

EVALUATION OF THE EFFECTIVENESS OF DECREASING THE DOSE OF GNRH USED
FOR SYNCHRONIZATION OF OVULATION AND TIMED AI
AT FIRST SERVICE IN DAIRY COWS

by

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(Under the Direction of William M. Graves)

ABSTRACT

The objective was to determine the effectiveness of decreasing the dose of GnRH used for synchronization of ovulation. First service lactating Holstein cows (n=100) at the University of Georgia Dairy in Athens were randomly assigned to 1 of 4 treatment groups using different doses of GnRH (100 µg and 100 µg; 50 µg and 50 µg; 100 µg and 50 µg; 50 µg and 100 µg). All cows received 25 mg of PGF_{2a} 11 days (d -11) prior to starting Ovsynch. Blood samples were collected on days -11 and 0 for progesterone analysis. Cows received GnRH on day 0, PGF on day 7, and GnRH on day 9. Cows were artificially inseminated (AI) 16-20 hours after the second GnRH injection. Pregnancy was checked via ultrasound at 35-40 days and 55-60 days after AI. Pregnancy rates did not differ among treatments. Cows that were cycling were more likely to be pregnant.

INDEX WORDS: Ovulation Synchronization, Timed AI, GnRH, PGF, Dairy Cow

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CHAPTER 1

LITERATURE REVIEW

Pregnancy rate is defined as the AI submission or estrus detection rate multiplied by the conception rate (Stevenson et al., 1999). Pregnancy rates for AI in dairy cows have decreased steadily since the 1950's. In 1951 the average pregnancy rate was 66%; in 1975 it was 50%, and in 2001 it was 40% (Lucy, 2001). Average services per conception has also increased from 1.62 in 1972 to 2.91 in 1996 (Lucy, 2001). Dairy cows today have higher rates of embryonic loss, greater days to first ovulation, higher anestrus rates, and lower blood progesterone concentrations when compared to cows many years ago (Lucy, 2001). There are many factors that negatively affect reproductive performance, including heat stress, negative energy balance, increased milk yield, vitamin and mineral deficiencies, increased herd size, labor shortage, and increased use of confinement housing (Pursley et al., 1997a; Lucy, 2001).

The normal estrous cycle in the dairy cow is 21 days and is divided into four phases. Estrus is day 21 or 0 of the cycle and is the time when estrogen peaks and the cow will stand to be mounted. Progesterone is at its lowest at this time. Estrogen causes a surge of luteinizing hormone (LH) that will result in ovulation 10 to 12 hours later. Metestrus occurs on days 1 to 5. It is the phase during which ovulation occurs. Diestrus lasts from day 6 to 17. During diestrus a functional corpus luteum (CL) is present on the ovary. The CL releases progesterone which inhibits the release of gonadotropin releasing hormone (GnRH). GnRH is the hormone responsible for the release of LH and follicle stimulating hormone (FSH), which together cause follicular development and ovulation. If the cow is not pregnant at the end of this phase,

prostaglandin (PGF_{2a}) from the uterine endometrium will cause regression of the CL. Proestrus occurs on days 18 to 20. During this time the follicle is maturing and increasing its release of estrogen. The cow may start to mount other cows and have cervical mucous secretions (Senger, 1999).

A major factor that limits reproductive performance and increases days open is failure to detect cows in estrus. As dairy farm size and milk production per cow continue to increase, estrus detection has become an increasing problem (Nebel and Jobst, 1998). Lucy (2001) reported that estrus in the average dairy cow lasts only 7 hours with only 8.5 stands per estrus. Almost one-fourth of cows had estruses that were less than 7 hours with less than 1.5 stands (Lucy, 2001). This makes detection of estrus difficult, even if it is done frequently. The average estrus detection rate on most dairy farms is less than 50% (Fricke et al., 1998). Cows must be bred back by 85 days in milk in order to achieve a 12 month calving interval and optimal milk production (Nebel and Jobst, 1998; Cordoba and Fricke, 2001). When cows fail to become pregnant early in lactation, they spend more time in the less productive stage of late lactation and have a higher chance of being culled from the herd (Pursley et al., 1997a; Nebel and Jobst, 1998).

Synchronization of ovulation (Ovsynch) can eliminate the need for estrus detection and makes it possible to inseminate a large number of cows on a set time schedule (Fricke et al., 1998). Ovsynch can also decrease days to first service, days open, and calving intervals (Keister et al., 1999; Jobst et al., 2000). The Ovsynch protocol includes an injection of 100 µg GnRH on day 0, followed by 25 mg PGF_{2a} on day 7, and second 100 µg GnRH injection on day 9. Cows are then time bred 16 to 20 hours after the second GnRH (Pursley et al., 1997b; Fricke et al., 1998). The first GnRH injection causes a new follicular wave and dominant follicle to emerge.

