COMPARISON OF REPRODUCTIVE PERFORMANCE AND COST OF NATURAL SERVICE AND TIMED ARTIFICIAL INSEMINATION IN LACTATING DAIRY COWS IN FLORIDA

By

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To Gaspar and Dolores, my brother and sisters, and God that unconditionally supported me under all circumstances and provided me all the strength necessary to keep my focus to accomplish my goals and happily conquer this dream

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LIST OF ABBREVIATIONS

AI	Artificial insemination
BCS	Body condition score
BSE	Breeding soundness evaluation
CL	Corpus luteum
CR	Conception rates
DNA	Deoxyribonucleic acid
E_2	Estradiol
GnRH	Gonodorelin release hormone
LH	Luteinizing hormone
mRNA	Messenger ribonucleic acid
NAHMS	National animal health monitoring system
NRR	Non-return rates
NS	Natural service
P4	Progesterone
PGE_2	Prostaglandin E 2
$PGF_{2\alpha}$	Prostaglandin F-2 alpha
US	Ultrasonography examination
U.S.A.	United States of America
VWP	Voluntary waiting period

Abstract of Thesis Presented to the Graduate School
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By

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Objectives were to compare reproductive performance, cost and profitability of lactating dairy cows bred by natural service and timed AI. One thousand and fifty-five cows were blocked by parity and randomly enrolled to receive either NS or TAI. Cows in both groups were pre-synchronized with 2 injections of $PGF_{2\alpha}$, given 14 d apart. TAI group receveid an Ovsynch protocol and were re-synchronized with CIDR inserted 18 d after TAI and removed 7 d later, when GnRH was given. Cows were examined by US on d 32 after TAI; non-pregnant cows received $PGF_{2\alpha}$ and GnRH 56 h later followed by TAI 16 h after the GnRH injection. Non-pregnant cows in TAI group were re-inseminated up to 5 times using the same scheme. Cows in the NS group were exposed to bulls 14 d after the end of the pre-synch protocol and US was performed 42 d after exposure to bulls. Non-pregnant cows in the NS group were re-examined by US every 28 d until diagnosed pregnant or 223 d post partum (pp), whichever occurred first. Cows diagnosed pregnant in TAI or NS were re-confirmed 28 d later to determine pregnancy loss. All bulls underwent a BSE and were rested for 14 d after 14 d of cow exposure. Health disorders, BCS and cyclic status were evaluated. An economical model was created using

Microsoft Excel 2007 (Microsoft® Corporation, Redmond, WA, U.S.A.) to account for all costs, return and opportunity cost involving NS and TAI. The proportion of pregnant and 21-d cycle pregnancy rate cows in the first 21 d of breeding did not differ between groups. The daily rate of pregnancy was 15% greater for NS than TAI because cows in NS had a greater pregnancy rate, which resulted in fewer median d open (111 vs. 116 d). Proportion of pregnant cows at 223 d postpartum was greater in the NS than TAI group (84.2% vs. 74.8%, respectively). The daily rate of pregnancy tended to increase with a concurrent increase in milk yield. Cows with BCS ≥ 2.75 cows had greater proportion of pregnant cows in the first 21 d of breeding and daily pregnancy rate in the first 223 d post partum Primiparous cows had greater proportion of pregnant cows and daily pregnancy rate than multiparous cows at 223 d post partum. Direct net cost of the NS and TAI program during the trial were $100.53/cow/yr and $61.84/cow/yr, respectively. Costs per eligible day were estimated $1.44 for NS program and $0.90 for the TAI program. The advantage of the TAI program was $38.69/cow/yr. When the differences in voluntary waiting periods and PR obtained were accounted the economic advantage of TAI over NS was $10.00. The advantage of the TAI program was $22.75 if genetic advantage of TAI was not considered. Sensitivity analysis revealed that if the marginal feed cost increase from $2.00 to $8.00 the profit of TAI over NS rose for $144.81. Considering opportunity cost, if each bull was replaced by an additional cow the advantage of the TAI program was $68.82/slot/yr. Changing PR for each program for 12.0% resulted in an advantage of $75.39/cow/yr for the TAI program.

In conclusion, NS had a better reproductive performance than TAI. The higher proportion of pregnant cows in the NS group can be attributed to a greater opportunity for breeding than in TAI. The TAI breeding program was less expensive than NS even when the benefits on reproductive performance and VWP for NS obtained in our study were accounted.

CHAPTER 1
GENERAL INTRODUCTION

Reproductive performance of dairy cows has declined during the last 50 years in the US and other countries, as shown in studies that compared pregnancy per artificial insemination (AI) from the 1950 s to recent years (Lucy et al. 2001, Jorristma and Jorristma 2000 and López-Gatius 2003), which has impacted profitability of dairy producers. Concurrently, AI has been established as the preferred method to breed cows since its commercial development in the 1950s (Vishwanah, 2003). Dairy producers worldwide have benefitted from the use of semen from progeny tested bulls used to breed dairy cows. With the use of AI, the risk for venereal disease is eliminated and the incidence of dystocia is reduced, as well as human accidents caused by bulls with bad temperament. Furthermore, a better dry cow management occurs with AI because of more accurate drying off and calving dates (Vishwanah, 2003). Moreover, increasing rate of genetic change in dairy breeds, and in particular, increases in milk yield with the use of AI has been reported. Daughters of AI sires produced 113 to 136 Kg more of milk per year than daughters from sires used for NS (Norman and Powell, 1992).

De Vries (2008) reported that in a U.S.A. herd producing 20,000 lbs of milk, 1 unit of pregnancy rate is valued between $22 and $35 when pregnancy rate range from 15 to 19%. Similarly, an extra day open beyond 90 days costs between $1 and $5, with an increasing cost as days in lactation progresses (De Vries, 2008).

Despite these considerable advantages for AI, a significant number of dairy producers use NS for their breeding program. In a survey on bull management practices in California, 84% of the producers reported use of NS as a component of their breeding program (Champagne et al. 2002). The most common use of NS was after unsuccessful AI attempts. In dairy herds located

in the northeast region of the US, reported use of NS, as a component of the breeding system, varied from 55% to 74% (NAHMS, 2002; Smith et al. 2004). In a study that compared pregnancy rates (PR) between AI and NS in Georgia and Florida dairy herds, the use of NS alone or in combination with AI was reported to be around 70% (De Vries et al. 2005). A survey that examined management practices in 103 herds participating in the Alta Genetics (Watertown, WI) Advantage Progeny Testing Program, reported that 43% of herds used a clean-up bull (Caraviello et al. 2006). Non-pregnant cows were moved to the clean-up pen after six unsuccessful AI's or 232 d post partum (pp). A common perception among these dairy producers that use NS is that NS is comparable to AI because human errors in estrous detection are avoided when bulls are used.

Estrous detection is a major factor that impairs reproductive performance and profitability of lactating dairy cows (Pursley et al. 1997a). Timed artificial insemination and NS are two breeding systems that are used by dairy producers to mitigate the reduced fertility caused by inefficient low rates of estrous detection. Natural service, notwithstanding all the recognized advantages of AI previously mentioned still is used widely throughout the U.S.A. Dairy producers have the common perception that NS is the cheapest and easiest strategy to improve problems related to estrous detection. However, a paucity of research exists to support this premise. Overton (2005) compared the cost of AI and NS using a partial budget approach to stochastically model the expected costs and returns of reproductive management of NS or AI. Option one was NS managed using currently recommended approaches including breeding soundness evaluations, bull vaccination, and a rotational breeding system and option two was an AI system using a modified Presync-Ovsynch timed AI program in conjunction with estrus detection and inseminations. Under the model's assumptions, the use of NS sires averaged U.S.

$10 more in cost per cow per year as compared to an AI program. Nevertheless, that study considered a combination of TAI and estrus detection, which does not exclude estrus detection in the equation of reproductive performance. Furthermore, several assumptions were done in attempting of obtain the closest scenario of the predominant dairy farm in the Western region of the U.S.A., which not necessarily reflects the reality in the several different scenarios throughout of the U.S.A. In addition, the use of TAI solely has been reported to be more economical than AI at detected estrus (Risco et al., 1998; LeBlanc 2001) due to reductions in days non-pregnant and cows culled due to infertility. However, studies that compare costs and profitability and reproductive performance of NS and TAI, two breeding systems where estrous detection is not required, using a random allocation design are lacking. The hypothesis was that the use of TAI would result in a greater proportion of pregnant cows than NS. Our biological justification and rationale for improved reproductive performance were that TAI cows undergo ovulation and synchronization of follicular wave and all eligible cows are inseminated at a fixed time, which results in greater proportion of pregnancy right after voluntary waiting period where the value of the pregnancy is greater increasing the chance for profit. Therefore, the objectives of this study were to compare reproductive performance, costs and profitability of lactating dairy cows bred by TAI or NS, two breeding systems that do not require estrus detection, as the sole reproductive program on a commercial dairy farm located in north central Florida.

CHAPTER 2
LITERATURE REVIEW

Reproductive Performance of Lactating Dairy Cows

Reproductive performance in lactating dairy cows is characterized as the ability of obtaining one live calf as soon as possible after the end of the voluntary waiting period. Reproductive performance is affected by several factors that include: fertility, embryonic and fetal development, calving and calf survival. Fertility generally is assessed by reproductive responses such as: conception rate (CR), pregnancy rate (PR), cyclic status and non-return rates (NRR).

Consistently throughout the world several studies have reported a remarkable decline in fertility of dairy cows in the past few decades. In the U.S.A., the conception rate at first artificial insemination (AI) has decreased by 0.45% per year over a 20-year period (Butler and Smith, 1989; Beam and Butler, 1999). In Spain, PR to cows AI between days 45-70 postpartum decline from 42.3% to 33.1% with an increase of 4.6% in ovarian inactivity between 1991 and 2000 (Lopez-Gatius et al. 2003). In England, this reduction has been in the magnitude of 1% per year in the last 40 years (Royal et al. 2000a; Royal et al. 2000b). In the Netherlands, the success rate to first AI decreased from 55.5% to 45.5% in 10 years (Jorristma et al. 2000.) In the U.S.A. Lucy (2001) reported an increase in the number of AI's required for conception, from 1.75 to more than 3 over a period of 20 years. In Ireland, Mee et al. (2004) reported a higher number of AIs per cow 1.75 vs. 1.54 and a decrease in CR from 64.9% to 57.1% between 1990 and 2000 in pasture based system. In France, Bousquet et al. (2004) reported that from 157,630 first AIs per year, there was a 15% decrease in the NRR. In Canada the analysis of insemination data of Canadian Holstein cows between 1995 and 2001 showed a decrease in the NRR rate at 56 days from above 69% down to 67% (VanDoormal, 2002) and CR at first and second AI, respectively,

has been continually on the decrease from 44% to 39% and 47% to 41% between 1990 and 2000 (Bouchard et Du Tremblay, 2003).

The impairment in fertility is a combination of a diverse physiological and management factors that have an additive effect on reproductive efficiency. Amongst these factors the possible modernization of the dairy cow and farm to meet the industry required demands for a more efficient production of milk possibly have affected reproductive performance of dairy cows.

This modernization was achieved by a rapid progress in animal genetic improvement towards milk production and management. The genetic advance was obtained through the selection of more productive animals facilitated by the use of AI. However, the intense use of AI until the middle 90's relied on intensity and accuracy of estrus detection and conversely cows that produce more milk are less likely to show signs of estrus. In addition, the increase of herd size has made more difficult to monitor lactating dairy cows daily on an individual basis for estrus behaviour.

Timed Artificial Insemination (TAI) was a program reported by Pursley et al. (1995) with the purpose of synchronizing ovulation and inseminate at a fixed time and consequently eliminate estrus detection in the equation of reproductive efficiency. Despite the success of the TAI program to eliminate difficulties with estrus detection, which results in economic and reproductive performance advantages when compare to AI at detected estrus (Pursley et al. 1997a; Burke et al. 1996; Pursley et al. 1997b; Cartmill et al. 2001, Risco et al. 1998 and LeBlanc 2001), the use of NS to breed dairy cows is still widely used throughout the U.S.A. to mitigate the decline in fertility related to poor estrus detection (NAHMS, 2002; Smith et al. 2004; De Vries et al. 2005; and Caraviello et al. 2006). The range of reported use of NS in the

U.S.A. varies from 43% (Caraviello et al. 2006) as clean up bulls in herds using high quality genetics to 84% when used as a component of the breeding program in California (Champagne et al. 2002) and 70 % in Florida and Georgia (De Vries et al, 2005)

Estrous Cycle of Dairy Cattle

Period, Stages and Classifications of the Estrous Cycle

The estrous cycle is a period of reproductive cyclicity initiated after puberty that continues for the entire life of dairy cows only being interrupted by pregnancy, nursing, nutrition deficiency and inadequate environmental conditions. In addition, pathologic conditions of the reproductive tract such as: pyometra, uterine infection, follicular cyst, persistent corpus luteum (CL) and mummified fetus also interrupt the estrous cycle. The estrous cycle is composed of a series of correlated and expected events that start and end between two consecutive estrous events periods marked by behavioral events of sexual receptivity and copulation. The estrous cycle provides females repeated opportunities to become pregnant in intervals of 21 days. Dairy cattle are categorized as polyestrus and consequently can become pregnant independent of the season of the year (Asdel, 1964).

The estrous cycle can be divided in follicular and luteal phases that are classified according to the predominant structure present in the ovaries. The luteal phase takes place after ovulation and lasts until regression of the CL, which generally corresponds to 80% of the estrous cycle duration (Senger, 2003). During the luteal phase progesterone (P_4) is the predominant hormone produced and controls physiological events of the cycle. In addition, during the luteal phase, it is noticeable that follicular growth continues as well as atresia of follicles that do not ovulate. The production of estradiol (E_2) by the follicles is inhibited by P_4 during the luteal phase. The follicular phase starts after regression or luteolysis of the CL with a concomitant reduction in P_4 concentration. With the reduction of P_4, the gonodotropins FSH and LH are released from the

hypothalamus, which stimulate the production of E_2 and is the predominant hormone during the follicular phase in association with low levels of P_4. As E_2 increases an LH peak occurs which results in ovulation between 24 to 30 hours. The follicular phase ends with the behavioral event of estrus.

The estrous cycle is classified in four stages, proestrus, estrus that are considered subdivision of the follicular phase and metestrus and diestrus that are subdivision of the luteal phase. Proestrus starts when P_4 declines as a result of luteolysis caused the action of $PGF_{2\alpha}$ and it lasts 2 to 5 days.

Proestrus is the stage where the major endocrine transition events take place and is marked by the final maturation of the dominant follicle and a significant concentration increase of E_2. In addition, this is the stage in which P_4 dominance is replaced by E_2. FSH and LH orchestrate the recruitment and selection of the ovulatory follicle. The pre-ovulatory follicle produces increased concentrations of E_2 and prepares the uterus for the onset of estrus and mating.

Estrus is the most recognizable stage of the estrous cycle because the behavioral symptoms of sexual receptivity are expressed. Estradiol is the hormone responsible for the behavior of estrus (stand to be mounted), which characterizes this stage. Females that are in estrus are not initially receptive to other females. The acceptance of mounting only occurs at the second half of estrus. Initially, cows increase locomotion, display phonation (vocal expression), nervousness and attempts to mount other animals without accepting the male for matting. After certain progress in the continuity of estrus the female accepts the male for matting.

Metestrus is the transition between ovulation and formation of the CL. Progesterone and E_2 are relatively low during this stage of the estrous cycle. The recently ovulated follicle develops into a CL through the process of luteinization, which is accomplished by cellular and structural

renovation. Before formation of the CL there is a formation of a transitory structure named the corpus hemorrhagicum. Metestrus has P_4 concentration detectable soon after ovulation, but the concentration will not increase significantly until 2 to 5 days after ovulation.

Diestrus is the longest stage of the estrous cycle in cows lasting about 10 to 14 days in dairy cows and it is characterized by a fully functional CL with high concentration of P_4 (3-6 ng/ml). This high P4 concentration prepares the uterus for early embryonic development and eventual attachment of the conceptus to the endometrium. The diestrus phase ends when the CL is lyzed by endometrial $PGF_{2\alpha}$.

Anestrous is a condition where cows do not cycle, which is characterized by inactive ovaries and neither ovulatory follicles nor functional CL are present (Senger, 2005).

Bartolome et al. (2005), summarized the stages of the estrous cycle based on clinical findings of the uterus and ovaries and are shown in Table 2-1.

Hodgen (1982) described the process of follicular growth and development in primates as recruitment, selection, and dominance. Recruitment is a process whereby a cohort of follicles begins to mature in a milieu of sufficient pituitary gonadotropic stimulation to permit progress toward ovulation. Selection is the process by which a single follicle is chosen and avoids atresia and undergoes further development and dependent upon which follicular wave will achieve ovulation. Dominance is the means by which the selected follicle is through inhibits the growth and the recruitment of a new cohort of follicles.

Wiltbank et al. (2002) classified critical follicular sizes accordingly with evaluation of follicular growth patterns by ultrasound combined with measurement of circulating reproductive hormones. The three functionally critical follicular sizes during the final stages of follicular growth are: emergence (4 mm), deviation (9 mm), and ovulation (variable from 10 to 20 mm).

Follicular deviation was defined as the beginning of the greatest difference in growth rates (diameter changes between successive ultrasound examinations) between the largest follicle (i.e. dominant follicle) and the second largest follicle (i.e. largest subordinate follicle) at or before examination when the second largest follicle reaches its maximum diameter (Ginther et al. 1996). Selection of the dominant follicle either occurs at the time of follicular diameter deviation or is closely associated with this process (Wiltbank et al. 2002). This classification based on the three critical points is logical and provides a rational diagnosis and treatment of the underlying physiological condition such as: anovulatory conditions.

Endocrine and Molecular Inter-relationship of the Estrous Cycle

Follicular development

The endocrine control of the estrous cycle in cattle is made up by the interface among the hypothalamus, anterior pituitary and reproductive tract. The hypothalamus has a central role in controlling the follicular phase, because it is where gonodotropin release hormone (GnRH) is produced and released. The hypothalamus is divided in two different centers according to the different patterns of GnRH release. The first center of GnRH release is located at the ventromedial and arcuate nuclei and is called the tonic GnRH center. This tonic center is responsible for basal secretion of GnRH and is characterized by various small pulses of GnRH of different frequencies and amplitudes. Conversely, the other GnRH center located at the preoptic and suprachiasmatic nuclei at the superior anterior hypothalamic area are responsible for the preovulatory release of GnRH that causes an LH surge and consequently ovulation. This center is called the surge center and is also referred to as the “preovulatory center” (Senger, 2005).

The release of GnRH by the surge center is related to high estrogens concentrations in blood and is accompanied by low P4. In contrast, the release of GnRH from the tonic center is spontaneous in nature, however it is influenced by high P4.

The mechanism of how E_2 and P4 influence the release of GnRH in cattle is not completely understood. Dungan et al. (2006) reviewing studies in several mammalian species reported that kisspeptin stimulates the secretion of gonodotropins from the pituitary by stimulating the release of GnRH from the forebrain after the activation of G protein-coupled 54, which is expressed by GnRH neurons. Kisspeptin is a family of peptides encoded by the gene Kiss 1, which bind to G protein-coupled receptor 54. Kisspeptin is expressed in large quantities in the Arcuate nucleus and the anteroventral periventricular nuclei of the hypothalamus. Both E_2 and testosterone regulate the expression of the Kiss1 gene in the Arcuate nucleus and anteroventral periventricular nuclei; however, the response of the Kiss1 gene to these steroids is exactly opposite between these two nuclei. Estradiol and testosterone down-regulate Kiss1 mRNA in the Arcuate nucleus and up-regulate its expression in the anteroventral periventricular nuclei. Thus, kisspeptin neurons in the Arcuate nucleus may participate in the negative feedback regulation of gonadtropin secretion, whereas kisspeptin neurons in the anteroventral periventricular nuclei may contribute to generating the preovulatory gonadotropin surge in the female.

GnRH released by the tonic center early in the follicular phase stimulates the release of FSH and LH in the anterior lobe of the pituitary. These two gonodotropins act directly in the ovaries stimulating the recruitment of a new follicular wave. The mechanism that controls recruitment of these small follicles and determines which follicles are recruited is unknown, but increased concentrations of FSH in plasma after ovulation may stimulate this process (Walters and Schallenberger, 1984). In addition, McNatty et al. (2006) reported that once follicular growth has been initiated, at least two oocyte-derived growth factors, namely growth differentiation factor 9 and bone morphogenetic protein 15, are critical for ongoing development to ovulation. This occurs most likely by regulating the proliferative and differentiates functions

of adjacent follicular cells. In sheep, the granulosa cell populations doubles some 12-14 times, and a well-defined thecal layer differentiates before antrum formation and the time taken to complete this process varies between 50 -150 days with very little follicular atresia. During preantral growth, FSH and LH receptors coupled to the cyclic adenine monophosphate second messenger system develop in granulosa and thecal cells, respectively. From the late preantral stage, growth differentiation factor 9, bone morphogenetic protein 15 and perhaps other factors are thought to regulate gene expression in cumulus cells to enhance metabolic cooperatively with the oocyte and mural granulosa cells to regulate their responses to pituitary hormones. In sheep, antral follicular development is characterized by a much faster rate of growth, additional increases in the numbers of granulosa (4-5 more doublings) and theca cells, an increased level of steroid and inhibin secretion in response to FSH and LH, but also by most follicles undergoing atresia. The final number of follicles that go on to ovulate is dependent upon FSH, as well as the intrafollicular concentrations of growth differentiation factor 9 and BMP15.

The selection of the dominant follicle involves various factors. Cows that develop a dominant follicle have higher concentrations of E_2 and inhibin A and lower concentration of FSH than cows without a dominant follicle (Buttler et al. 2004). Fortune et al. (20040 summarized the role of intrafollicular insulin growth factor system in the selection process of the dominant follicle in cattle. The concentrations of E_2 in the follicular fluid are the hallmark of dominant and preovulatory follicles and are associated with lower concentrations of low molecular weight (MW) insulin-like growth factor binding proteins -2, -4, and -5, which can prevent binding of insulin growth factor to its receptor. In addition, Fortune et al. (2004) reported that dominant and preovulatory follicles also have much higher levels of an insulin-like growth factor binding proteins -4/-5 protease activity, which is the bovine equivalent of the human insulin-like growth

factor binding proteins -4 protease and pregnancy-associated plasma protein-A. Analysis of the temporal sequence of changes in, E_2 low molecular weigh insulin-like growth factor binding proteins, free insulin growth factor, and pregnancy-associated plasma protein-A in the follicular fluid suggested that an increase in pregnancy-associated plasma protein-A is the earliest biochemical difference detected in the eventual dominant follicle and that follicular selection is the result of a progressive series of changes beginning with the acquisition of pregnancy-associated plasma protein-A, which leads to a decrease in insulin growth factor binding protein-4 and -5 and an increase in free insulin growth factor, which synergizes with FSH to increase estradiol production. Co-dominant follicles, induced by injection of small doses of recombinant bovine, FSH, contained levels of pregnancy-associated plasma protein-A similar to the single dominant follicle of control heifers in both follicles, supporting the hypothesized role of FSH in the induction of pregnancy-associated plasma protein-A in the dominant follicle. Together, these results suggest a critical role for FSH-induced pregnancy-associated plasma protein-A, and thus for free insulin growth factor, in the selection of the dominant follicle.

Ireland et al. (2000) reviewed findings of several studies regarding follicular turnover and number of follicular waves in the bovine and concluded that either 2 or 3 follicular waves can occur and the assumption that the first follicular wave occurs right before estrus, the follicle of the last follicular wave during an estrous cycle results in the ovulatory follicle.

Moore and Thatcher, (2006) described that early estrogenic follicles (i.e. d 3 of the first wave) contained mRNAs for the FSH receptor and the aromatase is elevated within the granulosa layer, and theca cells have increased abundances of LH receptor and 17α-hydroxylase, an enzyme required for production of androgen precursors for estrogen biosynthesis. However, there is an absence of LH receptor mRNA within the granulosa cells. Dynamic changes are

evident within the inhibin family in that dimeric inhibins (i.e. >160 kDa) are elevated and the smaller (32- to 34-kDa) dimer of inhibin is low in estrogen-active follicles. However, in estrogen-inactive follicles, the smaller inhibin dimer increases and the larger molecular weight forms are reduced. The increased secretions of E_2 and ovarian inhibin reach the pituitary gland through the circulation and lead to a decrease in pituitary secretion of FSH. Lack of FSH prevents further growth of subordinate follicles, which are also non-estrogenic due to low concentrations of free insulin growth factor -I. Once the dominant follicle reaches 10 mm approximately in dairy cows (i.e. after deviation), its granulosa cells begin to express LH receptors, and it can be induced to ovulate at approximately 12 mm in size. Continued growth and dominance of the dominant follicle beyond 10 mm appears to be dependent upon LH secretion. Eventually, in the absence of an increase in pulse frequency of LH, the dominant follicle undergoes functional atresia that permits an increase in FSH secretion.

Luteal function and Corpus Luteum regression

Progesterone produced by the CL plays a key role in regulation of the length of the estrous cycle in cattle and in the implantation of the blastocyst (Niswender et al. 2000). Cholesterol, which can be derived from the diet in rare occasion in dairy cows and is actually in mostly of the time is synthesized de novo (Block, 1965 and Schoroepfer, 1982) is transported to the ovaries by lipoproteins (high density lipoprotein and low density lipoprotein) and is a common precursor for steroids synthesis. Progesterone among others steroid hormones is the most important physiological regulator involved in the CL life span and implantation of the blastocyst. Ovarian steroidogenesis is regulated by several factors playing modulatory role during the estrous cycle. Centrally and locally produced factors modulate expression of genes encoding synthesis of steroidogenic enzymes and consequently influence the secretory function of the CL. Preovulatory surge of LH is crucial for the luteinization of granulosa and theca follicular cells

and CL maintenance; however, the CL is less dependent on LH stimulation during the early luteal phase. Since the early CL requires luteotropic support for its growth and development, there are other factors that support the role of LH to maintain CL development and function. Indeed, hormones of luteal origin i.e. prostaglandins (PG) I_2 and E_2, oxytocin, noradrenaline and growth factors stimulate P4 synthesis in the bovine early CL (Niswender et al. 2000; Miszkiel and Kotwica, 2001). Therefore, it is accepted that despite hormonal and neural signals, which are fundamental in the estrous cycle, the CL has a broad area of autonomy. Corpus luteum self-regulates synthesis of P4 (Kotwica et al. 2002), which truly affects transcription of genes encoding steroidogenic enzymes (Rekawiecki et al, 2005 and Rekawiecki and Kotwica, 2007). Moreover, high P4 concentrations in luteal cells protect them against apoptosis, while disruption or impairment of steroidogenesis or reduced ability of P4 production induces luteal cells death.

