CSIRO PUBLISHING

Reproduction, Fertility and Development, 2012, **24**, 267–271
http://dx.doi.org/10.1071/RD11917

Impact of circulating concentrations of progesterone and antral age of the ovulatory follicle on fertility of high-producing lactating dairy cows

J. Richard Pursley[A,B] *and João Paulo N. Martins*[A]

[A]Department of Animal Science, Michigan State University, East Lansing, MI 48824, USA.
[B]Corresponding author. Email: pursleyr@msu.edu

Abstract. Dairy cow infertility negatively affects profit of dairy production enterprises around the world, and enhancing conception rates of dairy cows is a critical management issue to resolve. It appears that conception rates of dairy cows are attenuated due to reduced progesterone concentrations in circulation during growth of the ovulatory follicle. It is not clear how reduced progesterone influences fertility, but data presented in this brief review suggest that it can be somewhat reversed through increasing concentrations of progesterone during the growth of the ovulatory follicle before luteolysis. Ovsynch protocols may be utilised to enhance progesterone concentrations through the induction of an accessory corpus luteum (CL) following the initial gonadotropin-releasing hormone (GnRH) treatment. Cows at Day 13 of the oestrous cycle with a 7-day-old accessory CL had ~50% more progesterone at the time of prostaglandin injection of Ovsynch compared with cows with only a Day 13 CL. Ovsynch can consistently induce an accessory CL following the initial GnRH treatment if cows are on Days 6 or 7 of the oestrous cycle at the time of treatment. Pre-synchrony strategies are critical to enhance the probability that cows will be on Days 6 or 7 at first GnRH treatement of Ovsynch.

Additional keywords: accessory corpus luteum, infertility, gonadotropin-releasing hormone, ovary, Ovsynch, prostaglandin $F_{2\alpha}$.

Introduction

Infertility of the lactating dairy cow continues to be a critical problem limiting profitability and sustainability of dairy farms (Lucy 2001; Washburn *et al.* 2002). Reproductive performance of lactating dairy cows is dependent on service rate (or oestrus detection rate), fertility of the service sire and maternal fertility. Service rate can be controlled utilising gonadotropin-releasing hormone (GnRH)-based (Ovsynch) technology (Fig. 1; Pursley *et al.* 1995, 1997). The majority of dairy producers in the USA regulate time to first and subsequent artificial inseminations (AI) with Ovsynch technology (USDA 2009). High fertility sires can be chosen utilising the USDA-ARS sire conception rate summaries. Yet maternal fertility, defined as the mother's ability to ovulate a competent oocyte and provide an oviducal and uterine environment capable of fertilisation and complete embryonic and fetal development, continues to be the key limiting factor for profitable reproductive performance in lactating dairy cows.

Conception rates of lactating dairy cows are ~30% (J. R. Pursley, unpubl. data) compared with 60% in virgin dairy heifers (Pursley *et al.* 1997; Roth *et al.* 2008) when inseminated following a detected oestrus. Increasing conception rates of lactating cows to that of heifers would allow producers to employ the most profitable calving interval strategies for cows with different production levels, and increase profit. Aspects of maternal fertility that limit conception and embryonic/fetal development are not well understood. Yet it appears that Ovsynch technology may be able to be modified to address these issues. A significant change in circulating concentrations of steroid hormones takes place following the transition from heifer to lactating dairy cow. Circulating concentrations of progesterone (P4) in lactating dairy cows are nearly half the levels detected in nulliparous heifers (Sartori *et al.* 2004). This difference in circulating P4 appears to influence follicle growth by prolonging the age of the ovulatory follicle via reduced negative feedback of P4 on pulses of luteinising hormone (LH; Roche and Ireland 1981). Allowing more LH pulses to occur may drive the growth of a dominant follicle (DF) similarly, but likely not to the extreme, as with a persistent follicle. Oocytes from this potentially LH over-stimulated ovulatory follicle may have similar characteristics to a persistent follicle and may be less competent to fertilise and/or develop into a competent embryo compared with oocytes from animals with higher concentrations of P4 (Sartori *et al.* 2002*a*).

This paper will focus on how increasing circulating concentrations of P4 before prostaglandin $F_{2\alpha}$ (PGF)-induced luteolysis during Ovsynch may significantly enhance fertility of lactating dairy cows. Our data strongly suggest that initiating Ovsynch on Days 6 or 7 of the oestrous cycle induces ovulation to the first GnRH more than 90% of the time and induces an accessory corpus luteum (CL), thereby increasing concentrations of P4 before PGF-induced luteolysis 7 days later (Bello *et al.* 2006).

