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Causas de infertilidad en vacas de leche en lactación

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Characterization of Reproductive Inefficiency in Lactating Dairy Cows

Fertility in lactating dairy cows has been decreasing in the past 50 years. This decrease in fertility has been associated with a steady increase in milk yield, which is a consequence of significant genetic selection toward milk production.

According to the National Animal Health Monitoring System (2007) reproductive failure is the most important cause of involuntary culling. The costs of reproductive inefficiency to the cattle industry are extremely important and have been recognized as such since the beginning of the 80s. In 1994, Senger et al. (1994) suggested that the dairy industry loses approximately $ 300 million per year because of poor estrous detection rates and accuracy. The estimated average value of a pregnancy is $ 275 (DeVries, 2006) and that of an abortion is between $ 555 and $ 640 (Thurmond et al., 1990; DeVries, 2006). These values are dependent on lactation number, milk yield, days in milk (DIM), price of milk, and cost of replacement animals.

In technical terms, compromised reproductive inefficiency involves, fertilization failure – observed from the day of AI to 5-6 d after AI, early embryonic loss – observed from 5-6 d after AI to 17-24 d after AI, late embryonic loss – observed from 17-24 d after AI to 42 d after AI, and fetal loss – observed from 42 d after AI to term (Santos et al., 2004a). Reduced fertilization and increased early embryonic loss are usually observed as increased return to estrus after AI. Increased late embryonic loss is observed as altered inter-AI interval or increased abortions if the first pregnancy diagnosis takes place before 42 d after AI. Increased fetal losses are observed as increased number of abortions after 42 d after AI (Figure 1). Ultimately, reproductive failure in large dairy herds is usually observed as reduced pregnancy per AI (**P/AI**;

number of pregnancies divided by the number of cows inseminated) and increased number of abortions.

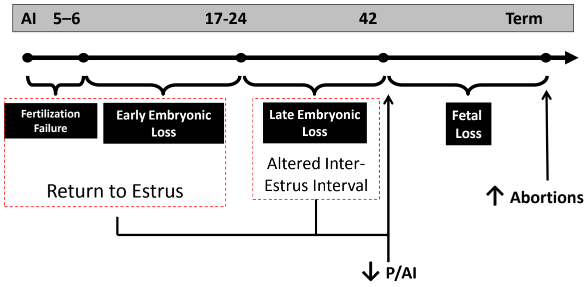


Figure 1. Characterization of reproductive failure in cattle.

Different studies have evaluated the fertilization of oocytes and quality of embryos from lactating dairy cows, heifers, and non-lactating dairy cows (DeJarnette et al., 1992; Dalton et al., 2001; Sartori et al., 2002; Cerri et al., 2009a; Cerri et al., 2009b; Cerri et al., 2009c). Although not all studies compared directly production and quality of embryos by lactating dairy cows with heifers or non-lactating dairy cows, it is estimated that non-superovulated lactating dairy cows have approximately 76% of recovered structures fertilized, whereas non-lactating cows have 78% of recovered structures fertilized and heifers have 100 of recovered structures fertilized. Further, approximately 66% of fertilized ova recovered from lactating dairy cows are classified as excellent/good quality embryos but approximately 74% and 72% of fertilized ova recovered from non-lactating dairy cows and heifers, respectively, are classified as excellent/good quality embryos. Consequently, among all ova-embryo recovered from lactating dairy cows, 50% are classified as excellent/good embryos, whereas 58% of all ova-embryo recovered from non-lactating cows and 72% of all ova-embryo recovered from heifers are classified as excellent/good embryos. In recent studies conducted by our group in several herds across the U.S. we observed P/AI of lactating dairy cows between 35 and 40% at 30 to 38 d after first postpartum AI (Chebel et al., 2010; Chebel and Santos, 2010; Santos et al., 2010).

Therefore, it is expected that 25% of all excellent/good quality embryos will be lost between 6 and 30 d after first postpartum AI, representing 1.78% of embryonic losses per day.

Summarization of data from 15 different studies conducted in the U.S. that reported late embryonic loss demonstrates that pregnancy losses from 27-31 to 38-50 d after AI is approximately 13% with a range of 3 to 43% (Table 1). This represents pregnancy losses of approximately 0.85% per day during this period. Further, late embryonic/fetal losses from approximately 40 to 120 d after AI have been reported to range from 8.3 to 10.7%, which represents daily losses of approximately 0.11% of pregnancies diagnosed at 40 d after AI (Table 2). On the other hand, P/AI at 38 d after first AI in virgin heifers ranges from 55 to 70% and only approximately 3% of heifers lose pregnancy from 38 to 120 d of gestation, resulting in daily pregnancy loss of approximately 0.05%.

From these data it is obvious that the stages of greatest risk for reproductive inefficiency are fertilization, embryo development, maternal recognition of pregnancy, and placentation. Further, it is clear that lactating dairy cows are less likely to conceive and to carry out the pregnancy to term than virgin heifers. Although the factors associated with reduced fertility in lactating dairy cows are multiple and multi-faceted, they all originate from the ability or lack thereof of lactating dairy cows to adapt to the nutritional demands associated with the extremely elevated milk yield. In the companion manuscript entitle “Manejo de la vaca en transición para maximizar la salud y reproducción” we discuss at length the consequences of onset of colostrum/milk production on energy balance, metabolic diseases, immune function, and health. In this manuscript we will discuss the effects of increased milk yield on physiological alterations associated with it that affect reproductive efficiency.