Luteolysis is the process of destruction of the CL mediated by the action of mainly $PGF_{2\alpha}$ that reaches the ovaries through a venous and arterial counter current system described by Mapletoft et at 1976. Due to anastomoses of the uterine vein to the contralateral ovary and ovarian artery ipsilateral to the CL, $PGF_{2\alpha}$ produced in the uterus reach the CL bearing ovary and cause luteolysis.

Skarzynski ank Okuda (2000) proposed that the mechanism controlling development, maintenance and secretory function of the CL might involve factors that are produced both within the CL and outside the ovary. Some of these regulators appear to be prostaglandins and other arachidonic acid metabolites (PGE_2, $PGF_{2\alpha,}$ leukotrienes), neuropeptides (noradrenaline), peptide hormones (Oxytocin, Endothelin 1), growth factors and hormones (vascular endothelial growth factor, fibroblast growth factor, growth hormone, prolactin), and steroids (P_4 and E_2) that act as autocrine and/or paracrine factors. Although $PGF_{2\alpha}$ is known to be the principal luteolytic

factor, it is action on the CL is mediated by other intra-ovarian factors: cytokines, nitric oxide, Endothelin 1. Nitric oxide, Tumor necrosis factor alpha in combination with interferon tau reduced P_4 secretion, increased luteal $PGF_{2\alpha}$ concentration, and induced apoptosis of the luteal cells (Skarzynski et al. 2008).

Manipulation of the Estrous Cycle

The implementation of AI became the preferred method to breed dairy cows since it's commercial development in the 1950 s (Vishwanah, 2003). However, the decline in fertility in dairy cattle (Lucy et al., 2001) associated with an increased difficulty to detect cows in estrus (Pursley et al., 1996) has required a better understanding of manipulation of the estrous cycle to synchronize estrus.

The aim of a successful estrous synchronization program is the control of ovulation permitting fixed-time AI without the need for estrous detection. Reproductive efficiency of lactating dairy cows measured by pregnancy rates (PR; defined as the proportion of pregnant cows relative to all eligible cows inseminated or not, in a given period of time) is greatly affected by poor estrous detection and anestrous (Thatcher and Santos, 2007).

Thatcher et al. (2004) reported that a simultaneous expansion of the knowledge of the estrous cycle and appropriate implementation of physiological methods to control sequential follicle turnover, CL regression and induction of ovulation have been achieved successfully in the past few years.

In the 1970 s, Lauderdale et al. (1974) and Hafs and Manns (1975), reported reproductive management protocols that synchronized the time of estrus using $PGF_{2\alpha}$. Synchronization with $PGF_{2\alpha}$ was successful in cows bred to a detected estrus, because estrus detection rates increased and management of AI was more efficient than daily detection of estrus alone (Stevenson et al. 1994). Nevertheless, this manipulation of the estrus detection using $PGF_{2\alpha}$ did not control the

time of AI, because estrus detection continued to be necessary. Lauderdale et al. (1974), Archbald et al. (1992), Lucy et al. (1986) and Stevenson et al. (1987) evaluated fixed-time breeding - TAI after $PGF_{2\alpha}$ in lactating dairy cows. Pregnancy rates per AI were substantially lower than those AI at detected estrus. Low pregnancy rates from timed AI using $PGF_{2\alpha}$ can be explained by the fact that $PGF_{2\alpha}$ alone does not result in an optimal synchronization of ovulation in relation to AI because time of ovulation depends on the stage of dominant follicle development in concert with the prostaglandin-induced regression of the CL. Therefore, strategies were developed in an attempt to resolve the problem of TAI in dairy cows without the need for estrus detection. Pursley et al. (1995) reported the development of the Ovsynch protocol, a TAI breeding strategy that resulted in acceptable pregnancy rates. The Ovsynch protocol is composed of an injection of GnRH at a random stage of the estrous cycle to induce ovulation of the dominant follicle and synchronize the emergence of a new follicular wave. Seven days later, $PGF_{2\alpha}$ is given to regress both the original and induced CL, followed by a second GnRH injection 48 h later to induce ovulation approximately 28 to 32 h later. A timed AI is performed 12 to 16 h after the second GnRH injection. Pregnancy rates obtained from the Ovsynch program were comparable to those of cows inseminated at detected estrus (Pursley et al. 1997a). This protocol has been implemented successfully in commercial dairy farms throughout the world as a strategy for TAI to the first postpartum AI, as well as for re-insemination of non-pregnant cows. Although, the Ovsynch protocol allows for TAI without the need for estrus detection, approximately 10 to 15% of cows will come in estrus during the protocol and should be AI to optimize PR.

Schimitt et al. (1996) also contributed to development and consolidation of TAI as an option to inseminate cows at a fixed time without estrous detection. Schimitt conducted a series

of three experiments to evaluate the efficacy, timing and replacement of the last GnRH of the TAI protocol. In experiment 1, dairy heifers were assigned randomly to two groups: 1) TAI, consisting of GnRH on d 0, $PGF_{2\alpha}$ injection on d 7, and second GnRH injection on d 8 and AI on d 9; and group 2) AI at estrus (AIE), consisting of GnRH on d 0, $PGF_{2\alpha}$ injection on d 7 and AI at detected estrus. Pregnancy rate was 25.8% for TAI compared with 48.7% for AIE ($P < .001$). Experiment 2 was comparable to experiment. 1, but the second GnRH in TAI was given 48 h after injection of $PGF_{2\alpha}$. Heifers in TAI were inseminated at detected estrus if estrus occurred within 39 h after administration of $PGF_{2\alpha}$. In experiment 2, PR were 45.5% for TAI and 48.0% for AIE (P>0.1) and CR rate was greater (P < .005) in AIE (61.2%) than TAI (45.5%). In experiment 3, the second injection of GnRH agonist, given at 48 h after injection of PGF2a, was replaced with hCG. No differences in PR were detected for TAI (52.9%) vs AIE (56.1%) and CR was worse (P < .005) for TAI (52 %) than AIE 72.3%. Delaying the second GnRH agonist injection by 24 h improved pregnancy rate, but replacing the second injection of GnRH with an injection of hCG did not prevent a reduction in conception rate

Despite the relative success of the Ovsynch protocol that eliminates the need for estrus detection, the poor reproductive performance continued to be observe in lactating dairy cows warranted further research to optimize pregnancy rate with the use of Ovsynch.

Pre-synchronization Programs

Vasconcelos et al. (1999) reported that initiation of the Ovsynch protocol between days 5 and 9 of the estrous cycle resulted in the highest frequency of ovulation to the first GnRH injection. Fertility was decreased when the duration of dominance of the ovulatory follicle was longer than 5 days (Austin et al. 1999) or the Ovsynch program was initiated in the early stages of the estrous cycle (Vasconcelos et al. 1999). Ovulation to the first GnRH injection and initiation of a new follicular wave should improve PR per AI because an ovulatory follicle with a

reduced period of dominance is induced to ovulate. Based on that theory, the concept of presynchronization was developed by Moreira et al. (2001) to enhance the likelihood of having a dominant follicle (≥ 10mm) capable to ovulate to the first GnRH injection in the Ovsynch protocol and assurance that a CL would be present throughout the synchronization period i.e. the CL will not regress prior to the injection of $PGF_{2\alpha}$. The Presynch-Ovsynch program by Moreira et al. (2001) employs two injections of $PGF_{2\alpha}$ 14 days apart, with the second injection given 12 days prior to the first GnRH of the Ovsynch protocol. This program increased PR per AI 18 percentage units (25% to 43%) in lactating dairy cows that were cycling cyclic. Similarly, El-Zarkouny et al. (2004) also reported improvement in PR when cows were pre-synchronized prior to the Ovsynch protocol. In addition to the potential benefit of optimizing the stage of the cycle by pre- synchronization, the prior repeated injections of $PGF_{2\alpha}$ might have a therapeutic benefit on the uterine environment by stimulating re-occurring proestrous/estrous phases allowing for improved uterine defense mechanisms. Navanukraw et al. (2004) demonstrated that pre-synchronizing cows with 2 injections of $PGF_{2\alpha}$ the second given 14 days prior to initiation of the Ovsynch protocol, improved PR compared to Ovsynch alone.

Considering the same rationale for enhancing ovulation to the first GnRH of the Ovsynch program to improve CR, Bello et al. (2006) compared three different pre-synchronization protocol before the Ovsynch. Prostaglandin was given and then 2 days later GnRH was given either 4, 5 or 6 days before starting Ovsynch. The ovulation rates to the first GnRH of Ovsynch were 56.0, 66.7, 84.6, and 53.8% for presynchronization protocols given with GnRH given either 4, 5 and 6 days prior Ovsynch, and no presynchronization, respectively, and was greater for GnRH given 6 d before Ovsynch than for control cows. Luteolytic response to PGF2α in the Ovsynch protocol was greater in all treated groups than for control (92.0, 91.7, 96.2, and 69.2%

for presynchronization protocols with GnRH given either 4, 5 and 6 days prior Ovsynch, and controls, respectively,). Synchronization rate to Ovsynch was greater (92 vs. 69%) in G6G than in control cows, respectively. In addition, cows that ovulated in response to the first GnRH had greater response to PGF2α (Ovulation: 92.7; No ovulation: 77.1%) and a greater synchronization rate to the overall protocol (Ovulation: 87.9; No ovulation: 62.9%,). Concentrations of progesterone on the day of $PGF_{2\alpha}$ injection in the Ovsynch protocol, and estradiol and follicle size at the final GnRH of Ovsynch, were identified as significant predictors of probability of pregnancy 35 d after artificial insemination.

Galvão et al. (2007) compared three different pre-synchronization protocols with outcome of interest being ovulation to the first GnRH, presence of a CL at $PGF_{2\alpha}$ and PR per AI in a TAI protocol. Galvão et al. (2007) used the presynch-Ovsynch protocol with two injections of $PGF_{2\alpha}$ given 14 days a part and the TAI protocol started either at 11 or 14 d later (Control). Also, a third group received the two $PGF_{2\alpha}$ 14 d a part, 4 d later an injection of GnRH followed by a TAI protocol 7 d later.

Altering the interval between presynchronization and the first GnRH of the TAI program did not affect the proportion of cows with a CL at 1st GnRH, but GnRH 7 d before the 1st GnRH of the TAI program increased the proportion of cows with a CL. Ovulation to the first GNRH of the TAI program was greater for 11 d interval compared with the 14 d interval, but GnRH did not further improve ovulation. The increased ovulation to 1st GnRH of the TAI when the interval was reduced from 14 to 11 d was observed only in cows with a CL at 1st GnRH of the TAI protocol. Treatment did not affect ovulation in cows without a CL at the 1st GnRH of the TAI program. Treatment affected the pregnancy per AI on d 38 and 66 after insemination, and they were greater for the 11 compared with 14-d interval, but addition of GnRH did not improve

further pregnancy per AI. Cows ovulating to the first GnRH had greater pregnancy per AI regardless of whether or not they had a CL at the first GnRH Reducing the interval from presynchronization to initiation of the timed AI protocol from 14 to 11 d increased ovulation to the first GnRH and pregnancy per AI in lactating dairy cows. The benefit of reducing the interval from presynchronization to the timed AI protocol from 14 to 11 d was likely the result of improved ovulatory response to the first GnRH of TAI program because cows that ovulated had increased pregnancy per AI.

Timed Artificial Insemination Protocol

The difficulties in identifying lactating dairy cows in estrus made crucial the development of new strategies to improve fertility. A major advancement in optimizing fertility in lactating dairy cows was the development of the Ovsynch TAI program by Pursley et al. (1997a). Timed AI protocols such as Ovsynch, Cosynch 48 and Cosynch 72 have been successful in improving pregnancy rates by increasing AI submission rates. Ovsynch utilizes a final treatment with GnRH that synchronizes the time of ovulation within an 8-h period (Pursley et al., 1995). This precise timing of ovulation allows optimization of TAI in relation to the time of ovulation. A number of studies have provided information relating to the optimal time for AI in a TAI protocol. A pivotal study on the optimal time for AI utilized the original Ovsynch protocol and AI at 0, 8, 16, 24, or 32 h after the second GnRH treatment (Pursley et al., 1998). This study found a quadratic effect of time of AI on number of pregnancies per AI with CR increasing from 0 to 16 h with subsequent decreases from 24 to 32 h the expected time of ovulation. Nevertheless, only AI after the expected time of ovulation (32 h after final GnRH) resulted in a significant decrease in both percentage calving per AI and CR determined at the first pregnancy diagnosis (Pursley et al., 1998).

Analysis of calving data suggested that AI at any time between 0 and 24 h after the final GnRH resulted in similar rates of calving. Thus, the study by Pursley et al., 1998, indicates that there may be an optimal time for AI (<16 h after final GnRH) but also that there may be substantial flexibility in the time for AI in relation to ovulation, provided that AI is performed before ovulation. Insemination near or after the time of ovulation may provide insufficient time for optimal sperm capacitation and transport but results in an aged oocyte which compromises conception (Hunter and Wilmut, 1983; Wilmut and Hunter, 1984; Hawk, 1987).

Alternatively, excessive time from insemination to ovulation (>24 h) also appears to reduce fertility. Indeed, results from the earliest (Trimberger and Davis, 1943; Trimberger, 1944) up to the most recent studies (Dransfield et al., 1998; Pursley et al., 1998; Saackeet al., 2000; Dalton et al., 2001) on time for AI have generally shown a decline in CR in cows inseminated at the onset of estrus or at the time of the LH surge (induced by GnRH treatment; Pursley et al., 1998) compared with later times. Decreases in fertilization rate have been reported when cows were inseminated at the onset of estrus compared with breeding 12 or 24 h later (Dalton et al., 2001). Therefore, a loss of sperm viability is likely responsible for the declines in fertilization rate and CR that have been observed in various (Trimberger and Davis, 1943; Trimberger, 1944; Pursley et al., 1998; Dalton et al., 2001) but not all (Portaluppi and Stevenson, 2005) studies when a long intervals between AI and ovulation occurred.

Regardless of these results, the Cosynch protocol developed by Geary and Whittier (1998) has become a popular TAI program among dairy producers. In the Cosynch protocol, AI is performed concurrently with the second GnRH injection, which requires one less time for cow handling, thereby potentially decreasing labor costs, as well as other cow-handling problems associated with the utilization of a TAI protocol for reproductive management. Some studies

have compared Cosynch to an Ovsynch protocol in which cows were inseminated 24 h after the GnRH treatment. Vasconcelos et al. (1997) found that inseminating at 24 h after GnRH improved pregnancy compared with Cosynch at 48 h (Cosynch-48). In contrast, two recent studies using only presynchronized first-service animals found no difference in CR for animals receiving Cosynch at 48 h (AI 48 h after PGF) compared with Ovsynch with a 24-h interval between the second GnRH and AI (Portaluppi and Stevenson, 2005; Cornwell et al., 2006). Portaluppi and Stevenson (2005) not only compared these 2 protocols in their study with presynchronized first-service animals, but also included a treatment group that received Cosynch at 72 h (Cosynch-72: AI 72 h after PGF). Cows in the Cosynch-72 h group had better CR than cows in the other 2 groups combined, suggesting that delaying the time of final GnRH as well as the time of AI may improve results from TAI, at least during the first AI after Presynch. Nevertheless, the authors cautioned that this protocol has not been evaluated for synchronizing cows at second or later services (Resynch). Although, Co-synch has become popular in the dairy industry, there are controversial results regarding whether this protocol produces CR that are similar to TAI protocols that AI at a time closer to ovulation. Therefore, Cosynch are definitely not the best option to increase conception rates in a TAI program and is not recommended if the aim is of the TAI breeding system is to maximize reproductive performance.

Brusveen et al. (2007) compared the Cosynch 48 and 72 to a modified Ovsynch protocol, were the interval between the second GnRH and $PGF_{2\alpha}$ was 56 h instead of the regular 48 hours and TAI was 16 hours after the second GnRH instead of 12 or 16 h as in the original Ovsynch. In addition, Brusveen et al. (2007) included cows presynchronized for all groups and used resynchronized services as well. The author found an overall CR similar for the Cosynch-48 (29.2%) and Cosynch-72 (25.4%) groups. The Ovsynch-56 group had a greater CR (38.6%) than

Cosynch-48 or Cosynch-72. Presynchronized first-service animals had greater CR than cows at later services in Cosynch-48 (36.2 % vs. 23.0%) and Ovsynch-56 (44.8 vs. 32.7%) but not in Cosynch-72 (24.6 vs. 26.2%). Similarly, primiparous cows had greater CR than multiparous cows in the Cosynch-48 (34.1 vs. 22.9%) and Ovsynch-56 (41.3 vs. 32.6%), but not Cosynch-72 (29.8 vs. 25.3%). Therefore, the author reported no advantage to Cosynch at 72 h vs. 48 h. Conversely, a clear advantage of treating with GnRH at 56 h to Cosynch 48 and 72 was observed, probably because of more-optimal timing of AI before ovulation.

Resynchronization Programs

The high AI submission rate to first TAI is often followed by a time lag for the re-insemination of non-pregnant cows that depends on estrus detection efficiency and when pregnancy diagnosis is performed after AI. Because AI CR of high producing dairy cows are reported to be 40% or less (Pursley et al., 1997a, 1997b; Fricke et al., 1998; Jobst et al., 2000), 60% or more of cows that are TAI fail to conceive and thus require a resynchronization strategy for re-insemination. Combining diagnosis of non-pregnant cows with a management strategy for a timely re-insemination improves reproductive efficiency by decreasing the interval between AI services (Fricke, 2002).

Moreira et al. (2000) reported an aggressive resynchronization protocol, in which cows received GnRH on d 20 after TAI followed by transrectal ultrasound and $PGF_{2\alpha}$ administration to non-pregnant cows on d 27. However, the authors found an interaction for cows resynchronized with GnRH on d 20 after TAI that were treated with bovine somatotropin treatment and embryonic losses. From d 20 to 27 embryonic losses increased for bovine somatotropin-treated cows that received GnRH but not for non-bovine somatotropin-treated cows. Because of this observation, this resynchronization strategy was discontinued. GnRH at 20 d after insemination adversely affected embryonic survival. The author cogitated the possibility of an injection of

GnRH induces an immediate increase in plasma estradiol concentrations, which may trigger the production of $PGF_{2\alpha}$ by the endometrium and cause the demise of the CL. Moreover, Moreira et al. (2000) mentioned there is evidence that LH receptors are found in the uterine endometrium and uterine vein and that LH may stimulate $PGF_{2\alpha}$ secretion. Hence, it is possible that an LH surge induced by an injection of GnRH may induce luteolysis and did not ovulate to the first injection of GnRH.

Fricke (2002) proposed a resynchronization protocol in which groups of cows beyond the voluntary waiting period received their first postpartum TAI after synchronization of ovulation. On d 18 after TAI, all cows received an injection of GnRH regardless of their pregnancy status. Non-pregnant cows received a PGF2α injection on d 25 after TAI based on a non-pregnancy diagnosis using ultrasound and continue with Ovsynch protocol, whereas pregnant cows would discontinue the protocol. Three different times to initiate the first GnRH of the Ovsynch protocol (100 µg GnRH, d 0, 25 mg $PGF_{2\alpha}$, d 7, 100 µg GnRH + TAI, d 9) for resynchronization were compared at 19 (D19), 26 (D26), or 33 d (D33) after first TAI to set up a second TAI service for cows failing to conceive. Conception rate was assessed 26 d after TAI for D19 and D26 cows and 33 d after TAI for D33 cows. Overall CR to resynchronization was 32%. However, the CR for D26 (34%) and D33 (38%) were greater than for D19 cows (23%). Cows with a CL at the $PGF_{2\alpha}$ injection (D19 cows) or at the first GnRH injection (D26 + D33 cows) of resynchronization had greater CR to compared to cows without a CL. Survival analysis (failure time) of cows in the D26 and D33 treatment groups across the first three TAI services did not differ statistically. Although administration of GnRH to pregnant cows 19 d after first TAI service did not appear to induce embryonic loss, initiation of resynchronization 19 d after TAI resulted in a lower CR compared to 26 or 33 d after TAI.

Chebel et al. (2003) compared resynchronization with GnRH on day 21 after a pre-enrollment of AI; animals assigned to the resynchronization (RES) group received 100 µg of GnRH, whereas animals in the control (CON) group received no treatment. For RES and CON, pregnancy at day 21 based on P_4 was 70.9% vs. 73.0% (P < 0:56), at day 28 33.1% vs. 33.6% (P < 0:80) and at 42 days 27.0% vs. 26.8% (P < 0:98), respectively after the pre-enrollment AI. Administration of GnRH on Day 21 after AI had no effect on pregnancy loss in either the RES or CON group from days 21 to 28 (53.2% versus 53.5%; P < 0:94) and days 28 to 42 (17.9%; P < 0:74) after AI. Pregnancy after the resynchronization period was similar for both treatment groups. In this study, Chebel et al. (2003) did not find an effect of resynchronization with GnRH given on Day 21 after AI for initiation of a TAI protocol prior to pregnancy diagnosis on pregnancy or pregnancy loss in lactating dairy cows.

Sterry et al. (2006) compared two resynchronization TAI strategies at different days post AI; cows were assigned to receive the first GnRH injection of Ovsynch at 26 (D26) or 33 (D33) days after TAI in cows that did not conceive. Cows in the D26 group received GnRH 26 d after TAI and continued resynchronization when diagnosed non-pregnant by using US at 33 d after TAI. Similarly, cows in the D33 diagnosed non-pregnant received GnRH 33 days after TAI. Cows were classified based on the presence or absence of a CL at the non-pregnant diagnosis, and cows without a CL received an intravaginal progesterone-releasing insert during the resynchronization protocol. When analyzed as a systematic strategy, CR was greater for cows assigned to the D33 than the D26 resynchronization strategy (39.4 vs. 28.6%). These results demonstrate that delaying the initiation of resynchronization until 33 d after TAI increased PR/AI for primiparous cows. Silva et al. (2007) use $PGF_{2\alpha}$ 12 d before initiation of a protocol for resynchronization of ovulation using Ovsynch. Lactating Holstein cows diagnosed not pregnant

31 d after TAI were randomly assigned to initiate the resynchronization protocol 32 d after TAI (RES), or receive 25 mg of $PGF_{2\alpha}$ 34 d after TAI and initiate the resynchronization protocol 12 d later at 46 d after TAI ($PGF_{2\alpha}$ +RES). Cows in the $PGF_{2\alpha}$ +RES group had better CR than RES cows 66 d after TAI (35.2 vs. 25.6%), whereas pregnancy loss from 31 to 66 d after TAI was greater for RES than for PGF+RES cows (17.1 vs. 7.6%). These results show that pretreatment with $PGF_{2\alpha}$ 12 d before initiation of the resynchronization Ovsynch protocol improved P/ AI and decreased pregnancy loss from 31 to 66 d after TAI. However, this approach for resynchronization took longer time until re-insemination was performed.

Economics of Timed Artificial Insemination in the Dairy Industry

Protocols for synchronizing ovulation in lactating dairy cows such as Cosynch and Ovsynch allows AI submission rates close to 100%, which improves pregnancy rate and reduces costs (Pursley et al., 1997a; Risco et al., 1998). Various studies compared Ovsynch to AI at detected estrus and several focused on conception and pregnancy rates after the first synchronization (Britt and Gaska, 1998). In some studies, Ovsynch was only used for the first AI and then started observation of cows for estrus (De la Sota et al., 1998; Jobst et al., 2000; Klindworth et al., 2001). It has been recognized that Ovsynch is more beneficial in herds with poor estrous detection (Risco et al., 1998, Mialot et al., 1999, Tenhagen et al. 2004).

However, the use of Ovsynch or other TAI program is completely dependent upon economic viability of the program, and the change of workload when TAI is introduced is difficult to assess with accuracy, as well as time spent on detection of estrus, cow selection, organization and documentation.

Risco et al. (1998) evaluated the economics of Ovsynch TAI to insemination at detected estrus by simulating PR for each breeding system using computerized models. An economic advantage to Ovsynch TAI was attributed to a reduction in days non-pregnant and cows culled

for infertility. Further, the Ovsynch protocol was found to be more economical when applied during the warm season when estrus detection efficiency is low.

Britt and Gaska (1998) compared PR, seasonal effects, and economic benefits of 2 estrus synchronization programs for a confinement-housed dairy herd. These author included cows eligible for breeding after palpation per rectum and randomly assigned to 2 treatment groups during 4 seasonal periods. Cows in one group (Ovsynch) received injections of GnRH on day 0, prostaglandin F2 alpha on day 7, and a second injection of GnRH on day 8. Cows in the other group (PP) that had a palpable CL were given $PGF_{2\alpha}$. Estrus detection was not performed on the Ovsynch cows, which were artificially inseminated at a predetermined time after the second GnRH injection. Cows in the PP group were observed for signs of estrus, and only those that were detected in estrus were inseminated. Pregnancy rates and insemination rates were significantly improved for cows in the Ovsynch group, compared with cows in the PP group. The Ovsynch program was an economically advantageous method for controlling reproduction that resulted in more pregnancies without the need for estrus detection.

Similarly, LeBlanc et al. (2001) evaluated Ovsynch for repeated inseminations and reported an economic profit for TAI as a result from a reduction in the number of days open and decreased number of cows culled for reproductive problems. Further benefit occurred because fewer sub estrous cows were inseminated and diagnosed per pregnancy status.