Prostaglandin causes regression of the CL and formation of a new dominant follicle. The second GnRH synchronizes ovulation of the dominant follicle (Pursley et al., 1997a; Nebel and Jobst, 1998). Synchronization occurs in 87 to 100 % of cows treated, and pregnancy rate per AI for Ovsynch is similar to that after detected estrus (Nebel and Jobst, 1998; Pursley et al., 1998; Cartmill et al., 2001b). Synchronization is possible because the follicles are at a similar stage of development, making them responsive to a LH surge (Pursley et al., 1997a).

Starting Ovsynch on day 5 to 12 of the estrous cycle has shown improved synchronization and conception rates compared with other days (Keister et al., 1999; Cordoba and Fricke, 2001). A dose of PGF_{2a} 12 days prior to starting Ovsynch should increase the number of cows at day 5 to 12 of their cycle by stimulating luteal regression in cows with a CL. Cordoba and Fricke (2001) found no benefit to the PGF_{2a}, while Cartmill et al. (2001a) reported increased conception rates.

Cyclicity in lactating dairy cows has been determined by analysis of serum progesterone levels prior to initiating the Ovsynch protocol. A progesterone sample of ≥ 1 ng/ml is defined as high and indicates a functional corpus luteum (CL). A sample of < 1 ng/ml is defined as low (Cartmill et al., 2001b). Cordoba and Fricke (2001) collected blood samples 12 days apart for progesterone analysis. A cow with two low samples was classified as acyclic. Cows with any combination of high and low for the two samples were considered to be cycling (Cordoba and Fricke, 2001).

The voluntary waiting period (VWP) is the time a producer elects to wait after calving before rebreeding his cows. It is estimated that 5 to 20% of cows are not cycling at the end of the voluntary waiting period (Cartmill et al., 2001a, 2001b). Cows must be bred back by 85 days in milk (DIM) in order to achieve a twelve month calving interval. A VWP of 50 to 60 days

only gives producers two cycles to get a cow pregnant. Ovsynch has been helpful in getting problem breeders and cystic cows pregnant by inducing ovulation. Graves et al. (1994) reported that GnRH injected at time of breeding increased conception rates in third and fourth service cows by over 10% compared to those not injected. Since GnRH is labeled for treatment of follicular cysts, and PGF_{2a} causes regression of luteal cysts, Ovsynch should readily help these cows to start cycling (Cordoba and Fricke, 2001). Graves et al. (2001) reported that in third service cows, higher pregnancy rates were obtained in cows treated with Ovsynch compared to those treated with a PGF and heat detection program. They observed that Ovsynch may increase fertility in problem breeders (Graves et al., 2001).

Two Ovsynch studies (Cartmill et al., 2001b; Cordoba and Fricke, 2001) found no difference in conception rates between cycling and noncycling cows. This indicated that Ovsynch does cause ovulation in these cows, and they have as good a chance of becoming pregnant as the previously cycling cows (Cartmill et al., 2001b; Cordoba and Fricke, 2001). Another study reported lower pregnancy rates and lower embryonic survival for noncycling cows treated with the Ovsynch protocol (Cartmill et al., 2001a).

High milk yield in early lactation is associated with a negative energy balance and failure to conceive. Vasconcelos et al. (1999) reported a positive correlation between high milk yield and larger follicles, which led to lower fertility. It is thought that higher milk yield increases the frequency of LH pulses. Decreased progesterone concentrations found in high producing cows may be related to higher dry matter intake in these cows. High dry matter intake increases blood flow to the liver resulting in increased metabolism of progesterone (Vasconcelos et al., 1999). Lucy (2001) suggested that cows in negative energy balance need a larger sized follicle in order to produce enough estradiol to trigger ovulation. The additional time required to increase the

size of these follicles may result in lowered fertility, much like a cystic cow (Lucy, 2001). Cows selected for milk yield tend to have differences in endocrine systems and follicular function compared to genetically inferior milkers (Al-Katanani et al., 1999).

A study by Peters and Pursley (2002) reported that cows with higher than the average milk yield within their herd were the most fertile animals in the herd. These cows tended to be high producers because of their lack of health problems, such as lameness, mastitis, retained placentas, ketosis, and metritis. The study also reported that double ovulations occur more frequently in high producing cows, allowing for a greater chance to conceive (Peters and Pursley, 2002). Lucy (2001) indicated that milk yield had little effect on reproductive performance, except in the very highest producing cows. The high producing cows are not necessarily the ones with the greatest negative energy balance. Usually these cows have high DMI to compensate for their high milk production. Low producing cows often have low DMI and are more likely to be anestrus than higher producers (Lucy, 2001).

