HUMAN CHORIONIC GONADOTROPIN AND GONADOTROPIN-RELEASING HORMONE INFLUENCE PREGNANCY SURVIVAL AND RESYNCHRONIZED OVULATION BEFORE TIMED ARTIFICIAL INSEMINAITON IN HOLSTEIN CATTLE

by

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Abstract

A study was performed to determine the minimum effective dose of human chorionic gonadotropin (hCG) needed to induce ovulation of follicles in cattle (Exp. 1). Another study determined the effects of replacing the first injection of GnRH (d -7) with hCG or saline in a Resynch-Ovsynch protocol [injection of GnRH 7 d before and 48 h after $PGF_{2\alpha}$ before a resynchronized fixed-timed AI (TAI)] on pregnancy rates in cows diagnosed not pregnant and pregnancy survival in cows diagnosed pregnant (d 0; Exp. 2). A final study determined the ovulation potential of hCG compared with GnRH and saline (Exp. 3). In Exp. 1, ovaries of Holstein cows were mapped by using transrectal ultrasonography 7 d before pregnancy diagnosis. Cows were assigned to treatments of saline, 100 μg of GnRH, or 500, 1,000, 2,000, or 3,000 IU of hCG. Ovarian structures were monitored 7 d later and proportion of cows and follicles that ovulated were recorded. In Exp. 2, cows in 4 herds were assigned to treatments of 1,000 IU of hCG, 100 μg of GnRH, or left as untreated controls 7 d before pregnancy diagnosis. Nonpregnant cows were given $PGF_{2\alpha}$ (d 0), then inseminated 72 h later, concurrent with a GnRH injection. Pregnancy rates tended ($P = 0.08$) to be increased by GnRH (17.9%; n = 703) compared with control (12.9%; n = 505), but not hCG (16.5%; n = 541). Incidences of ovulation in nonpregnant cows (Exp. 3) were: hCG (51.6%; n = 126), GnRH (46.1%; n = 102), and control (28.1%; n = 96), whereas those in pregnant cows were: hCG (59.3%; n = 59), GnRH (24.5%; n = 49), and control (6.9%; n = 58). We concluded that: 1) a minimum dose of 1,000 IU of hCG resulted in a greater ovulatory response than saline, GnRH, or 500 IU of hCG (Exp. 1); 2) initiating a Resynch-Ovsynch protocol 7 d before pregnancy diagnosis with saline reduced timed AI pregnancy rates (Exp. 2); and 3)

incidence of new CL was greater after hCG than GnRH in pregnant cows, but not in nonpregnant cows (Exp. 3).

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Dedication

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CHAPTER 1 - REVIEW OF LITERATURE

INTRODUCTION

Fertility of the high-producing dairy cow has declined significantly during the past half century (Lucy, 2001). This reduction in reproductive efficiency has occurred during the same period in which researchers have learned more about reproductive biology than any other time in history. This conundrum may be partially explained when we consider that the US dairy herd, as-well-as the dairy cow, is evolving.

Dairy herds are becoming larger. With the increase in size comes an increase in management challenges. There is less time to devote to detection of estrus. Estrus is displayed less frequently among cattle confined to concrete flooring compared with natural surfaces (Britt et al., 1986). More is expected from the lactating dairy female today than ever before. According to the USDA National Agricultural Statistics Service (http://www.nass.usda.gov), milk production per cow has increased 16% during the past 10-year period. Much of this increase in production can be attributed to genetic selection; the same selection that is responsible for increased inbreeding coefficients (Hansen, 2000). Fertility is one of the traits most affected by this inbreeding suppression (Hansen, 2000). The preceding items are just a few examples of the many changes within the dairy industry that have had a negative impact on fertility and proved to be formidable barriers for scientists working in this area. Still, there is good news because much advancement has been made to offset the antagonistic effects of these factors on reproductive efficiency.

Moore and Thatcher (2006) pointed out that poor fertility is a function of 2 major factors: low pregnancy rates and high rates of pregnancy loss. Each will be discussed in turn.

Further dissection reveals that pregnancy rate is also a function of 2 separate factors: service risk and conception risk (Sterry et al., 2006). That is, the probability that a female will become eligible for service multiplied by the chance that the service will result in pregnancy. Historically, detection of estrus has been considered the most limiting factor in achieving a pregnancy (Barr, 1975).

Hormonal applications as a method to control the estrous cycle have long been a reality. Most early systems still required at least some period of detected estrus, however, because fixed-time artificial insemination (TAI) failed to yield acceptable pregnancy rates. Britt et al. (1981) recognized the need for protocols that synchronized not only estrus, but ovulation of follicles. This need was met with the advent of the Ovsynch protocol (Pursley et al., 1995). A recent survey by Caraviello et al. (2006) showed that hormonal synchronization of estrus or ovulation before TAI were being used in 87% of responding herds with Ovsynch being the most popular system.

Although TAI allows producers to pre-determine the day that the cow herd will be presented for the first postpartum service, those that do not conceive must be presented for second service as quickly as possible to minimize days open. When one considers that conception rates of high-producing dairy cows have been reported to be 40% or less (Pursley et al., 1997), the need exists for reliable resynchronization methods.

Fricke (2002) proposed a resynchronization protocol in which cows receive an injection of GnRH on d 18 after AI regardless of pregnancy status. All cows diagnosed

not-pregnant 7 d later via ultrasound complete the resynchronization of ovulation and TAI, whereas the program is discontinued in pregnant cows. Therefore, open cows are presented for another service 28 d after initial AI. This aggressive strategy completely eliminates the dependency on detection of estrus.

Recently more emphasis has been placed on improving the second portion of the fertility equation: the risk of conception per AI. Methods include: altering the timing of insemination after a synchronization regimen, administering presynchronization treatments that optimize ovulation incidence at AI, and searching for more efficacious hormones. The most highly researched of these hormones is human chorionic gonadotropin (hCG).

The glycoprotein hormone, hCG, is produced by the blastocyst soon after fertilization and maintains the corpus luteum (CL) of pregnancy in women. Chorionic gonadotropin shares an identical α-subunit with TSH, FSH, and LH, whereas the β-subunit is distinct and responsible for its biological specificity (Jameson and Hollenberg, 1993). Luteinizing hormone and hCG bind to the same receptors on the CL (Jameson and Hollenberg, 1993), and when administered to bovine females, hCG has been shown to prolong CL life (Wiltbank et al., 1961), ovulate follicles (Price and Webb, 1989; Diaz et al., 1998), and increase plasma progesterone concentrations (Rajamahendran and Sianangama, 1992; Santos et al., 2001). Many of the mechanisms however, through which hCG affects ovarian physiology in the bovine are still poorly understood.

According to Vasconcelos et al. (1999), 10 to 30% of Ovsynch-treated cows failed to synchronize ovulation in response to final GnRH. As previously mentioned, presynchronization treatments have proven effective in increasing the number of females

that have a synchronized ovulation and thus become pregnant (Bello et al., 2006). Most of these protocols however require the use of $PGF_{2\alpha}$, restricting themselves to usage in females that are known to be not pregnant. An effective method to increase the incidence of ovulation to the first GnRH injection of Resynch has not been developed.

The other factor comprising reproductive efficiency is pregnancy loss. Pregnancy loss can be classified as early embryonic death (≤ d 15 to 17), late embryonic death (d 17 to approx. d 42), or fetal death (≥ d 50; Santos et al., 2004). Much of the blame for pregnancy loss in high-producing dairy cows has been placed on reduced concentrations of progesterone in the blood. According to Lucy et al. (1998), a link has been found between genetic selection for milk production and reduced serum concentrations of progesterone. This may be an indirect effect caused by high DMI. As intake increases so does metabolization of the steroid hormone as it passes through the liver. Based on this assumption, researchers (Lopez-Gatius et al., 2004, Stevenson et al., 2007) have attempted to reduce pregnancy loss by administering exogenous progesterone at various stages post-insemination. Others (Sterry et al., 2006, Bridges et al., 2000) have relied on induction of ancillary CL to increase endogenous concentrations of progesterone.

Today's TAI protocols consistently yield acceptable first-service pregnancy rates. The major areas that need improvement are the management of non pregnant cows after first service and prevention of pregnancy losses. An ovulation resynchronization system that provides acceptable pregnancy results for second and greater service females while aiding in pregnancy maintenance would be ideal. Such a system could greatly improve reproductive management within the dairy industry.

GONADOTROPIN-RELEASING HORMONE

Mechanism of Action

Gonadotropin-releasing hormone is a decapeptide that is released from both the tonic and surge centers of the hypothalamus. The first reports on the action of GnRH revealed that the hormone stimulated the release of LH from the pituitary in small mammals (McCann et al., 1965; Schally et al., 1967). Later studies (Amoss and Guillemin, 1969; Niswender, 1969; Reeves et al., 1971) revealed the same action in sheep. Reeves et al. (1971) recognized the need to understand the mechanism whereby GnRH caused the release of the preovulatory surge of LH and saw the possible practical applications of GnRH in domestic animals. Later, researchers (Kittok et al., 1973; Thompson et al., 1980; Milvae et al., 1984) observed that treating cattle with natural GnRH or with a GnRH analog (Milvae et al., 1984) during the luteal phase caused an increase in serum LH and progesterone.