Nebel and Jobst 1998, in a review that evaluated systematic breeding programs for lactating dairy cows focused on a hypothetical use of $PGF_{2\alpha}$ and GnRH for estrus detection by conducting a survey of bovine practitioners. Using their survey results, they calculated an estimated cost per pregnancy for Ovsynch and Targeted Breeding (Pharmacia-Upjohn, Kalamazoo, MI). The costs per pregnancy for drugs alone ranged from $5.75 for Targeted

Breeding with a 70% estrus detection rate to the least cost for drugs of $17.84 for Ovsynch at the mean costs for drugs. The authors advocated that as herd sizes and milk yield continue to increase, reproductive efficiency is pertinent to maximizing profit. In addition, the authors suggested that systematic breeding programs have the potential to increase the reproductive performance of lactating dairy herds while maintaining AI as the primary breeding option. Primary benefits of systematic breeding programs include the convenience and efficiency of estrus detection; however, reduced labor costs from less time spent on estrus detection may be offset by the cost of the drug used in the protocols. They concluded that cost effectiveness must be calculated for each herd to decide whether a systematic breeding program is the appropriate choice. Lastly, Nebel and Jobst speculated that perhaps the cost of the programs could be recovered through increased convenience and decreased time spent observing cows for estrus.

Tenhagen et al. (2004) compared the reproductive efficiency and economic benefit of Ovsynch protocols with conventional reproductive management in a field trial in two large dairy herds in Germany. These authors compared a TAI breeding protocol to insemination at detected estrus. Cows were synchronized for began the TAI program at 62 and 42 d in milk in herds 1 and 2, respectively. After TAI, cows seen in estrus received AI, whereas cows diagnosed not pregnant were resynchronized for TAI. Control cows received AI based on detected estrus after a voluntary waiting period of 72 d in herd 1 and 50 d in herd 2. Use of Ovsynch reduced intervals to first AI and days open in both herds and reduced culling for infertility in herd 2. CR for first AI at detected estrus were significantly higher compared to TAI in both herds and for overall AI at estrus in herd 2. For groups assigned to AI at estrus, mean 21-d submission rates over 200 d for AI were higher in herd 1 than in herd 2 (55.6 vs. 28.6%). Days open and culling were the major cost factors. Although Ovsynch improved reproduction in both herds, AI based on

detected estrus was economically superior in herd 1, whereas Ovsynch was superior in herd 2. This was consistent across the ranges of cost factors evaluated. Tenhagen et al. (2004) suggested that an evaluation of synchrony protocols should include reproductive performance along with appropriate costs associated with treatments. The authors concluded that such costs might balance benefit to reproduction in herds with good estrus detection rates.

Olynk and Wolf, 2008 reported in a survey from 57 farms throughout the U.S.A. that those farms with successful reproductive management programs will have less economic incentive to pursue reproductive management changes. When individual farms asses the potential economic benefit by changing the reproductive management program, the prior level of performance is a key determinant factor Farms that have experienced success with visual estrus detection, as measured by high levels of efficiency in detecting cows in estrus, for example, are more likely to find that the expected net present value of the visual estrus detection program remains greater than that of the potential benefit of a synchronization programs considering more labor cost scenarios than farms that have not experienced such a success. The program costs for visual estrus detection and Ovsynch for first-lactation animals, assuming a group size of 100, across labor costs ranging from $6.00 to $20.00/h is shown in figure 2-2. The cutoff criterion that they used for the programs was that the cow was bred for 6 AI. Specifically, Figure 2.2 shows a comparison of the value of an AI submission rate of 65% achieved with 2.15 labor h/d with the same program valued when using 2.6 labor h/d for visual estrus detection programs. The breeding periods assumed was a 21-d period for visual estrus detection and a 26-d period for synchronization. The difference between the expected net present value for the 2 visual estrus detection programs highlights the difference that labor efficiency has when selecting an estrus detection program. The scenario in which a 65% AI submission rate is obtained in 2.15-labor h/d

exhibits greater levels of labor efficiency in detecting estrus events than one using 2.6-labor h/d to attain the same 65% AI submission rate. Additionally, Ovsynch scenarios in which a 30% CR is achieved per injection times of 2.1 or 6.3 min are provided for comparison. Similarly, time for injections affects the total labor costs associated with the program, although the same injections are administered whether it takes 2.1 or 6.3 min/shot. In Figure 2.2, it is apparent that the visual estrus detection programs, which use a great deal of labor, have higher net present value at low labor costs, whereas the Ovsynch program has higher net present value at high labor costs. When looking at the visual estrus detection program, in which a 65% AI submission rate is achieved with 2.15 labor h/d, the visual estrus detection program yields a greater net present value than Ovsynch with injections taking 2.1 min, if labor costs are less than approximately $17.50/h. If injections for Ovsynch take 6.3 min, however, the visual estrus detection program remains the better value program when labor costs are less than approximately $28.00/h. When looking at the visual estrus detection program in which an AI submission rate of 65% is achieved with 2.6 labor h/d, the labor costs at which Ovsynch becomes the better value program are lower than in the previous case, in which only 2.15 labor h/d were used to achieve the same AI submission rate. In the case of 2.6 labor h/d being used to obtain a 65% AI submission rate, the visual estrus detection program is the better value program when labor costs are less than approximately $14.00 and $20.00/h for Ovsynch with injections taking 2.1 and 6.3 min, respectively.

Bull Aspects

Use of Bulls as Natural Service in U.S.A

Although, AI has been recognized as the preferred method to breed cows since its commercial development in the 1950s (Vishawanah, 2003) a significant number of dairy producers use NS for their breeding program.

Champagne et al. (2002), in a survey of bull management practices in California, reported that 84% of the producers reported use of NS at least as a component of their breeding program. In addition, this survey identified that the most common use of NS was after unsuccessful AI attempts. Two studies conducted in the Northeast region of the U.S.A. reported that the use of NS as component of dairy farms breeding program varies from 55% to 74% (NAHMS, 2002; Smith et al. 2004). In a study that compared pregnancy rates (PR) between AI and NS in Georgia and Florida dairy herds, the use of NS alone or in combination with AI was reported to be around 70% (de Vries et al. 2005).

Caraviello et al. (2006) reported in a survey that examined management practices in 103 herds participating in the Alta Genetics (Watertown, WI) Advantage Progeny Testing Program. The dairy producers aimed to capture the genetics benefits of AI, howeved 43% of herds used a clean-up bull. Furthermore, Caraviello indicated that in average non-pregnant cows were moved to the clean-up pen after 6 unsuccessful AI's or 232 d post partum.

Bull Behavior, Evaluation and Factors Affecting Fertility

Anderson (1945) differentiated sexual behavior in the bull into two components: libido (or sex drive) and ability to copulate (or mating ability). Libido has been defined as the willingness and eagerness to mount and complete service of a female and mating ability as the ability to complete service (Hultnas, 1959). Mating behavior is that behavior exhibited immediately before, during, and after service (Chenoweth, 1981). Both libido and mating ability are important in bulls, and there is ample evidence that these two traits are influenced strongly by genetic factors (Bane, 1954 and Blockey et al., 1978). Deficiencies in these traits represent major causes of bull wastage (Signoret, 1980 and Rawson, 1959).

Rawson (1959) also reported that the basic pattern of male sexual behavior in cattle appears to be innate in that animals reared in complete isolation often will exhibit normal mating

behavior when exposed to a female in estrus. However, Chenoweth (1981) described that rearing young postpuberal males in all-bachelor groups may delay or inhibit subsequent expression of heterosexual mating behavior. Although libido is believed to be influenced largely by genetic factors, mating ability has a learning component that could be influenced by rearing methods. The degree of error that this learning factor imposes upon the assessment of libido in young bulls has varied in different studies from negligible to significant (Aenelt et al., 1958; Chenoweth, 1979 and Osborne et al. 1971). Young male calves often display aspects of sexual behavior during play. The most common behave is the jumping impulse (Bonadonna, 1944). This impulse is not, however, sex-limited as it also is seen in females under steroid influences and in castrated males. In the intact bull, this behavior gradually develops into coordinated mating behavior under the mediation of nervous and hormonal influences (Galloway, 1970).

In the bull, different indicators have been used to identify puberty, including ejaculation of the first viable spermatozoa and ejaculation of the first semen sample containing a minimum of 50×10^6 spermatozoa with at least 10% showing progressive motility (Wolf et al., 1965). These definitions do not describe development of sexual behavior, which often does not reach full proficiency until some time after the spermatogenic definitions of puberty are achieved. When both spermatogenic and behavioral components are developed adequately for natural procreation, full puberty (or sexual maturity) is achieved. Breed differences occur in the time required for development of both components and in the time relationship between them (Chenoweth, 1979).

Social interactions between animals often have been differentiated into amicable and agonistic categories. Amicable behavior is seen most commonly in young animals with a stable dominance hierarchy and is expressed by such actions as sham fighting and bunting, mounting,

and licking of the head, neck, and preputial regions (Blockey, 1975)). Agonistic behavior is more evident in older animals during the formation or reestablishment of the social order and includes all those activities associated with conflict (Scott, 1956). Social hierarchies are established quickly (within 10 to 60 min) in animals placed suddenly together in a group, and such hierarchies are more prominent with animals that have had considerable experience with such encounters (generally older animals) than in those, which have not (Hafez and Boissou, 1975).

Changes occur in social behavior, as bulls get older. With dairy bulls, agonistic behavior greatly increases when they reach 3.5 to 4.5 yr of age (Kilgour and Campin, 1973). Although, horns and physical size may influence achievement of dominance of an individual within a group of bulls, age and seniority within the group appear to be of greatest importance in mixed-age groups (Blockey, 1975 and Chenoweth, 1979).

Chenoweth et al. (1993) revised the breeding soundness evaluation used by the majority of veterinarians worldwide. The two pages that summarized the guidelines are at the end of this chapter (Figures 2-3 and 2-4). Chenoweth's model for breeding soundness evaluation was published at the proceedings for annual meeting from Society for Theriogenology in San Antonio, Texas after 3 years of discussion for new guidelines. This revised guideline by Chenoweth et al. (1993) replaced the breeding soundness evaluation by Bierchwal, (1976) incorporating important findings of current research at the time that made this guideline more appropriate.

Parkinson et al. (2004) in a review about evaluation of fertility and infertility in natural service bulls points out that in essence the breeding potential of a bull can be considered to depend upon both its ability to mate and its ability to fertilize. Breeding soundness evaluation allows field assessments of a bull's ability to mate, its libido and of its physical capability to

mount, achieve intromission, ejaculate and quality of the semen that the bull produces, which is, in turn, related to physical characteristics of its genitalia. Although it is relatively easy to assess such traits in the field, their value as predictors of bulls' fertility remains the subject of considerable debate.

Bull management can severely affect bull fertility. Macmillan (1998) reported that bull: cow ratio can be underestimated if PR to AI were worse than expected. This would result in overuse of bulls, a situation that is not uncommon amongst dairy farms in Australia and New Zealand, where the AI period is short and the breeding season is highly compact.

Other management factor that plays an important role is the period length of exposure of bulls to dairy cows. One strategy suggested to improve the libido and performance of NS sires as well as reduce the risk of lameness is a rotational management system (Overton et al., 2003). This management system involves maintaining two groups of bulls on the dairy farms, at all times. One set is considered as the ''working group'' and is commingled with lactating cows. The second set is held in reserve and is referred to as the ''resting group''. The two groups are rotated every 1–4 week.

Among all parameters used to evaluate breeding soundness, scrotal circumference is emphasized because it is a good indicator of sperm output. Testis size is correlated highly with daily sperm output, since sperm production per unit of testis volume is a constant figure. Thus, sperm output is primarily determined by the size of the testes; a figure which is easily ascertained in the live bull by measurement of the scrotal circumference (Almquist et al., 1976; Coulter and Keller, 1982).

Measuring scrotal circumference is particularly important in the examination of yearling bulls (Brinks, 1994), since it is a good indicator of whether the animal is pubertal. Puberty occurs

when scrotal circumference is between 28 and 30 cm, 52% of bulls are pubertal when their scrotal circumference has reached 28 cm and 97% by the time it is 30 cm (Spitzer and Hopkins, 1997). The major factors affecting the age at which the testis reaches these threshold values are the genetic and nutritional effects which determine the rate of testicular growth, but age or breed per se are relatively unimportant (Spitzer and Hopkins, 1997). Consequently, the American Society of Theriogenologists (Hopkins and Spitzer, 1997) recommends that all breeding bulls should have a minimum scrotal circumference of 30 cm. Kasari et al. (1996) demur slightly from this opinion, suggesting that slightly higher figures (32–33 cm) should be used in breeds such as the Simmental, Angus and Maine-Anjou. Whether it is correct to increase the minimum acceptable testis size for some breeds is open to debate, it is the clear opinion of Hopkins and Spitzer (1997) that no figure lower than 30 cm should be allowed, even for breeds that are of smaller stature.

Testis size (and, hence, scrotal circumference) continues to increase after puberty, so the scrotal circumference of bulls that are 2 years old should exceed 33–34 cm (Coulter, 1991; Hopkins and Spitzer, 1997). Changes in testis size after 2 years of age are breed-dependent (Chenoweth et al. 1984).

Scrotal circumference has also been related to semen quality parameters such as number of sperm, percentage of motile sperm and percentage of morphologically normal sperm (Coulter and Foote, 1979; Jakubiec, 1983; Gipson et al., 1987; Madrid et al., 1988; Chacon et al., 1999). Consequently, many reports have demonstrated that scrotal circumference is positively related to conception and/or pregnancy rates (e.g. Mateos et al., 1978; Makarechian and Farid, 1985; Coulter and Kozub, 1989; McCosker et al., 1989). However, a number of other reports call into question the relationship between scrotal circumference, semen quality and fertility

(Makarechian et al., 1983; Raadsma et al., 1983; Lunstra, 1985; Williams, 1988; McGowan et al., 2002). Holroyd et al. (2002) also noted that breeding soundness traits were correlated poorly with fertility. Furthermore, Thompson and Johnson (1995) failed to find a relationship between scrotal circumference and calving interval. Some of the discrepancies between results can be explained with reference to the populations of animals that were examined. Coulter and Kozub (1989), for example, found that whilst scrotal circumference of mixed age bulls was related to fertility, within each individual year-group there was no relationship. Only when data across year-groups were pooled (thereby increasing the range of values) the effect of scrotal circumference on fertility became significant. Nevertheless, it may be that the relationship is based upon probability of normality rather than a direct correlation, since bulls with low scrotal circumference are less likely to have normal semen quality or fertility than bulls with a normal circumference (Cates, 1975; Smith et al., 1981). On the other hand, low scrotal circumference is not completely inimical to adequate semen quality or fertility, nor is it guaranteed by high circumference. Scrotal circumference can also be affected by the fatness of the bull (Barth, 1997), further confounding its relationship with fertility. Moreover, even in the studies that have shown a relationship between scrotal circumference and fertility, the relationship may be too imprecise to allow the prediction of actual pregnancy rates.

Coulter and Kozub (1989) incorporated scrotal circumference into a multi-factorial predictor for fertility; yet even when factors such as libido and bull:cow ratio were also included, the model was unable to accurately predict fertility outcomes. Likewise, even though Makarechian and Farid (1985) were able to relate scrotal circumference to fertility, the relationship between the two was not strong enough to be predictive of achieved pregnancy rates.

On the other hand, scrotal circumference is highly heritable (Coulter et al., 1987; Gipson et al., 1987; Graser and Raznozik, 1992; and see Brinks, 1994), and is related both to age of puberty of male and female progeny (Brinks et al., 1978; Lunstra et al., 1978; Smith et al., 1989; Brinks, 1994; Moser et al., 1996) and to subsequent fertility of female progeny (Jakubiec, 1983; Werre and Binks, 1986). Hence, advantages would accumulate from selecting bulls of higher-than-average scrotal circumference, even if it were unrelated to the fertility of the bulls themselves.

Despite all the controversial findings regarding breeding soundness evaluation and fertility, Alexander (2008) reported that breeding soundness evaluation has been well accepted by the veterinary profession and the cattle industry as a standard for determining if a bull is a good potential breeder and is a satisfactory method of identifying subfertile breeders.

Kastelic and Thundathil (2008) reported that traditional breeding soundness evaluation would usually identify bulls that are grossly abnormal. However, a comprehensive approach, including assessing sperm function and fertility at the molecular, cellular and whole-animal levels, is needed to predict fertility of bulls that are producing apparently normal sperm. Kastelic and Thundathil et al. (1999) discussed several factors that can be involved with the prediction of fertility such as: sperm plasma membrane, sperm motility, sperm-oviduct interaction, sperm capacitation, sperm zona pellucida interaction, sperm oolema fusion and sperm DNA decondensation.

Semen Quality

Semen quality standards for natural service sires mirror in general accepted ideas about minimum thresholds that have to be attained if a bull has an acceptable fertility (Parkinson et al., 2004). Minimum of 30% motility, 70% normal sperm and age-dependent minimal scrotal circumference probably represent a consensus of opinion over the standards that are acceptable

to veterinary andrologists. Using such criteria, bulls that fail a breeding soundness evaluation generally have more sperm abnormalities than those who pass (Spitzer et al., 1988), largely as a result of the strong emphasis that is placed upon motility/morphology in the evaluation process (Higdon et al., 2000). It is a fact that the relationship between semen quality and fertility is contradictory, although many authors have reported that percentages of morphologically normal or abnormal sperm are related to conception rate (Jaczewski and Kazimirow, 1997; Aguilar, 1978; Chenoweth, 1980b; Coulter and Kozub, 1989; Pangewar and Sharma, 1989; Larsen et al., 1990; Gotschall and Mattos, 1997; Fitzpatrick et al., 2002; Padrik and Jaakma, 2002; Holroyd et al., 2002). The significance of initial motility of the ejaculate is less clear, with some reports (Aguilar, 1978; Chenoweth, 1980b; Pangewar and Sharma, 1989; Gotschall and Mattos, 1997) finding that it is related to fertility, others (Fitzpatrick et al., 2002; Holroyd et al., 2002) did not report a relationship between semen quality and fertility. On the other hand, there are reports that none of the commonly assessed parameters of semen quality are reliably or consistently related to fertility (Smith et al., 1981; Lunstra, 1985; Makarechian et al., 1985; Makarechian and Berg, 1988). Despite these latter findings, many bulls that are presented for infertility examination have abnormalities of semen that can explain their poor results (Dirks, 1983), so examination of semen is obviously an essential component of a breeding soundness examination.

Sperm Pathologies

Sperm abnormalities have traditionally been classified by site (head, tail, midpiece, cytoplasmic droplets) or site of origin (primary: testis; secondary: epididymis; tertiary: accessory glands/post ejaculation). Blom (1983) advanced understanding of the significance of sperm abnormalities by classifying them according to their effect on fertility into major and minor defects. Major defects include most abnormalities of the head and midpiece, proximal cytoplasmic droplets and single abnormalities that are present in a high percentage. Minor

defects include looped tails, detached sperm heads and distal cytoplasmic droplets. More recently, the significance of specific sperm abnormalities has become better understood from the results of mating trials, analysis of non-return rates to artificial insemination and in vitro fertilization with semen with high percentages of sperm with individual classes of abnormalities. In particular, the notion of compensable and uncompensable abnormalities has been developed from such studies (Saacke et al., 1988). Barth (1997) described the two forms of abnormalities; abnormal sperm which are not transported to the uterine tube, or which are unable to penetrate the oocyte can be compensated by increasing sperm dose. Such defects are therefore 'compensable'. Sperm cells, which are capable of penetrating the zona, but they fail to cause cleavage or result in non-viable embryos, cannot be compensated by increasing sperm dose. These defects are therefore uncompensable. Research is still required to elucidate how these categories relate to classical sperm morphology assessments. However, some abnormalities, such as chromatin defects are unequivocally uncompensable (Evenson, 1999; Saacke et al., 2000) and other abnormalities (as discussed below) have effects in vitro fertilization systems that indicate that they are also likely to be uncompensable.

Head abnormality of sperm

The head of the sperm consists of the genetic material (in the form of highly condensed chromosomes) and key effectors of fertilization (i.e. binding and passage through the zona pellucida). Hence, most abnormalities of the head are associated with a significant impairment of fertility (Wilmington, 1981; Soderquist et al., 1991). Abnormal condensation of chromatin (Johnson, 1997) and abnormal nuclear shape (Ostermeier et al., 2000, 2001) are closely associated with reduced fertility. Many defects of the head can be observed through careful examination of simple eosin–nigrosin stained smears, although some require special stains or phase- contrast microscopy to be detected.

The most common defect, the pyriform (pear shape) head, is readily recognizable through the narrow, elongated appearance of the head and the 'pinching' in of its post-acrosomal region. The defect commonly appears as an acquired defect during testicular degeneration. Fertility is affected by the degree of deformity of the sperm head (Petac and Kosec, 1989), probably as a consequence of a reduction of its total surface area (Barth et al., 1992). Nothling and Arndt (1995) described that a bull with 36% pyriform heads achieved a 46% pregnancy rate (versus 75% for a normal bull). This abnormality impairs both fertilization rate and subsequent embryonic development (Thundathil et al., 1999), with failure of cleavage being the main problem. Some bulls normally produce spermatozoa that are relatively pyriform in shape and, as a consequence, can be relatively subfertile.

Acrosomal defects are also associated with reduced fertility. Of these, the knobbed acrosome defect is best known. It can be difficult to observe (Barth and Oko, 1989), yet the present author has found it relatively easy to detect in carefully made eosin–nigrosin smears. It is a common incidental finding at low percentages, but can occur at high percentages (25–100%; Barth, 1986) as a familial, or, in the case of the Friesian, inherited defect. Moderate percentages of abnormal sperm are associated with reduced CR (Andersson et al., 1990), but bulls with high percentages are virtually sterile. Thundathil et al. (2000) used sperm with knobbed acrosome defects for in vitro fertilization, finding that no sperm with the defect penetrated the zona pellucida and that embryos that were fertilized by 'normal' sperm from affected bulls had a slower rate of cleavage than those from control bulls.

Nuclear vacuoles are the most difficult of the head abnormalities to visualize in eosin–nigrosin smears, but can usually be seen in wet preparations or with phase contrast microscopy. The best known is the diadem defect, which is seen as a series of refractile lesions at the base of

the acrosome. Other vacuoles can appear as single or multiple lesions of the nucleus. It is common to observe occasional sperm with vacuoles in normal semen, but bulls with a high percentage of vacuolated sperm are severely infertile or sterile (Miller et al., 1982; Saacke et al., 1995). Some vacuoles are undetectable by light microscopy, but can be found by electron microscopy in bulls with unexplained low fertility (Barth and Oko, 1989; Parkinson et al., 1993).

Non-specific acrosomal abnormalities are variably associated with reduced fertility; for example, Meyer and Barth (2001) found that bulls with high percentages of abnormal acrosomes achieved normal CR except in competitive mating trials. Other acrosomal defects were classified by Blom (1983) as major defects, reflecting the relatively large effects that they have upon fertility.

Midpiece and tail abnormalities of Sperm

Detached heads occur commonly at low to moderate percentages in normal ejaculates and are only associated with infertility if present at high percentages. The abnormality was classified as minor by Blom (1983) and the presence of 30–40% of detached heads in an ejaculate is not associated with infertility (Johnson, 1997). However, a rather more serious defect occurs in the Hereford, Guernsey and Brown Swiss breeds (Blom and Birch-Anderson, 1970; Blom, 1977a; Kozumplik, 1990), in which nearly 100% of sperm are decapitated. Interestingly, the detached tails are motile, so the gross motility of the semen appears normal to a cursory examination. This defect is probably inherited. Similarly, the ‘tail-stump’ defect, in which morphologically normal

heads are attached to a vestigial structure which appears like a protoplasmic droplet, occurs as an inherited condition of several breeds of bull (see Blom, 1976; Blom and Birch-Anderson, 1980). On electron microscopy, this droplet-like structure can be seen to consist of small segments of flagellar material, which represent a vestigial tail that if present will make bulls sterile.

Defects of the midpiece and tail generally arise as defects of spermatogenesis, and sperm with such abnormalities are either non-motile or have subnormal motility (Barth and Oko, 1989). Consequently, the presence of such abnormalities is generally associated with subfertility (Blom, 1983). It is also common to find sperm with gross deformities of the tail and midpiece in association with a wide range of other abnormalities (i.e. abnormal heads, detached heads, proximal droplets, etc.) in animals that are suffering from testicular degeneration (Parkinson, 1987, 2001). It is rare for the coiled tail to occur at high percentage, but the apparently-similar Dag defect, in which the tail appears as a loose coil, occurs at low to moderate percentages as an acquired defect, or at high percentage (50–100%) as an inherited defect of the Jersey (Barth, 1997). Bulls with a high percentage of Dag defects are either sterile or of very low fertility (Blom, 1966). The distal midpiece reflex abnormality resembles a looped tail with a droplet enclosed within the loop. It has little effect upon fertility in AI, but causes subfertility in natural service sires (Barth, 1992; Johnson, 1997). The simple looped tail (i.e. with no enclosed droplet) is generally regarded as a tertiary (post ejaculation) defect, which arises when sperm are subjected to osmotic or temperature shock. The defect can also be present when the pH of seminal plasma is abnormal (e.g. in animals with seminal vesiculitis), or in the company of mixed abnormalities in animals with testicular degeneration. Abaxial implantation of the tail is no longer regarded as a significant abnormality, since it does not affect fertility (Barth, 1989; Pant et al., 2002). The presence of accessory tails is not significant at low percentage, however when present at high percentage cause infertility (Williams and Savage, 1925).

