Animal (2014), **8:s1**, pp 70–81 © The Animal Consortium 2014
doi:10.1017/S1751731114000585

animal

Physiological and practical effects of progesterone on reproduction in dairy cattle

M. C. Wiltbank[1†], A. H. Souza[1,2], P. D. Carvalho[1], A. P. Cunha[1], J. O. Giordano[1,3], P. M. Fricke[1], G. M. Baez[1] and M. G. Diskin[4]

[1]*Department of Dairy Science, University of Wisconsin-Madison, Wisconsin, USA;* [2]*University of California Cooperative Extension, University of California-Davis, Tulare, California, USA;* [3]*Department of Animal Science, Cornell University, New York, USA;* [4]*Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Teagasc, Ireland*

(Received 3 January 2014; Accepted 14 February 2014; First published online 4 April 2014)

The discovery of progesterone (P4) and elucidation of the mechanisms of P4 action have an important place in the history of endocrinology and reproduction. Circulating P4 concentration is determined by a balance between P4 production, primarily by the corpus luteum (CL), and P4 metabolism, primarily by the liver. The volume of luteal tissue and number and function of large luteal cells are primary factors determining P4 production. Rate of P4 metabolism is generally determined by liver blood flow and can be of critical importance in determining circulating P4 concentrations, particularly in dairy cattle. During timed artificial insemination (AI) protocols, elevations in P4 are achieved by increasing number of CL by creating accessory CL or by supplementation with exogenous P4. Dietary manipulations can also alter circulating P4, although practical methods to apply these techniques have not yet been reported. Elevating P4 before the timed AI generally decreases double ovulation and increases fertility to the timed AI. Near the time of AI, slight elevations in circulating P4, possibly due to inadequate luteal regression, can dramatically reduce fertility. After AI, circulating P4 is critical for embryo growth and establishment and maintenance of pregnancy. Many studies have attempted to improve fertility by elevating P4 after timed AI. Our recent meta-analysis and manipulative study indicated small fertility benefits (3% to 3.5%) mostly in primiparous cows. Thus, previous research has provided substantial insight into mechanisms regulating circulating P4 concentrations and actions. Understanding this prior research can focus future research on P4 manipulation to improve reproductive success.

Keywords: progesterone, lactating dairy cows, fertility

Implications

This manuscript reviews effects of circulating progesterone (P4) on dairy cattle reproduction. Various methods to elevate P4 during growth of the preovulatory follicular wave have been shown to increase pregnancies/ artificial insemination (AI) and reduce double ovulation, providing methods to improve fertility and reduce twinning rate in lactating dairy cattle. Conversely, very low concentrations of P4 near AI are needed to optimize fertility. Finally, elevations of P4 after AI can impact embryonic development and also may elevate fertility. Thus, innovative strategies to optimize circulating P4 concentrations during selected reproductive periods enhance our management tools for improving reproductive efficiency of lactating dairy cows.

Discovery of Progesterone (P4)

The discovery of P4 begins with a clear description by Regnier deGraaf (1641–1673) of the corpus luteum (CL), calling them 'globules' and correctly surmising (for rabbits) that 'the number of globules equals the number of offspring from a particular mating' (deGraaf, 1672 in (Jocelyn and Setchell, 1972)). A key discovery came in the laboratory of Gustav Born (1851–1900), an excellent histologist, who observed that the CL was a ductless gland and correctly advanced the idea that it was a gland of internal secretion that could be involved in pregnancy (see excellent reviews by Simmer (1971); Magnus and Simmer (1972)). Two of his students later tested this hypothesis in their own laboratories. In Germany, Ludwig Fraenkel performed bilateral ovariectomy or electrocautery of all corpora lutea in mated rabbits and found that they did not maintain their pregnancies (Fraenkel and Cohn, 1901). In Norway, Vilhelm Magnus also performed galvano-cautery of all CL as well as bilateral ovarian removal in mated rabbits and also reported that pregnancy was not maintained (Magnus, 1901). Both researchers reached the same conclusion that the CL was essential for maintenance of pregnancy. Additional experiments by Magnus showed that unilateral oophorectomy did not alter pregnancy. He also

† E-mail: Wiltbank@wisc.edu

reported that bilateral oophorectomy produced atrophy of the uterus but that destruction of the CL did not produce atrophy of the uterus, in spite of lack of pregnancy in both treatments. He concluded that the ovarian stroma produced an endocrine substance that maintained the uterine structure but that the CL produced an endocrine secretion that maintained pregnancy, called 'differentieringsstofe' (differentiating stuff), the first name given to the hormone later known as P4 (Magnus, 1901). In addition, Magnus correctly predicted the differing functions of ovarian estrogens and gestagens. The initial reports were met with some skepticism, however, Fraenkel continued his research during the next decade on more than 160 rabbits eventually concluding that, 'Thus by the power of large numbers my thesis is proven: The ovary, in particular the CL, regulates the implantation and initial development of the embryo' (Fraenkel, 1910).

In order to isolate the luteal hormone, a bioassay was needed. Initial development was based on research that showed that the presence of the CL was required for the distinctive changes in the uterus that occurred during pregnancy in the rabbit (Bouin and Ancel, 1910). George Corner and Willard Allen (Corner and Allen, 1929) used a similar bioassay to evaluate the chemical nature of the luteal hormone. They found that an alcohol extract of the CL that was subsequently depleted of phospholipids could be injected into ovariectomized, mature rabbits and would result in distinctive 'progestational proliferation' when the uterus was removed after 5 days of treatment and evaluated grossly and histologically. Further, they demonstrated that this hormone was distinct from estrogen since follicular fluid did not produce these progestational changes. A second study demonstrated that daily administration of this CL extract could maintain pregnancy in rabbits that were ovariectomized at about 18 h after mating and the authors stated: 'The evidence is now complete that… the corpus luteum is an organ of internal section which has for one of its functions the production of a special state of the uterine mucosa (progestational proliferation)… to nourish or protect the free blastocysts and to make possible their implantation'. By 1934, four different laboratories independently isolated the crystalline hormone that maintained pregnancy (Butenandt and Westphal, 1934; Fels *et al.*, 1934; Hartmen and Wettstein, 1934; Wintersteiner and Allen, 1934) and reported the steroidal structure of the molecule (Allen, 1974). Two names were being used for the hormone, progestin and luteosterone. Through a series of personal communications the name of P4 was chosen and accepted during an international conference on hormonal nomenclature (Allen *et al.*, 1935).

Factors regulating circulating P4 concentrations

P4 is a steroid hormone primarily secreted by the CL and placenta. Production of P4 from cholesterol utilizes only two enzymes and is considered the simplest steroidogenic pathways to produce a biologically active steroid (Figure 1). All steroids are derived from the common precursor cholesterol. Cholesterol for steroidogenesis can come from multiple

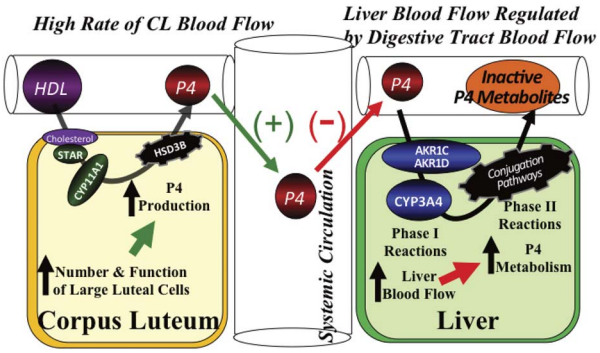

Figure 1 Physiological model of the factors that regulate circulating P4 concentrations in the lactating dairy cow (center of model). Production of P4 (left side of model) is primarily a function of production in the corpus luteum (CL) through pathways that convert cholesterol from high density lipoprotein (HDL) into P4. The cholesterol transport protein, StAR, and the two steroidogenic enzymes, CYP11A1 and HSD3ß, are critical for this conversion. Although stimulators, such as LH, and inhibitors, such as PGF2α, can regulate P4 production by CL, it is emphasized in the model that the primary factor determining greater P4 production is likely to be CL volume and number of large luteal cells due the constitutive nature of P4 production in the bovine CL. On the right side of the model are pathways involved in P4 metabolism tending to decrease circulating P4. The Phase I pathways involve an aldo-ketoreductase (AKR) step, catalyzed by enzymes from the family of AKR enzymes, such as AKR1C and AKR1D, and a hydroxylation step catalyzed by a cytochrome P450 enzyme such as CYP3A4. The Phase II conjugation pathways are also important for the final production of hydrophilic P4 metabolites. Although stimulation and inhibition of the P4 metabolizing enzymes has been reported, this model emphasizes the critical role of the elevated liver blood flow in producing the extraordinary rates of P4 metabolism in high producing lactating dairy cows.

sources within a cell including: cellular membranes, cholesterol ester stores, circulating lipoproteins, or *de novo* cholesterol biosynthesis (Diaz *et al.*, 2002). The major source of cholesterol for luteal cells is circulating lipoproteins and in particular high-density lipoprotein (HDL) in ruminants (Grummer and Carroll, 1988; Wiltbank *et al.*, 1990). Receptors for HDL and LDL have been identified in the CL. The HDL receptor increases dramatically after luteinization of granulosa cells *in vitro* and *in vivo* (Rajapaksha *et al.*, 1997; Li *et al.*, 1998). However, changes in luteal abundance of mRNA for LDL or HDL receptor did not explain the effects of various luteotrophic and luteolytic treatments on P4 production from ovine CL (Tandeski *et al.*, 1996).

