Characterization of Early Embryonic Death and Prevention of Pregnancy Wastage

W.W. Thatcher[1]**; J.E.P. Santos**[2]
[1]*Department of Animal Sciences, University of Florida, Gainesville, FL 32611-0910*
[2]*Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA 93274*

Abstract

In lactating dairy cows, fertilization rates of 88% were comparable to nonlactating cows when estimated at day 5; however, embryo quality was significantly less. Measurements of both progesterone and Pregnancy Specific Protein B coupled with rectal palpation indicate that rates of Early Embryonic Mortality (EEM) and Late Embryonic Mortality (LEM) are 32 and 15%, respectively in lactating cows. Estimates of pregnancy losses between ~31 and 45 days are ~ 14% for timed insemination and do not appear to be different from cows inseminated at detected estrus. Several physiological windows are critical to achieve good pregnancy rates. Follicular development in the periovulatory period prior to insemination, luteal phase progesterone concentrations both prior to and after insemination, as well as number of follicular waves, have effects on pregnancy rates. Reproductive management programs will be discussed that attempt to optimize these critical windows in a manner that increases pregnancy rates. With our understanding of the dialogue between the developing embryo and the maternal reproductive tract, pharmaceutical and nutritional strategies also have increased pregnancy rates. For example, injection of bovine somatotrophin at the time of insemination increases pregnancy rates in lactating dairy cows. The lactational state in modern day dairy cows contributes to a subfertile condition that the practitioner needs to manage based upon a thorough understanding of the underlying physiological causes. These limitations are partially amenable to improvement as part of a production medicine program.

Introduction

In high producing dairy cows, herd pregnancy rates are reduced due to poor estrus expression and/or detection, anestrus, low conception rates and increased embryo mortality. Furthermore, these impediments to optimal reproductive performance are exacerbated under stressful environmental conditions such as heat stress, which is even more detrimental in higher milk producing cows. Reproductive performance has decreased in North America, Europe, Israel and Australia. Reasons for the decline are multi-factorial and not entirely associated with an increase in milk production.[20] Epidemiological studies indicate that other factors such as reproductive diseases (i.e., retained placenta, metritis and ovarian cysts) or season of calving were relatively more important than milk yield on reproductive performance.[15,20] In fact, higher producing herds may have a greater reproductive performance because of better feeding, reproductive management and healthier cows. Nevertheless, the physiological state of lactation is associated with a lower reproductive rate compared to heifers.[4] The challenge to characterize the factors compromising embryo development and developing strategies to improve embryo survival is complex, involving steroidogenesis, cell proliferation, follicle development, ovulation, fertilization, corpus luteum development and maintenance, oviductal and uterine functions, embryo development and function, implantation and subsequent fetal growth. Indeed our current day production and reproductive management systems impact on all of these coordinated events which need to be optimized if reproductive efficiency in lactating dairy cows is to be enhanced. Objectives of this presentation are to characterize embryo development and losses, to identify physiological windows that may be impaired and associated with embryo wastage, and to identify strategies to improve pregnancy rates.

Embryo Development and Losses

Estimates of fertilization rates in dairy heifers fall within a range of 97-100%. Estimates in dairy cows are more variable within a range of 85 to 100%, but these estimates were determined over 25 years ago, and it was not always clear whether the cows were lactating or not, and if so, what was the level of production.[43] During the early cleavage stages from 1-cell through to the early blastocyst stage, at day 8, the embryo stays within the zona pellucida.[43] Between 3 and 4 days after fertilization, the embryo migrates from the oviduct to the uterus at the 8-16 cell stage. At 5 to 6 days (16-32 cell stage), the embryo undergoes compaction forming cell to cell contacts, development of tight junctions and begins to function as an organism designated as the morula. At 8 days of age, the blastocyst develops the blastocoel cavity and cells (~120 cells) associated with the inner cell mass (25%) and trophoectderm (75%). At approximately 9 to 10 days (~160 cells), the blastocyst hatches from the zona pellucida and undergoes expansion until it begins to elongate at approximately 13 days of age. Elongation represents a transition in appearance from spherical to ovoid to filamentous, with embryo length increasing from 5.25 mm at day 13 to 52 mm on day 16. On day 17, it is not uncommon to see embryos plus extraembryonic membranes with lengths of 30-40 cm that occupy the major part of the uterine horn ipsilateral to the CL. However, there is considerable variation in size of embryos. Early attachment of the conceptus occurs at day 19 with visible caruncular-cotyledonary attachment points faintly visible on day 21. At day 42, the embryonic period ends with completion of differentiation. The embryo is now called a fetus in which the major tissues, systems and organs are formed.