Body condition score (BCS) and days in milk also play an important role in fertility. Cartmill et al. (2001a) observed that cyclicity increased 9.3% for every 1 unit increase in BCS. Cyclicity increased 6.1% for every 10 day increase in DIM (Cartmill et al., 2001a). One study (Stevenson et al., 1999) showed that BCS was positively related to conception rate and to progesterone levels 7 days following a GnRH injection. For each unit increase in BCS, progesterone concentrations increased by 0.5 ng/ml and conception rate increased by 8.6% (Stevenson et al., 1999).

A study by Pursley et al (1998) looked at time of ovulation to determine the ideal time for AI after GnRH. Inseminations were done at 0, 8, 16, 24, and 32 h post GnRH injection. Pregnancy rates were similar for all times except for 32 h, which was lower. Ovulation occurred

24 to 32 h after GnRH, which was similar to the time of ovulation after natural estrus (Pursley et al., 1997b; Cartmill et al., 2001a). Insemination at 32 h occurred after ovulation in most cases. Pregnancy rates were highest for cows inseminated at 16 h after the second GnRH injection, followed closely by 8 and 24 h (Pursley et al., 1998).

The major problem with Ovsynch is its total cost to producers. According to Nebel and Jobst (1998), the average cost of one 100 µg GnRH injection is \$7.27, and the average cost of one 25 mg injection of PGF_{2a} is \$3.30. Total drug cost for one cow averages \$17.84, and the average cost per pregnancy is \$46.10. In comparison, a PGF_{2a} only program has a total drug cost of \$8.58 with a 40% heat detection rate and \$7.59 with a 70% heat detection rate. Average cost per pregnancy is \$29.61 and \$21.11 for a 40% and 70% heat detection rate respectively. Similar pregnancy rates were reported for Ovsynch (37.8%) and PGF_{2a} (38.9%) cows (Nebel and Jobst, 1998). The estimated cost of nonpregnant cows to the producer ranges from \$1 to \$10 per day for each day over 100 DIM that she is open (Keister et al., 1999).

As previously mentioned, GnRH is labeled for treating follicular cysts at a dosage of 100 µg (Merial Animal Health, Duluth, GA). This is why the Ovsynch protocol utilizes a 100 µg dose. A study conducted in 1998 by Fricke et al. compared 100 µg doses of GnRH to a lower 50 µg dose. Results suggest that half the recommended dose of GnRH could be used at both injections of the Ovsynch protocol without any decrease in conception rate. Synchronization rate was no different for the full versus half dose groups (84.9% versus 83.1%). Overall conception rates were 41% at 28 days and 34.4% at 56 days. Embryonic death from day 28 to 56 was 13.5% for both groups. Using half doses of GnRH decreased total hormone cost from \$16.10 to \$9.70. Total cost per pregnancy was reduced from \$47.88 to \$27.61, making it comparable to the cost of a PGF_{2a} only program (Fricke et al., 1998).

A study by Navanukraw et al. (2002) reported a 37.5% pregnancy rate at 42 days post AI using Ovsynch with two half doses of GnRH. Another study by Fricke and Welle (2003) found similar pregnancy rates using half dose Ovsynch on second service animals. Leslie et al. reported in 2003 that cows bred before 75 DIM and receiving 200 μg of GnRH at the first injection were three times more likely to become pregnant than cows receiving the full 100 μg dose. This was attributed to more cows ovulating in response to the higher first dose. After 75 DIM the higher dose appeared to have no effect on pregnancy (Leslie et al., 2003). It seems likely, based on this study, that using 100 μg and 50 μg for the first and second GnRH injections respectively may result in higher pregnancy rates than using 50 μg and 50 μg (Leslie et al., 2003).

Heat stress can be defined as the sum of external forces that raise body temperature from its resting state (Hansen and Arechiga, 1999). Continued selection for higher milk yield has reduced the dairy cow's ability to regulate body temperature during heat stress (Al-Katanani et al., 1999; Hansen and Arechiga, 1999). Heat stress causes a decrease in estrogen secretion, poor follicular growth, decreased conception rates due to decreased expression of estrus, and poor embryonic survival (Cartmill et al., 2001b). Decreased estrogen secretion in follicles damaged by heat stress, results in a longer luteal phase (Lucy, 2001). When heat stress occurs on the day a cow ovulates, the subsequent dominant follicle has decreased diameter and volume (Hansen and Arechiga, 1999).