Cholesterol is primarily a hydrophobic molecule and this makes it difficult for cholesterol to freely diffuse through hydrophilic environments such as the cytoplasm. In addition, cholesterol has a hydroxyl group at the three position that produces a discrete hydrophilic region making it difficult for 'flip-flop' of cholesterol between membrane surfaces within the lipid bilayer of cellular membranes. Therefore, movement of cholesterol in the circulatory system (lipoproteins) or within the cell is dependent upon transport proteins. Provision of another hydroxyl-group at the other end of the cholesterol molecule alleviates the need for transport proteins. For example, when large or small luteal cells are treated with hydroxylated cholesterol, there is a dramatic increase in P4 production (25-OH > 10-fold; 20-OH or 22-OH > 100-fold) and factors that

normally stimulate or inhibit P4 production by luteal cells no longer have effects on P4 production (Wiltbank *et al*., 1993). Thus, regulation of P4 production in luteal cells is related more to transport of cholesterol within the cell than to changes in the activity of steroidogenic enzymes (Wiltbank *et al*., 1993, Belfiore *et al*., 1994). A number of proteins have been postulated to be involved with transport of cholesterol (sterol carrier protein-2, peripheral benzodiazepine receptor), however the key role of StAR has been the best characterized (Pescador *et al*., 1996; Stocco and Clark, 1996; Stocco *et al*., 2005). It is now clear that changes in StAR transcription, protein and activity account for increases in P4 production in response to trophic stimulation and acute decreases in P4 production after luteolytic treatments (Diaz *et al*., 2002).

The P450 cholesterol side chain cleavage enzyme (P450scc) is located on the inner mitochondrial membrane and catalyzes the conversion of cholesterol to pregnenolone. Three oxidation steps are catalyzed by this enzyme: hydroxylations at the 20 and 22 positions and then cleavage between these two carbons. The mRNA and protein for P450scc dramatically increase in CL after the LH surge and luteinization (Belfiore *et al*., 1994; Niswender *et al*., 2000). Pregnenolone has two hydrophilic residues that increase the mobility through cellular membranes. Pregnenolone diffuses from the mitochondria to the smooth endoplasmic reticulum where it is converted to P4 by the enzyme 3-β hydroxysteroid dehydrogenase (3βHSD). This final reaction produces a double bond between the 4 and 5 carbon of the molecule and is the basis for the abbreviation of progesterone as P4. P4 then diffuses from the luteal cells to the bloodstream for transport to target tissues. The levels of 3βHSD mRNA and protein increase dramatically following the LH surge and subsequent ovulation to a maximum at days 8 to 11 after estrus in cattle (Couet *et al*., 1990). After prostaglandin F2α (PGF2α) treatment, there was an abrupt and dramatic decrease in 3βHSD mRNA abundance (35% of control concentrations at 4 h; 15% of control at 12 h). However, there is no decrease in protein for P450scc or in 3βHSD enzyme activity even at 24 h after PGF2α treatment (Hawkins *et al*., 1993; Juengel *et al*., 1998; Niswender *et al*., 2000; Atli *et al*., 2012). Given that circulating P4 is decreased by 12 h after PGF2α treatment it seems unlikely that inhibition of P450scc and 3βHSD are a critical part of the acute inhibition of P4 production during luteolysis. Both small and large luteal cells have large amounts of P450scc and 3βHSD activity and neither enzyme appears to be the key step that acutely regulates luteal P4 production from these cells (Wiltbank *et al*., 1993).

In ruminants, the role of LH in luteal function has been acknowledged for more than 3 decades (Niswender *et al*., 1994 and 2000) but there is still controversy on the precise role of LH in luteal function (Diaz *et al*., 2002; Wiltbank *et al*., 2012a; Haughian *et al*., 2013). Indeed in our recent study we treated heifers with a potent gonadotropin releasing hormone (GnRH) receptor antagonist, acyline, for the first 6 days after the LH surge. Treatment with GnRH antagonist stopped follicle growth at the time of dominant follicle deviation, about 8 mm in diameter. In contrast, there was no change in development of the CL or in circulating P4 during the first 6 days of luteal development (Haughian *et al*., 2013). It should be stated that all cows had the GnRH-induced LH surge in this study before initiation of acyline treatment. It seems clear that the LH surge is essential for ovulation and luteinization of the ovulated follicular cells into the luteal cells, however, the role for LH pulses, after the LH surge, is still controversial. Consistent with our results, other researchers have also found that treatment with a GnRH antagonist eliminated pulsatile LH secretion but had little or no effect on circulating P4 and CL function (Baird, 1992; McNeilly *et al*., 1992). In contrast, when sheep are hypophysectomized on day 5 after estrus and CL recovered on day 12, circulating P4, luteal P4, and weight of CL are lower than expected for a day 12 CL but similar to a day 5 CL (Kaltenbach *et al*., 1968; Denamur *et al*., 1973; Farin *et al*., 1990). However, acute treatments with LH have surprisingly small effects on circulating P4 (reviewed in Wiltbank (1994)) and in contrast to primates, circulating P4 in ruminants is greatest at times of the cycle when LH pulses are least (Wiltbank *et al*., 2012a). Thus, the acute or even chronic effects of LH pulses on CL function in ruminants remain to be unequivocally defined in the scientific literature. In general, P4 production by the ruminant CL appears to be very high, due to high constitutive P4 production by the large luteal cells (Wiltbank, 1994; Diaz *et al*., 2002; Bogan and Niswender, 2007; Wiltbank *et al*., 2012a).

The other key factor regulating circulating P4 is the rate of metabolism of P4, primarily by the liver (Figure 1). Primary regulation of P4 metabolism is due to changes in blood flow to the liver (Wiltbank *et al*., 2006). For example, if liver blood flow increased from 1000 to 2000 l/h then circulating P4 concentrations will decrease to 50% even though P4 production and P4 metabolizing enzymes have not changed (Sangsritavong *et al*., 2002; Wiltbank *et al*., 2006 and 2012b). The role of feed intake in regulating liver blood flow, P4 metabolism, and circulating P4 was initially demonstrated in studies with pigs and sheep (Christenson *et al*., 1985; Parr *et al*., 1993; Prime and Symonds, 1993). For example, the studies in sheep showed convincingly that as feed intake increased, there was a clear increase in liver blood flow with a corresponding decrease in circulating P4 (Parr *et al*., 1993). In addition, these studies demonstrated that the reduced P4 concentrations caused by overfeeding produced the reduction in fertility in overfed ewes (Parr *et al*., 1987). Our results extended the results from previous studies to the lactating dairy cow and clearly demonstrated the relationship between dry matter intake, liver blood flow, and circulating concentrations of P4 (Sangsritavong, 2002; Sangsritavong *et al*., 2002; Vasconcelos *et al*., 2003; Wiltbank *et al*., 2006).

In conclusion, circulating P4 concentrations represent a balance between production of P4 and metabolism of P4 (Figure 1). Production of P4 is regulated by development of the CL after the LH surge, number of granulosa cells that luteinize into large luteal cells, and constitutive production of P4 by the large luteal cells. Metabolism of P4 is primarily related to amounts of blood flow to the liver due to the

abundance in the bovine liver of the enzymes that mediate metabolism of P4. Thus, practical regulation of circulating P4 will probably be most productive by focusing on increasing luteal tissue volume to increase P4 production and/or limiting liver blood flow and perhaps blocking P4 metabolizing enzymes.

Cellular mechanisms regulating P4 action

The receptors for P4 and the intracellular mechanisms of action have been extensively reviewed (Ellmann *et al*., 2009). The classical P4 receptors (PR) are members of the nuclear receptor superfamily. In other words, these receptors work through actions in the nucleus that regulate expression of specific genes. There are two types (isoforms) of the nuclear PR, termed PR-A and PR-B. These PR are products of the same gene with the key distinction that PR-B contains an additional 165 amino acids at the N-terminal end of the protein. Studies in mice in which PR-A or PR-B are selectively eliminated (knockout mice) show that these two receptors are functionally distinct and both are critical for successful reproduction (Arck *et al*., 2007). The PR-A isoform alone is sufficient for establishment and maintenance of pregnancy; whereas, PR-B is not sufficient for establishment or maintenance of pregnancy but is essential for fertility, possibly through actions on tissues other than the uterus (Fernandez-Valdivia *et al*., 2005). Surprisingly, there are also multiple types of plasma membrane P4 receptors that act through activation of intracellular signal transduction systems that are not related to the nuclear PR systems (Peluso, 2006).

The P4 concentration that reaches the receptors within each particular cell is the key determinant of the physiological actions of P4 in an animal. Almost all tissues have sufficient blood supply that the circulating P4 concentration is the primary determinant of cellular P4 concentrations. Therefore, factors regulating circulating P4 concentrations and intracellular P4 receptors primarily determine the magnitude of P4 responses throughout the body.

Studies on the effects of low *v*. high P4 during follicular growth before AI

Incidence of double ovulation

The incidence of increased twinning in lactating dairy cows is primarily due to a high percentage of cows with double ovulation as evidenced by over 93% of twins being non-identical (del Rio *et al*., 2006). There are numerous factors that regulate twinning rates, including age of dam, season, genetics, use of reproductive hormones or antibiotics, ovarian cysts and days open; however, there is increasing interest in the key role of peak milk production and circulating P4 concentrations on the incidence of double ovulation (Kinsel *et al*., 1998; Wiltbank *et al*., 2000 and 2012b). For example, in a study with cows that were evaluated near natural estrus (Lopez *et al*., 2005b), almost 50% of cows that were above 50 kg/day of milk production had double ovulation, whereas <10% had double ovulation for cows producing below 40 kg/day. These effects were similar for multiparous or primiparous cows (Fricke and Wiltbank, 1999; Lopez *et al*., 2005b).