Gossypol appears to exert unique and selective effects upon the male reproductive system, which includes reduced spermatogenesis and sperm motility, associated with morphological aberrations of the sperm midpiece (Chenoweth et al. 1994). Risco et al. (1993) reported an

increase in midpiece abnormality and erythrocyte fragility in Brahman bulls fed 2.75 kg day^{-1} of cottonseed meal (8.2 g day^{-1} of free gossypol). Chase et al. (1994) reported a similar damage to seminiferous epithelium for Brahman bulls fed either 1.8g day^{-1} of free gossypol from cottonseed meal or 16 g day^{-1} of free gossypol from whole cottonseed indicating that the type of cottonseed product (whole versus meal) might play a fundamental role in toxicological effect of gossypol. It has been suggested that detoxification of gossypol in the rumen is more efficient with whole cottonseed that with cottonseed meal.

Cytoplasmic droplets of Sperm

Spermatozoa are released from the seminiferous epithelium with their residual cytoplasm in the form of a droplet just behind the head (proximal droplet). During passage of the epididymis, the droplet first migrates to the distal end of the midpiece (distal droplet), and then is lost entirely during sperm maturation (Rao et al., 1980). Distal cytoplasmic droplets are not generally regarded as serious abnormalities (Blom, 1983; Johnson, 1997). Although they can occur at high percentages in animals with defects of sperm maturation, such as epididymal dysfunction, or, more commonly in young bulls and overuse (Barth, 1997). On the other hand, proximal droplets are a more serious abnormality, usually regarded as a defect of spermatogenesis (Johnson, 1997), especially where they occur with other, mixed, abnormalities. When proximal droplets are present at significant percentages, substantial impairment of fertility results. For example, Blom (1977b) suggested that ejaculates containing as little as 5–10% of proximal droplets are associated with poor fertility, whilst Nothling and Arndt (1995) showed that a bull with a high percentage of proximal droplets achieved a low pregnancy rate in a mating trial. Likewise, Soderquist et al. (1991) and Saacke et al. (1995) showed that the proportion of sperm with proximal droplets is related to nonreturn rate to AI and to pregnancy rate in superovulated heifers, respectively. Furthermore, when sperm with proximal droplets have been

used in vitro fertlization, cleavage rates of embryos are poor (Amann et al., 2000). Proximal droplets are, however, common in the first few ejaculates from peri-pubertal bulls. Several studies have shown that immature bulls have high percentages of proximal droplets in the peri-pubertal period, but that these rapidly decline to normal levels thereafter (Evans et al., 1995; Johnson et al., 1998; Arteaga et al., 2001; Padrik and Jaakma, 2002). Most older bulls display low (but quite repeatable) percentages of proximal droplets (Soderquist et al., 1996), and the mean percentages of affected sperm increases during warmer seasons of the year (Sekoni and Gustafsson, 1987). Where a high proportion of proximal droplets occur in mature bulls (often with other, mixed, abnormalities) it reflects a perturbation of the spermatogenesis process.

Venereal Diseases in Bulls

Venereal diseases in bulls cause reproductive wastage and result in enormous economic losses to the dairy industry. Among the venereal diseases reported in the United Sates campylobacteriosis and trichomonosis are the most prevalent and most significant venereal causes of reproductive loss (Ball et al. 1987). In both, losses are caused by infertility, embryonic death, and abortions due to salpingitis, endometritis, and cervicitis. (Parsonson et al. 1976 and Roberts, 1986).

Campylobacteriosis and trichomonosis are nearly identical in their clinical presentation in dairy cows, however their control is completely different. Control of either, as with other diseases, must includes attempts to stop transmission, eliminate the infection in affected animals, and prevent reintroduction of the organism (Abbit and Ball, 1978. Abbit, 1980 and Abbit, 1981). Although epidemiologic aspects are similar for the two diseases, control of campylobacteriosis is easier because effective vaccines are available.

There are commercially available vaccines that are effective for the control of Campylobacteriosis that should be used as the principal method of prevention and control in

bull-bred herds (Ball et al., 1987). It is important to emphasize that vaccines are only effective if used according to label directions. In general, vaccines adjuvinated with modified Freund's (oil) adjuvant give better immunity (Vasquez et al., 1983).

Trichomonosis must be controlled by considering epidemiological aspects of its transmission. Trichomonosis is caused by the protozoan parasite, *Tritrichomonas foetus*. The bovine disease is characterized by pregnancy loss, usually early and occult, although abortions of fetuses up to 7 months' gestation have been reported (Abbitt, 1980; Fitzgerald, 1986; Skirrow, 1987; BonDurant and Honigberg, 1994; BonDurant, 1997, 2005). It is highly prevalent in naturally bred cattle; in California, an estimated 16% of beef herds are infected (BonDurant et al., 1990) and infection in dairy herds that use bulls is not uncommon (Goodger and Skirrow, 1986). A larger survey of Florida beef cattle reported a herd prevalence of 30.4% (Crews et al., 2000).

The disease is insidious in that neither infected cows nor bulls show overt signs but tremendous economic losses are realized due to the reduction in calving rate following a herd infection (Rae, 1989). Estimates of the national cost of bovine trichomoniasis, based on limited prevalence surveys and market prices of more than 15 years ago, range as high as $600 million or more (Speer and White, 1991).

Only one species of the bovine pathogen is described (Honigberg, 1978). In cattle, the organism is considered an obligate parasite of the reproductive tract (BonDurant, 1997), so transmission to the female is presumed to occur solely during coitus with an infected bull (Goodger and Skirrow, 1986).

Trichomonad organisms live on the epithelium and the epithelial crypts of the gland penis and prepuce of bulls (Ball et al., 1984 and Porsonson el al. 1974). Since these crypts develop as

the bull ages, the habitat for trichomonads probably is more favorable in older bulls (Samuelson, 1966). Several authors reported episodes where young bulls of most breeds have been found sporadically infected and bulls older than two years are persistent carriers (Abbit and Meyerholz, 1979, Christensen et al., 1986, Clark et al., 1974 and Kimsey et al., 1980). Thousands of trichomonads are required for a consistently infective dose (Clark et al. 1974). Under heavy breeding pressure, the population of trichomonads on the penis of carrier bulls may be depleted to the extent that some cows do not receive an infective dose when they are bred, but when carrier bulls are under reduced breeding pressure transmission is likely to approach 100 per cent (Clark et al., 1983).

Some old and most young bulls are resistant to infection with Tritrichomonas foetus. However, even resistant bulls may mechanically transmit trichomoniasis by first breeding an infected and then susceptible female within 20 to 30 minutes (Clark et al., 1977). Mechanical transmission of trichomonads does not occur naturally between cows by means other than venereal disease and thus does not occur when infected cow are not in estrus. Artificial mechanical transmission can occur under some conditions (Goodger and Skirrow, 1986).

Risk of chronic (often life-long) trichomonosis infection in the bull increases with age (Abbitt, 1980; Fitzgerald, 1986; Skirrow and BonDurant, 1988; BonDurant et al., 1990; BonDurant, 2005) contrast, yearling bulls are relatively resistant to chronic infection with ‘‘true’’ T. foetus. Even though trichomonosis is thought to be only transmitted sexually, several studies have reported trichomonad isolations from virgin bulls (BonDurant et al., 1999; Hayes et al., 2003; Grahn et al., 2005).

Trichomonads infections are usually self-limiting in the cow, and very few infected cows maintain the infection throughout gestation. Cows that do carry the organism through gestation

may have the ability to infect susceptible bulls during the subsequent breeding period (Bartlett, 1947, Bartlett and Dickmans, 1949 and BonDurant, 1985). Nevertheless, most cows that have calved normally are not infected. Therefore, with adequate management the impact of occasional carrier cow on reproduction should be small (Ball et al., 1987).

After initial infection, cows are convalescent for approximately 3 ½ months and then become temporarily immune to *T. fetus.* Most immune cows can be reinfected after approximately 1 year, but the subsequent convalescent period is only about 3 weeks long (Clark et al., 1974). Consequently, infection confers a degree of immunity and resistance that can be advantageous in some management schemes.

Campylobacteriosis is caused by *Campylobacter fetus* subspecies *venerealis* and is an infectious cause of infertility in cattle that is spread venerealy by asymptomatically infected bulls. Infection of cows can result in a uterine environment unsuitable for embryo implantation, leading to early embryonic loss (Ware 1980).

Exposure of bulls positive for Trichomonosis to a large proportion of an uninfected herd inevitably results in a marked decrease in pregnancy rate, approximately 20% of cows becoming pregnant in a mating period of 60 days (Carroll and Hoerlein 1972). Chronically infected herds may show suboptimal reproductive performance in subsequent years caused by transmission of infection to susceptible young stock entering the herd (Hoerlein et al. 1964).

The average pregnancy rate in such herds varies from year to year depending on the relative proportions of carrier, susceptible and immune breeding-age females, and of infected and non-infected bulls within the herd (Carroll and Hoerlein 1972).

Ball et al. (1987) reported that the most satisfactory way to control Trichomoniasis should be based on a stringent surveillance program of both bulls and cows and then to cull positive animals.

Comparison of Natural Service and Artificial Insemination

In the U.S.A., the combination of natural service and AI is the preferred breeding method in 43% to 84% of dairy producers (Caraviello et al. 2006; NAHMS 2002, de Vries et al. 2005, Smith et al. 2004 and Champagne et al. 2002).

Although, these producers understand the benefits of AI, they also recognize that detection of estrus in order to inseminate cows is inefficient because not all cows are identified in estrus due to: human errors, attenuated expression of estrus in high producing cows, and adverse responses to heat stress. Therefore, producers that use NS maintain that more cows are bred by NS compared to AI because human errors in estrus detection are avoided when bulls are used. The most common method for NS use is to comingle bulls with lactating cows and thus allow bulls to assume the role of both estrus detector and inseminator. This reduces perceived labor costs and eliminates human errors associated with estrus detection. Despite the popular use of natural service in dairy farms there has been limited research on the economics of this breeding program.

A study by Hillers et al. (1982) utilized systems analysis to study costs and returns of breeding dairy cows artificially or by natural service. In their analysis, semen cost, estrus detection cost, days open, and bull maintenance costs were included. In addition, genetic merit of sires, discount rates on expected returns, and probability of a female offspring reaching lactation were also included. Transmission of genetic advantage of sires used by artificial insemination over sires used by natural service to both daughter and grand- daughter was considered. Daughters of sires used for artificial insemination were superior to daughters of natural service

sires by 227, 454, or 682 kg (mature equivalent) milk for a necessary calving interval of 374, 384 and 394, respectively. Breeding was started 60 days into lactation and continued for 15 cycles (21 days each) or until 90% of the cows would be expected to have conceived. Conception rate from artificial service was decreased by 5% of total rate for the subsequent breeding. For comparing artificial insemination and natural service, an initial conception rate for artificial insemination of 50%, cost of keeping a bull of $15 per cow per year, semen cost of $8 per unit, costs for detection of estrus of $10 per cow per year, discount rate of 10%, a 40% chance of obtaining a lactating heifer replacement per conception, a cost of an additional day open of $1.50, and income over feed costs of $.099 per kg were applied. Calving interval was 365 days for breeding by natural service. Each increase in genetic advantage superiority of bulls used artificially over natural service bulls of 227 kg meant that the longer calving interval obtained with AI days longer resulted in the with the same expected returns. Other factors in the analysis had less effect on the comparison of artificial insemination with natural service than the genetic abilities of sires. Investment in herd health (reproductive) programs to increase breeding efficiency appeared economically sound.

Johnston et al. (1987) calculated the annual cost associated with maintaining a single herd sire for 2–2.5 years in a 40-cow Wisconsin dairy. Keeping a NS sire for breeding management costs US$ 22.03 per cow in 1987, which was less expensive than using AI, but the study did not consider the value of the genetic differences between the herd sire and AI sires.

Shaw and Dobson (2000) assessed the impact of a change from standard bull breeding to on-farm do-it-yourself AI in small dairies in the United Kingdom during a period of three years. According to their work, the cost of using natural service sires was approximately 76% of the cost of the on-farm AI program, once all of the cash costs were considered. However, by the

second and third year, the AI program was more profitable due to the improved reproductive efficiency that resulted in increased milk production and more calves. These differences in cost and profitability were reported without any evaluation in potential benefit from genetic improvement that was likely in the AI program.

Overton (2004) estimated the explicit and implicit costs of natural service and AI including loss of genetic progress associated with the use of natural service sires in dairy herds. Overton used a partial budget approach to stochastically model the expected costs and returns of reproductive management options in large, western, Holstein dairies. Option one was comparison of natural service sires managed using currently recommended methods including breeding soundness evaluations, bull vaccination, and a rotational breeding system. Option two was an AI system using a modified Presync-Ovsynch timed AI program in conjunction with estrus detection and inseminations performed by a commercial route breeder. Stochastic variables in the model included the cost of the lactating ration and purchased bulls, as well as the value received for milk, market bulls, and net merit gains. All other variables were treated deterministically. Under his model's assumptions, the use of natural service sires averaged approximately US$ 10 more in cost per cow per year when compared to the AI program. Sixty percent of the time, AI was less expensive than using bulls. However, there was a wide variation in expected differences in cost between the two systems with net merit estimates having the largest impact, followed by prices received for milk sold and market bulls.

Valergakis et al. (2007) compared the costs associated with breeding cattle by do-it-yourself AI and NS in dairy farming conditions in Greece. A simulation study was designed based on data from 120 dairy cattle farms that differed in size (range 40 to 285 cows) and milk production level (4000 to 9300kg per cow per year). Different scenarios were employed to

estimate costs associated directly with AI and NS as well as potentially extended calving intervals due to AI. Results showed that bull maintenance costs for NS were €1440 to €1670 per year (US$1,820 to US$2,111). Direct AI costs were higher than those for NS for farms with more than 30 cows and extended calving interval constituted a considerable additional inconvenience. In fact, amongst the factors that affected the amount of milk needed to cover total extra AI costs, number of days open was the dominant one. Semen, feed and heifer prices had a very small effect. Hypothetically, use of NS bulls resulted in a calving interval of 12 months; AI daughters with a calving interval of 13.5 months have to produce about 705kg of additional milk in order to cover the extra cost. Their actual milk production, however, exceeds this limit by more than 25%. When real calving intervals were considered (13.0 v. 13.7 months for NS and AI, respectively) AI daughters turn out to produce more than twice the additional amount of milk needed. Valergakis concluded that even under less than average management conditions, AI is more profitable than the best NS scenario.

Reproductive performance of natural service versus AI has not been evaluated extensively. The first report comparing the aspects of fertility of cows bred by natural service or AI was by Elving and Govers (1975), where the difference between the non-return percentage (60-90 days) following 17,086 first inseminations and after 9,776 first natural matings in Dutch-Friesian cattle was studied in a random sample of the Netherlands Herd Book records in 1969. The effects of the following factors on this difference in the cases studied were considered: age of bulls and cows, frequency of natural service and artificial insemination during each estrus, use of deep-frozen semem, housing and grazing periods. After corrections of the difference for these factors, it was estimated that no more than 9 per cent of non-return 60-90 days in favor of natural service.

Williamson et al. (1978) used information routinely supplied during the conduct of a dairy herd health program to evaluate the performance of artificial breeding in Victoria province Australia. A comparison was made between 60 to 90-day non-return rates (supplied by artificial breeding centers) and pregnancy rates (determined by manual pregnancy diagnosis) for first artificial inseminations in 108 herd years in which both responses were available in the 1973 and 1974 breeding seasons. The values were 69.3% and 58.2% respectively ($P < 0.001$). Non-return rates and CR were found to decline as herd size increased. Pregnancy rates to first artificial and natural services did not differ significantly from each other, but pregnancy rates were significantly more variable to natural than artificial service ($P < 0.001$). The mean pregnancy rate to artificial insemination for all herd years studied was 57.5% and the pregnancy rate to natural service was 58.0%.

De Vries et al. (2005) evaluated the effects of artificial insemination and natural service breeding systems on pregnancy rates by stage of lactation, season, and changes in milk production over time using lactation and herd DHIA records of Holstein cows in dairy herds located in Florida and Georgia. The reported genetic profile of service sires of the herd was used to determine the percentage of cows bred to natural service bulls (%NS). Two seasons were considered: winter (November–April) and summer (May–October) from 1995 to 2002 (16 periods). Herd-periods were assigned 1 of 3 breeding systems: AI (0 to 10% NS), mixed (11 to 89% NS) and NS (90 to 100% NS). Seventy percent of the herds used NS bulls as a component of their breeding system during the study period. The PR during winter (17.9%) was greater than that during summer (9.0%). During winter, PR for AI herds (17.9%) did not differ from that for mixed (17.8%) and NS herds (18.0%). During summer, PR for AI herds (8.1%) was slightly less than that for mixed (9.3%) and NS herds (9.8%). During winter, PR for cows at 71 to 91 d, 92 to

112 d, and 113 to 133 d in milk were 1.4% lower for mixed herds compared with AI and NS. Pregnancy rate for NS herds was 2.6% lower during late lactation compared with AI and mixed herds. During summer, PR for cows at 71 to 91 and 92 to 112 days in milk were 2.6% and 1.8% greater, respectively, for NS herds compared with AI. However, from 260 to 364 d in milk, PR for NS herds was lower than that for AI and mixed herds. No significant interaction was detected between breeding system and lactation number. Rolling herd average milk production during the study period was lower in the NS herds (7180 kg) than mixed AI plus NS herds. However, the annual change in milk production over the seven year study period was not different among breeding systems. The results indicated that use of NS bulls did not result in meaningful disadvantages in terms of PR and changes in milk production overtime.

Overton and Sischo (2005) compared the calving to conception intervals for cows in AI pens with cows exposed to natural service sires, controlling for milk production, mastitis occurrence, parity and calving month effects. Records from ten western U.S.A. dairy herds (mean herd size = 2058 cows) were evaluated retrospectively over an 18-month period. Eight bull breeding analysis cohorts were created (the first cohort 0–50 days in milk and the remaining cohorts at 25 days in milk intervals through 226 days). The cohorts contained non-pregnant cows that were first moved into bullpens during the described cohort period. Equal numbers of non-pregnant cows only exposed to AI during the cohort period were selected randomly from the pool of eligible non-pregnant cows. An AI cow was used only once in the data analysis but was included in a bull breeding cohort at a later date if she remained non-pregnant and was transferred to a bullpen. Cows in AI groups had higher hazard rates for pregnancy across all cohorts. Parity and milk production were significantly associated with risk for pregnancy resulting in decrease days to pregnancy. Overton and Sischo (2005) concluded that herds that

practice a combination of AI and bull breeding, overall herd reproductive performance might be improved by allowing cows more AI opportunities prior to moving them into the clean-up bullpens.

Table 2-1. Criteria for determination of the stage of the estrous cycle, or presence of ovarian cysts or anestrus based on ultrasonography and per rectum palpation of the genital tract (These criteria were adapted from Zemjanis, 1962; Pierson and Ginther, 1984 and 1987)

Stage	Clinical Findings	
	Ovaries	Uterus
Diestrus	Functional CL, follicle > 10 mm	Slight tonus
Metestrus	Corpus hemorrhagicum, follicle < 10 mm	Edema and moderate tonus
Proestrus/Estrus	Follicle ~18 mm, regressing CL	High tonus
Ovarian cysts	Multiple follicles ~18 mm, absence of CL	Flaccid
Anestrus	Follicle < 18 mm	Flaccid

(Adapted from Bartolome et al., resynchronization of ovulation and timed insemination in lactating dairy cows use of the ovsynch and heatsynch protocols after non-pregnancy diagnosis by ultrasonography. 2005.Theriogenology 63: page 1620).

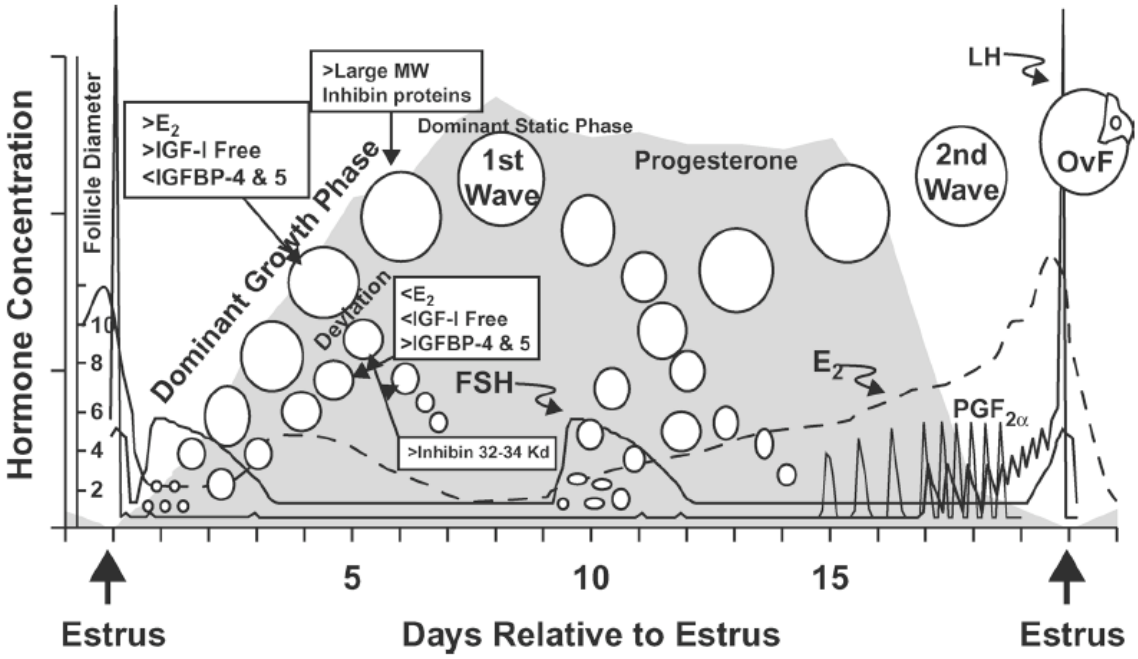

Figure 2-1. Depicts the continuity and interaction of events occurring during the estrous cycle in cows with substantial detail of variation of hormones involved. (Adapted from Moore and Thatcher, Major advances with reproduction of dairy cattle. 2006. Journal of Dairy Science. 89:page 1255)

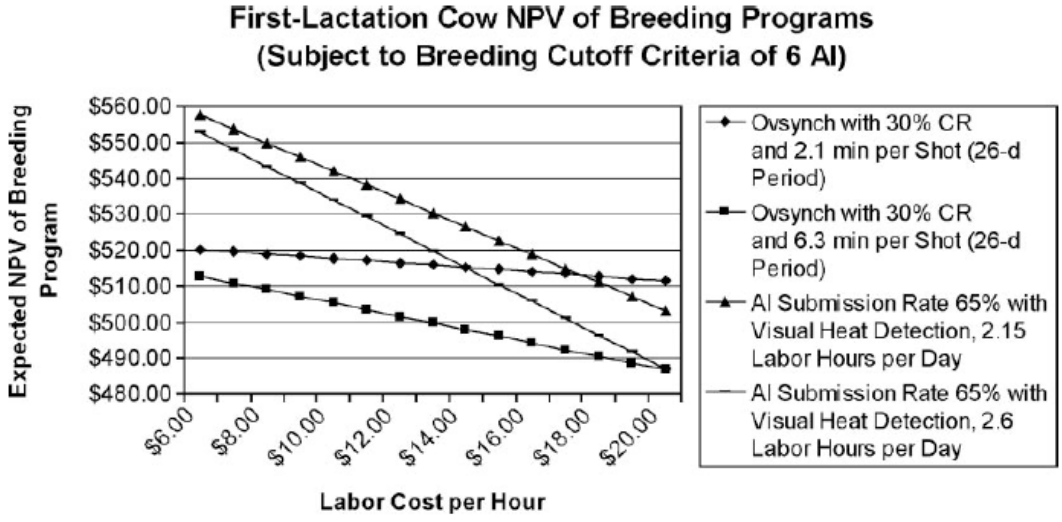

Figure 2-2. Net present values (NPV) of reproductive management programs for first-lactation cattle. CR = conception rate (Adapted from Olynk and Wolf, 2008 Analysis of Reproductive Management Strategies on US Commercial Dairy Farms. Journal of Dairy Science. 91:4088)

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Bull Breeding Soundness Evaluation

Guidelines Established by Society for Theriogenology
530 Church Street, Suite 700 • Nashville, TN 37219
Phone 615/244-3060 • FAX 615/254-7047 • www.therio.org

OWNER	CASE NO.	DATE
ADDRESS	BULL NAME	BREED
ZIP	I.D. NO.	Brand ❑ Tattoo ❑ Ear Tag ❑
TELEPHONE ( )	BIRTH DATE	AGE (MO.)
HISTORY: Previous BSE — DATE	CASE NO.	CLASSIFICATION

PHYSICAL EXAMINATION

Body Condition Score _____ Thin ❑ Moderate ❑ Good ❑ Obese ❑
Beef 1, 2, 3, 4, 5, 6, 7, 8, 9 Pelvic Ht. _____ Width _____ Area _____
Dairy 1, 2, 3, 4, 5

Feet/Legs	❑
Eyes	❑
Vesicular Glands	❑
Ampullae/Prostate	❑
Inguinal Rings	❑
Penis/Prepuce	❑
Testes/Spermatic Cord	❑
Epididymides	❑
Scrotum (Shape)	❑
Other	

SCROTAL CIRCUMFERENCE (CM) _____ _____

This bull has been examined for physical soundness and quality of semen only. Unless otherwise noted, no diagnostic tests were undertaken for libido, mating ability or infectious disease status of this bull.