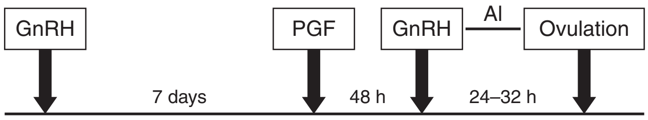

Fig. 1. Description of the original Ovsynch programme utilising GnRH and prostaglandin $F_{2\alpha}$ (PGF) to control the time of ovulation in lactating dairy cows. AI, artificial insemination.

Inducing ovulation and a new accessory CL also induces a new follicular wave and the growth of a new DF. This allows for greater control of ovulation of a young, yet mature ovulatory follicle.

Effect of low P4 on ovarian dynamics and fertility of lactating dairy cows

The problem

During the past 50 years, conception rates of lactating dairy cows in the USA have decreased from ~60% (Foote 1952; Herman 1956) to 30% (López-Gatius 2003; Macfarlane 2003), while conception rates in heifers remained steady at ~60% (Herman 1956; Pursley *et al.* 1997). During that time milk production per cow and dry matter intake have increased linearly (Washburn *et al.* 2002). Published data now indicate that high dry matter intake in high-producing dairy cows results in increased blood flow to the liver and increased steroid metabolism (Sangsritavong *et al.* 2002). Even though lactating dairy cows have larger ovulatory follicles and CL than nulliparous heifers, circulating concentrations of oestradiol (E2) and P4 are significantly less in cows compared with heifers (Sartori *et al.* 2002*b*). In addition, there are clearly greater concentrations of E2 and P4 in mature beef cows compared with mature lactating dairy cows (Twagiramungu *et al.* 1992). This difference can only be explained by differences in level of milk production and dry matter intake. Thus, the modern high-producing, lactating dairy cow is deficient in E2 and P4. This deficiency may be partly responsible for the decline in conception rates.

Evidence that low P4 may be responsible for the decline in conception rates of lactating dairy cows

Treatments that maintain low concentrations of P4 (1–2 ng mL^{-1}) for an extended period cause prolonged growth and dominance of the DF, and can be made to persist longer than the lifespan of a normal DF (Sirois and Fortune 1990; Adams *et al.* 1992; Savio *et al.* 1993; Mihm *et al.* 1994; Bridges and Fortune 2003). When the persistent DF is allowed to ovulate, fertility is decreased in comparison with younger ovulatory follicles (Savio *et al.* 1993; Stock and Fortune 1993; Mihm *et al.* 1994; Cooperative Regional Research Project 1996; Revah and Butler 1996). Sub-luteal levels of P4 during a prolonged period increase frequency of LH pulsatility (Roberson *et al.* 1989; Kojima *et al.* 1992). As a result, the oocyte housed in this persistent DF may resume meiosis while still contained in the follicle and could undergo premature nuclear maturation. Histological characteristics of these persistent DFs indicate that the oocyte undergoes early germinal vesicle breakdown and continues a progression through the cell cycle towards metaphase I or II (Revah and Butler 1996; Mihm *et al.* 1999). By the time of ovulation of the persistent follicle the oocyte has already matured and aged resulting in reduced fertility, which may be explained either by low fertilisation rates (Fugo and Butcher 1966; Butcher and Fugo 1967), high early embryonic mortality (Ahmad *et al.* 1995) or both. In a normal oestrous cycle, lactating dairy cows have about half the circulating concentrations of P4 to that of heifers (Sartori *et al.* 2004). We hypothesise that follicles that grow in this environment may have similar characteristics to persistent follicles that grow under slightly lower concentrations of P4. Thus, increasing P4 by utilising Ovsynch technology to induce an accessory CL may resolve this problem. Our data indicate that, compared with a normal oestrous cycle, inducing an accessory CL during Ovsynch increases serum P4 from 3.5 to 5.2 ng mL^{-1}, which increases the likelihood that the ovulatory follicle will emerge (J. R. Pursley, unpubl. data), deviate from subordinates and develop into a DF. These data indicate that the higher the P4 before PGF-induced luteolysis, the higher the conception rates.