Many results are consistent with reduced P4 concentrations being the key factor that links high milk production with increased double ovulation and twinning in lactating dairy cattle. For example, cattle that grow the ovulatory follicular wave in the absence of P4 have an increased percentage of cows with co-dominant follicles and double ovulation (Hayashi *et al*., 2008). Manipulative studies have now been performed that have demonstrated a decrease in the percentage of cows that double ovulate when circulating P4 is increased (Stevenson *et al*., 2007a; Cunha *et al*., 2008; Cerri *et al*., 2011a). For example, a study from our laboratory found that cows with high P4 during follicular growth, had a much lower percentage of cows with double ovulation than cows with low P4 (7.0% *v*. 20.6%; $P < 0.01$; Cunha *et al*., 2008). Similarly, in cows synchronized with the Ovsynch protocol (Fricke and Wiltbank, 1999), double ovulation was much greater in cows that were above average milk production (40.7 kg/day) than below (20.2% *v*. 6.9%; $P < 0.05$). Alternatively, analysis of cows with co-dominant follicles during the first follicular wave demonstrated that these cows had greater milk production, lower circulating P4, and a distinct elevation in circulating FSH and LH concentrations during the 24 h before dominant follicle deviation (Lopez *et al*., 2005a). Thus, greater milk production may produce decreased circulating P4 concentrations due to the increased feed intake and corresponding increases in P4 metabolism. The depressed P4 concentrations may underlie a delay in the FSH nadir and increased LH pulses near dominant follicle deviation and thus selection of two or even three dominant follicles.

Fertility in dairy cows

The key role of greater P4 before AI on fertility of lactating dairy cows was recognized in early studies that compared fertile to non-fertile inseminations (Folman *et al*., 1973; Erb *et al*., 1976; Meisterling and Dailey, 1987). In addition, concentrations of P4 12 days before first AI had a positive relationship with conception rate at first AI in lactating Holstein and Jersey dairy cattle (Fonseca *et al*., 1983). Similarly, concentrations of P4 on the day of PGF2α-induced luteolysis had a positive linear correlation with subsequent fertility (Diskin *et al*., 2006). More recent manipulative studies have shown some improvements (~5% to 7% increase in percentage pregnant) by using a controlled internal drug release (CIDR; releases P4) during the Ovsynch program before AI (Stevenson *et al*., 2006 and 2008; Chebel *et al*., 2010; Bilby *et al*., 2013). Nevertheless, the results from these studies may not be due exclusively to the elevation in P4 produced by the CIDR, because improvements in synchronization may also accompany the use of a CIDR in the Ovsynch protocol. In the study mentioned above we tested the effects of elevated P4 on fertility to a timed AI during the Double Ovsynch program (Cunha *et al*., 2008). Cows ($n = 564$) were randomly assigned to have either high or low P4 during the Ovsynch protocol. The cows with low P4 had increased

double ovulation rate, which would be expected to potentially increase fertility in these cows (ovulation of more follicles should result in greater chance for pregnancy). However, cows with lower P4 before AI had much lower fertility (37.1% pregnant at day 29 pregnancy diagnosis) compared with cows with high P4 (51.0%; $P < 0.001$). This indicates that increasing P4 before timed AI can result in a substantial improvement in fertility, suggesting that the reason for the lower fertility in lactating dairy may be, at least partly, due to reduced P4 concentrations during the time period before AI. Obviously, the reduction in P4 in our study was done by hormonal manipulations in a commercial farm setting; however, physiologically, P4 concentrations may also be reduced in high-producing dairy cows due to high feed intake that produces increased gastro-intestinal/liver blood flow and increased P4 metabolism. A number of studies have targeted an elevation of circulating P4 in order to increase fertility.

Studies by Bisinotto *et al.* (2010a) demonstrated the importance of higher P4 during growth of the final follicular wave. Two studies were performed in which the effect of P4 during the Ovsynch protocol was evaluated. In the first study, cows were evaluated for P4 concentration at the time of the first GnRH of Ovsynch and 7 days before this first GnRH. Cows were classified as anovular (both samples low in P4), or as beginning Ovsynch with high P4 or low P4 (first sample had high P4, second had low P4). Cows that began Ovsynch with high P4 had greater pregnancies per AI (P/AI = 43.0%) than cycling cows that had low P4 (31.3%) or that were anovular (29.7%) at the time of initiation of Ovsynch (Bisinotto *et al*., 2010a). In a second manipulative study, cows received two PGF2α treatments and Ovsynch was initiated either 3 or 10 days after the second PGF2α. This design would produce cows that ovulated a dominant follicle from either the first (3 days) or second (10 days) follicular wave near the timed AI. Similar to the first study, cows ovulating a follicle of the second follicular wave (high P4 concentrations) had greater P/AI that cows ovulating the first follicular wave (low P4) (41.7% *v.* 30.4%) (Bisinotto *et al*., 2010a). Other studies have also reported that low P4 at the beginning of the Ovsynch protocol results in decreased P/AI compared with cows with high P4 concentrations (Silva *et al*., 2007; Denicol *et al*., 2012; Giordano *et al*., 2012a, 2012b and 2013a). In addition, a recent study (Bisinotto *et al*., 2013) compared cows with a CL at the initiation of a synchronized ovulation program to cows either without a CL, that were untreated, or cows without a CL that were treated with two intravaginal P4 implants (two CIDRs). The cows treated with two CIDRs had similar fertility to cows with a CL and both of these groups were greater than untreated cows without a CL (Bisinotto *et al*., 2013). Thus, it seems clear that increasing P4 during growth of the preovulatory follicular wave increases fertility more than 10% to the subsequent timed AI.

Pregnancy loss in dairy cows

One further important observation related to low P4 before AI is that there is generally an increase in pregnancy loss following the subsequent AI. Pregnancy loss averages 6% to 20% in high producing dairy cows when pregnancy loss is evaluated between day 28 after AI (by ultrasound) and day 60 after AI. This time period is critical for implantation and development of the embryo. We observed a decrease ($P = 0.05$) in pregnancy loss between day 29 and day 57 when cows had high P4 (6.8% loss) *v.* low P4 (14.3% loss) during growth of the follicular wave that produces the ovulatory follicle before AI (Cunha *et al*., 2008). Thus, the high P4 group not only had increased number of pregnancies at the day 29 pregnancy diagnosis, these cows also had less risk of subsequent pregnancy loss after this time. This was not due to differences in circulating P4 after AI because P4 concentrations after AI were actually somewhat higher in the cows that had low P4 before AI (2.88 ng/ml) compared with the cows with high P4 before AI (2.49 ng/ml). Other studies have also reported increased pregnancy loss in anovular cows compared with cyclic cows (Santos *et al*., 2004 and 2009; Sterry *et al*., 2006; Stevenson *et al*., 2006). Thus, there was a positive effect of elevated P4 before AI on subsequent maintenance of pregnancy even after 29 days following AI.

Effect of pre-AI P4 on embryo quality

An elegant study in superstimulated cows (Rivera *et al*., 2011) showed that high P4 concentrations during superstimulation increased the subsequent quality of embryos flushed on day 7 after superovulation. Cows began superstimulation during the second follicular wave (high P4), during the first follicular wave (low P4), or during the first follicular wave with P4 supplementation using two CIDRs to increase P4 concentrations. Although, total structures that were collected (embryos/oocytes) was not different between groups, the percentage of structures that were transferable embryos were much less ($P = 0.01$) for cows superstimulated during the first follicular wave (55.9%), than during the second follicular wave (88.5%) or during the first follicular wave with P4 supplementation (78.6%). This result is consistent with an effect of elevated P4 during follicle growth on embryo development before day 7. It is postulated that effects of P4 during growth of the follicles may allow production of a better oocyte for subsequent fertilization and embryo development (Rivera *et al*., 2011). However, a recent study that flushed embryos from single ovulating cows that had follicles grow during low or high P4 did not find a difference in embryo quality on day 7 (58.3% *v.* 53.3%) (Cerri *et al*., 2011a). A companion study (Cerri *et al*., 2011b) indicated that although cows with low P4 had increased basal LH concentrations and altered follicular dynamics and follicular fluid composition that could alter oocyte quality, a particularly distinct difference in cows with low P4 was the premature development of pathways leading to uterine PGF2α secretion. Thus, altered uterine function could also have an important role in reducing fertility in cows that have low P4 concentrations before AI. Our laboratory (Wiltbank *et al*., unpublished) has recently completed a study evaluating day 7 embryo quality in single ovulating cows with follicle development occurring in low *v.* high P4. We found a

greater percentage of cows with Grade 1 and two embryos in cows with high P4 than low P4 before AI (86.5% *v.* 61.5%; $P = 0.02$). Thus, our recent results are consistent with the effects of low P4 on fertility being evident by day 7 after AI even in single-ovulating lactating cows, consistent with the results of Rivera *et al.* (2011) in superovulated cows.

Importance of low P4 near time of AI

Inadequate luteolysis can result in an elevation in circulating P4 near AI and a reduction in fertility. This has been clearly shown in numerous studies using timed AI programs (Souza *et al.*, 2007; Brusveen *et al.*, 2008; Bisinotto *et al.*, 2010b; Giordano *et al.*, 2012b and 2013b). In addition, studies on cows that receive AI after detection of estrus, have generally reported that minor elevations in P4 near AI are also detrimental to fertility (De Silva *et al.*, 1981; Waldmann *et al.*, 2001; Ghanem *et al.*, 2006), although some studies did not obtain this result (Erb *et al.*, 1976; Plym Forshell *et al.*, 1991). During Ovsynch, the percentage of cows that do not have complete regression following the PGF2α treatment before timed AI has been reported to range from 5% to 30% (Moreira *et al.*, 2000; Gumen *et al.*, 2003; Souza *et al.*, 2007; Brusveen *et al.*, 2009; Martins *et al.*, 2011b; Giordano *et al.*, 2013b). A recent extensive study of incomplete luteolysis evaluated multiple blood samples in cows at first AI ($n = 652$) and second or greater AI ($n = 394$) (Martins *et al.*, 2011b). They defined complete luteolysis and low P4 (<0.5 ng/ml) at 56, 72 and 96 h after PGF2α. At first AI, 80% of cows underwent complete luteolysis, whereas at second+ AI only 71% underwent complete luteolysis. Surprisingly, greater P4 concentrations at the time of PGF2α were associated with greater probability of luteolysis after PGF2α treatment and greater fertility (50% *v.* 28%).