Ovulation Incidence

Researchers also reported the induced ovulation of dominant follicles by GnRH with varying degrees of success. Macmillan et al. (1985) reported no incidence of accessory CL in dairy cows treated on d 12 to 16 post-estrus with 5 μg of buserelin, a GnRH agonist. Martin et al. (1990) also reported no ovulations in cows treated with 100 μg of GnRH on d 2 and 8 post-estrus. Another report (Thatcher et al., 1989), however, demonstrated that 67% of dairy cows treated from d 12 to 48 with 8 to 10 μg of buserelin every 3 d had an induced CL. More recently, Bello et al. (2006) reported an 80% incidence of ovulation in dairy cows given GnRH 2 d after an injection of $PGF_{2\alpha}$. A single injection of GnRH once between 4 and 9 d after AI induced 60% of dairy cows to

form at least 1 accessory CL (Stevenson et al., 2007). Clearly, formation of an accessory CL post-GnRH is dependent on several physiological factors.

Corpus Luteum Function

A review of several studies indicates that "the LH release induced by an injection of GnRH is not a sufficient luteotropic stimulus to sustain elevated concentrations of progesterone in serum for more than a few hours" (Rettmer, 1991). This is in agreement with a later study by Stevenson et al. (2007) in which dairy cows treated once with GnRH on d 4 to 9 post-AI showed no more increase in serum progesterone concentrations between day of treatment and 7 d later than did untreated controls. Surprisingly, treatment with GnRH has been shown (Ford and Stormshak, 1978; Rodger and Stormshak, 1986; Lokhande et al., 1981) to reduce long-term concentrations of progesterone.

HUMAN CHORIONIC GONADOTROPIN

Mechanism of Action

Human chorionic gonadotropin is produced by the trophoblast of the blastocyst and can be detected in the peripheral circulation as soon as d 8 to 10 of gestation. The hormone maintains the CL of pregnancy and is used to detect early pregnancy (Jameson and Hollenberg, 1993). Once hCG binds to the LH-CG receptor it directs the CL to produce different hormones including progesterone and estrogen. Over time, the CL becomes less sensitive to hCG, but increasing concentrations of the hormone maintain its functional capacity until approximately 7 wk of pregnancy (Jameson and Hollenberg, 1993).

This glycoprotein is composed of 2 polypeptide chains with carbohydrates attached to each. These chains have been designated as alpha and beta subunits. The alpha subunit is shared among the glycoprotein hormones LH, FSH, TSH, and CG but the beta unit differs for each. This distinction of the beta subunit is responsible for the biological specificity among hormones. For example, hCG bound to the LH receptor in luteal cells is internalized 50 times slower than LH (Niswender et al., 1985).

The longer half-life of hCG compared with that of LH results from 4 sites of O-linked glycosylation that largely accounts for the fact that hCG is more heavily glycosylated than LH. The glycosylated extension probably serves an important function either for hormone biosynthesis or hormone function (Jameson and Hollenberg, 1993).

Ovulation Incidence

Much like GnRH, researchers have reported much variation in the ability of hCG to induce follicles to ovulate. Wiltbank et al. (1961) reported that 18 of 27 (67%) beef heifers formed accessory CL when treated with 1,000 IU of hCG daily from 15 to 35 d post-estrus. A later study (de los Santos-Valadez et al., 1982) reported only 29 of 114 (25%) of heifers ovulated in response to 5,000 IU of hCG on d 15 post-estrus. Similar results [51 of 193 (26%)] were found when dairy cows were treated with 3,300 IU on d 15 post-estrus (McDermott et al., 1986). Price and Webb (1989) observed a large variation in number of dairy heifers with accessory CL when they were treated with 1,550 IU of hCG once from d 0 to 16 post-estrus. Five-thousand IU yielded an ovulation incidence of 81% (Howard and Britt, 1990), whereas 10,000 IU induced 100% (Howard et al., 1990) of dairy heifers to ovulate when cattle in both groups were treated 10 d post-estrus. A later study (Stevenson et al., 2007) compared the ovulation potential of hCG

with that of GnRH and showed accessory CL formation in 77.5% and 60.0% of lactating dairy cows, respectively, on d 4 to 9 post-AI.

Bovine follicles develop in a wave-like pattern during the estrous cycle with most cattle having 2 or 3 waves per cycle (Pierson and Ginther, 1984). Under normal conditions the final wave of each cycle yields the follicle that will ovulate in response to the pre-ovulatory surge of LH, whereas the dominant follicles of the preceding wave or waves are destined to become atretic in the high progesterone milieu of diestrus. An exogenous source of LH release or LH-like activity, however, can induce these follicles to ovulate when administered at certain stages of their development. This explains the wide variation observed in ovulation incidence on different days of the estrous cycle.

The dosage of hCG used in bovine experiments varies greatly. There is no published dose titration report revealing the minimum effective dose of hCG needed to induce the formation of accessory CL in cattle. From a practical perspective this information is needed to determine the cost that hCG would represent in an ovulation-synchronization program. This information also would prove useful from a scientific perspective as it would provide future researchers with an effective, consistent dose needed to conduct studies.

Corpus Luteum Function

Numerous researchers (Donaldson and Hansel, 1965; Hansel and Seifart, 1967; Moody and Hansel, 1971) have reported that hCG administration during the luteal phase of cattle increased the size and weight of the already existing CL as well as serum concentrations of progesterone. A review of several studies revealed that progesterone concentrations increase by 24 h after treatment with hCG and remain elevated above

controls until the onset of luteolysis (Rettmer, 1991). This agrees with a later study (Rajamahendran and Sianangama, 1992) that also reported an increase in total CL diameter from 7 to 42 d post-AI in hCG-treated cows. Progesterone concentrations also were significantly greater in cows treated with hCG on d 7 or 14 post-AI than in those treated on d 0 or those not treated. Yet another study (Stevenson et al., 2007), more induced CL were observed and more total CL were detected in cows treated with either GnRH or hCG after insemination. Increased concentrations of progesterone, however, were observed only in hCG-treated cows. This may have occurred because total CL volume was increased only after hCG, suggesting a luteotropic effect of hCG.

Researchers have reported varying responses of the CL in hCG-treated cattle to exogenous $PGF_{2\alpha}$. Several authors [Bolt (1979), McDermott et al. (1986), and Shipley et al. (1988)] reported delayed luteolysis and fewer animals displaying estrus when receiving $PGF_{2\alpha}$ after hCG treatment. Shipley et al. (1988) also reported reduced fertility in cattle bred after treatment with 2,500 IU of hCG. Bolt (1979) and McDermott et al. (1986) both showed that hCG-treated cows responded to $PGF_{2\alpha}$ with a drastic decline in blood concentrations of progesterone by 24 h, but progesterone still remained > 1 ng/mL for several days. Howard and Britt (1990) however, reported decreased serum progesterone concentrations in heifers that were given $PGF_{2\alpha}$ 2 to 5 d after 5,000 IU of hCG were given on d 10. In addition to lysing the original CL, $PGF_{2\alpha}$ also lysed the accessory CL formed in response to the hCG treatment. This is especially interesting when we consider that the hCG-induced CL was regressed before d 5 of its existence.

MANAGEMENT OF THE NON PREGNANT COW

Management of non pregnant dairy cows has changed dramatically in the last half-century. The classic a.m.-p.m. rule was first established by Trimberger (1948). At approximately the same time, researchers (Christian and Casida, 1948; Willett, 1950; Ulberg et al., 1951; Hansel and Trimberger, 1952) began exploring the effects of progesterone on the ovaries and estrous cycles of cattle. These studies laid the foundation for the first phase of estrus-synchronization research using progestational compounds beginning about 1960 (Hansel and Convey, 1983). Although estrus occurred in a large percentage of these early study cattle, conception rates were not equal to that of controls (Britt et al., 1981). The second phase of research combined progestational treatments with estrogen or gonadotropins in order to more strictly regulate the timing of estrus. By and large, these programs also were not successful very (Hansel and Convey, 1983).

In the early 1970's the luteolytic properties of $PGF_{2\alpha}$ were demonstrated (Rowson et al., 1972). This finding provided several options to synchronize estrus in cattle before AI. Use of $PGF_{2\alpha}$ to induce estrus by regression of the CL, is ineffective before d 5 of the estrous cycle. One program calls for insemination of cattle as they come into spontaneous estrus during a 5-d period, after which those not inseminated are treated with $PGF_{2\alpha}$ and inseminated at the resulting induced estrus (Hansel and Convey, 1983). Another protocol calls for 2 treatments of $PGF_{2\alpha}$ 10 to 12 d apart with an insemination at detected estrus after the second injection (Britt et al., 1981). Yet another method involves an injection of $PGF_{2\alpha}$ after palpation of a functional CL (Stevenson, 2001). This may be done at any time for first-service cows or at pregnancy diagnosis for those

already inseminated. All methods yield acceptable pregnancy results when cattle are inseminated after a detected estrus.