Physiological Changes Associated with Reduced Fertility

Importance of Progesterone and Estradiol for Reproductive Function

There are several hormones that are extremely important to the reproductive function of ruminants such as progesterone, estradiol, GnRH, LH, and FSH. In this section, we will focus on the importance of progesterone and estradiol, their concentrations, and metabolism.

Table 1. Incidence of late embryonic mortality in lactating dairy cows

	Days of gestation at diagnosis					
No. Preg.	First	Second	Interval, d	Preg. loss, %	Preg. Loss, %per d	Reference
256	28	38-58	~ 20	28.0	1.40	Cartmill *et al.* (2001)
110	27-30	40-50	~ 16	42.7	2.67	Cartmill *et al.* (2001)
261	30	44	14	12.5	0.89	Cerri *et al.* (2003)
195	28	42	14	17.9	1.28	Chebel *et al.* (2003)
74	31	45	14	10.8	0.77	Chebel *et al.* (2003)
1465	31	45	14	12.5	0.89	Chebel *et al.* (2004)
231	30	66	28	22.1	0.79	Chebel and Santos (2010)
251	27	41	14	17.5	1.25	Galvão *et al.* (2004)
167	28	39	11	11.4	1.04	Juchem *et al.* (2002)
139	27	45	18	20.7	1.15	Moreira *et al.* (2001)
172	28	45	17	9.3	0.55	Santos *et al.* (2001)
372	31	45	14	11.4	0.82	Santos *et al.* (2004b)
215	27	41	14	9.9	0.71	Santos *et al.* (2004c)
705	28	42	14	3.2	0.23	Silke *et al.* (2002)
488	28	42	14	10.5	0.75	Vasconcelos *et al.* (1999)
Overall: 5101	27-31	38-50	~15	13.2 (3.2-42.7)	0.88 (0.23-2.67)	

Estradiol is produced by antral ovarian follicles. Under reduced concentrations of progesterone (< 1 ng/ml) estradiol is responsible for signs of estrus and a positive feed-back on the hypothalamus, which stimulates secretion of GnRH that causes the pituitary gland to produce an ovulatory LH-peak. Further, priming of the uterus with estradiol during the proestrus is expected to reduce the binding capacity of oxytocin to its endometrial receptors (Mann and Lamming, 2000). Reduced affinity of oxytocin to its receptors in the endometrium is expected to reduce the production of prostaglandin (PG) $F_{2\alpha}$ by the endometrium. For example, ovariectomized cows treated with exogenous estradiol, mimicking concentrations of estradiol during the proestrus, had smaller concentrations of $PGF_{2\alpha}$ metabolite after oxytocin challenge compared with ovariectomized cows not treated with estradiol (Mann and Lamming, 2000).

Table 2. Incidence of late embryonic and fetal mortality in lactating dairy cows

	Days of gestation at diagnosis					
No. Preg.	First	Second	Interval, d	Preg. loss, %	Preg. loss, % per d	Reference
1547	35-48	180	~139	9.9	0.07	Ettema and Santos (2004)
1107	38	66	28	8.8	0.31	Chebel et al. (2010)
601	38-44	90-96	~52	10.7	0.21	Lopez-Gatius *et al.* (2002)
3162	41	120-150	~84	9.6	0.11	Labèrnia *et al.* (1996)
156	45	90	45	8.3	0.18	Santos *et al.* (2001)
Overall: 6573	35-48	90-180	45-139	9.6 (8.3-10.7)	0.11	

Several studies have found a link between metestral and early diestral P4 concentrations, embryo development and elongation and the subsequent establishment of pregnancy. Mann and Lamming (2001) demonstrated that cows that had the largest embryos at 16 d after AI were also the cows that had the greatest P4 concentration starting at approximately d 5 after AI and that larger embryos produced greater quantities of interferon-τ, indicating that increased P4 concentrations should result in hastened development of embryos and improved signaling from the embryo for maternal recognition of pregnancy (Mann and Lamming, 2001). Despite the fact that mRNA for P4 receptors can be identified in nuclei of cells of early bovine embryos, in vitro exposure to elevated P4 concentrations did not affect subsequent development to the blastocyst stage, nor recovery rates of 14 d old in vitro-produced embryos 7 d after transfer (Clemente et al., 2009). On the other hand, although supplementation with P4 between d 3 and 7 of pregnancy did not alter the morphology of embryos recovered in the morula to blastocyst stage, conceptuses from heifers supplemented with P4 were significantly larger at d 13 and 16 after AI (Carter et al., 2008). Similarly, when multiple embryos were transferred into superstimulated or non-superstimulated recipient heifers, it was observed that embryos transferred into superstimulated heifers were significantly larger at d 13 than embryos transferred into non-superstimulated heifers, indicating a strong association between P4 concentration and embryo development post-transfer (Lonergan et al., 2007). The establishment of a uterine environment conducive to embryo growth and elongation appears to be P4 dependent, because alterations in uterine gene expression are induced by increased P4 concentrations. Messenger RNA expression for transport and secretory proteins, thought to contribute to uterine histotroph and thus conceptus elongation, was quantified and localized within the bovine endometrium using quantitative real-time PCR (Forde et al., 2010). Two proteins in particular, lipoprotein lipase (LPL) and connective tissue growth factor (CTFG), were expressed earlier and at higher levels in cows with elevated P4 concentrations.