Remarks and Interpretation (diagnosis, prognosis, recommendations)

SEMEN EXAMINATION

Collection Method: EE ❑ AV ❑ Massage ❑
Response: Erection ❑ Protrusion ❑ Ejaculation ❑

Semen Characteristics	Ejaculate 1	Ejaculate 2
Motility — Gross		
Motility — (or) Individual (%)		
% Normal Cells		
% Primary Abnormalities		
% Secondary Abnormalities		
WBC, RBC, Other		

CLASSIFICATION

Interpretation of data resulting from this examination would indicate that on this date, this bull is a:

❑ Satisfactory potential breeder
❑ Unsatisfactory potential breeder
❑ Classification Deferred

Re-examination recommended on _____
DATE

Signed: _____
MEMBER—SOCIETY FOR THERIOGENOLOGY

Clinic:

Figure 2-3. First page of guidelines established by the Society for Theriogenology for breeding soundness evaluation in bulls. Chenoweth, P.J. 1992. A new bull breeding soundness evaluation form. Society for Theriogenology. In Proc. Annual Meeting, August 14-15, San Antonio, Texas, page. 69)

Reference Tables for Evaluation of Scrotal Circumference and Spermiogram

Minimum Recommended Scrotal Circumference Age	SC(CM)
≤ 15 MO	30
> 15 ≤ 18 MO	31
> 18 ≤ 21 MO	32
> 21 ≤ 24 MO	33
> 24 MO	34

Minimum Recommended Motility is: 30% or FAIR (F)		
Mass Activity (Gross)	Rating	Individual
Rapid Swirling	Very Good (VG)	≥ 70%
Slower Swirling	Good (G)	50 - 69%
Generalized Oscillation	Fair (F)	30 - 49%
Sporadic Oscillation	Poor (P)	< 30%

Sperm Morphology

Minimum Recommended Morphology is: 70% Normal Cells

Primary sperm Abnormalities
- Underdeveloped
- Double forms
- Acrosome defect (e.g. knobbed acrosome)
- Narrow heads
- Crater/Diadem defect
- Pear-shaped defect
- Abnormal contour
- Small abnormal heads
- Free abnormal heads
- Abnormal midpiece
- Proximal droplet
- Strongly folded or coiled tail
- Accessory tails

Secondary sperm Abnormalities
- Small normal heads
- Giant and short broad heads
- Free normal heads
- Detached, Folded, Loose acrosomal membranes
- Abaxial implantation
- Distal droplet
- Simple bent tail
- Terminally coiled tail

Other cells
- Epithelial cells
- Erythrocytes
- Medusa formations
- Sperm precursor cells
- Round cells
- White blood cells

For more information on sperm morphology refer to:
Abnormal Morpology of Bovine Spermatozoa.
A.D. Barth and R.J. Oko. 1989. Iowa State University Press, Ames.

To be classified a Satisfactory Potential Breeder, requires a satisfactory Physical Examination and minimum values for Scrotal Circumference, Motility and Morphology. Any bull not meeting minimums is classified either as an Unsatisfactory Potential Breeder or classification may be Deferred at the discretion of the evaluator.

*It should be noted that it is common for yearling bulls, due to immaturity, to require a second fertility examination to achieve satisfactory potential breeder status.

Figure 2-4. Second page of guidelines established by the Society for Theriogenology for breeding soundness evaluation in bulls. Chenoweth, P.J. 1992. A new bull breeding soundness evaluation form. Society for Theriogenology. In Proc. Annual Meeting, August 14-15, San Antonio, Texas, page 70).

CHAPTER 3
COMPARISON OF REPRODUCTIVE PERFORMANCE IN LACTATING DAIRY COWS BRED BY NATURAL SERVICE OR TIMED ARTIFICIAL INSEMIANTION

Introduction

Despite considerable advantages for AI, a significant number of dairy producers use natural service (**NS**) for their breeding program. In a survey on bull management practices in California, 84% of the producers reported use of NS as a component of their breeding program (Champagne et al., 2002). The most common use of NS was after unsuccessful AI attempts.

Several studies have compared reproductive performance between AI and NS breeding systems. Pregnancy rates (PR) obtained from dairy herd records of cows bred by AI or NS were not different (Niles et al., 2002; Williamson et al., 1978), but PR from NS were more variable in NS herds (deVries et al., 2005).

A breeding program that synchronizes ovulation and AI at a fixed time (timed AI; TAI) has been developed which allows for 100% of the cows to be submitted to AI, without the need for estrous detection (Pursley et al., 1995). There have been ample studies that have clearly demonstrated an advantage in pregnancy rate of TAI over insemination at detected estrus (Pursley et al., 1997a; Pursley et al., 1997b; Cartmill et al., 2001). However, studies that compare reproductive performance between NS and TAI, two breeding systems where efficiency of estrous detection is not a factor, are lacking. It was hypothesized that the use of TAI would result in a greater 21-d cycle pregnancy rate than NS because in TAI, cows undergo ovulation synchronization and all eligible cows are inseminated at a fixed time. Therefore, the objective of this study was to compare reproductive performance of lactating dairy cows bred by TAI or NS, two breeding system in which estrous detection is not required.

Materials and Methods

Animals, Housing and Diets

The University of Florida Institutional Animal Care and Use Committee approved all procedures involving cows and bulls. The study was conducted between November 2006 and March 2008 in a commercial dairy farm of 2,200 Holstein cows located in north central Florida. Cows were housed in free-stall barns with fans and sprinklers for forced evaporative cooling during the warm season. A total of four barns (2 TAI; 2 NS) each with a maximum capacity of 180 cows were used in the study. These barns were similar in design, size, and number of animals housed and were switched between and within seasons to avoid environmental bias.

Resting bulls were housed in bermudagrass pasture lots with portable shades and trees for heat abatement. Lactating cow diets were formulated using the CPM-Dairy cattle ration analyzer (Cornell-Pen-Miner Ver. 3.0.7a) to meet or exceed the nutrient requirements established by NRC (2001) for lactating Holstein cows weighing 650 kg, consuming 24 kg of DM per d, and producing 45 kg/d of milk containing 3.5% fat and 3.1% true protein during the first 80 d of lactation. The composition of the diets consisted of corn silage, alfalfa hay, ground corn, citrus pulp, cottonseed hulls, expeller soybean meal (SoyPlus, West Central Soy, Ralston, IA) and solvent extracted soybean meal. Bulls were fed the lactating cow diet during the 2 wk cow exposure period and lactating cow diet weigh back that averaged 17.2 kg of DM per bull per d during the 2 wk rest period.

Study Design, Treatments and Exclusion Criteria

Lactating Holstein dairy cows were blocked by parity (primiparous and multiparous) and within each block randomly allocated at 42 ± 3 d post partum into two groups, TAI (n=543) and NS (n = 512), once a week until completion of the study. Prior to study enrollment cows underwent a reproductive tract examination and health record evaluation. Cows with uterine

infection or adhesion, a displaced abomasum, c-section or fetotomy, and cows that missed any part of their experimental protocol were not included in the study. After enrollment cows that were sold or died or missed any part of their protocol were removed from the study. Eighty nine cows from the TAI group and 118 cows from NS cows were removed accordingly.

Timed Artificial Insemination Reproductive Management

Cows in the TAI group were presynchronized with 2 injections of $PGF_{2\alpha}$ (500 µg cloprostenol sodium; Estroplan®, Pfizer Animal Health, New York, NY) given at 42 ± 3 and 56 ± 3 d post partum. Fourteen d after the second injection of $PGF_{2\alpha}$ cows were given an injection of GnRH (100 µg gonadorelin; Fertagyl®, Intervet Inc, Millsboro, DE) followed 7 d later by an injection of $PGF_{2\alpha}$, and a second injection of GnRH 56 h after the last dose of $PGF_{2\alpha}$. The TAI was performed 16 h after the second injection of GnRH. Eighteen d after TAI, cows received a controlled internal drug-releasing insert (CIDR Eazi-Breed®; Pfizer Animal Health; New York, NY) followed by insert removal and GnRH administration 7 d later, on d 25 after TAI. Cows were evaluated for pregnancy by ultrasonography examination at 32 d after TAI. The reproductive program for TAI cows is illustrated in figures 3-1 and 3-2. The presence of an embryo with a heartbeat was the criterion used to determine pregnancy as previously described (Ginther, 1998). The re-synchronization chosen aimed to maximize reproductive efficiency and to allow cows to be re-inseminated immediately after the diagnosis of non-pregnancy. Based on ovarian dynamics at 18 d after AI, a pre-ovulatory follicle is potentially present and insertion of a CIDR device will maintain this follicle and not allow ovulation in cows with a regressed CL. When GnRH is administered 7 d later, at CIDR insert removal (d 25 after insemination in TAI cows), ovulation of the follicle is expected and a new follicular wave initiated. Therefore, cows found non–pregnant at 32 d after AI would be ready to receive $PGF_{2\alpha}$ and complete the modified Ovsynch protocol at 35 d from their previous breeding. In this manner, the modified Ovsynch

protocol begins 7 d before the diagnosis of non-pregnancy, which shortens the interval between inseminations.

Cows diagnosed pregnant were re-examined by transrectal palpation of the uterus and its contents 28 d later (i.e., 60 d gestation) to reconfirm pregnancy status and to identify pregnancy loss. Cows diagnosed not pregnant at 32 d after TAI were administered $PGF_{2\alpha,}$ followed with an injection of GnRH at 56 h after $PGF_{2\alpha}$ and TAI was performed 16 h after GnRH. Non pregnant cows were re-synchronized again with the same TAI protocol until diagnosed pregnant or at a maximum of 223 d post partum.

Natural Service Reproductive Management

Cows in the NS group received $PGF_{2\alpha}$ at d 42 ± 3 and 56 ± 3 and were moved to a bull pen at 70 ± 3 d post partum. Cows were moved to the bull pen 14 d after the last $PGF_{2\alpha}$ (70 ± 3 d post partum) to improve synchronized estrus and to have the bull breeding at around 80 d post partum, i.e. similar to the first service in the TAI group. After 42 d of being exposed to the bulls, cows underwent an ultrasonography and trans rectal examination to determine pregnancy status. This allowed a diagnosable gestation length in pregnant cows to vary from 28 to 42 d. The reproductive program for NS cows is illustrated at figures 3-1 and 3-2. Gestation age was estimated by measurement of embryo size, presence of a heartbeat by ultrasound (Ginther, 1998), and the diameter of the pregnant uterine horn and length of the amniotic vesicle by transrectal palpation (Zemjanis, 1970). Gestation age from 28 to 34 d was determined by ultrasound, from 35 to 56 d by ultrasound in combination with transrectal palpation.

Cows diagnosed non pregnant were re-examined for pregnancy status by ultrasound 28 d later to detect pregnancy in cows that were less than 28 d of gestation at the previous ultrasound diagnosis (i.e. 28 to 56 d pregnant), utilizing the same criteria described above for ultrasound and transrectal palpation. This procedure was similar for subsequent groups assigned weekly to the

NS up to 223 d post partum. Cows diagnosed pregnant were re-confirmed 28 d later to identify pregnancy loss. The bull to cow ratio in the NS herds was one bull per 20 non-pregnant cows. Day post partum when pregnancy occurred in NS bred cows was calculated by subtracting the d of pregnancy from the d post partum when pregnancy was diagnosed. For example, a cow diagnosed pregnant 32 d at 130 d post partum was pregnant at 98 d post partum (i.e. 130 to 32 d). The interval between services in the timed AI group was 35 d due to the process of carrying out the re-synchronization protocol. Therefore, for cows in the TAI group, d post partum when pregnancy occurred to first, second, third, fourth or fifth service were classified as follows: d 80 ± 3, first service; d 115 ± 3, second service; d 150 ± 3, third service; 185 ± 3 d, fourth service, and 220 ± 3 d fifth service. For cows in the NS group, when pregnancy was diagnosed from 28 to 56 d, first, second, third, fourth, fifth, sixth, seventh or eighth services were classified at d 70 to 90, d 91 to 111, d 112 to 132, d 133 to 153, d 154 to 174, d 175 to 195, d 196 to 216 and d 217 to 223 post partum, respectively. A cow in the NS group diagnosed 40 d pregnant at 150 d post partum would have conceived at 110 d (i.e. 150 - 40 d) post partum or at her second service.

Pregnancy Loss

Because of the reproductive management schemes, NS cows were at different stages of gestation when pregnancy was diagnosed compared to TAI cows, which were consistently diagnosed pregnant at 32 d. Consequently, stage of gestation when pregnancy was diagnosed in the NS group was categorized by 4 d intervals (28 to 32, 33 to 36, 37 to 40, 41 to 44, 45 to 48, 49 to 52 and 53 to 56) in an attempt to discern the effect of embryonic age on pregnancy loss.

Bull Management

Twenty-six bulls 18-months old at the beginning of the study were used. All bulls underwent a breeding soundness evaluation according to the guidelines of the Society for Theriogenology (Chenoweth, 1992) before cow exposure. In addition, bulls were tested for

Tritrichomonas foetus using a smegma sample cultured in a modified diamond media (InPouch™ TF, Biomed Diagnostics, White City, OR). Both of these tests were performed every 3 mo for a total of five evaluations for each bull. All bulls were rested for 14 d after 14 d of cow exposure. The breeding soundness evaluation included a physical examination, testicular evaluation, measurement of scrotal circumference and evaluation of sperm morphology and motility following electro ejaculation. Only bulls classified as potential satisfactory breeders were used. This classification requires: a minimum of 31 cm for scrotal circumference for bulls between 15 to 18 mo of age; a minimum of individual sperm motility of 30%; and 70% normal spermatozoa. In addition, the bulls were tested for bovine viral diarrhea by imunohistochemistry of skin using an ear notch sample. All bulls were vaccinated according to farm operational practices for infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, bovine respiratory syncytial virus, leptospirosis, clostridial diseases, and campylobacteriosis.

Milk Production Data and Body Condition Score

Milk weights were recorded once a month for 988 cows (n = 484 and n = 504 for NS and TAI, respectively). First measurements occurred at different d after calving for the first monthly sample depending upon d of parturition and d of the monthly milk test for cow and herd, respectively. Because cows were randomly assigned to the treatments on a weekly basis, the first d post partum measurement was balanced between groups. Data for the first 3 mo of lactation were obtained from the Dairy Herd Improvement association (Raleigh, NC). Cows in both the NS and TAI groups underwent a BCS evaluation at 70 ± 3 d post partum before introduction to the bulls in NS or receiving the GnRH injection in TAI using a scale of 1 to 5 according to Ferguson et al. (1994).

Temperature Humidity Index

The temperature (°F) and relative humidity (%) data were obtained from the Florida Automated Weather Network (http://fawn.ifas.ufl.edu/scripts/reportrequest.asp). The data were collected from January 2007 to March 2008. The weather station is located in Alachua, FL, approximately 30 miles from the experimental location. Average daily temperature humidity index (**THI**) was calculated as described by West (1993): THI = temperature (◦F) − [0.55− (0.55 x relative humidity)] x (temperature - 58). The THI was the criterion used to determine effect of season (warm or cold) on reproductive performance. The maximum daily THI was categorized as cool, when THI < 72, or warm when THI ≥ 72. The THI on the d of the first TAI or the first d of exposure to bulls was used in the statistical analysis.

Blood Sampling and Evaluation of Cyclicity

Blood samples (~6 mL) were collected from a subset of 608 cows (NS = 302 and TAI = 306) before the second $PGF_{2\alpha}$ of the presynchronization program and again 9 d later, which corresponded to 56 ± 3 and 65 ± 3 d post partum. Sampling was collected by puncture of the median coccygeal vein or artery using evacuated tubes containing K_2 EDTA for plasma separation (Becton Dickinson, Franklin Lakes, NJ). Samples were placed immediately in ice and transported to the laboratory within 5 h of collection. Blood tubes were centrifuged at 1,500 × *g* for 15 min, and plasma frozen at −25°C until analysis. Analysis of progesterone in plasma was determined using a solid phase radioimmunoassay (Coat-A-Count, Progesterone In-vitro Diagnostic Test Kit, Diagnostic Products Corporation, Los Angeles, CA). Plasma concentrations of progesterone of known values were used in duplicates in every assay to calculate inter- and intra assay coefficients of variation. The known samples were plasma from an ovariectomized cow (0.7 ng/mL), a low progesterone sample (1.0 ng/mL) and high progesterone sample (5.0 ng/mL. The inter- and intra-assay CV were 5.74% and 11.1%, respectively. Cows were reported

as cyclic if they had progesterone plasma concentration equal to or greater than 1.0 ng/mL in at least one of the two samples or non-cyclic if both samples were below 1.0 ng/mL.

Health Disorder Monitoring Program and Treatments

Farm personnel were trained and farm operational procedures were created by the herd veterinarian (corresponding author) to provide a reliable source for health monitoring and prompt treatment. All cows underwent a post partum health monitoring program consisting of daily evaluation of rectal temperature and attitude from d 2 to 10 pp. Rectal temperature was determined with the use of a digital thermometer (GLA M500HPDT, Agricultural Electronics, San Luis Obispo, CA) between 0700 to 0900 h after milking. Retained placenta was defined as the presence of fetal membranes 24 h after calving. Cows that either appeared sick (inappetence, depressed, sunken and/or tented eyes) or had a rectal temperature ≥ 39.4^{o}C were examined for vaginal discharge, urine ketones, displacement of abomasum, and respiratory disorder. The criterion for diagnosis of metritis was the presence of a watery, brown, fetid discharge from the vulva (i.e., noted after palpation per rectum of the uterus) with rectal temperature ≥ 39.4^{o}C. Urine was evaluated for ketonuria using urine test strips (Ketostix^{TM}, Bayer Diagnostics, Tarrytown, NY). Clinical ketosis was characterized by inappetence, depressed attitude and presence of ketonuria according to the degree of color change in the test strips. Cows were examined for clinical mastitis by herd personnel during each milking. A case of mastitis was characterized by either the presence of abnormal milk or by signs of inflammation in one or more quarters or by both situations. Respiratory disorder was categorized by the presence of abnormal lung sounds, cough, increased respiratory rate, decreased milk production and fever. Cows were examined for clinical lameness on a weekly basis as they walked out of the milking parlor to barns. Cows affected with lameness exhibited an arched back posture while standing and walking and an abnormal gait. Lame cows were examined on a tilt table for lesions and treatment

by a professional hoof trimmer. Morbidity was defined as the occurrence of at least one health disorder in the first 70 d post partum.

Statistical Analysis

The outcomes for comparison of reproductive performance between NS and TAI were the proportion of cows pregnant in the first 21 d of breeding, 21-d cycle pregnancy rate, daily rate of pregnancy in the first 223 d post partum, median d non pregnant, and proportion of pregnant cows at 223 d post partum. In order to evaluate the 21-d cycle pregnancy rate in both NS and TAI cows, an assumption was made that cows were eligible to be bred every 21 d in both groups, despite the fact that TAI cows had a breeding opportunity only every 35 d. Therefore a breeding eligibility period was considered every 21 d up to 223 d post partum for both groups.

The proportions of pregnant cows during the first 21 d of breeding and at the end of 223 d post partum were analyzed by logistic regression using the PROC LOGISTIC of SAS. The model included the effects of group (NS vs. TAI), parity (primiparous vs. multiparous), BCS categorized as ≤ 2.75 or > 2.75, season of breeding (warm vs. cool), morbidity, and interactions of group with parity, BCS, season of breeding and morbidity. Modeling was performed using backward stepwise selection with the significance level of stay when $\alpha \leq 0.10$. The model fit statistics were performed by comparison of the difference in the deviances by the likelihood-ratio statistic test. Adjusted odd ratio (AOR) and 95% confidence intervals (CI) were calculated.

The 21-d cycle pregnancy rate was analyzed using the GLIMMIX procedure for generalized linear mixed models from SAS (SAS/STAT, ver. 9.1, SAS Institute Inc.). The model included the effects of group, parity, BCS, season of breeding, and morbidity. The rate of pregnancy in the first 223 d post partum (daily pregnancy rate) and median d non pregnant were analyzed using Cox's proportional hazards regression model (PROC PHREG) and the Kaplan Meyer survival curves (PROC LIFETEST) of SAS. The Cox's model included effects of group,

parity, BCS categorized, season of breeding, morbidity, and the interactions of group and season of breeding, and group and morbidity. When interactions were non significant ($P > 0.10$), they were dropped from the model. The adjusted hazard ratios (AHR) and the 95% CI were calculated.

In order to determine the impact of milk yield in the first 3 mo post partum on reproductive performance of dairy cows, additional analyses were performed with 988 cows that had milk yield data of the 1055 cows in the study. The models were the same described above, but included milk yield either as a continuous variable or categorized into quartiles within primiparous and multiparous cows.

Cows d at risk was calculated as the cumulative number of d for cows between exposure to bulls and pregnancy or end of study in NS, and between first AI and pregnancy or end of study for the TAI group. The proportion of NS and TAI bred cows affected by health disorders were analyzed by chi-square. Pregnancy loss was analyzed by chi-square analysis using SAS, and it was categorized accordingly with the age of the embryo at the time pregnancy was diagnosed.

Differences with $P \leq 0.05$ were considered significant, and those with $0.05 < P < 0.10$ were considered a tendency.

Results

Assessment of Bulls

Six bulls (23.1%; 6/26) were culled during the study: two became lame, one developed a bad temperament, one had a positive test for *T. foetus*, and two were classified as unsatisfactory potential breeders at the completion of the breeding soundness evaluation.

Reproductive Performance

The proportion of pregnant cows in the first 21 d of breeding did not differ ($P = 0.17$) between groups (Table 3-1). The overall 21-d cycle pregnancy rate, which included a total of 8

and 5 service opportunities for NS and TAI, respectively, was not different between groups and they were 25.7 and 25.0 % for NS and TAI, respectively as shown in table 3-2. However, the rate of pregnancy differed (P = 0.05) between groups and was 15% greater (AHR = 1.15; 95% CI = 1.00 to 1.31) for NS than TAI (Figure 3-3.). The median and mean d to pregnancy for NS bred cows were, respectively, 111 (95% CI = 105 to 126) and 131.5 ± 2.3 d, and for TAI cows 116 (95% CI = 115 to 118) and 137.9 ± 2.9 d, respectively. The survival curves did not differ until 150 d post partum, when they began to separate. To reinforce this observation, an additional survival analysis was performed for the interval to pregnancy up to 150 d postpartum, in which time non-pregnant cows were censored. In the latter scenario, group did not influence (P = 0.44) the rate of pregnancy (AHR = 1.06; 95% CI = 0.91 to 1.23). Nevertheless, at 223 d post partum, which was the end point of the study, the proportion of pregnant was greater (P = 0.001) for NS than for TAI (NS = 84.8% and TAI = 76.4%). Cow d at risk for pregnancy was not different between NS and TAI, and they were 30,978 and 29,424 d, respectively.

Parity, Body Condition Score and Milk Production

Parity did not (P = 0.15) affect the proportion of cows pregnant in the first 21 d of breeding. However, a greater proportion (P < 0.001) of primiparous cows were pregnant at the end of 223 d of breeding than multiparous cows (87.7 vs. 77.8%) because primiparous cows had a 27% greater (P = 0.002) daily pregnancy rate than multiparous cows (AHR = 1.27; 95% CI = 1.09 to 1.48) showing in figure 3-4.

At 70 ± 3 d post partum, the distribution among BCS categories was not different (P = 0.66) between TAI and NS: BCS ≤ 2.75 were 52.5% for NS and 53.9% for TAI; BCS > 2.75 were 47.5% for NS and 46.1% for TAI. Cows with BCS greater than 2.75 had increased (P = 0.004) proportion of pregnant cows in the first 21 d of breeding (39.9 vs. 32.4%; AOR = 1.46; 95% CI = 1.26 to 1.89) as shown in figure 3-5 and also had an increased (P = 0.02) daily

pregnancy rate in the first 223 DIM (AHR = 1.18; 95% CI = 1.03 to 1.35). Despite those effects, no effect (P = 0.33) of BCS was observed for the overall cumulative proportions of pregnant cows at the end of the study which were 78.6% for cows with BCS ≤ 2.75 and 82.4% for cows > 2.75.

When additional analyses were performed with the subset of 988 cows with milk production data, the daily rate of pregnancy tended (P = 0.08) to increase with a concurrent increase in milk yield (AHR = 1.01; 95% CI = 1.00 to 1.01). At the end of 223 DIM, the proportion of pregnant cows was not (P = 0.23) affected by milk production in the first 3 months of lactation.

Cyclic Status, Seasonality and Health Disorders

Progesterone analyses indicated that 83.1 % (251/302) of cows in the NS group and 87.2 % (267/306) of cows in the TAI group were cyclic (progesterone > 1ng/mL in at least one sample) before exposure to NS or onset of the TAI protocol. The percentages of cyclic cows were not different (P=0.17) between groups, and cyclic status did not affect (P = 0.53) the proportions of pregnant cows in the first 21 d of breeding, which were 35.4% and 38.6% for non-cyclic and cyclic cows respectively.

Cows receiving their first breeding during the cool season had increased (P < 0.01) pregnancy in the first 21 d of breeding (41.2 vs. 27.7%), 21-d cycle pregnancy rate (27.5 vs. 22.5%), and daily pregnancy rate (AHR = 1.22; 95% CI = 1.06 to 1.41) as shown in table 3-6. It is important to indicate that no interaction between treatment and heat stress was observed for the proportion of cows pregnant in the first 21 d of breeding or pregnancy rates.

The only health disorder that was not (P = 0.03) distributed equally between TAI and NS was metritis (TAI = 6.6 % and NS = 10.3%). However, metritis did not affect the proportion of pregnant cows in the first 21 d of breeding (Table 3-3.). On the other hand, the proportion of

pregnant cows in the first 21 d of breeding was less in cows with mastitis ($P < 0.01$), respiratory disorder ($P < 0.05$), ketosis ($P < 0.01$), and morbidity ($P < 0.01$). Morbidity influenced ($P = 0.002$) the rate of pregnancy and healthy cows had a 28% greater daily pregnancy rate than cows affected by at least one health problem (AHR = 1.28; 95% CI = 1.09 to 1.49) as shown in table 3-7.

Pregnancy Loss

The overall pregnancy loss considering gestation period from d 28 to 56 in the NS group and 32 d for TAI, was lower ($P=0.02$) for NS than TAI bred cows, 10.4 % and 15.2%, $P <0.05$; respectively. However, in the NS group, pregnancy loss for cows diagnosed pregnant between 28 - 32 d of gestation was the same as for TAI cows at 32 d of gestation (14.9% and 15.2%, respectively) as shown in table 3-4.