Accurate detection of estrus is a problem (Foote, 1975), and may even be the most limiting factor to producing a pregnancy (Barr, 1975) on many farms. Because of this limitation, attempts were made to inseminate cattle at a fixed-time after $PGF_{2\alpha}$ treatments. Researchers observed reduced conception rates when cattle were inseminated once at 80 h after a second injection of $PGF_{2\alpha}$ (Fogwell et al., 1986). Two timed inseminations (72 and 96 h) after prostaglandin yielded conflicting results (Seguin et al., 1978; Plunkett et al., 1984). Timing of $PG_{F2\alpha}$ injection relative to the stage of follicular development is responsible for this variation in response (Moore and Thatcher, 2006). Britt et al. (1981) recognized the need for a protocol that would reduce this variation by synchronizing, not only estrus, but ovulation.

Later work (Hansel and Beal, 1979; Roche et al., 1981) combined a progesterone releasing intra-vaginal device (PRID) with $PGF_{2\alpha}$ treatments (Hansel and Convey, 1983). Administration of a PRID to Holstein heifers for 7 d, with $PGF_{2\alpha}$ given on d 6 and one fixed-time AI at 84 h after PRID removal, yielded pregnancy rates (66%) similar to those in controls bred after a detected estrus (73%); (Smith et al., 1984).

Early work with GnRH (Cumming et al., 1977; Fernandez-Limia et al., 1977) demonstrated the decapeptide's ability to induce ovulation in cattle. Turnover of the dominant follicle resulting from GnRH-induced LH release and subsequent ovulation led to recruitment of a new follicular wave, and thus, a new dominant follicle was present 7 d later (Moore and Thatcher, 2006). This sequence of events served as the basis for the timed insemination program referred to as the Ovsynch protocol (Pursley et al. 1995,

1997). Until that time, no protocol was available that would "consistently synchronize estrus with sufficient precision to permit high levels of success with fixed-time insemination" (Larson and Ball, 1992).

A major advantage of Ovsynch is that it allows a producer to inseminate all cows on the first day following the voluntary waiting period (VWP) by beginning the synchronization program 10 d earlier. This option can reduce days to first service, or at least, reduce variation in days to first AI. In one study (Pursley et al., 1997), the median time to conception was reduced by 19 d for Ovsynch-treated cows compared with cows managed using typical reproductive strategies. In that study, Ovsynch was re-initiated on d 32 post-insemination in cows diagnosed open at this time and second service was performed 10 d later. The authors concluded that in such a scenario, early pregnancy diagnosis becomes more critical in order to re-submit cows for insemination as soon as their not-pregnant status is known.

To further reduce days open Fricke (2002) proposed a more aggressive reproductive management strategy. In this scenario the first GnRH injection of Ovsynch would be administered to all cows 7 d before pregnancy diagnosis despite their unknown pregnancy status. Nonpregnant cows then receive $PGF_{2\alpha}$ on the day of pregnancy examination, whereas the program is discontinued in pregnant females thus reducing the days to subsequent service by 7. One study (Moreira et al., 2000), reported increased embryonic loss for bST-treated cows receiving GnRH on d 20 after TAI. Other researchers (Chebel et al., 2003; Fricke et al., 2003) however, did not confirm that observation.

Ovulation in response to the first GnRH injection of Ovsynch is the major determinant for successful synchronization (Bello et al., 2006). Because of this limitation, several researchers (Moreira et al., 2001; Navanukraw et al., 2002; El-Zarkouny et al., 2004; Bello et al., 2006) have developed presynchronization programs that precede Ovsynch and optimize ovulation. Although these protocols have proved successful, unfortunately they can not be implemented in cattle of unknown pregnancy status because they require the use of $PGF_{2\alpha}$. Waiting until pregnancy diagnosis often increases days to re-insemination. Therefore, presynchronization protocols eliminate themselves from usage before applying resynchronization regimens. A substance with greater ovulatory capacity than GnRH could potentially increase pregnancy rates in cattle synchronized with the Ovsynch protocol.

Human chorionic gonadotropin has seen limited use in estrus- and ovulation-synchronization protocols. Schmitt et al. (1996) saw no increase in conception rates of dairy heifers when the second GnRH of Ovsynch was replaced with an injection of hCG (3,000 IU). Another study (De Rensis et al., 1999) compared GnRH with hCG 6 or 9 d before $PGF_{2\alpha}$ treatment in dairy cows. Again, similar outcomes were observed in estrus synchronization and conception rates between GnRH- and hCG-treated cattle. Suckled beef cows were used to examine the effects of replacing GnRH with hCG on pregnancy rates when using the CO-Synch protocol (Geary et al., 2001). The authors observed that primiparous cows had greater pregnancy rates when treated with hCG than when they were treated with GnRH. The opposite, however, was true for multiparous cows.

The general consensus has been that hCG shows no major advantage over GnRH in estrus- or ovulation-synchronization protocols. Effects of replacing the first GnRH

injection in the Ovsynch protocol with hCG, while still using GnRH for the second treatment, have not been evaluated.

PREVENTION OF PREGNANCY LOSS

Much of the blame for low fertility in high-producing dairy cows can be placed on pregnancy loss. According to Sartori et al. (2002) fertilization rates in lactating dairy cows averaged 76.2% (ranging from 55.3 to 87.8%) on d 6 post-breeding. This is almost depressing when one considers that by d 27to 31 after AI, conception rates are usually 35 to 45% in dairy cattle (Santos et al., 2004).

Causes of pregnancy loss are varied. They include, but are not limited to diet, body condition score, disease, milk yield, cycling status, heat stress, oocyte quality, insemination protocol, resynchronization method, CL maintenance, concentrations of progesterone, and the uterine environment (Santos et al., 2004). A large portion of the research aimed at reducing pregnancy loss has focused on the latter 3 factors. A resynchronization protocol that aids in CL maintenance and increases progesterone concentrations is desirable. Researchers have attempted to accomplish this in 2 ways: 1) provide a supplemental, exogenous source of progesterone or 2) increase exogenous concentrations of progesterone by inducing formation of accessory CL or enhancing the endogenous function of the existing CL.

Progesterone from the CL is essential to successful gestation (Inskeep, 2004). According to Inskeep (2004), progesterone concentrations have been implicated in embryonic deaths during the following periods: before d 6 post-mating, d 4 through 9 post-mating, d 14 through 17 during the maternal recognition of pregnancy, and d 28 through 42 while placentation and attachment are in progress.

Reduced concentrations of progesterone in the peripheral circulation in high-producing dairy cows could be a function of reduced secretion from the CL, increased metabolization of the steroid, or both. The available data are conflicting. Feed intake influenced metabolism of progesterone in lactating dairy cows in some studies (Wiltbank et al., 2000; Rabiee et al., 2001c), but not in others (Rabiee et al., 2002a). Increased feed intake decreased circulating progesterone concentrations in nonlactating intact (Rabiee et al., 2001b) or in ovariectomized cows with injected progesterone (Rabiee et al., 2001a) or exposed to a PRID (Rabiee et al., 2002b). In a study by Sangsritavong et al. (2002), progesterone clearance persisted longer in cows given greater amounts of feed and was correlated ($r = 0.92$) with liver blood flow. Gombe and Hansel (1973) observed that Holstein heifers fed low energy diets had lighter CL with reduced progesterone content than heifers fed normal energy diets.

Exogenous Progesterone

Mann and Lamming (1999) observed increased conception rates in lactating dairy cows treated with supplemental progesterone before d 6 after AI. Mann et al. (2006) supplemented dairy cows with progesterone from d 5 to 9 or d 12 to 16 post-breeding. Although both periods of supplementation resulted in marked increases in plasma progesterone, an increase in trophoblast length and uterine concentration of interferon-τ were observed only in the earlier treatment. Researchers (Stevenson et al., 2007) observed mixed results when dairy cows were treated with another progesterone-releasing intravaginal controlled internal drug release (CIDR) insert for 7 d, starting 4 to 9 d after insemination. Volume of luteal tissue was reduced by progesterone compared with controls. Although the CIDR tended to increase conception rates in 2 herds, it also

decreased conception rate in 1 herd. Sterry et al. (2006) observed no effect on pregnancy rate when a CIDR was in place from d 5 to 12 post-AI. Lopez-Gatius et al. (2004) demonstrated the benefit of supplemental progesterone during the early fetal period. Pregnancy diagnosis was performed between 36 and 42 d after AI. Treated cows were then administered a PRID for 28 d. Pregnancy loss was recorded in 12% of controls and 5.3% of treated cows on d 90 of gestation. Treated cows were 2.4 times less likely to miscarry (Lopez-Gatius et al., 2004).