To study whether P4 near AI had an effect on fertility during timed AI protocols, our laboratory (Souza *et al.*, 2007; Brusveen *et al.*, 2009) evaluated fertility in lactating cows synchronized with Ovsynch on three commercial dairy herds. We collected blood samples for P4 measurement near the time of the last GnRH of Ovsynch. The combined results from these two studies are shown in Figure 2. There was a dramatic decrease in P/AI as P4 increased above 0.4 ng/ml near the time of the second GnRH of Ovsynch (16 h before AI). Thus, slight elevations in circulating P4 near AI result in dramatic decreases in fertility to the AI. This decrease was observed even when only cows that ovulated to the second GnRH were evaluated for fertility. The decrease that we observed was similar to the decrease described in the pioneering study of De Silva *et al.* (1981) using visual detection of estrus in lactating cows and heifers.

In the two studies analyzed for Figure 2 there was no effect of parity on incidence of luteal regression. However, in a different study from our laboratory (Giordano *et al.*, 2012b) lower rates of CL regression were observed for multiparous (83.9%) than primiparous (89.7%) cows. Other studies have also reported lower rates of luteal regression in multiparous than in primiparous lactating dairy cows (Martins *et al.*, 2011a).

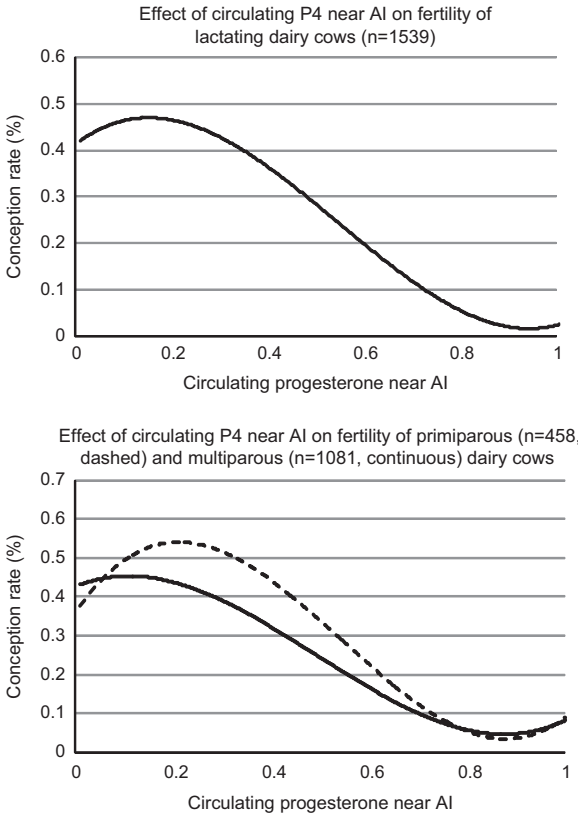

Figure 2 Relationship between circulating P4 near AI and conception results in lactating dairy cows receiving Ovsynch (data from Souza *et al.* (2007) and Brusveen *et al.* (2009) combined).

Two methods have been used to increase regression of the CL during timed AI protocols, increasing the dose of PGF2α or increasing the number of PGF2α treatments. This is particularly important in cows treated with the 5-day Ovsynch protocol (Santos *et al.*, 2010; Ribeiro *et al.*, 2012; Nascimento *et al.*, 2013a). Fertility was reduced if only a single PGF2α was given (Santos *et al.*, 2010) or if two PGF2α treatments were given on the same d (5 days after GnRH) compared with giving one treatment on day 5 and a second on day 6 (Ribeiro *et al.*, 2012). Using the 7-day Ovsynch protocol, an increased percentage of cows with complete CL regression (<0.4 ng/ml 56 h after PGF2α) was observed after two (days 7 and 8; 326/341 = 95.6%) compared with one (day 7; 301/356 = 84.6%) PGF2α treatment (Brusveen *et al.*, 2009). In a recent study from our laboratory (Giordano *et al.*, 2013b), increasing the dose of cloprostenol from 500 to 750 μg on day 7 of a 7-day Ovsynch protocol increased CL regression in multiparous (122/154 = 79.2% *v.* 135/154 = 87.7%; $P = 0.025$) but not primiparous (131/146 = 89.7% *v.* 129/139 = 92.8%; $P = 0.181$) cows. An indication of improved fertility ($P = 0.054$) was observed at the 39 days pregnancy diagnosis with the 750 (247/544 = 45.4%) compared with the 500 (221/540 = 40.9%) μg dose of cloprostenol (Giordano *et al.*, 2013b). Consistent with this idea, treatment with PGF2α one day earlier provided greater time for reduced P4 at the time of AI and

improvements in fertility (Pereira *et al.*, 2013). In this study, cows with P4 concentrations above 0.1 ng/ml near the time of AI had reduced fertility (Pereira *et al.*, 2013). Thus, a small elevation in P4 near AI can reduce fertility. Treatment with an increased dose of PGF2α or increased number of PGF2α treatments can alleviate or reduce this problem. Based on the above results, it appears that two doses of PGF2α, 24 h apart, may be the most consistent method for causing CL regression and lowering P4 concentrations near AI. Nevertheless, in certain on-farm situations two treatments may be difficult, and treatment with a higher dose or leaving a longer time from PGF2α treatment until removal of the P4 vaginal implant is likely to enhance but possibly not optimize fertility.

There may be multiple physiological mechanisms that result in the reduced fertility observed when P4 is elevated near AI. First, P4 may alter sperm or oocyte transport by altering uterine or oviductal contractility and thus reduce fertilization (Hunter, 2005). Second, addition of P4 to *in vitro* fertilization (IVF) media reduced blastocyst rate (Silva and Knight, 2000) suggesting that there may be direct effects of P4 during IVF on subsequent embryo development. This detrimental effect was reversed with a P4 receptor antagonist (mifepristone – RU486) indicating a specific role for P4 receptors in this action. Elevated P4 *in vitro*, also increased total α-inhibin production by the cumulus–oocyte complex, which may reduce embryo development after cleavage (Silva *et al.*, 1999). Further, the reduced endometrial thickness that accompanies slight elevations in P4 (Souza *et al.*, 2011) may indicate other major effects of P4 on the blood flow and functionality of the uterus and these changes could underlie the reduced embryo development.

In summary, circulating P4 near AI, $>$0.1 ng/ml in a study using estradiol to induce ovulation (Pereira *et al.*, 2013) or $>$ 0.3 to 0.5 ng/ml in studies using GnRH to induce ovulation (Souza *et al.*, 2007; Brusveen *et al.*, 2009; Santos *et al.*, 2010; Martins *et al.*, 2011b; Ribeiro *et al.*, 2012; Giordano *et al.*, 2012b), is detrimental to fertility in dairy cattle, but the underlying physiological mechanisms that reduce fertility are not well understood. An additional PGF2α treatment in cows receiving Ovsynch and more precise detection of estrus may help to minimize the percentage of cows with elevated circulating P4 near AI.

Importance of high P4 after AI

The role of the 'CL hormone' after ovulation in maintaining pregnancy was described more than 100 years ago and was recognized 30 years before the chemical structure of P4 was identified (Fraenkel and Cohn, 1901; Magnus, 1901). Recent studies continue to characterize the uterine effects of P4, inducing a uterine environment compatible with embryo growth, implantation, and maintenance of pregnancy (Arck *et al.*, 2007; Forde *et al.*, 2009; Bazer *et al.*, 2012; Mullen *et al.*, 2012a and 2012b).

Although there is unequivocal evidence that there is an absolute requirement for P4 in pregnancy maintenance, results have been somewhat more equivocal about the relationship between levels of circulating P4 after AI and fertility in lactating dairy cows. A number of studies have reported lower P4 in non-pregnant than pregnant cows, whereas, other studies reported no relationship between post-AI P4 and fertility (Bulman and Lamming, 1978; Larson *et al.*, 1997; Mann and Lamming, 1999; Gumen *et al.*, 2003; Stronge *et al.*, 2005; Lonergan *et al.*, 2007; Morris and Diskin, 2008). More extensive logistic regression modeling demonstrated a relationship in dairy cows between P/AI with circulating P4 on days 5, 6 and 7 after AI or with rate of P4 increase after AI (Stronge *et al.*, 2005). They reported that 60% to 85% of dairy cows had sub-optimal circulating P4 for pregnancy, based on absolute P4 concentrations during the early luteal phase or rate of P4 increase (Stronge *et al.*, 2005). For example, a milk P4 concentration of 7.4 ng/ml on day 5 was optimal for embryo survival, however 60% of cows had lower P4 concentrations than this optimum. A particularly intriguing recent study reported that one of the major hormonal differences found in cows with genetics for high fertility was 34% greater circulating P4 concentrations than cows with poor genetic merit for fertility (Cummins *et al.*, 2012). Many recent studies have attempted to unravel the mechanisms involved in the complex relationship between circulating P4 concentrations and levels of fertility in lactating dairy cows.

