a field study. J. Dairy Sci. 77:3073-3080.

Van Hoeck, V., R. G. Sturmey, P. Bermejo-Alvarez, D. Rizos, A. Gutierrez-Adan, H. J. Leese, P. E. J. Bols, and J. L. M. R. Leroy. 2011. Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. PLoS ONE 6:823183.

Vanholder, T., J. L. M. R. Leroy, A. Van Soom, G. Opsomer, D. Maes, M. Coryn, and A. de Kruif. 2005. Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. Anim. Reprod. Sci. 87:33-44.

Van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, S. Tamminga, and B. Kemp. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders, and reproduction in lactating dairy cattle. Reprod. Nutr. Dev. 45:665-688.

Volman, J. J., J. D. Ramakers, and J. Plat. 2008. Dietary modulation of immune function by β-glucans. Physiol. Behav. 94:276-284.

Wakefield, S. L., M. Lane, and M. Mitchell. 2011. Impaired mitochondrial function in the preimplantation embryo perturbs fetal and placental development in the mouse. Biol. Reprod. 84:572-580.

Wang, Z., M. L. Eastridge, and X. Qiu. 2001. Effects of forage neutral detergent fiber and yeast culture on performance of cows during early lactation. J. Dairy Sci. 84:204-212.

Wathes, D. C., M. Fenwick, Z. Cheng, N. Bourne, S. Llewellyn, D. F. Morris, D. Kenny, J. Murphy, and R. Fitzpatrick. 2007. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology 68:S232-S241.

Webb, R., R. G. Gosden, E. E. Telfer, and R. M. Moor. 1999. Factors affecting folliculogenesis in ruminants. Anim. Sci. 68:257-284.

Wohlt, J. E., A. D. Finkelstein, and C. H. Chung. 1991. Yeast culture to improve intake, nutrient digestibility, and performance by dairy cattle during early lactation. J. Dairy Sci. 74:1395-1400.

Zeleňák, I., D. Jalč, V. Kmet, and P. Siroka. 1994. Influence of diet and yeast supplement on in vitro ruminal characteristics. Anim. Feed Sci. Tech. 49:211-221.

Chapter 2 - Relationships of metabolites and hormones in follicular fluid and blood serum in transition dairy cows supplemented with a *Saccharomyces cerevisiae* fermentation product

ABSTRACT

Metabolites (–4, –2, +1, +2, +5, and +7 wk relative to calving) and steroid hormones in ovarian follicular fluid (FF) and blood serum (BS) were examined in Holstein cows (n = 59) individually fed *Saccharomyces cerevisiae* fermentation product from –4 through +7 wk (calving = wk 0). Blood was collected before injections of GnRH, prostaglandin $F_{2\alpha}$, and GnRH at 32 ± 3, 39 ± 3, and 42 ± 3 days in milk (DIM), respectively. Dominant follicle recovery (DFR) occurred by transvaginal aspiration at 49 ± 3 DIM. Luteal status (luteal = progesterone > 1 ng/mL) on 32 ± 3 DIM was determined. After DFR, cows received GnRH at 53 ± 3 and 60 ± 3, prostaglandin $F_{2\alpha}$ at 67 ± 3, and AI at 70 ± 3 DIM. Neither metabolite nor hormonal concentrations in BS or FF differed between treatments. Non-luteal cows had greater concentrations of beta-hydroxybutyrate (BHB; –4 and +1 wk; $P < 0.01$) and free fatty acids (FFA; +2 and +5 wk; $P < 0.05$) in BS compared with luteal cows. Non-luteal cows had greater ($P < 0.05$) BHB in BS and FF at +7 wk than luteal cows. Pregnant cows had less ($P = 0.03$) BHB in BS at +1 wk and less ($P = 0.05$) FFA in BS at +7 wk than nonpregnant cows. Concentrations in BS and FF of BHB ($r = 0.61$; $P < 0.001$) and glucose ($r = 0.56$; $P = 0.003$) were correlated. Differences in BHB and FFA in BS were predictive of luteal and pregnancy status.

Metabolites, Steroids, Fertility

1. Introduction

Reproductive success is critical to survivability in a dairy herd. Failure to produce a calf at regular intervals decreases profitability (Royal et al., 2000). Milk production combined with reduced dry matter intake (DMI) causes dairy cows to experience a period of negative energy balance (NEB) and increased metabolic stress in early lactation that is associated with decreased fertility (Leroy et al., 2012). Energy balance was found to be the most influential factor in a model that predicted days to first ovulation (Francisco et al., 2003) partly because is associated with the LH pulse frequency (Canfield and Butler, 1990). Physiological adaptations that occur in lactating dairy cows to cope with NEB include increased concentrations of free fatty acids (FFA), hypoglycemia, or ketonemia (Beam and Butler, 1997). The metabolic stress associated with NEB is a contributing factor to decreased ovarian follicle and oocyte development, resulting in subfertility (Britt, 1994; Dupont et al., 2014).

The intrafollicular environment in which a preovulatory oocyte develops may impact reproductive success (Leroy et al., 2005). Oocytes exposed to metabolic changes early postpartum do not ovulate until 60 to 80 days later, which coincides with the time of first insemination (Britt, 1994). Follicular fluid originates from thecal capillaries and crosses the thecal interstitium, follicular basal lamina, and the mural granulosa (Rodgers and Irving-Rodgers, 2010). Fluid accumulates in the antrum of the follicle as development advances and submerges the oocyte providing the environment for oocyte maturation (Leroy et al., 2012).

Composition of follicular fluid is impacted by metabolic changes during the early postpartum period (Leroy et al., 2004). Elevated concentrations of FFA are toxic for bovine granulosa cell growth and function in vitro (Vanholder at al., 2005). In vitro maturation of oocytes exposed to long-chain fatty acids demonstrated reduced rates of maturation, fertilization, cleavage, and blastocyst formation (Leroy et al., 2012).

The yeast *Saccharomyces cerevisiae* is a commonly used direct-fed microbial (Dann et al., 2000). Improvements in dry matter intake (DMI) and milk production have been reported when cows were supplemented with yeast culture in some (Wohlt et al., 1991, 1998), but not all studies (Robinson et al., 1997; 1999). Responses of dairy cows to supplemental yeast culture depend on stage of lactation, type of forage fed, feeding strategy, and forage-to-concentrate ratio (Piva et al., 1993).

Although understanding the micro-environment of a follicle can explain some subfertility problems, the collection of follicular fluid is an invasive and expensive process. Changes in BHB, glucose, and urea were similar in blood serum and follicular fluid (Leroy et al., 2004) and FFA concentrations may be up to 60% lower in follicular fluid when compared with blood serum (Leroy et al., 2005).

The hypothesis of the present study is supplementation with a *Saccharomyces cerevisiae* fermentation product (SCFP) will improve reproductive outcomes by improving steroid hormone concentrations and decrease metabolites associated with negative nutrient balance in blood serum and follicular fluid. Therefore, the primary objective of the present study was to investigate the relationships of metabolites and steroid hormones in follicular fluid and blood serum in transition dairy cows supplemented with a SCFP. Secondary objectives were to: (1) evaluate the metabolic and hormonal profile of cows that have early postpartum luteal function and conceive at first AI, and 2) investigate further the correlation between follicular fluid and blood serum of metabolite and steroid hormone concentrations.

2. Material and methods

An experiment at the Kansas State University Dairy Research and Teaching Center was conducted under Kansas State University Institutional Animal Care and Use Committee protocol 3759.1. Sixty-four Holstein cows (50 multiparous, 14 primiparous) were used in a completely randomized block design. Cows were blocked by parity, expected calving date, and previous 305ME yield, and then assigned randomly to treatment within block (Olagaray et al., 2018). Treatments were either control or supplementation with a *Saccharomyces cerevisae* fermentation product (NutriTek, Diamond V Mills, Inc., Cedar Rapids, IA) at a rate of 18 g/d, fed from −29 ± 5 to +42 ± 5 days relative to calving. Prepartum cows (n = 64) were fed treatment diets using an electronically gated feeding system (Roughage Intake System; Insentec B. V., Marknesse, The Netherlands). All cows receiving a given treatment diet were allowed access to 4 feed bins assigned to that treatment, and no more than 6 cows shared the 4 bins at any given time. After calving, cows were housed in a tie-stall facility and fed individually. Both feeding systems recorded individual feed consumption and meal patterns. Cows were fed thrice daily prepartum. Postpartum cows were fed and milked twice daily. Cows were fed a TMR calculated to meet nutritional requirements of a gestating cow, or a lactating dairy cow producing 50 kg of 3.5% milk, respectively (NRC, 2001).

2.1. Dominant follicle recovery (DFR)

Of the 64 cows, only 47 cows were enrolled in a reproductive protocol (Figure 2-1). Cows (n = 47) remained on dietary treatments until dominant follicle recovery (DFR) at 49 ± 3 DIM. Beginning at 32 ± 3 DIM, cows were subjected to an ovulation-synchronization program. Briefly, cows were injected with GnRH (Factrel; Zoetis, Kalamazoo, MI, USA), prostaglandin $F_{2\alpha}$ (Lutalyse HighCon; Zoetis, Kalamazoo, MI, USA), and GnRH at 32 ± 3, 39 ± 3, and 42 ± 3

DIM, respectively. Ovarian structures assessed by transrectal ultrasonography (7.5 MHz linear-array transducer, Ibex EVO; E.I. Medical Imaging, CO) were recorded using electronic calipers at 32, 39, and 42 ± 3 DIM for the assessment of dominant follicle size and days to detection of the first CL and progesterone concentration > 1 ng/mL.

At 49 ± 3 DIM, dominant follicles > 8 mm in diameter were subjected to ultrasound-guided transvaginal aspiration. The rectum of the cow was emptied, and perineum and external genitalia were cleaned carefully. An ovum pickup device equipped with a 5.0-MHz mechanical multi-angle probe transducer and a needle guidance system was inserted vaginally. Both ovaries were visualized by ultrasound through transrectal manipulation. Before aspiration, dominant follicle size was estimated and presence of a corpus luteum (CL) was recorded. The dominant follicle was punctured, and follicular fluid was recovered. The aspiration needle was attached by means of a stainless-steel connector to silicon tubing. An aspiration pump was used to recover follicular fluid. Follicular fluid from the largest follicle with a diameter > 8 mm was recovered. The collected follicular fluid was cooled immediately in an ice bath. Follicular fluid samples were centrifuged within 10 min after collection at 10,000 *x g* for 15 min. The supernatant was snap-frozen in liquid nitrogen and stored for later analysis.

2.2. Breeding protocol

After DFR, cows were moved to free stall pens and enrolled in an ovulation-synchronization program. Briefly, cows received injections of GnRH at 53 ± 3 and 60 ± 3 days in milk (DIM) and an injection of prostaglandin $F_{2\alpha}$ at 67 ± 3 DIM. An injection of GnRH was administered 56 h after prostaglandin $F_{2\alpha}$, and artificial insemination was performed approximately 16 h later.

2.3. Measurements

Blood samples were collected at −4 and −2 wk from expected parturition date in addition to +1, +2, +5, and +7 wk postpartum to determine concentrations of free fatty acids (FFA) and beta-hydroxybutyrate (BHB) in blood. At 32 ± 3 DIM, a blood sample was collected to determine concentrations of progesterone. Blood samples were collected concurrently with DFR at 49 ± 3 DIM to measure FFA, BHB, glucose, progesterone, androstenedione, and estradiol. Blood samples were placed on ice and transported to the laboratory. Samples were separated by centrifugation (1,500 *x g* for 15 min) and stored in microcentrifuge tubes at −20°C until analyses. Samples were analyzed for FFA (NEFA-HR; Wako Chemicals USA INC., Richmond, VA), BHB (kit #H7587-58; Pointe Scientific Inc., Canton, MI), and glucose (kit #439-90901; Wako Chemicals USA Inc.) by enzymatic assays.

Concentrations of progesterone in blood sera were measured in one assay by direct quantitative (nonextracted) RIA using ImmuChem Double Antibody progesterone 125I kits (MP Biomedicals LLC, Orangeburg, NY) previously validated for bovine serum (Hill et al., 2016). Intra-assay coefficients of variation for a low (1.28 ± 0.07 ng/mL) and high (17.2 ± 1.3 ng/mL) concentration pool were 4.93 and 5.34%, respectively. Calculated assay sensitivity averaged 2 ± 0.5 pg/mL, and progesterone standard concentrations in the assay were 0.05, 0.1, 0.2, 0.5, 2.0, 5.0, 10.0, and 25.0 ng/mL.

Concentrations of estradiol-17β were measured in blood sera and follicular fluid samples in one RIA previously validated for bovine serum (Stevenson, 2011). Follicular fluid samples were diluted 1:1,000 or 1:10,000 in assay buffer before extraction with methyl t-butyl ether. Standard concentrations of estradiol in the assay were 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, and 10.0 pg/mL. Recovery of added estradiol in triplicate to 100 µL of 3 different follicular fluid samples (4.8, 7.7, and 17.8 pg/mL) averaged 101.5%. Parallelism was demonstrated by assaying 50-, 100- and 200-µL aliquots of diluted follicular fluid. Assay sensitivity was 0.25 ± 0.03 pg/mL. Intra-assay coefficient of variation averaged 11.2%.

Concentrations of androstenedione were measured by quantitative extracted RIA using ImmuChem Double Antibody androstenedione 125I kits (MP Biomedicals LLC, Orangeburg, NY). The radioligand was ^{125}I-labeled androstenedione (1,500 to 2,000 µCi/µg). The anti-androstenedione antibody was generated in rabbits using 4-androstene-3, 17-dione-13-carboxy-methylene-BSA. Kit standard concentrations of androstenedione were 0.1, 0.25, 0.50, 1.0, 2.5, 5.0, and 10.0 ng/mL. Samples (0.3 mL) were extracted by using 6 mL of ethyl acetate, frozen, decanted into 16- by 100-mm glass tubes, and dried under air. The dried residue was reconstituted in 2.5 mL assay diluent and incubated for a minimum of 30 min at room temperature. After incubation, 0.5 mL was aliquoted into 12- by 75-mm plastic conical-bottom tubes in duplicate. Recovery of androstenedione added in triplicate to 0.50 mL of three different bovine serum and follicular fluid samples (1.88, 3.75, and 7.50 ng/mL) averaged 96.3 and 90.8%. Parallelism was demonstrated by assaying selected samples at 1:2 and 1:4. Assay sensitivity was 0.56 ± 0.05 ng/mL. Intra-assay coefficient of variation averaged 5.24%.

2.4. Statistical analyses

Of the 47 cows enrolled, follicular fluid was analyzed from only 38 cows because of inappropriate sample storage. Blood serum was analyzed from 47 cows. To assess the primary objective all continuous variables (concentrations of metabolites, and concentrations of steroid hormones, ratio of estradiol to androstenedione, and days to progesterone > 1 ng/mL) were analyzed using the procedure MIXED in SAS (SAS studio, SAS Inst. Inc., Cary, NC, USA). The initial model included the fixed effects of treatment (control vs. SCFP), parity (primiparous vs.

multiparous), and the interaction of treatment with parity, in addition to the random effect of parity nested within block. The option DDFM= RESIDUAL was included in the MODEL statement to adjust the degrees of freedom. All binomial variables (luteal status at 32 ± 3 DIM and pregnancy per AI) were analyzed using procedure GLIMMIX in SAS. Options used in the model statement included LINK = LOGIT, DIST = BINOMIAL, and the least square means option of ILINK and DIFF.

To evaluate the metabolic and steroid hormone concentrations of cows according to luteal or pregnancy status, cows were retrospectively categorized by progesterone concentration (≥ 1 ng/mL) at 32 ± 3 DIM (luteal vs. non-luteal), and by first service pregnancy status (pregnant vs. nonpregnant). The data were analyzed using procedure MIXED in SAS. The model included the fixed effects of pregnancy status (pregnant vs. not pregnant), luteal status (luteal vs. non-luteal), parity, and the interaction of parity with luteal or pregnancy status. Options used in the model statement included DDFM=KR, SOLUTION, and the least square means option of DIFF. The option REPEATED = week was included in the model statement when analyzing FFA and BHB.

To assess partial correlation of metabolites (FFA, BHB, and glucose) and steroid hormones (progesterone, androstenedione, and estradiol) in follicular fluid and blood serum data were analyzed using the MANOVA option in procedure GLM in SAS. The GLM model included the fixed effects of pregnancy status, luteal status, treatment, and parity.

3. Results and Discussion

3.1. Impact of yeast (SCFP) on metabolites, steroid hormones, and reproductive outcomes

The portion of luteal cows was not affected (*P* = 0.86) by treatment with SCFP (Table 2-1). Mean days in milk to the first CL were 37.4 and 40.7 days for control and SCFP, respectively, and did not differ ($P = 0.33$) between treatments (Table 2-1). Pregnancy per AI at first service was 44 and 50% for SCFP and control, respectively (Table 2-1). Metabolite concentrations did not differ between SCFP and control for FFA, glucose, or BHB in follicular fluid and blood serum (Table 2-2). Concentrations of progesterone and androstenedione in blood serum tended ($P < 0.10$) to be greater in SCFP than control cows (Table 2-3). Previous researchers reported ewes supplemented with *S. cerevisiae* had increased blood serum concentrations of estradiol, progesterone, blood urea nitrogen, insulin, glucose, cholesterol, and total protein compared with non-supplemented ewes (Ahmadzadeh et al., 2018). In the current study, androstenedione tended ($P = 0.10$) to be less in follicular fluid of SCFP than control cows. Treatment did not affect concentrations of progesterone in follicular fluid or estradiol concentrations in follicular fluid or in serum (Table 2-3). In addition, the ratio of estradiol to androstenedione was not different in blood serum ($P = 0.79$) or follicular fluid ($P = 0.96$) when SCFP cows were compared with controls (Table 2-3).

The present experiment was designed to determine if supplementing SCFP during the transition period could alter traits associated with follicular health and early ovulation. The transition from late gestation to early lactation is one of the most challenging stages in the production cycle (Lehloenya et al., 2007). The changes necessary for parturition and lactogenesis inflict extreme metabolic and physiologic changes that often disrupt the homeostatic mechanisms of the cow as the liver adapts from a minimal glucose demand to an astounding demand for glucose (DeFrain et al., 2005). During early lactation, many dairy cows undergo a period of negative energy balance because energy output in milk exceeds energy input from feed consumption (Spicer et al., 1990). By 4 days after parturition, the demand for glucose, amino acids, and fatty acids for milk synthesis is several-fold greater than those of the gravid uterus

(Bell, 1995). To compensate for the increase in nutrient demand, rates of hepatic gluconeogenesis, adipose lipolysis and muscle proteolysis mobilization are greatly accelerated (Bell, 1995; Reynolds et al., 2003).

Because of increased adipose triglyceride mobilization, FFA and BHB increase in blood serum (Ospina et al., 2010). The circulating metabolites FFA and BHB are commonly used indices of negative energy balance in transition cows. An increase in these metabolites is normal as the lactating dairy cow must balance energy intake and energy demands; however, excessive elevation in FFA and BHB indicate poor adaptation to galactopoeisis (Herdt, 2000). In the current experiment, FFA and BHB increased from 4 wk before parturition, reached a peak 2 wk after parturition, and declined by 5 wk after parturition (Olagaray et al., 2018). In the current experiment, DMI did not differ ($P = 0.84$) between SCFP-supplemented and control cows (Olagaray et al., 2018). Supplementation with SCFP also did not impact measures of negative energy balance [FFA ($P = 0.36$), BHB ($P = 0.57$), or glucose ($P = 0.60$)] in blood serum in supplemented cows compared with controls (Olagaray et al., 2018).

Reproductive performance and postpartum ovarian activity have been closely related to energy balance (Beam and Butler, 1999), including a temporal association with increased energy balance and greater LH pulse frequency necessary to cause ovulation (Canfield and Butler, 1990). Mechanisms that regulate nutrient and energy distribution in the somatotropic system may impact the reproductive system at various levels of the hypothalamus-pituitary-ovarian axis (Chagas et al., 2007). The preoptic region of the hypothalamus is where releasing hormones are produced, and where interactions may occur between the gonadotropic and somatotropic system (Blache et al., 2007). Metabolic inputs in the hypothalamus may have differing impacts on the somatotropic and gonadotropic axes (Leroy et al., 2012). Decreased concentrations of glucose and insulin in the postpartum cow suppresses secretion of GnRH from the hypothalamus and LH from the pituitary (Diskin et al., 2003). An increase in LH pulse frequency is detected before first ovulation (Schams et al., 1972) and is associated with fewer days to resumption of normal cyclicity (Lamming et al., 1981).

3.2. Metabolite and steroid hormones as an indicator of luteal status

Metabolites in blood serum were compared between luteal and non-luteal cows. Luteal cows had reduced ($P < 0.01$) concentrations of FFA at −4, 1, and 2 wk relative to calving (Figure 2-2) and reduced ($P < 0.05$) concentrations of BHB at 2 and 5 wk relative to calving (Figure 2-3) compared with non-luteal status cows.

Blood serum and follicular fluid were analyzed for metabolite and steroid hormone concentrations at 49 ± 3 DIM. Concentrations of FFA in blood serum were 0.28 ± 0.04 mEq/L regardless of luteal status (Table 2-4) and were 0.27 ± 0.04 and 0.35 ± 0.05 mEq/L in follicular fluid for luteal and non-luteal cows, respectively (Table 2-5). No differences were detected in FFA concentrations in blood serum ($P = 0.97$) or follicular fluid (P = 0.17) when luteal cows were compared with non-luteal. Concentrations of glucose were 61.6 ± 1.5 and 64.2 ± 1.4 mg/dL in blood serum, and 47.8 ± 3.8 and 52.7 ± 2.8 mg/dL in follicular fluid for non-luteal and luteal cows, respectively (Table 2-4 and 2-5). Concentrations of glucose did not differ between luteal and non-luteal cows in blood serum ($P = 0.13$) or follicular fluid ($P = 0.26$). Non-luteal cows had increased BHB concentrations in blood serum ($P = 0.04$; Table 2-4) and in follicular fluid ($P = 0.04$; Table 2-5) compared with luteal cows. Progesterone concentrations in blood serum and follicular fluid were not different by luteal status. Concentrations of androstenedione in blood serum tended ($P = 0.09$) to be greater in luteal cows (Table 2-4) but did not differ ($P = 0.66$) in follicular fluid (Table 2-5). Estradiol concentrations were 2.9 ± 0.7 pg/mL and 2.0 ± 0.5 in blood serum (Table 2-4), and 436 ± 102 and 580 ± 76 ng/mL in follicular fluid (Table 2-5) for non-luteal and luteal cows, respectively. The estradiol to androstenedione ratio in blood serum tended ($P = 0.06$) to be reduced in luteal cows (Table 2-4), but did not differ ($P = 0.93$) in follicular fluid (Table 2-5).

In the current study, luteal cows had decreased BHB in blood serum and follicular fluid at 49 ± 3 DIM relative to non-luteal cows. Previous research has demonstrated that elevated BHB in blood serum is reflected in the follicular fluid (Leroy et al., 2004). Elevated concentrations of BHB generally reflect hypoglycemic conditions (Ospina et al., 2010). Because BHB is found in varying concentrations in follicular fluid, hypoglycemic conditions impact the microenvironment of the oocyte. During lactogenesis, the demand for glucose increases exponentially. The mammary gland uses 60 to 85% of the glucose available to ruminants, with 50 to 85% of glucose available to the mammary gland used for lactose synthesis (Knowlton et al., 1998). Glucose is also essential for proper oocyte maturation and expansion of surrounding cumulus cells (Sutton-McDowall et al., 2010). Decreased concentrations of glucose in follicular fluid caused decreased oocyte competence (Leroy et al., 2006). In addition, non-luteal cows had increased concentrations of FFA in blood serum at −4, +1 and +2 wk relative to parturition. Previous research has shown that increased concentrations of FFA during negative energy balance are reflected in follicular fluid (Vanholder et al., 2005). In *in vivo* maturation models, addition of long-chain fatty acids reduced maturation, fertilization, and embryo development (Aardema et al., 2011).

Non-luteal cows tended to have decreased androstenedione concentrations. Ovarian androgen synthesis is dependent on the expression of CYP11A1, CYP17A1, and HSD3β (Conley et al., 1997). Others have speculated that there may be an increase in protein abundance and/or enzyme activity to promote an accumulation of androstenedione in the follicular fluid (Summers et al., 2014). Luteinizing hormone and FSH mediate the regulation of steroidogenic enzyme expression. Cows with greater androstenedione also had greater mRNA abundance for the LH and chorionic gonadotropin receptor, indicating a change in LH pulse frequency or sensitivity to LH that may contribute to excess androgen synthesis by the follicle (Summers et al., 2014). In addition, previous researchers suggested that cows with increased androstenedione may promote partial luteinization of granulosa cells because of altered steroid production (Summers et al., 2014). Furthermore, excess ovarian-derived androgens in follicular fluid may reduce oocyte quality (Carmina et al., 1999). In the current study, non-luteal cows tended to have increased estradiol to androstenedione ratios in blood serum, which contradicts previous observations in which cows with increased androstenedione in follicular fluid had reduced estradiol to androstenedione ratios (Summers et al., 2014).

3.3. Metabolites and steroid hormones as an indicator of pregnancy outcome

Metabolites (BHB and FFA) in blood serum were compared between cows that conceived and did not conceive at first insemination (Figures 2-4 and 2-5). Cows that became pregnant had decreased ($P < 0.05$) BHB concentrations at +1 and +5 weeks relative to calving compared with cows that failed to conceive (Figure 2-5). No differences were detected in concentrations of FFA in blood serum between pregnant and non-pregnant cows (Figure 2-4).

Metabolite and steroid hormone concentrations in blood serum at the time of DFR were compared between cows that conceived to first service and those that did not. Cows that conceived tended ($P = 0.08$) to have lesser FFA concentrations in blood serum (Table 2-4), but no differences ($P = 0.21$) in follicular fluid FFA were detected. In addition, no differences were detected in concentrations of glucose and BHB in blood serum (Table 2-4) or in follicular fluid

(Table 2-5) when cows that conceived to first service were compared with cows that did not. Furthermore, no differences were detected in steroid hormones in blood serum (Table 2-4) or in follicular fluid (Table 2-5) between cows that became pregnant and those that failed to conceive.

Cows with excessive negative energy balance (assessed by elevated concentrations of BHB and FFA) have decreased risk of pregnancy by 70 d after the end of the voluntary waiting period (Ospina et al., 2010). It is normal for blood concentrations of BHB to increase in response to lactation; however, BHB concentrations greater than a given threshold has been associated with poor reproductive performance, reduced milk yield, and increased risk of transition disease (McArt et al., 2013). Cows with elevated concentrations of BHB take longer to establish a viable pregnancy than their healthy counterparts (Rutherford et al., 2016). In the present experiment, cows that failed to conceive to first service had increased concentrations of BHB in blood serum at +1 and +5 weeks relative to parturition compared with cows that became pregnant.

Furthermore, non-pregnant cows in the current experiment also tended to have greater FFA concentrations in blood serum at DFR compared with cows that conceived to first service. Previous research demonstrated that maturation of oocytes *in vitro* in the presence of elevated concentrations of FFA caused negative carryover effects on blastocyst quality (Van Hoeck et al., 2011). Oocytes in mice and cattle exposed to increased FFA concentrations during final oocyte maturation have compromised mitochondrial function (Igosheva et al., 2010; Van Hoeck et al., 2011). Restricted mitochondrial activity has been related to suboptimal fetal and placental development in mice (Wakefield et al., 2008).

3.4. Relationship between Follicular Fluid and Blood Serum

Blood serum and follicular fluid collected at DFR were analyzed to determine the correlation of metabolites and steroid hormones. Concentrations of FFA in blood serum tended (P = 0.07) to be correlated with concentrations of FFA in follicular fluid (Table 2-6). Metabolites in blood serum (BHB and glucose) were correlated ($P < 0.01$) to their respective concentrations in follicular fluid (Table 2-6). Steroid hormone concentrations in blood serum were not correlated with those in follicular fluid (Table 2-6).

It has been established that metabolic changes caused by negative energy balance can impact the microenvironment of the oocyte (Leroy et al., 2012). Collecting follicular fluid is an invasive and costly process. The purpose of this analysis was to determine if metabolites and steroid hormones in blood serum correlated to those in follicular fluid as reported previously (Leroy et al., 2004). The current experiment supports the previous findings; however, the current experiment did not find a correlation for steroid hormones between blood serum and follicular fluid.

4. Conclusions

In conclusion, supplementation with SCFP did not significantly affect reproductive characteristics, metabolite concentrations, or steroid hormone concentrations in blood serum and follicular fluid. Non-luteal cows and cows that failed to conceive to first service had increased concentrations of FFA and BHB in blood serum. Concentrations of BHB and glucose in blood serum were significantly correlated with concentrations in follicular fluid, and concentrations of FFA in blood serum tended to be correlated with those concentrations in follicular fluid. These results fail to support the primary hypothesis that supplementation with SCFP impacts the relationship of metabolites and hormones in follicular fluid and blood serum in lactating dairy cows. The findings from this study support previous observations that circulating metabolites during the transition period predict reproductive success (luteal status and pregnancy outcomes).

Our results also support the correlation of concentrations of metabolites in blood serum with those in follicular fluid.

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References

1. AAFCO., 2011. Official Publication. Association of the Animal Feed Control Officials, Oxford, IN.

2. Aardema, H., Vos, P. L. A. M., Lolicato, F., Roelen, B. A. J., Knij, H. M., Vaandrager, A. B., Helms, J. B., Gadella, B. M., 2011. Oleic acid prevents detrimental effects of saturated fatty acids on bovine oocyte developmental competence. Biol. Reprod. 85, 62-69.

3. Ahmadzadeh, L., Hosseinkhani, A., Daghigh Kia, H., 2018. Effect of supplementing diet with monensin sodium and *Saccharomyces cerevisiae* on reproductive performance of Ghezel ewes. Anim. Reprod. Sci. 188, 93-100.

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

5. Beam, S. W., Butler, W. R., 1999. Effects of energy balance on follicular development and first ovulation postpartum dairy cows. J. Reprod. Fertil. 54, 411-424.

6. Bell, A. W., 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73, 2804-2819.

7. Blache, D., Chagas, L. M., Martin, G. B., 2007. Nutritional inputs into the reproductive neuroendocrine control system- a multidimensional perspective. Reproduction, Suppl. 64, 123-139.

8. Britt, J. H., 1994. Follicular development and fertility: potential impacts of negative energy balance. In Proceedings of the National Reproduction Symposium, pp 103-112. Ed. ER Jordan. Pittsburgh, PA, USA.

9. Canfield, R.W., and Butler, W. R. 1990 Energy balance and pulsatile LH secretion in early postpartum dairy cattle. Domest. Anim. Endocrinol. 7, 323-330.

10. Carmina, E., Lobo, R. A., 1999. Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J. Clin. Endocrinol. Metab. 84, 1897-1899.

11. Chagas, L. M., Bass, J. J., Blanche, D., Burke, C. R., Kay, J. K., Lindsay, D. R., Lucy, M. C., Martin, G. B., Meier, S., Rhodes, F. M., Roche, J. R., Thatcher, W. W., Webb, R., 2007. New perspectives on the role of nutrition and metabolic priorities in subfertility of high producing dairy cows. J. Dairy Sci. 90, 4022-4032.

12. Conley, A. J., Bird, I.M., 1997. The role of cytochrome P450 17 alpha-hydroxylase and 3 beta-hydroxysteroid dehydrogenase in the integration of gonadal and adrenal steroidogenesis via the delta 5 and delta 4 pathway of steroidogenesis in mammals. Biol. Reprod. 56, 789-799.

13. Dann, H. M., Drackley, J. K., McCoy, G. C., Hutjes, M. F., and Garrett, J. E., 2000. Effects of yeast culture (*Saccharomyces cerevisiae*) on prepartum intake and postpartum intake and milk production of Jersey cows. J. Dairy Sci. 83, 123-127.

14. DeFrain, J. M., Hippen, A. R., Kalscheur, K. F., Patton, R. S., 2005. Effects of feeding propionate and calcium salts of long-chain fatty acids on transition dairy cow performance. J. Dairy Sci. 88, 983-993.

15. Diskin, M. G., Machey, D. R., Roche, J. F., Sreenan, J. M., 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim. Reprod. Sci. 78, 345-370.

16. Dupont, J., Scaramuzzi, R. J., Reverchon, M., 2014. The effect of nutrition and metabolic status on the development of follicles, oocytes, and embryos in ruminants. Animal 8, 1031-1044.

17. Francisco, C. C., Spicer, L. J., Payton, M. E., 2003. Predicting cholesterol, progesterone, and days to ovulation using postpartum metabolic and endocrine measures. J. Dairy Sci. 86, 2852-2863.

18. Herdt, T. H., 2000. Ruminant adaptation to negative energy balance influence on the etiology of ketosis and fatty liver. Vet. Clin. North Am. Food Anim. Pract. 16, 215-230.

19. Hill, S. L., Grieger D. M., Olson, K. C., Jaeger, J. R., Dahlen, C. R., Crosswhite, M. R., Negrin Pereira, N., Underdahl, S. R., Neville, B. W., Ahola, J., Fischer, M. C., Seidel, G. E., Stevenson, J. S., 2016. Gonadotropin-releasing hormone increased pregnancy risk in suckled beef cows not detected in estrus and subjected to a split-time artificial insemination program. J. Anim. Sci. 94, 3722–3728.

20. Igosheva, N., Abramov, A. Y., Poston, L., Eckert, J. J., Fleming, T. P., Duchen, M. R., McConnell, J. 2010. Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. PLoS ONE 5(4), E10074. doi:10.137/JOURNAL.PONE.0010074

21. Knowlton, K. F., Dawson, T. E., Glenn, B. P., Huntington, G. B., Erdman, R. A., 1998. Glucose metabolism and milk yield of cows infused abomasally or ruminally with starch. J. Dairy Sci. 81, 3248-3258.

22. Lamming, G. E., Wathes, D. C., and Peters, A. R. 1981. Endocrine patterns of the post-partum cow. J. Reprod. Fertil. Suppl. 30, 155-170.

23. Lehloenya, K. V., Stein, D. R., Allen, D. T., Selk, G. E., Jones, D. A., Aleman, M. M., Rehberger, T. G., Mertz, K. J., Spicer, L. J., 2007. Effects of feeding yeast and propionibacteria to dairy cows on milk yield and components, and reproduction. J. of Anim. Phys. and Anim. Nutr. 92, 190-202.
24. Leroy, J. L. M. R., Vanholder, T., Delanghe, J. R., Opsomer, G., Van Soom, A., Bols, P. E. J., Dewulf, J., de Kruif, A., 2004. Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early postpartum. Theriogenology 62, 1131-1143.
25. Leroy, J. L. M. R., Vanholder, T., Mateusen, B., Christophe, A., Opsomer, G., de Kruif, A., Genicot, G., Van Soom, A., 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. Reproduction 130, 485-495.
26. Leroy, J. L. M. R., Vanholder, T., Opsomer, G., Van Soom, A., de Kruif, A., 2006. The in vitro development of bovine oocytes after maturation in glucose and beta-hydroxybutyrate concentrations associated with negative energy balance in dairy cows. Reprod. Domest. Anim. 41, 119-123.
27. Leroy, J. L. M. R., Rizos, D., Sturmey, R., Bossaert, P., Gutierrez-Adan, A., Van Hoeck, V., Valckx, S., Bols, P. E. J., 2012. Intrafollicular conditions as a major link between maternal metabolism and oocyte quality: a focus on dairy cow fertility. Reprod. Fertil. Dev. 24, 1-12.
28. McArt, J. A. A., Nydam, D. V., Oetzel, G. R., Overton, T. R., Ospina, P. A., 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. Vet. J. 198, 560-570.