Increase in Endogenous Progesterone Production

Both GnRH and hCG have been used to induce accessory CL after insemination and reduce pregnancy loss. Sterry et al. (2006) observed that GnRH treatment 5 d after TAI increased conception rates for noncycling, but not for cycling cows. Stevenson et al. (2007) observed that while GnRH and hCG both induced ovulation in more than 60% of cows when administered 4 to 9 d after AI, pregnancy survival was reduced slightly after GnRH compared with controls. Treatment with hCG increased serum progesterone, but conception rates were increased only in some herds. Other researchers have observed more consistent results with hCG. Treatment with hCG on d 5 (Santos et al., 2001) or d 7 (Rajamahendran and Sianangama, 1992) after breeding induces accessory CL, enhances plasma progesterone concentration, and improves conception rate of high-producing dairy cows, but not nulliparous heifers (Schmitt et al., 1996).

CONCLUSIONS

Poor fertility is a real and significant problem in high-producing US dairy cows. Reproductive management can improve fertility in 2 ways: 1) increase the fertilization rate/AI or 2) reduce pregnancy losses after fertilization. An ovulation resynchronization

program that would accomplish both of these goals simultaneously while presenting non-pregnant cows for subsequent service soon after pregnancy exam would dramatically improve reproductive management of the dairy herd.

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CHAPTER 2 - HUMAN CHORIONIC GONADOTROPIN AND GONADOTROPIN-RELEASING HORMONE INFLUENCE PREGNANCY SURVIVAL AND RESYNCHRONIZED OVULATION BEFORE TIMED ARTIFICIAL INSEMINATION IN HOLSTEIN CATTLE

ABSTRACT

Experiments have shown human chorionic gonadotropin (hCG) to be more effective than GnRH as a means to ovulate follicles. Dosages used, however, have varied greatly among experiments. A study was performed to determine the minimum effective dose of hCG needed to induce ovulation of ovarian follicles in dairy females (Exp. 1). Another study determined the effects of replacing the first injection of GnRH (d -7) with hCG or saline in a Resynch-Ovsynch protocol [injection of GnRH 7 d before and 48 h after $PGF_{2\alpha}$ before a resynchronized fixed-timed AI (TAI)] on pregnancy rates in cows subsequently diagnosed not pregnant and pregnancy survival in cows subsequently diagnosed pregnant (d 0; Exp. 2). A final study determined the ovulation potential of hCG compared with GnRH and saline (Exp. 3). In Exp. 1, ovaries of Holstein cows were mapped by using transrectal ultrasonography 7 d before a biweekly pregnancy diagnosis. Cows were assigned randomly to treatments of saline, 100 μg of GnRH, or 500, 1,000, 2,000, or 3,000 IU of hCG. Ovarian structures were monitored again 7 d later and proportion of cows and proportion of follicles ≥ 8 mm in diameter that ovulated were recorded. In Exp. 2, cows in 4 herds were assigned randomly based on lactation number, number of previous AI, and last test-day milk yield to treatments of 1,000 IU of hCG,

100 µg of GnRH, or left as untreated controls 7 d before pregnancy diagnosis. Cows found not pregnant were given $PGF_{2\alpha}$ (d 0), then inseminated 72 h later, concurrent with a GnRH injection (3 herds) or given GnRH 16 to 24 h before AI at 72 h (1 herd). Pregnancy rates tended ($P = 0.08$) to be increased by GnRH (17.9%; n = 703) compared with control (12.9%; n = 505), but not hCG. Among pregnant cows treated, pregnancy survival 4 to 9 wk after initial pregnancy diagnosis differed among herds ($P < 0.001$), but a treatment × herd interaction ($P = 0.004$) also was detected. In 1 herd, GnRH reduced pregnancy survival, whereas hCG seemed to increase survival compared with control. Only small differences were detected in the other 3 herds except for a slight negative effect of hCG compared with control in 1 herd. Ovarian structures were monitored in herd 1 by using transrectal ultrasonography 0 and 7 d after treatment with hCG, GnRH, or saline (Exp. 3). A tendency for a treatment x pregnancy status interaction ($P = 0.07$) was detected. Incidences of ovulation in nonpregnant cows were: hCG (51.6%; n = 126), GnRH (46.1%; n = 102), and control (28.1%; n = 96), whereas those in pregnant cows were: hCG (59.3%; n = 59), GnRH (24.5%; n = 49), and conrol (6.9%; n = 58). We concluded that: 1) a dose of at least 1,000 IU of hCG resulted in a greater ovulatory response than saline, GnRH, or 500 IU of hCG (Exp. 1); 2) initiating a Resynch-Ovsynch protocol 7 d before pregnancy diagnosis with saline compared with GnRH reduced timed AI pregnancy rates (Exp. 2); 3) in pregnant cows treated with GnRH, pregnancy survival was slightly reduced in 1 of 4 herds (Exp. 2); and 4) incidence of new CL was greater after hCG than GnRH in pregnant cows, but not in nonpregnant cows (Exp. 3).

INTRODUCTION

Ovulation synchronization protocols that facilitate fixed-time artificial insemination (TAI) have been a reality for several years. Many producers utilize these programs with 77% of respondents to a recent survey resynchronizing repeat services (Caraviello et al., 2006). Although these programs offer the opportunity to facilitate the use of TAI without detection of estrus, conception rates have historically been compromised. According to Vasconcelos et al. (1999), 10 to 30% of Ovsynch-treated cows failed to have synchronized ovulation. Although presynchronization treatments have proven effective in increasing the number of females with a synchronized ovulation (Bello et al., 2006), they are not suitable for use before resynchronization.

Traditionally, most ovulation synchronization schemes use GnRH to control follicular development and induce ovulation of a dominant follicle. Research has shown, however, that human chorionic gonadotropin (hCG) is more effective than GnRH at causing these follicles to ovulate (Stevenson et al., 2007). A minimum effective dose of hCG to induce ovulation however has not been documented.

We hypothesized that replacing the first injection of GnRH in a Resynch-Ovsynch protocol with hCG would induce more follicles to ovulate subsequently improving synchronization and pregnancy rate at TAI. In addition, we hypothesized that the greater number of ancillary CL would increase progesterone concentrations in pregnant cows, thus reducing the incidence of pregnancy loss. Our overall objective was to develop an ovulation resynchronization protocol that increases the risk of conception, reduces the risk of pregnancy loss, and allows for TAI in dairy cattle.

MATERIALS AND METHODS

Herd Management

Experiments 1 and 3 were conducted at the Kansas State University Dairy Teaching and Research Center, Manhattan. Experiment 2 was conducted at Kansas State University as well as at 3 commercial northeast Kansas locations. All research at Kansas State University was conducted from October 2005 until October 2006. Research at the 3 commercial locations was performed between March and November 2006. Cows were housed in covered freestalls bedded with sand at all locations. All pens were covered and water was applied by sprinklers during summer months. Fans over freestalls, feed lines, or both were also in place at the 3 commercial locations. All cows were fed twice or thrice daily a TMR that met or exceeded National Research Council (NRC, 2001) requirements for lactating cows. Diets consisted primarily of chopped alfalfa hay, wet corn gluten meal, corn silage, whole cottonseed, soybean meal, and corn grain, plus a vitamin-mineral premix. Cows had ad libitum access to fresh water. Table 1 summarizes other herd management information.

Experimental Approach

Experimental approach is presented in Figure 1. Seven d before pregnancy diagnosis, dairy cows, along with a few nulliparous dairy heifers (herd 1 only), were assigned randomly to treatments of hCG, GnRH, or saline. Treatments were assigned based on lactation number, number of previous AI, and last test-day milk yield (cows only). Pregnancy was diagnosed 1 wk later (d 0).

Experiment 1

Ovaries of Holstein cows and heifers in herd 1 were examined by transrectal ultrasonography (5.0 MHz linear-array transducer, Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT) and structures were mapped, sized, and recorded. Cattle received a treatment of saline, GnRH (100 μg; 2 mL of Fertagyl, Intervet Inc., Millsboro, NJ), or either of 4 doses (500, 1,000, 2,000, or 3,000 IU) of hCG (0.5, 1, 2, or 3 mL of Chorulon, Intervet Inc.). Cows were then re-examined 1 wk later and those follicles that were induced to ovulate were noted.

All variables (ovulation incidence, number of females having at least 1 follicle ≥ 8 mm in diameter, number of follicles ≥ 8 mm, follicles ≥ 8 mm that ovulated and pregnancy rate) were analyzed by using ANOVA (procedure GLM; SAS Inst. Inc., Cary, NC) in a model that included treatment (n = 6), stage (d 22 to 28 or d 29 to 35 post-AI) at treatment injection, and the interaction of treatment × stage. A priori contrast was constructed to test all hCG doses ≥ 1,000 IU vs. other treatments.