Sreenan *et al*,[43] summarized the literature for staged estimates of embryo losses from the earlier literature (i.e., > 21 years ago). Fertilization rates were estimated to be 90%, and average calving rates about 55%. This suggests an embryonic and fetal mortality rate of about 39%. Very few embryos are lost in the days immediately after fertilization and up to day 8 of gestation. A significant increment of the total losses (27 -31%) occurred between days 8 and 16 after insemination, 3.8% of the total occurred between days 16 and 42, and a further 1.9 to 3.1% occurred between day 42 and parturition. A major question is whether the temporal pattern of embryonic and fetal losses has changed in the current populations of high producing lactating Holstein dairy cows.

Several recent reports have evaluated fertilization rates in modern day high producing dairy cows whose overall fertility is low. In studies involving the use of the 6-day old bovine embryo (i.e. morula) as a biomonitor, it was demonstrated in non-lactating dairy cows that fertilization rates were 66, 74 and 82% when inseminations were made at 0, 12 and 24 h after onset of estrus, respectively.[12] The percentages of excellent to good embryos were 77, 52 and 47% for the 0, 12 and 24 h time periods. Consequently, AI 12 h after onset of estrus is the recommended time for insemination, which is a compromise between a potentially lower fertilization rate at 0 h AI and lowered embryo quality with AI at 24 h. A recent report[38] vividly demonstrates the negative impact of lactation on early embryo development compared to nonlactating dairy cows. In a temperate environment, fertilization rates, estimated at day 5 after ovulation, were 87.8 and 89.5% for lactating and nonlactating cows, respectively. However, day 5 embryos from lactating dairy cows were detectably inferior (lower quality embryo score, and lower percentage of excellent-good-fair embryos [52.8%] compared to embryos from nonlactating cows (82.3%).

Losses beyond the period of hatching from the zona are more difficult to determine relative to percent recovery and determining well-being of the embryo. From a practical perspective, we can further divide embryonic losses (EL) into early (EEL) and late (LEL) embryonic losses that can be monitored with such techniques as milk progesterone (P_4), pregnancy specific protein B (PSPB; a protein secreted by the binucleate cells of the trophoectoderm), ultrasound (US) and rectal palpation. Removal of the embryo on day 17 causes an extension of the CL, and intrauterine infusion of recombinant bovine IFN-τ (i.e., protein secreted by trophectoderm that inhibits luteolytic secretion of $PGF_{2\alpha}$) into cyclic cows extends CL lifespan until day 28 and the interestrus interval until day 31.[45] Humblot[17] proposed that luteolysis within 24 days after AI could be associated with either a lack of fertilization or to early embryonic mortality (EEM) that did not allow the CL to be maintained. In contrast, extended CL maintenance and return to estrus after 24 days could be associated with late embryonic mortality (LEM) occurring at or more than 16 days after AI. With this rationale, cows were classified pregnant (i.e., milk P_4 was <3.5 ng/ml on day 0 and >5 ng/ml on days 21 to 24, PSPB detectable on day 35, absence of second service and/or pregnant at rectal palpation later on); EEM or no fertilization (i.e., P_4 <5 ng/ml on days 21 to 24, second service or nonpregnant at rectal palpation) and LEM (i.e., P_4 was <3.5 ng/ml on day 0 and >5 ng/ml on Days 21 to 24, nonpregnant based on PSPB assay on day 35 or later rectal palpation). In a study involving 44 dairy herds (1395 Holstein cows) of low fertility in a temperate environment of France, pregnancy rate, EEM and LEM rates after first AI were 42.9 (599/1395), 31.6 (441/1395) and 14.7% (209/1395), respectively.[17] Several factors were associated with pregnancy rate (interval to first AI: >90 day, 46.6% vs < 90 days, 41.5%; parity: primiparous, 47.5% vs lactation 2+3, 42.7% vs > 4 lactations, 34.6%; milk yield: ≥ 85.8 lb [39 kg]/d, 34.9%, < 85.8 lb [39 kg]/d, 45.5%). Interestingly, LEM was greater in high producing dairy cows with BCS ≥ 2.5, but there was no association with BCS in lower producing cows.