Overview of Ovsynch

This overview of Ovsynch intended to describe the potential outcomes of each injection of hormone within the Ovsynch protocol and the reasons for each outcome. It provides the basis for why cows must be on Day 6 or 7 of the oestrous cycle when Ovsynch is initiated, and thus why an efficient pre-synchronisation programme is essential to achieve the goal to induce accessory CL and increase P4 before PGF-induced luteolysis (Fig. 2). Ovsynch is based on three treatments: the first treatment, 100 μg of GnRH, in absence of pre-synchrony programmes, is administered at a random stage of the oestrous cycle (Day 0). The intent of the first GnRH-induced LH surge is to induce ovulation of a mature functional DF (Pursley *et al.* 1995). Cows with two follicular waves have an approximately 70% chance that a functional DF capable of ovulation is present in the ovaries at a random stage of the oestrous cycle. Ovulation of the DF induces the subsequent emergence of a new follicular wave ~1.5 days later (Pursley *et al.* 1995) followed by the growth and development of both a new DF and an accessory CL during the next 7 days. The new DF has an approximately 97% chance of remaining functional during the 7 days leading up to the PGF injection on Day 7, then continuing on to ovulation following the final GnRH-induced LH surge on Day 9, even if luteolysis following the PG is not complete (Pursley *et al.* 1995). At random stages of the oestrous cycle, cows have an ~30% chance of being in early stages of follicular development (first or second wave) at a time when granulosa cells have not yet acquired sufficient LH receptors to respond to an LH surge (Garverick *et al.* 1985). In this case, the first GnRH does not induce ovulation and the potential DF continues to grow, deviate from subordinates and develop as a DF (Pursley *et al.* 1995). This DF has an approximately 80% chance of remaining functional before the PGF-induced luteolysis and subsequent increase in LH pulsatility that allows further development (Pursley *et al.* 1995). If this DF remains functional until the time of PGF injection, it will continue to develop into a pre-ovulatory follicle and has a 97% chance of ovulation following the final injection of GnRH (Bello *et al.* 2006). However, this follicle could be as

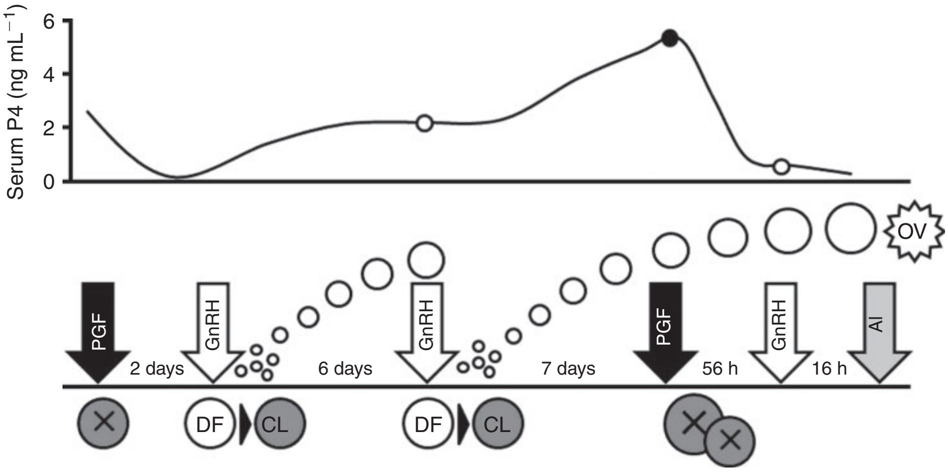

Fig. 2. Description of dominant follicle (DF) and corpus luteum (CL) development and concentrations of progesterone in lactating dairy cows that responded (new CL and new follicular wave following GnRH or luteolysis following prostaglandin $F_{2\alpha}$ (PGF)) to each treatment of a PGF (2 days) GnRH pre-synchronisation scheme 6 days before the initiation of Ovsynch (GnRH, 7 days later PGF, 56 h later GnRH, 16 h later AI). ×, luteolysis; OV, ovulation; AI, artificial insemination.