Concentrations of Estradiol and Progesterone

Studies have compared the concentrations of estradiol and progesterone and the diameter of follicles and corpora lutea (CL) volume between lactating dairy cows and non-lactating cows or heifers.

Lopez et al. (2004) demonstrated that high producing lactating dairy cows (47 kg/day) had smaller peak concentration of estradiol during estrus (6.8 pg/ml) compared with low producers (32 kg/day - 8.6 pg/ml) and heifers (11.3 pg/ml), despite having larger ovulatory follicles (high producers = 18.6 mm, low producers = 17.4 mm, and heifers = 15 mm). Consequently, high producing dairy cows had shorter duration of estrus (high producers = 7 h, low producers = 11.9 h, and heifers = 11.3 h) and had fewer standing events during estrus (high producers = 6.5, low producers = 9.8, and heifers = 16.8 mounts). Further, Sartori et al. (2004) demonstrated that lactating dairy cows had smaller concentration of estradiol during estrus than non-lactating dairy cows (7.9 vs. 11.3 pg/ml). This reduced estradiol concentration is expected to result in prolonged interval from luteolysis to ovulation (lactating cows = 5.2 d, non-lactating cows = 4.6 d; Sartori et al., 2004) because the estradiol threshold necessary to stimulate an LH surge would take longer to be reached in lactating dairy cows. Extended interval from luteolysis to ovulation compromises oocyte quality because pre-ovulatory follicles are exposed to reduced progesterone (P4) concentration (< 2 ng/ml) for prolonged period, to increasing LH pulsatility, and premature maturation.

Similarly, lactating dairy cows have reduced concentrations of P4 compared with heifers starting as early as d 5 of the estrous cycle (Sartori et al., 2004). Before ovulation, the reduced concentrations of P4 may result in increased exposure of follicles to pulsatile release of LH, which causes premature oocyte maturation and reduced embryo quality (Calder et al., 2000; Inskeep, 2004). Oocytes collected on d 8 of the estrous cycle, from cows with P4 concentration declining from 1.7 to 0.6 ng/ml from estrous cycle d 6 to 9, were more likely to be in stage II of meiosis compared with oocytes from cows that had P4 concentration increasing from 1.4 to 3.1 ng/ml during the same period (Inskeep, 2004). Further, cows exposed to P4 < 1 ng/ml before ovulation are at higher risk for short luteal phase, because the lack of P4 priming results in premature increase in E2 receptors in the endometrium following ovulation and consequently premature expression of oxytocin receptors in the endometrium, which leads to premature secretion of $PGF_{2\alpha}$ and luteolysis. Exposure of cows to reduced P4 concentration after AI may affect embryo growth and consequently production of IFN-τ, compromising maternal recognition of pregnancy and pregnancy establishment.

It is not clear whether reduced production of E2 and P4 or increased metabolism of E2 and P4 or both are the cause for reduced E2 and P4 concentrations in lactating dairy cows, but the latter is more likely. Studies conducted in Wisconsin have demonstrated that the rate of metabolism of steroidal hormones in lactating dairy cows is greater than that of non-lactating dairy cows. This seems to be directly correlated with the increased feed intake of lactating dairy cows and the consequent hyperthrophy and hyperplasia of the liver and organs of the gastrointestinal tract. This results in increased blood flow through the liver and greater metabolism of steroidal hormones. Sangsritavong et al. (2002) demonstrated that unfed cows have reduced blood flow through the liver compared with cows fed 7.8 lb/d, 23.4 lb/d, and 33.4 lb/d (Figure 2). This resulted in faster decrease in progesterone and estradiol concentrations after feeding (Sangsritavong et al., 2002). Similarly, cows receiving 100 and 50% of NRC (2001) recommendations had significantly reduced P4 concentrations compared with cows receiving 25% of NRC recommendations or unfed cows (Figure 3; Vasconcelos et al., 2003).

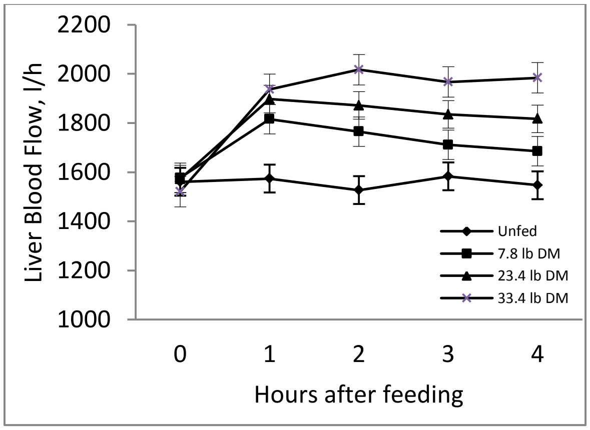


Figure 2. Effect of feed intake on liver blood flow. Adapted from Sangsritavong et al. (2002).

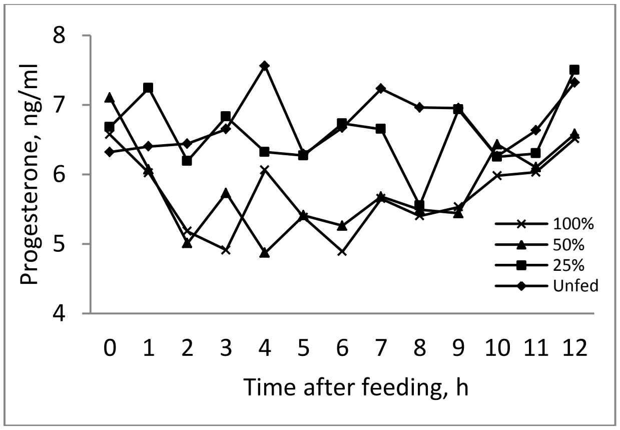


Figure 3. Effect of feed intake on progesterone concentration. Adapted from Vasconcelos et al. (2003).