29. National Research Council, 2001. Nutrient requirements of dairy cattle, 7th rev. ed. Natl. Acad. Sci., Washington, D. C.

30. Olagaray, K. E., Sivinski, S. E., Saylor, B. E., Sauls, J. A., Yoon, I., and Bradford, B. J., 2018. Impacts of *Saccharomyces cerevisiae* fermentation product (SCFP) on feed intake parameters and lactation performance of transition dairy cattle. J. Dairy Sci. 101 (Suppl. 2), 307 (Abstr.).

31. Ospina, P. A., Nydam, D. V., Stokol, T., Overton T. R., 2010. Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93, 1596-1603.

32. Piva, G., Belladonna, S., Fusconi, G., Sicbaldi, F., 1993. Effects of yeast on dairy cow performance, ruminal fermentation, blood components, and milk manufacturing properties. J. Dairy Sci. 76, 2717-2722.

33. Reynolds, C. K., Aikman, P. C., Lupoli, B., Humphries, D. J., Beever, D. E., 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86, 1207-1217.

34. Robinson, P. H., 1997. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to diets postpartum. J. Dairy Sci. 80, 1119-1125.

35. Robinson, P. H., Garrett, J. E., 1999. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to postpartum diets and on lactational performance. J. Anim. Sci. 77, 988-999.

36. Rodgers, R. J., Irving-Rodgers, H. F., 2010. Formation of the ovarian follicular antrum and follicular fluid. Biol. Reprod. 82, 1021-1029.

37. Royal, M., Mann, G. E., Flint, A. P. F., 2000. Strategies for reversing the trends toward subfertility in dairy cattle. Vet. J. 160, 53-60.

38. Rutherford, A. J., Oikonomou, G., Smith, R. F., 2016. The effect of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle. J. Dairy Sci. 99, 4808-4815.

39. Schams, D., Hoffmann, B., Fischer, S., Marz, E., Karg, H., 1972 Simultaneous determination of LH and progesterone in peripheral bovine blood during pregnancy, normal and corticoid-induced parturition and the post-partum period. J. Reprod. Fertil. 29, 37-48.

40. Spicer, L. J., Tucker, W. B., Adams, G. D., 1990. Insulin-like growth factor-1 in dairy cows: relationship among energy balance, body condition score, ovarian activity and estrous behavior. J. Dairy Sci. 73, 929-937.

41. Stevenson, J. S. 2011. Alternative programs to presynchronize estrous cycles in dairy cattle before a timed artificial insemination program J. Dairy Sci. 94, 205-217.

42. Summers, A. F., Pohlmeier, W. E., Sargent, K. M., Cole, B. D., Vinton, R. J., Kurz, S. G., McFee, R. M., Cushman, R. A., Cupp, A. S., Wood, J. R., 2014. Altered theca and cumulus oocyte complex gene expression, follicular arrest, and reduced fertility in cows with dominant follicle follicular fluid androgen excess. PlosONE 9, e110683.

43. Sutton-McDowall, M., Gilchrist, R., Thompson, J., 2010. The pivotal role of glucose metabolism in determining oocyte developmental competence. Reproduction 139, 685-695.

44. Van Hoeck, V., Sturmey, R. G., Bermejo-Alvarez, P., Rizos, D., Gutierrez-Adan, A., Leese, H. J., Bols, P. E. J., and Leroy, J. L. M. R., 2011. Elevated non-esterified fatty

acid concentrations during bovine oocyte maturation compromise early embryo physiology. PlosONE 6, e23183.

45. Vanholder, T., Leroy, J. L. M. R., Van Soom, A., Opsomer, G., Maes, D. Coryn, M., de Kruif, A., 2005. Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. Anim. Reprod. Sci. 87, 33-44.
46. Wakefield, S. L., Lane, M., Schulz, S. J., Hebart, M. L., Thompson, J. G., Mitchell, M., 2008. Maternal supply of omega-3 polyunsaturated fatty acids alter mechanisms involved in oocyte and early embryo development in the mouse. Am. J. Physiol. Endocrinol. Metab. 294, E425-E434.
47. Wohlt, J. E., Corcione, T. T., Zajac, P. K., 1998. Effects of yeast on feed intake and performance of cows fed diets based on corn silage during early lactation. J. Dairy Sci. 81, 1345-1352.
48. Wohlt, J. E., Finkelstein, A. D., Chung, C. H., 1991. Yeast culture to improve intake, nutrient digestibility, and performance by dairy cattle during early lactation. J. Dairy Sci. 74, 1395-1400.

Table 2-1. Reproductive characteristics of cows supplemented with *Saccharomyces cerevisiae* fermentation product (SCFP) compared with control.

	Treatment[1]		
Item	Control	SCFP	*P*-value
Cows, no.	24	23	
Progesterone > 1 ng/mL at 32 ± 3 DIM, %	41	45	0.86
DIM at first CL[2]	37.4 ± 2.4	40.7 ± 2.4	0.33
Pregnancy per AI, %	50	44	0.75

[1] Cows were assigned randomly to be supplemented with *Saccharomyces cerevisiae* fermentation product or no supplement (control) beginning 4 weeks before expected calving and continuing until 7 weeks postpartum.
[2] First corpus luteum was detected by transrectal ultrasonography.

Table 2-2. Metabolite profiles of free fatty acids (FFA), glucose, and beta-hydroxybutyrate (BHB) in follicular fluid (FF) and blood serum (BS) after nutritional supplementation with *Saccharomyces cerevisiae* fermentation product (SCFP).

	Treatment (T)[1]		*P*-value		
Item	Control	SCFP	T	Parity	T × parity
FFA in FF[2], mEq/L	0.26 ± 0.04	0.23 ± 0.04	0.69	0.48	0.52
FFA in BS[3], mEq/L	0.28 ± 0.04	0.26 ± 0.04	0.63	0.82	0.84
Glucose in FF, mg/DL	53.7 ± 4.3	54.6 ± 4.2	0.88	0.45	0.31
Glucose in BS, mg/DL	64.1 ± 2.0	64.2 ± 2.0	0.97	0.20	0.39
BHB in FF, mmol/L	0.68 ± 0.1	0.83 ± 0.1	0.38	0.67	0.98
BHB in BS, mmol/L	0.74 ± 0.1	0.81 ± 0.1	0.67	0.89	0.70

[1] Cows were assigned randomly to be supplemented with *Saccharomyces cerevisiae* fermentation product or no supplement (control) beginning 4 wk before expected calving and continuing until 7 wk postpartum.
[2] Follicular fluid was aspirated from the dominant follicle at 49 ± 3 DIM.
[3] Blood serum was collected at 49 ± 3 days DIM.

Table 2-3. Steroid hormone concentrations (LSM ± SE) in follicular fluid (FF) and blood serum (BS) after nutritional supplementation with *Saccharomyces cerevisiae* fermentation product (SCFP).

	Treatment (T)[1]			*P*-value		
Item	Control	SCFP	SEM	T	Parity	T × parity
Progesterone in FF[2], ng/mL	138	87	54	0.91	0.71	0.19
Progesterone in BS[3], ng/mL	3.2	5.2	1.2	0.10	0.93	0.77
Androstenedione in FF, ng/mL	50	78	17	0.10	0.95	0.29
Androstenedione in BS, ng/mL	0.42	0.32	0.04	0.09	0.56	0.29
Estradiol in FF, ng/mL	583	518	418	0.63	0.29	0.63
Estradiol in BS, pg/mL	2.2	2.2	0.7	0.97	0.97	0.88
E:A ratio in BS	6.35	7.00	2.39	0.84	0.79	0.94
E:A ratio in FF	11.17	7.72	5.48	0.22	0.96	0.15

[1] Cows were assigned randomly to be supplemented with *Saccharomyces cerevisiae* fermentation product or no supplement (control) beginning 4 wk before expected calving and continuing until 7 wk postpartum.
[2] Follicular fluid was aspirated from the dominant follicle at 49 ± 3 DIM.
[3] Blood serum was collected at 49 ± 3 DIM.

Table 2-4. Concentrations (LSM ± SEM) of metabolites (free fatty acids [FFA], glucose, and beta-hydroxybutyrate [BHB]) and hormones in blood serum assessed at 49 ± 3 DIM in lactating dairy cows according to prebreeding luteal status and subsequent pregnancy status after timed artificial insemination[1].

	Pregnancy status		P-	Luteal status[2]		P-
Item	Pregnant	Not pregnant	value	Luteal	Non-luteal	value
FFA, mEq/L	0.26 ± 0.03 (18)[3]	0.31 ± 0.02 (23)	0.08	0.28 ± 0.02 (20)	0.28 ± 0.03 (21)	0.97
Glucose, mg/dL	61.8 ± 1.6 (18)	64.1 ± 1.2 (23)	0.20	64.2 ± 1.4 (20)	61.6 ± 1.5 (21)	0.13
BHB, mmol/L	0.52 ± 0.07 (18)	0.48 ± 0.05 (23)	0.60	0.42 ± 0.06 (20)	0.58 ± 0.7 (21)	0.04
Progesterone, ng/mL	3.0 ± 1.0 (12)	3.8 ± 0.7 (20)	0.42	3.8 ± 0.7 (18)	3.1 ± 1.0 (14)	0.50
Androstenedione, ng/mL	0.37 ± 0.05 (11)	0.36 ± 0.03 (17)	0.72	0.40 ± 0.03(17)	0.33 ± 0.04 (11)	0.09
Estradiol, pg/mL	2.7 ± 0.7 (11)	2.3 ± 0.5 (17)	0.59	2.0 ± 0.5 (17)	2.9 ± 0.7 (11)	0.19
E:A ratio	8.6 ± 2.6 (11)	7.2 ± 1.7 (17)	0.59	5.5 ± 1.8 (17)	10.3 ± 1.8 (11)	0.06

[1] Cows were supplemented with *Saccharomyces cerevisiae* fermentation product or no supplement (control) beginning 4 wk before expected calving and continuing until 7 wk postpartum.
[2] Luteal status was determined by progesterone ≥ 1 ng/mL at 32 ± 3 DIM.
[3] Number of cows.

Table 2-5. Concentrations (LSM ± SEM) of metabolites (free fatty acids [FFA], glucose, and beta-hydroxybutyrate [BHB]) and hormones in follicular fluid assessed at 49 ± 3 DIM in lactating dairy cows according to prebreeding luteal status and subsequent pregnancy status after timed artificial insemination[1]

	Pregnancy status		*P*-	Luteal status		*P*-
Item	Pregnant	Not pregnant	value	Luteal	Non-luteal	value
FFA, mEq/L	0.35 ± 0.05 (14)[3]	0.27 ± 0.04 (21)	0.21	0.27 ± 0.04 (19)	0.35 ± 0.05 (16)	0.17
Glucose, mg/dL	47.5 ± 3.8 (12)	52.7 ± 2.8 (18)	0.22	52.7 ± 2.8 (18)	47.8 ± 3.8 (12)	0.26
BHBA, mmol/L	0.56 ± 0.09 (14)	0.47 ± 0.06 (21)	0.33	0.42 ± 0.07 (19)	0.61 ± 0.08 (16)	0.04
Progesterone, ng/mL	275 ± 145 (14)	298 ± 109 (20)	0.88	227 ± 119 (18)	346 ± 135 (16)	0.43
Androstenedione, ng/mL	67.2 ± 14.3 (11)	64.2 ± 9.3 (17)	0.83	62.8 ± 10.0 (17)	68.7 ± 13.6 (11)	0.66
Estradiol, ng/mL	436 ± 108 (10)	580 ± 72 (15)	0.20	554 ± 76 (16)	462 ± 102 (9)	0.39
E:A ratio	7.4 ± 2.3 (10)	10.5 ± 1.5 (15)	0.19	9.1 ± 1.6 (16)	8.9 ± 2.2 (9)	0.93

[1] Cows were supplemented with *Saccharomyces cerevisiae* fermentation product or no supplement (control) beginning −4 wk before expected calving and continuing until 7 wk postpartum.
[2] Luteal status was determined by progesterone ≥ 1 ng/mL at 32 ± 3 DIM.
[3] Number of cows.

Table 2-6.Partial correlations between blood serum and follicular fluid in metabolites (free fatty acids [FFA], glucose, and beta-hydroxybutyrate [BHB]) and hormones in follicular fluid assessed at 49 ± 3 DIM in lactating dairy cows.

Item	FFA	BHB	Glucose	Progesterone	Androstenedione	Estradiol
Correlation	0.38	0.91	0.46	0.10	0.32	0.05
P-value	0.07	<0.01	0.03	0.65	0.13	0.82

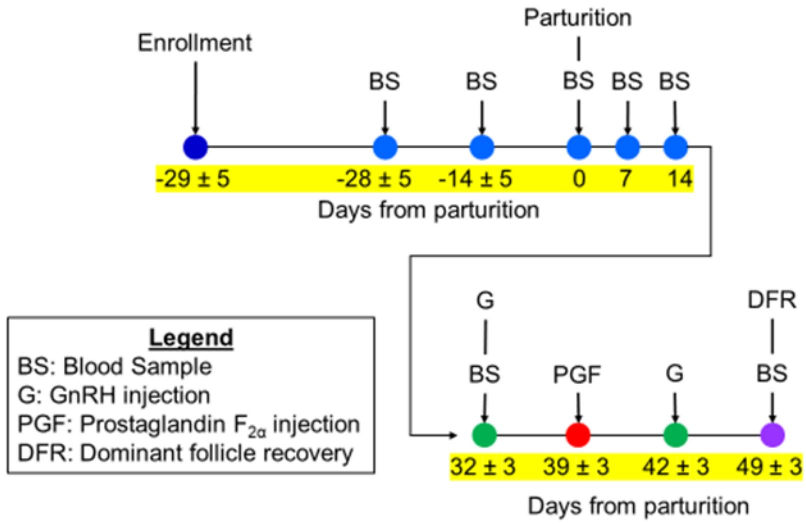

Figure 2-1. On day -29 relative to parturition cows were randomly assigned to treatments (Olagaray et al., 2018). Blood samples (BS) were collected for analysis of metabolites and steroid hormones. On day 32 ± 3 ovaries were scanned to determine follicle and corpus luteum location. At 49 ± 3 DIM after ovulation synchronization, cows were subjected to ultrasound-guided transvaginal aspiration for dominant follicle recovery (DFR).

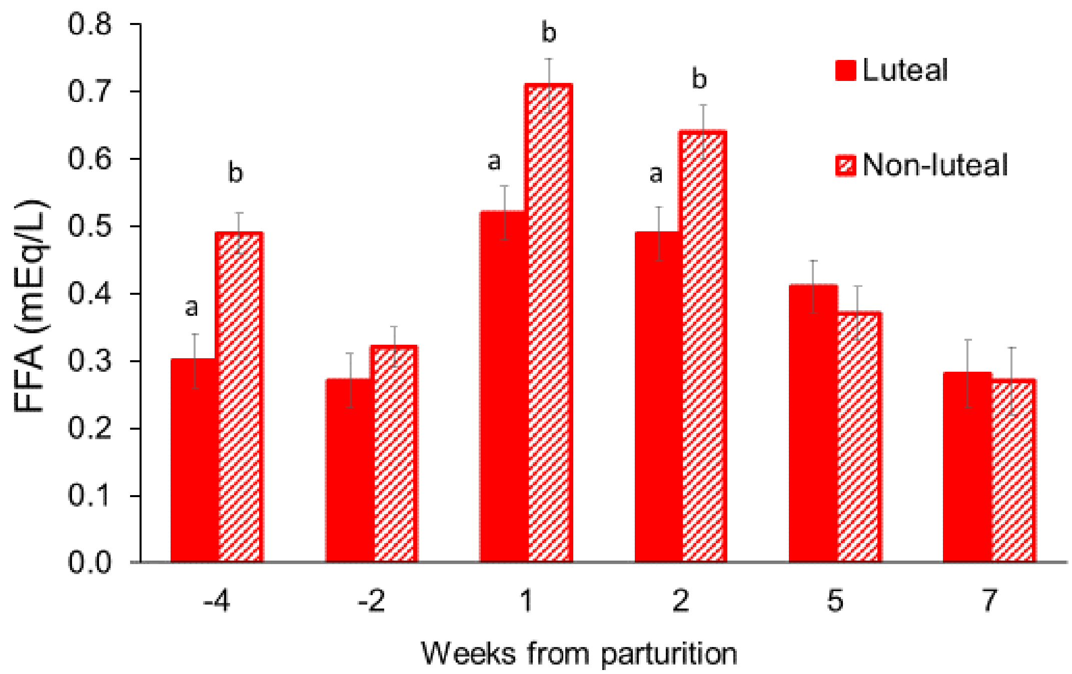

Figure 2-2. Concentrations of free fatty acids (FFA) in blood serum of dairy cows retrospectively categorized by luteal status at 32 ± 3 days in milk. Luteal is defined as progesterone ≥ 1 ng/mL, and non-luteal status is defined as progesterone < 1 ng/mL. Error bars represent SE. Least square means bearing different letters within week differ (*P* < 0.01).

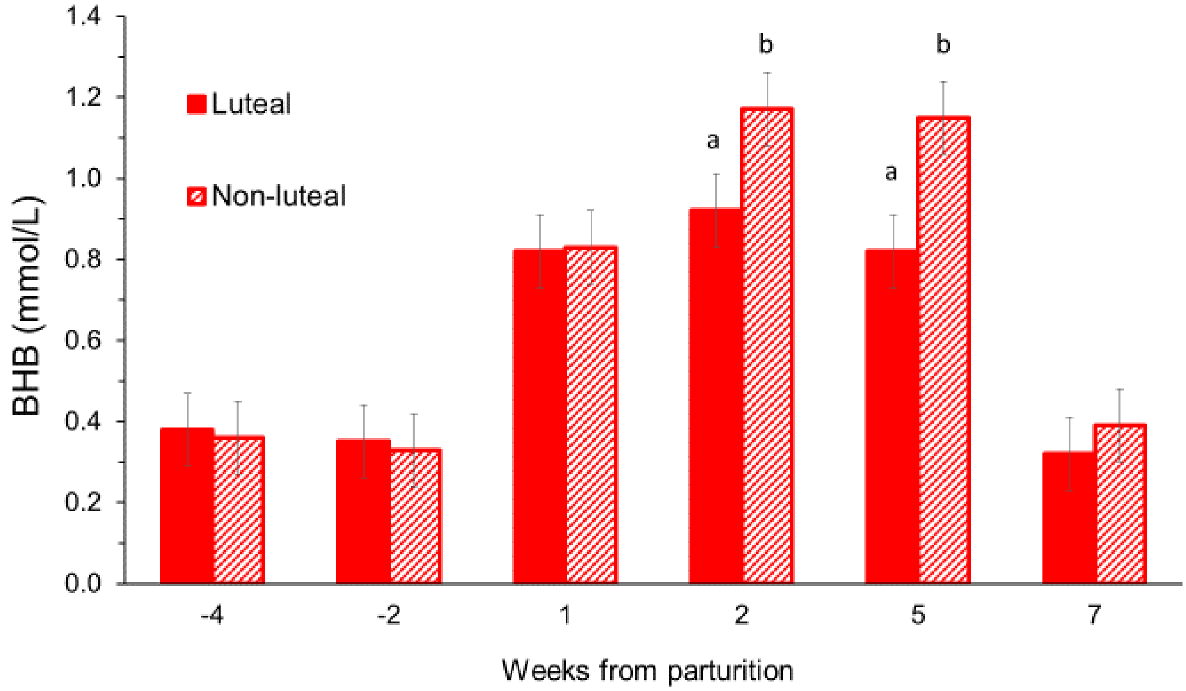

Figure 2-3. Concentrations of beta-hydroxybutyrate (BHB) in blood serum of dairy cows retrospectively categorized by luteal status at 32 ± 3 days in milk. Luteal is defined as progesterone ≥ 1 ng/mL, and non-luteal status is defined as progesterone < 1 ng/mL. Error bars represent SE. Least square means bearing different letters within week differ (*P* < 0.05).

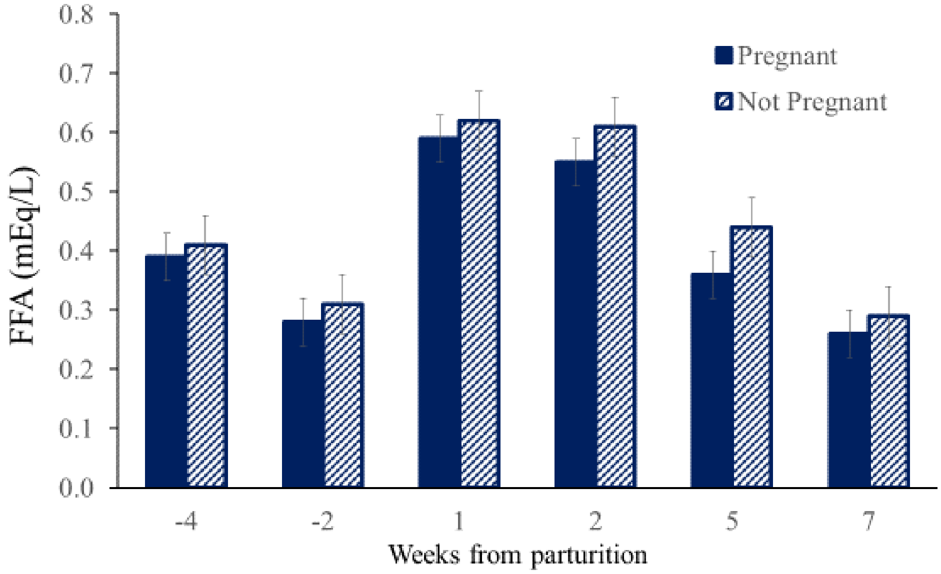

Figure 2-4. Concentrations of free fatty acids (FFA) in blood serum of dairy cows retrospectively categorized by first service conception. Error bars represent SE.

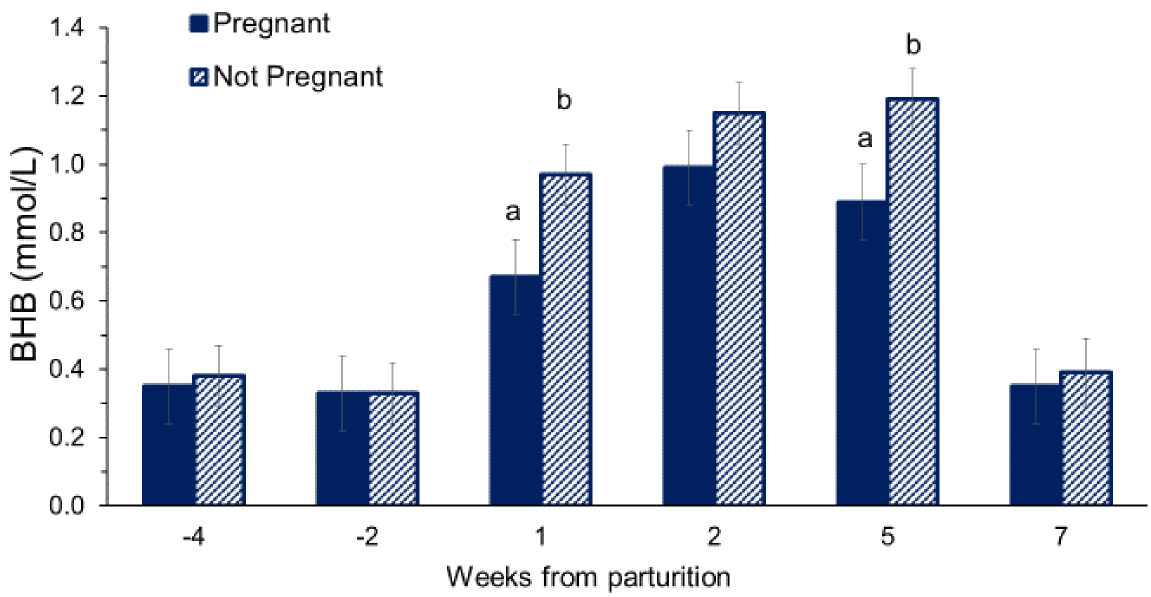

Figure 2-5. Concentrations of beta-hydroxybutyrate (BHB) in blood serum of dairy cows retrospectively categorized by first service conception. Error bars represent SE. Least square means bearing different letters within week differ (*P* < 0.05).

Chapter 3 - Review of Literature- Rumen Protected Glucose

Introduction

Fertility of dairy cattle is considered a multifactorial trait (Butler, 2003). Successful reproduction in dairy cattle occurs by a succession of events that consist of initiation or resumption of estrous cycles after parturition in replacement heifers and cows, respectively, development and ovulation of a viable oocyte, conception, embryo development, implantation in the uterus, maintenance of pregnancy, and eventual parturition (Garnsworthy et al., 2008). A disruption in any of these steps results in failure to conceive or to maintain the pregnancy to survive (Leroy et al., 2008a). Progesterone is a steroid hormone required for achieving puberty, resumption of estrous cycles, and the establishment and maintenance of pregnancy (Gonzalez-Padilla et al., 1975; Spencer and Bazer, 2002; Looper et al., 2003). Previous researchers have reported that increased progesterone concentrations during the estrous cycle before insemination at estrus (Fonseca et al., 1983) or during the 7 to 10 days preceding a timed insemination (Stevenson, 2016) are predictive of subsequent pregnancy risk in lactating dairy cows. Peripheral concentrations of progesterone are affected by the amount of progesterone produced as well as level of milk yield and rate of metabolism in the liver (Lemley and Wilson, 2010). Selection for increased milk yield during several decades has led to feeding cows less roughage and more high energy diets to support milk production. High energy diets are used to improve fertility in lactating dairy cows by altering energy sources.

Progesterone Production and Clearance

Discovery of progesterone began with the discovery of the corpus luteum (CL) by Regnier deGraaf (Jocelyn and Setchell, 1972). The CL was first referred to as “globules” by deGraaf and he concluded that the number of globules equaled the number of offspring resulting from a mating (Jocelyn and Setchell, 1972). Magnus and Simmer (1972) performed bilateral ovariectomy or cautery with electrical heat in mated rabbits. They that the CL was essential for the maintenance of pregnancy in rabbits (Wiltbank et al., 2014). The researchers continued these studies into the next decade and concluded that the CL regulates implantation and initial development of the embryo. By 1934, four laboratories independently isolated crystalline progesterone (Wiltbank et al., 2014).

Progesterone is a steroid hormone primarily secreted by the CL and the placenta in cattle (Wiltbank et al., 2014). Progesterone production from cholesterol utilizes two enzymes: CYP11A1 and HSD3β. Progesterone production is the simplest steroidogenic pathway to produce a biologically active steroid (Wiltbank et al., 2014). Progesterone exits the cell and enters the blood stream where it is transported to target tissue (Hansel and Convey, 1983). The primary target organs for progesterone in females are the hypothalamus, mammary glands, and uterus. Progesterone targets two components of the uterus: the glandular endometrium and the muscular myometrium. Progesterone not only stimulates secretion of histotroph from the endometrial glands to support development of the "free-floating" conceptus, but initiates lactation by causing final development of the alveolar cells in the mammary gland (Hansel and Convey, 1983).

Circulating progesterone concentrations are a balance between progesterone production and clearance. Although progesterone production by the CL is partly regulated by luteinizing hormone (LH), circulating progesterone is greatest in ruminants at times of the estrous cycle when LH pulse frequency is least (Wiltbank et al., 2012). Production of progesterone is associated with the number of granulosa cells that luteinize into large luteal cells. Progesterone production in luteal cells is related to transport of cholesterol within the cell (Wiltbank et al., 1993; Wiltbank et al., 2014).

Clearance rate of progesterone is a function of hepatic blood flow and liver enzyme activity (Sangsritavong et al., 2002; Lemley and Wilson, 2010). Until recently, genetic selection for high milk yield has been the standard practice in sire selection for artificial insemination (AI; Egger-Danner et al., 2015). Cows having greater daily milk production have greater luteal tissue, but have reduced circulating concentrations of progesterone compared with lesser milk-producing cows (Lopez et al., 2005). Because high-yielding cows have greater metabolic rates to meet their nutritional requirements, dairy producers must feed a diet with more energy. As a result of these high-energy diets in lactating dairy cattle, feed intake and liver blood flow are chronically increased and metabolic clearance rate (inactivation) of progesterone also is increased (Sangsritavong et al., 2002).

Inactivation of progesterone occurs by the liver and can be divided in two phases. Both phases utilize steroid-inactivating enzymes. The cytochrome P450 (CYP) superfamily of enzymes is involved in several physiological pathways including endogenous vitamin D_3 activation, metabolism of cholesterol to bile acids, xenobiotic metabolism (Anzenbacher and Anzenbacherova, 2001), and metabolism of all major classes of steroid hormones (Waxman et al., 1991). Previous researchers found that the mixed function monooxygenase cytochrome P450 2C (CYP2C) and cytochrome P450 3A (CYP3A) metabolize progesterone to 21-hydroxyprogesterone and 6β-hydroxyprogesterone in sheep (Murray, 1991, 1992). The aldo-keto reductase (AKR) enzymes are involved in prostaglandin metabolism, reduction of glucose, generation of bile acids, and reduction of steroids containing aldehyde or ketone groups (Penning et al., 2000; Barski et al., 2008; Kabututu et al., 2009). In humans and rodents, the AKRC1 subfamily converts progesterone to 3α-hydroxyprogesterone or 20α-hydroxyprogesterone, respectively (Penning et al., 2000). In cattle, phase one of progesterone inactivation occurs by progesterone being metabolized to hydroxyprogesterone metabolite using enzymes CYP2C, CYP3A, and AKR1C (Lemley and Wilson, 2010). In phase two of steroid inactivation hydroxyprogesterone metabolite is conjugated with glucuronic acid using uridine diphosphate-glucuronic acid (UGT) enzymes resulting in a more hydrophilic metabolite (Lemely and Wilson, 2010). Enzymes UGT1A and UGT2B are used in glucuronidation of hydroxylated derivatives of C18, C19, and C21 steroids (Lemely and Wilson, 2010).

Insulin and Cytochrome P450 Enzyme Relationship

A relationship between CYP enzymes and insulin was first proposed in the early 1990s (Barnett et al., 1990). Insulin-dependent diabetes increases the expression of cytochrome P450 2E1, 2B, 3A, and 4A, and administration of insulin to diabetic rats reduces the expression of these cytochrome P450s to basal concentration (Barnett et al., 1990). Likewise, Shimojo et al. (1993) observed an increase in CYP3A expression in insulin-dependent diabetic rats which was reversed when administered insulin. Insulin and glucagon were associated with an increase or decrease in the expression of CYP3A in rat hepatocytes (Saad et al., 1994). A murine hepatocyte cell-line was challenged with increasing physiological concentrations of insulin and a dose-dependent decrease in the fractional rate of progesterone decay was observed (Smith et al., 2006). In a different study utilizing the same cell line, a dose-dependent decrease in CYP2C and CYP3A activity was reported after challenging the hepatocytes with increasing physiological concentrations of insulin (Lemley et al., 2009).

In other research, ewes were orally drenched with a glucogenic substrate (sodium propionate) and had decreased progesterone inactivation compared with ewes that were drenched with an energy control (sodium acetate; Smith et al., 2006). Drenching lactating dairy cows daily with propylene glycol (500 mL/cow per day) during the transition period resulted in increased plasma concentrations of insulin and reduced cytochrome CYP3A expression (Butler et al., 2004; Lemley et al., 2008). Similarly, lactating dairy cows receiving a continuous i.v. infusion of insulin had increased plasma concentrations of insulin during the infusion period as well as reduced expression of CYP2C and CYP3A compared with the cohorts that were infused with saline (Butler et al., 2004; Lemley et al., 2008). Because insulin modulates hepatic expression of the cytochrome P450 enzymes that are associated with the catabolism of progesterone in cattle, insulin may impact reproductive success (Vieira et al., 2010).

Importance of Glucose to Lactating Dairy Cows

Insulin is a hormone that is vital in the regulation of blood glucose. As blood glucose concentrations increase, insulin is secreted from beta islet cells in the pancreas. There is always a basal secretion of insulin, but insulin secretion is directly proportional to glucose concentrations in the blood.

Glucose is an essential nutrient in dairy cows because glucose is necessary for milk synthesis (Bell, 1995). Glucose is also necessary for other tissues, including those involved in reproduction (Clark et al., 2001; Nishimoto et al., 2006; Berlinguer et al., 2012). In monogastric animals, glucose is a major product from carbohydrate digestion (Lucy et al., 2013). In ruminants, however, dietary nutrients such as fiber, protein, and starch are readily fermented in the rumen to volatile fatty acids (Van Knegsel et al., 2005). The main volatile fatty acids include acetate, butyrate, and propionate. Acetate and butyrate are compounds that either are or can be broken down into two-carbon compounds and are considered lipogenic, whereas propionate is a glucogenic three-carbon compound (Van Knegsel et al., 2005). The volatile fatty acids are absorbed from the rumen and can be oxidized for energy in peripheral tissues (Lucy et al., 2013). Propionate, however, is primarily utilized for gluconeogenesis in lactating ruminants.

Bell (1995) reported that 72 g of glucose are required for each kg of milk produced. In cattle, because carbohydrates are fermented to volatile fatty acids in the rumen, a cow must produce several kilograms of glucose daily via de novo gluconeogenesis in hepatic tissue (Drackley et al., 2001). Unfortunately, an early lactating cow can only meet approximately 85% of her glucose requirements, which leaves her lacking approximately 500 g of glucose daily to meet demands for maintenance and milk synthesis (Drackley et al., 2001; Lucy et al., 2013). Low blood glucose during early lactation may have a negative impact on reproductive success in dairy cows (Lucy et al., 2013). Green et al. (2012) found that cows becoming pregnant after first insemination had greater concentrations of glucose early postpartum compared with cows that failed to conceive after first AI. Blood glucose concentrations 3 d after parturition were associated with the probability of pregnancy later in lactation (Garverick et al., 2013). The extensive demand for glucose by the mammary gland for milk synthesis may decrease the amount of glucose readily available to other body tissues, including those tissues involved in reproductive processes (Wathes et al., 2001).

Dietary Manipulation of Starch

Cereal grains are primarily fed to provide energy to dairy cattle with most of the digestible energy coming from starch (Ali et al., 2012). Diets with large amounts of rumen degradable starch and a small amount of fiber decrease the acetate-to-propionate ratio (Bannink et al., 2006), and propionate is the major precursor for gluconeogenesis in cattle (Van Knegsel et al., 2007c). Glucogenic-type ingredients in a diet consist of cereal grains and their milling by-products, molasses, beet and citrus pulps, roots and tubers and their by-products, and dried reclaimed bakery products (Mosavi et al., 2012; Steyn et al., 2017). Previous studies have focused on manipulating the glucogenic-based ingredients in a diet to increase glucose and better fertility (Santos et al., 2008; Roche et al., 2011).