Early embryos express different types and concentrations of P4 receptors (Clemente *et al.*, 2009) raising the possibility that P4 may be acting directly on the embryo to improve embryo development. An elegant series of experiments found no effect of P4 supplementation after fertilization, *in vitro*, on blastocyst yield, in the presence or absence of bovine oviductal epithelial cells (Clemente *et al.*, 2009). In a surprising twist, these researchers treated recipient cows with an intravaginal P4-releasing device (PRID) starting on day 3 after estrus with *in vitro* produced blastocysts transferred on day 7. Circulating P4 concentrations were elevated in the recipient cows from days 3 to 6 but were not elevated in treated cows after that time. Thus, the rise in P4 concentrations in treated cows occurred before the transfer of embryos. Nevertheless, embryos that were transferred into recipients that had received prior P4 exposure (days 3 to 6 increased P4) had longer embryos on day 14 and embryo area was much greater (Clemente *et al.*, 2009). The authors concluded that 'P4-induced changes in the uterine environment are responsible for the advancement in conceptus elongation reported previously in cattle and that, interestingly, the embryo does not need to be present during the period of high P4 in order to exhibit advanced elongation' (Clemente *et al.*, 2009). These results are consistent with the studies of (Larson *et al.*, 2011) that also failed to find a direct effect of P4 during either days 1 to 3 or days 4 to 7 of culture on percentage of embryos that developed to the morula or blastocyst stage; although small differences in glucose metabolism were observed. Further evidence for a lack of P4 effect in early embryo is found in the studies of Carter *et al.*, 2008 and 2010. In the first experiment, 210 crossbred beef

heifers were used to analyze the effects of *in vivo* supplementation with P4 on embryo development. These researchers observed no difference in early embryo development by day 5 or 7 after AI, however dramatic effects of P4 supplementation on embryonic length could be observed on day 13 and 16 after AI (Carter *et al.*, 2008). In an elegant study that continued this research focus (Carter *et al.*, 2010), *in vitro* produced embryos were transferred to the oviduct of beef heifers that received or did not receive a PRID on day 3 after estrus. There was no detectable effect of P4 on the proportion of embryos that developed to the blastocyst stage by day 7 when embryos were recovered or during subsequent culture of the embryos *in vitro*. However, there were subtle but intriguing differences in gene expression, detected by microarray, in the embryos recovered from recipients that received P4 supplementation (Carter *et al.*, 2010). Thus, it seems clear that increased P4 during days 3 to 7 induces changes in the uterus that increase embryo elongation by day 14. Whether a P4-induced increase in embryo development can improve fertility in lactating dairy cows continues to be an area of investigation, as discussed below.

Many studies have investigated the P4-induced changes in gene expression that occur in the endometrial tissue and these will not be extensively reviewed in this manuscript. However, it seems clear that there are dramatic differences in endometrial gene expression as the luteal phase progresses and that early supplementation with P4 can induce earlier expression of this P4 program (McNeill *et al.*, 2006; Forde *et al.*, 2009 and 2011). The P4-induced changes in uterine gene expression can have dramatic consequences for the development of the embryos (Forde *et al.*, 2011).

There have been numerous studies that have evaluated the effects of P4 supplementation on fertility in cattle with the earliest experiments conducted in the 1950s (Herrick, 1953; Wiltbank *et al.*, 1956). Throughout the last 60 years (reviewed by Mann and Lamming, 1999), there have been numerous methods to increase P4 including: treatment with exogenous P4 (injectable P4; PRID; or CIDR) or by treatments attempting to ovulate a follicle and produce an accessory CL (human chorionic gonadotropin (hCG); or GnRH). These experiments have varied considerably in regard to type of animal (beef *v.* dairy, heifers *v.* cows), supplementation/administration day relative to AI, utilization of synchronization before AI, and number of animals in the trial (*n*). Of the 30 trials that we evaluated, the vast majority (25/30) showed a numeric improvement in fertility with P4 supplementation, although only six of these trials showed significance ($P < 0.05$). Only two (Stevenson *et al.*, 2007b; Nascimento *et al.*, 2013c) of these trials that found significance used >100 animals per comparison.

The most extensive trials to increase P4 have been done by inducing formation of an accessory CL with hCG or GnRH treatment. When hCG or GnRH is administered on day 5 after AI, there is generally formation of an accessory CL and increased P4 during the mid-luteal phase (Fricke *et al.*, 1993; Santos *et al.*, 2001). In our recent study we found that 93% of dairy cows treated with hCG on day 5 had ovulation (Nascimento *et al.*, 2013b). Cows treated with hCG showed an increase in circulating P4 by 3 days after hCG treatment, day 8, until day 16 (Nascimento *et al.*, 2013b). However, circulating P4 remained lower, even after hCG treatment, in lactating cows compared with normally ovulating heifers. Eight studies that evaluated the effect of hCG on P4 concentrations from days 4 to 7 after AI in lactating dairy cows reported significant increases in circulating P4 concentrations after treatments with different doses of hCG: 3300 IU (Schmitt *et al.*, 1996; Santos *et al.*, 2001), 2500 IU (Stevenson *et al.*, 2007b; Vasconcelos *et al.*, 2011); 1500 IU (Walton *et al.*, 1990; Hanlon *et al.*, 2005; Kendall *et al.*, 2009) or 1000 IU (Rajamahendran and Sianangama, 1992). Thus, treatment with hCG on day 5 after AI produces a consistent, fairly rapid (3 days delay) increase in circulating P4 in lactating dairy cows.

The effect of hCG treatment on subsequent fertility in lactating dairy cows has been the subject of many previous studies. Recently we did a meta-analysis of 10 previous trials that analyzed a total of 4397 lactating cows (Nascimento *et al.*, 2013c). There was a modest ($P = 0.04$) increase of 3% comparing hCG (37.0%; 808/2,184) to control cows (34.0%; 752/2,213). Based on these results, we designed a manipulative study that included data from 2979 lactating dairy cows on six commercial dairies (Nascimento *et al.*, 2013c). Treatment with hCG 5 days after AI increased ($P = 0.01$) fertility by 3.5% from 37.3% in controls (566/1519) to 40.8% in hCG-treated cows (596/1460). These results are consistent with some improvement due to the hCG treatment, however, it is unclear why the effect is not of a larger magnitude. It seems possible that formation of the CL and the increase in P4 following hCG or GnRH administration is not sufficiently early in the cycle to induce the uterine changes that are needed to optimize fertility. A particularly surprising result from this study was that all of the effect of hCG on fertility was due to a dramatic increase in primiparous cows (39.5%; 215/544 Control primiparous; 49.7%; 266/535 hCG-treated primiparous) with no change due to hCG treatment in multiparous cows (36.0%; 351/975 Control multiparous; 35.7%; 330/925 hCG-treated multiparous). Numerous other studies have reported that first parity cows generally have higher fertility than older cows particularly when cows are bred after protocols that provide excellent synchrony of ovulation for fixed time AI (Brusveen *et al.*, 2008; Souza *et al.*, 2008; Giordano *et al.*, 2012b). However, our experiment does not provide a clear explanation for the differences between parities in the fertility effects of hCG. Further research is clearly needed to clarify whether the timing of the P4 increase or other factors can explain the relatively low effect of hCG on fertility and the unexpected parity influence on the hCG effect.

Conclusions

This manuscript has attempted to describe the underlying physiology that produces the changes in circulating P4 in lactating dairy cows and the potential reproductive

challenges associated with sub-optimal P4 concentrations. Metabolism of P4 appears to be the primary cause of lowered P4 in lactating dairy cows, although increases in P4 production by the CL may be important in higher fertility genotypes of dairy cows. This manuscript reviewed the scientific literature on P4 and fertility with clear evidence for effects of P4 at all three-time periods that were analyzed. Before AI, there were very dramatic effects observed when the P4 concentrations before AI were compared with subsequent fertility or when manipulative studies were performed to increase P4 before AI. Improvements of more than 10% in P/AI were observed by increasing P4 concentrations. In addition, insufficient P4 at this time may, at least partially, underlie the high double ovulation rate as well as the lowered fertility that is characteristic of high-producing dairy cows. Near the time of AI, it is critical that P4 concentrations reach a nadir concentration. Even small increases in P4 near the time of AI were associated with dramatic reductions in fertility, either in cows bred to natural estrus or after timed AI protocols. Following AI, there are dramatic effects of increasing P4 on embryo elongation. In addition, there have been relationships found between P4 concentrations after AI and subsequent fertility when linear regression analyses were performed. However, manipulative studies have not provided consistent or dramatic effects of P4 supplementation on fertility in most studies that have evaluated large numbers of cows. Thus, although substantial research has investigated the role of P4 on fertility in lactating dairy cows for more than 6 decades, it seems clear that future research is needed to fully understand the physiology that underlies previous research observations and to unlock the practical improvements in fertility that are expected by consistent management of P4 concentrations in lactating dairy cows.

Acknowledgements

The work has been supported by grants from Wisconsin Experiment Station Hatch project WIS01240 to M.C.W., Pfizer Animal Health Global Academic Consortium, and FAPESP, Brazil.

References

Allen W 1974. Recollections of my life with progesterone. Gynecological Investigation 5, 142–182.

Allen WM, Butenandt A, Corner GW and Slotta KH 1935. Nomenclature of corpus luteum hormone. Nature 136, 303.

Arck P, Hansen PJ, Jericevic BM, Piccinni M-P and Szekeres-Bartho J 2007. Progesterone during pregnancy: endocrine-immune cross talk in mammalian species and the role of stress. American Journal of Reproductive Immunology 58, 268–279.

Atli MO, Bender RW, Mehta V, Bastos MR, Luo W, Vezina CM and Wiltbank MC 2012. Patterns of gene expression in the bovine corpus luteum following repeated intrauterine infusions of low doses of prostaglandin F2 alpha. Biology of Reproduction 86, 1–13.

Baird DT 1992. Luteotrophic control of the corpus luteum. Animal Reproduction Science 28, 95–107.

Bazer FW, Kim J, Song G, Ka H, Tekwe CD and Wu G 2012. Select nutrients, progesterone, and interferon tau affect conceptus metabolism and development. Annals of the New York Academy of Sciences 1271, 88–96.