much as 12 days from emergence. Thus, conception rates following the ovulation of this follicle could be attenuated due to the antral age of the follicle and oocyte. Approximately 20% of these follicles become atretic before the PGF injection. If this happens, a new wave will emerge generally just before the PGF and the largest follicle may deviate from subordinates, but this follicle may not have deviated from subordinates and acquired LH receptors before the final GnRH-induced LH surge (Garverick *et al.* 1985). In this scenario, cows will likely have a natural oestrus that may or may not be detected several days after the timed-AI as the new DF develops under basal concentrations of progesterone into an ovulatory follicle. Conception rates from the timed-AI in this case would be near 0%. Thus, it is critical to control ovulation of a DF in response to the first GnRH not only to induce an accessory CL and increase circulating P4, but to control the age of the DF and control ovulation to the final GnRH-induced LH surge and avoid the asynchrony of ovulation as described. To ensure that cows respond to the first GnRH-induced LH surge of Ovsynch, cows must be on Days 6 or 7 of the oestrous cycle (Bello *et al.* 2006). Thus, it is absolutely imperative that cows be pre-synchronised before Ovsynch so that cows are on Days 6 or 7 of the oestrous cycle at the first GnRH of Ovsynch.

The second treatment in the Ovsynch protocol, a luteolytic dose of PGF, is administered to induce luteolysis, thus enabling the DF of the new follicular wave to develop into a pre-ovulatory follicle. Our recent data indicate that lactating dairy cows only have a 77% chance of complete luteolysis before the final GnRH (Martins *et al.* 2011). Even though cows have a high likelihood of responding to the final GnRH and ovulating, these data indicate that the chance of a pregnancy is 5% if circulating P4 at the time of the final GnRH is not less than 0.5 ng mL^{-1}. Also, when Ovsynch is initiated late in the oestrous cycle, there is a high likelihood that the CL may undergo natural luteolysis before the PGF injection. If this happens, cows may have a natural oestrus and ovulate early. If cows are not detected in oestrus and inseminated accordingly, conception rates may be significantly reduced following timed-AI due to the asynchrony of AI and ovulation, i.e. timed-AI may occur well after ovulation.

The third treatment in the Ovsynch protocol is 100 μg of GnRH. This additional GnRH treatment is administered 48–60 h after PGF injection to induce a pre-ovulatory LH surge, trigger ovulation of the functional DF 24–32 h later (Pursley *et al.* 1995) and release the oocyte to be fertilised following timed-AI. The chance of ovulation to this treatment is >95% even if luteolysis is not complete before this injection (Pursley *et al.* 1995). As mentioned above, cows can have a synchronised ovulation but still not have a chance to become pregnant due to incomplete or prolonged luteolysis.

Evidence that P4 concentrations at time of PGF-induced luteolysis of Ovsynch are positively associated with fertility

Fonseca *et al.* (1983) were the first to report that Holstein and Jersey dairy cows that became pregnant had higher concentrations of P4 in a 12-day period before AI compared with cows that did not become pregnant. In two studies conducted recently in our laboratory (Strickland *et al.* 2010; Martins *et al.* 2011), P4 concentrations at the time of the PGF-induced luteolysis of Ovsynch had a substantial impact on the probability of a pregnancy in Holstein dairy cows (Fig. 3). Thus, enhancing P4 concentrations before PGF-induced luteolysis is expected to enhance fertility. To induce an accessory CL following the first GnRH of Ovsynch, cows must be pre-synchronised (before Ovsynch) to ensure that cows are in a stage of the oestrous cycle that has both a high probability of ovulation of a DF to the first GnRH and control of subsequent luteolysis with PGF, i.e. before endogenous luteolysis. In two previous studies (Bello *et al.* 2006; Bello and Pursley 2007) we tested and compared the rate

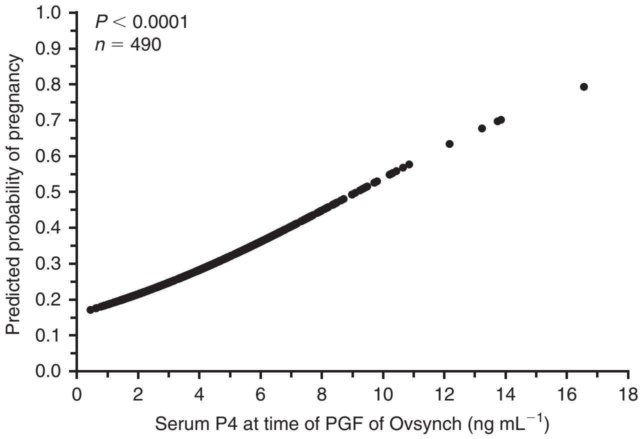

Fig. 3. The predicted probability of pregnancy 39 days post artificial insemination based on circulating concentrations of progesterone (P4) at time of final prostaglandin $F_{2\alpha}$ (PGF) of Ovsynch in lactating dairy cows ($n = 490$) treated with pre-synchronisation + Ovsynch and with functional corpus luteum (CL) at time of PGF of Ovsynch.