This is clear evidence that high yield lactating dairy cows have reduced E2 and P4 concentrations as a consequence of increased feed intake, which is a consequence of increased milk yield. This poses significant challenges to the reproductive efficiency of these animals because of the importance of E2 and P4 for reproductive efficiency, explaining in part the significant decreases in reproductive efficiency observed in the past decades.

Postpartum Diseases and Reproductive Inefficiency

Most of the economic losses due to peripartum diseases are usually considered to be related to reduced milk yield, increased culling, cost of treatment, and milk withhold. Cows experiencing any postpartum disease, however, are susceptible to having reduced P/AI after first postpartum AI or long term reproductive inefficiency. Therefore, herds with deficient management of transition cows and consequent increase in incidence of postpartum diseases are expected to have compromised reproductive performance.

Multiple mechanisms are candidates to be the cause of reproductive inefficiency of lactating dairy cows affected by postpartum diseases. Among them are reduced feed intake (Maltz et al., 1997), pyrexia or fever (Wenz et al., 2001), production of cytokines and pro-inflammatory factors (Soto et al., 2003; Pampfer et al., 1994; Wuu et al., 1999; Soto et al., 2003; Chen et al., 2001; Hobbs et al., 1999; Buford et al., 1996; Skarzynski et al., 2000; Davidson et al., 1995), and compromise of the hypothalamic-pituitary-ovarian axis (McCann et al., 2000; Stoebel et al., 1982; Li et al., 1983; Padmanabhan et al., 1983; Alpizar et al., 1994; Fairchild et al., 1991; Petroff et al., 2001).

Therefore, cows that have peripartum diseases may or may not have pyrexia and fever, but in general all of them will have reduced feed intake and likely some extension of the negative energy balance. Because postpartum cows are already experiencing significant negative energy balance the diseases caused-reduced feed intake may further reduce negative energy balance, which could delay resumption of ovarian cyclicity (Buttler, 2000). The interval from calving to first ovulation is clearly associated with reproductive inefficiency as will be discussed below.

Postpartum Diseases effects on Reproductive Performance

Several studies have evaluated the effects of postpartum diseases (e.g. retained fetal membranes, metritis, endometritis, ketosis, and displacement of the abomasum) on fertility. Table 3 depicts data from a study in which we evaluated, among other things, the effect of postpartum diseases on reproductive efficiency in two dairy herds and over 8,500 lactations (Mendonça and Chebel, 2011). From this data, it is clear that any postpartum disease is likely to significantly reduce reproductive efficiency and profoundly affect profitability of dairy herds.

Table 3. Effects of peripartum diseases and mastitis on reproductive performance of lactating dairy cows (Mendonça and Chebel, 2011).

	Disease Outcome					
	Yes			No		
	AOR (95% CI)	MIC, d	Pregnant 305 DIM, %	AOR (95% CI)	MIC, d	Pregnant 305 DIM, %
Diseases						
Metritis	1.23 (1.14, 1.32)	168.5 ± 2.7	66.3	Referent	151.8 ± 1.1	73.8
Ketosis	1.79 (1.37, 2.35)	191.7 ± 9.7	42.4	Referent	153.5 ± 1.0	73.3
Stillbirth	1.58 (1.37, 1.82)	187.3 ± 5.2	52.3	Referent	152.6 ± 1.0	73.7
Mastitis	1.26 (1.19, 1.33)	167.7 ± 1.7	68.7	Referent	147.0 ± 1.2	74.9

AOR = Adjusted odds ratio
95% CI = 95% confidence interval
MIC = mean interval to conception

Considering that the cost of 1 d increase in the interval from parturition to conception is approximately $ 3/cow/d, occurrence of the diseases described in table 3 can result in economic losses of approximately $ 50 to $ 115 per cow per lactation.

As mentioned in the companion manuscript entitled “Manejo de la vaca en transición para maximizar la salud y reproducción” the key to the reproductive success of dairy cows starts with good and sound management of transition cows.

Mastitis effects on Reproductive Performance

Clinical and sub-clinical mastitis occurring at any time during lactation is expected to have a profound effect on reproductive performance of lactating dairy cows. In a large study performed in the Central Valley of California (Santos et al., 2004b), 1,001 lactating Holstein cows from two dairy herds were also classified according to the timing of occurrence of the first mastitis case during the lactation as: no clinical mastitis (CON), mastitis occurring before FPAI (MG1), mastitis occurring between FPAI and pregnancy diagnosis (MG2), or mastitis occurring after pregnancy confirmation (MG3). The P/AI after first postpartum AI and the percentage of cows pregnant after 320 DIM were significantly smaller for MG1 and MG2 cows compared with CON and MG3 cows (Figure 4). Cows in the MG1 and MG2 groups had extended interval from calving to conception compared with CON and MG3 cows (CON = 139.7 ± 3.7, MG1 = 165.0 ± 5.7, MG2 = 189.4 ± 6.4, and MG3 = 118.4 ± 6.4 d). When cows were grouped as either not experiencing mastitis (CON) or experiencing mastitis (MG1, MG2, and MG3), those that experienced mastitis had longer interval from calving to conception (Figure 5).