Temperature humidity index (THI) is a good indicator of the level of stress faced by lactating dairy cows. It is calculated from ambient temperature and relative humidity. A THI of 72 or greater indicates heat stress (Ravagnolo et al., 2000; Cartmill et al., 2001b). One study by Hansen and Arechiga (1999) found that fertility decreased 4.1 to 4.7% and embryo survival

decreased by 7.1% for each 10 unit increase in THI on day of calving. Average THI on the day before breeding was found to have no effect, however pregnancy rate decreased by 2.2 to 2.5% for each 10 unit increase in THI near the time of AI (Hansen and Arechiga, 1999). Al-Katanani et al. (1999) also indicated that heat stress prior to ovulation can negatively affect embryonic survival.

Ovsynch has been shown to help improve pregnancy rates during times of heat stress (Cartmill et al., 2001b). In the summer months cows are less likely to show estrus, and duration of estrus may be decreased. This may be due in part to physical lethargy associated with the heat. There is some speculation that heat stress causes varying levels of hormone concentrations which effects estrus expression and proper cycling. Studies have reported a reduced LH surge and lower level of progesterone secretion in heat stressed cows (Ullah et al., 1996; Hansen et al., 1999). With the Ovsynch protocol, GnRH causes the release of LH, ensuring that the cow will ovulate and then form a CL to release progesterone (Ullah et al., 1996; Willard et al., 2003). Hansen and Arechiga (1999) indicated that Holsteins in the summer months averaged 4.5 mounts per estrus period compared to 8.6 mounts per estrus period during the winter months.

Timed AI eliminates problems associated with estrus detection since all cows are inseminated. Ovsynch cows typically do not show signs of estrus. This eliminates problems related to mounting and riding, such as cows falling and injuring themselves or having elevated body temperatures due to activity (Cartmill et al., 2001b; Pursley, 2003). In about two thirds of Ovsynch cows, the follicle that ovulates is not mature enough to produce estrogen to cause signs of estrus. While the ovulatory follicle may be slightly less mature in these cows compared to those that do show estrus, chances of becoming pregnant are the same (Pursley, 2003). However,

Jobst et al. (2000) reported that Ovsynch cows that showed signs of estrus had higher pregnancy rates than those that did not show estrus.

Early embryonic death between breeding and 56 days post breeding has been reported at rates of 10 to 20% in lactating dairy cows (Fricke, 2003). Heat stress contributes a great deal to these numbers due to alterations in oocyte quality and follicular function as well as reductions in steroid hormone concentrations in the blood. Heat stress causes a redistribution of blood flow from the viscera to the periphery to allow the animal to release body heat. This in turn reduces blood flow to the placenta, resulting in compromised fetal growth (Hansen and Arechiga, 1999). Heat stress can also cause endometrial secretion of prostaglandin, resulting in embryonic death (Al-Katanani et al., 1999). Most embryonic death occurs prior to 14 days old, which is before maternal recognition of pregnancy (17-19 days post insemination). The effects of heat stress on the embryo decrease as it develops (Hansen and Arechiga, 1999).

A study by Cartmill et al. (2001b) compared Ovsynch to a PGF_{2a} and estrus detection method in cows exposed to THI's of >72 or <72. Results of this trial indicate that Ovsynch had higher conception rates for cows exposed to high THI's, while PGF_{2a} had higher rates for cows exposed to low THI's. However, by 40 to 50 days post insemination, conception rates between the two were similar due to higher embryonic death in the Ovsynch cows. The higher rate of embryonic death was attributed to the fact that some (average of 15%) of the Ovsynch cows were not cycling prior to treatment at the end of the VWP. These cows were induced to ovulate and conceive but then had a decreased ability to maintain that pregnancy (Cartmill et al., 2001b). Other hot weather studies have reported lower conception rates with Ovsynch cows compared with cows bred on detected estrus. However, due to decreased estrus detection rates associated

with heat stress, pregnancy rate (the overall number of pregnancies) is greater with Ovsynch (Pursley, 2003).

The objectives of this study were to determine the effectiveness of decreasing the dose of GnRH used in the Ovsynch protocol at first service in lactating dairy cows during high and low THI's, and to estimate the cost associated with four different combinations of GnRH doses (100 µg and 100 µg; 50 µg and 50 µg; 100 µg and 50 µg; 50 µg and 100 µg). We hypothesized that the treatments with one or two half doses would have similar pregnancy rates to the full dose treatment.

CHAPTER 2

MATERIALS AND METHODS

First service, primiparous and multiparous lactating Holstein cows (n=100) from the University of Georgia Dairy Farm in Athens were used in this study. Cows ranged from first to seventh lactation, with an average of 2.29 lactations. These cows were milked twice daily, and the rolling herd average for the duration of this study (February 2003 to December 2003) was 9,722 kg. A TMR was fed twice daily, and cows were housed in a free-stall barn bedded with sawdust.