Feeding larger amounts of dietary starch to lactating dairy cows increased insulin and glucose concentrations and reduced free fatty acid (FFA) and β-hydroxybutyrate (BHB) concentrations during negative energy balance (Van Knegsel et al., 2007b). Feeding a glucogenic diet during early lactation increased the proportion of cows ovulating before 50 DIM, and therefore initiated earlier postpartum cyclicity (Gong et al., 2002; Van Knegsel et al., 2005). Earlier postpartum resumption of estrous activity was attributed to an improvement in energy balance status resulting from synergies between the somatotropic axis and effects of gonadotropins on ovarian cells, enabling ovulation of the dominant follicle (Useni et al., 2018). Feeding increased dietary starch also increased conception risk to first insemination (Gong et al., 2002). The published literature concerning manipulation of dietary starch to improve fertility, however, is conflicting. Garnsworthy et al. (2009) reported no effect on days to first increase in concentrations of progesterone or first estrus in cows fed a diet designed to stimulate increased insulin compared with a control diet. Feeding a high-starch diet did not hasten the onset of postpartum luteal activity or improve conception risk compared with an industry standard lactation diet (Gilmore et al., 2011).

Several factors could contribute to the inconsistency in the effects of dietary starch on reproductive success of dairy cattle in the previous studies (Useni et al., 2018). The rate of fermentation in the rumen, as well as the proportion of starch that bypasses the rumen, directly affect the nutrient profile absorbed post-rumen and indirectly affect the energy balance status of the cow (Van Knegsel et al., 2007a). Nutrient profile and energy balance status impact the ability of a cow to generate and maintain a pregnancy (Leroy et al., 2008b; Roche et al., 2011). The energy density of the diet, defined by nutrient content, has a significant effect on reproductive efficiency (Van Knegsel et al., 2005); therefore, another factor that is crucial to understanding is the non-isocaloric or isocaloric nature of diet comparisons in previous studies. The duration of starch supplementation could influence fertility. Garnsworthy et al. (2009) reported improved conception risk when feeding a diet that increased glucose and insulin during early lactation, and then switched to a diet that reduced insulin concentrations during the mating or insemination period. Differences in number of animals, reproduction management programs, and interpretations of experimental results among studies is also a source of variation that could explain the inconsistency in the published literature (Gilmore et al., 2011; Useni et al., 2018).

Summary

Progesterone is essential for maintenance of pregnancy, inhibiting expression of estrus, and overall reproductive success in dairy cattle. Increased peripheral concentrations of progesterone can be achieved by decreasing the activity of hepatic enzymes CYP2C and CYP3A, which are responsible for progesterone inactivation, by increasing the secretion of insulin (Lemley and Wilson, 2010). An increase in circulating glucose or propionate induces an increase in secretion of insulin. Dairy cattle are unable to produce sufficient glucose for maintenance and milk synthesis via gluconeogenesis, thus leaving tissues required for reproductive processes deficient in glucose (Drackley et al., 2001; Bell, 1995). Researchers have investigated different sources of starch and feeding rates to increase concentrations of glucose and consequently insulin in diets of lactating dairy cattle (Lemley et al., 2010). Improved pregnancy risk has been observed when feeding additional dietary starch to dairy cows (Burke et al., 2010), whereas other studies have found no differences (Gilmore et al., 2011). The starch sources supplemented to cows to improve fertility in the published literature have been readily fermentable in the rumen. Research is warranted to determine the optimal energy source and feeding strategy to improve fertility though diet manipulation of starch.

References

Ali, M., M. R. Weisbjerg, J. W. Cone, G. Van Duinkerken, M. C. Blok, M. Bruinenberg, and W. H. Hendriks. 2012. Postruminal degradation of crude protein, neutral detergent fiber and starch of maize and grass silages in dairy cows. Anim. Feed Sci. Technol. 177:172-179.

Anzenbacher, P., and E. Anzenbacherová. 2001. Cytochrome P450 and metabolism of xenobiotics. Cell. Mol. Life Sci. 58:737-747.

Bannink, A. J., J. Kogut, J. Dijkstra, J. France, E. Kebreab, A. M. Van Vuuren, and S. Tamminga. 2006. Estimation of the stoichiometry of volatile fatty acid production in the rumen of lactating cows. J. Theor. Biol. 238:36-51.

Barnett, C. R., G. G. Gibson, C. R. Wolf, P. R. Flatt, and C. Ioannides. 1990. Induction of cytochrome P450III and P450IV family proteins in streptozotocin-induced diabetes. Biochem. J. 268:765-769.

Barski, O. A., S. M. Tipparaju, and A. Bhatnagar. 2008. The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. Drug Metab. Rev. 40:553-624.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804–2819.

Berlinguer, F., A. Gonzalez-Bulnes, I. Contreras-Solis, A. Spezzigu, L. Torres-Rovira, S. Succu, S. Naitana, and G. G. Leoni. 2012. Glucogenic supply increases oocyte developmental competence in sheep. Reprod. Fertil. Dev. 24:1055-1062.

Burke, C. R., J. K. Kay, C. V. C. Phyn, S. Meier, J. M. Lee, and J. R. Roche. 2010. Short communication: Effects of dietary non-structural carbohydrates prepartum and postpartum on reproduction of grazing dairy cows. J. Dairy Sci. 93:4292-4296.

Butler, S. T., S. H. Pelton, and W. R. Butler. 2004. Insulin increases 17 β-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. Reproduction 127:537-545.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 83:211-218.

Clark, A. R., Y. M. Stokes, and J. G. Thompson. 2011. Estimation of glucose uptake by ovarian follicular cells. Ann. Biomed. Eng. 39:2654-2667.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptions of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84(E. Suppl.):E100–112.

Egger-Danner C., J. B. Cole, J. E. Pryce, N. Gengler, B. Heringstad, A. Bradley, and K. F. Stock. 2015. Invited review: overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. Animal 9:191-207.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk, and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66:1128–1147.

Garnsworthy, P. C., A. A. Fouladi-Nashta, G. E. Mann, K. D. Sinclair, and R. Webb. 2009. Effect of dietary-induced changes in plasma insulin concentrations during the early postpartum period on pregnancy rate in dairy cows. Reprod. 137:759-768.

Garnsworthy, P. C., K. D. Sinclair, and R. Webb. 2008. Integration of physiological mechanisms that influence fertility in dairy cows. Animal 2:1144-1152.

Garverick, H. A., M. N. Harris, R. Vogel-Bluel, J. D. Sampson, J. Bader, W. R. Lamberson, J. N. Spain, M. C. Lucy, and R. S. Youngquist. 2013. Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. J. Dairy Sci. 96:181–188.

Gilmore, H. S., F. J. Young, D. C. Patterson, A. R. G. Wyile, R. A. Law, D. J. Kilpatrick, C. T. Elliot, and C. S. Mayne. 2011. An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and estrous behavior oh high yielding Holstein Friesian dairy cows. J. Dairy Sci. 94:3510-3526.

Gong, J. G., W. J. Lee, P. C. Garnsworthy, and R. Webb. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reprod. 123:491-427.

Gonzalez-Padilla, E., J. N. Wiltbank, and G. D. Niswender. 1975. Puberty in beef heifers. The interrelationship between pituitary, hypothalamic, and ovarian hormones. J. Anim. Sci. 40:1091-1104.

Green, J. C., J. P. Meyer, A. M. Williams, E. M. Newsom, D. H. Keisler, and M. C. Lucy. 2012. Pregnancy development from day 28 to 42 of gestation in postpartum Holstein cows that were either milked (lactating) or not milked (not lactating) after calving. Reproduction 143:699–711.

Hansel, W., E. M. Convey. 1983. Physiology of the estrous cycle. J. Anim. Sci. 57:404-424.

Jocelyn, H. D., B. P. Setchell. 1972. An annotated translation of Regnier deGraaf’s new treatise concerning the generative organs of women (1672). J. Repro. Fertil. Suppl. 17:77-206.

Kabututu, Z., M. Manin, J. C. Pointud, T. Maruyama, N. Nagata, S. Lambert, A. M. Lefrançois-Martinez, A. Martinez, and Y. Urade. 2009. Prostaglandin F2alpha synthase activities of aldo-keto reductase 1B1, 1B3, and 1B7. J. Biochem. 145:161-168.

Lemley, C. O., J. M. Koch, K. P. Blemings, K. M. Krause and M. E. Wilson. 2008. Concomitant changes in progesterone catabolic enzymes, cytochrome P450 2C and 3A, with plasma insulin concentrations in ewes supplemented with sodium acetate or sodium propionate. Animal 2:1223-1229.

Lemley, C. O., J. M. Koch, K. P. Blemings, and M. E. Wilson. 2009. Alterations in progesterone catabolic enzymes, CYP2C, and CYP3A, in hepatocytes challenged with insulin and glucagon. J. Anim. Vet. Adv. 8:39-46.

Lemley, C. O., and M. E. Wilson. 2010. Effect of cytochrome P450 and aldo-keto reductase inhibitors on progesterone inactivation in primary bovine hepatic cell cultures. J. Dairy Sci. 93:4613–4624.

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

Leroy, J. L. M. R., T. Vanholder, A. T. M. Van Knegsel, I. Garcia-Ispierto, and P. E. J. Bols. 2008b. Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part II Mechanisms linking nutrition and reduced oocyte and embryo quality in high-yielding dairy cows. Reprod. Domest. Anim. 43:623-632.

Looper, M. L., C. A. Lents, and R. P. Wettemann. 2003. Body condition at parturition and postpartum weight changes do not influence the incidence of short-lived corpora lutea in postpartum beef cows. J. Anim. Sci. 81:2390-2394.

Lopez, H., D. Z. Caraviello, L. D. Satter, P. M. Fricke, and M. C. Wiltbank. 2005. Relationship between level of milk production and multiple ovulations in lactating dairy cows. J. Dairy Sci. 88:2783-93.

Lucy, M. C., R. C. Escalante, D. H. Keisler, W. R. Lamberson, and D. J. Mathew. 2013. Short communication: glucose infusion into early postpartum cows defines an upper physiological set point for blood glucose and causes rapid and reversible changes in blood hormones and metabolites. J. Dairy Sci. 96:5762–5768.

Magnus V., H., and H. Simmer. 1972. The first experiments to demonstrate an endocrine function of the corpus luteum. II. Ludwig Fraenkel versus Vilhelm magnus. Sudhoffs Archiv. 56:76–99.

Mosavi, G. R., F. Fatahnia, H. R. M. Alamouti, A. A. Mehrabi, and H. D. Kohi. 2012. Effect of dietary starch source on milk production and composition of lactating Holstein cows. S. Afr. J. Anim. Sci. 42:201-209.

Murray, M. 1991. Microsomal cytochrome P450-dependent steroid metabolism in male sheep liver. Quantitative importance of 6β-hydroxylation and evidence for the involvement of a P450 from the IIIA subfamily in the pathway. J. Steroid Biochem. Mol. Biol. 38:611-619.

Murray, M. 1992. Participation of a cytochrome P450 enzyme from the 2C subfamily in progesterone 21-hydroxylation in sheep liver. J. Steroid Biochem. Mol. Biol. 43:591-593.

Nishimoto, H., R. Matsutani, S. Yamamoto, T. Takahashi, K. G. Hayashi, A. Miyamoto, S. Hamano, and M. Tetsuka. 2006. Gene expression of glucose transporter (GLUT) 1, 3, and 4 in bovine follicle and corpus luteum. J. Endocrinol. 188:111-119.

Penning, T. M., M. E. Burczynski, J. M. Jez, C. F. Hung, H. K. Lin, H. Ma, M. Moore, N. Palackal, and K. Ratnam. 2000. Human 3α-hydroxysteroid dehydrogenase isoforms

(AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: Functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. Biochem. J. 351:67-77.

Roche, J. R., C. R. Burke, S. Meier, and C. G. Walker. 2011. Nutrition x reproduction interaction in pasture-based systems: Is nutrition a factor in reproductive failure? Anim. Prod. Sci. 51:1045-1066.

Saad, B., H. Thomas, H. Schawalder, F. Waechter, and P. Maier. 1994. Oxygen tension, insulin, and glucagon affect the preservation and induction of cytochrome P450 isoforms cultured rat hepatocytes. Toxicol. Appl. Pharmacol. 126:372-379.

Sangsritavong, S., D. K. Combs, R. Sartori, L. E. Armentano, and M. C. Wiltbank. 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J. Dairy Sci. 85:2831-2842.

Santos, J. E. P., T. R. Bilby, W. W. Thatcher, C. R. Staples, and F. T. Silvestre. 2008. Long chain fatty acids of diet as factors influencing reproduction in cattle. Reprod. Domest. Anim. 43:23-30.

Shimojo, N., T. Ishizaki, S. Imaoka, Y. Funae, S. Fuji, and K. Okuda. 1993. Changes in amounts of cytochrome P450 isozymes and levels of catalytic activities in hepatic and renal microsomes of rats with streptozotocin-induced diabetes. Biochem. Pharmacol. 46:621-627.

Smith, D. L., B. M. Stinefelt, K. P. Blemings, and M. E. Wilson. 2006. Diet-induced alterations in progesterone clearance appear to be mediated by insulin signaling in hepatocytes. J. Anim. Sci. 84:1102-1109.

Spencer, T. E., and F. W. Bazer. 2002. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. Front. Biosci. 7:1879-1898.

Stevenson, J. S. 2016. Physiological predictors of ovulation and pregnancy risk in a fixed-time artificial insemination program. J. Dairy Sci. 99:10077–10092.

Steyn, L., R. Meeske, and C. W. Cruywagen. 2017. Replacing maize grain with dried citrus pulp in a concentrate feed for Jersey cows grazing ryegrass pasture. S. Afr. J. Anim. Sci. 47:553-564.

Useni, B. A., C. J. C. Muller, and C. W. Cruywagen. 2018. Pre- and postpartum effects of starch and fat in dairy cows: A review. S. Afr. J. Anim. Sci. 48:413-426.

Van Knegsel, A. T. M., H. Van den Brand, J. Dijsktra, W. M. Van Straalen, M. J. W. Heetkamp, S. Tamminga, and B. Kemp. 2007a. Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. J. Dairy Sci. 90:1467-1476.

Van Knegsel, A. T. M., H. Van den Brand, J. Dijkstra, W. M. Van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007b. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. J. Dairy Sci. 90:3397-3409.

Van Knegsel, A. T. M., H. Van den Brand, J. Dijlstra, S. Tamminga, and B. Kemp. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Reprod. Nutr. Dev. 45:665-688.

Van Knegsel, A. T. M., H. Van den Brand, E. A. M. Graat, J. Dijkstra, R. Jorritsma, E. Decuypere, S. Tamminga, and B. Kemp. 2007c. Dietary energy source in dairy cows in early lactation: Metabolites and metabolic hormones. J. Dairy Sci. 90:1477-1485.

Vieira, F. V. R., C. N. Lopes, B. I. Cappellozza, A. B. Scarpa, R. F. Cooke, and J. L. M. Vasconcelos. 2010. Effects of intravenous glucose infusion and nutritional balance on serum concentrations of nonesterified fatty acids, glucose, insulin, and progesterone in nonlactating dairy cows. J. Dairy Sci. 93:3047-3055.

Wathes, D. C., Z. Cheng, M. A. Fenwick, R. Fitzpatrick, and J. Paton. 2001. Influence of energy balance on somatotropic axis and metalloproteinase expression in the endometrium of the postpartum dairy cow. Reproduction 141:269–281.

Waxman, D. J., D. P. Lapenson, T. Aoyama, H. V. Gelboin, F. J. Gonzalez, and K. Korzekwa. 1991. Steroid hormone hydroxylase specificities of eleven cDNA-expressed human cytochrome P450s. Arch. Biochem. Biophys. 290:160-166.

Wiltbank M. C., C. J. Belfiore, G. D. Niswender. 1993. Steroidogenic enzyme activity after acute activation of protein-kinase (PK) A and PKC in ovine small and large luteal cells. Mol. Cell. Endocrinol. 97:1-7.

Wiltbank M. C., S. M. Salih, M. O. Atli, W. Luo, C. L. Bormann, J. S. Ottobre, C. M. Vezina, V. Mehta, F. J. Diaz, S. J. Tsai, and R. Sartori. 2012. Comparison of endocrine and cellular mechanisms regulating the corpus luteum of primates and ruminants. Anim. Repro. 9:242-259.

Wiltbank, M. C., A. H. Souza, P. D. Carvalho, A. P. Cunha, J. O. Giordano, P. M. Fricke, G. M. Baez, M. G. Diskin. 2014. Physiological and practical effects of progesterone on reproduction in dairy cattle. Anim. Consort. 8:70-91.

Chapter 4 - Physiologic responses to feeding rumen-protected glucose to lactating dairy cows

ABSTRACT

We hypothesized that supplementing rumen-protected glucose (RPG) in the diet of lactating dairy cows would increase concentrations of glucose and insulin resulting in decreased activity of liver cytochromes P450 2C and P450 3A, thus increasing blood progesterone concentrations. Estrus and ovulation were synchronized, and data was used from 59 Holstein cows using GnRH and $PGF_{2\alpha}$ (d 0 = ovulation). Cows were milked thrice daily and assigned randomly to be fed individually a basal diet supplemented with 0, 1, 2, or 4 kg of a RPG product in place of corn grain, top-dressed in the diet beginning on d −3. Blood was collected pre-feeding and 8 h after feeding on d 0, 2, and 4 to determine glucose and insulin concentrations and daily from d 2 through 12 to assess progesterone concentrations. Blood was collected every 4 h for 24 h on d 8 to assess a diurnal pattern in progesterone. Crude protein and energy-soluble carbohydrates intake increased linearly with dose, whereas starch intake decreased linearly with dose. Diameter of the corpus luteum (CL) was determined by ultrasonography on d 8. On d 8, dry matter intake (DMI), energy-corrected milk (ECM), somatic cell count and percentages of milk fat and lactose did not differ among dietary treatments. A quadratic effect was detected for ECM:DMI, with a concave-upward effect resulting from greater efficiency for the 1- and 2-kg doses. Neither pre-feeding nor post-feeding concentrations of glucose differed among treatments; however, post-feeding glucose decreased from d 0 through 4. Pre-feeding insulin did not differ among treatments, but a difference in the change of insulin (postprandial minus pre-prandial) was detected. The increase in insulin was greater in control cows than the combined mean for the 3 RPG doses. Milk urea nitrogen increased linearly with RPG dose and pregnancy risk at first AI was reduced by RPG treatment. Volume of the CL on d 8 did not differ among treatments. Concentrations of progesterone increased from d 2 to 12 but were unaffected by RPG treatment. The pattern of progesterone assessed every 4 h on d 8 fit a 4th-order polynomial curve ($R^2 = 0.97$) for all concentrations during the 24-h period (0800 through 0400 h). We conclude that the insulin response to the RPG diets was diminished relative to the control. Supplementation with RPG caused a linear increase in CP intake and MUN with increasing dose, but did not impact concentrations of progesterone, milk yield, or DMI.

Key Words: Glucose, Insulin, Progesterone

INTRODUCTION

Reproductive performance resulting in timely pregnancies during early lactation is critical to profitability of dairy farms. Progesterone is essential for the maintenance of pregnancy because it inhibits uterine contractions until parturition (Csapo, 1956). Progesterone also plays an important role in modulating amino acids in the histotroph, a potentially critical factor for early embryonic survival (Mullen et al., 2014). In fact, reduced concentrations of progesterone because of inadequate luteal function, increased rates of steroid inactivation, or both, contribute to early embryonic loss (Inskeep and Dailey, 2005). Peripheral concentration of progesterone is a function of progesterone synthesis and secretion by the corpus luteum (CL) and its subsequent inactivation by hepatic enzymes (Hart et al., 2018). Supplementing cows having a functional CL with progesterone after AI in the form of a controlled internal drug-release (CIDR) insert, which increased peripheral progesterone by approximately 1 ng/mL, improved first-service conception risk in lactating dairy cows (Larson et al., 2007). Even though supplementing progesterone artificially increased the amount of progesterone, it failed to decrease the rate of steroid inactivation in the liver by progesterone-inactivating enzymes (Lemley et al., 2010).

Several researchers have investigated manipulating the diet by increasing the glucogenic components to improve fertility (Van Knegsel et al., 2005; Lemley et al., 2010). Insulin is secreted in response to increased concentrations of circulating glucose and is potentially a key metabolic mediator between nutrition and reproduction (Vieira et al., 2010). Insulin has been shown to decrease the expression of cytochrome P450 enzymes (Lemley et al., 2010). Adding more dietary starch (Gong et al., 2002), maize gluten (Armstrong et al., 1990), ground shelled corn (Carroll et al., 1990), abomasal glucose infusion (Oldick et al., 1997), or propylene glycol supplementation (Miyoshi et al., 2001) are means of achieving increased glucogenic nutrients.

Glucose is a key nutrient required during lactation for milk synthesis and maintenance of other body tissues (Bell, 1995; Lucy et al., 2013). In dairy cattle, dietary starch is converted to glucose and then rapidly converted to VFA, which are then oxidized as an energy source in body tissues or utilized for gluconeogenesis in the case of propionate (Lucy et al., 2013). Because glucose is converted to VFA in the rumen, a cow must synthesize glucose de novo via gluconeogenesis in the liver (Drackley et al., 2001; Lucy et al., 2013). An early lactating cow may only achieve 85% of her glucose requirement via gluconeogenesis, which leaves her lacking approximately 500 g of glucose per day to meet demands for maintenance and milk synthesis (Drackley et al., 2001; Lucy et al., 2013). The extensive demand for glucose by the mammary gland for milk synthesis may decrease the amount of glucose readily available to other body tissues including those tissues involved in reproductive processes (Wathes et al., 2001; Green et al., 2012; Garverick et al., 2013).

Previous research has focused on increasing dietary starch that is readily fermented in the rumen because no rumen-protected glucose product was available. A rumen-protected glucose product could facilitate more glucose being delivered to the abomasum and small intestine for absorption without relying solely on its de novo synthesis in the liver from propionate. Because circulating glucose induces secretion of insulin, the resulting increase in insulin may decrease the activity of hepatic enzymes involved in clearance of progesterone (Lemley et al., 2010), and thus, increase peripheral concentrations of progesterone. Increased concentrations of progesterone during the estrous cycle before insemination at estrus (Fonseca et al., 1983) or during the 7 to 10 d preceding a fixed-time insemination (Stevenson, 2016) are predictive of subsequent pregnancy risk in lactating dairy cows.

Therefore, the objective of the current study was to determine the effect of supplementing a rumen-protected glucose (RPG) product on concentrations of glucose, insulin, and progesterone. We hypothesized that supplementing RPG would increase concentrations of glucose and insulin, resulting in decreased activity of liver cytochromes P450 2C and P450 3A, thus increasing circulating concentrations of progesterone.

MATERIALS AND METHODS

An experiment at the Kansas State University Dairy Research and Teaching Center was conducted under the Kansas State University Institutional Animal Care and Use Committee protocol 3988. Sixty-four Holstein cows (24 primiparous and 40 multiparous) were enrolled in a completely randomized design before first insemination. Cows calved in a maternity barn on a straw-bedded pack and were subsequently housed in a sand-bedded free stall facility. Cows were then moved to a tie-stall barn (58 ± 3 DIM) and enrolled in the experiment. Cows were paired by calving date, blocked by parity, and then randomly assigned to a dietary treatment. Treatments included daily supplementation with 0 (control; n = 16), 1 (n = 16), 2 (n = 16), or 4 (n = 16) kg of a rumen-protected glucose product (Soybest; West Point, NE) replacing finely-ground corn grain. Appropriate sample size was evaluated using the power procedure (PROC POWER) in

SAS 9.4 software (SAS Inst. Inc., Cary, NC) to detect difference in concentrations of progesterone in response to RPG supplementation. A variance of 0.2 ng/mL was used for progesterone concentrations from past in-house data. The power test revealed 60 cows (15/treatment) would provide 80% power to detect a 0.21 ng/mL difference in concentrations of progesterone at an alpha value of 0.05.

Ovulation Synchronization

To meet the starting criteria of 58 ± 3 DIM, 10 clusters of cows were subjected to an ovulation-synchronization program to synchronize ovulation (d 0; Figure 4-1) between September and December 2017. Briefly, cows received $PGF_{2\alpha}$ at 48 ± 3 DIM, an injection of GnRH at 51 ± 3 DIM, and $PGF_{2\alpha}$ at 58 and 59 ± 3 DIM. An injection of GnRH was administered 56 h after the injection of $PGF_{2\alpha}$ on d 58 (d −0.5) to induce ovulation of the dominant follicle. The products used were 100 μg GnRH (Factrel) and 25 mg $PGF_{2\alpha}$ (dinoprost tromethamine) from Zoetis Inc. (Kalamazoo, MI). Cows were eligible to continue in the experiment when ovulation (d 0) was detected by the appearance of a new CL by d 2 and serum progesterone concentrations exceeded 1 ng/mL by d 4. Frozen images of ovarian structures were measured by electronic calipers upon transrectal ultrasonography (Ibex Evo, 7.5 MHz probe, E.I. Medical Imaging, Loveland, CO) on d −3, −0.5, 0, 2, and 8. Total volume of luteal tissue measured on d 8 was calculated ($4/3 \times r^3 \times \pi$, where W = largest width and H = largest height of the structure; r = radius [W/2 + H/2]/2, and $\pi = 3.14159$). When a luteal structure contained a fluid-filled cavity, volume of the cavity was subtracted from the total luteal volume. Cows were reintroduced in the herd at 72 ± 3 DIM (d 12) and estrous cycles were resynchronized in cows for subsequent insemination (GnRH on 72 ± 3, $PGF_{2\alpha}$ on 79 ± 3 and 80 ± 3, GnRH on 81 ± 3, and timed AI on 82 ± 3 DIM).

Dietary Treatments and Production Measures

Dietary supplements were initiated 3 d before expected ovulation. Half of the daily allocation of dietary supplements was hand-mixed into the top third of the basal diet at each feeding (0600 h and 1600 h). Control cows were fed 4 kg ground corn (2 kg in the a.m. feeding and 2 kg in the p.m. feeding). Treated cows receiving the RPG product were fed the appropriate RPG dose plus enough ground corn to equal a total of 2 kg at each of two daily feedings. The RPG product was rumen protected by mixing a reducing carbohydrate source and a non-protein nitrogen source and heating the mixture under appropriate conditions to cause sufficient Maillard reaction product to form thus limiting or preventing ruminal degradation of the carbohydrate (Russi, 2013). The diets were balanced to be isocaloric (Table 4-1). Samples of the basal diet were collected once weekly, composited across 2 wk ($n = 5$), and analyzed by near infrared reflectance spectroscopy (Dairy One, Ithaca, NY). Individual feed intake was recorded daily.

Cows were milked thrice daily at 6:00, 11:00, and 18:00 h. Milk samples were collected once 3 d before initiation of treatment (d −6) and again on d 8 of the estrous cycle, and were analyzed for concentrations of fat, true protein, and lactose (B-2000 Infrared Analyzer, Bentley Instruments, Chaska, MN), MUN (MUN spectrophotometer, Bentley Instruments), and somatic cells (SCC 500, Bentley Instruments) by MQT labs (Kansas City, MO). Somatic cell score was calculated as described by Schukken et al. (2003): $SCS = Log_2(SCC/100) + 3$. Energy-corrected milk (3.5% fat and 3.2% protein basis) was calculated from d 8 sampling according to Dairy Records Management Systems as (0.3246 × milk yield) + (0.1286 × milk × fat %) + (0.704 × milk × protein %). Daily milk production was recorded from d 0 through d 12 of the estrous cycle (Boumatic Global, Madison, WI). Body condition score and body weight were recorded at enrollment (58 ± 3 DIM).

Blood Traits

Blood samples were collected via coccygeal puncture on d 0, 2, and 4 to analyze concentrations of glucose and insulin. Samples were collected 1 h before and 8 h after the morning feeding to determine pre-prandial and postprandial concentrations of glucose and insulin. Progesterone was measured in blood serum samples collected on d 2 and daily from d 4 through 12. Blood samples also were collected every 4 h during 24 h on d 8 to determine the diurnal pattern of progesterone concentration.

Concentrations of glucose were measured by a colorimetric kit (kit #439-90901; Wako Chemicals USA Inc.). Assay sensitivity for glucose is 0.413 mg/dL. Concentrations of insulin were measured by a bovine-specific sandwich ELISA (#10-1201-01; Mercodia AB, Uppsala, Sweden), and assay sensitivity is 0.025 μg/L. Concentrations of progesterone in blood serum were measured in an assay by direct quantitative (nonextracted) RIA using ImmuChem Double Antibody progesterone ^{125}I kits (MP Biomedicals LLC, Orangeburg, NY) previously validated for bovine serum (Hill et al., 2016). Intra- and inter- assay coefficients of variation for 13 assays for a low (1.28 ± 0.07 ng/mL) and high (17.2 ± 1.3 ng/mL) concentration pool were 4.93 and 5.34%, respectively. Calculated assay sensitivity averaged 20 ± 0.5 pg/mL, and progesterone standard concentrations in the assay were 0.05, 0.1, 0.2, 0.5, 2.0, 5.0, 10.0, and 25.0 ng/mL.

Statistical Analyses

Of the 64 cows enrolled, data were analyzed from 59 cows because 5 cows (control: n = 3; 1 kg: n = 1; 2 kg: n = 1) failed to ovulate. All analyses were performed using SAS 9.4 software (SAS Inst. Inc., Cary, NC). Daily concentrations of progesterone, insulin, glucose, milk yield, and DMI were analyzed by using a mixed model procedure (MIXED procedure) to account for repeated measures. The model consisted of the fixed effects of parity (primiparous vs.

multiparous), treatment (0, 1, 2, or 4 kg), day, and the interaction of treatment and day, in addition to the random effect of cow nested within treatment. Treatment was tested by the cow with treatment variance (split-plot error), whereas all other model variables were tested by the residual error (whole-plot error). The covariance structure varied with model. The final model chosen for each variable produced the smallest Akaike's information criteria (AIC). The covariance structures included: autoregressive with and without heterogeneity, compound symmetry with and without heterogeneity, and Toeplitz.

Non-repeated traits including ECM, milk components, feed efficiency (ratio of ECM to DMI), and CL volume on d 8 were analyzed by ANOVA (GLM procedure) by applying a model that consisted of treatment, parity, and their interaction. The models for ECM and all milk components also included a pretreatment milk component sample (covariate) collected 3 d before the onset of dietary treatments. A priori orthogonal contrasts (for unevenly spaced treatment doses) were constructed to determine linear and quadratic effects of treatment. In addition, an a priori contrast of the control vs. the combined 3 RPG doses was tested.

Binomial data (pregnancy risk) were analyzed by logistic regression (procedure GLIMMIX) using the model of treatment, parity, and their interaction. Options used in the model statement included LINK = LOGIT, DIST = BINOMIAL, and the least square means option of ILINK and DIFF. A priori contrasts were conducted to compare the control with each of the 3 treatment doses.

In all cases, statistical significance of treatment effects was set as $P < 0.05$, with tendencies as $0.05 < P < 0.10$.

RESULTS

Feed Intake, Milk Production, and Milk Composition

Dry matter intake, milk yield, and feed efficiency are summarized in Table 4-2. As anticipated, starch intake decreased ($P < 0.01$) linearly with increasing dose of RPG. In contrast, crude protein and ethanol-soluble carbohydrate intake increased ($P < 0.01$) linearly with increasing dose of RPG. Neither milk nor ECM yield were impacted by RPG dose, however, a day effect was detected ($P < 0.01$) for milk production increases and decreases throughout the trial. On d 8, DMI did not differ among treatments, however, a concaved-up quadratic effect ($P = 0.02$) for ECM:DMI was detected indicating that the control and 4-kg dose of RPG produced the poorest feed efficiency (Table 4-2).

Milk components are summarized in Table 4-3. Dose of RPG had no effect on yields of milk fat, lactose, or SCC. Milk protein content, however, tended ($P = 0.10$) to differ among treatment doses. A treatment dose effect and linear effect of dose was observed ($P < 0.01$) for MUN concentration.

Metabolic Blood Traits

The difference in post- and pre-prandial concentrations of glucose and insulin are shown in Figure 4-2. As anticipated, pre-prandial concentrations of glucose ($P = 0.81$) and insulin ($P = 0.49$) did not differ among treatments; however, postprandial glucose decreased ($P < 0.01$) from d 0 through 4. The change in concentration of insulin (postprandial minus pre-prandial) on d 0, 2, and 4 was greater ($P < 0.01$) for control cows compared with cows supplemented with any dose of RPG (Figure 4-2A), but there was no effect of day ($P = 0.40$) or the interaction of treatment and day ($P = 0.66$). No effect of treatment on glucose concentration was detected ($P = 0.30$), but there tended ($P = 0.07$) to be a day effect for change in glucose on d 0, 2, and 4 (Figure 4-2B). No interaction of treatment and day was detected ($P = 0.66$) for post-feeding change in glucose.

Reproductive Traits

Concentrations of progesterone increased from d 2 to 12 but did not differ among treatment doses (Figure 4-3). The pattern of multiple samples assessed for concentrations of progesterone during a 24-h period on d 8 fit a 4^{th}-order polynomial curve ($R^2 = 0.97$; Figure 4-4). Treatment did not differ during the 24-h period, but an hour effect ($P < 0.01$) was observed. Volume of the CL on d 8 of the estrous cycle averaged 9.5 ± 1.5, 10.3 ± 1.5, 9.4 ± 1.5, and 12.0 ± 1.4 cm^3 for 0, 1, 2, and 4 kg RPG treatments, respectively, but did not differ among treatments. Although this experiment was not designed with sufficient power to detect differences in pregnancy risk, pregnancy risk (69.2%) was greater ($P < 0.01$) for control cows compared with all RPG-treated cows; Pregnancy risk was lesser for 1 kg (14.2%), 2 kg (42.9%), and 4 kg (25%) doses of RPG.

DISCUSSION

The present experiment tested the hypothesis that circulating progesterone concentrations would be increased by replacing corn grain with a rumen-protected glucose product. The intent was to increase insulin concentrations and thereby inhibit activity of hepatic progesterone-inactivating enzymes. Progesterone inactivation is a two-phase process in hepatocytes. Phase one of steroid biotransformation involves the cytochrome P450 superfamily of enzymes and aldo-keto reductase enzymes that add hydroxyl groups to the steroid nucleus (Lemley et al., 2010). During phase two of steroid inactivation, uridine diphosphate-glucuronosyl transferase (UGT) conjugates the hydroxyl-steroid metabolites with glucuronic acid (Hart et al., 2018). Because steroids are extremely lipophilic, they are difficult for animals to excrete in urine or feces. The process of steroid inactivation allows an animal to excrete more easily the inactivated steroid (Hart et al., 2018).