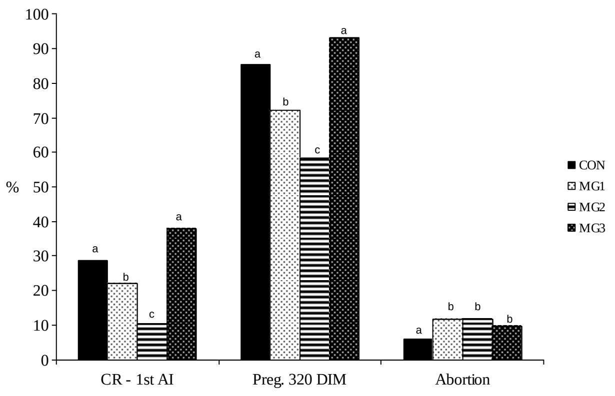


Figure 4. Association between mastitis and P/AI after first postpartum AI (CR-1st AI), percentage of cows pregnant after 320 DIM (Preg. 320 DIM), and incidence of abortions (Abortion) in Holstein cows. Adapted from Santos et al. (2004b).

Cows diagnosed with mastitis at any stage of the lactation are also at greater risk of having abortion (Figure 4). Chebel et al. (2004) evaluated factors that affect P/AI and pregnancy loss in lactating Holstein cows from three different dairy herds in the Central Valley of California. In this study a total of 7,633 AI were used for evaluation of factors affecting CR and 1,465 cows diagnosed pregnant by ultrasonography at 31 d after AI and re-examined 14 d later were used for evaluation of factors affecting pregnancy loss. Among the factors evaluated was occurrence of mastitis between AI and pregnancy confirmation at approximately 45 d after AI. Cows that had mastitis during this interval were 2.8 times more likely to experience pregnancy loss between 31 and 45 d after AI compared with those cows that did not have mastitis. In a subsequent study, the same group evaluated the correlation between subclinical mastitis and pregnancy maintenance (Moore et al., 2005). Cows were classified as experiencing subclinical mastitis when they had LSCC > 4.5 in the test day immediately prior to the AI but had no clinical signs of mastitis (Moore et al., 2005). Pregnancy was diagnosed at 28 d after AI by ultrasonography and at 35 d after AI by palpation per rectum. Cows classified as experiencing subclinical mastitis immediately prior to AI were 2.40 times more likely to lose the pregnancy between 28 and 35 d after AI compared with those cows that had LSCC ≤ 4.5. Therefore, occurrence of mastitis or sub-clinical mastitis is expected to affect reproductive performance of lactating dairy cows throughout their lactation (Figure 5).

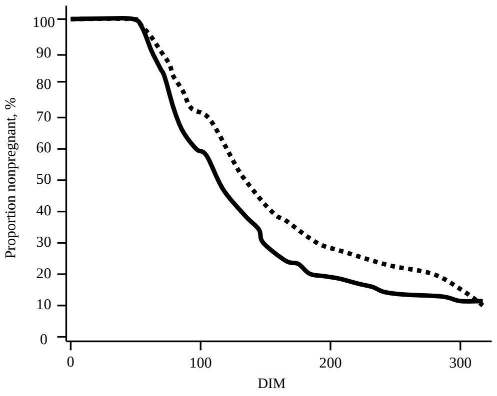


Figure 5. Association between mastitis and speed at which lactating dairy cows became pregnant. (No Mastitis – solid line, Mastitis – doted line). Adapted from Santos et al. (2004b).

Anovular Condition

Constant selection for increased milk yield has resulted in changes in the physiology and metabolism of lactating dairy cows so that energy demands for milk production are met in detriment of other physiological functions such as reproduction. Studies from the late 70's and early 80's in England and France indicated that only 5.0 to 6.5% of lactating dairy cows were anovular by approximately 50 d postpartum according to either milk progesterone concentration and/or lack of behavioral estrus (Bulman and Lamming, 1978; Martinez and Thibier, 1984). In later studies, the proportion of cows classified as anovular by two examinations of ovarian structures by palpation per rectum was 8.5% (Markusfeld, 1987), whereas in Israel the proportion of cows not observed in estrus within 60 d postpartum ranged from 17.0 to 18.6% (Francos and Mayer, 1987). More recently, studies have reported incidence of anovular condition based on sequential plasma progesterone (P4) concentrations varying from 20 to 50% (Moreira et al., 2001; Santos et al., 2004c; Chebel et al., 2006; Chebel et al., 2010). This indicates that selection for higher milk yield has resulted in larger postpartum increases in energy demands that are not fully met by feed intake making necessary body fat mobilization and suppression of other physiologic non-vital functions.

Several studies have demonstrated that early resumption of cyclicity after parturition is associated with improved reproductive performance (Thatcher and Wilcox, 1973; Darwash et al., 1997). Earlier resumption of normal ovarian cycles after parturition and before first postpartum AI is generally associated with improved energy balance (Beam and Butler, 1997), reduced incidence of short luteal phase (Zollers et al., 1993), and reduced incidence of sub-clinical endometritis (Galvão et al., 2009), which are likely to improve reproductive performance.