Experiment 2

One wk before pregnancy diagnosis, dairy cows at 4 Kansas locations were assigned to receive 100 μg of GnRH (Fertagyl, Intervet Inc.), 1,000 IU of hCG (Chorulon, Intervet Inc.), or left as untreated controls based on lactation number, number of previous AI, and last test-day milk weight. Cows were diagnosed for pregnancy by transrectal ultrasonography on d 30 to 43 (herd 1) or by transrectal palpation on d 37 to 45 (herds 2 to 4) post-insemination. The same veterinary practitioner diagnosed pregnancy in herds 2, 3, and 4. When cows (n = 1,235) were diagnosed pregnant, the resynchronization protocol was discontinued and pregnancy status was reassessed 4 to 9

wk later in herds 2, 3, and 4 and 9 wk later in herd 1 to determine pregnancy survival. Cows diagnosed not pregnant (n = 1,748) were given $PGF_{2\alpha}$ at diagnosis and received one TAI 72 h later. Cows at 3 locations were administered 100 μg of GnRH (Fertagyl, Intervet Inc.) at the time of AI, whereas cows in herd 1 were given GnRH 16 to 24 h before TAI. Following TAI, all cows not detected in estrus and inseminated were again diagnosed for pregnancy 30 to 45 d later. Some nonpregnant cows in the 3 commercial dairies were inseminated early based on activity, standing estrus, and chalk rubs. These cows were eliminated from the results and were not included in analyses.

Palpation pregnancy rate was calculated as the number of pregnant cows at each diagnosis divided by the number of cows presented for pregnancy diagnosis. Pregnancy rate was calculated as the number of pregnant cows at each diagnosis divided by the number of cows previously inseminated and treated. Pregnancy survival between the first and second pregnancy diagnosis (4 to 9 wk later) also was determined. Pregnancy rates and pregnancy survival were analyzed by using ANOVA (procedure GLM; SAS Inst. Inc.). The model for pregnancy survival consisted of treatment (n = 3), lactation number (1, 2, or ≥3), herd (n = 4), interaction of treatment × lactation number, interaction of treatment × herd, season of treatment nested within herd, and most recent test-day milk weight (covariable). A priori contrasts were constructed for each treatment vs. control. The model for pregnancy rate also included sire and technician, each nested within herd.

Experiment 3

At 1 location (herd 1), transrectal ultrasonography was conducted at the initiation of the resynchronization protocol before treatment. Ovarian structures were mapped and follicles were sized. Follicular diameter was determined by averaging the largest cross-

sectional width and height measured by ultrasound electronic calipers. Structures were monitored again 7 d later and new corpora lutea (CL) which were not present or visible at the first ultrasound exam were noted. New CL corresponding to large follicles at the first ultrasound were assumed to have ovulated in response to saline, 100 μg of GnRH, or 1,000 IU of hCG.

Blood samples were collected from a coccygeal blood vessel at the time of each ultrasonography exam. Samples were stored on ice until transported to the laboratory for centrifugation 24 h later. The serum portion was retained, frozen, and serum concentrations of progesterone were later quantified by RIA (Skaggs et al., 1986). Intra- and interassay coefficients of variation for 11 assays were 8.9 and 9.5%, respectively, for a pooled sample that averaged 3.85 ± 0.1 ng/mL.

Incidence of ovulation was analyzed by using ANOVA (procedure GLM; SAS Inst. Inc.) with a model consisting of treatment (n = 3), lactation number (1, 2, or ≥3), pregnancy status, stage of follicular development (d 22 to 28 or d 29 to 35 post-AI), follicle class (≤10, 11-12, 13-14, or >14 mm), number of CL before treatment (0, 1, or ≥2), number of follicles ≥ 8 mm at treatment (0, 1, 2, or 3), breeding cluster (n = 28), interaction of treatment × lactation, treatment × pregnancy, treatment × stage, and treatment × beginning number of CL. A priori contrasts were constructed for the combined treatments vs. control and GnRH vs. hCG. Incidence of ovulation based on probable days of the cycle at treatment also was analyzed using ANOVA (procedure GLM; SAS Inst. Inc.) with a model similar to that above. This reduced model condensed stage to 2 groups, each consisting of 3 d (d 23 to 25 and d 31 to 33 post-AI at treatment

injection) in which the majority of cows had a follicle with a high probability of ovulating.

Concentrations of progesterone from 7 d after treatment also were analyzed by using ANOVA (procedure GLM; SAS Inst. Inc.). The model was similar to the one used to analyze incidence of ovulation, but did not include follicle class, beginning number of CL, or number of follicles ≥ 8mm. The model included however, the ending number of CL (original + induced CL) and the interaction of treatment × ending number of CL, plus the concentration of progesterone at pregnancy diagnosis as a covariable adjustment. A priori contrasts were constructed for both treatments vs. control and GnRH vs. hCG.

RESULTS

Experiment 1

Results of this experiment are summarized in Table 2. More than 95% of the females had at least 1 follicle ≥ 8 mm in diameter. Average number of follicles at least 8 mm or more in diameter per female ranged from 1.4 ± 0.2 to 1.9 ± 0.2 in each group of females before treatment. Ovulatory responses (shown as the percentage of cows with a new CL in Table 2) per female treated with saline, GnRH, and 500 IU of hCG were exceeded ($P < 0.05$) by the larger doses (1,000 IU or greater) of hCG. When the combined hCG doses ≥ 1,000 IU were compared with only controls, the P value was 0.06. When ovulatory response was calculated based on the total numbers of follicles, percentage responses were similar to those on a per-female basis. Compared with , control, GnRH, and 500 IU of hCG, the greater doses of hCG produced more ($P < 0.05$) ovulations. A tendency ($P = 0.12$) occurred for more follicles to ovulate after at least 1,000 IU of hCG than after saline alone.

Experiment 2

Herd palpation pregnancy rates ranged from 33.5 to 39.6% in the 4 herds (Table 3). Herd 4 had greater palpation pregnancy rates than herds 2 ($P = 0.04$) and 3 ($P = 0.003$). Pregnancy survival 4 to 9 wk after initial pregnancy diagnosis by treatment based on postpartum insemination number is illustrated in Table 4 for 1,236 cows. Overall, no difference in pregnancy survival was detected between cows treated with hCG (93.6%; n = 420) 7 d before pregnancy diagnosis and those left as untreated controls (95.3%; n = 403). Pregnancy survival, however, tended ($P = 0.06$) to be reduced in those cows treated with GnRH (93.0%; n = 413) compared with controls. Herd ($P = 0.004$) and season ($P < 0.05$) affected pregnancy survival. Herd tended to have or had an effect on pregnancy survival at the first ($P = 0.10$), second ($P < 0.01$), and third ($P = 0.002$) inseminations post-AI. Herds 1, 2, 3, and 4 had survival rates of 85.1, 99.6, 91.2, and 94.2%, respectively. Neither lactation number nor last test-day milk weight had an effect on pregnancy survival. Lactation number, however, tended ($P = 0.11$) to affect pregnancy survival for cows that conceived at their first postpartum insemination, with older cows having less survival (89%) than first-lactation cows (95%).

A treatment × herd interaction ($P = 0.004$) is illustrated in Figure 2. In herd 1, cows treated with hCG exhibited the greatest pregnancy survival; whereas survival was compromised in cows treated with GnRH compared with controls. Herd 3, however, exhibited reduced survival in females treated with hCG compared with those treated with GnRH and those left as untreated controls. Herds 2 and 4 responded similarly to treatment with survival rates being comparable across all treatments.

Resynchronized pregnancy rate by treatment based on postpartum insemination number is illustrated in Table 5 for a total of 1,749 inseminations in 4 herds. Overall, no

significant difference ($P = 0.17$) in pregnancy rate was detected between cows treated with hCG (16.5%; n = 541) and those treated with GnRH (17.9%; n = 703) or left as untreated controls (12.9%; n = 505) 7 d before pregnancy diagnosis. Pregnancy rate for GnRH- treated cows, however, tended ($P = 0.08$) to be greater than that of controls. Treatment, herd, lactation, nor treatment × lactation interaction had any effect on the risk of pregnancy. In contrast, for each 10 kg increase in last test-day milk weight, pregnancy rate decreased ($P < 0.05$) by 2.2 ± 1%. Sire nested within herd ($P = 0.007$) and season nested within herd ($P = 0.018$) had an effect on pregnancy rate, whereas technician nested within herd did not ($P = 0.79$).

Figure 3 illustrates a treatment × herd interaction ($P < 0.05$) on pregnancy rate. Herds 1 and 4 responded similarly to treatment as did herds 2 and 3. Cows treated with hCG and those treated with GnRH had the greatest pregnancy rates numerically in 2 herds each. Untreated controls seemed to have reduced pregnancy rates in 1 herd, but fertility was comparable with either GnRH or hCG in the other 3 herds.

Experiment 3

Ovaries of 490 cows were monitored for ovulation 7 d after treatment (pregnancy diagnosis) with hCG, GnRH, or saline. Treatment affected ($P < 0.001$) incidence of ovulation with 52.4% (n = 185), 39.1% (n = 151), and 20.1% (n = 154) of hCG, GnRH, and control cows ovulating, respectively. Treatment with hCG did not result in more ($P = 0.20$) cows ovulating than treatment with GnRH. Treatment with hCG or GnRH resulted in more ($P < 0.001$) cows ovulating than treatment with saline. Percentage of cows having at least 1 new CL by 7 d after treatment is summarized in Figure 4. Among nonpregnant cows, no difference was detected between hCG and GnRH treatments in the

appearance of new CL. Treatment with hCG ($P = 0.07$) tended to produce more accessory CL in pregnant cows as indicated by a treatment × pregnancy status interaction (Figure 4).