Barnett et al. (1990) were first to propose a relationship between cytochrome P450 expression and insulin when they observed insulin-dependent rats had increased expression of cytochrome P450 3A, which was reversed by administering insulin. When a murine hepatocyte cell-line was challenged with increasing physiological concentrations of insulin, a dose-dependent decrease in progesterone decay was observed (Smith et al., 2006). Similarly, Lemley et al. (2009) observed a decrease in activities of cytochrome P450 3A and cytochrome P450 2C when hepatocytes were challenged with insulin. Results from in vivo research showed decreased progesterone clearance when anestrous ewes were orally drenched with a glucogenic substrate compared with those drenched with an isoenergetic control (Smith et al., 2006). Others have observed approximately a 50% decrease in hepatic cytochrome P450 2C and cytochrome P450 3A activity 1 h after supplementing a glucogenic compound to ovariectomized ewes (Lemley et al., 2008).

A previous study indicated a 33% increase in insulin concentrations, a 27% increase in concentrations of progesterone, and a 33% increase in pregnancy risk in cattle drenched daily with 250 ml of propylene glycol (Hidalgo et al., 2004). Another report demonstrated an increase in plasma progesterone concentrations 1 h post-feeding in cows with increased insulin compared with low insulin cows, but at 5 h post-feeding this effect was reversed (Moriel et al., 2008). In the current experiment, concentrations of progesterone increased linearly with day of the estrous cycle but did not differ among treatments. A possible explanation for not observing a treatment effect could be that the RPG was more ruminally available than anticipated. The primary goal of the current study was not to test the rumen degradability of RPG. The RPG product was produced by combining a carbohydrate with non-protein nitrogen under appropriate conditions to cause a Maillard Reaction to occur (Russi, 2013). A Maillard reaction involves the condensation of sugar residues with amino acids followed by polymerization to form a brown-colored substance that possesses many of the same physical properties of lignin (van Soest and Mason, 1991). Proteins are rendered indigestible during the condensation reaction.

Diurnal variation in progesterone was observed in the current experiment. This finding contradicts another report in which heifers were sampled for concentrations of progesterone and no diurnal variation was observed (Hannan et al., 2010). A possible explanation for this difference could be the effect of greater metabolism and elevated feed intake associated with lactation and the observed increase in liver blood flow and metabolism of progesterone and estradiol in lactating dairy cattle (Sangsritavong et al., 2002).

Increasing endogenous progesterone may prevent pregnancy loss during early gestation. Corpus luteum volume on d 8 did not differ among treatments in the current study. Day 8 was selected to measure CL volume because CL reach maximum growth between d 8 and 12 of the estrous cycle (Ginther et al., 1989). This is the first study to report CL volume when manipulating the energy source in a diet. A previous study demonstrated greater pregnancy retention to week 7 of gestation in dairy cows classified as having increased rather than decreased progesterone at week 5 of gestation (Starbuck et al., 2004). Furthermore, progesterone concentrations are predictive of future pregnancy loss between d 26 and 71 of gestation in lactating dairy cows (Stevenson et al., 2008). Others have reported increased pregnancy risk when cows were supplemented with progesterone via an intravaginal progesterone insert during the second week of pregnancy or after treatment with hCG on d 5 to 7 post-AI, which induces a new CL (Stevenson et al., 2007; Nascimento et al., 2013; Garcia-Ispierto and Lopez-Gatius, 2017).

It has been well established that an increase in insulin decreases the expression of cytochrome P450 enzymes, which are responsible for clearance of blood progesterone (Barnett et al., 1990; Saad et al., 1994). Previous research found increased peripheral concentrations of progesterone in repeat-breeding cows treated with long-acting bovine insulin (Selvaraju et al., 2002). Researchers have administered increasing glucogenic nutrients by various methods to increase circulating insulin. Vieira et al. (2010) found infusing glucose i.v. increased insulin concentrations in cows in positive or negative energy balance. Other research has indicated that feeding high-moisture corn increased insulin concentrations compared with cows offered finely or coarsely ground corn (Moriel et al., 2008). Garnsworthy et al. (2008) altered energy sources in isoenergetic and isonitrogenous dairy rations resulting in greater concentrations of insulin in cows fed starch from 9 to 16% in the form of wheat. Likewise, Lemley et al. (2010) found feeding a diet with 35.3 ± 1.0 % starch compared with 19.2 ± 0.4% caused an increase in insulin, probably because of increased propionate availability for hepatic gluconeogenesis.

Providing a rumen-available starch source in the present study resulted in a greater change in concentrations of insulin compared with rumen-protected glucose for post-ruminal absorption. A possible explanation for this response suggests that a slower passage of protected glucose into the small intestine for absorption occurred compared with a rumen-available starch source that was more rapidly fermented to propionate. It is known that propionate is a strong insulin secretagogue (Leuvenink et al., 1997; Bradford et al., 2006), and is the primary glucose precursor in ruminants for gluconeogenesis (Bergman, 1990).

Previous research focused on increasing rumen-available starch, which is then available for fermentation to propionate, to increase concentrations of insulin. Pereira et al. (1999) replaced dry, cracked corn with different nonforage fiber sources and reported an increase in DMI, milk yield, milk fat yield, and milk fat percentage. Lemley et al. (2010) reported an increase in milk protein percentage when cows were fed a high corn starch compared with a high fiber diet. Mammary epithelial cells exhibit responsiveness to insulin, even though insulin does not regulate glucose uptake by the mammary gland (Shukla et al., 2009). Previous research observed an increase in milk protein content and a tendency to have increased milk protein yield when cows were treated with long-acting insulin (Winkelman and Overton, 2013). In the current experiment, RPG supplementation did not impact DMI, milk yield, milk fat, or milk protein. A possible explanation for this observation could be that majority of the RPG was available to the lower gut, which might have had a slower absorption rate as it passed into the small intestine, resulting in a less intensive insulin response compared with the readily fermentable starch sources tested in the previous literature.

Urea can be used as a sensitive index of protein utilization efficiency and is a good indicator of energy or protein imbalance (Kenny et al., 2002). The small molecular size of urea allows it to easily circulate in all fluids, and concentrations are well correlated in milk and blood (Butler et al., 1996). Evaluating the nitrogen utilization in a diet for cattle can be done using both milk and blood urea concentrations (Oltner and Wiktorsson, 1983; Baker et al., 1995). A linear increase in MUN in the present study was observed with increasing dose of RPG supplementation, which resulted in compromised pregnancy risk for cows fed the RPG compared with control cows, most likely resulting from increased BUN (Butler et al., 1996).

Fermentable carbohydrates are necessary for improved utilization of non-protein nitrogen (Johnson, 1976). Rumen-protected glucose replaced a portion of the fermentable carbohydrate source in the current experiment. In addition, the process of protection for the glucose product likely contributed to the increase in non-protein nitrogen observed in the current experiment (Russi, 2013). Previous research indicated urea concentrations ≥ 19.3 mg/dL resulted in 43% lesser odds of conception, and this association is stronger when the cow is exposed to elevated urea concentrations before AI (Raboisson et al., 2017).

CONCLUSIONS

Fertility continues to be a leading economic concern for the dairy industry. In the current study we focused on reducing progesterone clearance by supplementing a rumen-protected glucose source. The insulin response was diminished with RPG diets relative to the control. Supplementation with RPG caused a linear increase in CP intake and MUN with increasing dose. Rumen-protected glucose did not impact concentrations of progesterone, milk yield, or DMI.

REFERENCES

Armstrong, J. D., E. A. Goodall, F. J. Gordon, D. A. Rice, and W. J. McCaughey. 1990. The effects of levels of concentrate offered and inclusion of maize gluten or fish meal in the concentrate on reproductive performance and blood parameters of dairy cows. Anim. Prod. 50:1-10.

Baker, L. D., J. D. Ferguson, and W. Chalupa. 1995. Responses in urea, and true protein of milk to different protein feeding schemes for dairy cows. J. Dairy Sci. 78:2424-2434.

Barnett, C. R., G. G. Gibson, C. R. Wolf, P. R. Flatt, and C. Ioannides. 1990. Induction of cytochrome P450II and P450IV family proteins in streptozotocin-induced diabetes. Biochem. J. 268:765-769.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804–2819.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70:567-590.

Bradford, B. J., A. D. Gour, A. S. Nash, and M. S. Allen. 2006. Propionate challenge tests have limited value for investigating bovine metabolism. J. Nutr. 136:1915-1920.

Butler, W. R., J. J. Calaman, and S. W. Beam. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J. Anim. Sci. 81:2533-2539.

Carroll, D. J., M. J. Jerred, R. R. Gummer, D. K. Combs, R. A. Pierson, and E. R. Hauser. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratio on plasma progesterone, energy balance, and reproductive traits of dairy cattle. J. Dairy Sci. 73:2855-2863.

Csapo, A. 1956. Progesterone ‘block.’ Am. J. Anat. 98:273-291.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptions of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84(E. Suppl.):E100–112.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk, and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66:1128–1147.

Garcia-Ispierto, I., and F. López-Gatius. 2017. Progesterone supplementation in the early luteal phase after artificial insemination improves conception rates in high-producing dairy cows. Theriogenology 90:20-24.

Garnsworthy, P. C., A. Lock, G. E. Mann, K. D. Sinclair, and R. Webb. 2008. Nutrition, metabolism, and fertility in dairy cows: 1. Dietary energy source and ovarian function. J. Dairy Sci. 91:3814-3823.

Garverick, H. A., M. N. Harris, R. Vogel-Bluel, J. D. Sampson, J. Bader, W. R. Lamberson, J. N. Spain, M. C. Lucy, and R. S. Youngquist. 2013. Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. J. Dairy Sci. 96:181–188.

Ginther, O. J., L. Knopf, and J. P. Kastelic. 1989. Temporal associations among ovarian events in cattle during estrous cycles with two and three follicular waves. Reproduction 87:223-230.

Gong, J. G., W. J. Lee, P. C. Garnsworthy, and R. Webb. 2002. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 123:419-427.

Green, J. C., J. P. Meyer, A. M. Williams, E. M. Newsom, D. H. Keisler, and M. C. Lucy. 2012. Pregnancy development from day 28 to 42 of gestation in postpartum Holstein cows that were either milked (lactating) or not milked (not lactating) after calving. Reproduction 143:699–711.

Hannan, M. A., M. J. Fuenzalida, M. A. R. Siddiqui, M. Shamsuddin, M. A. Beg, and O. J. Ginther. 2010. Diurnal variation in LH and temporal relationships between oscillations in LH and progesterone during the luteal phase in heifers. Theriogenology 74:1491-1498.

Hart, C. G., B. E. Voelz, K. E. Brockus, and C. O. Lemley. 2018. Hepatic steroid inactivating enzymes, hepatic portal blood flow and corpus luteum blood perfusion in cattle. Reprod. Dom. Anim. 53:751-758.

Hidalgo, C. O., E. Gómez, L. Prieto, P. Duque, F. Goyache, L. Fernández, I. Fernández, N. Facal, and C. Díez. 2004. Pregnancy rates and metabolic profiles in cattle treated with propylene glycol prior to embryo transfer. Theriogenology 62:664-676.

Hill, S. L., D. M. Grieger, K. C. Olson, J. R. Jaeger, C. R. Dahlen, M. R. Crosswhite, N. Negrin Pereira, S. R. Underdahl, B. W. Neville, J. Ahola, M. C. Fischer, G. E. Seidel, and J. S. Stevenson. 2016. Gonadotropin-releasing hormone increased pregnancy risk in suckled beef cows not detected in estrus and subjected to a split-time artificial insemination program. J. Anim. Sci. 94:3722–3728.

Inskeep, E. K., and R. A. Dailey. 2005. Embryonic death in cattle. Vet. Clin. Food Anim. 21:171-174.

Johnson, R. R. 1976. Influence of carbohydrate solubility on non-protein nitrogen utilization in the ruminant. J. Animal Sci. 43:184-191.

Kenny, D. A., P. G. Humpherson, H. J. Leese, and D. G. Altma. 2003. Measuring inconsistency in meta-analyses. Brit. Med. J. 327:557-560.

Larson, S. F., W. R. Butler, and W. B. Currie. 2007. Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. Anim. Reprod. Sci. 102:172-179.

Lemley, C. O., J. M. Koch, K. P. Blemings, K. M. Krause, and M. E. Wilson. 2008. Concomitant changes in progesterone catabolic enzymes, cytochrome P450 2C and 3A, with plasma insulin concentrations in ewes supplemented with sodium acetate or sodium propionate. Animal 2:1223-1229.

Lemley, C. O., J. M. Koch, K. P. Blemings, and M. E. Wilson. 2009. Alterations in progesterone catabolic enzymes, CYP2C and CYP3A, in hepatocytes challenged with insulin and glucagon. J. Anim. Vet. Adv. 8:39-46.

Lemley, C. O., T. A. Wilmoth, L. R. Tager, K. M. Krause, and M. E. Wilson. 2010. Effect of a high cornstarch diet on hepatic cytochrome P450 2C and 3A activity and progesterone half-life in dairy cows. J. Dairy Sci. 93:1012-1021.

Leuvenink, H. G., E. J. Bleumer, L. J. Bongers, J. van Bruchem, D. van der Heide. 1997. Effect of short-term propionate infusion on feed intake and blood parameters in sheep. Am. J. Physiol. 272:E997-1001.

Lucy, M. C., R. C. Escalante, D. H. Keisler, W. R. Lamberson, and D. J. Mathew. 2013. Short communication: glucose infusion into early postpartum cows defines an upper physiological set point for blood glucose and causes rapid and reversible changes in blood hormones and metabolites. J. Dairy Sci. 96:5762–5768.

Miyoshi, S., J. L. Pate, and D. L. Palmquist. 2001. Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. Anim. Reprod. Sci. 68:29-43.

Moriel, P., T. S. Scatena, O. G. Sa Filho, R. F. Cooke, and J. L. M. Vasconcelos. 2008. Concentrations of progesterone and insulin in serum of nonlactating dairy cows in response to carbohydrate source and processing. J. Dairy Sci. 91:4616-4621.

Mullen, M. P., F. W. Bazer, G. Wu, M. H. Parr, A. C. O. Evans, M. A. Crowe, and M. G. Diskin. 2014. Effects of systemic progesterone during the early luteal phase on the availabilities of amino acids and glucose in the bovine uterine lumen. Reprod. Fertil. Dev. 26:282-292.

Nascimento, A. B., R. W. Bender, A. H. Souza, H. Ayres, R. R. Araujo, J. N. Guenther, R. Sartori, and M. C. Wiltbank. 2013. Effect of treatment with human chorionic gonadotropin on day 5 after timed artificial insemination on fertility of lactating dairy cows. J. Dairy Sci. 96:2873-2882.

Oldick, B. S., C. R. Staples, W. W. Thatcher, and P. Gyawu. 1997. Abomasal infusion of glucose and fat-Effect on digestion, production, and ovarian and uterine functions of cows. J. Dairy Sci. 80:1315-1328.

Oltner, R., and H. Wiktorsson. 1983. Urea concentrations in milk and blood as influenced by feeding varying amounts of protein and energy to dairy cows. Livest. Prod. Sci. 10:457-467.

Pereira, M. N., E. F. Garrett, G. R. Oetzel, and L. E. Armentanto. 1999. Partial replacement of forage with nonforage fiber sources in lactating cow diets. I. Performance and health. J. Dairy Sci. 82:2716-2730.

Raboisson, D., A. Albaaj, G. Nonne, and G. Foucras. 2017. High urea and pregnancy or conception in dairy cows: A meta-analysis to define the appropriate urea threshold. J. Dairy Sci. 100:7581-7587.

Russi, J. P. 2013. Energy supplement for ruminant animals. United States Patent 8,507,025 B2.

Saad, B., H. Thomas, H. Schawalder, F. Waechter, and P. Maier. 1994. Oxygen tension, insulin, and glucagon affect the preservation and induction of cytochrome P450 isoforms in cultured rat hepatocytes. Toxicol. Appl. Pharmacol. 126:372-379.

Sangsritavong, S., D. K. Combs, R. Sartori, L. E. Armentano, and M. C. Wiltbank. 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J. Dairy Sci. 85:2831-2842.

Schukken, Y. H., D. J. Wilson, F. Welcome, L. Garrison-Tikofsky, and R. N. Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell counts. Vet. Res. 34:579-596.

Selvaraju, S., S. K. Agarwal, S. D. Karche, S. K. Srivastava, A. C. Majumdar, and U. Shanker. 2002. Fertility responses and hormonal profiles in repeat breeding cows treated with insulin. Anim. Reprod. Sci. 73:141-149.

Shukla, A., J. Grisouard, V. Ehemann, A. Hermani, H. Enzmann, and D. Mayer. 2009. Analysis of signaling pathways related to cell proliferation stimulated by insulin analogs in human mammary epithelial cell lines. Endocr. Relat. Cancer 16:429-441.

Smith, D. L., B. M. Stinefelt, K. P. Blemings, and M. E. Wilson. 2006. Diet-induced alterations in progesterone clearance appear to be mediated by insulin signaling in hepatocytes. J. Anim. Sci. 84:1102-1109.

Starbuck, M. J., R. A. Dailey, and E. K. Inskeep. 2004. Factors affecting retention of early pregnancy in dairy cattle. Anim. Reprod. Sci. 84:27-39.

Stevenson, J. S. 2016. Physiological predictors of ovulation and pregnancy risk in a fixed-time artificial insemination program. J. Dairy Sci. 99:10077–10092.

Stevenson, J. S., M. A. Portaluppi, D. E. Tenhouse, A. Lloyd, D. R. Eborn, S. Kacuba, and J. M. DeJarnette. 2007. Interventions after artificial insemination: Conception rates, pregnancy survival, and ovarian responses to gonadotropin-releasing hormones, human chorionic gonadotropin, and progesterone. J. Dairy Sci. 90:331-340.

Stevenson, J. S., S. M. Tiffany, and E. K. Inskeep. 2008. Maintenance of pregnancy in dairy cattle after treatment with human chorionic gonadotropin or gonadotropin-releasing hormone. J. Dairy Sci. 91:3092-3101.

Van Knegsel, A. T. M., H. van den Brand, J. Dijkstra, W. M. van Straalen, R. Jorritsma, S. Tamminga, and B. Kemp. 2007. Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. J. Dairy Sci. 90:3397-3409.

Van Soest, P. J., and V. C. Mason. 1991. The influence of the Maillard reaction upon the nutritive value of fibrous feeds. Anim. Feed Sci. and Tech. 32:45-53.

Vieira, F. V. R., C. N. Lopes, B. I. Cappellozza, A. B. Scarpa, R. F. Cooke, and J. L. M. Vasconcelos. 2010. Effects of intravenous glucose infusion and nutritional balance on serum concentrations of nonesterified fatty acids, glucose, insulin, and progesterone in lactating dairy cows. J. Dairy Sci. 93:3047–3055.

Wathes, D. C., Z. Cheng, M. A. Fenwick, R. Fitzpatrick, and J. Paton. 2011. Influence of energy balance on somatotrophic axis and metalloproteinase expression in the endometrium of the postpartum dairy cow. Reproduction 141:269–281.

Winkelman, L. A., and T. R. Overton. 2013. Long-acting insulins alter milk composition and metabolism of lactating dairy cows. J. Dairy Sci. 96:7565-7577.

Table 4-1. Ingredient and nutritional composition of the basal diet[1]

Ingredients	% DM		
Corn silage	22.5		
Triticale silage	15.0		
Alfalfa hay[2]	3.1		
Alfalfa hay[3]	3.1		
Corn gluten feed[4]	22.8		
Whole cottonseed	4.0		
Corn grain, finely ground	13.4		
Concentrate mix[5]	16.1		
Nutrient, % of DM (unless otherwise specified)			
	Basal diet	Ground corn	RPG[6]
DM, % as-fed	47.8	86.3	82.9
CP	18.2	9.8	43.2
ADF	23.9	3.9	4.9
aNDF	36.5	9.8	20.2
Starch	12.3	69.7	1.1
Ethanol-soluble carbohydrates (simple sugars)	8.1	5.5	38.6
TDN	70.0	88.0	81.0
NE_L, Mcal/kg	1.65	2.08	1.90

[1]Nutrient composition values presented are results of NIR analysis of the basal diet.
[2] Lower quality alfalfa with 22.1% CP.
[3] Higher quality alfalfa with 23.9% CP.
[4] Sweet Bran (Cargill Inc., Blair, NE).
[5] Postpartum concentrate premix consisted of 59.9% expeller soybean meal (SoyBest, Grain States Soya, West Point, NE), 12.0% limestone, 10.5% sodium bicarbonate, 7.48% Ca salts of long-chain fatty acids (Megalac R, Arm & Hammer Animal Nutrition, Princeton, NJ), 2.40% magnesium oxide, 2.14% of a 1.50% stock salt, 1.50% trace mineral salt, 1.50% potassium chloride, 1.50% vitamin E (20 kIU/g), 0.94% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), 0.25% selenium premix (0.06%), 0.23% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.15% vitamin A premix (30 kIU/g), 0.12% Zinpro 120 (Zinpro Corp.), 0.06% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.04% vitamin D premix (30,000 IU/g), 0.01% ethylenediamine dihydriodide premix (3.65% I).
[6] Rumen-protected glucose product.

Table 4-2. Dry matter intake, ECM, and feed efficiency in cows supplemented with varying doses of rumen-protected glucose (RPG) in place of ground corn.

	Treatment[1], kg					*P*-value		
	0	1	2	4				
Item	n = 13	n = 15	n = 15	n = 16	SEM	RPG[2]	L[3]	Q[3]
Starch intake, kg/d	5.47	4.93	4.26	3.30	0.11	<0.01	<0.01	0.06
Crude protein intake, kg/d	5.19	5.61	5.82	6.84	0.17	<0.01	<0.01	0.09
ESC[4] intake, kg/d	1.79	2.13	2.41	3.15	0.06	<0.01	<0.01	<0.01
DMI, kg	24.3	24.8	24.0	25.5	0.74	0.62	0.42	0.51
Milk[5], kg/d	49.9	50.6	48.9	50.5	1.27	0.96	0.88	0.57
ECM[6], kg/d	47.8	49.5	48.9	49.0	1.10	0.29	0.60	0.46
ECM:DMI	1.56	1.60	1.72	1.57	0.05	0.21	0.79	0.02

[1] Lactating dairy cows were supplemented with either 0 (control), 1, 2, or 4 kg of a rumen-protected glucose product in replacement of finely ground corn grain.
[2] A priori contrasts of the 0 kg (control) were compared with the combined 3 treatment means.
[3] A priori orthogonal contrasts for unevenly spaced treatment doses to determine linear (L) and quadratic (Q) effects.
[4] Free ethanol-soluble carbohydrates.
[5] Mean milk production from day 0 through d 12. Treatment did not impact milk production, but a day effect was detected ($P < 0.01$).
[6] Energy-corrected milk (ECM) was calculated from milk samples collected on d 8 of the estrous cycle.

Table 4-3. Milk components for cows supplemented with varying doses of rumen-protected glucose (RPG) in place of ground corn.

	Treatment[1], kg					*P*-value		
Item	0	1	2	4	SEM	RPG[2]	L[3]	Q[3]
Fat, %	3.8	3.9	4.0	4.0	0.1	0.20	0.36	0.72
Fat, kg/d	1.8	2.1	1.9	2.1	0.1	0.29	0.28	0.65
Lactose, %	5.0	5.0	5.0	5.0	0.1	0.81	0.78	0.49
Lactose, kg/d	2.5	2.7	2.4	2.5	0.1	0.48	0.76	0.87
MUN, mg/dL	15.3	16.7	17.4	20.1	0.6	< 0.01	<0.01	0.73
Protein, %	2.7	2.6	2.7	2.6	0.1	0.10	0.15	0.74
Protein, kg/d	1.3	1.4	1.3	1.3	0.1	0.79	0.67	0.95
SNF, %	8.5	8.4	8.5	8.4	0.1	0.19	0.29	0.66
SNF, kg/d	4.2	4.5	4.1	4.2	0.1	0.74	0.50	0.81
SCLS[4]	3.5	3.1	3.4	3.2	0.2	0.94	0.83	0.73

[1] Lactating dairy cows were supplemented with either 0 (control), 1, 2, or 4 kg of a rumen-protected glucose product in replacement of finely-ground corn grain.
[2] A priori contrasts of the 0 kg (control) were compared with the combined 3 treatment means.
[3] A priori orthogonal contrasts for unevenly spaced treatment amounts to determine linear (L) and quadratic (Q) effects of treatment.
[4]Somatic cell linear score (SCLS) = $\log_2$(somatic cell count/100) + 3

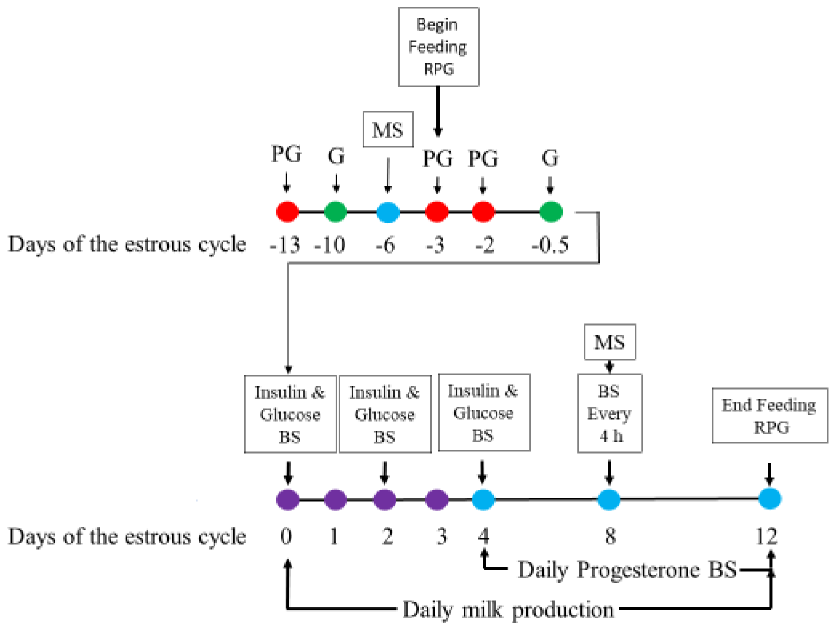

Figure 4-1. Illustration of the ovulation-synchronization scheme, blood sampling (BS) and milk sampling (MS) schedule for cows supplemented with rumen-protected glucose at varying doses in place of ground corn. Ovulation was synchronized with an injection of $PGF_{2\alpha}$ (PG) on d –13, GnRH (G) on d –10, and PG on d –3 and –2. Another injection G was given on d –0.5 to cause ovulation (PG = 25 mg of $PGF_{2\alpha}$; G = 100 µg of GnRH). Blood samples were collected on d 0, 2, and 4 before the morning feeding and again 8 h later to measure insulin and glucose. Progesterone was measured on d 2, and daily from d 4 through 12. Blood also was collected every 4 h on d 8 to assess a potential diurnal pattern of progesterone concentration. Corpus luteum size was measured on d 8. Milk samples were collected on d –6 and 8 to determine milk components. Daily milk production was recorded from day 0 through 12

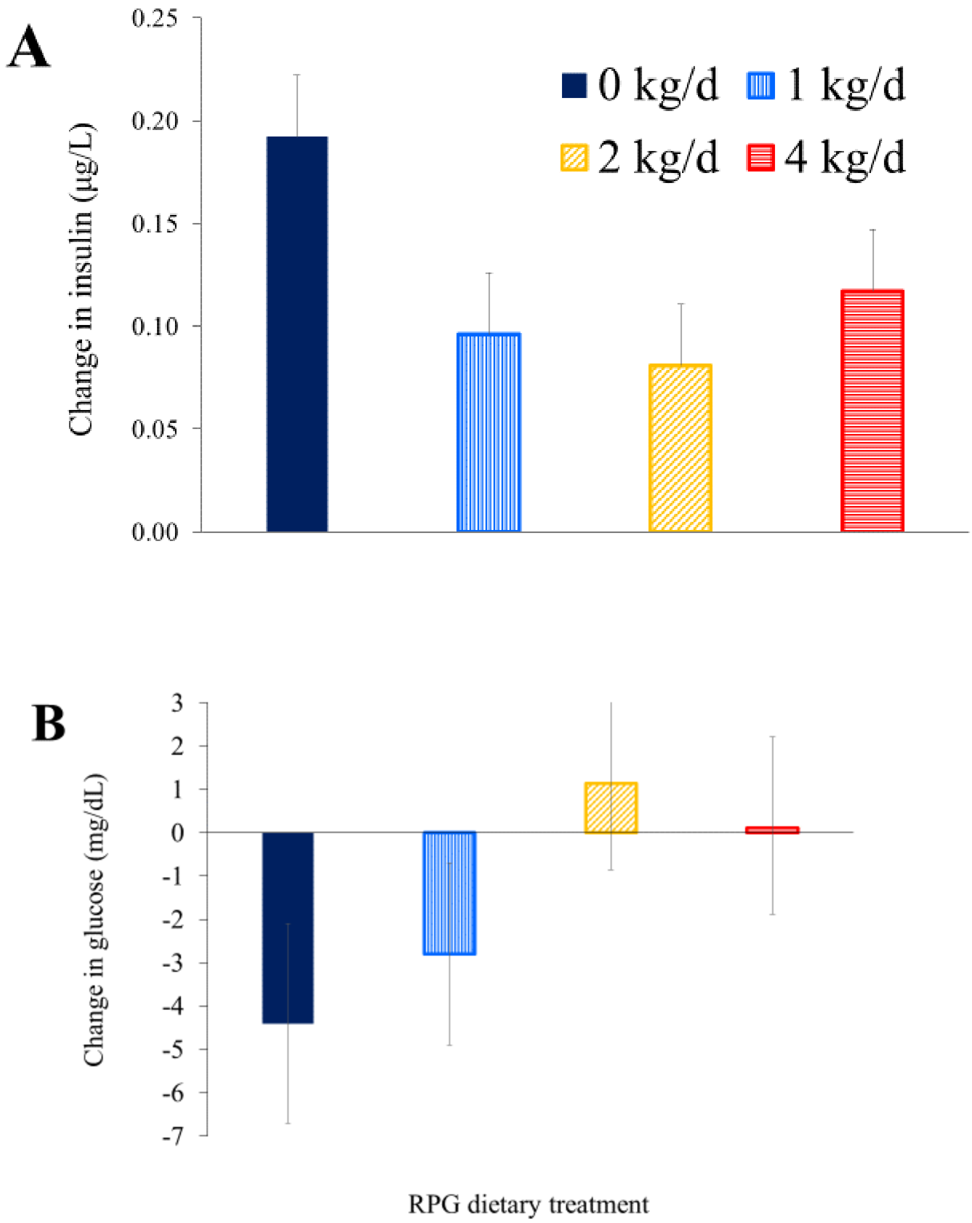

Figure 4-2. Composite change (post feeding "minus" prefeeding) in least squares mean concentrations (± SEM) of insulin (A) and glucose (B) measured in blood samples collected on d 0, 2, and 4 prefeeding and 8 h later (postfeeding) from cows supplemented with rumen-protected glucose at varying doses in place of ground corn. A difference was detected (*P* < 0.01) for the a priori contrasts of LSM for the 0 kg (control; 4 kg of ground corn) compared with the combined LSM of the other 3 treatments, but no effect of day was detected (*P* = 0.30) or an interaction of treatment and day (*P* = 0.84). No differences were detected (*P* = 0.40) among treatments for change in glucose concentration but a day effect tended (*P* = 0.07) to occur. No interaction was detected (*P* = 0.26) between treatment and day for change in glucose concentration.

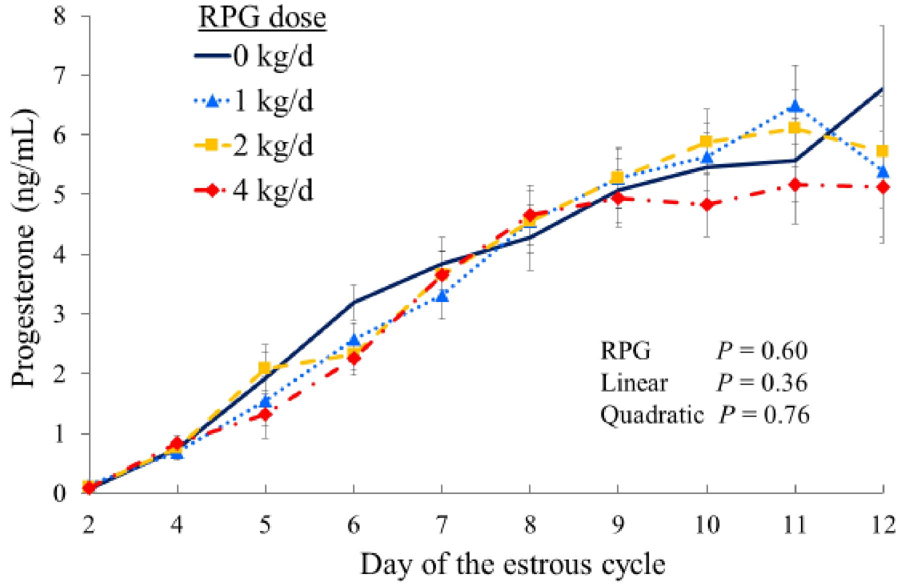

Figure 4-3. Least squares means (± SEM) of daily progesterone in cows supplemented with rumen-protected glucose (RPG) in place of ground corn. Progesterone increased (*P* < 0.01) from d 2 through 12 of the estrous cycle, but was not affected by treatment.

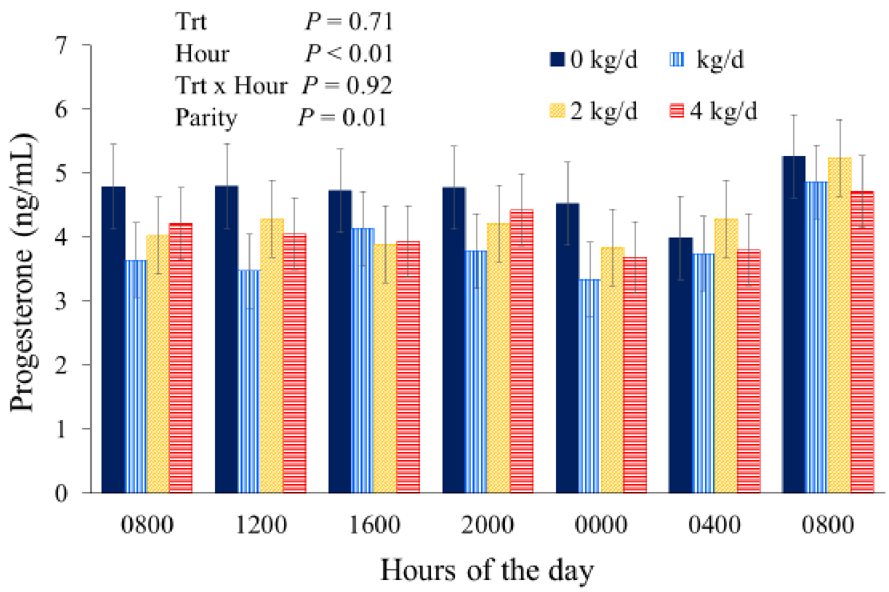

Figure 4-4. Least squares mean (± SEM) of progesterone sampled on d 8 of the estrous cycle in cows supplemented with rumen-protected glucose at varying doses in place of ground corn. The pattern of progesterone on d 8 fit a 4th-order polynomial curve (R^2=0.97). Treatment did not differ during the 24-h period, but there was an effect of hour.