In a recent study, we demonstrated that cows that resumed cyclicity by 49 DIM became pregnant at the fastest rate compared with cows that resumed cyclicity by 62 DIM and cows that remained anovular by 62 DIM (Figure 6; Chebel and Santos, 2010). Further, the cost of the reproductive program for first AI was smallest for the cows that were cyclic by 49 DIM because a greater percentage of them was inseminated in estrus. Further, because fewer cows that were cyclic by 49 DIM were not pregnant at 305 DIM, they have the best economic outcome after 305 DIM (approximately $ 130 and $ 160 greater for cows cyclic by 49 DIM than cows cyclic by 62 DIM and anovular cows, respectively; Chebel and Santos, 2010).

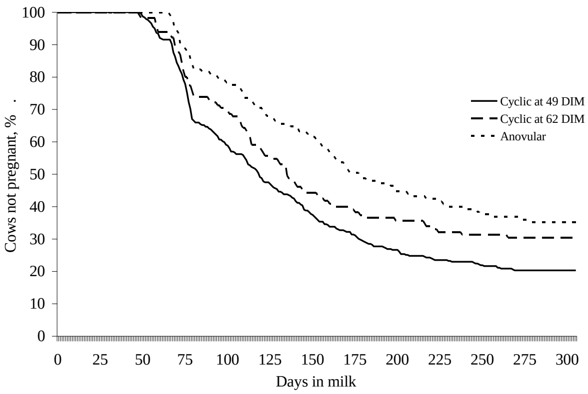


Figure 6. Effect of interval to resumption of cyclicity on the speed at which cows become pregnant (Chebel and Santos, 2010).

The average interval from parturition to resumption of ovarian cycles is approximately 25 d in Holstein cows. The resumption of ovarian cycles after parturition is associated with the interval from parturition to the nadir of negative energy balance, and in general it occurs approximately 10 d after the nadir of negative energy balance (Butler, 2000). Postpartum cows in negative energy balance have reduced LH pulsatile release, which limits the development of ovarian follicles. Therefore, follicles of postpartum cows develop in wave like pattern, but do not reach pre-ovulatory size. Further, because during states of profound negative energy balance and reduced P4 concentration, observed in cows without a CL, the hypothalamus is hyper-sensitive to a negative feed-back of E2 on GnRH secretion, which blocks ovulatory LH peaks. When cows are past the nadir of negative energy balance the concentrations of IGF-I start to increase, resulting in increased pulsatile release of LH and further growth of follicles and a change in responsiveness of the hypothalamus to the increasing E2 from negative to positive feed-back, allowing the peak of estradiol to result in a peak of LH.

Resumption of cyclicity far in advance to first postpartum AI is important because after first postpartum ovulation cows are at greater risk for short luteal phases, which may last for 6 to 8 d after ovulation. This occurs primarily because before first ovulation anovular cows are not exposed to P4 concentrations > 1 ng/ml, which results in premature down-regulation of P4 receptors in the uterus after first ovulation, allowing an earlier increase in E2 and oxytocin receptors in the endometrium, which results in early secretion of $PGF_{2\alpha}$ and luteolysis. Therefore, peripartum cows should be managed in order to minimize incidence of postpartum disease and expedite the return to positive energy balance in order to hasten the resumption of ovarian cycles.

Timed AI Protocols and Fertility

Timed AI protocols were developed in 1995 with the goal of synchronizing follicular growth, luteolysis, and ovulation. The first protocol developed was the Ovsynch, which consists of one injection of GnRH on d 0, one injection of prostaglandin $PGF_{2\alpha}$ on d 7, a second GnRH injection approximately 56 h after the $PGF_{2\alpha}$ injection and TAI at 12-16 h later (Pursley et al., 1995). The first GnRH injection synchronizes a new follicular wave, whereas the $PGF_{2\alpha}$ injection controls CL lifespan, and the last GnRH injection synchronizes ovulation. Subsequent studies demonstrated that the ideal time to initiate the Ovsynch protocol is between d 5 and 9 of the estrous cycle, because at this stage of the estrous cycle more cows ovulate in response to the first GnRH injection of the protocol (Vasconcelos et al., 1999). Later it was demonstrated that the ovulation to the first GnRH injection of the Ovsynch protocol is critical for embryo quality (Cerri et al., 2009a) and pregnancy per AI (Chebel et al., 2006) of lactating dairy cows, as cows that do not ovulate in response to the first GnRH injection have prolonged dominance period of the ovulatory follicle (Cerri et al., 2009a) and ovulate aged oocytes (Mihm et al., 1999).

Presynchronization

In an attempt to maximize the number of cows that ovulate in response to the first GnRH injection of the Ovsynch a presynchronization protocol based on two injections of $PGF_{2\alpha}$ given 14 d apart (Presynch) was developed (Moreira et al., 2001). In this study, cows submitted to the Ovsynch 12 d after receiving the Presynch had P/AI approximately 12 percentual points greater than those not presynchronized (Moreira et al., 2001). By giving 2 injections of $PGF_{2\alpha}$ 14 d apart the proportion of cows that display estrus from 2 to 6 d after the second injection is over 65% (Chebel et al., 2006). Therefore, by starting the Ovsynch protocol 10 to 12 d after the last $PGF_{2\alpha}$ injection the majority of cows would be between d 4 and 10 of the estrous cycle.

In an attempt to simplify the Presynch-Ovsynch protocol by giving most injections on the same of the week, Navanukraw et al. (2004) compared the Ovsynch protocol alone with a Presynch-Ovsynch with the PGF2α injections of the Presynch given 14 d apart and the last PGF2α injection given14 d before the start of the Ovsynch. In this study, cows receiving the Presynch-Ovsynch (14-14) had greater P/AI than cows receiving the Ovsynch alone.