Table 1 shows numerous studies that have used US for early pregnancy diagnoses followed by a subsequent confirmation such that pregnancy losses can be estimated between ~28 and ~45 days after AI. Losses between these two points are estimated to be between 10 to 20% (Table 1). It has been suggested that use of TAI programs may have increased LEL.[18,20] Chebel *et*

Table 1. Rate of late embryonic loss between days 27 and 45 of pregnancy.

Reference	Number of cows	Day of first diagnosis	Day of second diagnosis	Pregnancy Loss %
8	253	28	38-58	25.7
9	176	31	45	9.7
10	195	28	42	17.9
11	1,503	31	45	13.2
19	167	28	39	11.4
28	139	27	45	20.7
34	220	27	41	10.0
35	360	31	45	11.1

al[11] studied factors involved in losses of pregnancy between 31 and 45 days after AI on three commercial dairy farms in California. Cows inseminated either following estrus detection or at fixed time with an OvSynch protocol had similar losses of pregnancy (13.7 vs 11.7%). Estimates in two other studies,[9,35] involving AI following estrus detection or TAI with Heatsynch or OvSynch protocols, reported that pregnancy losses between 31 and 45 days did not differ. Controlled breeding programs that are properly implemented do not seem to affect pregnancy losses when compared to insemination following estrus detection. This does not mean that induction of ovulations in follicles that are too small does not reduce pregnancy rates, or that the current systems cannot be improved. However, disproportional losses attributed to TAI do not seem warranted.

In a study in Ireland,[42] pregnancy losses between 28 and 84 d after AI were similar for grazing lactating cows producing 15,943 lb (7247 kg) of milk/year compared to heifers (7.2 vs 6.1%, respectively). Of the losses detected, 47.5% occurred between days 28 and 42 of gestation. It is interesting that in this production system of grazing and lower absolute milk production, that embryonic losses are considerably lower than reported above in intensely managed dairy herds. The extent of embryonic loss was greater in cows that lost body condition between days 28 and 56 of pregnancy compared to cows maintaining or gaining in body condition.

Physiological Windows That May Be Impaired and Contribute To Pregnancy Losses

In current production systems, it is imperative to recognize the multiplicity of factors that may influence reproductive performance.

Cycle prior to insemination and periovulatory period. Several reports indicate that low plasma progesterone concentrations during the luteal phase of the estrous cycle preceding AI was associated with lower fertility than high plasma progesterone concentrations. Progesterone concentrations can influence several physiological events such as ovarian follicular dynamics and subsequent uterine function. Sangsritavong *et al*,[33] demonstrated in classical experiments that liver blood flow was elevated following feeding in nonlactating and lactating dairy cows. Both progesterone and estradiol metabolism were acutely elevated with feed consumption. Higher rates of liver blood flow and steroid metabolism in lactating dairy cows may reflect the chronic effects of higher feed intakes leading to lower steroid concentrations. The lower concentrations of progesterone and estradiol in lactating dairy cows compared to nonlactating dairy cows[13,37] appears to influence ovarian follicular dynamics. Evidence across these reports support the concept that lactating dairy cows have a larger number of larger follicles, a larger ovulatory follicle, lower concentrations of estradiol and a longer interval to ovulation. Of critical importance is the subsequent ability of the oocyte arising from such follicles to form normal embryos. Sartori[38] demonstrated that day 5 embryo quality was reduced in lactating dairy cows. Possible greater clearance of estradiol in high producing lactating dairy cows may result in less inhibition of FSH secretion that would alter follicular deviation, leading to a greater occurrence of double ovulations.[48] Furthermore, lower concentrations of progesterone may influence LH pulsatility and lead to longer persistence of the dominant follicle.