Number of CL already present at the time of treatment affected ($P < 0.001$) the incidence of ovulation. Cows with no CL present (n = 98) ovulated 62.2% of the time, whereas cows with 1 (n = 261) and ≥ 2 (n =112) CL ovulated 36.0 and 24.1% of the time, respectively. A tendency ($P = 0.08$) for a treatment × beginning number of CL interaction also was detected (Figure 5). Interestingly, GnRH produced a greater incidence of ovulation when no spontaneous CL was present at the time of treatment.

Neither lactation number, pregnancy status, nor treatment × lactation number influenced incidence of ovulation. Diameter of the follicles present at the time of treatment had no significant effect on the incidence of ovulation. Also not significant was the putative follicular wave as described for each cow at treatment based on days since timed AI (Table 6).

Control cows ovulated fewer ($P < 0.05$) follicles than those cows treated with GnRH or hCG during the first follicular wave (d 23 to 25; Table 7). Proportion of cows that ovulated more than 1 follicle in response to treatment was 24.6% (n = 69) when treatments occurred at d 29 to 35 compared with 16.5% (n = 121) at d 22 to 28. Average CL per cow was greater ($P < 0.05$) for the latter treatment period (1.3 ± 0.08 vs. 1.1 ± 0.06) for cows that ovulated.

Blood samples were collected from 486 cows at the time of treatment 7 d before pregnancy diagnosis and again at pregnancy diagnosis (d 0). Table 8 summarizes blood serum concentrations of progesterone 7 d after treatment, based on treatment, pregnancy

status, and number of CL. Concentrations of progesterone 7 d after treatment were adjusted for concentrations of progesterone before treatment. Treatment had no effect on concentrations of progesterone. As expected, pregnant cows (n = 166) had greater ($P <$ 0.001) concentrations of progesterone than nonpregnant cows (n = 320). Also not surprising is the observation that concentrations of progesterone increased as the number of CL present at collection of the second blood sample increased from 0 to ≥ 2 CL. Stage post-AI (n = 2) did not affect concentrations of progesterone for cows in which treatments were initiated at d 22 to 28 or d 29 to 35.

DISCUSSION

Experiment 1

Many researchers (Wiltbank et al., 1961; de los Santos-Valadez et al., 1982; McDermott et al., 1986; Price and Webb, 1989; Howard and Britt, 1990; Howard et al., 1990; Stevenson et al., 2007) have used hCG to induce ovulation in dairy and beef cattle. Our study, however, was first to determine the minimum effective dose needed to induce ovulation in cattle. A distinct increase in number of females that ovulated and percentage of total follicles that ovulated is evident in those cows treated with ≥ 1,000 IU of hCG. No advantage, however, was detected for the larger doses (2,000 or 3,000 IU) of hCG.

Some ovulations in each treatment were spontaneous due to the stage of cycle at treatment and some CL were immature at the time of first observation and were not visible until the second examination occurred 7 days later. These occurrences are accounted for by detected ovulations after saline treatment and provide some evidence as to what percentages of ovulations were actually induced by treatment with GnRH or

hCG. From Exp. 1, we concluded that a dose of 1,000 IU of hCG exceeded the ovulatory induction of saline, GnRH, and the smallest dose of hCG (500 IU) in these dairy females.

Experiment 2

Pregnancy survival tended to be reduced in cows treated 7 d before pregnancy diagnosis with GnRH compared with untreated controls. This reduction is similar to another report in which pregnancy survival was reduced when third or greater lactation cows were treated with GnRH, but at 4 to 9 d after insemination (Stevenson et al., 2007). Because treatment with GnRH did not increase concentrations of progesterone over controls, no change in pregnancy survival should be expected. The mechanism behind a possible increase in pregnancy mortality, however, is unknown. Careful interpretation of this reduction in pregnancy survival by GnRH should be noted because it was clearly evident only in 1 herd, although in 2 of the remaining herds, pregnancy survival was reduced by 0 to 3.5% units (Figure 2). Pregnancy survival in hCG treated cows did not differ statistically from GnRH treated cows or controls. This is surprising because the results from Exp. 3 revealed that more accessory CL were formed after hCG in pregnant cows than after GnRH or saline. Perhaps accessory CL formation was too late (d 23 to 38) to benefit pregnancy survival. As a result of differences in genetics and management practices among herds, a herd effect on pregnancy survival is not surprising. Cavestany et al. (1985) demonstrated the effects of heat stress on fertility in Holstein cattle. Because the experiment encompassed all seasons, the seasonal effect on pregnancy survival also is to be expected.

Pregnancy rate tended ($P = 0.08$) to be greater in cows initiating the resynchronization protocol with GnRH than in untreated controls. The logical

explanation for this is simply that more follicles ovulated in response to GnRH, and thus, more cows had a synchronized follicle at the time of AI. By this logic, however, pregnancy rate in hCG treated cows also should be greater than that of controls because more follicles were induced to ovulate after hCG than in controls. Howard and Britt (1990) discovered that an hCG-induced CL behaves differently than a spontaneously induced CL when they observed that the former was induced by $PGF_{2\alpha}$ to regress before d 5 of its existence. Fricke et al. (1993) also observed that hCG-induced CL differed in size and color from spontaneously formed CL and produced less progesterone when cultured in vitro in the presence of LH. It is a reasonable assumption that physiological differences in hCG-induced CL could hinder pregnancy rates at a later insemination.

As with pregnancy survival, a treatment × herd interaction was detected for pregnancy rate. Because the pregnancy diagnosis schedule of herd 1 differed from that of the other 3 herds, one might expect the first herd to respond differently to treatment than the others. This, however, is not the case because pregnancy rates in herds 1 and 4 were similar and those of herds 2 and 3 were similar (Figure 3).

We conclude that there is no distinct advantage to hCG over GnRH, or vice versa, in pregnancy survival or pregnancy rate in this resynchronization protocol. Cows in 3 of 4 herds exhibited increased pregnancy rates when Resynch-Ovsynch was initiated with hCG. Economically, a 100 μg dose of GnRH is similar in cost to 1,000 IU of hCG. We conclude, however, that from a dairy producer viewpoint the evidence does not merit a change from GnRH to hCG. Even though untreated controls exhibited the numerically greatest incidence of pregnancy survival, it is not a feasible option to initiate the Resynch-Ovsynch protocol with saline because pregnancy rates in those females

subsequently diagnosed not pregnant and resubmitted for insemination were reduced by more than 38% compared with GnRH.

Experiment 3

The treatment × pregnancy status interaction was a result of many more accessory CL formed in pregnant cows treated with hCG than with GnRH. This is probably a function of hCG's increased half-life compared with GnRH-induced LH release in response to hCG's 4 sites of O-linked glycosylation (Jameson and Hollenberg, 1993). For example, hCG bound to the LH receptor in luteal cells is internalized 50 times slower than LH (Niswender et al., 1985). This allows hCG to be more potent when inducing ovulation. A portion of the new CL detected in nonpregnant control cattle is probably a result of extended estrous cycles (> 22 d). Spontaneous CL in these cattle would have been undetectable at the time of first ultrasound diagnosis. Some of these CL also were detected because cycles in cows were not synchronized at the initial AI and ovulated spontaneously between scans. Seven percent of pregnant cows left as untreated controls ovulated between ultrasound examinations. It is not known what percentage of cows ovulate spontaneously after pregnancy is initiated. A portion of this 7% likely resulted from technician error.

A novel finding of this study was that in cows with no spontaneous CL present at the time of treatment, GnRH induced more follicles to ovulate than hCG. A similar number of ovulations were observed between hCG treated cows and controls when no CL were present at the time of treatment. When 1 or more CL were already present, however, hCG induced more ovulations than GnRH or saline. When all treatments are pooled, the incidence of ovulation decreased as the beginning number of CL increased.

Table 6 provides an in depth look at the incidence of ovulation based on days since AI at treatment. Days 23 to 25 and 31 to 33 were considered to be the probable first and second follicular waves since the first eligible estrus after AI in nonpregnant cows. Interestingly, follicular wave dynamics also can be observed in pregnant cows as well. In first-wave cattle, the incidence of ovulation was reduced in control cows compared with cows receiving treatment. Once again, reasons for ovulation in these cows are varied and include: lengthened estrous cycles, previously unsynchronized cycle at AI, and technician error. When only cattle that fall into the expected wave categories are considered, or in all days since previous AI, incidence of ovulation is greater for GnRH and hCG than for controls.