Discussion

Following the recommendations of the Society for Theriogenology (Chenoweth, 1992) for breeding soundness, a culling rate of 23.1% occurred in bulls used in this study. Kastelic el al. (2000) reported that 20 to 40% of bulls from an unselected population might have reduced fertility. Because these bulls were classified as potential satisfactory breeders prior to cow exposure and were removed from the study and replaced with sound bulls, the potential for using sub-fertile bulls in the NS group was minimized. Despite the fact that bulls used in this study had repeatedly breeding soundness evaluations (every 3 mo for a total of 5 evaluations) which is more than what is commonly done, our culling rates were not greater than what is expected for service bulls in a random population (Kastelic et al., 2000). Our approach to bull evaluation and management was chosen to assure that sub-fertile bulls could be identified rapidly and removed from the herd avoiding a negative impact on reproduction.

The overall 21-d cycle pregnancy rate in the NS group of 25.7% was substantially greater than previously reported by de Vries et al. (2005) in NS herds in the states of Florida and Georgia where 21-d cycle pregnancy rate was 14.0%. Our results can be attributed to the stringent bull management program employed (periodic resting and breeding soundness evaluation) and early removal of unsound bulls, which reduced the potential for deviation in bull fertility during cow exposure. Cows in the TAI group had a first 21- d cycle pregnancy rate of 37.4%, similar to a pregnancy rate to first TAI of 37% and 37.9%, reported by Pursley et al. (1997) and Santos et al. (2009), respectively. The greater proportion of pregnant cows observed in the NS group at the end of the study is attributed to differences in breeding dynamics between groups. In the NS group, bulls had the potential for daily detection of estrus and breeding of non-pregnant cows. On the other hand, due to the TAI re-synchronization scheme, non-pregnant cows in this group required 35 d to be re-inseminated and thus the number of d to become pregnant increased. However, within this scenario, up to 223 d post partum cows in the TAI group had only five opportunities to be bred compared with a potential eight times for cows in the NS group. The increased median number of d to pregnancy observed for TAI cows can also be attributed to this difference in breeding opportunities. A greater number of non-pregnant cows in the NS group had earlier opportunities to be bred than TAI cows under the same 21-d cycle pregnancy rate; consequently the final outcome for median time to pregnancy favored the NS group.

A greater proportion of pregnant cows and daily pregnancy rate at 223 d post partum was observed for primiparous cows than multiparous cows. This finding indicates that primiparous cows are more fertile than multiparous cows, which agrees with the study of Santos et al. (2009) that reported a greater pregnancy rate to first service in primiparous cows. In the present study**, a**

greater proportion of pregnant cows in the first 21 d of breeding was found for cows with a BCS > 2.75 at 70 d post partum. This is in agreement with the report by Santos et al. (2009) and Moreira et al. (2000) where BCS around 70 d post partum positively influenced pregnancy rate. Results from the present study support the concept that BCS at 70 d post partum is of paramount importance for the establishment and maintenance of pregnancy in high producing dairy cows.

Cyclicity did not affect the proportion of pregnant cows in the first 21 d of breeding in the current study. In contrast, Santos et al. (2009) reported a greater PR 58 d post AI to first service in cyclic compared to non-cyclic cows. However, in our study the percentage of cyclic cows was greater than expected which diminished sample size for a valid comparison between cyclic and non-cyclic cows and likely limits the power to identify an effect of cyclicity in this study. A bull effect also may have occurred in cows bred by NS inducing non-cyclic animals to ovulate. These factors may have contributed to our inability to detect a difference in the proportion of pregnant cows in the first 21 d of breeding between cyclic and non-cyclic cows.

Cows bred during the warm season had a lower proportion of pregnant cows in the first 21 d of breeding in both NS and TAI bred cows. This agrees with a previous report (De Vries et al., 2005) that showed conception rates of lactating cows located in Florida decrease to 20% during summer months (June through August). Furthermore, in the present study pregnancy rate continued to be low during fall (September and October) when THI decreased. During periods of heat stress (summer), overall PR dropped for cows bred by either AI or NS, but no difference in PR was found between groups. In contrast, de Vries et al. (2005) reported that during summer cows bred by NS had greater 21-d cycle pregnancy rate than cows bred by AI. However, in the de Vries et al. (2005) study, 21-d cycle pregnancy rate included all cows eligible for AI during summer. The fact that some dairy producers in Florida choose not to breed cows during summer,

may have biased the estimates reported by De Vries et al. (2005). During hot weather, some reduction of bull fertility may be expected due to lowered semen quality associated with an increase in abnormal heads, abnormal acrosome, proximal droplets and a corresponding decrease in motility (Ott, 1986). In our study, bulls were housed and managed using heat stress abatement conditions designed for lactating dairy cows (shade, fans, evaporative cooling) and changes in bull semen quality may not have occurred or were less severe. Therefore, the depression in PR observed during summer in this study with NS and TAI could be attributed to the effect of heat stress on cow fertility.

The study herd employed a post partum health monitoring program with the aim to treat disorders promptly. Among the health disorders evaluated, metritis did not affect PR to first service. This result supports the finding by Benzaquen et al. (2007) who reported that cows with metritis did not have impaired fertility. Our finding and those of Benzaquen et al. (2007) reinforced the concept that a post partum health-monitoring program, which allows for early diagnosis and treatment of metritis may mitigate impairment in fertility related to metritis. On the other hand, the proportion of pregnant cows in the first 21 d of breeding was less in cows with mastitis, respiratory disorder, ketosis and morbidity.

The greater pregnancy loss observed in TAI bred cows in our study can be attributed to the earlier time of gestation when pregnancy was diagnosed in this group. In a report by Santos et al. (2004) late pregnancy losses were characterized by CL maintenance to the end of the differentiation stage, at approximately 42 d of gestation, and were more frequent than pregnancy losses after this stage. This pattern of pregnancy loss agrees with our lower pregnancy losses after 40 d of gestation. Therefore, we attribute the greater pregnancy loss observed in TAI compared to NS bred cows to the d of gestation when diagnosis of pregnancy occurred. Indeed

pregnancy loss was the same when compared at comparable stages of pregnancy in the present study.

The final logistic regression model revealed that the variables affecting the proportion of pregnant cows in the first 21 d of breeding were BCS and an interaction between seasonality and morbidity. The effect of BCS on fertility in dairy cows has been well documented previously and possible reasons are clearly described (Domecq et al., 1997; López-Gatius et al., 2002; Santos et al., 2009).

Conclusion

The TAI breeding program did not result in a greater 21-d cycle pregnancy rate compared with NS as initially hypothesized. A greater proportion of pregnant cows at the end of the study occurred in NS bred cows because they had more opportunities for breeding to occur compared with the TAI reproductive management program. When reproductive performance was compared to the same breeding eligibility period of 21-d cycle pregnancy rate, there was no difference between NS and TAI. In conclusion, NS and TAI are two breeding systems that can be used strategically to minimize problems related to detection of estrus, but the extended inter-insemination interval in TAI reduces daily pregnancy rate because these cows have fewer opportunities for breeding. Whether or not TAI is more economical than NS warrants investigation.

Table 3-1. Reproductive responses in cows bred by natural service (NS) or timed artificial insemination (TAI) throughout the study

	Treatment	
Variables	NS	TAI
Cows inseminated or exposed to the bulls by 223 DIM	512	543
Proportion of cows pregnant in the 1st 21 d breeding	34.4%	37.4
Proportion of pregnant by 223 DIM	434 (84.8%)	415 (76.4%)
Mean d open in pregnant cows (± SEM)	115.9 ± 1.9	113.2 ± 2.0
Median d non-pregnant	111[b]	116[a]
Cow-d at risk[1]	30,978	29,424
Pregnancies/1,000 cow-d at risk	14.00	14.10
21-d cycle pregnancy rate, %[2]	25.7	25.0

[a,b] Superscripts in the same row differ ($P < 0.05$).

[1] Cumulative number of d for cows between exposure to the bulls and pregnancy or end of study in NS, and between first service and pregnancy or end of study in TAI.

[2] In order to evaluate the 21-d cycle pregnancy rate in both NS and TAI cows, an assumption was made that cows were eligible to be bred every 21 d despite the fact that TAI cows had a breeding opportunity only every 35 d.

Table 3-2. Pregnancy rates calculated for every 21-d cycle for cows bred by natural service (NS) or timed artificial insemination (TAI) throughout the study

21-d cycle number[1]	Treatment	
	NS	TAI
	---------- % (no./no.) ----------	
1st	34.2 (175/512)	37.4 (203/543)
2nd	24.3 (82/337)	28.7 (97/338)
3rd	13.9 (35/251)	0.0 (0/241)
4th	26.8 (56/209)	28.9 (63/218)
5th	31.1 (47/151)	27.0 (38/141)
6th	15.8 (16/101)	0.0 (0/117)
7th	11.8 (10/80)	14.4 (14/97)
8th	19.4 (13/67)	----
Total[2]	25.7 (421/1641)	25.0 (417/1669)

[a,b] Superscripts in the same row differ ($P < 0.05$).

[1] In order to evaluate the 21-d cycle pregnancy rate in both NS and TAI cows, an assumption was made that cows were eligible to be bred every 21 d despite the fact that TAI cows had a breeding opportunity only every 35 d.

[2] Data from the 8th 21-d cycle were not included in the analysis.

Table 3-3. Effect of health disorders on proportion of pregnant cows in the first 21 d of breeding for cows bred by natural service (NS) or by timed artificial insemination (TAI)

Type of disease	Disease		P
	Yes	No	
	---------- % (no./no.) ----------		
Metritis[a]	31.5 (28/89)	35.7 (345/966)	0.49
Lameness	25.6 (11/43)	35.8 (362/1012)	0.19
Retained placenta	28.5 (15/52)	35.7 (358/1003)	0.37
Respiratory	17.2 (5/29)	35.9 (368/1026)	<0.05
Mastitis	22.7 (20/88)	36.5 (353/967)	<0.01
Ketosis	15.4 (6/39)	36.1 (367/1016)	<0.01
Morbidity	26.4 (76/288)	38.7 (297/767)	<0.01

[a] Variable not distributed equally between NS and TAI.

Table 3-4. Pregnancy losses according to age of gestation at the initial pregnancy diagnosis in cows bred by natural service (NS) or timed artificial insemination (TAI)

Treatment		Age of pregnancy at first diagnosis (d)	Pregnancy loss (%)
TAI		32	15.2 (63/415)[a]
NS		28-56	10.4 (45/434)[b]
1	NS	28-32	14.9 (18/121)
2		33-36	11.6 (11/95)
3		37-40	14.9 (10/67)
4		41-44	5.3 (2/38)
5		45-48	5.7 (3/53)
6		49-52	0.0(0/11)
7		> 52	2.0(1/49)
Total		28-56	12.7 (108/849)

[a,b] Superscripts in the same column differ ($P < 0.05$).

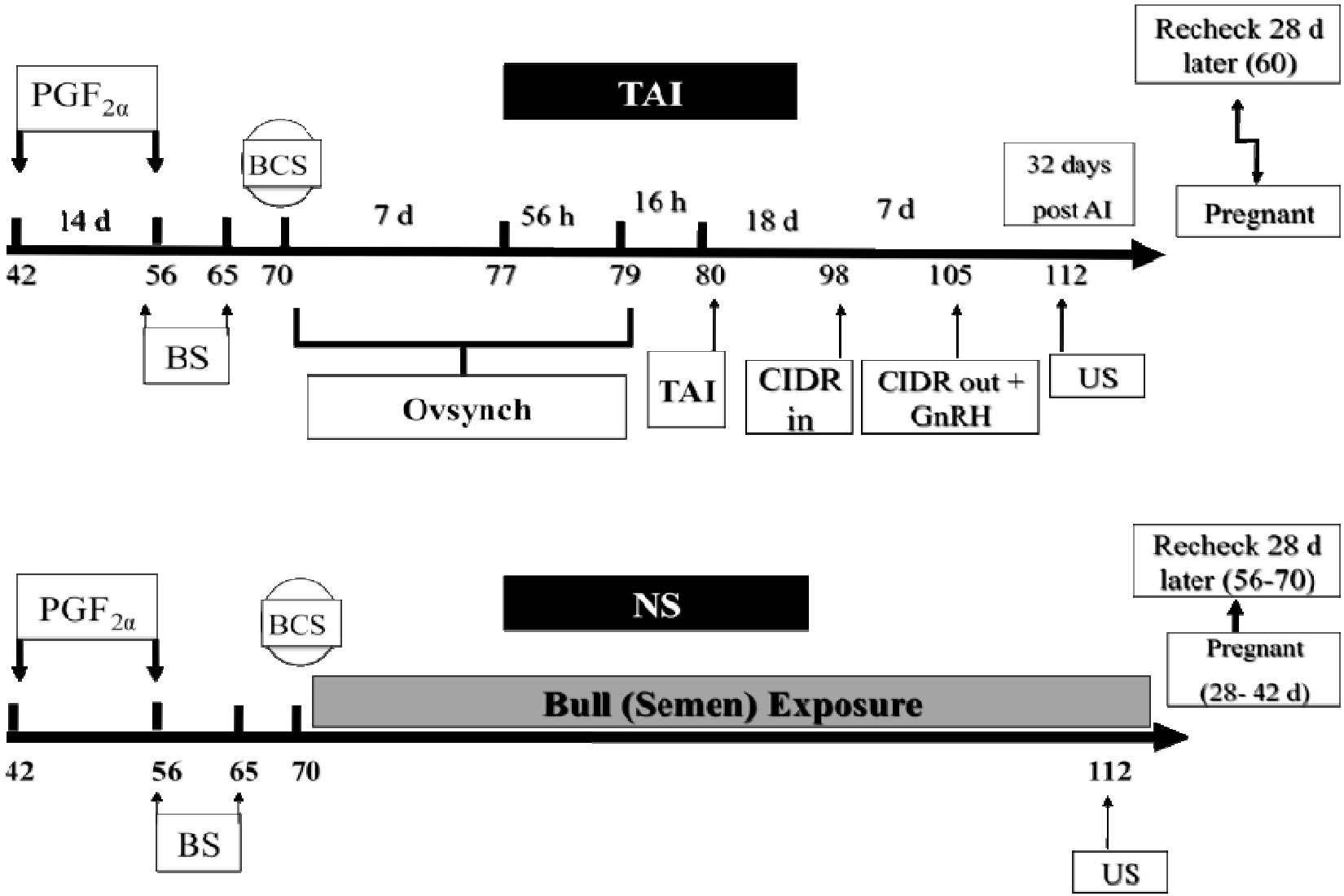

Figure 3-1. Timeline of reproductive events to the 1st service, blood sample collection and body score evaluation for cows enrolled at TAI and NS breeding program

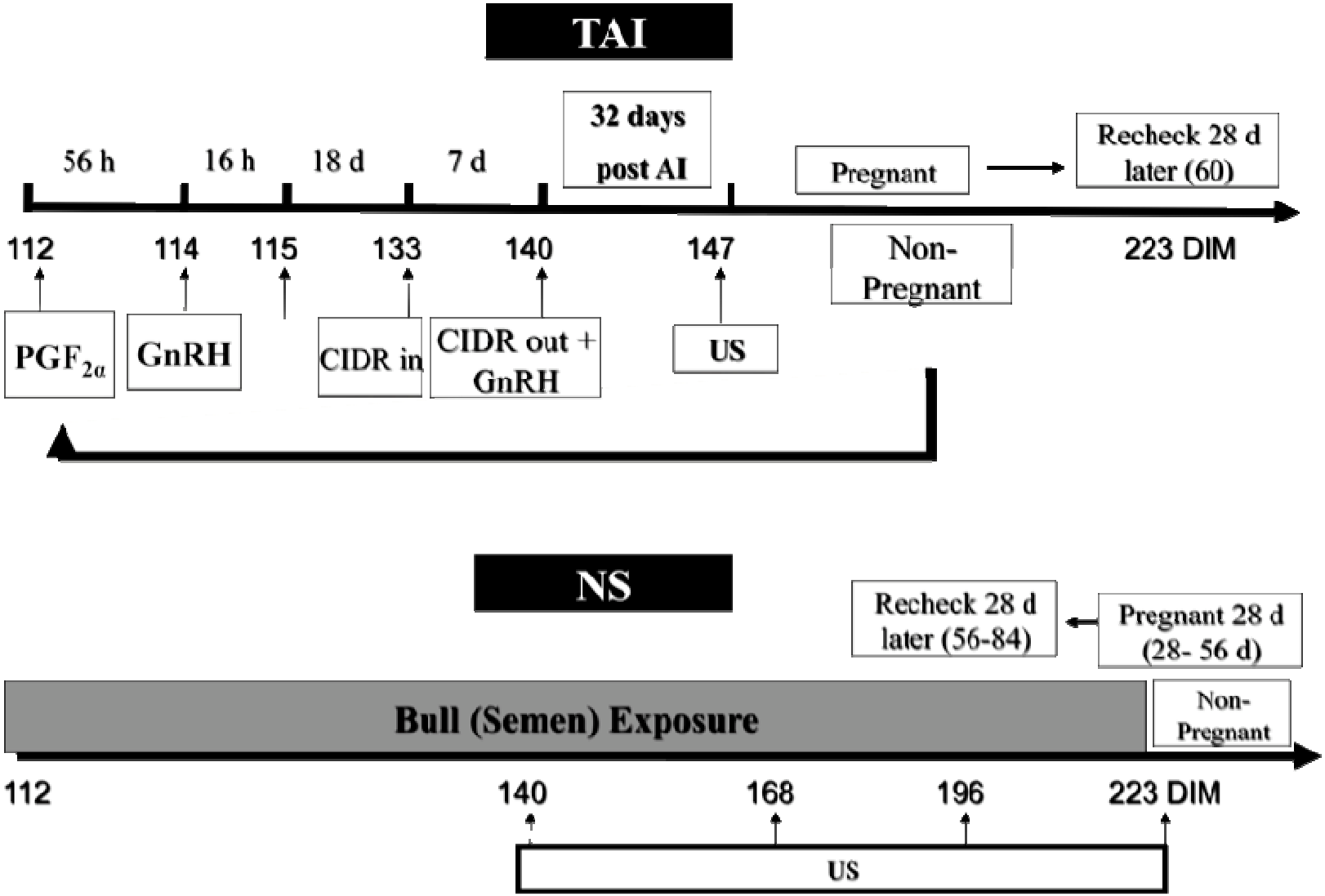

Figure 3-2. Timeline of reproductive events for non-pregnant cows to the 1st service and respective services in TAI and NS breeding program.

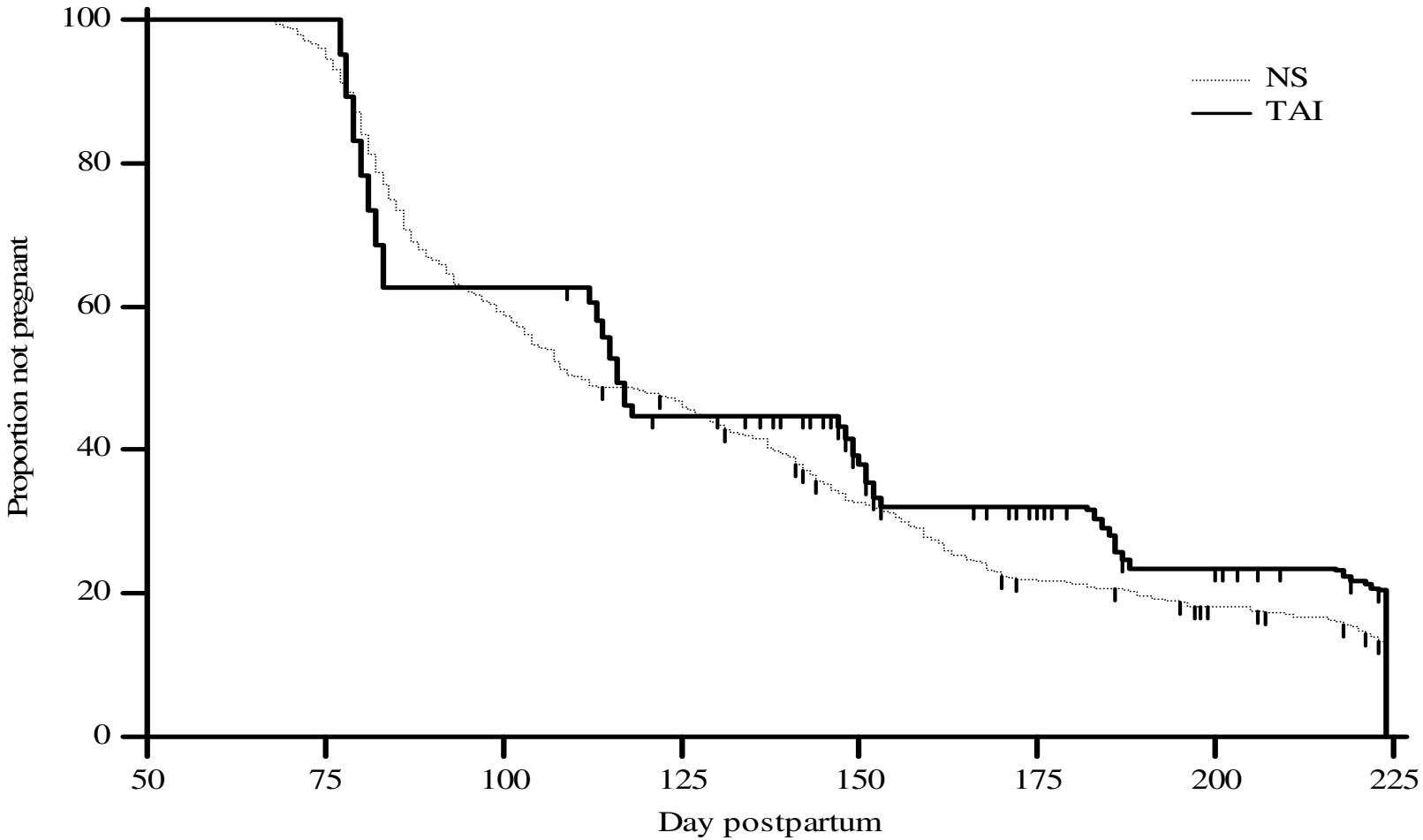

Figure 3-3. Survival curves for proportion of non-pregnant cows by d post partum for cows bred by natural service (NS) or timed artificial insemination (TAI) in the first 223 d post partum. Median interval to pregnancy for NS and TAI groups were 111 d (95 % CI = 104 to 125) and 116 d (95 % CI = 115 to 117), respectively. The rate of pregnancy in the 223 d post partum was greater (P = 0.05) for NS than TAI (adjusted hazard ratio = 1.15; 95% CI = 1.00 to 1.31).

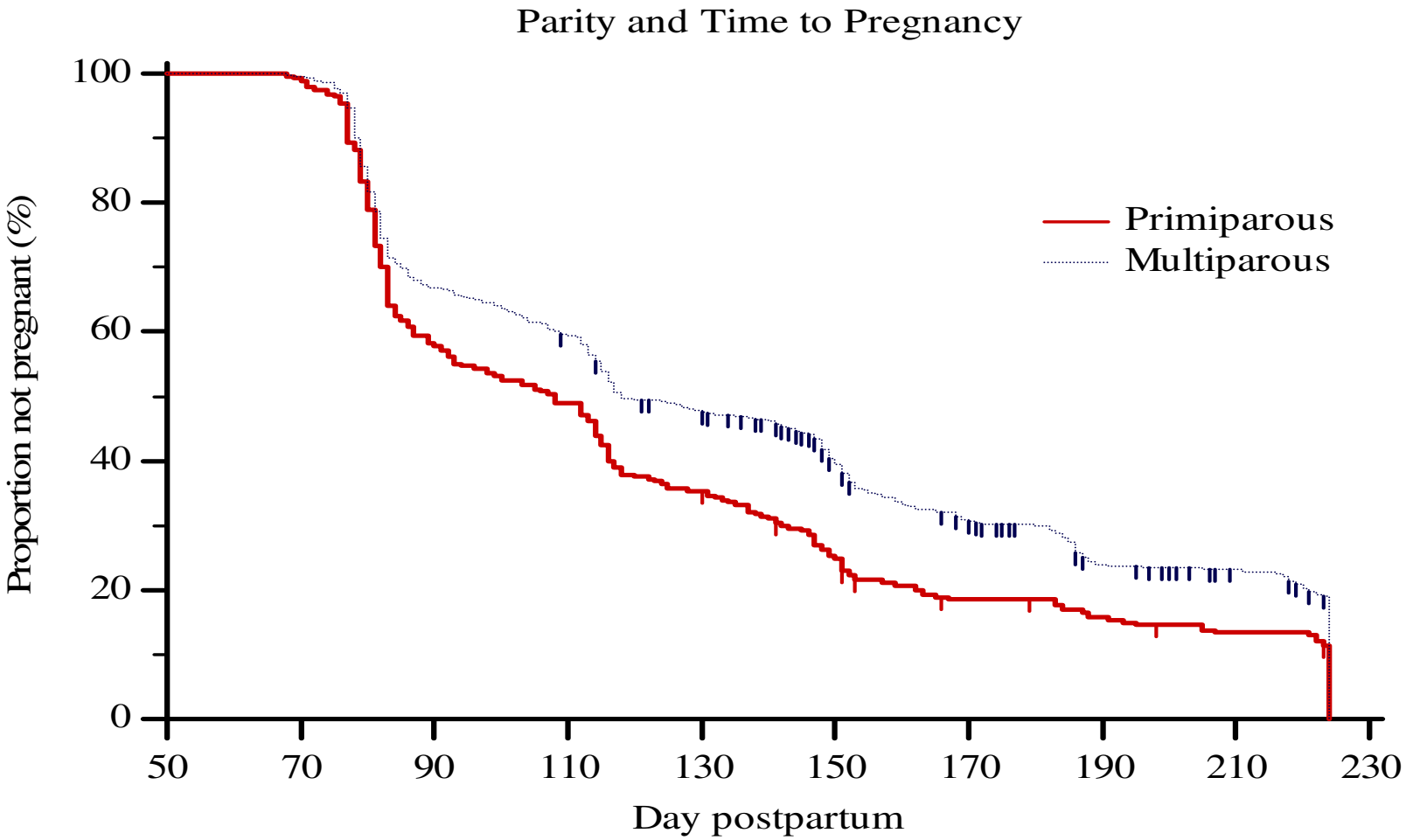

Figure 3-4. Survival curves for proportion of non-pregnant cows by d post partum for primiparous cows and multiparous cows. The hazard for pregnancy in the 223 d post partum was greater (P = 0.002) for primiparous than in multiparous cows (adjusted hazard ratio = 1.27; 95% CI = 1.09 to 1.48).