Blood samples were collected on days -11 and 0 via coccygeal venipuncture for progesterone analysis. Blood was allowed to clot for = 24 hours at 4°C and was then centrifuged at 3500 X g for 15 minutes. Serum was collected and stored at -20 °C until samples were analyzed (Cartmill et al., 2001b). Progesterone concentrations were analyzed using the ImmuChem Progesterone RIA Kit (ICN Pharmaceuticals, Inc., Diagnostics Division, Costa Mesa, CA). The samples were run in duplicates, and the result was reported as the average of the two tubes. Readings were classified as either high (= 1 ng/mL) or low (< 1 ng/mL). Cows with two low readings were considered to be acyclic. Cows with two high readings or one high and one low were considered to be cycling.

Cows were randomly assigned to one of 4 treatment groups (Table 1). Cows were started on their treatment at a random stage of the estrous cycle and were bred after 54 days in milk. All cows received a shot of PGF_{2a} (Lutalyse®, Pfizer Animal Health, New York, NY) 11 days (d - 11) prior to day 0. Intramuscular injections of GnRH (Cystorelin®, Merial Limited, Duluth,

GA) and PGF_{2a} were given as follows: Day 0, GnRH; Day 7, PGF_{2a}; Day 9, GnRH (Pursley et al., 1997b). The day 9 GnRH was given in the late afternoon and cows were bred the following morning 16-20 hours after the injection. All GnRH injections were given using 20 g, 1 ½ inch needles to ensure that the animals received the entire dose.

Table 1. Protocol for synchronization of ovulation and timed AI in lactating dairy cows using PGF_{2a} and one of 4 combinations of half (50 µg) or full (100 µg) doses of GnRH.

Treatment 1: Full Dose Ovsynch			
Day 0	Day 7	Day 9	16-20 hours later
100 µg GnRH	25 mg PGF _{2a}	100 µg GnRH	AI

Treatment 2: Half/Full Dose Ovsynch			
Day 0	Day 7	Day 9	16-20 hours later
50 µg GnRH	25 mg PGF _{2a}	100 µg GnRH	AI

Treatment 3: Full/Half Dose Ovsynch			
Day 0	Day 7	Day 9	16-20 hours later
100 µg GnRH	25 mg PGF _{2a}	50 µg GnRH	AI

Treatment 4: Half/Half Dose Ovsynch			
Day 0	Day 7	Day 9	16-20 hours later
50 µg GnRH	25 mg PGF _{2a}	50 µg GnRH	AI

Pressure activated heat mount detectors (Kamar®, Kamar, Inc., Steamboat Springs, CO) were applied on day 7 to determine which cows showed signs of estrus. No cows were bred before day 10. Cows were body condition scored by the herd manager at time of breeding. A scale of 1 to 5 was used, with 1 being thin and 5 being obese (Wildman et al., 1982).

All cows were bred by one of two AI technicians at the farm. Reproductive tract tone was evaluated by the AI technicians and scored as either toned or lacking tone. Service sires were selected by the herd managers according to the standard mating procedures. Estimated

relative conception rates (ERCR) for all bulls used were recorded for evaluation at the end of the trial.

Pregnancy determination was made using an ultrasound machine (Aloka 500, Wallingford, CT) with 5 MHz linear transrectal probe. Pregnancy diagnosis was made by detection of fluid and a fetal heartbeat. Cows were checked at 35 to 40 days post AI and again at 55 to 60 days post AI. Pregnancy rates were calculated at 35 to 40 days and at 55 to 60 days by the number of cows pregnant, expressed as the percentage of cows receiving that treatment. Pregnancy loss was calculated as the number of cows open at 55 to 60 days, expressed as a percentage of cows diagnosed pregnant at the first check.

Temperature humidity index (THI) was recorded on days cows were bred to determine the effect of heat stress on reproductive performance. THI was obtained from the University of Georgia Climatology Research Laboratory. The high THI for the day, as well as the THI at time of breeding were recorded on the days cows were bred. A THI of 72 to 79 indicates mild stress. A THI of 80 to 89 indicates medium stress. A THI of 90 or greater is indicative of severe heat stress (Wiersma, 1990). A comparison was made between pregnancy rates during high THI periods versus low ones.

Differences in pregnancy rate at 35-40 days and 55-60 days post AI were analyzed using chi-square (Steele and Torrie, 1960) and the PROC GEN MODE procedures of SAS (1989). A bivariate analysis was conducted between independent and dependent variables. A multivariate model including treatment, lactation number, season bred, and cyclicity was then developed. The significance level was set at $P < 0.05$.