Israeli investigators[41] established an experimental model to induce CL that secreted ascending concentrations of progesterone that were substantially lower than control cows. During the next cycle when progesterone concentrations were normal, both groups were challenged with oxytocin to evaluate $PGF_{2\alpha}$ secretion (i.e., 13, 14, dihydro-15keto $PGF_{2\alpha}$ [PGFM; metabolite of $PGF_{2\alpha}$]) on day 15 of the cycle. The PGFM response in the low progesterone group was markedly higher. One interpretation of these findings is that low progesterone concentrations during an estrous cycle may have a delayed stimulatory effect on uterine responsiveness to oxytocin during the late luteal phase of the subsequent cycle that approaches the time when the embryo initiates maintenance of the CL. A series of experiments in beef cattle indicate that shortened CL lifespan associated with first ovulation is associated with premature uterine secretion of $PGF_{2\alpha}$ that is also embryo toxic to the developing embryo when in the uterus between days 5 to 8.[18] Oxytocin secretion from the CL may be contributing to this phenomenon. However, treatment of these cows with a progestin during the synchronization period eliminates this problem. Whether such a phenomenon is contributing to early embryo death in lactating dairy cows that have anovulatory follicle turnover before induction of first ovulation warrants investigation. It is clear that the preovulatory/periovulatory changes in steroids regulate subsequent estradiol and progesterone receptor concentrations that influence subsequent functions of the oviduct, uterus and ovary.

An additional factor associated with the preovulatory period that reduces fertility is the development of persistent follicles. Again this is coupled with the lower concentrations of progesterone, which predisposes the cow to a higher LH pulse frequency and maintenance of the dominant follicle. When a persistent follicle ovulates, the oocyte is at a later stage of maturation.[24] The oocyte undergoes fertilization but early embryonic death occurs. It is important to recognize this phenomenon when we deal with types of synchronization systems. We need to use synchronization and/or ovulatory control systems that do not entail long periods of low progesterone exposure and promote induction of a new dominant follicle that is induced to ovulate before expressing dominance beyond a 5-day period. Period of follicular dominance longer than 8 days is associated with reduced fertility.[28] Normally cows have two or three wave cycles. A recent report[47] indicated that fertility was greater in lactating cows inseminated after ovulation of the third-wave follicle that had developed for fewer days of the estrus cycle (81% pregnancy rate) as compared with two-wave cows (63% pregnancy rate). The longer developed ovulatory follicle of the second wave group should not be considered a persistent follicle, but it does emphasize the potential importance of recruiting a fresh follicle as part of a synchronization or timed-insemination program.

Post ovulatory cycle. Elevation of progesterone soon after ovulation may advance maturation of the uterine endometrium and accelerate growth of the developing embryo.[5] Indeed size of the ovulatory follicle in dairy heifers was positively related to subsequent increases in plasma progesterone concentrations.[27] A very interesting association was shown among lactational statuses between ovulatory follicle volume, subsequent CL volume on day 7 of the cycle, and plasma progesterone concentration on day 7.[37] Basically a clear positive association existed between ovulatory follicle and CL volume that appeared to be similar between heifers, dry cows and lactating cows. However, due to lactation state the ng/ml increase in progesterone concentration per mm^3 volume of the CL was 2x greater at day 7 for dairy heifers, and 1.38 times greater for dry cows compared to lactating dairy cows. It is clear that lactation is reducing luteal phase progesterone concentrations. The reduced postovulatory concentrations in progesterone of lactating dairy cows may reduce embryo development[21] and thereby reduce interferon τ production by the subsequent filamentous embryo that would contribute to both EEL and LEL.

In beef cattle, approximately equal numbers of animals had two waves or three waves of follicular development during the equivalent period of one estrous cycle after insemination.[1] However, fertility was greater for animals with three waves (96% > 70%, $P < 0.05$). At the time of pregnancy recognition (i.e., day 17 after estrus), cows in the third follicle wave would have an atretic second wave dominant follicle and a newly recruited third wave follicle that was approximately 7mm and not yet capable of secreting appreciable estradiol. In contrast, cows with a two follicular wave cycle would have a 10-12 mm second wave dominant follicle that would be capable of producing more estradiol. No differences were detected in estradiol concentrations in the peripheral circulation. However, this does not preclude the possibility of differences in estradiol concentrations between the two follicular statuses at the localized ovarian-uterine tissue level close to the site of production that may have differential affects on the luteolytic mechanism within the uterus. Hernandez-Ceron and Morales[16] reported that lactating dairy cows with a follicle < 15 mm between 12 to 14 days after insemination had a higher conception rate (49.7%, 106/211) than cows with follicles ≥15 mm (37.1%, 78/124).