Galvão et al. (2007a) compared the Presynch-Ovsynch (14-14) to a Presynch-Ovsynch in which the interval between the last PGF2α injection of the Presynch and the start of the Ovsynch was 11 d. In this study, cows receiving the 14-11 Presynch had P/AI 6 percentual points higher than cows receiving the 14-14 Presynch. The main reason for this improvement in fertility was the increased percentage of cows that ovulated in response to the first GnRH injection of the Ovsynch protocol when the interval between the last PGF2α injection of the Presynch and the Ovsynch was 11 d (Galvão et al., 2007a).

Therefore, the recommended protocol for first postpartum AI for herds with good estrous detection rate is the Presynch-Ovsynch, with the interval between the last PGF2α injection of the Presynch and the start of the Ovsynch of 10 to 12 d.

Resynchronization

Most researchers agree that resynchronization protocols of non-pregnant cows have to be optimized. The resynchronization protocols most commonly used in western U.S. states are to treat non-pregnant cows with $PGF_{2\alpha}$ if they have a CL at the time of non-pregnancy diagnosis and inseminate at detected estrus and to initiate a timed AI protocol in cows not bearing a CL, or to initiate the timed AI protocol in all cows at non-pregnancy diagnosis regardless of ovarian structures. Because accuracy of diagnosis of CL by manual palpation is only 60% and estrous detection accuracy is poor in most dairy operations, the use of TAI protocols for resynchronization has grown. However, little control of the estrous cycle of non-pregnant cows prior to the start of the resynchronization protocol has been possible.

Although the indicated label use of CIDR inserts is to improve return to estrus when used from 14 to 21 d after initial AI, the use of CIDR inserts as a resynchronization tool has proven to be inefficient, as the interval to re-insemination and proportion of cows re-inseminated prior to pregnancy diagnosis is not improved (Chebel et al., 2006; Galvão et al., 2007b).

Recently (Silva et al., 2007), non-pregnant cows were either resynchronized with the Ovsynch protocol at non-pregnant diagnosis (CON) or with an injection of $PGF_{2\alpha}$ 2 d after non-pregnant diagnosis followed 12 later with the Ovsynch protocol (POVS). Cows in the POVS group had ($P = 0.01$) greater P/AI than CON cows at 66 d after re-insemination (35.2 vs. 25.6%). Although there was no ($P = 0.49$) difference in the proportion of cows ovulating in response to the first GnRH injection of the Ovsynch (CON = 49.3, POVS = 53.9%), the number of cows may have been too small to determine this difference statistically significant. Nonetheless, the extended inter-AI interval observed in the POVS group could negate the benefits of improved P/AI in this protocol.

Our laboratory has recently conducted several clinical trials evaluating different resynchronization protocols. In the most recent study (Dewey et al., 2010), cows at 31±3 d after initial AI were randomly selected to receive one of three resynchronization protocols: CON = Cosynch72 starting at non-pregnancy diagnosis; CIDR = Cosynch72+CIDR for 7 d starting at non-pregnancy diagnosis; or G7G = GnRH injection at enrollment and start the Ovsynch at non-pregnancy diagnosis. All cows were examined for pregnancy 7 d after enrollment, at 38±3 d after initial AI. Throughout this study, cows that were observed in estrus were re-inseminated on the same day. Among cows re-inseminated at fixed (after the completion of the resynchronization protocol), cows in the G7G and CIDR treatment had ($P < 0.05$) the greatest P/AI (CON = 22.1, G7G = 31.2%, CIDR = 29.5).

When we evaluated the data from all cows enrolled in the study in the CA site, including those re-inseminated in estrus, the differences in overall P/AI after re-insemination were smaller (Mendonça et al., 2011b). That was mainly because the presynchronizing GnRH injection given to G7G cows and the treatment with CIDR during the resynchronization protocol, reduced the percentage of G7G cows and CIDR cows that were re-inseminated in estrus (Figure 7). Among control cows the P/AI of those re-inseminated in estrus was significantly better than that of cows re-inseminated at fixed time, which increased the overall P/AI.

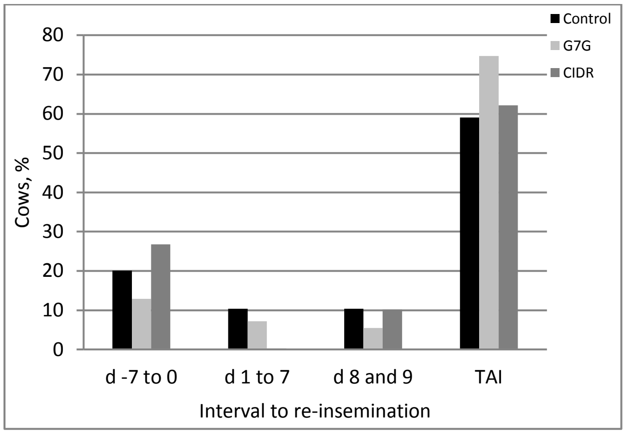


Figure 7. Percentage of cows re-inseminated according to interval to start of the resynchronization (day 0).