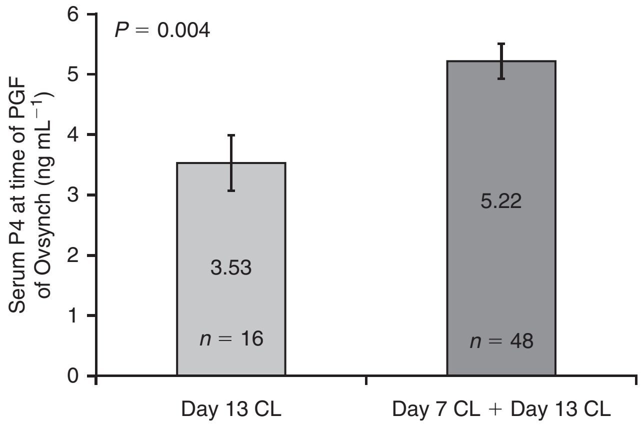

Fig. 4. Average circulating concentrations of progesterone (P4) at prostaglandin $F_{2\alpha}$ (PGF) of Ovsynch in lactating dairy cows ($n = 64$) with a Day 13 corpus luteum (CL) versus a Day 13 CL with an additional Day 7 accessory CL.

of induction of accessory CL at Days 4, 5, 6, 7 or 8 of the oestrous cycle at the time of the first GnRH of Ovsynch. The Day 6 interval (referred to as G6G) was found to result in a significantly higher percentage of cows ovulating to the first GnRH and inducing an accessory CL compared with the other intervals. In cows that responded to both PGF and GnRH pre-synchrony treatments and were on Day 6 of the cycle at time of first GnRH of Ovsynch, 97% developed an accessory CL, had significantly higher circulating P4 concentrations and a greater probability of a pregnancy (Bello *et al.* 2006).

Evidence that GnRH induction of accessory CL during Ovsynch increases P4 concentrations at time of PGF-induced luteolysis of Ovsynch

Multiple strategies have been tested previously to increase P4 before the PGF injection of Ovsynch. In studies that used exogenous P4 (progestin-releasing vaginal devices) to achieve higher concentrations of circulating P4 before AI, there was no significant increase in P4 on the day of PGF-induced luteolysis of Ovsynch or in resulting conception rates of cycling cows (Stevenson *et al.* 2006). In cycling cows receiving progesterone-releasing vaginal devices, i.e. controlled internal drug release (Pfizer), concentrations of P4 were 2.7 versus 2.8 ng mL^{-1} in cows without a progesterone-releasing vaginal device (Stevenson *et al.* 2006). Thus, use of a progesterone-releasing vaginal device does not represent a viable strategy to increase P4 concentrations and enhance fertility in lactating dairy cows. We therefore examined the possibility that GnRH inducement of an accessory CL during Ovsynch could be used to more effectively enhance P4 concentrations. As mentioned above, when cows were on Day 6 of the oestrous cycle at the time of the first GnRH of Ovsynch, 97% ovulated a first wave DF and formed an accessory CL. When the PGF of Ovsynch was administered 7 days later, cows that ovulated had both a primary Day 13 CL and a Day 7 accessory CL and correspondingly higher circulating concentrations of P4 at that time, compared with cows that did not receive GnRH and only had a Day 13 CL (Fig. 4). Thus, presence of a young (Day 7) accessory CL during Ovsynch positively impacts P4 concentrations before AI.

Summary

These data provide a basis for the concept that low progesterone in lactating dairy cows due to enhanced steroid metabolism may be the underlying cause of the low fertility that has plagued dairy herds for the past two decades. We have developed synchronisation strategies that partially solve this problem. Enhancing the percentage of cows that respond to the first GnRH of Ovsynch allows for more cows with an accessory CL, higher concentrations of circulating progesterone at time of induced luteolysis and a greater chance of the establishment of pregnancy.