An additional factor influencing both EEL and/or LEL is the occurrence of mastitis. Cows that had clinical mastitis during the first 45 d of gestation were 2.7 times at higher risk of abortion within the next 90 d.[32] Both days open and services per pregnancy were increased in cows with clinical mastitis that occurred between first service and establishment of pregnancy.[40]

Strategies to Improve Pregnancy Rates

Development and Optimization of Timed Insemination Programs. Effective estrus synchronization programs provide a number of advantages: cows or heifers are in estrus at a predicted time which facilitates AI, and embryo transfer; time and labor expense for detection of estrus are reduced; AI becomes more practical under extensive conditions; and precise control of ovulation permits a timed insemination without the need for detection of estrus.

The ability to control the time of ovulation precisely permits a timed insemination, following a period in which follicular development and CL regression have been programmed sequentially. With the implementation of fixed timed inseminations, specific timed treatments to improve embryo survival can be implemented effectively and strategies implemented to promptly program re-synchronization of non-pregnant cows for timed insemination. Such programs are essential in high producing dairy cows that experience a reduction in estrus intensity that contributes to undetected heats, re-occurring luteal phases without estrus expression, or re-occurring waves of follicles that fail to ovulate. Development of timed insemination programs has been based upon a thorough understanding of the factors controlling ovarian follicular growth.

OvSynch. One program that has been extremely successful for insemination of cows at a fixed time for first service without the need for detection of estrus is the Ovynch program in which injections of GnRH are given 7 d before and 48 h after an injection of $PGF_{2\alpha}$, and cows are inseminated 12 to 16 h after the second injection of GnRH. This system synchronizes follicle maturation with regression of the corpus luteum before the GnRH-induced ovulation and timed insemination. Numerous studies indicate that pregnancy rates (proportion of all treated cows that were pregnant) to the OvSynch program were comparable and in some studies greater than the appropriate control group.[46]

There are several stages of the estrous cycle when initiation of the OvSynch program causes reduced pregnancy rates. Initiation of the program between days 13 to 17 of the cycle is a time in which spontaneous regression of the CL occurs prior to the time that $PGF_{2\alpha}$ is injected at 7 days after the injection of GnRH. These cows will be asynchronized in that they may ovulate prior to the time of insemination and insemination will be to late for the cow to conceive. During the early stages of the cycle (e.g., days 2 to 4), the recruited dominant follicle is not sufficiently developed to ovulate in response to GnRH. As a consequence, the dominant follicle at the second injection of GnRH is considered aged and has expressed dominance for 5 days or longer. Follicles that have periods of dominance longer than 5 days are less fertile, and some of these aged follicles fail to ovulate in response to the ovulatory injection of GnRH. An additional point to consider is that GnRH-induced turnover of follicles or induction of a new follicular wave is most efficient if ovulation is induced in response to the first injection of GnRH. Collectively, these findings indicate that presynchronization of cows prior to implementation of the OvSynch program should improve pregnancy rates if cows enter the OvSynch program at the most favorable period of the estrous cycle (i.e., days 5 to 12 of the cycle).

Presynch-OvSynch. A program defined as Presynch-OvSynch was developed in which pre-synchronization is achieved with a standard estrous synchronization protocol ($PGF_{2\alpha}$ given twice at a 14-day interval) with the OvSynch program initiated 12 days after the second injection of $PGF_{2\alpha}$.[28] A Presynch-OvSynch program increased pregnancy rates 18 percentage units (i.e., 25% to 43%;) in lactating cyclic cows.[28] This stimulation in pregnancy rates was attributed to manipulation of the estrous cycle such that the OvSynch, timed insemination program was initiated at the most favorable stages of the estrous cycle. Future programs for further optimization of fertility likely will consider programs that manipulate ovarian function such that follicular turnover via ovulation or induced follicular atresia occurs in all cows, and luteal phase-like progesterone concentrations are sustained until the time of induced CL regression. These future systems in lactating dairy cows may include insertion of intravaginal devices containing progesterone and acute injections of estrogens. Success of the OvSynch program is dependent on whether lactating dairy cows are anestrus or cycling. Pregnancy rates were less in cows that were not cycling at the time the OvSynch program was initiated (e.g., 22% versus 42%). Overall, the TAI protocol was able to induce cycles in 75% of anestrous cows, based upon the number of anestrous cows which were classified as ovulating to either the first and/or second injection of GnRH. If anestrous cows ovulate to the first and second GnRH injections of the OvSynch program, then pregnancy rates appeared to be normal (e.g., 39%).