CHAPTER 3

RESULTS AND DISCUSSION

Cows in this study were bred at an average of 68 days in milk (DIM) and averaged a body condition score (BCS) of 2.7. Average daily milk yield for cows in the trial was 40 kg based on DHIA test weights recorded on the monthly test date closest to AI. Somatic cell count (SCC) for each cow was also recorded on the same date and averaged 334,340 cells/ml. Means and standard errors (SE) by treatment for the independent variables in this study are shown in Table 2. Means, ranges, and SE for all cows used in the study are shown in Table 3. In tests for randomization, no difference was found among the treatments for any of the following variables.

Table 2. Means and standard errors (SE) for each treatment (25 cows/treatment).

Item ^a	Treatment 1 Full/Full		Treatment 2 Half/Full		Treatment 3 Full/Half		Treatment 4 Half/Half	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Lactation #	2.16	0.22862	2.4	0.258	2.48	0.337	2.12	0.247
ERCR of Breeding Sire	-0.458	0.38999	-0.6	0.365	-0.79	0.385	-0.72	0.414
# Milk Last Test (kg)	87.68	4.4742	91.48	1.11986	87.32	3.8604	85.96	3.7423
SCC Last Test (cells/ml)	246.88	99.382	542.6	158.381	274.48	112.612	273.4	100.162
DIM	69.52	4.812	68.52	2.2	69.2	3.943	64.88	1.3
BCS	2.716	0.075	2.664	0.06	2.688	0.06	2.644	0.06
THI at Time Bred	63.588	2.6113	63.196	2.78674	62.54	2.6608	62.776	2.412
High THI on Day Bred	73.648	3.3726	73.296	3.4751	71.612	3.0308	72.18	3.0633
Progesterone Day -11 (ng/ml)	2.447	0.583	1.8064	0.4978	3.204	0.6704	1.9132	0.5446
Progesterone Day 0 (ng/ml)	3.1608	0.38083	2.7464	0.4661	3.3296	0.501	2.9772	0.355

^a No difference was found among the treatments for any of these variables.

Table 3. Means, ranges, and standard errors (SE) of data collected. (n=100 cows)

Item	Mean	Minimum	Maximum	SE
Lactation #	2.29	2	7	0.1343
ERCR of Breeding Sire	-0.63	-4	2	0.20516
# Milk Last Test (kg)	40	20	61.7	2.088
SCC Last Test (cells/ml)	334,340	13,000	2,787,000	60.36
DIM	68.03	55	180	1.662
BCS	2.678	2.0	3.5	0.0308
THI at Time Bred	63.03	41.8	90.2	1.267
High THI on Day Bred	72.68	43.6	105.1	1.598
Progesterone Day -11 (ng/ml)	2.34	0	10.84	0.2897
Progesterone Day 0 (ng/ml)	3.05	0	11.3	0.2128

^a No difference was found among the treatments for any of these variables.

Kamars were classified on the morning of breeding as either red, white, or lost. Red indicated that the cow was in standing estrus, while white indicated that the cow did not show signs of estrus. Lost kamars could have been due to cows being ridden or possibly to the kamar not adhering properly to the cow. Therefore, it was often difficult to be sure if these cows were in standing estrus or not. There was no difference in number of estruses shown among the treatments (Table 4). Cows that were considered to be in estrus (those with red kamars) were no more likely to be pregnant than those that did not show estrus (those with white kamars). This is consistent with results reported by Pursley (2003), that Ovsynch cows who did not show estrus had similar pregnancy rates as those that did show estrus.

Table 4. Number of Kamars classified as red, white, and lost for each treatment group.

Treatment	Red	White	Lost
1 (full/full)	9	15	1
2 (half/full)	7	15	3
3 (full/half)	8	17	0
4 (half/half)	8	16	1

Pregnancy rate did not differ ($P > 0.05$) among the four treatment groups at either 35-40 days or 55-60 days post AI (Table 5). Overall pregnancy rate at 35-40 days was 46%. At 55-60 days the overall pregnancy rate was 37%. Embryonic death between 35 and 60 days was similar among the treatments, with an overall loss rate of 9%. This is just slightly below the average rate for lactating dairy cows of 10-20% as indicated by Fricke (2003).

Table 5. Pregnancy rates for each of the 4 treatment groups at 35-40 days and 55-60 days post AI and embryonic death between 35 and 60 days.

Item^a	35-40 days	55-60 days	Embryonic Death
Treatment 1 (full/full) (number/total)	52% (13/25)	36% (9/25)	16% (4/25)
Treatment 2 (half/full) (number/total)	32% (8/25)	28% (7/25)	4% (1/25)
Treatment 3 (full/half) (number/total)	44% (11/25)	36% (9/25)	8% (2/25)
Treatment 4 (half/half) (number/total)	56% (14/25)	48% (12/25)	8% (2/25)
Standard Error	0.78 (0.4)	0.6776 (0.339)	0.839 (0.419)

^a For each item, no statistical difference was found ($P > 0.05$).