References

Adams, G. P., Matteri, R. L., and Ginther, O. J. (1992). Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone in heifers. *J. Reprod. Fertil.* **96**, 627–640. doi:10.1530/JRF.0.0960627

Ahmad, N., Schrick, F. N., Butcher, R. L., and Inskeep, E. K. (1995). Effect of persistent follicles on early embryonic losses in beef cows. *Biol. Reprod.* **52**, 1129–1135. doi:10.1095/BIOLREPROD52.5.1129

Bello, N. M., and Pursley, J. R. (2007). Strategies to maximize ovulation to first GnRH of Ovsynch in lactating dairy cows. *J. Dairy Sci.* **90**(Suppl. 1), 327–328.

Bello, N. M., Steibel, J. P., and Pursley, J. R. (2006). Optimizing ovulation to 1st GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J. Dairy Sci.* **89**, 3413–3424. doi:10.3168/JDS.S0022-0302(06)72378-5

Bridges, P. J., and Fortune, J. E. (2003). Characteristics of developing prolonged dominant follicles in cattle. *Domest. Anim. Endocrinol.* **25**, 199–214. doi:10.1016/S0739-7240(03)00053-5

Butcher, R. L., and Fugo, N. W. (1967). Over-ripeness and the mammalian ova. II. Delayed ovulation and chromosome anomalies. *Fertil. Steril.* **18**, 297–302.

Cooperative Regional Research Project (1996). Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *J. Anim. Sci.* **74**, 1943–1952.

Fonseca, F. A., Britt, J. H., McDaniel, B. T., Wilk, J. C., and Rakes, A. H. (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. doi:10.3168/JDS.S0022-0302(83)81910-9

Foote, R. H. (1952). Survey of breeding efficiency on 2,700 New York state dairy herds. Cornell University, Mimeo.

Fugo, N. W., and Butcher, R. L. (1966). Over-ripeness and the mammalian ova. I. Over-ripeness and early embryonic development. *Fertil. Steril.* **17**, 804–814.

Garverick, H. A., Smith, M. F., Elmore, R. G., Morehouse, G. L., Agudo, L. S., and Zahler, W. L. (1985). Changes and interrelationships among luteal LH receptors, adenylate cyclase activity and phosphodiesterase activity during the bovine estrous cycle. *J. Anim. Sci.* **61**, 216–223.

Herman, H. A. (1956). Age-fertility relationships in cattle serviced by artificial insemination. In: '3rd Int. Congr. Anim. Reprod. AI'. pp. 56.

Kojima, N., Stumpf, T. T., Cupp, A. S., Werth, L. A., Roberson, M. S., Wolfe, M. W., Kittok, R. J., and Kinder, J. E. (1992). Exogenous progesterone and progestins as used in estrous synchrony regimens do not mimic the corpus luteum in regulation of luteinizing hormone and 17 beta-estradiol in circulation of cows. *Biol. Reprod.* **47**, 1009–1017. doi:10.1095/BIOLREPROD47.6.1009

López-Gatius, F. (2003). Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. *Theriogenology* **60**, 89–99. doi:10.1016/S0093-691X(02)01359-6

Lucy, M. C. (2001). Reproductive loss in high-producing dairy cattle: where will it end? *J. Dairy Sci.* **84**, 1277–1293. doi:10.3168/JDS.S0022-0302(01)70158-0

Macfarlane, M. (2003). Effects of timing of artificial insemination and site of semen deposition on fertility in lactating dairy cows and gender ratio of resulting offspring. MS Thesis, Michigan State University, East Lansing.

Martins, J. P. N., Policelli, R. K., Neuder, L. M., Raphael, W., and Pursley, J. R. (2011). Effects of cloprostenol sodium at final $PGF_{2\alpha}$ of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. *J. Dairy Sci.* **94**, 2815–2824. doi:10.3168/JDS.2010-3652

Mihm, M., Baguisi, A., Boland, M. P., and Roche, J. F. (1994). Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. *J. Reprod. Fertil.* **102**, 123–130. doi:10.1530/JRF.0.1020123

Mihm, M., Curran, N., Hyttel, P., Knight, P. G., Boland, M. P., and Roche, J. F. (1999). Effect of dominant follicle persistence on follicular fluid oestradiol and inhibin and on oocyte maturation in heifers. *J. Reprod. Fertil.* **116**, 293–304. doi:10.1530/JRF.0.1160293

Pursley, J. R., Mee, M. O., and Wiltbank, M. C. (1995). Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. *Theriogenology* **44**, 915–923. doi:10.1016/0093-691X(95)00279-H