Somewhat surprising is the fact that treatment had no effect on concentrations of progesterone 7 d after treatment. Several authors (Fricke et al., 1993; Schmitt et al., 1996; Santos et al., 2001; Stevenson et al., 2007) have reported that treatment with hCG increases progesterone in 2 ways. First is the induction of accessory CL and second by an increase in number of progesterone-secreting luteal cells in existing CL. We observed a numerically greater number of induced ovulations in hCG treated cattle. Although luteal volume was not measured in this study, it is a plausible assumption that CL size increased after treatment with hCG as reported elsewhere (Stevenson et al., 2007). Reasons why serum concentrations of progesterone were not increased in hCG-treated cattle are unexplained.

In summary, doses of hCG of 1,000 IU and greater produced more ovulations than saline, GnRH, or 500 IU of hCG (Exp. 1). No difference was detected in pregnancy survival among cows treated 7 d before pregnancy diagnosis with hCG and those left as

untreated controls. Cows treated with GnRH, however, tended to have reduced pregnancy survival compared with controls. Herd had an effect on survival and a treatment × herd interaction occurred (Exp. 2). Pregnancy rate for GnRH treated cows tended to be greater than controls. For every 10-kg increase in test-day milk weight, a 2.2% decrease in pregnancy rate was detected. A treatment × herd interaction occurred as herds 1 and 4 and herds 2 and 3 responded similarly to treatments (Exp. 2). Treatment 7 d before pregnancy diagnosis with hCG or GnRH resulted in a similar number of induced ovulations. Both treatments induced more accessory CL than treatment with saline. Among pregnant cows treated, however, hCG tended to produce more ovulations than GnRH or saline. Treatment had no effect on concentrations of progesterone (Exp. 3).

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Table 1. Herd characteristics

Herd	Pregnancy diagnosis frequency	Range of days at pregnancy diagnosis	Method of pregnancy diagnosis	Daily milking frequency
1	Biweekly	30 to 43	Ultrasonography	2×
2	Weekly	38 to 44	Palpation	3×
3	Weekly	37 to 43	Palpation	3×
4	Weekly	39 to 45	Palpation	3×

Table 2. Ovulatory response 7 d after saline, GnRH, and hCG (Exp. 1)[1]

Item	Control	GnRH	Dose of hCG, IU			
			500	1,000	2,000	3,000
Dairy females[2], no.	18	18	18	18	17	16
			Pretreatment			
Females having at least 1 follicle ≥ 8 mm, no.	15	18	17	17	16	15
Follicles ≥ 8 mm per female[3], no.	1.4	1.9	1.7	1.6	1.8	1.8
			Post-treatment			
Females having a new corpus luteum, %	44.4	44.4	44.4	66.7[a]	64.7[a]	68.8[a]
Follicles ≥ 8 mm that ovulated, %	27.6 (29)[4]	31.4 (35)	28.1 (32)	40.5[b] (37)	43.8[b] (32)	48.4[b] (31)

[a]Combined doses of hCG (≥ 1,000 IU) differed ($P < 0.05$) from combination of saline, GnRH, and 500 IU of hCG ($\chi^2 = 4.68$).
[b]Combined doses of hCG (≥ 1,000 IU) differed ($P < 0.05$) from combination of saline, GnRH, and 500 IU of hCG ($\chi^2 = 5.18$).
[1]Treatment injections were administered 7 d before pregnancy diagnosis (d 30 to 43 since last AI).
[2]Included a few nulliparous heifers.
[3]Standard errors ranged from 0.21 to 0.23.
[4]No. of follicles per group.

Table 3. Herd palpation pregnancy rates (Exp. 2)

Herd	Palpation pregnancy rate[1]
	---- % (no.) ----
1	35.3 (434)
2	35.3 (881)
3	33.5 (932)
4	39.6 [a] (1,264)

[a] Herd 4 differed from herds 2 ($P = 0.04$) and 3 ($P = 0.003$).

[1]Number of cows diagnosed pregnant divided by the number of cows presented for weekly or biweekly pregnancy diagnosis.

Table 4. Pregnancy survival 4 to 9 wk after initial pregnancy diagnosis by treatment in response to postpartum insemination number (Exp. 2)

	Postpartum insemination number			
Treatment[1]	1	2	≥ 3	Total
		---------- % (no.) ----------		
hCG	91.1 (158)	93.5 (78)	95.7 (184)	93.6 (420)
GnRH	90.8 (152)	94.2 (69)	94.3 (192)	93.0[a] (413)
Control	94.6 (147)	98.4 (63)	94.8 (193)	95.3 (403)
Total	92.1 (457)	95.2 (210)	94.9 (569)	

[a]Tended ($P = 0.06$) to differ from control.

[1]Cows were treated once 7 d before pregnancy diagnosis (d 23 to 38) with hCG, GnRH, or served as untreated controls.

Table 5. Resynchronized pregnancy rate by treatment in response to postpartum insemination number (Exp. 2)

Treatment[1]	Postpartum insemination number				Total
	2	3	4	≥5	
	---------- % (no.) ----------				
hCG	14.9 (161)	19.5 (118)	8.9 (79)	19.1 (183)	16.5 (541)
GnRH	19.2 (198)	19.6 (143)	15.7 (115)	17.0 (247)	17.9[a] (703)
Control	11.8 (144)	13.7 (102)	12.4 (89)	13.5 (170)	12.9 (505)
Total	15.7 (503)	17.9 (363)	12.7 (283)	16.7 (600)	

[a]Tended ($P = 0.08$) to differ from control.

[1]Cows were treated once with hCG, GnRH, or served as untreated controls 7 d before pregnancy diagnosis.

Table 6. Incidence of ovulation in response to GnRH or hCG based on days since AI and pregnancy status at the time of treatment (Exp. 3)[1]

Days since timed AI	Nonpregnant[2]			Pregnant[2]		
	Control	GnRH	hCG	Control	GnRH	hCG
22	50.0 (2)	0.0 (1)	66.7(3)	…	…	…
23	50.0 (4)	100 (2)	60.0 (5)	0.0 (3)	100.0 (2)	100.0 (1)
24	25.0 (24)	50.0 (24)	51.4 (37)	4.8 (21)	46.2 (13)	60.9 (23)
25	50.0 (12)	53.9 (13)	65.0 (20)	20.0 (5)	16.7 (6)	66.7 (6)
26	0.0 (1)	66.7 (3)	33.3 (3)	0.0 (5)	0.0 (4)	80.0 (10)
27	0.0 (2)	0.0 (1)	25.0 (4)	0.0 (1)	0.0 (1)	50.0 (2)
28	66.7 (3)	50.0 (2)	66.7 (3)	0.0 (3)	…	…
29	…	66.7 (6)	0.0 (3)	50.0 (2)	…	100.0 (1)
30	100 (2)	0.0 (1)	100.0 (1)	0.0 (1)	…	0.0 (1)
31	33.3 (3)	50.0 (2)	80.0 (5)	0.0 (1)	0.0 (4)	50.0 (2)
32	13.5 (37)	35.1 (37)	45.7 (35)	0.0 (11)	17.7 (17)	55.6 (9)
33	0.0 (2)	60.0 (5)	50.0 (4)	0.0 (2)	0.0 (2)	0.0 (2)
34	...	50.0 (4)	0.0 (2)	0.0 (1)	…	0.0 (1)
35	50.0 (4)	0.0 (1)	100.0 (1)	50.0 (2)	…	0.0 (1)
Total	28.1 (96)	46.1 (102)	51.6 (126)	6.9 (58)	24.5 (49)	59.3 (59)
Wave 1[3]	35.0 (40)	53.9 (39)	56.5 (62)	6.9 (29)	42.9 (21)	63.3 (30)
Wave 2[4]	14.3 (42)	38.6 (44)	44.0 (44)	0.0 (14)	13.0 (23)	46.2 (13)
Nonpregnant vs. pregnant		42.9* (324)			30.7 (166)	

*Differed ($P = 0.05$) from pregnant cows.

[1]Cows were treated once with hCG, GnRH, or served as untreated controls 7 d before pregnancy diagnosis.

[2]Includes cows with no follicles at first ultrasound examination.

[3]Wave 1 = Putative first dominant follicle since first eligible estrus (d 23 to 25) in nonpregnant cows.

[4]Wave 2 = Putative second dominant follicle since first eligible estrus (d 31 to 33) in nonpregnant cows.

Table 7. Incidence of ovulation in response to treatment based on putative wave since first eligible estrus at treatment (Exp. 3)

	Treatment[2]			
Wave[1]	Control	GnRH	hCG	Total
1	23.2 (69)	50.0 (60)	58.7 (92)	45.2 [x] (221)
2	10.7 (56)	29.9 (67)	49.1 (57)	30.0 [y] (180)
Total	17.6[a] (125)	39.4[b] (127)	55.0[c] (149)	

[a,b,c] Treatment means having different superscript letters differ ($P < 0.05$).

[x,y] Wave means having different superscript letters differ ($P < 0.05$).

[1]Wave 1 = Putative first dominant follicle since estrus (d 23 to 25) in nonpregnant cows.

Wave 2 = Putative second dominant follicle since estrus (d 31 to 33) in nonpregnant cows.

[2]Cows were treated once with hCG, GnRH, or served as untreated controls 7 d before pregnancy diagnosis.