Chapter 5 - Review of Literature- Hormonal strategies to improve fertility

Introduction

Reproductive efficiency is of major importance to the dairy industry (Yaniz et al., 2006). The average estrus-detection rate (< 50%) in most U.S. dairy herds has been identified as a major factor limiting reproductive efficiency (Lopez et al., 2005). Reproductive management strategies have changed during the past 20 years because of the development and implementation of fixed-time artificial insemination (AI) ovulation-synchronization programs (Bisinotto et al., 2014). Early research focused on control of follicle development and the lifespan of the corpus luteum (CL) with the objective to synchronize follicle growth, luteolysis, and ovulation at a predictable time so that timed insemination could occur to optimize pregnancy risk in cattle (Thatcher et al., 1996). Macmillan and Thatcher (1991) first studied the control of follicular waves with gonadotropin-releasing hormone (GnRH) and was partly the basis that led to the development of the Ovsynch program by Pursley et al. (1995). Timed AI programs improved insemination rates for producers struggling with estrus detection (Lopez et al., 2005), as well as optimizing fertility because of the ability to manipulate both follicle growth and luteal lifespan (Moreira et al., 2001; Souza et al., 2008). Timed AI programs have been widely adopted into reproductive management strategies because of the increased insemination risk and pregnancy risk (Caraviello et al., 2006). Ultrasonography is routinely used in the dairy industry to identify and quantify ovarian structures and to diagnose pregnancy (Fricke et al., 2002). Ultrasonography is currently being used to assess which ovulation synchronization program should be implemented based on ovarian structures in cows that require repeat inseminations.

Poor Estrus Detection Risk in Dairy Cows

A major limitation for efficient reproductive success in dairy operations is poor estrus-detection risk and therefore reduced insemination risk in lactating cows (Bisinotto et al., 2014). When estrus detection is the only method employed to inseminate cows, pregnancy risk generally is impacted compared with management programs that implement a systematic control of AI (Pursley et al., 1997; Tenhagen et al., 2004). In an evaluation of 2 dairies when cows were inseminated only after detected estrus, insemination risk averaged 28.6% and 55.6%, but after the incorporation of timed AI into the breeding management program, insemination risk increased to 65.4% and 69.6% (Tenhagen et al., 2004). When only timed AI was employed and there were no inseminations between pregnancy diagnoses, time to pregnancy was reduced by 19 d compared with insemination after estrus detection only (Pursley et al., 1997). Despite the widespread adoption of ovulation-synchronization programs, detection of behavioral estrus still plays an important role in overall reproductive management programs in most U. S. dairies (Caraviello et al., 2006; Fricke et al., 2014).

Detecting estrus is fraught with many challenges. A large portion of dairy cows in the U.S. are housed in free-stall barns with concrete flooring. One of the most important factors affecting sexual activity in lactating Holstein cows is the surface on which they are observed for estrus (Britt et al., 1986). Cows are more likely to express estrus when housed on dirt surfaces rather than on dry grooved concrete surfaces. When cows housed in dirt lots were observed during 30-min observation periods, they had increased estrus duration and showed more total mounts and standing events than when observed on concrete (Britt et al., 1986). Mounting activity has been shown to be 15-fold greater on dirt than on concrete surfaces (Vailes and Britt, 1990).

Genetic selection for high milk yield has been the standard practice in AI sire selection for many decades until recently (Egger-Danner et al., 2015). Milk production impacts estrus expression because cows producing greater quantities of milk have shorter and less intense periods of estrus (Lopez et al., 2005). High-yielding cows have increased metabolic rates and to meet their nutritional requirements they are fed a diet with greater amounts of energy. As a result of these high-energy diets in lactating dairy cattle, liver blood flow is chronically increased and metabolic clearance rate of estradiol and progesterone also is increased (Sangsritavong et al., 2002). Increased metabolic clearance of estradiol decreases expression of estrus as estradiol from the preovulatory follicle is responsible for the initiation of estrus. Concentrations of estradiol were less for high-producing cows ($\geq$ 39.1 kg) compared with low producing cows 1 d before estrus regardless of follicular diameter (Lopez et al., 2005). Therefore, high milk-producing cows are likely predisposed to decreased estrus expression because of decreased concentrations of estradiol. Furthermore, cows having greater daily milk production also have greater luteal tissue, but also have reduced circulating concentrations of progesterone compared with lower producing cows (Lopez et al., 2005).

During heat stress lactating dairy cows have shorter duration and less intense periods of estrus (Gwazdauskas et al., 1981; Younas et al., 1993). Long-term heat stress before day of insemination leads to fewer follicles, reduced concentrations of estradiol in follicles, and earlier emergence of the dominant follicle (Wolfenson et al., 1995; Wolfenson et al., 1997). During periods of heat stress cows are at a greater risk for anestrus and silent ovulation (Gwazdauskas et al., 1981). Because the ovary is regulated by gonadotropins, heat-stressed cows have decreased LH pulse amplitude and LH pulse frequency (Gilad et al., 1993; Wise et al., 1988). As a result of decreased concentrations of LH during times of heat stress, the dominant follicle develops in a compromised LH environment (De Rensis and Scaramuzzi, 2003). A compromised LH environment limits estradiol secretion from the dominant follicle resulting in poor estrus expression (De Rensis and Scaramuzzi, 2003). Because of the challenges associated with estrus expression, dairies have adopted reproductive management strategies that utilize estrus detection and ovulation-synchronization programs.

Development of Ovsynch

Many ovulation synchronization programs exist for timed AI in dairy cattle, but drug use limitations for food animals have restricted the choices to manipulate estrous cycles in many countries (Lane et al., 2008). Pursley et al. (1995) are credited with the first publication of the Ovsynch, an ovulation-synchronization program (GnRH-1 – 7 d – prostaglandin (PG) $F_{2\alpha}$ – 2 d – GnRH2 – 16 h – timed AI). Since this publication, ovulation-synchronization programs using GnRH and $PGF_{2\alpha}$ have been manipulated to improve the control of follicular development, luteal lifespan, and ovulation around the time of AI. Ovulation resulting from injection of GnRH-1 decreases the period of follicular dominance in the subsequently selected dominant follicle and improves synchronization of ovulation and luteolysis, which are associated with increased pregnancy risk (Vasconcelos et al., 1999; Bleach et al., 2004; Santos et al., 2010). Priming of the uterus for the establishment of pregnancy and follicle maturation occur during the period of proestrus (Bisinotto et al., 2014). Complete luteal regression after treatment with $PGF_{2\alpha}$ and timing of the second GnRH injection are vital to expose cows to an adequate period of proestrus for final follicle maturation and uterine priming to occur (Ribeiro et al., 2012). Fertility is associated with the time between the GnRH-2 injection (which induces ovulation) and timed AI (Pursley et al., 1997) because it regulates the time that oocyte and sperm cells are capable of fertilization (Saacke, 2008).

The Ovsynch program does have some constraints on its ability to synchronize follicle growth (Bisinotto et al., 2014). When the injection of GnRH-1 is administered at random stages of the estrous cycle, incidence of ovulation is usually 50 to 60% and can be up to 70% when a presynchronization program precedes the Ovsynch program (Bisinotto and Santos, 2012; Ribeiro et al., 2012). Even when cows were exposed to a Double-Ovsynch program, ovulation to the injection of GnRH-1 in the breeding program was < 72% (Souza et al., 2008; Giordano et al., 2013). To achieve increased pregnancy risk, the concentrations of progesterone on the day of AI need to be < 0.3 ng/mL (Santos et al., 2010). Unfortunately, another limitation of the Ovsynch program is the inability of a single dose of $PGF_{2\alpha}$ to induce complete luteal regression in all cows (Bisinotto et al., 2014). Administering a single dose of $PGF_{2\alpha}$ on d 7 (GnRH-1 = d 0) usually results in only 70 to 84% of the cows with progesterone < 0.3 ng/mL at timed AI (Giordano et al., 2013). Not all cows have synchronized ovulation to the GnRH-2 injection in the Ovsynch program. In fact, only 85% of the cows on average have ovulation and luteolytic success to the second injection of GnRH (Santos et al., 2010), and this number decreases with heat stress (López-Gatius et al., 2005). Disease during the peripartal period further decreases the subsequent response to ovulation-synchronization programs because of increased prevalence of anovulatory cows, reduced risk of fertilization, and impaired embryo development, resulting in decreased pregnancy risk and increased pregnancy loss (Ribeiro et al., 2013; Bisinotto et al., 2014).

Importance of Progesterone before Breeding

Concentrations of progesterone during growth of the dominant follicle influence subsequent pregnancy risk (Bisinotto et al., 2015a). Absence of a CL when an ovulation-synchronization program is initiated is a risk factor for decreased pregnancy risk compared with cows that begin an ovulation-synchronization program bearing a CL (Bisinotto et al., 2010). Nearly 30% of all cows entering an ovulation-synchronization program do not have a CL (Stevenson et al., 2008; Bisinotto et al., 2010). Even when a CL is present, plasma progesterone in high yielding lactating dairy cows is approximately 1.5 ng/mL less than that of heifers (Sartori et al., 2004). Increased growth rate of the dominant follicle, reduced embryo quality, and a decline in pregnancy risk in lactating cows have been associated with reduced concentrations of progesterone during growth of the dominant follicle (Rivera et al., 2011).

Early research showed cows with greater progesterone during the luteal phase before estrus and insemination had increased pregnancy risk compared with cows that had lesser progesterone (Folman et al., 1973; Fonseca et al., 1983). In addition, a decrease in pregnancy risk has been reported in cows enrolled in synchronization programs during the follicular phase of the estrous cycle or during anovulation compared with cows in diestrus (Stevenson et al., 2008; Wiltbank et al., 2011; Denicol et al., 2012). Cows supplemented with progesterone but lacking a CL having concentrations of progesterone > 2.5 ng/mL during the 7-d period after GnRH-1 in a timed AI program had similar pregnancy risk to that of cows with a CL (Bisinotto et al., 2013).

Supplementation of progesterone during an ovulation-synchronization program improves synchrony of ovulation by decreasing the risk of ovulation before the day of timed AI (Bisinotto et al., 2015a). A system of sustained delivery of progesterone is required to mimic that of the CL (Bisinotto et al., 2015b). Commercially available inserts that supplement progesterone, such as the controlled internal drug releasing (CIDR) insert, were initially designed for grazing heifers (Macmillan et al., 1991). The CIDR releases approximately 90 mg of progesterone per d, which causes plasma progesterone to increase between 0.8 to 1 ng/mL in lactating dairy cows bearing a CL (Cerri et al., 2009; Lima et al., 2009). A meta-analysis showed that administration of a CIDR during the 7 d between GnRH-1 and $PGF_{2\alpha}$ in the ovulation-synchronization program increased pregnancy risk by 8 and 10% on d 32 and 60, respectively (Bisinotto et al., 2015a). The primary benefit of progesterone supplementation was observed in cows without a CL at the beginning of a synchronization program when estrus detection was not being utilized (Bisinotto et al., 2015a). Previous research also has indicated that to achieve high fertility a minimum progesterone concentration of 2 ng/mL is required (Bisinotto et al., 2013; 2015b). Bisinotto et al. (2013) reported that providing 2 CIDR inserts to cows lacking a CL at the time of GnRH-1 increased plasma progesterone to 2.65 ng/mL and restored fertility like that of cows in diestrus when the ovulation-synchronization program was initiated. Other studies have reported similar findings (Lima et al., 2009; Denicol et al., 2012). When supplementing progesterone it is recommended that the resulting plasma concentrations should resemble that of a cow in diestrus to impact pregnancy risk, and to achieve that increase in concentration two CIDR inserts may be necessary (Bisinotto et al., 2014).

Addition of a Second $PGF_{2\alpha}$ Treatment

Cows that do not have a CL at the initiation of an ovulation-synchronization program are likely to ovulate to GnRH (Pulley et al., 2015) and have a single 6-d old CL at the induction of luteolysis. This poses a problem because a 6-d old CL may not be fully responsive to $PGF_{2\alpha}$. Achieving complete luteal regression is crucial to maximize subsequent pregnancy risk because progesterone concentrations at or near baseline at the injection of GnRH-2 in a timed AI program affect the GnRH-induced LH release, and consequently, pregnancy risk (Stevenson and Pulley, 2016). A few studies have evaluated the addition of a second treatment of $PGF_{2\alpha}$ 24 h after the initial dose.

Ribeiro et al. (2012) tested a single larger dose of cloprostenol (1 mg) in a 5-d Ovsynch program compared with two doses of cloprostenol (0.5 mg) given 24 h apart. Luteolysis (progesterone < 1 ng/mL 72 h after $PGF_{2\alpha}$) was greater in cows receiving 2 doses compared with 1 dose of cloprostenol (95.9 vs. 72.2%). In a second experiment utilizing dinoprost, cows exposed to a 5-d Ovsynch receiving 2 doses of 25 mg of $PGF_{2\alpha}$ 24 h apart had improved pregnancy risk compared with a single dose of 50 mg of $PGF_{2\alpha}$ (Ribeiro et al., 2012). Barletta et al. (2018) tested the ability of a single 50 mg dose of $PGF_{2\alpha}$ compared with 2 standard doses of $PGF_{2\alpha}$ to improve luteolysis in a 7-d Ovsynch program for cows diagnosed not pregnant. Overall, the proportion of cows with complete luteal regression (progesterone <0.4 ng/mL at 56 h after $PGF_{2\alpha}$) did not differ between treatments; however, an orthogonal contrast between the single 50-mg dose and two, 25-mg doses differed significantly (88 vs. 94%, respectively). Another study treated nonpregnant cows with a 5-d Ovsynch program and tested two standard 25-mg doses of $PGF_{2\alpha}$ 24 h apart compared with a single 50-mg dose (Stevenson et al., 2014). Complete luteal regression (progesterone < 0.5 ng/mL by 72 h after the first or only injection of $PGF_{2\alpha}$) was greater in cows receiving the two standard doses of $PGF_{2\alpha}$.

A review of the literature and meta-analytic assessment was performed to evaluate the effects an additional dose of $PGF_{2\alpha}$ during an Ovsynch program has on luteal regression and reproductive success (Borchardt et al., 2018). Reproductive outcomes of interest included luteal regression at the end of the Ovsynch program and subsequent pregnancy risk at 32 and 39 d after timed AI. The meta-analysis used data from 6 different studies including 5,356 cows, and information for luteal regression was available for 1,856 cows. The relative risk of luteal regression at the end of an Ovsynch program was increased (RR = 1.14; 95% CI = 1.10 to 1.17), in cows that received the second dose of $PGF_{2\alpha}$ on d 8 of an Ovsynch program. Likewise, relative risk for pregnancy 32 d after timed AI also was increased (RR = 1.14; CI = 1.06 to 1.22) in cows that received the second treatment of $PGF_{2\alpha}$. A study that was not included in the meta-analysis enrolled 406 lactating cows in a 2 x 2 factorial comparing the effects of an Ovsynch program duration (5- vs. 7-d) and the dose frequency of $PGF_{2\alpha}$ (2 x 25-mg vs. 1x 50-mg dose; Stevenson et al., 2018). A single dose (50 mg) of $PGF_{2\alpha}$ caused complete luteolysis as effectively as 2 standard doses administered 24 h apart in a 7-d Ovsynch program (Stevenson et al., 2018). In contrast, when utilizing a 5-d Ovsynch program, complete luteal regression determined 72 h after the initial or only $PGF_{2\alpha}$ injection was reduced in cows receiving the single larger dose of $PGF_{2\alpha}$ (85%) compared with the other 3 combinations of dose and Ovsynch duration (> 96%; Stevenson et al., 2018). In addition, pregnancy risk was reduced in cows administered a single dose of $PGF_{2\alpha}$ and enrolled in the 5-d Ovsynch program (Stevenson et al., 2018). The literature indicates a benefit of an additional dose of $PGF_{2\alpha}$ during an Ovsynch program on luteal regression (Borchardt et al., 2018), especially when a 5-d Ovsynch program is utilized (Stevenson et al., 2018).

Accurate Detection of Ovarian Structures

During the past several decades transrectal palpation and ultrasonography have been utilized for diagnosis of pregnancy and ovarian structures in beef and dairy cows (Bicalho et al., 2008). The ovulation-synchronization program chosen may vary depending on the presence of a CL; cows that lack a CL may be given supplemental progesterone (CIDR) and cows with a CL may be administered $PGF_{2\alpha}$ (Chebel et al., 2006; McDougall and Rhodes, 1999; McDougall, 2003). For cows that have been diagnosed not pregnant, administration of treatment based on the presence or absence of a CL is common; cows lacking a CL may receive GnRH and supplemental progesterone as part of the program to resynchronize estrus and ovulation, whereas cows with a CL may receive $PGF_{2\alpha}$ to induce a return to estrus (Chebel et al., 2006; McDougall, 2003). The CL is more responsive to $PGF_{2\alpha}$ to effectively induce luteolysis after d 6 of the estrous cycle (Ott et al., 1986). Therefore, ability to detect a functional CL is critical to the success of $PGF_{2\alpha}$ based ovulation-synchronization programs.

A functional CL has most often been defined by concentration of progesterone in blood serum or milk. Ott et al. (1986) determined the efficacy of two technicians to determine a mature CL by transrectal palpation with the gold standard being serum progesterone concentrations. When concentrations of progesterone were between 2.0 and 11.8 ng/mL, technician agreement on the presence of a CL was 97.2% (35 of the 36 cows). The agreement between technicians decreased (53.8%; 14 of 26 cows) when plasma progesterone concentrations were 0.1 to 1.5 ng/mL. The correlation between detecting a CL and increased progesterone were 91% and 97% for technicians A and B, respectively. These findings indicated, when concentrations of progesterone are increased, that identifying a mature CL by transrectal palpation is more accurate (Ott et al., 1986). When transrectal palpation was compared with an RIA for progesterone in skimmed milk, the overall sensitivity for palpation was 82.6% and specificity was 52.6% for 9 technicians employed to determine the presence of a functional CL (Kelton et al., 1991).

Transrectal ultrasonography has become widespread to diagnosis pregnancy and determine ovarian structures (Fricke, 2002). Previous researchers reported that the positive predictive and negative predictive values of ultrasonography were greater when compared with transrectal palpation (Hanzen et al., 2000). A study utilizing 5 technicians compared transrectal palpation with ultrasonographic examination using the gold standard of serum progesterone at 1.0 ng/mL as the cutoff (Bicalho et al., 2008). Sensitivity and specificity of transrectal palpation to detect a functional CL were 46.6 and 85.1%, respectively, whereas the sensitivity and specificity of ultrasonography was 89.4 and 45.7%, respectively (Bicalho et al., 2008).

In a second experiment, a minimum diameter cutoff for defining a CL as being functional was determined by transrectal ultrasonography (Bicalho et al., 2008). When technicians used a cutoff diameter of > 23 mm at 21 d and > 22 mm at 24 d after AI, both accuracy and specificity increased when identifying functional CL, whereas sensitivity decreased (Bicalho et al., 2008). Ultrasonography resulted in increased sensitivity with decreased specificity; however, implementing a cutoff for CL diameter to reclassify cows as having a CL increased accuracy (Bicalho et al., 2008). The accuracy of utilizing transrectal palpation or ultrasonography to determine ovarian structures varies considerably between technicians and this should be taken into consideration when assigning ovulation-synchronization treatments based on ovarian structures.

Decreasing the Interbreeding Interval

Despite improvements in ovulation-synchronization programs and estrus-detection methods, approximately 50 to 60% of lactating dairy cows fail to conceive after the initial postpartum AI (Cerri et al., 2004; Chebel et al., 2006; Galvão et al., 2007). Because < 60% of cows are re-inseminated before pregnancy diagnosis (depending on the estrus-detection risk), cows that do not become pregnant may have intervals between inseminations longer than the duration of 2 estrous cycles. To avoid an extended inter-insemination interval, increased DIM, and a reduction in milk yield, timely nonpregnancy diagnosis, and resynchronization programs are necessary (Dewey et al., 2010). Because of the need to re-inseminate a substantial proportion of cows after first, service many farms use reproductive management strategies for second and greater services that combine insemination after detected estrus and timed AI after resynchronization with an Ovsynch-type program (Pursley et al., 1995). Ovsynch-type resynchronization programs are usually started 7 d before or at the time of nonpregnancy diagnosis (Chebel et al., 2003; Giordano et al., 2012).

The overall goal of an ovulation-resynchronization program is to decrease the inter-insemination interval and maximize pregnancy risk. In dairy operations conducting pregnancy diagnoses by ultrasound at approximately 32 ± 3 d after AI and enrolling cohorts of cows in a resynchronization program weekly for timed AI, an effective strategy to decrease the interbreeding interval is to initiate the Ovsynch program 25 ± 3 d after AI (Wijma et al., 2017). Beginning an Ovsynch resynchronization program at 25 ± 3 d after AI results in an inter-insemination interval of 35 ± 3 d. Unfortunately, for farms also inseminating cows based on estrus detection, treatment with GnRH at 25 ± 3 d after AI may decrease estrus expression because of GnRH-induced LH release and subsequent ovulation (Wijma et al., 2017). A GnRH-induced LH surge causes ovulation of the dominant follicle (Komar et al., 2001; Jo and Fortune, 2003). After ovulation, the follicle forms a new CL, causing the cow to enter a new luteal phase which suppresses estrus. When GnRH has been administered 17 to 32 d after AI, fewer cows were detected in estrus compared with cows that did not receive GnRH (Chebel et al., 2003; Mendonça et al., 2012; Bruno et al., 2014). Another impact of administering GnRH at 25 ± 3 d after AI is a portion of the cows receiving the GnRH treatment are pregnant. Unnecessary treatment of pregnant cows not only increases the cost, but also increases the burden associated with reproductive management programs (Wijma et al., 2017).

The GnRH-1 injection in an Ovsynch program is intended to induce the emergence of a new follicular wave after ovulation of the dominant follicle and the formation of a new CL (Pursley et al., 1995). It is well established that cows ovulating to GnRH-1 have improved pregnancy risk compared with cows that fail to ovulate (Chebel et al., 2003; Rutigliano et al., 2008; Bisinotto et al., 2010; Giordano et al., 2012). When pregnancy diagnosis occurs at 32 ± 3 d after AI, 60 to 85% of the nonpregnant cows have a functional CL (Giordano et al., 2012, 2015; Bruno et al., 2014). A previous study evaluated removing GnRH-1 at 25 ± 3 d after AI and treating cows with $PGF_{2\alpha}$ at the time of nonpregnancy diagnosis when a CL was determined to be present by transrectal ultrasonography (Wijma et al., 2017). Administration of GnRH at 25 ± 3 d causes suppression of estrus because the resulting GnRH-induced ovulation prevents recurrence of estrus in nonpregnant cows (Mendonça et al., 2012). Pregnancy risk for cows that had a CL at nonpregnancy diagnosis was not different for cows that were administered GnRH at 25 ± 3 d after AI compared with cows that did not receive GnRH (Wijma et al., 2017).

Summary

Estrus detection is fraught with many challenges, and reproductive efficiency is reduced when estrus detection is the only method used to identify cows to inseminate. Ovulation-synchronization programs using GnRH and $PGF_{2\alpha}$ have been widely adopted in the dairy industry to improve reproductive success. Several alterations to Ovsynch can improve its outcomes. Supplementing progesterone to cows without a CL at the initiation of an Ovsynch program has increased pregnancy risk (Lima et al., 2009; Denicol et al., 2012; Bisinotto et al., 2014). An additional treatment with $PGF_{2\alpha}$ has improved the proportion of cows with complete luteal regression by timed AI and subsequent pregnancy risk (Borchardt et al., 2018; Stevenson et al., 2018). Selection of ovulation-synchronization program employed can occur after determining the presence or absence of a CL (Bicalho et al., 2008). Previous research has investigated decreasing the inter-insemination interval by beginning Ovsynch-type synchronization programs before pregnancy diagnosis or by eliminating the GnRH-1 injection and treating with $PGF_{2\alpha}$ when a CL is present at nonpregnancy diagnosis (Wijma et al., 2017). Further research is warranted to decrease the inter-insemination interval.

References

Barletta, R. V., P. D. Carvalho, V. G. Santos, L. F. Melo, C. E. Consentini, A. S. Netto, and P. M. Fricke. 2018. Effect of dose and timing of prostaglandin $F_{2\alpha}$ treatments during a Resynch protocol on luteal regression and fertility to timed artificial insemination in lactating Holstein cows. J. Dairy Sci. 101:1730-1736.

Bicalho, R. C., K. N. Galvão, C. L. Guard, and J. E. P. Santos. 2008. Optimizing the accuracy of detecting a functional corpus luteum in dairy cows. Theriogenology 70:199-207.

Bisinotto, R. S., L. O. Castro, M. B. Pansani, C. D. Narciso, N. Martinez, L. D. P. Sinedino, T. L. C. Pinto, N. S. Van de Burgwal, H. M. Bosman, R. S. Surjus, W. W. Thatcher, and J. E. P. Santos. 2015b. Progesterone supplementation to lactating dairy cows without a corpus luteum at initiation of the Ovsynch protocol. J. Dairy Sci. 98:2515-2528.

Bisinotto, R. S., R. Chebel, and J. Santos. 2010. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. J. Dairy Sci. 93:3578-3587.

Bisinotto, R. S., I. J. Lean, W. W. Thatcher, and J. E. P. Santos. 2015a. Meta-analysis of progesterone supplementation during timed artificial insemination programs in dairy cows. J. Dairy Sci. 98:2472-2487.

Bisinotto, R. S., E. S. Ribeiro, F. S. Lima, N. Martinez, L. F. Greco, L. F. Barbosa, P. P. Bueno, L. F. Scagion, W. W. Thatcher, and J. E. P. Santos. 2013. Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol. J. Dairy Sci. 96:2214-2225.

Bisinotto, R. S., E. S. Ribeiro, and J. E. P. Santos. 2014. Synchronization of ovulation for management of reproduction in dairy cows. Animal 8 (Suppl. 1):151-159.

Bisinotto, R. S., and J. E. P. Santos. 2012. The use of endocrine treatments to improve pregnancy rates in cattle. Reprod. Fertil. Dev. 24:258-266.

Bleach, E. C. L., R. G. Glencross, and P. G. Knight. 2004. Association between ovarian follicle development and pregnancy rates in dairy cows undergoing spontaneous estrous cycle. Reproduction 127:621-629.

Borchardt, S., A. Pohl, P. D. Carvalho, P. M. Fricke, and W. Heuwieser. 2018. Short communication: Effect of adding a second prostaglandin $F_{2\alpha}$ injection during the Ovsynch protocol on luteal regression and fertility in lactating dairy cows: A meta-analysis. J. Dairy Sci. 101:8566-8571.

Britt, J. H., R. G. Scott, J. D. Armstrong, and M. D. Whitacre. 1986. Determinants of estrous behavior in lactating Holstein cows. J. Dairy Sci. 69:2195-2202.

Bruno, R. G., J. Moraes, J. Hernández-Rivera, K. Lager, P. Silva, A. Scanavez, L. Mendonça, R. Chebel, and T. Bilby. 2014. Effect of an Ovsynch56 protocol initiated at different intervals after insemination with or without a presynchronization injection of gonadotropin-releasing hormone on fertility in lactating dairy cows. J. Dairy Sci. 97:185-194.

Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. L. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. J. Dairy Sci. 89:4723-4735.

Cerri, R. L., H. M. Rutigliano, R. G. Bruno, and J. E. P. Santos. 2009. Progesterone concentrations, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. Anim. Reprod. Sci. 110:56-70.

Cerri, R. L. A., J. E. P. Santos, S. O. Juchem, K. N. Galvão, and R. C. Chebel. 2004. Timed artificial insemination with estradiol cypionate or insemination at estrus in high producing dairy cows. J. Dairy Sci. 87:3704-3715.

Chebel, R. C., J. E. Santos, R. L. Cerri, K. N. Galvão, S. O. Juchem, and W. W. Thatcher. 2003. Effect of resynchronization with GnRH on day 21 after artificial insemination on pregnancy rate and pregnancy loss in lactating dairy cows. Theriogenology 60:1389-1399.

Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, H. M. Rutigliano, and R. G. S. Bruno. 2006. Reproduction on dairy cows following progesterone insert presynchronization and resynchronization protocols. J. Dairy Sci. 89:4205-4219.

De Rensis, F., R. J. Scaramuzzi. 2003. Heat stress and seasonal effects on reproduction in the dairy cow, a review. Theriogenology 60:1139-51.

Denicol, A. C., G. Lopez Jr., L. G. Mendonça, F. A. Rivera, F. Guagnini, R. V. Perez, J. R. Lima, R. G. Bruno, J. E. P. Santos, and R. C. Chebel. 2012. Low progesterone concentrations during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. J. Dairy Sci. 95:1794-1806.

Dewey, S. T., L. G. D. Mendonça, G. Lopes Jr., F. A. Rivera, F. Guagnini, R. C. Chebel, and T. R. Bilby. 2010. Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: 1. Pregnancy rates and ovarian responses. J. Dairy Sci. 93:4086-4095.

Egger-Danner C., J. B. Cole, J. E. Pryce, N. Gengler, B. Heringstad, A. Bradley, and K. F. Stock. 2015. Invited review: overview of new traits and phenotyping strategies in dairy cattle with a focus on functional traits. Animal 2015 9:191-207.

Folman, Y., M. Rosenberg, Z. Herz, and M. Davidson. 1973. The relationship between plasma progesterone concentrations and conception in post-partum dairy cows maintained on two levels of nutrition. J. Reprod. Fertil. 34:267-278.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk, and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66:1128-1147.

Fricke, P. M. 2002. Scanning the future-ultrasonography as a reproductive management tool for dairy cattle. J. Dairy Sci. 85:1918-1926.

Fricke, P. M., P. D. Carvalho, J. O. Giordano, A. Valenza, G. Lopes Jr., M. C. Amundson. 2014. Expression and detection of estrus in dairy cows: the role of new technologies. Animal 8:134-143.

Galvão, K. N., J. E. P. Santos, R. L. Cerri, R. C. Chebel, H. M. Rutigliano, R. G. Bruno, and R. C. Bicalho. 2007. Evaluation of methods of resynchronization for insemination in cows of unknown pregnancy status. J. Dairy Sci. 90:4240-4252.

Gilad, E., R. Meidan, A. Berman, Y. Graber, D. Wolfenson. 1993. Effect of heat stress on tonic and GnRH-induced gonadotrophin secretion in relation to concentration of estradiol in plasma of cyclic cows. J. Reprod. Fertil. 99:315-21.

Giordano, J. O., M. Stangaferro, R. Wijma, W. Chandler, and R. Watters. 2015. Reproductive performance of dairy cows managed with a program aimed at increasing insemination of cows in estrus based on increased physical activity and fertility of timed artificial inseminations. J. Dairy Sci. 98:2488-2501.

Giordano, J. O., M. C. Wiltbank, P. M. Fricke, S. Bas, R. Pawlisch, J. N. Guenther, and A. B. Nascimento. 2013. Effect of increasing GnRH and $PGF_{2\alpha}$ dose during double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. Theriogenology 80:773-783.

Giordano, J. O., M. Wiltbank, J. Guenther, R. Pawlisch, S. Bas, A. Cunha, and P. Fricke. 2012. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J. Dairy Sci. 95:639-653.

Gwazdauskas, F. C., W. W. Thatcher, C. A. Kiddy, M. J. Pape, C. J. Wilcox. 1981. Hormonal pattern during heat stress following $PGF_{2\alpha}$-tham salt induced luteal regression in heifers. Theriogenology 16:271-85.

Hanzen, C., M. Pieterse, O. Scenczi, and M. Dorst. 2000. Relative accuracy of the identification of ovarian structures in the cow by ultrasonography and palpation per rectum. Vet. J. 159:161-170.

Jo, M., and J. Fortune. 2003. Changes in oxytocin receptor in bovine preovulatory follicles between the gonadotropin surge and ovulation. Mol. Cell. Endocrinol. 200:31-43.

Kelton, D. F., K. E. Leslie, W. G. Etherington, B. N. Bonnett, and J. S. Walton. 1991. Accuracy of rectal palpation and of a rapid milk progesterone enzyme immunoassay for determining the presence of a functional corpus luteum in subestrous dairy cows. Can. Vet. J. 32:286-291.

Komar, C. M., A. K. Berndtson, A. C. Evans, and J. E. Fortune. 2011. Decline in circulating estradiol during the preovulatory period is correlated with decreases in estradiol and androgen, and in messenger RNA for p450 aromatase and p450 17alpha-hydroxylase, in bovine preovulatory follicles. Biol. Reprod. 64:1797-1805.

Lane, E. A., E. J. Austin, and M. A. Crowe. 2008. Estrous synchronization in cattle-current options following the EU regulations restricting use of estrogenic compounds in food producing animals: a review. Anim. Reprod. Sci. 109:1-16.

Lima, J. R., F. A. Rivera, C. D. Narciso, R. Oliveira, R. C. Chebel, and J. E. Santos. 2009. Effect of increasing amounts of supplemental progesterone in a timed artificial insemination protocol on fertility of lactating dairy cows. J. Dairy Sci. 92:5436-5446.

Lopez, H., D. Z. Caraviello, L. D. Satter, P. M. Fricke, M. C. Wiltbank. 2005. Relationship between level of milk production and multiple ovulations in lactating dairy cows. J. Dairy Sci. 88:2783-93.

López-Gatius, F., M. López Béjar, M. Fenech, and R. H. F. Hunter. 2005. Ovulation failure and double ovulation in dairy cattle: risk factors and effects. Theriogenology 63:1298-1307.

Macmillan, K. L., V. K. Taufa, D. R. Barnes, and A. M. Day. 1991. Plasma progesterone concentrations in heifers and cows treated with a new intravaginal device. Anim. Reprod. Sci. 26:25-40.

Macmillan, K. L., and W. W. Thatcher. 1991. Effects of an agonist of gonadotropin-releasing hormone on ovarian follicles in cattle. Biol. Reprod. 45:883-889.

McDougall, S. 2003. Resynchrony of previously anestrous cows and treatment of cows not detected in estrus that had a palpable corpus luteum with prostaglandin F2 alpha. NZ Vet J. 51:117-124.

McDougall, S., and F. M. Rhodes. 1999. Detection of a corpus luteum in apparently anestrous cows by manual palpation, transrectal ultrasonography, and plasma progesterone concentration. NZ Vet J. 47:47-52.

Mendonça, L. G., S. T. Dewey, G. Lopes, F. A. Rivera, F. S. Guagnini, J. P. Fetrow, T. R. Bilby, and R. C. Chebel. 2012. Effects of resynchronization strategies for lactating Holstein cows on pattern of reinsemination, fertility, and economic outcome. Theriogenology 77:1151-1158.

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

Ott, R. S., K. N. Bretzlaff, and J. E. Hixon. 1986. Comparison of palpable corpora lutea with serum progesterone concentrations in cows. JAVMA 188:1417-1419.

Pulley, S. L., D. Keisler, and J. Stevenson. 2015. Concentrations of luteinizing hormone and ovulatory responses in dairy cows before timed artificial insemination. J. Dairy Sci. 98:6188-6201.

Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80:301-306.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915-923.

Ribeiro, E. S., R. S. Bisinotto, M. G. Favoreto, L. T. Martins, R. L. A. Cerri, F. T. Silvestre, L. F. Greco, W. W. Thatcher, and J. E. P. Santos. 2012. Fertility in dairy cows following presynchronization and administering twice the luteolytic dose of prostaglandin $F_{2\alpha}$ as one or two injections in the 5-day timed artificial insemination protocol. Theriogenology 78:273-284.

Ribeiro, E. S., F. S. Lima, L. F. Greco, R. S. Bisinotto, A. P. A. Monteiro, M. Favoreto, H. Ayres, R. S. Marsola, N. Martinez, W. W. Thatcher, and J. E. P. Santos. 2013. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplements with concentrates. J. Dairy Sci. 96:5682-5697.

Rivera, F. A., L. G. D. Mendonça, G. Lopes Jr., J. E. P. Sanots, R. V. Perez, M. Amstalden, A. Correa-Calderón, and R. C. Chebel. 2011. Reduced progesterone concentrations during growth of the first follicular wave affects embryo quality but has no effect on embryo survival post transfer in lactating dairy cows. Reprod. 141:333-42.

Rutigliano, H. M., F. Lima, R. Cerri, L. Greco, J. Vilela, V. Magalhães, F. Silvestre, W. Thatcher, and J. Santos. 2008. Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. J. Dairy Sci. 91:3323-3336.

Saacke, R. G. 2008. Insemination factors related to timed AI in cattle. Theriogenology 70:479-484.

Sangsritavong, S., D. K. Combs, R. Sartori, L. E. Armentano, M. C. Wiltbank. 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J. Dairy Sci. 85:2831-42.

Santos, J. E. P., C. D. Narciso, F. Rivera, W. W. Thatcher, and R. C. Chebel. 2010. Effect of reducing the period of follicle dominance in a timed AI protocol on reproduction of dairy cows. J. Dairy Sci. 93:2976-2988.

Sartori, R. J. M. Haughian, R. D. Shaver, G. J. M. Rosa, and M. C. Wiltbank. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J. Dairy Sci. 87:905-920.

Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new pre-synchronization system (double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 70:208-215.

Stevenson, J. S., and S. L. Pulley. 2016. Feedback effects of estradiol and progesterone on ovulation and fertility after gonadotropin-releasing hormone-induced release of luteinizing hormone. J. Dairy Sci. 99:3003-3015.

Stevenson, J. S., S. L. Pulley, and S. L. Hill. 2014. Pregnancy outcomes after change in dose delivery prostaglandin $F_{2\alpha}$ in a 5-day timed artificial insemination program in lactating dairy cows. J. Dairy Sci. 97:7586-7594.

Stevenson, J. S., J. A. Sauls, L. G. D. Mendonca, and B. E. Voelz. 2018. Dose frequency of prostaglandin $F_{2\alpha}$ administration to dairy cows exposed to presynchronization and either 5- or 7- day Ovsynch program durations: Ovulatory and luteolytic risk. J. Dairy Sci. 101:9575-9590.

Stevenson, J. S., D. E. Tenhouse, R. L. Krisher, G. C. Lamb, J. E. Larson, C. R. Dahlen, J. R. Pursley, N. M. Bello, P. M. Fricke, M. C. Wiltbank, D. J. Brusveen, M. Burkhart, R. S. Youngquist, and H. A. Garverick. 2008. Detection of anovulation by heat-mount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol. J. Dairy Sci. 91:2901-2915.

Tenhagen, B. A., M. Drillich, R. Surholt, and W. Heuwieser. 2004. Comparison of timed AI after synchronized ovulation to AI at estrus: reproductive and economic considerations. J. Dairy Sci. 87:85-94.

Thatcher, W. W., R. L. de la Sota, E. J. Schmitt, T. C. Diaz, L. Badinga, F. A. Simmen, C. R. Staples, and M. Drost. 1996. Control and management of ovarian follicles in cattle to optimize fertility. Reprod. Fertil. and Devel. 8:203-217.

Vailes, L. D., and J. H. Britt. 1990. Influence of footing surface on mounting and other sexual behaviors of estrual Holstein cows. J. Anim. Sci. 68:2333-39.

Vasconcelos, J. L. M., R. W. Silcox, G. J. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067-1078.

Wijma, R., M. Stangaferro, and J. Giordano. 2015. Characterization of ovarian function in nonpregnant previously inseminated lactating dairy cows. J. Dairy Sci. 98(Suppl. 2):96. (Abstr.)

Wijma, R., M. L. Stangaferro, M. Masello, G. E. Granados, and J. O. Giordano. 2017. Resynchronization of ovulation protocols for dairy cows including or not including gonadotropin-releasing hormone to induce a new follicular wave: Effects on re-insemination pattern, ovarian responses, and pregnancy outcomes. J. Dairy Sci. 100:7613-7625.

Wiltbank, M. C., A. H. Souza, P. D. Carvalho, R. W. Bender, and A. B. Nascimento. 2011. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. Reprod. Fertil. Dev. 24:238-243.

Wise, M. E., D. V. Armstrong, J. T. Huber, R. Hunter, and F. Wiersma. 1988. Hormonal alterations in the lactating dairy cow in response to thermal stress. J. Dairy Sci. 71:2480-2485.

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

Wolfenson, D., W. W. Thatcher, L. Badinga, J. D. Savio, R. Meidan, B. J. Lew, R. Braw-Tal, and A. Berman. 1995. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. Biol. Reprod. 52:1106-1113

Yániz, J. L., P. Santolaria, A. Giribet, and F. López-Gatius. 2006. Factors affecting walking activity at estrus during postpartum period and subsequent fertility in dairy cows. Theriogenology 66:1943-1950.

Younas, M., J. W. Fuquay, A. E. Smith, A. B. Moore. 1993. Estrus and endocrine responses of lactating Holsteins to forced ventilation during summer. J. Dairy Sci. 76:430-4.

Chapter 6 - A shortened resynchronization treatment for dairy cows after a non-pregnancy diagnosis based on corpus luteum status

ABSTRACT

We tested: (1) a shortened version of Ovsynch (OVS: GnRH-1 – 7 d – $PGF_{2\alpha}$-1 – 24 h – $PGF_{2\alpha}$-2 – 32 h – GnRH-2 – 16 h – AI) that excluded GnRH-1 to resynchronize ovulation in cows bearing a corpus luteum (CL) after a non-pregnancy diagnosis (NPD); (2) the value of including progesterone-releasing intravaginal insert + OVS in absence of a CL compared with presence of a CL + OVS; and (3) the accuracy of detecting a functional CL (progesterone was ≥ 1 ng/mL) by transrectal ultrasonography. Lactating Holsteins (n = 1,697) in 3 herds were enrolled in an incomplete factorial design of 3 treatments at NPD (32 ± 3 d after AI). Cows bearing a visually detected CL were assigned randomly to OVS or Short Synch (SS; $PGF_{2\alpha}$-1 – 24 h – $PGF_{2\alpha}$-2 – 32 h – GnRH-2 – 16 h – AI), whereas cows with no CL were assigned to OVS + progesterone insert (OVS + CIDR). Blood collected at NPD (d 0) determined accuracy of treatment assignment based on progesterone (functional CL cut point > 1 ng/mL). In 1 herd, ovaries of SS cows (n = 108) were scanned at d 0, 2, and 9 to determine follicle diameters and ovulation risk to GnRH-2. Ovarian scans occurred on d 0, 7, 9, and 16 (ovulation risk to GnRH-2) in OVS (n = 97) and OVS + CIDR (n = 68) cows to determine follicle diameters, and ovulation risk to GnRH-1 and GnRH-2. Orthogonal treatment contrasts included: OVS vs. OVS + CIDR and OVS vs. SS. Ovulation risk after GnRH-1 was greater for CIDR (40.3%) than for OVS (27.1%) cows. Before $PGF_{2\alpha}$, the largest follicle diameter was greater for SS than OVS cows and progesterone was less in OVS + CIDR compared with OVS cows. No differences were detected in luteolytic risk (progesterone < 0.5 ng/mL at 72 h after $PGF_{2\alpha}$-1) after $PGF_{2\alpha}$ (> 96.6%) and ovulation risk after GnRH-2 was 94.2, 91.7, and 85.2% for SS, OVS, and OVS + CIDR, respectively. Technicians were more accurate in detecting a functional CL than a non-functional CL (81.2 vs. 61.1%). Accuracy and sensitivity of detecting a functional CL by technicians ranged from 73.6 to 77.8% and 80.9 to 91.3%, respectively. With herd as a random effect, pregnancy per AI (P/AI) at 32 ± 3 d after AI tended to be greater for OVS than SS but did not differ from OVS + CIDR at 32 d (30.5% [n = 644], 25.5% [n = 678], and 25.6% [n = 270]), respectively. When cows were categorized according to CL status based on progesterone concentration, P/AI for cows with nonfunctional CL was greater for OVS (29.1%) than SS (16.4%) cows. In contrast, when CL were accurately detected, P/AI did not differ between OVS and SS cows. Short synch is a viable alternative to the entire OVS treatment, but especially when CL status is accurately detected.

Key Words: Artificial insemination, Fertility, $PGF_{2\alpha}$

INTRODUCTION

Ovulation-synchronization programs have facilitated greater and more efficient reproductive performance in U.S. dairies during the past decade (Bisinotto et al., 2014). Even with ovulation-synchronization programs 50 to 70% of cows fail to conceive to first service (Stevenson et al., 2014). Well-managed dairy herds with effective estrus-detection programs will generally inseminate more than 50% of the nonpregnant cows after estrus detection (Giordano et al., 2015). In herds with poor estrus detection, timely nonpregnancy diagnosis (NPD) coupled with a resynchronization timed-AI program is a common strategy to decrease the interbreeding interval and maximize pregnancy risk for cows requiring repeat inseminations (Pursley et al., 1995; Fricke et al., 2016). Ovsynch (GnRH-1 – 7 d – $PGF_{2\alpha}$-1 – 24 h – $PGF_{2\alpha}$-2 – 32 h – GnRH-2 – 16 h – AI) programs can be initiated 7 d before or at the time of NPD (Bartolome et al., 2009; Giordano et al., 2012b). A pitfall of beginning an ovulation-synchronization program with GnRH at 25 ± 3 d after AI in herds employing transrectal ultrasonography at 32 ± 3 d after AI is that GnRH-1 injection coincides with a time many nonpregnant cows could express estrus (Remnant et al., 2015; Wijma et al., 2017) or is too early in the estrous cycle when a dominant GnRH-responsive follicle exists (Vasconcelos et al., 1999). An injection of GnRH is known to suppress estrus because the resulting GnRH-induced ovulation prevents recurrence of estrus in nonpregnant cows (Mendonça et al., 2012).

Several studies have evaluated ovarian dynamics and pregnancy risk of resynchronized cows classified as low or high fertility after timed AI (Stevenson and Tiffany, 2004; Giordano et al., 2012a; Lopes et al., 2013). A major factor that predicted the success of the resynchronized timed AI service was the presence of a functional corpus luteum (CL; progesterone ≥ 1 ng/mL) at the time of the $PGF_{2\alpha}$-1 injection (Giordano et al., 2016). Cows lacking a functional CL that were submitted to a timed AI had a 50% reduction in pregnancy risk compared with cows that had a functional CL (Stevenson and Tiffany, 2004). Reduced pregnancy risk of cows without a CL could be attributed to a lack of synchrony, inadequate endocrine milieu before AI, or both (Vasconcelos et al., 1999). It is important to maximize fertility of cows in response to ovulation-synchronization programs because those cows that remain not pregnant after a resynchronized timed AI incur the expenses of completing the protocol as well as a substantial delay in days to next service, which reduces herd profitability (Giordano et al., 2013). Pregnancy risk has been improved in cows without a functional CL at the initiation of an Ovsynch program by supplementing progesterone in the form of a CIDR insert, which likely improved ovulation synchrony and optimized the hormonal milieu before AI (Stevenson et al., 2006, 2008; Lima et al., 2009; Bilby et al., 2013).

The GnRH-1 injection of an Ovsynch program is intended to induce ovulation of a dominant follicle and formation of a new CL, in addition to inducing emergence of a new follicular wave that produces the next dominant follicle that could ovulate after GnRH-2 (Pursley et al., 1995). When nonpregnant cows were treated with $PGF_{2\alpha}$ 27 to 29 d after timed AI and then inseminated after detected estrus or administered GnRH 48 h after $PGF_{2\alpha}$ (before timed AI), pregnancy risk was not different from control cows treated with Ovsynch (Stevenson et al., 2003). Likewise, Wijma et al. (2018) reported similar pregnancy risk when GnRH-1 was removed from an Ovsynch program that was initiated 7 d before NPD compared with a standard Ovsynch program also initiated 7 d before NPD.

A potential strategy to resynchronize ovulation is to eliminate GnRH-1 in an Ovsynch program in cows with a functional CL. Transrectal ultrasonography is a common tool used to assess pregnancy in dairy herds and can be used by skilled technicians to assess ovarian structures and select an ovulation resynchronization program based on the presence or absence of a CL. A previous study tested the accuracy of ultrasonography for the detection of a functional CL using progesterone $\geq$ 1 ng/mL as the gold standard (Bicalho et al., 2008).

The hypotheses of the current experiment were: (1) pregnancy risk will be similar between a shortened version of Ovsynch that excludes GnRH-1 when compared with a standard Ovsynch program if cows have a CL at NPD; (2) cows lacking a CL at NPD and supplemented with progesterone will have comparable pregnancy risk with cows having a CL subjected to an Ovsynch program, and (3) with acceptable accuracy and sensitivity, technicians will be able to detect a functional CL using transrectal ultrasonography.

Therefore, objectives of the present study were to determine: (1) the pregnancy risk of a shortened version of Ovsynch that excluded GnRH-1 in cows bearing a CL at NPD compared with Ovsynch; (2) the value of including progesterone supplementation to Ovsynch in the absence of a CL compared with presence of a CL for cows starting Ovsynch; and (3) the accuracy of detecting a functional CL by transrectal ultrasonography.

MATERIALS AND METHODS

An experiment was conducted under the Kansas State University Institutional Animal Care and Use Committee protocol #3671 (Manhattan) from June 2016 through June 2017. In the current experiment, 1,697 lactating Holstein cows were enrolled in 3 herds. Cows were housed in naturally ventilated free stalls with roofs overhead and fed a TMR calculated to meet nutritional requirements for lactating dairy cows producing 45 to 50 kg of 3.5% milk fat (NRC, 2001). Sprinklers over the feed lane and fans over free stalls were employed during days in which ambient temperature exceeded 22°C. Other herd characteristics of cows enrolled in the experiment are in Table 6-1.

Treatments

Cows (n = 1,697) were enrolled in an incomplete factorial design consisting of 3 treatments at NPD (d 0) at 32 ± 3 d after AI (Figure 6-1). Cows bearing a visually detected CL were assigned randomly to: (1) Ovsynch (OVS; GnRH-1 – 7 d – $PGF_{2\alpha}$-1 – 24 h – $PGF_{2\alpha}$-2 – 32 h – GnRH-2 – 16 h – AI) or (2) Short Synch (SS; $PGF_{2\alpha}$-1 – 24 h – $PGF_{2\alpha}$-2 – 32 h – GnRH-2 – 16 h – AI); whereas cows with no CL were assigned to OVS plus a progesterone insert (CIDR; Eazi-Breed CIDR Cattle Insert; Zoetis, Inc., Kalamazoo, MI; OVS + CIDR) for 7 d beginning with the GnRH-1 injection. Standard doses of $PGF_{2\alpha}$ (25 mg; Lutalyse, dinoprost tromethamine, Zoetis, Inc.) and GnRH (100 µg; Factrel, Zoetis, Inc.) were administered.

At enrollment, cows were body condition scored (BCS; 1 = thin and 5 = fat) and their ovaries were assessed for the presence of a CL determined by transrectal ultrasonography (7.5 MHz linear-array transducer, Ibex EVO; E. I. Medical Imaging, Loveland, CO). Each farm utilized an on-farm technician for pregnancy diagnosis using transrectal ultrasound. All technicians had more than 4 years previous experience diagnosing pregnancy before involvement in this experiment. Training was conducted by the authors to distinguish the differences between follicles and CL. Emphasis was placed on visual contrasts between nonechogenic follicles and "salt and peppery" echogenic luteal structures (CL), some of which had nonechogenic central cavities. Size was not considered when identifying a CL in the present experiment in contrast to a previous study (Wijma et al.; 2018) in which cows were assigned to treatments when CL > 15 mm and at least 1 follicle ≥ 10 mm. Sensitivity and specificity reportedly were optimized for predicting a functional CL > 22 mm at 24 d after AI (Bicalho et al., 2008).

Inseminations administered to SS cows occurred 1 wk earlier (week of NPD) than those in OVS and OVS + CIDR-treated cows. Sires were applied randomly across treatments and not more than 2 technicians performed inseminations at each of the 3 farms. Initial pregnancy diagnoses using transrectal ultrasonography were conducted d 32 ± 3 after treatment AI to verify a live embryo and were confirmed at d 65 ± 3 after treatment AI.

Ovarian Mapping

Transrectal ovarian ultrasonography in herd 1 was performed at the time of NPD (d 0) to measure diameter and map location of follicles and location of CL. Scans were repeated on d 2 for SS cows to determine preovulatory follicle diameter, and on d 9 to determine ovulation risk to GnRH-2. Scans were repeated on d 7 for OVS and OVS + CIDR cows to determine ovulation response to GnRH-1, on d 9 to determine preovulatory follicle diameter, and on d 16 to determine ovulation risk after GnRH-2.

Blood Collection

Blood samples were collected from all cows via coccygeal vessel puncture on d 0 to determine progesterone concentrations. In herd 1, additional blood samples were collected from OVS and OVS + CIDR cows on d 7 and from all cows at timed AI. Samples were stored on ice and transported to the laboratory for storage at 5ºC until serum was harvested by centrifugation (1,200 x *g*). Sera samples were stored at −15 ºC until assayed for concentrations of progesterone by direct quantitative (nonextracted) RIA using ImmuChem Double Antibody progesterone ^{125}I kits (MP Biomedicals LLC, Orangeburg, NY) previously validated for bovine serum (Hill et al., 2016). Intra- and inter- assay coefficients of variation for 19 assays for a low (1.45 ± 0.03 ng/mL) and high (17.7 ± 0.89 ng/mL) concentration pool were 4.69 and 5.28%, respectively. Calculated assay sensitivity averaged 5.28 ± 0.45 pg/mL, and progesterone standard concentrations in the assay were 0.05, 0.1, 0.2, 0.5, 2.0, 5.0, 10.0, and 25.0 ng/mL.

Statistical Analyses

Pregnancy per AI. Of the 1,697 cows enrolled in this experiment, pregnancy results were available for only 1,572 cows because the remainder left the herd before pregnancy diagnosis. Pregnancy per AI (P/AI) at d 32 ± 3 and d 65 ± 3 after treatment AI, and intervening pregnancy loss were analyzed using the GLIMMIX procedure in SAS (SAS 9.4, SAS Inst. Inc., Cary, NC, USA). Options used in the model statement included LINK=LOGIT, DIST=BINOMIAL, and the least square means option of ILINK and DIFF. The model included the fixed effects of treatment (OVS, OVS + CIDR, and SS), parity (primiparous vs. multiparous), and their interaction, plus the random effects of herd, BCS, and DIM at enrollment. Pregnancy per AI at d 32 ± 3 also was analyzed separately for each herd using procedure GLIMMIX applying the same model without herd as a random effect. In addition, cows were retrospectively classified into functional CL and nonfunctional CL status groups using progesterone ≥ 1 ng/mL as a cut point. The same GLIMMIX analysis was executed using the previous model including treatment, progesterone status, their interaction, and parity as fixed effects, plus herd, BCS, and DIM as random effects to determine the effect of a functional CL at the time of NPD on subsequent fertility regardless of treatment scheme.

A priori treatment contrasts were constructed: OVS vs. OVS + CIDR and OVS vs. SS. The former contrast represented cows with a CL exposed to the entire Ovsynch treatment compared with cows without a CL exposed to Ovsynch and progesterone, whereas the latter contrast tested the entire Ovsynch treatment compared with its shortened version (SS) without GnRH-1.

Pregnancy per AI at d 32 ± 3 also was analyzed using procedure GLIMMIX applying the model that included the fixed effects of treatment (OVS, OVS + CIDR, and SS), CL status (functional vs. nonfunctional), and their interaction, plus the random effects of herd, BCS, and DIM at enrollment. A priori treatment contrasts were constructed as previously described.

Data are presented as mean percentages or LSM ± SEM where appropriate. Differences among comparisons were considered significant when $P < 0.05$, whereas differences between $0.05 < P < 0.10$ were defined as tendencies.

Technician Accuracy. Accuracy, sensitivity, specificity, positive predictive values, and negative predictive values were calculated for technician's visual ability to detect a functional CL by transrectal ultrasonography. The gold standard for comparison of a functional CL was defined as progesterone ≥ 1 ng/mL. Positive detection of a CL and serum progesterone ≥ 1 ng/mL was defined as a true positive (TP). Likewise, absence of a CL and serum progesterone < 1 ng/mL was considered to be a true negative (TN). A false positive (FP) was defined as detection of a CL when progesterone was < 1 ng/mL. In contrast, a false negative (FN) was defined as absence of a CL when serum progesterone was ≥ 1 ng/mL. Accuracy was calculated by dividing the true positives by all positives ((TP/ (TP + FP)). Sensitivity was determined by dividing the true positives by the sum of the true positives and false negatives (TP / [TP + FN]), whereas specificity was determined by dividing the true negatives by the sum of the false positives and true negatives (TN / [FP + TN]). The positive predictive value was determined by dividing the true positives by all test positives (TP / [TP + FP]). Likewise, the negative predictive value was calculated by dividing the true negatives by all test negatives (TN / [TN + FN]).

RESULTS

Pregnancy Risks

Characteristics of the herds at the time of enrollment are shown in Table 6-1. Concentrations of progesterone for all cows at the time of NPD were 5.1 ± 0.16, 4.9 ± 0.15, and 2.3 ± 0.24 ng/mL for OVS, SS, and OVS + CIDR treatments, respectively. As expected, cows initiating the OVS treatment had greater ($P < 0.01$) concentrations of progesterone when compared with CIDR treatment at the time of NPD. Concentrations of progesterone at the time of NPD did not differ ($P = 0.28$) between OVS and SS treatments. By design, days to re-insemination were less ($P < 0.001$) in SS than OVS cows (3.1 ± 0.05 vs. 9.4 ± 0.06 d) and cows in the OVS + CIDR treatment were re-inseminated slightly later ($P = 0.001$) than OVS cows (9.7 ± 0.08 d) compared with OVS cows.

Pregnancy per AI by herd is shown in Figure 6-2. In herds 1 and 3, no differences in P/AI were detected when SS or OVS + CIDR treatments were compared with OVS treatment. In herd 2, however, OVS treatment increased ($P < 0.03$) P/AI compared with the SS treatment but did not differ from the OVS + CIDR treatment. When herd was included as a random effect in the model, cows treated with OVS tended to have greater ($P = 0.09$) P/AI at 32 ± 3 and 65 ± 3 d after AI compared with SS (Figure 6-3). Pregnancy risk for OVS and OVS + CIDR treatments did not differ at 32 or 65 ± 3 d after AI. Pregnancy loss between at 32 ± 3 and 65 ± 3 d after AI ranged from 4.3 to 7.6% and did not differ for designed contrasts.

Cows were retrospectively categorized into functional and nonfunctional CL status groups to determine the effect of detecting a functional CL on pregnancy outcomes. Technicians more ($P < 0.001$) accurately detected a functional CL (83.0 and 79.3% for SS and OVS cows) than detecting a nonfunctional CL (61.1% for OVS + CIDR cows). For SS cows, P/AI at 32 ± 3 d after AI was less ($P = 0.01$) than that for OVS cows when the CL was inaccurately diagnosed, whereas for accurately detected CL, P/AI did not differ between SS and OVS cows (Figure 6-4). No differences in P/AI at 32 ± 3 d were detected between OVS + CIDR and OVS for either CL status. Similar differences and responses occurred for P/AI assessed at 65 ± 3 d after AI (data not shown).

Ovarian Responses

Ovarian responses to treatments in herd 1 are shown in Table 6-2. No differences were detected in follicle diameters at the time of GnRH-1 when OVS was compared with the OVS + CIDR treatment. Cows in the OVS + CIDR treatment had a greater ($P = 0.04$) ovulation risk to GnRH-1 than cows in the OVS treatment: however, no differences were detected for multiple ovulation risk. The largest follicle for SS cows was greater ($P = 0.05$) in diameter at the time of $PGF_{2\alpha}$ compared with that for OVS cows, whereas no difference was detected between the OVS + CIDR and OVS treatments.

Ovulation risk to GnRH-2 did not differ among treatments (Table 6-2). As expected, concentration of progesterone was greater ($P < 0.01$) for OVS cows on d 0 compared with OVS + CIDR treatment. This difference was no longer detectable by 72 h after $PGF_{2\alpha}$-1 injection. Progesterone concentrations did not differ between OVS and SS treatments at 0 or 72 h after $PGF_{2\alpha}$-1 injection. Luteolytic risk ranged from 96.6 to 97.4% and did not differ among treatments.

Although not an objective of the study and lacking power to determine differences, data from herd 1 was further examined based on the ovulatory response to GnRH-1. Luteolytic risk was greater ($P = 0.02$) for OVS cows that ovulated (99%; n = 50) after GnRH-2 compared with those that failed to ovulate (89%; n = 18). In contrast, luteolytic risk did not differ ($P = 0.25$) in the

OVS + CIDR treatment for cows that ovulated (100%; n = 17) compared with those that did not ovulate (94%; n = 16). Pregnancy risk did not differ (*P* = 0.14) for cows in the OVS treatment (n = 96) that ovulated to GnRH-1 compared with cows that failed to ovulate (45.3 vs. 23.1%), respectively. Cows in the OVS + CIDR treatment (n = 62) that ovulated in response to GnRH-1 achieved a pregnancy risk of 29.2%, which did not differ (*P* = 0.55) from that of cows that failed to ovulate (23.7%).

Technician Precision

Measures of technician precision for visual detection of a CL by transrectal ultrasound are in Table 6-3. Accuracy for all 3 herds averaged 75.4% with the lowest accuracy in herd 2 at 73.6%. Sensitivity of the test averaged 88.4%, whereas specificity averaged 36.4%. The positive predictive value was 1.6 times greater than the negative predictive value.

DISCUSSION

Programs used to resynchronize the estrous cycle in dairy operation routinely used in reproductive management have the goal of increasing reinsemination risk and reducing inter-insemination intervals (Sinedino et al., 2014). The rationale for the current experiment was to take advantage of the technician's skills of identifying a functional CL and then applying a treatment to shorten the reinsemination interval without compromising fertility. We evaluated the use of a new shortened resynchronization program to test the hypothesis that fertility could be maintained after applying it to cows at an NPD.

The two overall goals of a resynchronization program are to decrease the inter-insemination interval and to maximize pregnancy outcomes at the resynchronized AI. Dairy managers generally achieve these goals by reinseminating cows returning to estrus after AI based on estrus detection, ovulation-synchronization programs, or both. Ovsynch-type resynchronization programs are generally initiated 7 d before or at the time of NPD (Bartolome et al., 2009; Giordano et al., 2012b). The GnRH-1 injection in the Ovsynch program causes an LH surge but reduces estrus expression in previously inseminated cows that are not pregnant (Mendonça et al., 2012; Bruno et al., 2013). Reduced expression of estrus likely occurs because of GnRH-induced ovulation or by suppressing concentrations of estradiol responsible for triggering behavioral estrus (Jo and Fortune, 2003). In addition, when Ovsynch-type programs are initiated before pregnancy diagnosis the injection of GnRH-1 may be necessary because pregnancy status is unknown. When a cow has a functional CL at the time of NPD, then the GnRH-1 injection may not be necessary to cause ovulation and provide a source of additional progesterone during the 7 d period after GnRH-1. The current experiment demonstrated that eliminating the GnRH-1 injection (SS treatment) resulted in comparable pregnancy risk when compared with the OVS treatment in herds 1 and 3, and only a slight nonsignificant reduction in pregnancy risk in all herds combined.

Detection of a CL via ultrasonography is an important diagnostic tool for making decisions regarding treatment of nonpregnant cows (McDougall and Rhodes, 1999; McDougall, 2003). The ovulation-synchronization program chosen may vary depending on the presence of a CL. Cows that lack a CL may be given supplemental progesterone (CIDR) at the time of GnRH-1, whereas cows with a CL may be administered $PGF_{2\alpha}$ (Chebel et al., 2006; McDougall and Rhodes, 1999; McDougall, 2003). The CL is responsive to $PGF_{2\alpha}$ to effectively induce luteolysis after d 6 of the estrous cycle (Momont and Seguin, 1984; Ott et al., 1986). When cows were retrospectively categorized as having a functional or nonfunctional CL at NPD in the present study, pregnancy risk did not differ from that of OVS cows. Pregnancy risk was greater when a functional CL was diagnosed accurately in SS cows, but with an accuracy of more than 81% of

CL detection in all cows assigned to the SS treatment, P/AI was not significantly different from that of the standard OVS treatment. In addition, and equally important, is the 7-d reduction in the pregnancy establishment resulting from application of the SS treatment in lieu of the OVS treatment. Accuracy of detecting a nonfunctional CL was less at 61.1%, but because P/AI did not differ in the OVS + CIDR compared with the OVS cows, regardless the less accuracy in detecting a nonfunctional CL, the only downside was the additional cost of employing a CIDR insert when it was not needed.

Previous research found that sensitivity, specificity, negative predictive value, and positive predictive value of employing ultrasound to detect a functional CL changed minimally from a progesterone cut-point concentration ranging from 0.5 to 1.2 ng/mL (Silva et al., 2007), thus a cut-point of 1 ng/mL was selected for this experiment. Previous researchers reported that positive and negative predictive values of ultrasonography were greater compared with those achieved with transrectal palpation (Hanzen et al., 2000). A study utilizing 5 technicians compared transrectal palpation with ultrasonographic examination using the gold standard of serum progesterone > 1 ng/mL as the cut-point (Bicalho et al., 2008). The sensitivity and specificity of transrectal palpation to detect a functional CL were 46.6% and 85.1% respectively, whereas the sensitivity and specificity of ultrasonography was 89.4% and 45.7%, respectively (Bicalho et al., 2008). In the current experiment, using ultrasound to detect a functional CL resulted in increased sensitivity with poorer specificity. Implementing a cutoff for CL diameter of > 23 mm at 21 d after AI or > 22 mm at 24 d after AI to classify cows as having a functional CL increased accuracy when using transrectal ultrasonography (Bicalho et al., 2008). Imposing a cut-point for CL diameter may have improved on-farm technician’s accuracy in identifying a functional CL in the current experiment. This additional criterion could easily be accomplished using dimensional grids on the ultrasound monitor or goggle display.

Previous research evaluated supplementing progesterone during selection and growth of the preovulatory follicle. Early research showed cows with greater progesterone during the luteal phase before estrus and insemination had increased pregnancy risk compared with cows that had lesser progesterone (Folman et al., 1973; Fonseca et al., 1983). Similar responses for cows enrolled in a timed AI program demonstrated that cows having greater rather than lesser progesterone at the time of $PGF_{2\alpha}$ had greater pregnancy risk to the timed AI (Stevenson, 2016). In addition, a decrease in pregnancy risk has been reported in cows enrolled in synchronization programs initiated during the follicular phase of the estrous cycle or during anovulation compared with cows in diestrus (Stevenson et al., 2008; Wiltbank et al., 2011; Denicol et al., 2012). Bisinotto et al. (2013) reported that cows exposed to Ovsynch without a CL at GnRH-1 supplemented with 2 progesterone inserts achieved concentrations of progesterone > 2.5 ng/mL during the 7-d period after GnRH-1 in a timed AI program had similar pregnancy risk to that of cows starting Ovsynch with a CL.

Supplementing progesterone in cows without a CL relies on systems for sustained delivery of progesterone to mimic concentrations of that observed in diestrous cows (Bisinotto et al., 2015). Commercially available inserts that supplement progesterone, such as the CIDR, were originally developed for grazing heifers (Macmillan et al., 1991). The CIDR insert releases approximately 89 mg of progesterone daily (Rathbone et al., 2002), which causes concentration of progesterone in blood plasma of lactating dairy cows with no CL to increase to 0.8 ng/mL (Cerri et al., 2009). In the current experiment, even though progesterone was less in OVS + CIDR-treated cows at the onset of the treatment compared with cows having a CL and exposed to the OVS treatment, pregnancy risk did not differ. Previous research has indicated that to achieve high fertility a minimum concentration of progesterone of 2 ng/mL is required, and to achieve that concentration, supplementation of 2 CIDR inserts may be required for cows without a CL (Bisinotto et al., 2013; 2015). In the current experiment, one CIDR insert was supplemented in cows without a visually detected CL and concentrations of progesterone at initial $PGF_{2\alpha}$ injection (7 d after NPD) averaged 2.24 ± 0.3 ng/mL. Supplementing 1,072 cows in 6 herds with a single CIDR at the time of GnRH-1 during Ovsynch with no CL at the time of GnRH-1 resulted in progesterone concentrations of 2.48 ± 0.3 ng/mL at the time of $PGF_{2\alpha}$ and a pregnancy risk of 32.2% compared with cows started in diestrous with progesterone averaging 3.8 ± 0.3 ng/mL and a pregnancy risk of 38% (Stevenson et al., 2008).

Cows in the OVS + CIDR treatment had greater risk of ovulation to GnRH-1 compared with cows subjected to the OVS treatment. This greater ovulatory response is consistent with the fact that cows with lesser progesterone concentration at the initiation of a timed AI program were more likely to ovulate in response to GnRH (Lopes et al., 2013; Pulley et al., 2015; Wijma et al., 2018) because of a greater GnRH-induced LH response (Lima et al., 2013; Pulley et al., 2015). Furthermore, LH response to GnRH is related to concentrations of both estradiol and progesterone (Stevenson and Pulley, 2016). The OVS + CIDR-treated cows without a CL were more likely to have a younger CL at the time of $PGF_{2\alpha}$ treatment and the CL was less likely to regress in response to $PGF_{2\alpha}$. Even though OVS + CIDR cows did not have a CL at NPD, they had comparable fertility to the cows enrolled in the OVS treatment. Neither luteolytic risk nor pregnancy risk differed when OVS and OVS + CIDR treatments were compared regardless of ovulation risk to GnRH-1. Adding a second treatment of $PGF_{2\alpha}$ was likely important to induce complete luteolysis in no-CL cows that ovulated to GnRH-1 because exposure of cows to 1, 50-

mg dose of $PGF_{2\alpha}$ (Stevenson et al., 2018) or 2 standard doses of $PGF_{2\alpha}$ 24 h apart (Wiltbank et al., 2015; Stevenson et al., 2018) improved the proportion of cows with complete luteolysis before timed AI.