High THI on the day bred had no effect on overall pregnancy rates or embryonic death (Table 6). However, none of the cows in treatment 2 (half/full) bred on days with THI 80 or greater were pregnant at 35-40 days post AI. Pregnancy rates for the other three treatments at 35-40 days and THI 80+ were 43%, 50%, and 66.7% for treatments 1, 3, and 4 respectively. It is

unclear as to why treatment 2 (half/full) did not work in severely heat stressed cows, but had the lowest overall embryonic death.

Table 6. Overall pregnancy rates at 35-40 and 55-60 days post AI and embryonic loss between the pregnancy checks for 3 different THI levels.

High THI on Day^a Bred	35-40 days	55-60 days	Embryonic Death
80+ (number/total)	35.7% (10/28)	28.6% (8/28)	7.1% (2/28)
70-79 (number/total)	54.8% (17/31)	45.2% (14/31)	9.6% (3/31)
<70 (number/total)	46.3% (19/41)	36.6% (15/41)	9.7% (4/41)
Standard Error	0.618 (0.7)	0.791 (0.622)	0.287 (1.232)

^a For each item, no statistical difference was found ($P > 0.05$).

For analysis, cows were divided into high and low producers, with >31.8 kg/d considered high and <31.8 kg/d considered low. At the 35-40 day pregnancy check, cows that were low producers in the herd were more likely to be open than high producers ($P = 0.03$) (Table 7). This supports conclusions made by Peters and Pursley (2002) and Lucy (2001) that cows with higher than herd average milk yield are more fertile than cows with lower than herd average production. However, at 55-60 days there was no significant difference in pregnancy between high (>31.8 kg/d) and low producers (<31.8 kg/d). It should be noted that there were only 21 cows milking <31.8 kg/d compared to the 77 cows that were milking >31.8 kg/d.

Table 7. Pregnancy diagnosis at 35-40 days for cows milking >31.8 kg/d and <31.8 kg/d.

Item	>31.8	<31.8
Open	38 ^a	16
Pregnant	39 ^a	5
Standard Error	0	0.3704

^a Different from cows milking <31.8 kg/d ($P = 0.03$).

For further analysis, cows were divided into two milking groups based on the study average milk of 40 kg/d. Those milking >40 kg/d constituted one group, while those milking =40 kg/d made up the other group. No difference was detected in pregnancy rate between the two groups at either the 35-40 day pregnancy check (Table 8) or the 55-60 day pregnancy check. It should be noted that splitting the cows at 40 kg/d milk yield should be more accurate than the previous grouping at 31.8 kg/d because this divides the data into more equal numbers of cows in each group. There were 54 cows in the >40 kg/d group and 46 cows in the =40 kg/d group.

Table 8. Pregnancy diagnosis at 35-40 days for cows milking >40 kg/d and =40 kg/d.

Item ^a	>40	Standard Error	<40	Standard Error
Open	24	.4998	30	0.3799
Pregnant	22	.2132	24	0.4999

^a For each item, no statistical difference was found ($P > 0.05$).

A total of 14 of the 100 cows were considered to be noncyclic (both blood samples < 1 ng/ml progesterone), and only 2 of these were pregnant at 35-40 days (14.3% pregnant). Of the 86 cyclic cows (either or both samples = 1 ng/ml), 44 (51.2%) were pregnant at 35-40 days. The two noncyclic cows pregnant at 35-40 days were still pregnant at 55-60 days. Both cows were in treatment 4 (half/half). Pregnancy rate was greater in cycling cows than in noncycling cows at 35-40 days ($P = 0.01$). Noncycling cows were 6.2 times more likely to be open than cycling cows and had less than a 0.2 % chance of becoming pregnant. Cartmill et al. (2001a) also found that in cows treated with the Ovsynch protocol, noncycling cows were more likely to be open. This indicates that not all cystic or acyclic cows are helped by Ovsynch. At the 55-60 day pregnancy check, pregnancy rate tended to be greater in cycling cows, but was not significant.

According to DHIA records for this herd of 130 milking cows, days open decreased by 34 days (from 152 to 118) during the duration of this study (February 2003 to December 2003).

Days to first service decreased by 21 days (from 86 to 65). Calving interval decreased from 14.2 to 13.1 months. Percentage of estruses detected increased from 37 to 47%. This is not an actual reflection of the number of cows seen in standing estrus but shows that a greater number of animals were serviced due to the timed breeding. Conception rate over the duration of the study decreased from 42% to 36%. This may be associated with the slight decreased conception associated with timed AI, or it may be in part because of the lasting effects of heat stress (Hansen and Arechiga, 1999).