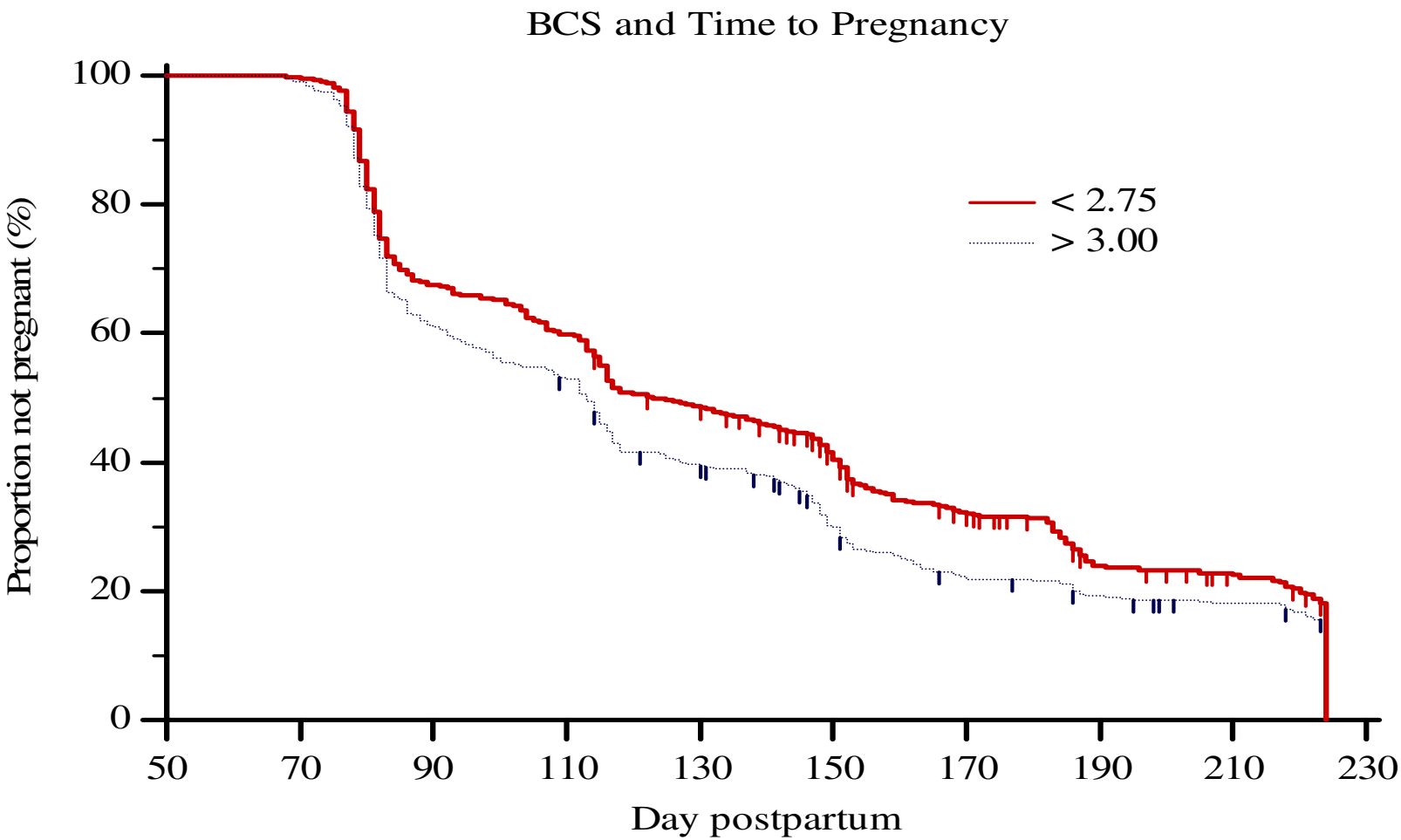

Figure 3-5. Survival curves for proportion of non-pregnant cows by d post partum for cows with body condition score (BCS) smaller or equal than 2.75 or greater than 3.00. The hazard for pregnancy in the 223 d post partum was greater (P = 0.004) for BCS greater than 3.00 that for BCS smaller or equal than 2.75 (adjusted hazard ratio = 1.18; 95% CI = 1.03 to 1.35).

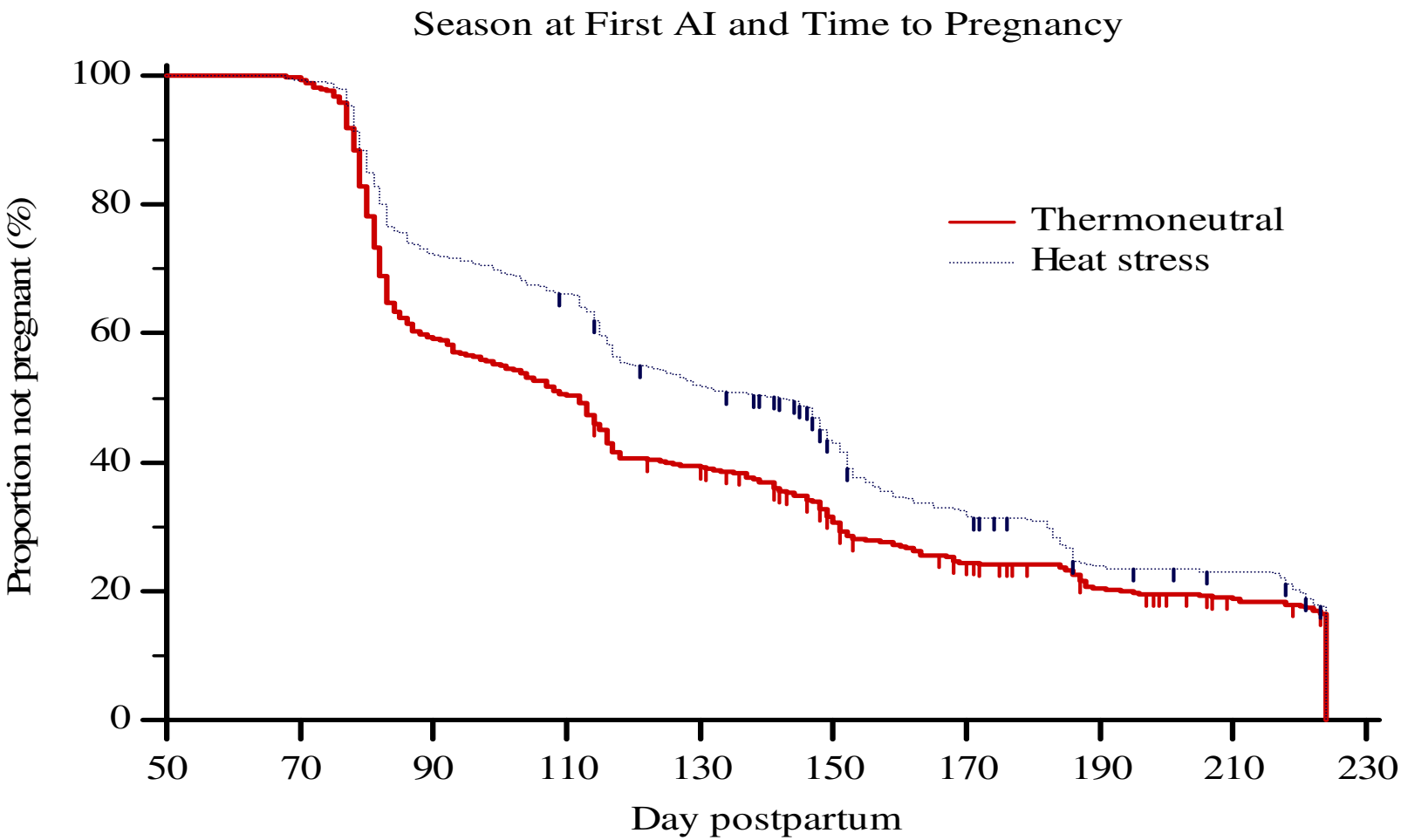

Figure 3-6. Survival curves for proportion of non-pregnant cows by d post partum for thermomeutral cows and heat stress cows. The hazard for pregnancy in the 223 d post partum was greater (P = 0.01) for thermoneutral than in heat stressed cows (adjusted hazard ratio = 1.22; 95% CI = 1.06 to 1.41).

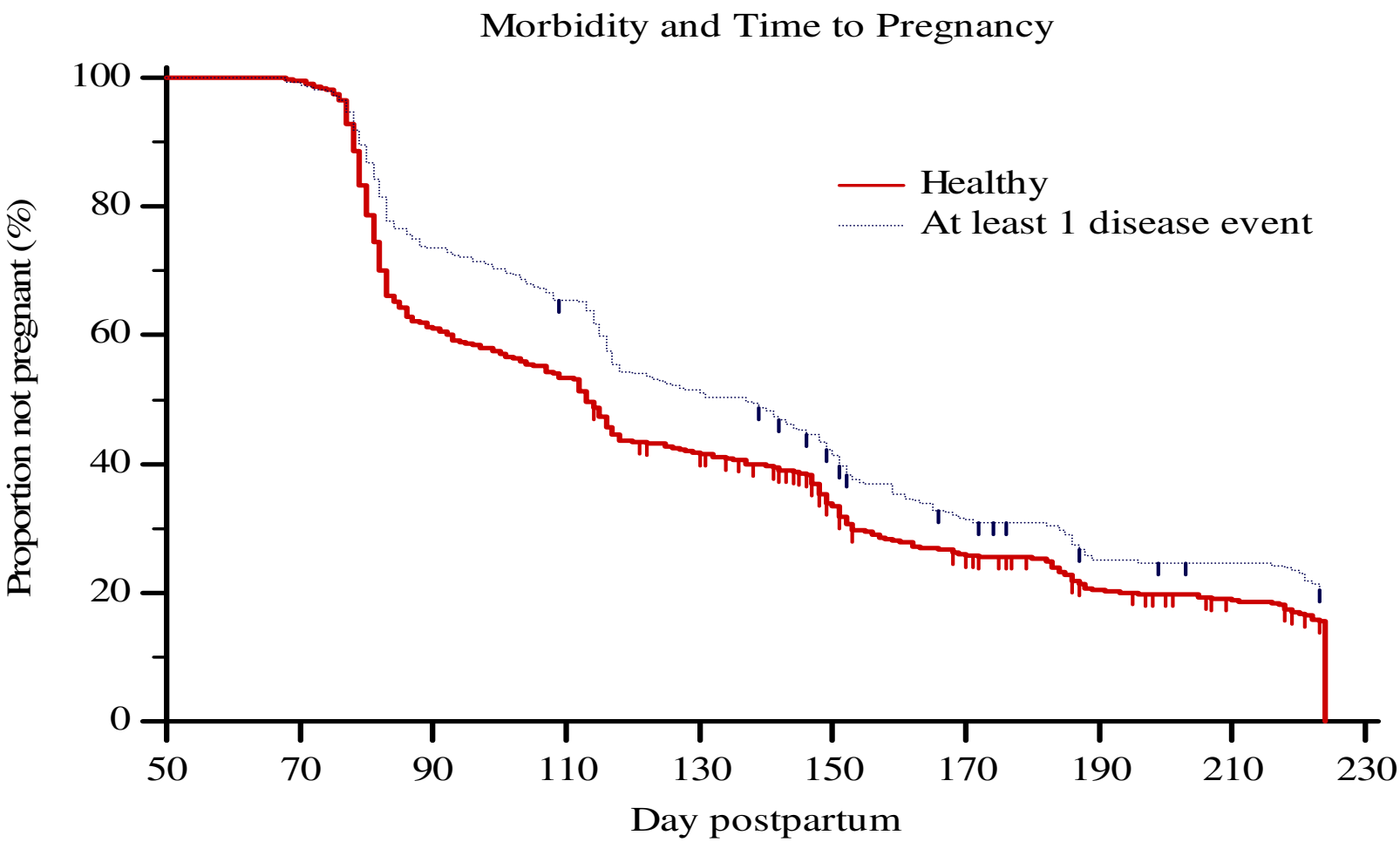

Figure 3-7. Survival curves for proportion of non-pregnant cows by d post partum for healthy cows and cows with the occurrence of at least one event of disease. The hazard for pregnancy in the 223 d post partum was greater ($P < 0.01$) for healthy cows than in cows with at least one event of disease (adjusted hazard ratio = 1.28; 95% CI = 1.09 to 1.49).

CHAPTER4
FINANCIAL ANALYSIS OF DIRECT COMPARISON OF NATURAL SERVICE SIRES AND TIMED ARTIFICIAL INSEMINATION IN A DAIRY HERD

Introduction

Reproductive efficiency plays a fundamental role in the economic viability of the dairy industry. De Vries (2008) reported that in a U.S.A. herd producing 20,000 lbs of milk, 1 percentage point pregnancy rate (PR) is valued between $22 and $35/cow per yr when PR varied from 15 to 19%. Similarly, an extra day open beyond 90 days cost between $1 and $5, with increasing value as the lactation progresses (De Vries, 2008). Lack of estrus detection is a major factor that impairs reproductive performance and profitability of lactating dairy cows (Pursley et al., 1997).

Timed artificial insemination and the use of natural service (**NS**) sires are 2 breeding programs that are used to overcome the problem of low estrus detection efficiency. Artificial insemination has many advantages such as the elimination of venereal diseases, more accurate dry-off dates, reduced incidence of dystocia, increased safety for farm employees (Vishwanah, 1993) and greater genetic improvement resulting in daughters that are more productivity and profitable (Norman and Powell, 1992). Nevertheless, NS is still a breeding program widely used throughout the US (NAHMS, 2002; Smith et al., 2004; De Vries et al., 2005; and Caraviello et al., 2006).

Recently, Lima et al. (chapter 3) compared the reproductive efficiency of NS and TAI without estrus detection side by side on a large commercial dairy farm in Florida. That study showed little differences in reproductive efficiency. The objective of this study was to compare the costs and profitability of the use of NS sires and TAI in the study of Lima et al. (chapter 3) and provide sensitivity analysis for differences in PR, feed cost, milk prices, semen prices,

genetic progress, and opportunity cost of replacing NS sires with additional cows.

Materials and Methods

The economic analysis was performed using data from the field study comparing NS and TAI as breeding programs from lactating cows that was conducted in north central Florida between November of 2006 and March of 2008 (Lima et al., Chapter 3). First, costs and revenues of both the NS and TAI program were collected and calculated. Secondly, differences in profitability due to differences in reproductive efficiency were calculated. Finally, a sensitivity analysis was carried out. The data was organized in a spreadsheet (Excel 2007, Microsoft® Corporation, Redmond, WA, U.S.A.).

Field Study

Cows in the field study were housed in free-stall barns with fans and sprinklers for forced evaporative cooling during the warm season (May to October). Four barns (2 TAI; 2 NS) each with a maximum capacity of 180 cows were used in this study. Lactating cow diets were fed a diet formulated using the CPM-Dairy cattle ration analyzer (Cornell-Pen-Miner Ver. 3.0.7a). After calving, cows were randomly assigned to the NS group or the TAI group.

All cows were presynchronized with injections of $PGF_{2\alpha}$ on day 42±3 and 56±3 after calving. The price of a dose of $PGF_{2\alpha,}$ was $2.03. Pregnancy diagnosis was performed by ultrasound. The cost of a pregnancy diagnosis was $3.00.

Cows that had a displacement of abomasum, c-section or fetotomy were not included in the study. Cows that missed any part of their protocol were removed from the study. Cows that were found in a pen of the other group (NS or TAI) were also removed from the study. Cows that were sold or died during the study were also excluded from the study. Pregnancy rates were determined for each group from the end of the VWP to 223 d post partum. The overall 21-d

pregnancy rates in the field study were 25.7 and 25.0 % for the NS and TAI groups, respectively.

Cows in the NS group were exposed to bulls 14 d after estrus presynchronization on day 70 after calving. Each pen of 180 cows was exposed to 5 bulls. Pens included both non-pregnant and pregnant cows. For each bull in a pen with cows, there were 1.17 bulls in the resting pen. Therefore, there were 0.028 bulls in breeding pen and 0.033 bulls in the resting pen per slot.

Pregnancy diagnosis was performed 42 d after first exposure to bulls to determine pregnancy status. Non-pregnant cows in the NS group were re-examined by pregnancy diagnosis every 28 d until diagnosed pregnant or up to 223 d post partum, whichever occurred first.

Twenty-six bulls, all 18 mo old at the beginning of the study, were used. After this period of one year the bulls were sold for an average price of $1,116.00. Bulls culled prematurely were sold for $670.00.

All bulls underwent a breeding soundness evaluation according to the guidelines of the Society for Theriogenology (Chenoweth, 1992) before first exposure to cows. In addition, bulls were tested for *Tritrichomonas foetus*. The breeding soundness evaluation (BSE) and *Tritrichomonas foetus* test were performed every 3 months for a total of 5 evaluations for each bull. In addition, the bulls were tested for bovine viral diarrhea by imunohistochemistry of skin using an ear notch sample. All bulls were vaccinated according to farm operational practices for infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, bovine respiratory syncitial virus, leptospirosis, clostridial diseases, and campylobacteriosis. Each bull was purchased by the amount of $1,148.00 staying for 1 year in the farm. The price of all breeding soundness evaluation plus trichommonosis testing was 152.50 per bull entering the herd.

All bulls were rested for 14 d after 14 d of cow exposure. Natural service sires were fed

the lactating cow diet during the 2 consecutive wk they were exposed to the cows. An assumption based in previous measurement at the specific dairy that bulls intake was 80% of the lactating cows were made. A "weigh back" lactating cow diet that averaged 17.2 kg of dry matter/bull per d was fed during a 2 wk rest period. Resting bulls were housed in Bermuda grass pasture lots with portable shade and trees for heat abatement. The cost of feeding each bull during the exposure and resting periods were calculated in $3.30 and $2.37, respectively.

Cows in the TAI group enrolled in a modified Ovsynch protocol 14 d after estrus presynchronization. Average VWP for first insemination was 80 d. Non-pregnant cows in the TAI group were re-synchronized with a CIDR inserted 18 d after TAI and removed 7 d later, when GnRH was given. Cows were examined for pregnancy on d 32 after TAI; non-pregnant cows received $PGF_{2\alpha}$ and GnRH 56 h later followed by TAI 16 h after the GnRH injection (Ovsynch). Non-pregnant cows in TAI group were re-inseminated up to 5 times using the same scheme before the end of the study (Resynch). The cost for each dose GnRH was $1.84. The cost of each CIDR device was $8.43. Each dose of semen cost on $6.00 and labor cost for each insemination was $3.00.

Herd Budget Calculator

A partial budget was developed to calculate the economic differences between the NS and TAI programs. Economic differences were caused by differences in reproductive costs, VWP, and pregnancy rates. The partial budget consisted of 3 steps.

In the first step, the actual net costs for the NS and TAI group in field study were enumerated per cow entering the experiment (breeding pool). Because in that study cows entered the breeding pool for 14 months, net costs were adjusted for the number of cows entering the breeding pool per year. This adjustment resulted in reproductive costs per cow per yr (Tables 4-1 and 4-2) for both programs.

In the second step, economic differences between both programs as a result of differences in VWP and pregnancy rates, but excluding reproductive costs, were calculated. A herd budget was developed to calculate the fraction cows by parity, day since calving and pregnancy status. The time step was 1 day. Inputs and prices were chosen to match those during the field study as close as possible.

Annual risk of forced culling for non-pregnant cows in parities 1 through 4 were set at 18, 28, 38, and 48%, respectively. For pregnant cows, the annual risk of forced culling was 10%. Daily risk of forced culling was calculated as $1-(1-\text{annual risk})^{(1/365)}$. Cows not pregnant after 320 days were culled on day 321. Culled cows were replaced with calving heifers. The price of a culled cow was set at $400 and the price of a calving heifer was $1,900. All calving heifers were purchased and assume to be the same for both the NS and TAI groups.

Cows that got pregnant moved to the next parity after gestations of 280 days. The VWP for the NS program was set at 70 d with a daily 4.76% service risk. This equals a 100% service risk in a 21-d period. Conception rates were varied to obtain the desired pregnancy rates. Pregnancy rates matched either those in the field study or were varied for sensitivity analyses. For the field study, the conception rate was set at 25.7% to obtain a 25.7% pregnancy rate. The VWP for the TAI group was set at 80 d with 35 days between breeding opportunities, 100% service risk, and 29.3% conception rate. This resulted in a 25.0% pregnancy rate.

Daily milk yield y_t for cows in both breeding programs was calculated as $y_t = a * t^b * \exp(-c/1000 * t) * d$ where t is the days since calving (Wood, 1969). For parity 1, a = 19.00, b = 0.34, and c = 2.80. For parities >1, a = 26.00, b = 0.34, and c = 5.00. Parameter d = 1.156 was a multiplier to obtain a 305 d herd milk yield of close to 22,000 lb/cow per yr observed during the field study. Herd milk yield was calculated by multiplying the daily milk yield by the faction

cows on day t. Cows were dry the last 60 days of gestation. The default milk price was set at $20/cwt.

Feed cost per cow was set at $1.50 maintenance cost per day and $0.03/lb milk produced. Calf prices were set at $200.

The advantage in genetic progress of the TAI program was calculated from the lifetime net merit of a TAI breeding (on average $361 in the field study) minus the estimated lifetime net merit of a NS breeding ($163). Assuming the value would be expressed 3 yr from the breeding, discounted at 8% interest per year, and lifetime is 3 yr, the annualized advantage of the TAI breeding was $(1/(1.08))^3$ * ($361 - $163) / 3 = $52. Further, this value applied only to heifer calves (48%) born from cows (0.75/cow per yr) that were raised and calved themselves (85%). Multiplied, the default genetic advantage of the TAI program was $15.94/cow per yr.

Performance of cows in parities >4 were equal to 4th parity cows. The fraction cows in parities >4 was calculated from the fraction cows entering the 4th parity and the fraction cows culled in the 4th parity (Handbook for Mathematics page 23). Results were calculated for the steady state situation where herd demographics were constant over time.

The herd budget also determined 6 factors that affected the reproductive costs. First, the number of cows entering the breeding pool and the number of pregnancy checks was calculated for both programs per slot per year. For the TAI program, the fraction of cows not pregnant after 223 d (the end of the experiment) was calculated. The TAI costs for eligible cows after 223 d until the end of the breeding period at 320 d after calving were added to the TAI cost per slot per yr. The fraction of calvings from ≥2nd parity cows was also calculated for the TAI program for the evaluation of the economic advantage of genetic progress due to AI. For the NS program, the number of slots per bull was calculated based on the number of eligible days per cow per yr. The

reason is that lower pregnancy rates would require more bulls per slot to maintain the same number of bulls per open cow in the breeding pen. Finally, the feed costs per bull per day were varied with the same magnitude as the marginal feed cost. For example, if marginal feed costs were doubled, then bull feed costs were doubled.

In the third step, net revenues per slot per yr were calculated as all revenues minus all costs. Revenues consisted of milk sales, calf sales, and cull cow sales. Costs consisted of feed cost, replacement costs, and reproductive costs, as show in Table 4-3. Any other costs, such as non-reproductive labor costs, were assumed to be the same and therefore not included in the analysis. The economic advantage of the TAI program was calculated as the net revenues for the TAI program minus the net revenues for the NS program.

Sensitivity Analysis

Three sets of scenarios were used to investigate the effects of variations in pregnancy rates, milk price, feed cost, semen cost, genetic advantage of TAI breedings, and opportunity cost of replacing bulls with cows.

In the first set, inputs from the field study were used with the following exceptions. The semen cost per dose was $2.00, $12.00 or $22.00. Marginal feed costs used were $2.00, $5.00 and $8.00/cwt of milk produced. Milk prices were $12.00, $18.00 or $24.00/cwt. The genetic advantage of pregnancies generated by TAI compared to NS was $0.00, $300.00 or $600.00. These inputs were varied one by one; with all other inputs the same as for the field study.

In the second set of scenarios, the VWP for both breeding programs was set to 80 d and conception rates adjusted to obtain pregnancy rates of 12%, 18% or 24% for both the TAI and NS breeding programs. Semen cost, marginal feed cost, milk prices and genetic advantage was varied as in the first set of scenarios.

The third set of scenarios evaluated opportunity costs of a fixed herd size of 1000 slots

where bulls replaced 0, 14, or 28 cows. In the field study, 0 cows were replaced. Pregnancy rates were varied as in the second set of scenarios.

Results

Field Study

The herd budget determined the fraction cows entering the breeding pool at 0.9586/slot per yr. The number of pregnancy diagnoses was 4.88/slot per yr. Costs and revenues of the NS program in the field study are shown in Table 4-1. Cost of the NS program was $163.3/cow per yr which included $71.48 for the purchase of the bulls, $3.90 for injections of prostaglandin, $28.21 for feeding bulls in the resting pen, $33.46 for feeding bulls exposed to the cows, $2.44 for labor including management of bulls and administration of injections for presynchronization, and $14.64 for pregnancy diagnosis. Revenues consisted of the sale of bulls at $63.10/cow per yr. Net cost of the NS program was therefore $100.53/cow per yr.

The fraction cows entering the TAI program was 0.9536/slot per yr. There were 9.6% more non-pregnant days from the end of the experiment at d 230 to the end of the breeding period at d 320 after calving. There were 0.75 calvings/cow per yr in the TAI program from cows starting their 2nd or greater parity. Calves from these calvings had an economic genetic advantage. The number of pregnancy diagnoses was 4.34/cow per yr for the TAI program. The average number of service per cow per yr was 2.59.