Pursley, J. R., Wiltbank, M. C., Stevenson, J. S., Garverick, H. A., and Anderson, L. L. (1997). Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.* **80**, 295–300. doi:10.3168/JDS.S0022-0302(97)75937-X

Revah, I., and Butler, W. R. (1996). Prolonged dominance of follicles and reduced viability of bovine oocytes. *J. Reprod. Fertil.* **106**, 39–47. doi:10.1530/JRF.0.1060039

Roberson, M. S., Wolfe, M. W., Stumpf, T. T., Kittok, R. J., and Kinder, J. E. (1989). Luteinizing hormone secretion and corpus luteum function in cows receiving two levels of progesterone. *Biol. Reprod.* **41**, 997–1003. doi:10.1095/BIOLREPROD41.6.997

Roche, J. F., and Ireland, J. J. (1981). The differential effect of progesterone on concentrations of luteinizing hormone and follicle-stimulating hormone in heifers. *Endocrinology* **108**, 568–572. doi:10.1210/ENDO-108-2-568

Roth, Z., Inbar, G., and Arav, A. (2008). Comparison of oocyte developmental competence and follicular steroid content of nulliparous heifers and cows at different stages of lactation. *Theriogenology* **69**, 932–939. doi:10.1016/J.THERIOGENOLOGY.2008.02.001

Sangsritavong, S., Combs, D. K., Sartori, R., Armentano, L. E., and Wiltbank, M. C. (2002). High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17beta in dairy cattle. *J. Dairy Sci.* **85**, 2831–2842. doi:10.3168/JDS.S0022-0302(02)74370-1

Sartori, R., Sartor-Bergfelt, R., Mertens, S. A., Guenther, J. N., Parrish, J. J., and Wiltbank, M. C. (2002*a*). Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. *J. Dairy Sci.* **85**, 2803–2812. doi:10.3168/JDS.S0022-0302(02)74367-1

Sartori, R., Rosa, G. J. M., and Wiltbank, M. C. (2002*b*). Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. *J. Dairy Sci.* **85**, 2813–2822. doi:10.3168/JDS.S0022-0302(02)74368-3

Sartori, R., Haughian, J. M., Shaver, R. D., Rosa, G. J. M., and Wiltbank, M. C. (2004). Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *J. Dairy Sci.* **87**, 905–920. doi:10.3168/JDS.S0022-0302(04)73235-X

Savio, J. D., Thatcher, W. W., Morris, G. R., Entwistle, K., Drost, M., and Mattiacci, M. R. (1993). Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *J. Reprod. Fertil.* **98**, 77–84. doi:10.1530/JRF.0.0980077

Sirois, J., and Fortune, J. E. (1990). Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. *Endocrinology* **127**, 916–925. doi:10.1210/ENDO-127-2-916

Stevenson, J. S., Pursley, J. R., Garverick, H. A., Fricke, P. M., Kesler, D. J., Ottobre, J. S., and Wiltbank, M. C. (2006). Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. *J. Dairy Sci.* **89**, 2567–2578. doi:10.3168/JDS.S0022-0302(06)72333-5

Stock, A. E., and Fortune, J. E. (1993). Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. *Endocrinology* **132**, 1108–1114. doi:10.1210/EN.132.3.1108

Strickland, J. M., Martins, J. P. N., Neuder, L. M., and Pursley, J. R. (2010). Effect of 14/11 Presynch/Ovsynch on 1st service conception rates of lactating dairy cows compared to AI following a detected estrus. In: '2010 American Association of Bovine Practitioners Meeting', 2010, Albuquerque, New Mexico, EUA.

Twagiramungu, H., Guilbault, L. A., Proulx, J., Villeneuve, P., and Dufour, J. J. (1992). Influence of an agonist of gonadotropin-releasing hormone (buserelin) on estrus synchronization and fertility in beef cows. *J. Anim. Sci.* **70**, 1904–1910.

USDA (2009). Reproduction practices on U.S. dairy operations. In: 'USDA-AFHIS-US C (ed.). (Fort Collins, CO.)

Washburn, S. P., Silvia, W. J., Brown, C. H., McDaniel, B. T., and McAllister, A. J. (2002). Trends in reproductive performance in southeastern Holstein and Jersey DHI herds. *J. Dairy Sci.* **85**, 244–251. doi:10.3168/JDS.S0022-0302(02)74073-3