Heatsynch. An alternative strategy to control the time of ovulation is the ability of exogenous estradiol to induce a LH surge by stimulating hypothalamic secretion of GnRH when given in a low progesterone environment during late diestrus and proestrus. An estradiol induced LH surge lasts for approximately 10 h, which is comparable to a spontaneous LH surge and longer than the LH surge induced by GnRH. Estradiol cypionate (ECP), an esterified form of estradiol-17β that is available commercially for use in cattle, has been used as part of a timed insemination program in lactating dairy cows. The ECP is used to replace the second GnRH injection of an OvSynch program and is called Heatsynch.[30,46] Cows are pre-synchronized with two injections of $PGF_{2\alpha}$ given 14 d apart with Heatsynch beginning 14 d after the second injection of $PGF_{2\alpha}$. Cows are then injected with GnRH followed by $PGF_{2\alpha}$ 7d later. The ECP (1 mg, IM) is injected 24 h after $PGF_{2\alpha}$, and cows are inseminated 48 h later. Pregnancy rates did not differ between Heatsynch and OvSynch programs.

In lactating dairy cows, the frequencies of detected estrus and ovulation after ECP were 75.7% and 86.5%, respectively.[30] Estrus occurred at 29.0 ± 1.8 h (n=28) after ECP. Mean intervals to ovulation were 55.4 ± 2.7 h after ECP and 27.5 ± 1.1 h after onset of estrus. Since 75% of the ovulations occurred between ≥48 h to ≤72 h after ECP, it is recommended that any cow detected in estrus by 1.5 days after ECP injection be inseminated at detected estrus, and all remaining cows be inseminated at 48 h. Based on synchronization of ovulation and pregnancy rates, ECP can be utilized as an alternative to induce ovulation in place of GnRH for a timed insemination. Since lactating dairy cows have reduced concentrations of plasma estradiol in the preovulatory period and reduced intensity of estrus, the elevation of estradiol following ECP injection supplements for a lactational induced deficiency, and our experience indicates that cows expressing estrus are fertile. The secretion of LH is regulated directly by GnRH whereas estradiol induces LH secretion indirectly via a positive feedback stimulation of hypothalamic GnRH secretion that then stimulates LH secretion. If cows are anovulatory (e.g., anestrus or have not developed positive estradiol feedback), the Heatsynch program may not be as effective as the GnRH based OvSynch program in which GnRH causes the direct secretion of LH. Greater uterine tone, ease of insemination and occurrence of estrus with the use of the Heatsynch program are well received by inseminators. Alternatively, in facilities with concrete flooring, the reduced estrous expression associated with the OvSynch program may be preferred. Since fertility between the two programs appears to be comparable, producers have a choice, which also includes relative costs of drugs (i.e., ECP < GnRH).