Costs and revenues of the TAI program in the field study are shown in Table 4-2. Cost for TAI was $76.32/slot per yr which included $3.90 for injections of prostaglandin for presynchronization, $4.94 for injections of prostaglandin in Ovsynch and Resynch, $10.37 for GnRH injections, $20.48 for CIDR inserts, $14.58 for semen, $2.82 for labor including administration of injections and CIDR insert and removal, $7.29 for AI labor, and $13.02 for pregnancy diagnosis. The economic value of the genetic advantage of calves sired by AI was

$15.94/slot per yr. Net cost of the TAI program was therefore $61.84/slot per pr.

Voluntary waiting periods and PR of the NS and TAI programs were different. The herd budget determined that at the end of the breeding period (320 d after calving), 96% of the cows in the NS program were pregnant and 92% of the cows in the TAI program. Average days to conception were 135 for the NS program and 137 for the TAI program. Consequently, costs and returns caused by differences in herd demographics were different (Table 4-3). The TAI program resulted in greater milk sales, cow sales, replacement cost and feed cost. The NS program resulted in greater calf sales. Revenues minus variable costs and reproductive cost were $2,201.19/slot per yr for the NS program and $2,172.50/slot per yr for the TAI program. This advantage of $28.69/slot per yr for the NS program was more than offset by the $38.69/slot per yr greater reproductive cost. Net economic advantage of the TAI program compared to the NS program was therefore $10.00/slot per yr in the field study.

Sensitivity Analysis

The first set of scenarios revealed large effects of semen price, marginal feed cost, and genetic advantage on the economic advantage of TAI over NS before adjustment for differences in VWP and pregnancy rates (Table 4-4). One dollar more expensive semen increased the cost of the TAI program by $2.51/cow per yr. One-dollar greater marginal feed cost/cwt increased the cost of the NS program by $19.22/cow per yr. One-dollar greater milk price/cwt increased the economic advantage of the TAI program by $1.34/slot per yr. An increased in net merit of $1 decreased the net cost of the TAI program by $0.08/slot per yr.

Table 4-5 shows the economic advantage of the TAI program including adjustment for differences in VWP and pregnancy rates. The difference between Table 4-4 and 4-5 for the same set of inputs is the advantage in profit per slot per yr of the TAI program due to differences in VWP and pregnancy rates. These varied from $23.34 to $39.38/slot per yr in the advantage of

the NS program. The effect of more expensive semen and genetic progress are the same as in Table 4-4 because they only affect the reproductive cost of the TAI program. One-dollar greater milk price/cwt increased the economic advantage of the TAI program by $1.34/slot per yr. One-dollar greater marginal feed cost/cwt increased the cost of the NS program by $19.22/slot per yr. The TAI program more profitable than the NS program for all evaluated inputs when semen price was $2. The NS program was more profitable for some inputs when semen price was $12 or greater.

The second set of scenarios calculated the effects of variations in PR. Conception rates for the TAI program were set at 0.2840 (24% PR), 0.2286 (18% PR) or 0.1645 (12% PR) depending on the desired PR. Conception rates for the NS program were the same as the desired PR. Voluntary waiting periods were set at 80 d after calving but differences remained in the daily probability of pregnancy between both programs caused by differences in service rates and conception rates.

When PR was set at 24%, 18% or 12% for the NS program, the herd budget determined that the number of cows per bull in a breeding pen were 35.2, 28.6, and 22.1. Eligible days per cow per year were 71.6, 88.1, and 114.1, respectively. The number of pregnancy diagnoses was 4.89, 5.56, and 6.60, respectively. Reproduction costs were $102.29 (24%), $123.08 (18%), and $155.80 (18%) for the NS program.

When PR for the TAI program was set at 24%, 18%, and 12%, the fraction cows not pregnant after 223 d was 0.135, 0.175, and 0.23.2, respectively. Eligible days per cow per year were 71.1, 87.5, and 113.2, respectively. The number of pregnancy diagnoses per slot per year was 4.38, 4.68, and 5.14, respectively. Reproduction costs were $64.27 (24% PR), $68.54 (18%), and $75.89 (18%) for the TAI program.

Not accounting for differences in PR (Table 4-6), one-dollar greater marginal feed cost/cwt increased the cost of the NS program by $21.03/slot per yr when the PR of the NS program was 24%. When the PR of the NS program was set at 18% or 12%, one-dollar greater marginal feed cost/cwt increased the cost of the NS program by $25.89 or $33.53, respectively.

For the same pregnancy rates for TAI and NS the profit of TAI over NS was consistent independent of use low pregnancy rates as 12% or high pregnancy rates as 24%. The only exception was for the use of semen of $22.00 for pregnancy rates of 24% that resulted an economic advantage for NS.

The same pattern of consistent profit of TAI over NS was observed when pregnancy rates were higher in TAI than in NS in this case with no exception. The profit of TAI over NS for pregnancy rates greater in NS than TAI was inconsistent. The economic advantage of NS over TAI in scenarios where NS had better reproductive performance was offset for greater costs with marginal feed cost and genetic advantage resulting in a profit for TAI over NS.

The advantage for different pregnancy rates reported in table 4-7 accounted for the different 21-d cycle pregnancy rates obtained at the study Lima et al. (Chapter 3). If those differences in 21-d cycle pregnancy rates and VWP were not accounted the economic of TAI over NS presented in table 6 would be consistently slightly smaller as it is depicted in table 5. However, the outcomes per slot per year would completely follow the same trends founds for variation of pregnancy rates per cow per year where difference 21-d cycle pregnancy rates and VWP are not accounted. Those numbers shown were consistently greater for an assumed same pregnancy rates for NS and TAI or lower pregnancy rates for TAI than for NS. If the assumed pregnancy rates were greater for TAI than for NS the economical advantage of TAI was

consistently lesser. Nevertheless, it is noticeable that for same pregnancy rates TAI always presented economically advantageous than NS.

Opportunity costs are shown in Table 4-8. The opportunity cost produced by the replacement of bulls for lactating cows for the ratio of 1 bull per a lactating cow or 0.5 bull per cow lactating at the same pregnancy rates cows generated a increase in profit of TAI over NS of $29.41 and $58.82 per slot per year, respectively, for the pregnancy rates obtained in the study. If an pregnancy rates of 12% for both NS and TAI it is assumed, the economic balance of TAI over NS for the replacement of 1 bull per lactating cow or 0.5 per lactating cow was slighter smaller being $26.19 and $52.37 per slot per year. When extreme low pregnancy rates of 12% were assumed for TAI and good pregnancy rates of 24% were assumed for NS the replacement of 1 bull per 1 lactating cow reduced the economic disadvantage in $57.86 per slot per year. Therefore, the replacement of bulls per lactating cow resulted in consistent considerable economic advantage for TAI independently of the pregnancy rates.

Discussion

Cow day at risk for pregnancy were not different between NS and TAI, and they were 30,978 and 29,424 days, respectively. Cow day at risk was used as input to calculate pregnancy rates in our economical model. In addition, the hazard for pregnancy was 15% greater for NS due the fact the cows in this breeding program had more opportunities for breeding what resulted in greater proportion of pregnant cows at the end of the Lima et al. (chapter 3) study.

The aims of the current study were to compare the cost and profitability of natural service (NS) and timed artificial insemination (TAI) two breeding system that do not require estrus detection as the sole reproductive program on a commercial dairy farm in random allocated field study. For the best of our knowledge this is the first time that TAI and NS two breeding system that do not require estrus detection are compared economically in controlled field trial. Final

outcome from this financial analysis show that TAI program was $38.69 per cow per year less expensive than NS. If increased hazard for pregnancy and earlier VWP for NS breeding program are accounted the economic advantage of TAI is reduced for $10.00 per slot per year what is similar with values reported by Overton (2005), however it is necessary to emphasize that study had model assumptions that did not account for differences in pregnancy rates and VWP. Among all the factors that contributed for this economic advantage of TAI the cost of feeding bulls was definitely the major component responsible for this difference. Feeding cost of bulls was 37.8% and 61.6% of total bull costs and net cost for NS, respectively. Similarly, Overton, 2005 reported feeding cost for bulls of 29.8% and 61.2% of total bull costs and net cost for NS, respectively. This important role played for the feeding cost is emphasized and expanded for other magnitude by simulated scenarios where marginal feeding cost increase had consistently the biggest impact in profitability of TAI over NS. Dairy producers are probably not familiar with what is the proportion of feeding cost in the accountability of a breeding program, which possibly generates the belief that NS is less expensive than AI or TAI (Overton, 2005). We are aware of that the bulls in this project were manage more intensively than what is normally done in United States, however, this differences are already accounted with the greater 21-days cycle pregnancy rates obtained in this study (25.7%) compared to NS 21-day cycle pregnancy rates reported in natural service of 9.0% in the summer and 18% in the winter, respectively. (De Vries et al., 2005). Differences in cost might be offset for different reproductive performance as was reported by Tenhagen et al. 2004, however as mentioned previously this was not problem in our budget, because reproductive performance was taken in consideration.

Simulated scenarios for different pregnancy rates with no difference in pregnancy rates for TAI and NS show that the profitability of TAI is even greater when low pregnancy rates are

compared. Possibly cows in those breeding program with lower pregnancy rates will take longer to become pregnant and the NS breeding program will require feeding bulls longer, which should add up for the already evident economic benefit of use TAI over NS.

Genetic advantage of TAI is also important factor in this financial analysis, which accounts for 47.1% of the profit of TAI over NS. The genetic advantage was also reported by Overton (2005) that point out under his model's assumptions this variable as the main variable driving variation of costs between NS herds and AI herds. This is future foregone income that will be generated by an increased milk production of heifers genetically superior produced by the use of AI. This is probably a key benefit attributed for the use of AI and should not be ignored, but if so the profit of TAI over NS still present. The simulated scenarios also follow a pattern of reliable increase of TAI economical advantage over NS for greater genetic advantage.

The possibility of replace bulls for lactating cows in the TAI program is other possible foregone income that should be accounted and if is has a significant impact on the profit of TAI over NS per slot per year. Bulls are occupying spaces that potentially could have a lactating cow generating more income for the dairy producers and when this taken in consideration the benefits of TAI are even more evident. This opportunity cost can make the profit of TAI over NS under same pregnancy rates reach high values such as: $128.36 of profit when a 1 bull is replaced by 1 cow for pregnancy rates of 12% for TAI and NS.

The economic analysis presented in this manuscript was based on the results of the study Lima et al. (2009 - chapter 3) where compliance allow 21-d cycle pregnancy rates that are considerably greater of what is reported in average for U.S.A. dairy herds (Devries et al., 2005) and certainly influenced the efficiency and profitability of both breeding programs.

The herd budget created for this financial analysis permitted accurately to report costs

and revenues of both breeding program accounting even for differences in 21-d cycle pregnancy rates. The differences in profitability due an inclusion of different 21-d cycle pregnancy rates in the herd budget calculator are in agreement with previous reports in the literature.

Conclusion

Timed artificial insemination resulted in the breeding program $38.69 less expansive than natural service breeding program per cow per year under the conditions of the present study contradicting the belief that the use of bulls is cheaper method of breeding cows to avoid problems with estrus detection. Even when the greater 21-d cycle pregnancy rates and shorter VWP from the Lima et al. (2009 – chapter 3) were accounted, which clearly benefit NS we had TAI economically advantageous than NS. The main variables that driving for this difference were feeding cost for bulls and genetic advantage of TAI. The differences in profit were even greater when opportunity cost provided by the replacement of bulls by lactating cows was taken in consideration. Therefore, TAI is a more economical option for breeding lactating cows eliminating problems with estrus than NS

Table 4-1. Cost and Returns for NS used in the economic analysis	NS - $/cow/year
Costs	
1) Purchase of bulls	
Price per bull = ($1,148.00)	
Average days bulls staying on the farm per year: 354	
Total bulls needed entering herd per cow per year: 0.062	
Calculation: 0.062 x ($1,148.00) =	$71.48
2) Additional expenses (BSE*, vaccines for bulls, trichomonosis test, other health costs) = $152.50	
Calculation: 0.062 x $152.50	$9.50
3) Feed costs (Cows/bull ratio in the breeding pen: 36/1)	
Bulls in the breeding pen per cow = 0.028 / Bulls in the resting pen per cow = 0.033	
Feed cost per day in the breeding pen = $3.30 / Feed cost per day in the resting pen = $2.37	
Feed cost per bull per year = ($1,021.00)	
Calculation: 0.028 x ($3.30) + 0.033 x ($2.37) x 365 =	$61.67
4) Labor costs (Fixed labor to manage bulls + labor to give presynch injections)	
Calculation ($2.06 + $0.38) =	$2.44
5) Pregnancy check expenses (4.50 times per cow per year at $ 3.0 each)	$14.64
6) Prostaglandin cost for pre-synchronization – ($2.035) – 2 doses per each cow	
Percentage of cows entering the breeding program year = 96.4%	
Calculation: 0.965 x 2 x 2.035 =	$3.90
Total costs	$163.63
Returns	
1) Sale of bulls	
Percentage of bulls culled prematurely = 23%	
Sale price for bull culled prematurely = $670.00	
Sale price for bull culled healthy = $1,116.00	
Total returns = *Calculation:* 0.23 x 0.062 x 670 + 0.67 x 0.060 x 1116 =	$63.10
Net costs for NS = Total returns NS - Total costs NS	
Net costs for NS = $63.10 - $163.63 =	($100.53)

*BSE = Breeding Soundness Evaluation

Table 4-2. Cost and Returns for TAI used in the economic analysis		$/cow/year
Costs		
1) Hormone and semen cost		
Cows entering the breeding program year = 95.4% / Cows non pregnant per year: 11.3%		
Number of doses per cow per year and cost of Prostaglandin Pre-synchronization: 2 and ($2.035)		
Number of doses per cow per year and cost of Prostaglandin Ovsynch/Resynch: 2.59 and ($2.035)		
Number of doses per cow per year and cost of GnRH: 6.01 and ($1.84)		
Number of inserts per cow per year and cost of CIDR: 2.59 and ($8.43)		
Number of breeding and price for each dose of semen: 2.59 and ($6.00)		
Calculation: Prostaglandin Pre-synchronization cost	0.954 x 2 x ($2.035) =	$3.88
Calculation: Prostaglandin Ovsynch/Resynch cost	0.954 x 2.59 x ($2.035) =	$5.18
Calculation: GnRH cost	0.954 x 6.01 x ($1.84) =	$10.88
Calculation: CIDR cost	0.954 x 2.59 x ($8.43) =	$21.48
Calculation: Semen cost	0.954 x 2.59 x ($6.00) =	$15.29
2) Labor cost TAI (labor for giving injections, labor for inserting and removal CIDR)		$2.82
3) Breeding expenses (2.59 services per cow per year at $ 3.0 each)		$7.29
3) Pregnancy check expenses (4.33 times per cow per year at $ 3.0 each)		$13.02
Total costs TAI		$76.32
Returns		
1) Genetic advantage of AI sires		
Cows calving per year from 2nd or greater lactation: 74.6%		
Single heifer calves: 48%		
Heifer calves surviving until freshening: 85%		
Calves with a genetic advantage: 75.3% x 48% x 85% = 30.4%		
Average net merit AI sires: $361 / Average net merit NS sires: $163 (estimated)		
Time adjusted advantage net merit AI sires: (361-163)/3 x (1/1.08)^3 = $52		
Calculation: 0.307 x 52 =		$15.94
Total returns TAI		$15.94
Net costs for TAI = Total returns TAI - Total costs TAI		
Net costs for TAI = $16.09 - $78.29 =		($61.84)

Table 4-3. Returns and cost ($/slot per year) for the default NS and TAI programs

	NS	TAI
Milk sales	4,359.76	4,386.48
Cow sales	102.15	114.33
Calf sales	154.42	148.70
Replacement cost	485.22	543.09
Feed cost	1,199.92	1,203.93
Other costs	730.00	730.00
Reproductive cost	100.53	61.84
Net returns	2,100.66	2,110.66

Table 4-4. Simulation of economic balance in dollars of TAI over NS considering different scenarios where arbitrary values for cost of semen are varying accordingly with feed cost, milk price and genetic advance using as baseline the cost obtained in this study not accounting the difference in reproductive performance.

ITEMS		Semen cost			
		$6.00 (Study Price)	$2.00	$12.00	$22.00
Results from the study		10.00	48.41	24.11	-0.19
Marginal Feed Cost /cwt	$ 2.00	38.69	27.85	3.56	-20.74
	$ 5.00	18.13	89.52	65.22	40.93
	$ 8.00	79.80	151.19	126.89	102.59
Milk Price ($/cwt)	$ 12.00	141.47	48.41	24.11	-0.19
	$ 18.00	38.69	48.41	24.11	-0.19
	$ 24.00	38.69	48.41	24.11	-0.19
Genetic Advantage of TAI ($)*	$ 0.00	38.69	32.47	8.17	-16.12
	$ 300.00	22.75	56.62	32.32	8.02
	$ 600.00	46.90	119.64	95.35	71.05

*Net merit dollars.

Table 4-5. Simulation of economic balance in dollars of TAI over NS considering different scenarios where arbitrary values for cost of semen are varying accordingly with feed cost, milk price and genetic advance using as baseline the cost obtained in this study and accounting for the difference in reproductive performance

ITEMS		Semen cost			
		$ 6.00 (Study Price)	$2.00	$12.00	$22.00
Results from the study		10.00	19.72	-4.58	-28.88
Marginal Feed Cost /cwt	$2.00	-9.22	0.50	-23.80	-48.10
	$5.00	48.44	58.16	33.86	9.57
	$8.00	106.10	115.82	91.52	67.23
Milk Price ($/cwt)	$12.00	-0.69	9.03	-15.27	-39.56
	$18.00	7.33	17.05	-7.25	-31.55
	$24.00	15.35	25.06	0.77	-23.53
Genetic Advantage of TAI ($)	$0.00	-5.94	3.78	-20.52	-44.81
	$300.00	18.21	27.93	3.63	-20.66
	$600.00	42.36	52.08	27.78	3.48

Table 4-6. Simulation of economic balance in dollars of TAI over NS considering different scenarios where arbitrary different pregnancy rates are varying accordingly with cost of semen, feed cost, milk price and genetic advance using as baseline the cost obtained in this study not accounting for the difference in reproductive performance.

Items	PR TAI (%)	24	24	24	18	18	18	12	12	12
	PR NS (%)	24	18	12	24	18	12	24	18	12
Results from the study		39.64	57.86	87.00	37.62	55.84	84.98	33.46	51.65	80.75
Marginal Feed Cost /cwt	$ 2.00	18.61	31.97	53.47	16.59	29.95	51.45	12.43	25.76	47.21
	$ 5.00	81.71	109.65	154.07	79.69	107.63	152.05	75.53	103.44	147.81
	$ 8.00	144.81	187.33	254.66	142.79	185.31	252.64	138.63	181.12	248.41
Milk Price ($/cwt)	$ 12.00	39.64	57.86	87.00	37.62	55.84	84.98	33.46	51.65	80.75
	$ 18.00	39.64	57.86	87.00	37.62	55.84	84.98	33.46	51.65	80.75
	$ 24.00	39.64	57.86	87.00	37.62	55.84	84.98	33.46	51.65	80.75
Genetic Advantage of TAI ($)	$ 0.00	23.85	42.07	71.21	22.97	41.19	70.34	20.72	38.90	68.00
	$ 300.00	47.77	66.00	95.14	45.17	63.39	92.53	40.03	58.22	87.31
	$ 600.00	71.70	89.92	119.06	67.36	85.58	114.72	59.35	77.54	106.63
Semen Cost/dose	$ 2.00	49.45	68.08	97.80	47.42	66.06	95.78	43.40	62.01	91.68
	$ 12.00	24.93	42.53	70.81	22.91	40.52	68.80	18.56	36.12	64.34
	$ 22.00	0.41	16.98	43.82	-1.60	14.97	41.82	-6.28	10.24	37.00

Table 4-7. Simulation of economic balance in dollars of TAI over NS considering different scenarios where arbitrary different pregnancy rates are varying accordingly with cost of semen, feed cost, milk price and genetic advance using as baseline the cost obtained in this study accounting for the difference in reproductive performance.

Items	PR TAI (%)	24	24	24	18	18	18	12	12	12
	PR NS (%)	24	18	12	24	18	12	24	18	12
Results from the study		36.17	108.34	226.44	-19.71	52.46	170.56	-114.19	-42.06	75.99
Marginal Feed Cost /cwt	$ 2.00	17.01	82.95	189.27	-37.11	28.83	135.15	-127.40	-61.50	44.78
	$ 5.00	74.51	159.13	300.79	15.11	99.73	241.39	-87.77	-3.18	138.43
	$ 8.00	132.01	235.32	412.31	67.33	170.64	347.62	-48.14	55.14	232.08
Milk Price ($/cwt)	$ 12.00	21.16	104.25	255.48	-48.79	34.29	185.53	-176.86	-93.82	57.37
	$ 18.00	32.42	107.32	233.70	-26.98	47.92	174.30	-129.86	-55.00	71.34
	$ 24.00	43.68	110.39	211.92	-5.17	61.55	163.08	-82.86	-16.18	85.30
Genetic Advantage of TAI ($)	$ 0.00	20.38	92.55	210.65	-34.35	37.82	155.91	-126.94	-54.81	63.24
	$ 300.00	44.31	116.48	234.58	-12.16	60.01	178.11	-107.63	-35.49	82.56
	$ 600.00	68.23	140.40	258.50	10.03	82.20	200.30	-88.31	-16.17	101.88
Semen Cost/dose	$ 2.00	45.98	118.56	237.24	-9.90	62.68	181.35	-104.26	-31.70	86.93
	$ 12.00	21.46	93.01	210.25	-34.41	37.14	154.37	-129.10	-57.59	59.59
	$ 22.00	-3.05	67.46	183.26	-58.92	11.60	127.39	-153.93	-83.47	32.25

Table 4-8. Simulation of economic balance in dollars of TAI over NS considering different scenarios where arbitrary different pregnancy rates and opportunity cost generated by replacement of bull for lactating cows accordingly with the number of slot available in one population of 100 animals.

TAI Pregnancy Rates	NS Pregnancy Rates	slots	1000	1000	1000
		cows	1000	986	972
		bulls	0	14	28
Results from the study	Results from the study		10.00	39.41	68.82
24%	24%		36.17	65.11	94.04
24%	18%		108.34	136.23	164.12
24%	12%		226.44	252.62	278.81
18%	24%		-19.71	9.23	38.16
18%	18%		52.46	80.35	108.24
18%	12%		170.56	196.75	222.93
12%	24%		-114.19	-85.26	-56.33
12%	18%		-42.06	-14.17	13.72
12%	12%		75.99	102.18	128.36

CHAPTER 5
GENERAL DISCUSSION AND CONCLUSIONS

Inefficient estrus detection is a major factor for the low reproductive performance observed in dairy farms throughout the U.S.A.. Consequently, research in dairy cattle reproductive management has focused intensely in methods to minimize the negative impact that inefficient estrus detection has on fertility. Arguably, NS or TAI are two breeding systems that can be used by dairy producers to mitigate the challenges of estrus detection. This is the first study that evaluated reproductive performance and economics of TAI and NS to reproductively managed dairy cows. Timed AI and NS were compared under a field trial where all possible variables were accounted and all conditions considered in order to optimize reproductive performance and to increase the likelihood of reasonable results with minimal possible confounding effects.

Both of the breeding program resulted in an excellent reproductive performance. The TAI breeding program did not result in a greater 21-d cycle pregnancy rate compared with NS as initially hypothesized. A greater proportion of pregnant cows at the end of the study occurred in NS bred cows because they had more breeding opportunities than TAI. The interval between breeding opportunities was 35 days for cows enrolled in the TAI program. On the other hand, if you consider length of an estrous cycle to be around 21 days, it is likely that cows bred by NS had a breeding opportunity every 21 days. When reproductive performance was compared to the same breeding eligibility period of 21-d cycle pregnancy rate, there was no difference between NS and TAI. Therefore, the greater proportion of pregnant cows at the end of the study and the greater daily hazards of pregnancy for NS can be attributed to the greater opportunity that NS cows had. Furthermore, a herd budget calculator was created, which allowed evaluation of costs and revenues of both breeding program accounting for the difference in 21-d cycle pregnancy rates. The economic analysis of this study revealed that the TAI breeding program was $38.69

less expensive than NS breeding program, contradicting the belief that the use of bulls is the cheapest method to breed dairy cows avoiding problems with estrous detection. The main variables for this difference were feeding cost for bulls and genetic advantage of TAI sires. The differences in profit were even greater when opportunity cost that result by the replacement of bulls by lactating cows was taken in consideration. Therefore, TAI is the most economical breeding program option to eliminate problems with estrus detection in lactating dairy.

In conclusion, NS and TAI are two breeding systems that can be used strategically to minimize problems related with detection of estrus, but the extended inter-insemination interval in TAI reduces daily pregnancy rate because these cow have fewer opportunities for breeding. However, the high price of feeding bulls and greater genetic advantage obtained with TAI makes this breeding program the most economical method to eliminate estrus detection problems in lactating dairy cows.

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BIOGRAPHICAL SKETCH

Fábio Soares de Lima was born on January 30, to Gaspar de Lima and Dolores Soares de Lima in Muzambinho, Minas Gerais, Brazil. He is the third of four children that grew up in a dairy farm and was surrounded by cows from an early age. After graduation from high school he was accepted and enrolled in the college of veterinary medicine at São Paulo State University, where he had an opportunity to work under the supervision of Dr José Luís Moraes Vasconcelos. In 2004, during the clinical year of his veterinary studies he spent 5 months at the University of Wisconsin, in Madison working with physiology of reproduction, health and reproductive management of dairy cows under the supervision of Dr Milo Wiltbank. After his graduation from São Paulo State University, he worked for 18 months at the University of California, Davis in the Veterinary Medicine Teaching and Research Center in Tulare, under the supervision of Dr José Eduardo P. Santos, conducting research in the areas of dairy cow nutrition, reproduction and health. In July 2006, he was accepted as resident in the Food Animal Reproduction and Medicine Program at University of Florida College of Veterinary Medicine. In 2007, he started a master degree program under the supervision of Dr Carlos Risco. After completion of his MS degree he will remain at University of Florida to pursue PhD degree.