Belfiore CJ, Hawkins DE, Wiltbank MC and Niswender GD 1994. Regulation of cytochrome P450scc synthesis and activity in the ovine corpus-luteum. Journal of Steroid Biochemistry and Molecular Biology 51, 283–290.

Bilby TR, Bruno RGS, Lager KJ, Chebel RC, Moraes JGN, Fricke PM, Lopes G, Giordano JO, Santos JEP, Lima FS, Stevenson JS and Pulley SL 2013. Supplemental progesterone and timing of resynchronization on pregnancy outcomes in lactating dairy cows. Journal of Dairy Science 96, 7032–7042.

Bisinotto RS, Chebel RC and Santos JE 2010a. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. Journal of Dairy Science 93, 3578–3587.

Bisinotto RS, Ribeiro ES, Martins LT, Marsola RS, Greco LF, Favoreto MG, Risco CA, Thatcher WW and Santos JEP 2010b. Effect of interval between induction of ovulation and artificial insemination (AI) and supplemental progesterone for resynchronization on fertility of dairy cows subjected to a 5-d timed AI program. Journal of Dairy Science 93, 5798–5808.

Bisinotto RS, Ribeiro ES, Lima FS, Martinez N, Greco LF, Barbosa L, Bueno PP, Scagion LFS, Thatcher WW and Santos JEP 2013. Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol. Journal of Dairy Science 96, 2214–2225.

Bogan RL and Niswender GD 2007. Constitutive steroidogenesis in ovine large luteal cells may be mediated by tonically active protein kinase A. Biology of Reproduction 77, 209–216.

Bouin P and Ancel P 1910. Recherches sur les fonctions du corps jaune gestatif. I. Sur le determinisme de la preparatiotion de l'uterus al fixation d l'oeuf. Journal de physiologie et de pathologie generale 12, 1–16.

Brusveen DJ, Souza AH and Wiltbank MC 2009. Effects of additional prostaglandin F-2 alpha and estradiol-17 beta during Ovsynch in lactating dairy cows. Journal of Dairy Science 92, 1412–1422.

Brusveen DJ, Cunha AP, Silva CD, Cunha PM, Sterry RA, Silva EP, Guenther JN and Wiltbank MC 2008. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during Ovsynch affects pregnancies per AI in lactating dairy cows. Journal of Dairy Science 91, 1044–1052.

Bulman DC and Lamming GE 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. Journal of Reproduction and Fertility 54, 447–458.

Butenandt A and Westphal U 1934. Zur isolierung und characterisierung des corpus-luteum-hormons. Berichte der deutschen chemischen Gesellschaft 67, 1440–1442.

Carter F, Forde N, Duffy P, Wade M, Fair T, Crowe MA, Evans ACO, Kenny DA, Roche JF and Lonergan P 2008. Effect of increasing progesterone concentration from day 3 of pregnancy on subsequent embryo survival and development in beef heifers. Reproduction, Fertility and Development 20, 368–375.

Carter F, Rings F, Mamo S, Holker M, Kuzmany A, Besenfelder U, Havlicek V, Mehta JP, Tesfaye D, Schellander K and Lonergan P 2010. Effect of elevated circulating progesterone concentration on bovine blastocyst development and global transcriptome following endoscopic transfer of in vitro produced embryos to the bovine oviduct. Biology of Reproduction 83, 707–719.

Cerri RLA, Chebel RC, Rivera F, Narciso CD, Oliveira RA, Thatcher WW and Santos JEP 2011a. Concentration of progesterone during the development of the ovulatory follicle: I. Ovarian and embryonic responses. Journal of Dairy Science 94, 3342–3351.

Cerri RLA, Chebel RC, Rivera F, Narciso CD, Oliveira RA, Amstalden M, Baez-Sandoval GM, Oliveira LJ, Thatcher WW and Santos JEP 2011b. Concentration of progesterone during the development of the ovulatory follicle: II. Ovarian and uterine responses. Journal of Dairy Science 94, 3352–3365.

Chebel RC, Al-Hassan MJ, Fricke PM, Santos JEP, Lima JR, Martel CA, Stevenson JS, Garcia R and Ax RL 2010. Supplementation of progesterone via controlled internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. Journal of Dairy Science 93, 922–931.

Christenson RK, Ford JJ and Redmer DA 1985. Metabolic-clearance and production-rates of estradiol and progesterone during pubertal and postpubertal development in gilts. Journal of Reproduction and Fertility 75, 247–253.

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

Corner GW and Allen WM 1929. Physiology of the corpus luteum. II. Production of a special uterine reaction (progestational proliferation) by extracts of the corpus luteum. American Journal of Physiology 88, 326–399.

Couet J, Martel C, Dupont E, Luuthe V, Sirard MA, Zhao HF, Pelletier G and Labrie F 1990. Changes in 3-beta hydroxysteroid dehydrogenase-delta-5-delta-4 isomerase mRNA, activity, and protein levels during estrous cycle in the bovine ovary. Endocrinology 127, 2141–2148.

Cummins SB, Lonergan P, Evans ACO and Butler ST 2012. Genetic merit for fertility traits in Holstein cows: II. Ovarian follicular and corpus luteum dynamics, reproductive hormones, and estrus behavior. Journal of Dairy Science 95, 3698–3710.

Cunha AP, Guenther JN, Maroney MJ, Giordano JO, Nascimento AB, Bas S, Ayres H and Wiltbank MC 2008. Effects of high vs. low progesterone concentrations during Ovsynch on double ovulation rate and pregnancies per AI in high producing dairy cows. Journal of Dairy Science 91 (suppl. 1), 246. (Abstract).

De Silva AWMV, Anderson GW, Gwazdauskas FC, McGilliard ML and Lineweaver JA 1981. Interrelationships with estrous behavior and conception in dairy-cattle. Journal of Dairy Science 64, 2409–2418.

del Rio NS, Kirkpatrick BW and Fricke PM 2006. Observed frequency of monozygotic twinning in Holstein dairy cattle. Theriogenology 66, 1292–1299.

Denamur R, Martinet J and Short RV 1973. Pituitary control of ovine corpus luteum. Journal of Reproduction and Fertility 32, 207–220.

Denicol AC, Lopes G Jr, Mendonça LGD, Rivera FA, Guagnini F, Perez RV, Lima JR, Bruno RGS, Santos JEP and Chebel RC 2012. Low progesterone concentration during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. Journal of Dairy Science 95, 1794–1806.

Diaz FJ, Anderson LE, Wu YL, Rabot A, Tsai SJ and Wiltbank MC 2002. Regulation of progesterone and prostaglandin F-2 alpha production in the CL. Molecular and Cellular Endocrinology 191, 65–80.

Diskin MG, Murphy JJ and Sreenan JM 2006. Embryo survival in dairy cows managed under pastoral conditions. Animal Reproduction Science 96, 297–311.

Ellmann S, Sticht H, Thiel F, Beckmann MW, Strick R and Strissel PL 2009. Estrogen and progesterone receptors: from molecular structures to clinical targets. Cellular and Molecular Life Sciences 66, 2405–2426.

Erb RE, Garverick HA, Randel RD, Brown BL and Callahan CJ 1976. Profiles of reproductive hormones associated with fertile and nonfertile inseminations of dairy cows. Theriogenology 5, 227–242.

Farin CE, Nett TM and Niswender GD 1990. Effects of LH on luteal cell populations in hypophysectomized ewes. Journal of Reproduction and Fertility 88, 61–70.

Fels E, Slotta KH and Ruschig H 1934. Preparation of pure hormones of the corpus luteum. Klinische Wochenschrift 13, 1207–1208.

Fernandez-Valdivia R, Mukherjee A, Mulac-Jericevic B, Conneely OM, DeMayo FJ, Amato P and Lydon JP 2005. Revealing progesterone's role in uterine and mammary gland biology: insights from the mouse. Seminars in Reproductive Medicine 23, 22–37.

Folman Y, Rosenber M, Herz Z and Davidson M 1973. Relationship between plasma progesterone concentration and conception in postpart dairy cows maintained on 2 levels of nutrition. Journal of Reproduction and Fertility 34, 267–278.

Fonseca FA, Britt JH, Mcdaniel BT, Wilk JC and Rakes AH 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. Journal of Dairy Science 66, 1128–1147.

Forde N, Beltman ME, Duffy GB, Duffy P, Mehta JP, O'Gaora P, Roche JF, Lonergan P and Crowe MA 2011. Changes in the endometrial transcriptome during the bovine estrous cycle: effect of low circulating progesterone and consequences for conceptus elongation. Biology of Reproduction 84, 266–278.

Forde N, Carter F, Fair T, Crowe MA, Evans ACO, Spencer TE, Bazer FW, McBride R, Boland MP, O'Gaora P, Lonergan P and Roche JF 2009. Progesterone-regulated changes in endometrial gene expression contribute to advanced conceptus development in cattle. Biology of Reproduction 81, 784–794.

Fraenkel L 1910. Neue experiment zur function des corpus luteum. Archiv fur Gynakologie 91, 705–730.

Fraenkel L and Cohn F 1901. Experimentelle untersuchungen des corpus luteum auf die insertion des eies (Theorie von Born). Anatomischer Anzeiger 20, 294–300.

Fricke PM and Wiltbank MC 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology 52, 1133–1143.

Fricke PM, Reynolds LP and Redmer DA 1993. Effect of human chorionic gonadotropin administered early in the estrous cycle on ovulation and subsequent luteal function in cows. Journal of Animal Science 71, 1242–1246.

Ghanem ME, Nakao T, Nakatani K, Akita M and Suzuki T 2006. Milk progesterone profile at and after artificial insemination in repeat-breeding cows: effects on conception rate and embryonic death. Reproduction in Domestic Animals 41, 180–183.