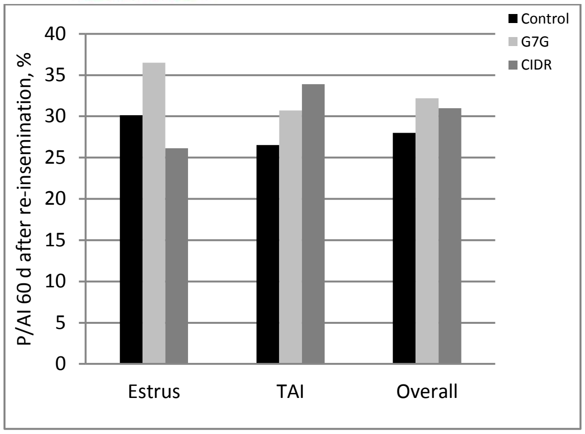


Figure 8. Pregnancy per AI according to re-insemination procedure.

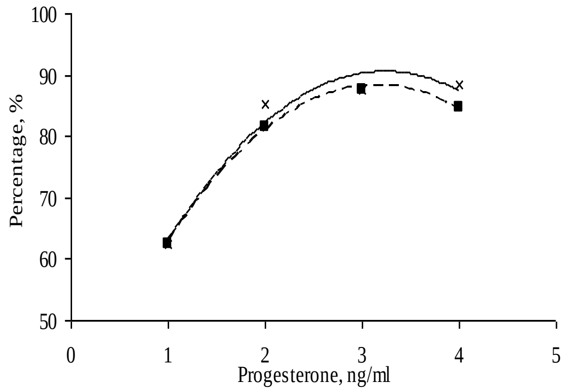
Reducing the Period of Dominance of the Ovulatory Follicle (5d-Cosynch)

Because it has become clear that the length of dominance of the ovulatory follicle is extended in lactating dairy cows because of the reduced circulating concentrations of estradiol during the proestrus, and this extended period of dominance compromises embryo quality and fertility (Cerri et al., 2009a), we have tested the hypothesis that reducing the interval from the first GnRH injection of the timed AI protocol to the time of insemination would improve fertility. To achieve this reduction in interval from the first GnRH injection to AI we would have to treat cows with $PGF_{2\alpha}$ 5 d after the GnRH injection, which could result in suboptimal luteolysis and compromised fertility. Therefore, in a pilot study, we compared the proportion of cows that experienced luteolysis when $PGF_{2\alpha}$ was given on d 7 (COS72; GnRH on d 0, $PGF_{2\alpha}$ on d 7, and GnRH+TAI on d 10), on d 5 (COS5d1; GnRH on d 0, $PGF_{2\alpha}$ on d 5, and GnRH+TAI on d 8), or on d 5 and 6 (COS5d2; GnRH on d 0, $PGF_{2\alpha}$ on d 5 and 6, and GnRH+TAI on d 8) after the first GnRH injection. As expected the proportion of cows that experienced luteolysis was ($P < 0.01$) smallest for those receiving one injection of $PGF_{2\alpha}$ on d 5 after the GnRH (COS72 = 79.0, COS5d1 = 59.1, COS5d2 = 95.7%; Santos et al., 2010).

In a subsequent study, 933 cows were submitted to the Presynch and 12 d later to either the COS72 or the COS5d2 (Santos et al., 2010). Cows receiving the COS5d2 had ($P < 0.01$) smaller ovulatory follicles compared with COS72 cows (18.4 ± 0.3 and 16.8 ± 0.3 mm), but progesterone (P4) concentration 7 d after TAI was not ($P = 0.18$) different (CoS72 = 2.32 ± 0.18, CoS5d2 = 1.98 ± 0.20 ng/mL). Finally, pregnancy per AI was ($P < 0.05$) greater for COS5d2 cows at 38 (39.3 and 33.9%) and 66 (36.7 and 32.5%) d after AI, indicating that by reducing the dominance period of the ovulatory follicle fertility could be significantly improved (Santos et al., 2010).

Low Progesterone Concentration and Fertility

The start of the timed AI protocols at 5 to 10 d after is not only important to maximize the percentage of cows that ovulate to the first GnRH injection of the protocol and to assure that synchronized luteolysis will occur, but also to assure that ovulatory follicles grow under elevated P4 concentrations. As mentioned above, reduced concentrations of P4 before ovulation may result in increased exposure of follicles to pulsatile release of LH, which causes premature oocyte maturation and reduced embryo quality (Calder et al., 2000; Inskeep, 2004). In recent studies conducted by my laboratory, we demonstrated that growth of ovulatory follicles under P4 concentration < 2 ng/ml results in compromised embryo quality and reduced P/AI (Figure 7 and 8).



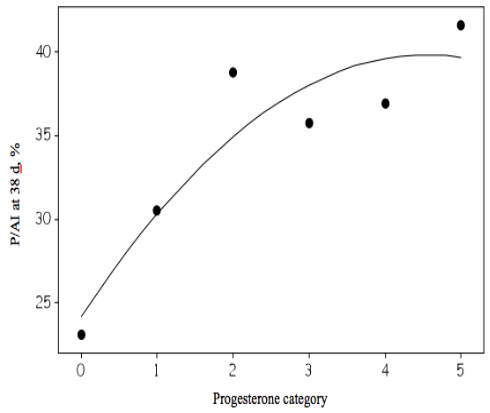


Figure 9. Percentage of cows producing excellent/good and fairy quality embryos (solid line) and percentage of cows producing excellent/good quality embryos (doted line). Adapted from Rivera et al. (2010).

Figure 10. Percentage of cows conceiving after first postpartum AI. Adapted from Denicol et al. (2009).

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