CONCLUSIONS

Fertility is a leading economic concern for the dairy industry. A reproductive management program that applies different resynchronization methods based on observed ovarian structures at a NPD is a viable alternative to reduce the interbreeding interval. Removal of the GnRH-1 injection in cows with a functional CL at NPD achieved similar pregnancy risk compared with a traditional Ovsynch program. Supplementing progesterone to cows without a functional CL achieved pregnancy risk compared with cows that had a functional CL. Even though ultrasound technicians were more accurate at detecting a functional CL compared with a nonfunctional CL, P/AI did not differ between treatments, but the accuracy of detecting a functional CL is critical to maximize pregnancy risk in the SS treatment. Therefore, we conclude SS is a viable resynchronization program to minimize the interbreeding interval by 7 d, and when a functional CL is accurately detected, pregnancy risk can be maximized compared with employing the OVS treatment.

REFERENCES

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

Bicalho, R. C., K. N. Galvao, C. L. Guard, and J. E. P. Santos. 2008. Optimizing the accuracy of detecting a functional corpus luteum in dairy cows. Theriogenology 70:199-207.

Bilby, T. R., R. G. Bruno, K. J. Lager, R. C. Chebel, J. G. Moraes, P. M. Fricke, G. Lopes Jr., J. O. Giordano, J. E. Santos, F. S. Lima, J. S. Stevenson, and S. L. Pulley. 2013. Supplemental progesterone on timing of resynchronization on pregnancy outcomes in lactating dairy cows. J. Dairy Sci. 96:7032-7042.

Bisinotto, R. S., L. O. Castro, M. B. Pansani, C. D. Narciso, N. Martinez, L. D. P. Sinedino, T. L. C. Pinto, N. S. Van de Burgwal, H. M. Bosman, R. S. Surjus, W. W. Thatcher, and J. E. P. Santos. 2015. Progesterone supplementation to lactating dairy cows without a corpus luteum at initiation of the Ovsynch protocol. J. Dairy Sci. 98:2515-2528.

Bisinotto, R. S., E. S. Ribeiro, F. S. Lima, N. Martinez, L. F. Greco, L. F. Barbosa, P. P. Bueno, L. F. Scagion, W. W. Thatcher, and J. E. P. Santos. 2013. Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol. J. Dairy Sci. 96:2214-2225.

Bisinotto, R. S., E. S. Ribeiro, and J. E. P. Santos. 2014. Synchronization of ovulation for management of reproduction in dairy cows. Animal 8:151-159.

Bruno, R. G., A. Farias, J. Hernandez-Rivera, A. Navarrette, D. Hawkins, and T. Bilby. 2013. Effect of gonadotropin-releasing hormone or prostaglandin $F_{2\alpha}$-based estrus synchronization programs for first or subsequent artificial insemination in lactating dairy cows. J. Dairy Sci. 96:1556-1567.

Cerri, R. L., H. M. Rutigliano, R. G. Bruno, and J. E. P. Santos. 2009. Progesterone concentrations, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. Anim. Reprod. Sci. 110:56-70.

Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, H. M. Rutigliano, and R. G. S. Bruno. 2006. Reproduction on dairy cows following progesterone insert presynchronization and resynchronization protocols. J. Dairy Sci. 89:4205-4219.

Denicol, A. C., G. Lopes Jr., L. G. Mendonça, F. A. Rivera, F. Guagnini, R. V. Perez, J. R. Lima, R. G. Bruno, J. E. P. Santos, and R. C. Chebel. 2012. Low progesterone concentrations during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. J. Dairy Sci. 95:1794-1806.

Folman, Y., M. Rosenberg, Z. Herz, and M. Davidson. 1973. The relationship between plasma progesterone concentrations and conception in post-partum dairy cows maintained on two levels of nutrition. J. Reprod. Fertil. 34:267-278.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk, and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66:1128-1147.

Fricke, P. M., A. Ricci, J. O. Giordano, and P. D. Carvalho. 2016. Methods for and implementation of pregnancy diagnosis in dairy cows. Vet. Clin. North Am. Food Anim. Pract. 32:165-180.

Giordano, J. O., P. M. Fricke, and V. E. Cabrera. 2013. Economics of resynchronization strategies including chemical tests to identify nonpregnant cows. J. Dairy Sci. 96:949-961.

Giordano, J. O., M. L. Stangaferro, R. Wijma, W. C. Chandler, and R. D. Watters. 2015. Reproductive performance of dairy cows managed with a program aimed at increasing insemination of cows in estrus based on increased physical activity and fertility of timed artificial inseminations. J. Dairy Sci. 98:2488-2501.

Giordano, J. O., M. J. Thomas, G. Catucuamba, M. D. Curler, M. Masello, M. L. Stangaferro, and R. Wijma. 2016. Reproductive management strategies to improve fertility of cows with a suboptimal response to resynchronization to ovulation. J. Dairy Sci. 2967-2978.

Giordano, J. O., M. C. Wiltbank, J. N. Guenther, M. S. Ares, G. Lopes Jr., M. M. Herlihy, and P. M. Fricke. 2012a. Effect of presynchronization with human chorionic gonadotropin-releasing hormone 7 days before resynchronization of ovulation on fertility in lactating dairy cows. J. Dairy Sci. 95:5612-5625.

Giordano, J. O., M. Wiltbank, J. Guenther, R. Pawlisch, S. Bas, A. Cunha, and P. Fricke. 2012b. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J. Dairy Sci. 95:639-653.

Hanzen, C, M. Pieterse, O. Scenczi, and M. Drost. 2000. Relative accuracy of the identification of ovarian structures in the cow by ultrasonography and palpation per rectum. Vet J. 159:161-70.

Hill, S. L., D. M. Grieger, K. C. Olson, J. R. Jaeger, C. R. Dahlen, M. R. Crosswhite, N. Negrin Pereira, S. R. Underdahl, B. W. Neville, J. Ahola, M. C. Fischer, G. E. Seidel, and J. S. Stevenson. 2016. Gonadotropin-releasing hormone increased pregnancy risk in suckled beef cows not detected in estrus and subjected to a split-time artificial insemination program. J. Anim. Sci. 94:3722–3728.

Jo, M., and J. Fortune. 2003. Changes in oxytocin receptor in bovine preovulatory follicles between the gonadotropin surge and ovulation. Mol. Cell. Endocrinol. 200:31-43.

Lima, F. S., E. Ribeiro, R. Bisinotto, L. Greco, N. Martinez, M. Amstalden, W. Thatcher, and J. Santos. 2013. Hormonal manipulations in the 5-day timed artificial insemination protocol to optimize estrous cycle synchrony and fertility in dairy heifers. J. Dairy Sci. 96:7054-7065.

Lima, J. R., F. A. Rivera, C. D. Narciso, R. Oliveira, R. C. Chebel, and J. E. Santos. 2009. Effect of increasing amounts of supplemental progesterone in a timed artificial insemination protocol on fertility of lactating dairy cows. J. Dairy Sci. 92:5436-5446.

Lopes, G. Jr., J. O. Giordano, A. Valenza, M. M. Herlihy, J. N. Guenther, M. C. Wiltbank, and P. M. Fricke. 2013. Effect of timing of initiation of resynchronization and presynchronization with gonadotropin-releasing hormone on fertility of resynchronized inseminations in lactating dairy cows. J. Dairy Sci. 96:3788-3798.

Macmillan, K. L., V. K. Taufa, D. R. Barnes, and A. M. Day. 1991. Plasma progesterone concentrations in heifers and cows treated with a new intravaginal device. Anim. Reprod. Sci. 26:25-40.

McDougall, S. 2003. Resynchrony of previously anoestrous cows and treatment of cows not detected in estrus that had a palpable corpus luteum with prostaglandin F (2 alpha). NZ Vet. J. 51:117-124.

McDougall, S., and F. M. Rhodes. 1999. Detection of a corpus luteum in apparently anestrous cows by manual palpation, transrectal ultrasonography and plasma progesterone concentration. NZ Vet. J. 47:47-52.

Mendonça, L. G., S. T. Dewey, G. Lopes, F. A. Rivera, F. S. Guagnini, J. P. Fetrow, T. R. Bilby, and R. C. Chebel. 2012. Effects of resynchronization strategies for lactating Holstein cows on pattern of reinsemination, fertility, and economic outcome. Theriogenology 77:2262-1158.

Momont, H. W., and B. E. Sequin. 1984. Influence of day of estrous cycle on response to PGF2 alpha products: implication for AI programs for dairy cattle. 10th Int. Cong. Anim. Reprod. AI. Champaign: Univ. Illinois at Urbana- Champaign, pp 336–338.

National Research Council (NRC). 2001. Nutrient requirements of dairy cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ott, R. S., K. N. Bretzlaff, and J. E. Hixon. 1986. Comparison of palpable corpora lutea with serum progesterone concentrations in cows. JAVMA 188:1417-1419.

Pulley, S. L., D. H. Keisler, and J. S. Stevenson. 2015. Concentrations of luteinizing hormone and ovulatory responses in dairy cows before timed artificial insemination. J. Dairy Sci. 98:6188-6201.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915-923.

Rathbone, M. J., C. R. Bunt, C. R. Ogle, S. Burggraaf, K. L. Macmillan, C. R. Burke, and K. L. Pickering. 2002. Reengineering of a commercially available bovine intravaginal insert (CIDR insert) containing progesterone. J. Control Release 85:105-115.

Remnant, J. G., M. Green, J. Huxley, and C. Hudson. 2015. Variation in the interservice intervals of dairy cows in the United Kingdom. J. Dairy Sci. 98:889-897.

Silva, E., R. A. Sterry, and P. M. Fricke. 2007. Assessment of a practical method for identifying anovular dairy cows synchronized for first postpartum timed artificial insemination. J. Dairy Sci. 90:3255-3262.

Sinedino, L. D. P., F. S. Lima, R. S. Bisinotto, R. L. A. Cerri, and J. E. P. Santos. 2014. Effect of early or late resynchronization based on different methods of pregnancy diagnosis on reproductive performance of dairy cows. J. Dairy Sci. 97:4932-4941.

Stevenson, J. S. 2016. Physiological predictors of ovulation and pregnancy risk in a fixed-time artificial insemination program. J. Dairy Sci. 99:10077–10092.

Stevenson, J. S., J. A. Cartmill, B. A. Hensley, and S. Z. El-Zarkouny. 2003. Conception rates of dairy cows following early not-pregnant diagnosis by ultrasonography and subsequent treatments with shortened Ovsynch protocol. Theriogenology 60:475-483.

Stevenson, J. S., S. L. Hill, R. L. Nebel, and J. M. DeJarnette. 2014. Ovulation timing and conception risk after automated activity monitoring in lactating dairy cows. J. Dairy Sci. 97:4296-4308.

Stevenson, J. S., and S. L. Pulley. 2016. Feedback effects of estradiol and progesterone on ovulation and fertility after gonadotropin-releasing hormone-induced release of luteinizing hormone. J. Dairy Sci. 99:3003-3015.

Stevenson, J. S., J. R. Pursley, H. A. Garverick, P. M. Fricke, D. J. Kesler, J. S. Ottobre, and M. C. Wiltbank. 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. J. Dairy Sci. 89:2567-2578.

Stevenson, J. S., J. A. Sauls, L. G. D. Mendonca, and B. E. Voelz. 2018. Dose frequency of prostaglandin $F_{2\alpha}$ administration to dairy cows exposed to presynchronization and either 5- or 7-day Ovsynch program duration: Ovulatory and luteolytic risk. J. Dairy Sci. 101:9575-9590.

Stevenson, J. S., D. E. Tenhouse, R. L. Krisher, G. C. Lamb, J. E. Larson, C. R. Dahlen, J. R. Pursley, N. M. Bello, P. M. Fricke, M. C. Wiltbank, D. J. Brusveen, M. Burkhart, R. S. Youngquist, and H. A. Garverick. 2008. Detection of anovulation by heatmount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol. J. Dairy Sci. 91:2901-2915.

Stevenson, J. S., and S. M. Tiffany. 2004. Resynchronizing estrus and ovulation after not-pregnant diagnosis and various ovarian states including cysts. J. Dairy Sci. 87:3658-3664.

Vasconcelos, J. L., R. W. Silcox, G. J. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067-1078.

Wijma, R., M. M. Perez, M. Masello, M. L. Stangaferro, and J. O. Giordano. 2018. A resynchronization of ovulation program based on ovarian structures present at nonpregnancy diagnosis reduced time to pregnancy in lactating dairy cows. J. Dairy Sci. 101:1697-1707.

Wijma, R., M. L. Stangaferro, M. Masello, G. E. Granados, and J. O. Giordano. 2017. Resynchronization of ovulation protocols for dairy cows including or not including gonadotropin-releasing hormone to induce a new follicular wave: Effects on re-insemination pattern, ovarian response, and pregnancy outcomes. J. Dairy Sci. 100:7613-7625.

Wiltbank, M. C., A. H. Souza, P. D. Carvalho, R. W. Bender, and A. B. Nascimento. 2011. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. Reprod. Fertil. Dev. 24:238-243.

Wiltbank, M. C., G. M. Baez, F. Cochrane, R. V. Barletta, C. R. Trayford, and R. T. Joseph. 2015. Effect of a second treatment with prostaglandin $F_{2\alpha}$ during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. J. Dairy Sci. 98:8644-8654.

Table 6-1. Characteristics of herds at the time of enrollment in the experiment.

Item	Herd 1	Herd 2	Herd 3
No. cows enrolled	273	794	630
Days in milk[1]	160 ± 56	155 ± 44	150 ± 56
Daily milking frequency	3×	3×	3×
First-lactation cows, %	37	32	38
Test-day milk, kg	40	39	38
Previous inseminations[1], no.	3.6 ± 1.5	3.5 ± 1.8	3.5 ± 1.5
Body condition score[1]	2.5 ± 0.4	2.9 ± 0.4	2.9 ± 0.4

[1]Mean ± SD.

Table 6-2. Ovarian responses to resynchronization treatments in herd 1.

	Treatment[1]			*P* value[2]	
Item	CIDR	Ovsynch	Short synch	C1	C2
GnRH-1					
Largest follicle, mm	13.8 ± 0.5	13.3 ± 0.5	…	0.404	
Second largest follicle, mm	11.5 ± 1.0	13.2 ± 1.9	…	0.487	
Ovulation, %	38.3	19.2	…	0.035	
Multiple ovulation, %	35.1	20.1		0.468	
$PGF_{2\alpha}$					
Largest follicle, mm	14.3 ± 0.3	14.3 ± 0.3	15.1 ± 0.3	0.863	0.054
Second largest follicle, mm	11.5 ± 0.6	11.7 ± 0.7	12.6 ± 0.6	0.790	0.340
GnRH-2					
Ovulation, %	85.2	91.7	94.2	0.224	0.509
Multiple ovulation, %	31.6	25.9	16.0	0.379	0.280
Progesterone, ng/mL					
0 h after $PGF_{2\alpha}$	2.24 ± 0.3	5.08 ± 0.4	5.00 ± 0.4	0.001	0.844
72 h after $PGF_{2\alpha}$	0.11 ± 0.06	0.22 ± 0.05	0.14 ± 0.05	0.166	0.271
Luteolysis[3], %	97.4	97.1	96.6	0.830	0.589

[1]Ovsynch = GnRH-1 – 7 d – $PGF_{2\alpha}$-1 – 24 h – $PGF_{2\alpha}$-2 – 56 h – GnRH-2 – 16 h – timed AI; CIDR = same as Ovsynch plus progesterone insert (CIDR) placed at GnRH1 and removed at PGF; Short synch = $PGF_{2\alpha}$-1 – 24 h – $PGF_{2\alpha}$-2 – 56 h - GnRH2 – 16 h – timed AI.
[2]C1 = Ovsynch vs. CIDR; C2 = Ovsynch vs Short synch.
[3]Proportion of cows with progesterone < 0.5 ng/mL at 72 h after $PGF_{2\alpha}$-1.

Table 6-3. Measures of technician precision to detect a functional CL by transrectal ultrasonography at time of non-pregnancy diagnosis in lactating dairy cows[1]

Item	Herd 1	Herd 2	Herd 3	All
Accuracy, %	74.9	73.6	77.8	75.4
Sensitivity, %	80.9	91.3	88.1	88.4
Specificity, %	51.9	20.1	50.0	36.4
Positive predictive value, %	86.6	77.6	82.6	80.6
Negative predictive value, %	51.9	20.1	50.0	36.4
Prevalence of a visual CL, %	74.1	88.5	77.8	82.2
Prevalence of P4 ≥ 1 ng/mL, %	79.3	75.2	72.9	74.9

[1]Functional CL is defined as blood serum P4 ≥ 1 ng/mL at enrollment.

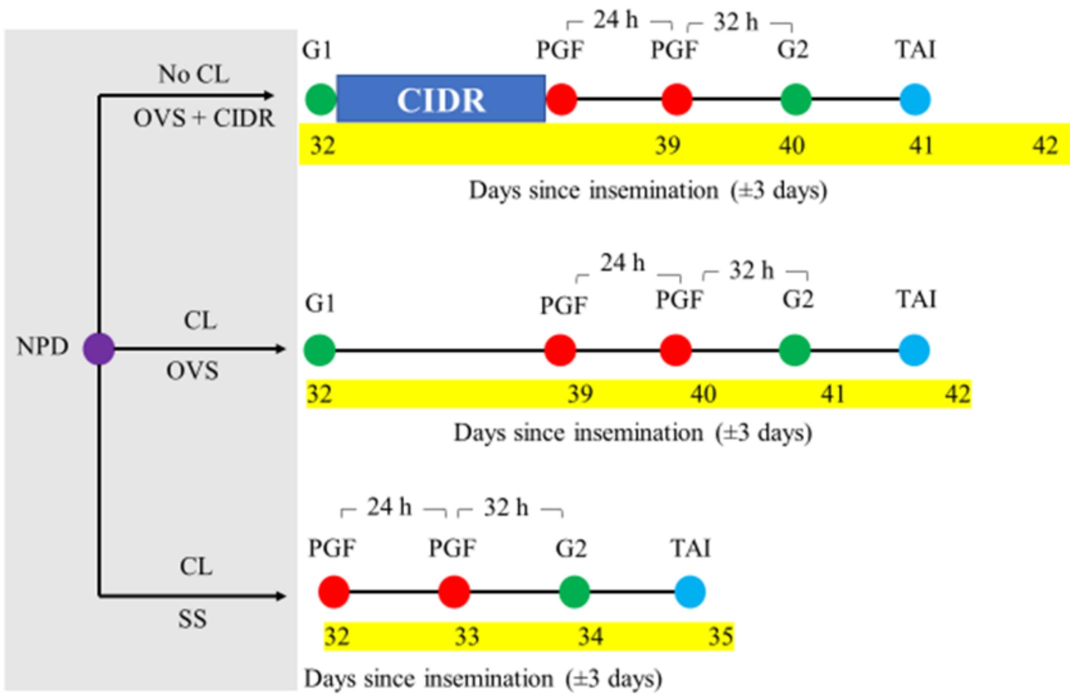

Figure 6-1. Illustration of the ovulation-resynchronization treatment schemes. At the time of nonpregnancy diagnosis (NPD) presence or absence of a corpus luteum (CL) was detected. Cows bearing a CL were assigned randomly to either the Ovsynch program (OVS; : GnRH-1 [G1] – 7 d – PGF$_{2\alpha}$-1 – 24 h – PGF$_{2\alpha}$-2 – 32 h – GnRH-2 [G2] – 16 h – timed AI [TAI]) or the Short Synch (SS) program: (PGF$_{2\alpha}$-1 – 24 h – PGF$_{2\alpha}$-2 – 32 h – GnRH-2 [G2] – 16 h – TAI). Cows lacking a CL received a modified Ovsynch program (OVS + CIDR) with supplemental progesterone via a controlled internal drug release (CIDR) insert (GnRH-1 [G1] + CIDR insertion – 7 d – PGF$_{2\alpha}$-1 + CIDR removal – 24 h – PGF$_{2\alpha}$-2 – 32 h – GnRH-2 [G2] – 16 h – TAI). A blood sample was collected from all cows at NPD to determine progesterone concentration.

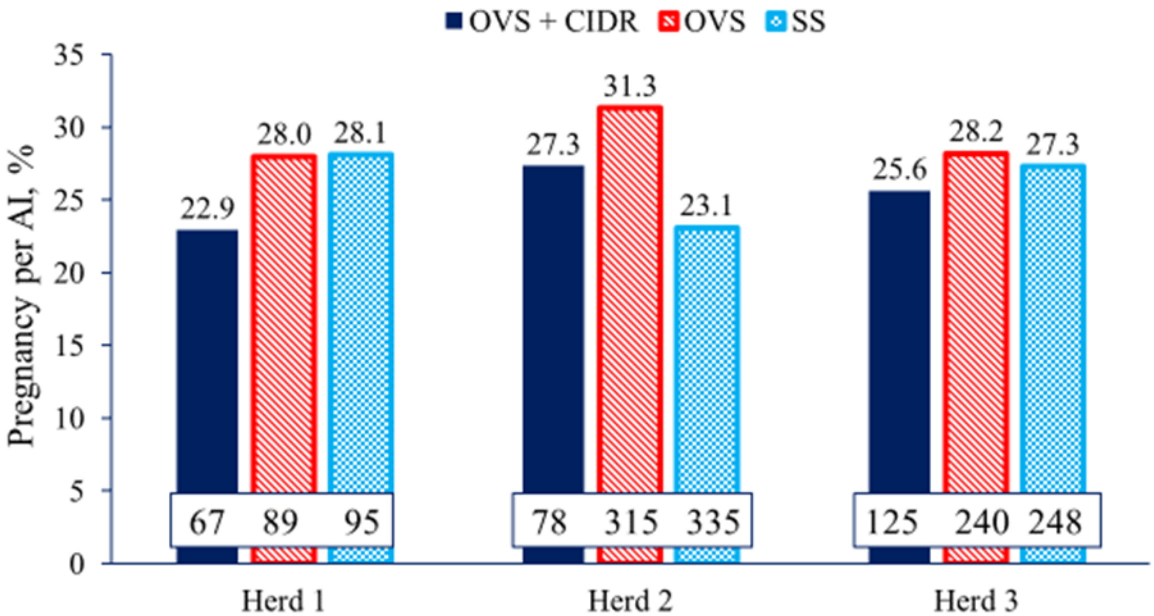

Figure 6-2. Pregnancy per AI (P/AI) determined at 32 ± 3 d after AI for each of 3 herds. No differences in P/AI were detected in herds 1 and 3 when the Ovsynch treatment (OVS) was compared with Short Synch (SS) or with Ovsynch treatment + progesterone insert (OVS + CIDR). In herd 2, P/AI was greater (*P* = 0.03) in OVS cows compared with SS, but OVS vs. OVS + CIDR did not differ. Number of cows per bar are shown above the horizontal axis.

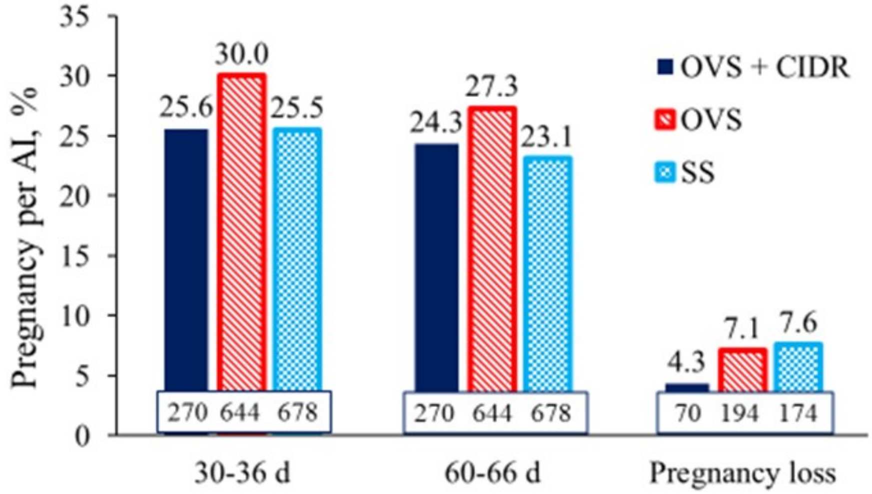

Figure 6-3. Pregnancy per AI (P/AI) for all herds at 32 ± 3 d and 65 ± 3 d after AI, and intervening pregnancy loss. Pregnancy per AI at both diagnoses tended (*P* = 0.09) to be greater in OVS cows compared with SS cows but OVS did not differ from OVS + CIDR. No differences in pregnancy loss were detected among treatments. Number of cows per bar are shown above the horizontal axis.

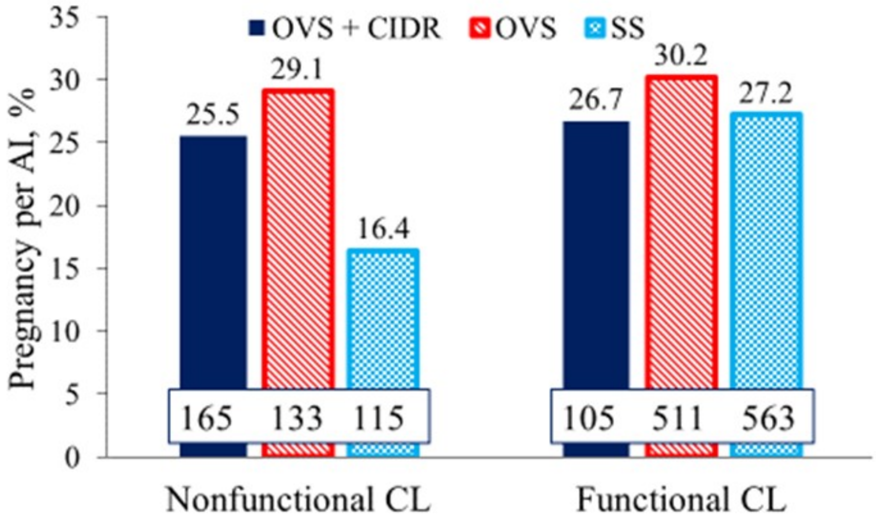

Figure 6-4. Pregnancy per AI (P/AI) for all herds at 32 ± 3 d based on correctly identifying a functional corpus luteum (CL; progesterone > 1 ng/mL) at nonpregnancy diagnosis. For SS cows, P/AI was less (*P* = 0.01) than that for OVS cows when the CL was not functional, whereas for accurately detected functional CL, P/AI did not differ between SS and OVS cows. No differences were detected between OVS + CIDR and OVS for either CL status. Number of cows per bar are shown above the horizontal axis.

Chapter 7 - Additional small dose of prostaglandin $F_{2\alpha}$ at timed artificial insemination failed to improve pregnancy risk of lactating dairy cows

Abstract

Two experiments were performed to test the hypothesis that administering $PGF_{2\alpha}$ concurrent with timed artificial insemination (AI) in lactating dairy cows would enhance pregnancy per AI (P/AI). In experiment 1, lactating Holstein cows (n = 289) in one herd were enrolled after a non-pregnancy diagnosis (30 to 36 d after AI) to synchronize subsequent ovulation before AI. Cows were assigned randomly to receive (im) 10 mg of $PGF_{2\alpha}$ concurrent with timed AI (Day 0; treatment) or no injection (control). Blood samples were collected on Days −3, 0, and 13 to determine serum concentrations of progesterone. Ovaries were scanned via transrectal ultrasonography to determine follicle diameters (Day −3), subsequent ovulation risk (Day 13), and total volume of luteal tissue (Day 13). Diagnosis of pregnancy occurred on Days 32 and 80 after AI. Ovulation risk post-AI exceeded 90% and did not differ between treatments. In addition, $PGF_{2\alpha}$ treatment only numerically increased progesterone (5.7 ± 0.3 vs. 6.2 ± 0.3 ng/mL) or luteal tissue volume (8.9 ± 0.4 vs. 9.8 ± 0.5 ng/mL) on Day 13 by 8.8% (P = 0.206) or 10.1% (P = 0.134) in control and treated cows, respectively. Pregnancy per AI at Days 32 (P = 0.50) and 80 (P = 0.33) did not differ between treatments. Cows with progesterone >0.5 ng/mL at timed AI had reduced (P < 0.001) ovulation risk but risk was unaffected by treatment. In experiment 2, lactating dairy cows (n = 1,828) in two commercial dairy herds were enrolled at time of insemination (Day 0), and assigned randomly to treatment or control as described in experiment 1. Initial (Days 32 to 35) and confirmed (Days 63 to 68) pregnancy diagnosis revealed no differences in P/AI or pregnancy loss. Pregnancy diagnosis on Days 32 to 35 produced percentage increases in P/AI for primiparous compared with multiparous cows (20.8%; P = 0.002), for first-service compared with repeat-service cows (26%; P = 0.001), and cows in one herd compared with the second herd (36%; P < 0.001). Pregnancy loss was greater (P = 0.001) for cows inseminated at first (10.0%) vs. later services (5.3%) but was unaffected by treatment. Cows treated with $PGF_{2\alpha}$ in one herd produced more twins than control cows (11.7 vs. 3.2%), whereas no treatment difference was detected in the second herd (5.6 vs. 5.6%), respectively. We conclude that im treatment of lactating dairy cows with 10 mg of $PGF_{2\alpha}$ concurrent with timed AI did not improve P/AI or embryo survival, but increased twinning in one herd.

***Keywords*:** artificial insemination, pregnancy per AI, prostaglandin $F_{2\alpha}$ at AI

1. Introduction

Treatment of domestic livestock with $PGF_{2\alpha}$ administered (iv, im, or iu) concurrent with artificial insemination (AI) to influence conception has been a subject of recurring research since the early 1990s. Pregnancy outcomes have been improved in some bovine studies [1,2,3], but not in others [4,5,6]. Furthermore, $PGF_{2\alpha}$ may successfully induce and synchronize ovulation similar to treatments with estradiol benzoate or estradiol cypionate [7]. Prostaglandin $F_{2\alpha}$ is produced by many cells, including endometrial cells, and is considered to be the main luteolysin in farm animals [8]. Because of the various biological activities of $PGF_{2\alpha}$, it has been used in reproductive management of cattle to induce parturition, synchronize estrus, and treat ovarian and uterine disease [9,10]. Administration of $PGF_{2\alpha}$ increased release of luteinizing hormone (LH) in postpartum cows that stimulated follicular growth and ovulation [11]. Treatment with a single iv injection of $PGF_{2\alpha}$ concurrent with AI improved synchrony of ovulation, increased ovulation risk, and pregnancy risk in dairy cows [2]. In addition, administering $PGF_{2\alpha}$ at the time of AI increased embryo numbers in rabbits [12] and improved farrowing rates and litter size in sows [13].

Approximately 20% of cows exposed to estrus- or ovulation synchronization programs have delayed or incomplete luteal regression [14]. A review of previous research demonstrated that cows with reduced concentrations of progesterone at AI had increased conception risk [15], in part, because basal concentrations of progesterone at AI are optimal for gamete transport. Administration of $PGF_{2\alpha}$ concurrent with AI could increase pregnancy rate by hastening luteal regression in cows that have delayed or incomplete luteolysis at AI. In addition, administration of $PGF_{2\alpha}$ concurrent with AI increased corpus luteum size and concentrations of progesterone 11 d after treatment and AI in buffaloes [16] and increased the number of cows with a corpus luteum after AI [2].

Sperm transport to the site of fertilization is a critical component to achieve pregnancy. Because smooth muscle contractions are most likely responsible for moving sperm to the uterus [17] as evidenced by limited sperm transport in ewes with reduced uterine contractions moving toward the oviduct [18]. Prostaglandin $F_{2\alpha}$ stimulated myometrial contractions in pigs [19] and cattle [20]. Previous research demonstrated improved sperm transport to the oviduct when compounds such as $PGF_{2\alpha}$, oxytocin, estradiol, phenylephrine, or ergonovine were added to semen or were administered to females [17]. In addition, ewes inseminated with $PGF_{2\alpha}$-supplemented semen or administered $PGF_{2\alpha}$ im at breeding had increased semen in all parts of their reproductive tract [21]. Therefore, administering $PGF_{2\alpha}$ concurrent with AI could increase pregnancy risk by inducing secretion of oxytocin that may stimulate uterine contractions and support semen transport.

The objectives of the present experiments were to test the hypothesis that administering im 10 mg of $PGF_{2\alpha}$ concurrent with timed AI will increase subsequent concentrations of progesterone, luteal volume, and pregnancy outcomes in lactating dairy cows receiving a fixed-time insemination.

2. Materials and methods

Two experiments were conducted under the Kansas State University Institutional Animal Care and Use Committee application #3671 (Manhattan) between January of 2015 through May of 2016.

2.1 Experiment 1

In experiment 1, 289 lactating Holstein cows in the Kansas State University Dairy Teaching and Research Center were enrolled. Cows were housed in sand-bedded free stalls with overhead roofs and fed twice daily a total mixed diet calculated to meet nutritional requirements for lactating dairy cows producing 50 kg of 3.5% milk [22]. The diet consisted of alfalfa hay, corn silage, soybean meal, whole cotton seed, corn or milo grain, corn-gluten feed, vitamins, and minerals. Feedline sprinklers and fans over the free stalls were employed during days in which ambient temperature exceeded 22°C. Other characteristics of cows enrolled in experiment 1 are summarized in Table 7-1 including monthly test-day milk.

2.1.1 Experimental design

Cows were enrolled in a completely randomized design with two treatments when diagnosed not pregnant (Day –3) at 30 to 36 d after AI. Beginning at the not-pregnant diagnosis, cows were exposed to either a 5- or 7-d Ovsynch timed AI program before AI (100 μg GnRH [2 mL Factrel, Zoetis, Kalamazoo, MI, USA] — 5 or 7 d — 25 mg $PGF_{2\alpha}$ [5 mL Lutalyse, Zoetis] — 56 h — 100 μg GnRH — 16 h — AI). A second dose of $PGF_{2\alpha}$ was administered 24 h after the first when the 5-d program was employed. Body condition scores (1 = thin and 5 = fat) were assigned to cows and cows were stratified by parity (primiparous vs. multiparous) and then assigned randomly to receive 10 mg $PGF_{2\alpha}$ (n = 140; 2 mL Lutalyse [dinoprost tromethamine], Zoetis) at timed AI (Day 0) or served as untreated controls (n = 149). The treatment dose of 10 mg $PGF_{2\alpha}$ was chosen because 10 mg, but not 5 mg, increased pregnancy risk at fixed-time AI in one North American Holstein herd [3]. Pregnancy was diagnosed by transrectal ultrasonography (7.5-MHz linear-array transducer, Aloka 500V; Corometrics Medical Systems Inc., Wallingford, CT) at Day 32 after timed AI.

2.1.2 Measurements

Blood samples were collected 3 d before timed AI (Day −3), at timed AI (Day 0), and at Day 13 to determine progesterone concentrations. Samples were stored on ice and transported to the laboratory for storage at 5º C until serum was harvested by centrifugation (1,200 x g). Sera samples were stored at −15º C until assayed for progesterone concentration by radioimmunoassay [23] using ImmuChem Double Antibody progesterone ^{125}I kits (MP Biomedicals, LLC, Orangeburg, NY, USA). Inter- and intra-assay coefficients of variation of 14 assays for a low (1.2 ± 0.1 ng/mL) and high concentration pool (17.4 ± 1.2 ng/mL) were 5.2 and 7.1%, respectively. Assay sensitivity averaged 60 ± 5.0 pg/mL and progesterone standard concentrations in the assay were 0.05, 0.1 0.2, 0.5, 2.0, 5.0, 10.0, and 25.0 ng/mL.