Bovine Somatortopin (bST) to Improve Embryo Development and Pregnancy rates. In cycling lactating dairy cows, injection of bovine Somatotropin (500 mg Posilac, Monsanto Co, St. Louis, Missouri) at the time of the first GnRH injection or at insemination in cows on a Presynch-OvSynch program increased pregnancy rates (57% > 42.6%).[28] Since bST was effective at insemination, it is likely that bST stimulated embryonic development and survival following insemination in lactating dairy cows. There was no evidence that bST given at the ninth week of lactation is detrimental to fertility when used with a timed breeding protocol such as OvSynch. A study in Mexico reported[25] that, in cows identified as having three or more prior services, bST given at estrus and again 10 days later stimulated pregnancy rates. The ability to detect this bST beneficial effect likely is attributable to the fact that bST was given at a physiologically important window in which occurrence of estrus and or ovulation was controlled. Several studies showed that bST stimulated bovine *in vitro* maturation of oocytes and embryonic development. Furthermore, administration of bST at AI to superovulated donor cows decreased the number of unfertilized ova, increased the percentage of transferable embryos and stimulated embryonic development to the blastocyst stage. Moreover, bST affected both early embryonic development and recipient components to increase pregnancy rates following embryo transfer.[26] Both bST and IGF-1 stimulated embryo development *in vitro*.[29] Our recent studies indicate that the beneficial effect of bST may be restricted to lactating dairy cows in contrast to non-lactating dairy cows. Metabolic and physiological differences between these two physiological states appear to make lactating cows more sensitive and responsive to bST and IGF-1 to improve embryo development and survival.

HCG Induction of Accessory CL, Three Wave Cycles and Pregnancy Rate. The opportunity to regulate ovarian function after insemination to improve pregnancy rates is an additional production management strategy. The ability to induce ovulation of the healthy first wave follicle on day 5 either of the cycle or after insemination results in two altered endocrine states. The administration of hCG induces ovulation with the subsequent formation of a functional accessory CL. The majority of the increase in plasma progesterone after hCG was due to the accessory CL. Size of CL, *in vitro* production of progesterone, and plasma concentrations of progesterone were greater in accessory CL induced by hCG than GnRH.[39] Due to the induction of ovulation at day 5, cows will experience three-follicular wave cycles due to the earlier emergence of the second wave.[14] Furthermore, the third follicular wave is delayed. Development of the conceptus is related to higher concentrations of progesterone and ability of the conceptus to secrete IFN-τ. Therefore, hCG induction of an accessory CL with increased progesterone may enhance embryo survival. Since a greater number of cows conceived that had three follicular waves after insemination compared with cows having two follicular waves, hCG induction of three-wave cycles also may contribute to higher pregnancy rates.

A study was designed to determine the effects of hCG (3,300 IU IM; Chorulon, Intervet Inc., Millsboro, DE) administered on d 5 after AI on accessory CL formation, plasma progesterone concentration, conception rate and pregnancy loss in high producing Holstein dairy cows.[36] Following synchronization of estrus (GnRH followed 7 d later by $PGF_{2\alpha}$) and AI at detected estrus, 406 lactating dairy cows (94.6 ± 1.01 lb/day at the time of AI) were injected with either hCG or saline on d 5 after AI (203/treatment). Blood sampling and ultrasonography of ovaries were conducted once between days 11 and 16 after AI. Pregnancy diagnosis was performed by ultrasonography on d 28 and by rectal palpation on days 45 and 90 after AI. The study was divided into two periods: period 1 of May 14 to September 16, 1999 when daily maximum temperatures ranged from 72 to 100° F (22 to 38° C warm period), and period 2 from October 5, 1999 to March 02, 2000, when daily maximum temperatures ranged from 48 to 84° F (9 to 29° C cool period). Treatment with hCG on d 5 induced formation of one or more accessory CL in 86.2% of the hCG-treated cows compared with 23.2% in the controls. Differences in progesterone concentrations between hCG and control cows were +6.3 ng/ml for primiparous cows, and +3.1 ng/ml for multiparous cows (treatment by parity; $P<0.02$). Accessory CL increased progesterone concentration in hCG-treated cows, but not in controls. Treatment with hCG increased ($P < 0.01$) conception rates on days 28 (45.8>38.7%), 45 (40.4>36.3%), and 90 (38.4>31.9%) after AI. Pregnancy losses between days 28 and 45, 45 and 90, and 28 and 90 were similar between the two groups. Progesterone concentration and number of CL after AI affected conception rate ($P < 0.01$) such that pregnant cows had higher progesterone concentrations and a greater frequency of accessory CL. Conception rates on day 28 were 34.4 and 48.7% for cows with low and moderate BCS ($P<0.01$), and this effect was similar for control and hCG-treated cows. Similar to the results observed for BCS at the day of AI, changes in body score from AI to day 28 were also associated with changes in CR. Cows that gained BCS from AI to day 28 had higher pregnancy than those that lost or maintained BCS (47.0% vs 42.7% vs 37.4%; $P < 0.03$). Interestingly, an interaction between treatment and BCS change was determined for pregnancy at day 28. Cows that lost BCS when treated with hCG had a CR of 57.1% compared with only 24.2% for those in the control group ($P < 0.05$). When pregnancy diagnosis was considered at day 45 and day 90 after AI, the effects of treatment, number of CL, plasma progesterone concentration, BCS at the day of AI and BCS change were similar to those observed for CR at day 28 after insemination. This study supports the concept that increased progesterone during the luteal phase increases embryo survival. However, this effect was not evident in the heat stress period where early embryo losses probably precluded any subsequent increases in embryo survival. The effect of hCG to improve pregnancy rates through possible increases in early embryo development and survival in the non-heat stress season are encouraging.