A comparison of actual hormone costs in this study per cow and per pregnancy for each of the four treatment groups is shown in Table 9. Each treatment consisted of two GnRH doses and one PGF_{2a} dose. In this study, GnRH cost per cow was \$6.80 less for treatment 4 (half/half) versus treatment 1 (full/full). Gonadotropin releasing hormone cost per pregnancy decreased by \$23.61 from treatment 1 (full/full) to treatment 4 (half/half). Prostaglandin cost per cow was the same for all four treatment groups since all cows received 25 mg of PGF. However, PGF_{2a} cost per pregnancy was less for treatment 4 (half/half) than for any of the others due to the higher pregnancy rate in that group.

Total hormone cost per cow was \$16.60 for treatment 1 (full/full) versus \$9.80 for treatment 4 (half/half). This was a reduction of \$6.80 in hormone cost per cow between the most expensive and least expensive treatments. The reduction in hormone cost per pregnancy with treatment 4 (half/half) was even more substantial. In treatment 4 (half/half) total hormone cost per pregnancy was only \$20.21 compared to \$46.11 in treatment 1 (full/full). Treatment 2 (half/full) was the most expensive in terms of cost per pregnancy with a total cost of \$47.14. In this study, the reduction in cost per pregnancy from using two 50 µg doses of GnRH instead of two 100 µg doses was \$25.70.

Table 9. Comparison of costs of hormones and pregnancies for each treatment group

Item	Treatment 1 Full/Full	Treatment 2 Half/Full	Treatment 3 Full/Half	Treatment 4 Half/Half
# Pregnant 60 days post AI	9	7	9	12
GnRH \$/cow	13.60	10.20	10.20	6.80
GnRH \$/pregnancy^a	37.78	36.43	28.33	14.17
PGF_{2a} \$/cow	3.00	3.00	3.00	3.00
PGF_{2a} \$/pregnancy^a	8.33	10.71	8.33	6.25
Total hormone \$/cow	16.60	13.20	13.20	9.80
Total hormone \$/pregnancy^a	46.11	47.14	36.67	20.41

^aCost per pregnancy was calculated as total hormone cost for treatment group divided by the number of cows pregnant at 60 days post AI. Each group contained 25 cows.

The average dairy herd in Georgia is currently 247 cows. Using half dose Ovsynch instead of full dose Ovsynch with all first service animals could lower total pregnancy costs by \$6,347 annually. Each extra day a cow is open costs the producer \$2 on the average (Graves and Montgomery, 1994). Based on our results, using Ovsynch could decrease days open by 34 days in a herd, saving each producer who uses half dose Ovsynch up to \$16,796 annually.

CHAPTER 4

CONCLUSION

No differences were observed in pregnancy rate among the four treatments. Similar pregnancy rates can be obtained using any combination of 50 µg and 100 µg of GnRH in the Ovsynch protocol. Cyclicity had a significant influence on pregnancy rate at 35-40 days. Cows that were cycling prior to Ovsynch were much more likely to become pregnant than noncycling cows. The Ovsynch protocol was not very effective in getting noncycling cows pregnant. Further research is needed to determine why this is and what can be done to help these problem cows conceive and maintain pregnancy.

While cows milking >31.8 kg/d were more likely to be pregnant than those milking <31.8 kg/d, the large difference in group size may have had an effect on this result. Cows milking higher than the average of cows in the study (40 kg/d) were just as likely to be pregnant as those milking less than the average. Since these two groups were of more equal size, it is likely that milk production had no effect on pregnancy rate.

Pregnancy rates were no different for the three THI levels, indicating that Ovsynch is just as effective in getting cows bred at high, moderate, and low temperatures. If it is necessary to breed cows in the summer months, Ovsynch may be beneficial. Less than half of the treated cows showed signs of standing estrus prior to breeding. Failure to show signs of estrus reduces the likelihood of cows injuring themselves and helps to prevent excess activity that may intensify the effects of heat stress.

The most economical was treatment 4 (half/half) both in terms of total hormone costs and cost per pregnancy. The GnRH dose in the Ovsynch protocol for synchronization of ovulation and timed AI in first service lactating dairy cows can effectively be decreased, while maintaining pregnancy rates. Based on this study and those by Fricke et al (1998), Navanukraw et al (2002), and Fricke and Welle (2003), producers could greatly benefit from a half dose Ovsynch program, particularly in the southeast where estrus detection rates are generally very low. This program could greatly reduce overall drug costs and decrease days open in their herds.

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