Giordano JO, Wiltbank MC, Guenther JN, Ares MS, Lopes G Jr, Herlihy MM and Fricke PM 2012a. Effect of presynchronization with human chorionic gonadotropin or gonadotropin-releasing hormone 7 days before resynchronization of ovulation on fertility in lactating dairy cows. Journal of Dairy Science 95, 5612–5625.

Giordano JO, Wiltbank MC, Guenther JN, Pawlisch R, Bas S, Cunha AP and Fricke PM 2012b. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. Journal of Dairy Science 95, 639–653.

Giordano JO, Wiltbank MC, Fricke PM, Bas S, Pawlisch R, Guenther JN and Nascimento AB 2013a. Effect of increasing GnRH and PGF2α dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. Theriogenology 80, 773–783.

Giordano JO, Wiltbank MC, Fricke PM, Bas S, Pawlisch RA, Guenther JN and Nascimento AB 2013b. Effect of increasing GnRH and PGF2α dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. Theriogenology 80, 773–783.

Grummer RR and Carroll DJ 1988. A review of lipoprotein cholesterol metabolism – importance to ovarian function. Journal of Animal Science 66, 3160–3173.

Gumen A, Guenther JN and Wiltbank MC 2003. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. Journal of Dairy Science 86, 3184–3194.

Hanlon DW, Jarratt GM, Davidson PJ, Millar AJ and Douglas VL 2005. The effect of hCG administration five days after insemination on the first service conception rate of anestrous dairy cows. Theriogenology 63, 1938–1945.

Hartmen M and Wettstein A 1934. Ein krystallisiertes hormon aus corpus luteum. Helvetica Chimica Acta 17, 878–882.

Haughian JM, Ginther OJ, Diaz FJ and Wiltbank MC 2013. Gonadotropin-releasing hormone, estradiol, and inhibin regulation of follicle-stimulating hormone and luteinizing hormone surges: implications for follicle emergence and selection in heifers. Biology of Reproduction 88, 165–177.

Hawkins DE, Belfiore CJ, Kile JP and Niswender GD 1993. Regulation of mRNA encoding 3-beta hydroxysteroid dehydrogenase delta(5)-delta(4) isomerase in ovine corpus luteum. Biology of Reproduction 48, 1185–1190.

Hayashi KG, Matsui M, Shimizu T, Sudo N, Sato A, Shirasuna K, Tetsuka M, Kida K, Schams D and Miyamoto A 2008. The absence of corpus luteum formation alters the endocrine profile and affects follicular development during the first follicular wave in cattle. Reproduction 136, 787–797.

Herrick JB 1953. Clinical observation of progesterone therapy in repeat breeding heifers. Veterinary Medicine 48, 489–490.

Hunter RHF 2005. The fallopian tubes in domestic mammals: how vital is their physiological activity? Reproduction Nutrition Development 45, 281–290.

Jocelyn HD and Setchell BP 1972. An annotated translation of Regnier deGraaf's new treatise concerning the generative organs of women (1672). Journal of Reproduction and Fertility Supplement 17, 77–206.

Juengel JL, Meberg BM, McIntush EW, Smith MF and Niswender GD 1998. Concentration of mRNA encoding 3 beta-hydroxysteroid dehydrogenase delta(5), delta(4) isomerase (3 beta-HSD) and 3 beta-HSD enzyme activity following treatment of ewes with prostaglandin F-2 alpha. Endocrine 8, 45–50.

Kaltenbach CC, Graber JW, Niswender GD and Nalbandov AV 1968. Effect of hypophysectomy on formation and maintenance of corpora lutea in ewe. Endocrinology 82, 753.

Kendall NR, Flint APF and Mann GE 2009. Incidence and treatment of inadequate postovulatory progesterone concentrations in repeat breeder cows. Veterinary Journal 181, 158–162.

Kinsel ML, Marsh WE, Ruegg PL and Etherington WG 1998. Risk factors for twinning in dairy cows. Journal of Dairy Science 81, 989–993.

Larson JE, Krisher RL and Lamb GC 2011. Effects of supplemental progesterone on the development, metabolism and blastocyst cell number of bovine embryos produced in vitro. Reproduction Fertility and Development 23, 311–318.

Larson SF, Butler WR and Currie WB 1997. Reduced fertility associated with low progesterone postbreeding and increased milk urea nitrogen in lactating cows. Journal of Dairy Science 80, 1288–1295.

Li XL, Peegel H and Menon KMJ 1998. In situ hybridization of high density lipoprotein (scavenger, type 1) receptor messenger ribonucleic acid (mRNA)

during folliculogenesis and luteinization: evidence for mRNA expression and induction by human chorionic gonadotropin specifically in cell types that use cholesterol for steroidogenesis. Endocrinology 139, 3043–3049.

Lonergan P, Woods A, Fair T, Carter F, Rizos D, Ward F, Quinn K and Evans A 2007. Effect of embryo source and recipient progesterone environment on embryo development in cattle. Reproduction, Fertility and Development 19, 861–868.

Lopez H, Sartori R and Wiltbank MC 2005a. Reproductive hormones and follicular growth during development of one or multiple dominant follicles in cattle. Biology of Reproduction 72, 788–795.

Lopez H, Caraviello DZ, Satter LD, Fricke PM and Wiltbank MC 2005b. Relationship between level of milk production and multiple ovulations in lactating dairy cows. Journal of Dairy Science 88, 2783–2793.

Magnus V 1901. Ovariets betydning for svangerskabet med saerligt hensyntil corpus luteum. Norsk Magazin for Laegevidenskaben 62, 1138–1142.

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

Mann GE and Lamming GE 1999. The influence of progesterone during early pregnancy in cattle. Reproduction in Domestic Animals 34, 269–274.

Martins JP, Policelli RK, Neuder LM, Raphael W and Pursley JR 2011a. Effects of cloprostenol sodium at final prostaglandin F2alpha of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. Journal of Dairy Science 94, 2815–2824.

Martins JPN, Policelli RK, Neuder LM, Raphael W and Pursley JR 2011b. Effects of cloprostenol sodium at final prostaglandin F-2 alpha of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. Journal of Dairy Science 94, 2815–2824.

McNeill RE, Sreenan JM, Diskin MG, Cairns MT, Fitzpatrick R, Smith TJ and Morris DG 2006. Effect of systemic progesterone concentration on the expression of progesterone-responsive genes in the bovine endometrium during the early luteal phase. Reproduction, Fertility and Development 18, 573–583.

McNeilly AS, Crow WJ and Fraser HM 1992. Suppression of pulsatile LH secretion by GnRH antagonist does not affect episodic progesterone secretion or corpus luteum function in ewes. Journal of Reproduction and Fertility 96, 865–874.

Meisterling EM and Dailey RA 1987. Use of concentrations of progesterone and estradiol-17-beta in milk in monitoring postpartum ovarian function in dairy cows. Journal of Dairy Science 70, 2154–2161.

Moreira F, de la Sota RL, Diaz T and Thatcher WW 2000. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. Journal of Animal Science 78, 1568–1576.

Morris D and Diskin M 2008. Effect of progesterone on embryo survival. Animal 2, 1112–1119.

Mullen MP, Elia G, Hilliard M, Parr MH, Diskin MG, Evans ACO and Crowe MA 2012a. Proteomic characterization of histotroph during the preimplantation phase of the estrous cycle in cattle. Journal of Proteome Research 11, 3004–3018.

Mullen MP, Forde N, Parr MH, Diskin MG, Morris DG, Nally JE, Evans ACO and Crowe MA 2012b. Alterations in systemic concentrations of progesterone during the early luteal phase affect RBP4 expression in the bovine uterus. Reproduction, Fertility and Development 24, 715–722.

Nascimento AB, Souza AH, Keskin A, Sartori R and Wiltbank MC 2013a. Lack of complete regression of the day 5 corpus luteum after one or two dose of PGF2α in nonlactating Holstein cows. Theriogenology (in press).

Nascimento AB, Souza AH, Guenther JN, Costa FPD, Sartori R and Wiltbank MC 2013b. Effects of treatment with human chorionic gonadotrophin or intravaginal progesterone-releasing device after AI on circulating progesterone concentrations in lactating dairy cows. Reproduction, Fertility and Development 25, 818–824.

Nascimento AB, Bender RW, Souza AH, Ayres H, Araujo RR, Guenther JN, Sartori R and Wiltbank MC 2013c. Effect of treatment with human chorionic gonadotropin on day 5 after timed artificial insemination on fertility of lactating dairy cows. Journal of Dairy Science 96, 2873–2882.

Niswender GD, Juengel JL, Mcguire WJ, Belfiore CJ and Wiltbank MC 1994. Luteal function – the estrous-cycle and early-pregnancy. Biology of Reproduction 50, 239–247.

Niswender GD, Juengel JL, Silva PJ, Rollyson MK and McIntush EW 2000. Mechanisms controlling the function and life span of the corpus luteum. Physiological Reviews 80, 1–29.

Parr RA, Davis IF, Fairclough RJ and Miles MA 1987. Overfeeding during early pregnancy reduces periperpheral progesterone concentration and pregnancy in sheep. Journal of Reproduction and Fertility 80, 317–320.

Parr RA, Davis IF, Miles MA and Squires TJ 1993. Liver blood flow and metabolic clearance rate of progesterone in sheep. Research in Veterinary Science 55, 311–316.

Peluso JJ 2006. Multiplicity of progesterone's actions and receptors in the mammalian ovary. Biology of Reproduction 75, 2–8.

Pereira MHC, Sanches CP, Guida TG, Rodrigues ADR, Aragon FL, Veras MB, Borges PT, Wiltbank MC and Vasconcelos JLM 2013. Timing of prostaglandin F-2 alpha treatment in an estrogen-based protocol for timed artificial insemination or timed embryo transfer in lactating dairy cows. Journal of Dairy Science 96, 2837–2846.