Administration of a Deslorelin (GnRH agonist) implant on day 27 of pregnancy increased the formation of accessory CL and elevated progesterone concentrations, but failed to reduce the occurrence of LEL.[6] Thus, EEL appears sensitive to hCG induced increases in progesterone,[36] but developmental and implantation processes after day 27 do not appear to be affected by an implant of a GnRH agonist.[6]

Effect of Lipids on Reproductive Performance. Several experiments performed *in vivo*[22,23] and *in vitro*[22,44] indicate that the omega-3 fatty acids are able to decrease secretion of $PGF_{2\alpha}$. Feeding fish meal (8% of dry matter is oil which contains two polyunsaturated fatty acids of the n-3 family, eicosapentaenoic acid [EPA, C20:5] and docosahexaenoic acid [DHA, C22:6]) increased fertility of lactating dairy cows. Bonnette *et al*[7] fed 82 lactating, primiparous beef cows a corn silage-based diet containing either 5% fish meal or 8.7% corn gluten meal (DM basis). Diets were initiated at 25 days prior to the breeding season and continued through the 90-d breeding season. Cows were artificially inseminated and pregnancy determined at 25-30 days post breeding using ultrasonography. First service conception rate tended to be greater for cows fed fish meal (75.6 vs. 61.5%; $P = 0.14$). Serum progesterone concentrations after insemination were similar between the two groups. In a study with Holstein cows (n=141), cows were allotted to one of three dietary treatments initiated at calving.[31] Diets were isonitrogenous, isoenergetic and isolipidic. Diets contained whole flaxseed, CaLCFA, or micronized soybeans. Flaxseeds are ~32% oil, 57% C18:3, 14% C18:2, and 18% C18:1. The diameter of the CL for cows fed flaxseed was larger than that of cows fed soybeans (19.7 vs.16.9 mm), but not larger than that of cows fed CaLCFA (17.5 mm). Embryo mortality from day 30 to 50 after AI tended to be lower ($P < 0.11$) when cows were fed flaxseed (0%) compared to CalCFA (15.4%) or soybeans (13.6%). More recently, Ambrose *et al*[2] assigned lactating Holstein cows (n=121) to diets supplemented with either rolled flaxseed (55% of lipid content is a-linolenic acid) or rolled sunflower seed (<1% of lipid content is a-linolenic acid), to provide approximately 750 g oil/cow/day. Diets were similar in metabolizable protein and net energy of lactation. The treatment diets were started 4 weeks prior to insemination following a Presynch/OvSynch protocol. Pregnancy diagnosis was conducted at 32 days after AI. Pregnancy rate to first AI at 32 d was higher in cows fed flax seed compared to cows fed sunflower seed (48.4% > 32.2%). Collectively, these results are encouraging in that selective nutrients (i.e., omega 3 fatty acids such as EPA, DHA, and a-linolenic acids) appear to improve pregnancy rates. These fatty acids are capable of decreasing the secretion of $PGF_{2\alpha}$ and compliment the antiluteolytic action of interferon-τ. EPA and DHA are known to have distinct anti-inflammatory and immunosuppressive effects that compliment the normal immunosuppressive and anti-inflammatory effects of progesterone and interferon-τ in early pregnancy. It would be interesting to determine if cows fed anti-inflammatory lipid diets would reduce the incidence of EEM and LEM associated with the occurrence of mastitis.

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