Ovaries were scanned via transrectal ultrasonography at not-pregnant diagnosis to characterize and map number and diameter of all ovarian follicles and any luteal structure(s). At Day −3, ovaries were re-examined to determine ovarian structures and ovulatory response to GnRH administered at the non-pregnant diagnosis. Ovaries were re-examined on Day 13 to determine ovulation risk (single or multiple) and total volume of luteal tissue (4/3 × r3 × π, where W = largest width and H = largest height of the structure; r = radius [W/2 + H/2]/2, and π = 3.14159). When a luteal structure contained a fluid-filled cavity, volume of the cavity was subtracted from the total luteal volume.

2.1.3 Statistical analyses

All binomial variables (incidence of single or multiple ovulation [proportion of ovulating cows having more than one luteal structure]), P/AI at Days 32 and 80 after AI, intervening pregnancy loss, and incidence of twin births) were analyzed using procedure GLIMMIX in SAS (SAS Enterprise 6.1, SAS Inst. Inc., Cary, NC, USA). Options used in the model statement included LINK = LOGIT, DIST = BINOMIAL, and the least square means option of ILINK and DIFF. The initial model included the fixed effects of treatment (control vs. 10 mg $PGF_{2\alpha}$ at AI), parity (primiparous vs. multiparous), season of AI (warm = May 1 through September 30 vs. cool = October 1 through April 30), and interactions of treatment with previous fixed effects, in addition to the random effects of days in milk and body condition score at time of enrollment. No interactions were detected, so they were deleted from the final model.

Continuous variables (progesterone and CL volume) were analyzed using the procedure MIXED in SAS to adjust for unequal variances. The REPEATED option added after the model statement, with GROUP = treatment allowed for estimating the individual treatment variances. Furthermore, including the option, DDFM = SATTERTHWAITE, to the MODEL statement adjusted the degrees of freedom for unequal variances. The model consisted of the same independent fixed effects described previously for the binomial variables.

2.2 Experiment 2

Experiment 2 was conducted in lactating Holstein cows on two commercial dairies in northeast Kansas. Characteristics of Herds B and C are summarized in Table 7-1. In both herds, cows were housed in sand-bedded free stall barns (2-row flushed or flushed cross-ventilated barns) and fed a total mixed diet calculated to meet nutritional requirements of lactating dairy cows [22]. Feedline sprinklers and fans over the free stalls were employed during days in which ambient temperature exceeded 22°C.

2.2.1 Experimental design

Cows were enrolled in a completely randomized design of two treatments after first or repeat timed AI as part of a 7-d Ovsynch program described for experiment 1. Cows with odd-numbered ear tags received no treatment (control; n = 911), whereas cows with an even-numbered ear tags received an im treatment of 10 mg of $PGF_{2\alpha}$ (2 mL Lutalyse, Zoetis; n = 917) concurrent with timed AI (Day 0) as in experiment 1. Initial positive pregnancy diagnoses between Days 32 and 35 were verified between Days 63 and 68 after timed AI via transrectal ultrasonography. Body condition scores (1 = thin and 5 = fat) were assessed biweekly corresponding to either 3 d before or 4 d after timed AI and treatment.

2.2.2 Statistical analyses

Binomial variables (P/AI at Days 32 to 35 and Days 63 to 68 after AI, intervening pregnancy loss, and incidence of twin births) were analyzed using procedure GLIMMIX in SAS as in experiment 1. The model included the fixed effects of treatment (control vs. 10 mg $PGF_{2\alpha}$ at AI), parity (primiparous vs. multiparous), number of inseminations (1 vs. ≥ 2), month of AI, and herd in addition to the random effects of body condition score at time of enrollment. Interactions of treatment with parity, number of inseminations, herd, and month were tested in initial models. Fixed herd effects were estimated initially in the final model that included one interaction (herd by treatment). A second model treated herd as a random effect and employed the statement

RANDOM INT/SUBJECT= HERD. Options used in the model statement included LINK = LOGIT, DIST = BINOMIAL, and the least square means option of ILINK and DIFF.

Factors influencing P/AI at Days 32 to 35, at Days 63 to 68, and intervening pregnancy loss were modeled using procedure LOGISTIC in SAS to determine odds ratios. The initial model consisted of the fixed effects of treatment, parity (primiparous vs. multiparous), number of inseminations (1 vs. ≥ 2), month of AI, and herd, and random effects of body condition score were included in the model in addition to all two-way interactions with treatment. A final model produced by backward stepwise selection of independent variables (eliminated month of AI, body condition score, and all interactions) entered or retained in the model was based on a Wald statistic ($P < 0.10$). Adjusted odds ratios (AOR) and 95% confidence intervals (CI) are reported.

3. Results

3.1 Experiment 1

Neither daily test-day (47.5 ± 0.6 vs. 46.9 ± 0.7 kg) nor energy-corrected (47.9 ± 0.6 vs. 47.7 ± 0.6 kg) milk yield differed ($P \geq 0.55$) between control and $PGF_{2\alpha}$-treated cows at the time of enrollment. Other pretreatment ovarian responses that preceded treatment at timed AI are summarized in Table 7-2. Although pretreatment concentrations of progesterone averaged < 0.3 ng/mL, they tended ($P = 0.089$) to be lesser in cows that received the treatment compared with controls (Table 7-2). When GnRH was administered at the non-pregnant diagnosis, 74.4% of the cows had a corpus luteum and ovulation response to GnRH exceeded 50%. At the time of $PGF_{2\alpha}$ preceding treatment, 89.3% of the cows had a corpus luteum. The proportion of cows with progesterone < 0.5 ng/mL at treatment averaged 91% (Table 7-2).

After treatment with $PGF_{2\alpha}$ at timed AI, ovulation risk averaged greater than 90% but did not differ between treatments (Table 7-3). Likewise, multiple ovulation risk (proportion of ovulating cows having more than one luteal structure) did not differ among treatments. Although season had no effect on either ovulation risk, multiple ovulation risk was greater ($P = 0.004$) in multiparous than primiparous cows (27.3 vs. 12.1%), respectively. Cows with progesterone > 0.5 ng/mL at timed AI had reduced ($P < 0.001$) ovulation risk compared with cows having progesterone < 0.5 ng/mL at timed AI (63.9 vs. 96.4%), but ovulation risk was unaffected by treatment.

None of the remaining outcome variables (mean concentration of progesterone and luteal tissue volume on Day 13, P/AI at Days 32 and 80 after AI, intervening pregnancy loss, or incidence of twins) differed between treatments (Table 7-3). Pregnancy risk at Day 32 (37.7 vs. 25.7%; $P = 0.048$) and Day 80 (34.2 vs. 21.6%; $P = 0.035$) was greater during the defined cool season compared with the warm season, respectively, but did not differ between primiparous and multiparous cows. Neither parity nor season influenced pregnancy loss. Although neither season ($P = 0.15$) nor treatment by season ($P = 0.87$) effects were detected, all incidences of twins occurred in cows that conceived during the defined cool season.

Progesterone concentration was greater ($P < 0.001$) in primiparous than multiparous cows (6.9 ± 0.3 vs. 5.0 ± 0.2 ng/mL), whereas luteal volume was greater ($P = 0.015$) in multiparous compared with primiparous cows (10.1 ± 0.4 vs. 8.7 ± 0.5 cm^3), respectively. Although season did not affect luteal volume, concentration of progesterone was greater ($P < 0.01$) during the defined cool vs. warm season (6.4 ± 0.2 vs. 5.5 ± 0.3 ng/mL), respectively.

Less than 9% of the cows had concentrations of progesterone ≥ 0.5 ng/mL at the time of timed AI and treatment, and based on that concentration cut point, P/AI at Day 32 did not differ ($P = 0.608$) between control and $PGF_{2\alpha}$-treated cows (13.6% [n = 22] vs.19.2% (n = 26]), respectively, indicating that the treatment did not further increase the proportion of cows with complete luteolysis. Likewise, P/AI at Day 32 for cows with progesterone < 0.5 ng/mL did not differ ($P = 0.583$) between control (34.8% [n = 149]) and $PGF_{2\alpha}$-treated cows (37.9% [n = 140]).

3.2 Experiment 2

Randomization of cows to treatment based on odd or even ear tag number produced no pretreatment differences (mean ± SE) between control and $PGF_{2\alpha}$-treated cows for days in milk, test-day milk, number of previous AI, or body condition score, respectively (Table 7-1).

Pregnancy per AI at Days 32 to 35 is summarized by month in Fig. 1. During 4 mo of the study, treatment with $PGF_{2\alpha}$ produced numerically more pregnancies compared with control cows. In contrast, during 3 mo, the reverse was true, and during 1 mo, no difference was noted. Neither treatment, month, nor treatment by month influenced P/AI at Days 32 to 35 (Fig. 1).

Pregnancy outcomes at Days 32 to 35 and Days 63 to 68, intervening pregnancy loss, and subsequent twin births are summarized by treatment and herd in Table 7-4. Herd C had greater ($P \leq 0.01$) P/AI at Days 32 to 35 and at Days 63 to 68. Pregnancy outcome was greater in primiparous than multiparous cows at Days 32 to 35 (41.2 vs. 33.8%) and at Days 63 to 68 (38.1 vs. 30.6%), respectively. Cows treated with $PGF_{2\alpha}$ at first AI had greater ($P < 0.001$) P/AI at Days 32 to 35 (42.3 vs. 32.7%) than cows treated after second or greater inseminations, respectively. A similar pattern was detected ($P = 0.001$) for P/AI at Days 63 to 68 (38.2 vs. 30.5%) for first vs. second or greater inseminations, respectively. Multiparous cows were less ($P = 0.002$; AOR = 0.74) likely to conceive at Days 32 to 35 than primiparous cows (Table 7-5). Pregnancy risk at Days 32 to 35 was less ($P = 0.001$; AOR = 0.69) likely for cows inseminated at repeat services compared with first inseminations (Table 7-5). Cows in herd C were more ($P = 0.001$; AOR = 1.46) likely to conceive at Days 32 to 35 than cows in herd B (Table 7-5). Similar findings (data not shown) were detected for factors influencing P/AI at Days 63 to 68 as described for Days 32 to 35.

Pregnancy loss tended ($P = 0.056$) to be greater in herd C than in herd B (Table 7-4) and greater ($P = 0.05$) in cows inseminated at first insemination compared with later inseminations (10.0 vs. 5.3%), respectively. Pregnancy loss was two times more ($P < 0.05$; AOR = 1.99; 95% CI = 1.06 to 3.73) likely after first services than after repeat services.

Twins produced by cows that calved subsequent to the treatment insemination resulted in an interaction ($P = 0.045$) between treatment and herd. In herd C, cows treated with $PGF_{2\alpha}$ produced 3.6 times more ($P < 0.05$) twins than control cows, whereas no treatment difference in twins was detected in herd B (Table 7-4). Neither parity, number of inseminations, season, nor body condition affected the incidence of twins.

4. Discussion

The current studies tested the hypothesis that administering im 10 mg of $PGF_{2\alpha}$ (dinoprost tromethamine) concurrent with timed AI would enhance pregnancy risk. Previous research in one study [3] reported an increase in P/AI in lactating dairy cows in one herd when 10 mg (dinoprost tromethamine), but not 5 mg, of $PGF_{2\alpha}$ were administered concurrent with timed AI in a second herd. Similar positive results were reported when 500 µg $PGF_{2\alpha}$ (cloprostenol) was administered (im or iv) to Italian Mediterranean buffaloes [16] and selected subpopulations of dairy cows in Spain [2]. Our experiments were conducted in three North-American style herds practicing a large percentage of fixed-time inseminations. Similar to the results of the present experiments, pregnancy risk was not enhanced when a standard luteolytic dose of cloprostenol (500 µg) was administered im concurrent with AI [5] in addition to two other studies reporting no positive effect of administering im standard luteolytic doses of $PGF_{2\alpha}$ (25 mg) at the time of insemination [4,6]. Results of the present study in multiple herds at a dose of 10 mg corroborate the results of the previous three studies [4,5,6] indicating that even a standard luteolytic dose of $PGF_{2\alpha}$ or one of its agonists administered im or iv at the time of insemination was incapable of increasing pregnancy risk. Based on inconsistent responses to treatment, the positive effects seem to be herd specific and depend on unknown factors for success.

In only two of three experiments [2], positive pregnancy outcomes were most consistent generally involved dairy cows inseminated at a fixed time during early lactation (near the peak of lactation and in negative energy balance) or in cows with less than acceptable reproductive performance such as repeat breeders, and where stress factors negatively impact ovulation. For example, studies with a $PGF_{2\alpha}$ analog (500 μg cloprostenol) delivered iv at timed AI significantly increased double ovulation (10.5 to 23.4%) during both winter and summer; increased P/AI in cows inseminated at estrus for second services (36 to 53%); and in repeat-breeding primiparous cows inseminated at estrus (35 to 66%) [2]. We failed to detect increased multiple ovulation in experiment 1 after treatment with $PGF_{2\alpha}$ at timed AI and did not observe any differences in P/AI in cows based on season, parity, or number of inseminations as reported by López-Gatius et al. [2].

Apart from its primary luteolytic role, $PGF_{2\alpha}$ has multiple actions in the bovine female reproductive system and in other farm animals [9]. Ambrose et al. [3] summarized evidence that might explain a positive role for treatment with $PGF_{2\alpha}$ concurrent with AI. Pregnancy risk could be enhanced directly or indirectly by: (1) increasing uterine contractility [24, 25] via $PGF_{2\alpha}$ stimulated oxytocin-induced myometrial contractions [20, 26], thereby enhancing sperm transport [17]; (2) inducing LH release via a luteolysis-independent mechanism [11], thereby facilitating the ovulatory process [27]; (3) hastening luteal regression in cows that may otherwise have delayed or incomplete luteolysis at AI, thereby creating a low progesterone environment conducive for optimal gamete transport; (4) inducing release of growth hormone [28, 29] and subsequent growth hormone-induced insulin-like growth factor-1 secretion known to increase fertility of lactating dairy cows during the pre- and peri-implantation periods resulting in improvements in conceptus development and a reduction in embryonic mortality [30]; (5) increasing the proportion of cows with a corpus luteum post-AI [2]; (6) increasing post-AI corpus luteum size and concentrations of progesterone [16]; or (7) inducing secretion of oxytocin that may stimulate uterine contractions and support sperm transport, because sperm transport has been improved by adding to semen or administering to females such compounds as $PGF_{2\alpha}$, oxytocin, estradiol, phenylephrine, or ergonovine [17].

An increase in prostaglandins in preovulatory follicles is essential for ovulation to occur [31]. Inhibiting follicular prostaglandin synthesis blocks the ovulatory process, but it can be restored by administering exogenous $PGF_{2\alpha}$ [31,32]. Treatment with $PGF_{2\alpha}$ concurrent with AI resulted in a 2.6-fold increase in multiple-ovulation risk [2] suggesting an intra-ovarian role for $PGF_{2\alpha}$ treatment at AI in which codominant follicles exist. Our results do not support increased multiple ovulation in addition to findings of another study [5]. Many factors influence the process of ovulation including the environment [2] and diet [33].

One theory proposes that the process of ovulation mimics an inflammatory response [31]. Prostaglandins mediate inflammatory responses, are self-propagating [34], and are destructive to connective tissue in acute inflammatory responses [35]. Previous research indicated that prostaglandins promote fibroblast proliferation because they mediate the later stages of the inflammatory response [31,35]. Fibroblasts are a source of latent collagenase [31,36] and secretions of pro-collagenase, activated to become collagenase, can occur by exposing fibroblasts to proteolytic enzymes [37]. Collagenase is a major enzyme that degrades thecal collagen, causing the follicle wall to weaken, and increasing follicular pressure to facilitate ovulation [31].

A greater incidence of twins was detected for cows treated with $PGF_{2\alpha}$ at timed AI in experiment 2 in one of two herds. Multiple ovulation was not increased in response to $PGF_{2\alpha}$ at timed AI in experiment 1 and no differences were detected in twinning risk. Furthermore, in herd C, in which more twins were detected after treatment, incidence of multiple ovulation was not determined. Incidence of twin births is generally undesirable in the dairy industry because it is associated with greater incidences of peripartum metabolic and reproductive disorders [39,40]. The majority of mixed-gender twins are freemartins [39] and cows bearing twins also have more abortions, stillbirths, calf mortality, and reduced birth weights compared with cows giving birth to singletons [39]. Other factors associated with twinning in cattle include genetics, parity, season of conception, cystic ovarian disease, milk production, ovulation risk, and use of hormone therapy [40,41]. Moreover, administration of $PGF_{2\alpha}$ concurrent with AI, however, increased embryo numbers in rabbits [12] and farrowing rates and litter size in sows [13].

Progesterone is partly responsible for maintaining pregnancy in cattle. A series of experiments in Italian Mediterranean buffaloes in which $PGF_{2\alpha}$ was administered (im or iv) at time of AI resulted in greater pregnancy risk, greater concentrations of progesterone 5 and 11 d later, and greater corpus luteum size, compared with controls [16]. Although numerically greater, results of experiment 1 failed to show greater luteal volume or progesterone on Day 13 after $PGF_{2\alpha}$ treatment and timed AI. Greater concentrations of progesterone in primiparous cows having smaller post-AI luteal volumes on Day 13 compared with multiparous cows with lesser concentrations of progesterone and larger luteal volumes are consistent with studies reviewed by

[15] indicating that older higher-milk producing cows may have greater metabolic clearance rate of progesterone because of greater dry matter intake and its subsequent effects on hepatic blood flow and hepatic enzymes responsible for steroid metabolism.

5. Conclusions

In conclusion, under the conditions of these studies, im treatment of lactating dairy cows with 10 mg $PGF_{2\alpha}$ concurrent with timed AI did not improve P/AI or embryo survival in three dairy herds. Treatment increased twining risk in one of three herds. These results do not support the hypothesis that administering $PGF_{2\alpha}$ to lactating dairy cows at the time of AI is a means to enhance fertility.

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References

[1] Prinzen R, Allgayer F, Bartz U, Huber E. Effects of prostaglandin $F_{2\alpha}$ on conception results of heifers and cows. Tierartzliche Umschau (Veterinary Review) 1991;46:27–35. [Article in German].

[2] López-Gatius F, Yaniz JL, Santolaria P, Murugavel K, Guijarro R, Calvo E. Reproductive performance of lactating dairy cows treated with cloprostenol at the time of insemination. Theriogenology 2004;62:677–89.

[3] Ambrose DJ, Gobikrushanth M, Zuidhof S, Kastelic JP. Low-dose natural prostaglandin $F_{2\alpha}$ (dinoprost) at timed insemination improves conception rate in dairy cattle. Theriogenology 2015;4:529–34.

[4] Archbald LF, Tran T, Massey R, Klapstein E. Conception rates in dairy cows after timed insemination and simultaneous treatment with gonadotropin releasing hormone and/or prostaglandin $F_{2\alpha}$. Theriogenology 1992;37:723–31.

[5] Kauffold J, Gommel R, Failing K, Beyon N, Wehrend A. Postinsemination treatment of primiparous and multiparous cows with cloprostenol failed to affect ovulation and pregnancy rate in dairy cattle. Theriogenology 2009;72:741–46.

[6] Gabriel H.G, Wallenhorst S, Dietrich E, and Holtz W. The effect of prostaglandin $F_{2\alpha}$ administration at the time of insemination the pregnancy rate of dairy cows. Anim Reprod Sci 2001;123:1–4.

[7] Pfeifer LF, Leonardi CE, Castro NA, Viana JH, Siqueira LG, Castilho EM, Singh J, Krusser RH, Rubin MI. The use of $PGF_{2\alpha}$ as ovulatory stimulus for timed artificial insemination in cattle. Theriogenology 2014;81:689–95.

[8] Lauderdale JW. ASAS centennial paper: Contributions in the Journal of Animal Science to the development of protocols for breeding management of cattle through synchronization of estrus and ovulation. J Anim Sci 2009;87:801–12.

[9] Weems CW, Weems YS, Randel RD. Prostaglandins and reproduction in female farm animals. Vet J 2006;171:206–228.

[10] Richterich R., Wehred, A. Application of prostaglandins in heifers and cows. Terarztl Prax 2009; 37(G):81–90.

[11] Randel RD, Lammoglia MA, Lewis AW, Neuendorff DA, Guthrie MJ. Exogenous $PGF_{2\alpha}$ enhanced GnRH-induced LH release in postpartum cows. Theriogenology 1996;45:643–54.

[12] Spilman CH, Finn AE, Norland JF. Effect of prostaglandins on sperm transport and fertilization in rabbits. Prostaglandins 1973;4:57–64.

[13] Peña FJ, Domínguez JC, Peláez J, Alegre B. Intrauterine infusion of $PGF_{2\alpha}$ at insemination enhances reproductive performance of sows during the low fertility season. Vet J 2000; 159:259–61.

[14] Martins JP, Policelli RK, Neuder LM, Raphael W, Pursley JR. Effects of cloprostenol sodium at final prostaglandin $F_{2\alpha}$ of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. J Dairy Sci 2011;94:2815–24.

[15] Wiltbank MC, Souza AH, Carvalho PD, Cunha AP, Giordano JO, Fricke PM, Baez GM, Diskin MG. Physiological and practical effects of progesterone on reproduction in dairy cattle. Animal 2014;8 Suppl 1:70–81.

[16] Neglia G, Natale A, Esposito G, Salzillo F, Adinolfi L, Campanile G. Effect of prostaglandin $F_{2\alpha}$ at the time of AI on progesterone levels and pregnancy rate in synchronized Italian Mediterranean buffaloes. Theriogenology 2008;69:953–960.

[17] Hawk HW. Sperm survival and transport in the female reproductive tract. J Dairy Sci 1983;66:2645–60.

[18] Hawk HW, Echternkamp SE. Uterine contractions in the ewe during progestogen-regulated estrus. J Reprod Fertil 1973;34:347–9.

[19] Yu H, Shin D-H, Hsu WH. Effects of oxytocin (OT),vasopressin (VP), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on porcine myometrial contractility in vitro and their dependence on Ca^{2+} influx. Biol Reprod 1993;48(Suppl):162.

[20] Patil RK, Sinha SN, Einarsson S, Settergren I. The effect of prostaglandin $F_{2\alpha}$ and oxytocin on bovine myometrium in vitro. Nord. Vet Med 1980;32:474–9.

[21] Edqvist S, Einarsson S, Gustafsson B. Effect of prostaglandin $F_{2\alpha}$ on sperm transport in the reproductive tract of the ewe. Acta Vet Scand 1975;16:149–51.

[22] National Research Council (NRC). Nutrient requirements of dairy cattle. 7th rev. ed. Natl Acad Press, Washington, DC. 2001.

[23] Hill SL, Grieger DM, Olson KC, Jaeger JR, Dahlen CR, Crosswhite MR, Negrin Pereira N, Underdahl SR, Neville BW, et al. 2016. Gonadotropin-releasing hormone increased pregnancy risk in sucked beef cows not detected in estrus and subjected to a split-time artificial insemination program. J Anim Sci 2016;94:3722–28.

[24] Eiler H, Hopkins FM, Armstrong-Backus CS, and Lyke WA. Uterotonic effect of prostaglandin F and oxytocin on the postpartum cow. Am J Vet Res 1984;45:1011–14.

[25] Stolla R, Schmid G. Effects of natural and synthetic $PGF_{2\alpha}$ preparations on the uterine contractility of cattle. Berl. Munch. Tierarztl. Wochenschr. 1990;103:198–202. [Article in German].

[26] Burns PD, Mendes JOB, Yemm RS, Clay CM, Nelson SE, Hayes SH, and Silvia WJ. Cellular mechanisms by which oxytocin mediates ovine endometrial prostaglandin $F_{2\alpha}$ synthesis: role of Gi proteins and mitogen-activated protein kinases. Biol Reprod 2001;65:1150–55.

[27] Leonardi CEP, Pfeifer LFM, Rubina MIB, Singh J, Mapletoft RJ, Pessoa GA. Prostaglandin $F_{2\alpha}$ promotes ovulation in prepubertal heifers. Theriogenology 2012;78:1578–82.

[28] Tucker HA, Vines DT, Stellflug JN, Convey EM. Milking, thyrotropin-releasing hormone and prostaglandin induced release of prolactin and growth hormone in cows. Proc Soc Exp Biol Med 1975;149:462–69.

[29] Renegar RH, Hafs HD, Britt JH, Carruthers TD. Luteolysis, growth hormone, glucocorticoids, prolactin, and milk production in lactating dairy cows given prostaglandin $F_{2\alpha}$. J Anim Sci 1978;47:532–37.

[30] Ribeiro ES, Bruno RGS, Farias AM, Hernández-Rivera JA, Gomes GC, Surjus R, Becker LFV, Birt A, Ott TL, Branen JR, Sasser RG, Keisler DH, Thatcher WW, Bilby TR, Santos JEP. Low doses of bovine somatotropin enhance conceptus development and fertility in lactating dairy cows. Biol Reprod 2014;90:1-12.

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

[32] Armstrong DT, Zamecnik J. Preovulation elevation of rat ovarian prostaglandins $F_{2\alpha}$, and its blockade by indomethacin. Mol Cell Endocrinol 1975;2:125–31.

[33] Webb R, Garnsworthy PC, Gong JG, Armstrong DG. Control of follicular growth: local interactions and nutritional influences. J Anim Sci 2004;82(E-Suppl):E63–74.

[34] Lewis G.P. Prostaglandins in inflammation. J Reticuloendothelial Soc. 1977;22:389–402.

[35] Bonta IL, Parnham MJ. Prostaglandins and chronic inflammation. Biochem Pharmacol 1978;27:1611–23.

[36] DeAsua LJ, Clingan D, Rudland PS. Initiation of cell proliferation in cultured mouse fibroblast by prostaglandin $F_{2\alpha}$. Proc Nat Acad Sci USA 1975;72:2724–28.

[37] Werb Z, Aggeler J. Proteases induce secretion of collagenase and plasminogen activator by fibroblast. Proc Nat Acad Sci USA 1978;75:1839–43.

[38] Kinsel ML, Marsh WE, Ruegg PL, Etherington WG. Risk factors for twinning in dairy cows. J Dairy Sci 1997;81:989–93.

[39] Fricke P. Twinning in dairy cattle. Prof Anim Sci 2001;17:61–7.

[40] Bendixen PH, Oltenacu PA, Anderson L. Case-referent study of cystic ovaries as a risk indicator for twin calvings in dairy cows. Theriogenology 1989;31:1059–66.

[41] Nielen M, Schukken YH, Scholl T, Wilbrink J, Brand A. Twinning in dairy cattle: a study of risk factors and effects. Theriogenology 1989;32:845–62.

Table 7-1 Characteristics of herd at the time of enrollment in experiment 1 and 2

Item	Experiment 1	Experiment 2	Experiment 2
Herd	A	B	C
No. cows treated	289	1,066	762
Days in milk[a]	145 ± 49	114 ± 48	100 ± 56
Milking frequency	3×	3×	3×
First-lactation cows, %	39	38	34
Test-day milk[a], kg	49 ± 9	42 ± 8	39 ± 10
Previous inseminations[a], no.	3.4 ± 1.5	1.5 ± 0.5	1.4 ± 0.5
Body condition score[a]	2.6 ± 0.4	2.8 ± 0.4	2.8 ± 0.4

[a] Mean ± SD.

Table 7-2. Pretreatment ovarian responses of lactating dairy cows (herd A) during the ovulation synchronization program initiated at the not-pregnant diagnosis preceding treatment with 10 mg $PGF_{2\alpha}$ at timed AI (experiment 1).

	Treatment[b]		
Item[a]	Control	$PGF_{2\alpha}$	P-value
Cows, no.	149	140	
GnRH			
Follicles ≥ 10 mm, no.	1.4 ± 0.07	1.4 ± 0.07	0.389
Corpus luteum, no.	0.9 ± 0.06	0.8 ± 0.06	0.107
Largest follicle, mm	13.9 ± 0.3	14.0 ± 0.3	0.669
Ovulation risk, %			
Single ovulation, %	48.7	52.2	0.543
Multiple ovulation, %	14.7	8.6	0.263
$PGF_{2\alpha}$			
Follicles ≥ 10 mm, no.	1.5 ± 0.07	1.5 ± 0.07	0.926
Largest follicle, mm	13.1 ± 0.2	13.0 ± 0.2	0.688
Corpus luteum, no.	1.3 ± 0.06	1.2 ± 0.06	0.243
Progesterone, ng/mL	4.1 ± 0.3	4.6 ± 0.3	0.248
Timed AI			
Progesterone, ng/mL	0.25 ± 0.04	0.15 ± 0.04	0.089
Progesterone < 0.5 ng/mL, %	90.1	93.3	0.332

[a] Five- or 7-d Ovsynch timed AI program before AI (GnRH administered upon not-pregnant diagnosis — 5 or 7 d — $PGF_{2\alpha}$ — 56 h — GnRH — 16 h — AI). A second dose of $PGF_{2\alpha}$ was administered 24 h after the first when the 5-d program was employed.

[b] Cows were treated with 10 mg $PGF_{2\alpha}$ at timed AI.

Table 7-3. Ovarian responses and pregnancy per AI (P/AI) after treatment of lactating dairy cows (herd A) with 10 mg $PGF_{2\alpha}$ at AI (experiment 1).

	Treatment[a]		
Item	Control	$PGF_{2\alpha}$	P-value
Cows, no.	149	140	. . .
Ovulation, %	91.9	89.8	0.514
Multiple ovulation[b], %	22.3	15.3	0.139
Progesterone[c], ng/mL	5.7 ± 0.3	6.2 ± 0.3	0.206
Volume of luteal tissue[c], cm^3	8.9 ± 0.4	9.8 ± 0.5	0.137
P/AI at Day 32, %	32.5	36.8	0.499
P/AI at Day 80, %	27.9	33.3	0.329
Pregnancy loss (Days 32 to 80), %	14.0	8.9	0.387
Incidence of twins, %	7.0	9.8	0.938

[a] Cows were treated im with 10 mg $PGF_{2\alpha}$ at AI.
[b] Percentage of cows that ovulated more than one follicle as a proportion of all cows that ovulated.
[c] Day 13 after treatment and AI.

Table 7-4. Pregnancy per AI (P/AI) after treatment of lactating dairy cows with 10 mg $PGF_{2\alpha}$ at AI[a] (experiment 2).

Item	Herd B	Herd C	Overall
	% (no. of cows)		
P/AI at Days 32 to35			
Control	33.9 (549)	43.1 (362)	37.6 (911)
$PGF_{2\alpha}$	30.2 (517)	44.0(400)	36.2 (917)
Herd mean	32.1 (1,066)	43.6[x] (762)	
P/AI at Days 63 to 68			
Control	32.2 (549)	38.1 (362)	34.5 (911)
$PGF_{2\alpha}$	27.7 (516)	38.9 (391)	32.5 (907)
Herd mean	30.0 (1,065)	38.5[x] (753)	
Pregnancy loss[b]			
Control	4.8 (186)	11.5 (156)	7.8 (342)
$PGF_{2\alpha}$	7.7 (155)	9.0 (167)	8.4 (322)
Herd mean	6.1 (341)	10.2[y] (323)	
Twins[c,d]			
Control	5.6 (177)	3.2 (126)	4.6 (303)
$PGF_{2\alpha}$	5.6 (143)	11.7[z] (137)	8.6 (280)
Herd mean	5.6 (320)	7.6 (263)	

[a] Cows were treated im with 10 mg $PGF_{2\alpha}$ at timed AI.
[b] One cow in herd B and 9 cows in herd C were culled before the second pregnancy diagnosis occurred.
[c] Interaction between treatment and herd ($P = 0.045$).
[d] Twenty seven cows from herd C did not calve: 5 aborted, 8 died, and 14 were sold.
[x] Differs ($P < 0.01$) from herd B.
[y] Tends ($P = 0.056$) to differ herd B.
[z] Differs ($P < 0.05$) from control in herd C.

Table 7-5. Factors affecting pregnancy per AI (P/AI) at Days 32 to 35[a] (experiment 2).

Item	n[b]	P/AI[c] (%)	P-value	AOR	CI
Treatment					
Control	911	37.6	0.392	Referent	
$PGF_{2\alpha}$	917	36.2		0.92	0.76-1.12
Parity					
Primiparous	770	41.2	0.002	Referent	
Multiparous	1,058	34.1		0.74	0.61-0.90
AI number					
1	965	42.0	0.001	Referent	
2+	863	33.4		0.69	0.55-0.82
Herd					
B	1,066	32.1	<0.001	Referent	
C	762	43.6		1.46	1.12-1.79

[a] Effects of either month of AI or body condition score (not shown) were eliminated by backward stepwise regression.
[b] Cows were treated with 10 mg $PGF_{2\alpha}$ at AI.
[c] Means determined by logistic regression model (GLIMMIX) with herd as a random effect. The same model was employed with herd as a fixed effect (LOGISTIC) to determine adjusted odds ratios (AOR) and 95% confidence intervals (CI).

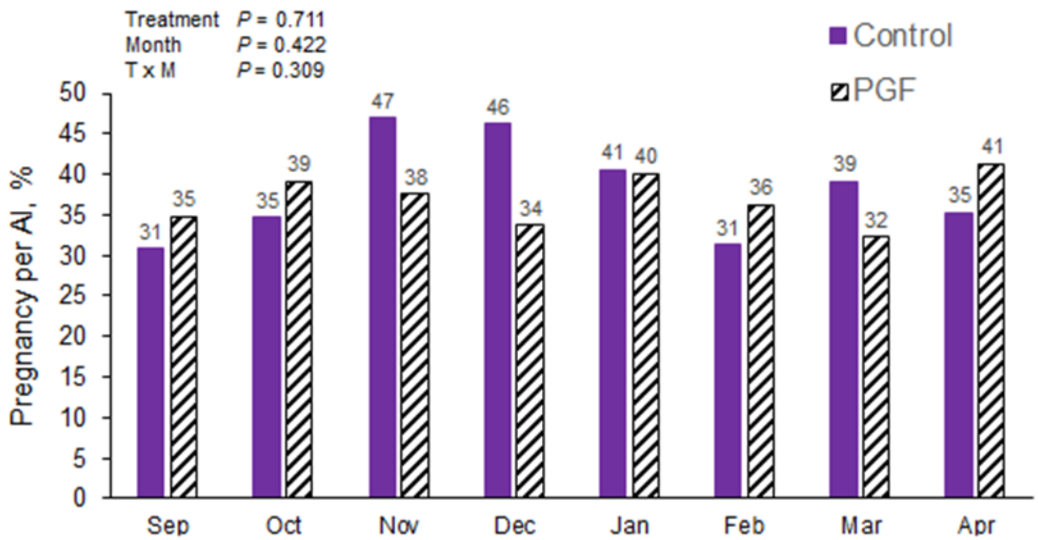

Figure 7-1. Monthly pregnancy per AI at Days 32 to 35 after AI and treatment (10 mg $PGF_{2\alpha}$ im at AI) of lactating dairy cows in two commercial herds (experiment 2).