Pescador N, Soumano K, Stocco DM, Price CA and Murphy BD 1996. Steroidogenic acute regulatory protein in bovine corpora lutea. Biology of Reproduction 55, 485–491.

Plym Forshell K, Andersson L and Pehrson B 1991. The relationships between the fertility of dairy cows and clinical and biochemical measurements, with special reference to plasma glucose and milk acetone. Zentralbl Veterinarmed A 38, 608–616.

Prime GR and Symonds HW 1993. Influence of plane of nutrition on portal blood-flow and the metabolic-clearance rate of progesterone in ovariectomized gilts. Journal of Agricultural Science 121, 389–397.

Rajamahendran R and Sianangama PC 1992. Effect of human chorionic gonadotrophin on dominant follicles in cows: formation of accessory corpora lutea, progesterone production and pregnancy rates. Journal of Reproduction and Fertility 95, 577–584.

Rajapaksha W, McBride M, Robertson L and Oshaughnessy PJ 1997. Sequence of the bovine HDL-receptor (SR-BI) cDNA and changes in receptor mRNA expression during granulosa cell luteinization in vivo and in vitro. Molecular and Cellular Endocrinology 134, 59–67.

Ribeiro ES, Bisinotto RS, Favoreto MG, Martins LT, Cerri RLA, Silvestre FT, Greco LF, Thatcher WW and Santos JEP 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.

Rivera FA, Mendonca LGD, Lopes G, Santos JEP, Perez RV, Amstalden M, Correa-Calderon A and Chebel RC 2011. Reduced progesterone concentration during growth of the first follicular wave affects embryo quality but has no effect on embryo survival post transfer in lactating dairy cows. Reproduction 141, 333–342.

Sangsritavong S 2002. Studies of steroid metabolism in dairy cattle. PhD Dissertation, University of Wisconsin, Madison, WI, USA.

Sangsritavong S, Combs DK, Sartori R, Armentano LE and Wiltbank MC 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17 beta in dairy cattle. Journal of Dairy Science 85, 2831–2842.

Santos JEP, Rutigliano HM and Filho MFS 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Animal Reproduction Science 110, 207–221.

Santos JEP, Thatcher WW, Pool L and Overton MW 2001. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. Journal of Animal Science 79, 2881–2894.

Santos JEP, Thatcher WW, Chebel RC, Cerri RLA and Galvão KN 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Animal Reproduction Science 82–83, 513–535.

Santos JEP, Narciso CD, Rivera F, Thatcher WW and Chebel RC 2010. Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. Journal of Dairy Science 93, 2976–2988.

Schmitt EJ, Barros CM, Fields PA, Fields MJ, Diaz T, Kluge JM and Thatcher WW 1996. A cellular and endocrine characterization of the original and induced corpus luteum after administration of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin on day five of the estrous cycle. Journal of Animal Science 74, 1915–1929.

Silva CC and Knight PG 2000. Effects of androgens, progesterone and their antagonists on the developmental competence of in vitro matured bovine oocytes. Journal of Reproduction and Fertility 119, 261–269.

Silva CC, Groome NP and Knight PG 1999. Demonstration of a suppressive effect of inhibin alpha-subunit on the developmental competence of in vitro matured bovine oocytes. Journal of Reproduction and Fertility 115, 381–388.

Silva E, Sterry RA, Kolb D, Wiltbank MC and Fricke PM 2007. Effect of pretreatment with prostaglandin F-2 alpha before resynchronization of ovulation on fertility of lactating dairy cows. Journal of Dairy Science 90, 5509–5517.

Simmer HH 1971. The first experiments to demonstrate an endocrine function of the corpus luteum. On the occasion of the 100th birthday of Ludwig Fraenkel (1870–1951). Sudhoffs Archiv 55, 392–417.

Souza AH, Ayres H, Ferreira RM and Wiltbank MC 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 70, 208–215.

Souza AH, Gumen A, Silva EPB, Cunha AP, Guenther JN, Peto CM, Caraviello DZ and Wiltbank MC 2007. Supplementation with estradiol-17 beta before the last gonadotropin-releasing hormone injection of the Ovsynch protocol in lactating dairy cows. Journal of Dairy Science 90, 4623–4634.

Souza AH, Silva EPB, Cunha AP, Gumen A, Ayres H, Brusveen DJ, Guenther JN and Wiltbank MC 2011. Ultrasonographic evaluation of endometrial thickness near timed AI as a predictor of fertility in high-producing dairy cows. Theriogenology 75, 722–733.

Sterry RA, Welle ML and Fricke PM 2006. Treatment with gonadotropin-releasing hormone after first timed artificial insemination improves fertility in noncycling lactating dairy cows. Journal of Dairy Science 89, 4237–4245.

Stevenson JS, Portaluppi MA and Tenhouse DE 2007a. Factors influencing upfront single- and multiple-ovulation incidence, progesterone, and luteolysis before a timed insemination resynchronization protocol. Journal of Dairy Science 90, 5542–5551.

Stevenson JS, Portaluppi MA, Tenhouse DE, Lloyd A, Eborn DR, Kacuba S and DeJarnette JM 2007b. Interventions after artificial insemination: conception rates, pregnancy survival, and ovarian responses to gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone. Journal of Dairy Science 90, 331–340.

Stevenson JS, Pursley JR, Garverick HA, Fricke PM, Kesler DJ, Ottobre JS and Wiltbank MC 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. Journal of Dairy Science 89, 2567–2578.

Stevenson JS, Tenhouse DE, Krisher RL, Lamb GC, Larson JE, Dahlen CR, Pursley JR, Bello NM, Fricke PM, Wiltbank MC, Brusveen DJ, Burkhart M, Youngquist RS and Garverick HA 2008. Detection of anovulation by heatmount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol. Journal of Dairy Science 91, 2901–2915.

Stocco DM and Clark BJ 1996. Role of the steroidogenic acute regulatory protein (StAR) in steroidogenesis. Biochemical Pharmacology 51, 197–205.

Stocco DM, Wang X, Jo Y and Manna P 2005. Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: more complicated than we thought. Molecular Endocrinology 19, 2647–2659.

Stronge AJH, Sreenan JM, Diskin MG, Mee JF, Kenny DA and Morris DG 2005. Post-insemination milk progesterone concentration and embryo survival in dairy cows. Theriogenology 64, 1212–1224.

Tandeski TR, Juengel JL, Nett TM and Niswender GD 1996. Regulation of messenger RNA encoding low density lipoprotein receptor and high density lipoprotein-binding protein in ovine corpora lutea. Reproduction Fertility and Development 8, 1107–1114.

Vasconcelos JL, Sa Filho OG, Justolin PL, Morelli P, Aragon FL, Veras MB and Soriano S 2011. Effects of postbreeding gonadotropin treatments on conception rates of lactating dairy cows subjected to timed artificial insemination or embryo transfer in a tropical environment. Journal of Dairy Science 94, 223–234.

Vasconcelos JLM, Sangsritavong S, Tsai SJ and Wiltbank MC 2003. Acute reduction in serum progesterone concentrations after feed intake in dairy cows. Theriogenology 60, 795–807.

Waldmann A, Reksen O, Landsverk K, Kommisrud E, Dahl E, Refsdal A and Ropstad E 2001. Progesterone concentrations in milk fat at first insemination - effects on non-return and repeat-breeding. Animal Reproduction Science 65, 33–41.

Walton JS, Halbert GW, Robinson NA and Leslie KE 1990. Effects of progesterone and human chorionic-gonadotropin administration 5 days postinsemination on plasma and milk concentrations of progesterone and pregnancy rates of normal and repeat breeder dairy cows. Canadian Journal of Veterinary Research 54, 305–308.

Wiltbank JN, Hawk HW, Kidder HE, Black WG, Ulberg LC and Casida LE 1956. Effect of progesterone therapy on embryos survival in cows of lowered fertility. Journal of Dairy Science 39, 456–461.

Wiltbank M, Lopez H, Sartori R, Sangsritavong S and Gumen A 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65, 17–29.

Wiltbank MC 1994. Cell types and hormonal mechanisms associated with mid-cycle corpus luteum function. Journal of Animal Science 72, 1873–1883.

Wiltbank MC, Belfiore CJ and Niswender GD 1993. Steroidogenic enzyme activity after acute activation of protein-kinase (PK) A and PKC in ovine small and large luteal cells. Molecular and Cellular Endocrinology 97, 1–7.

Wiltbank MC, Diskin MG, Flores JA and Niswender GD 1990. Regulation of the corpus luteum by protein kinase C 2. Inhibition of lipoprotein-stimulated steroidogenesis by prostaglandin-F2-alpha. Biology of Reproduction 42, 239–245.

Wiltbank MC, Fricke PM, Sangsritavong S, Sartori R and Ginther OJ 2000. Mechanisms that prevent and produce double ovulations in dairy cattle. Journal of Dairy Science 83, 2998–3007.

Wiltbank MC, Salih SM, Atli MO, Luo W, Bormann CL, Ottobre JS, Vezina CM, Mehta V, Diaz FJ, Tsai SJ and Sartori R 2012a. Comparison of endocrine and cellular mechanisms regulating the corpus luteum of primates and ruminants. Animal Reproduction 9, 242–259.

Wiltbank MC, Souza AH, Giordano JO, Nascimento AB, Vasconcelos JM, Pereira MHC, Fricke PM, Surjus RS, Zinsly FCS, Carvalho PD, Bender RW and Sartori R 2012b. Positive and negative effects of progesterone during timed AI protocols in lactating dairy cattle. Animal Reproduction 9, 231–241.

Wintersteiner O and Allen W 1934. Crystaline progestin. Journal of Biological Chemistry 107, 321–336.