Table 8. Blood serum concentrations of progesterone 7 d after treatment based on pregnancy status and number of CL at time of treatment (Exp. 3)[1]

Item	Mean ± SE (no.)
Treatment[2]	-----ng/mL-----
Control	4.8 ± 0.3 (154)
GnRH	5.3 ± 0.4 (151)
hCG	5.3 ± 0.3 (181)
Pregnancy status	
No	4.2 ± 0.2 [a] (320)
Yes	6.1 ± 0.3 [b] (166)
Corpora lutea, no.	
0	3.3 ± 0.4 [a] (68)
1	5.4 ± 0.2 [b] (234)
≥2	6.6 ± 0.2 [c] (184)

[a,b,c] Means having different superscript letters within item differ ($P < 0.001$).

[1]Adjusted for concentrations of progesterone at the time of treatment.

[2]Cows were treated once with hCG, GnRH, or served as untreated controls 7 d before pregnancy diagnosis.

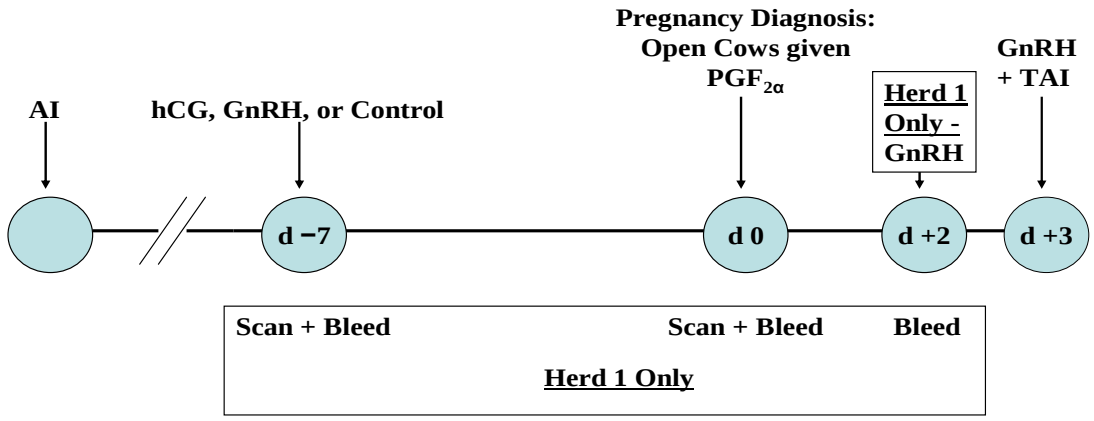

Figure 1. Experimental design

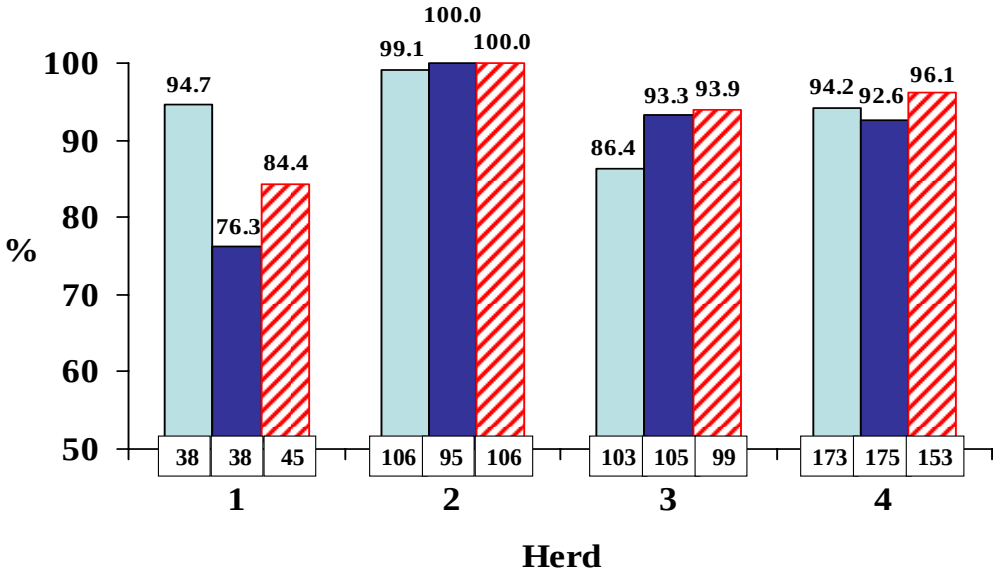

Figure 2. Pregnancy survival in lactating dairy cows treated with hCG (grey bars), GnRH (solid bars), or untreated control (cross-hatched bars). Cows were treated 7 d before initial pregnancy diagnosis (Exp. 2). Pregnancy survival was determined 4 to 9 wk after initial pregnancy diagnosis. A treatment × herd interaction ($P = 0.004$) was detected.

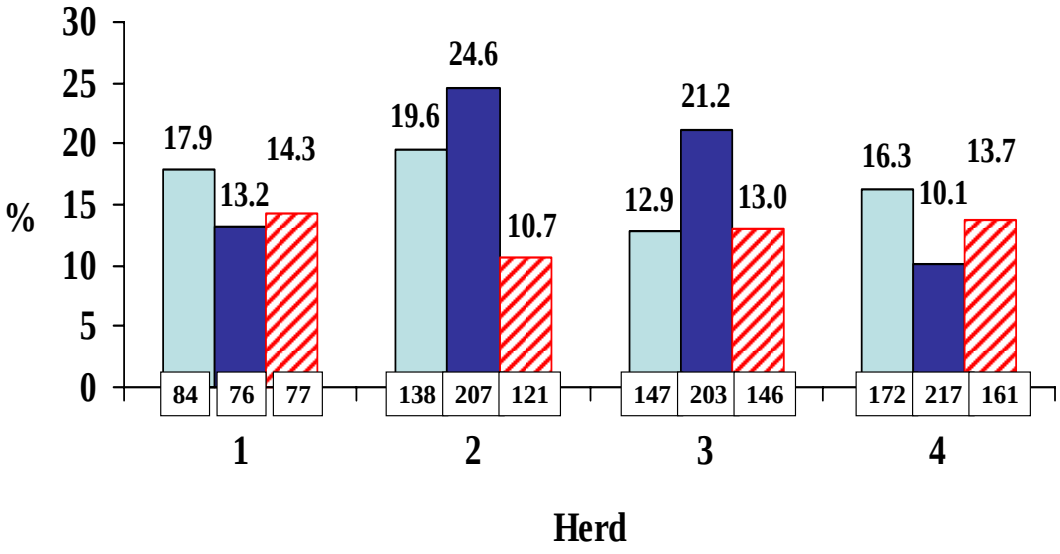

Figure 3 . Pregnancy rate in lactating dairy cattle treated with hCG (grey bars), GnRH (solid bars), or untreated control (cross-hatched bars) 7 d before not-pregnant diagnosis (Exp. 2). A treatment × herd interaction ($P < 0.05$) was detected.

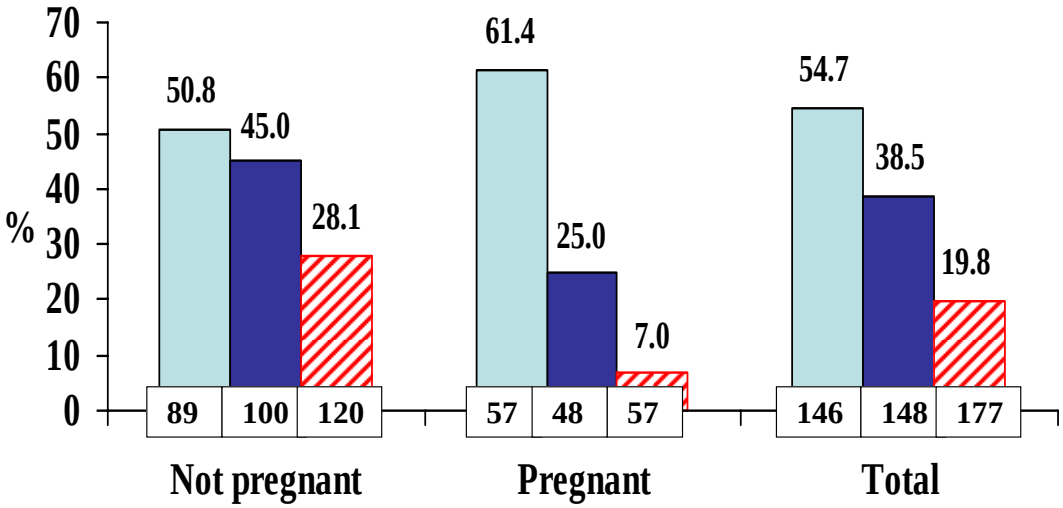

Figure 4. Percentage of cows having at least 1 new corpus luteum by 7 d after treatment with hCG (grey bars), GnRH (solid bars), or untreated control (cross-hatched bars) (Exp. 3). A tendency ($P = 0.07$) for a treatment × pregnancy status interaction was detected.

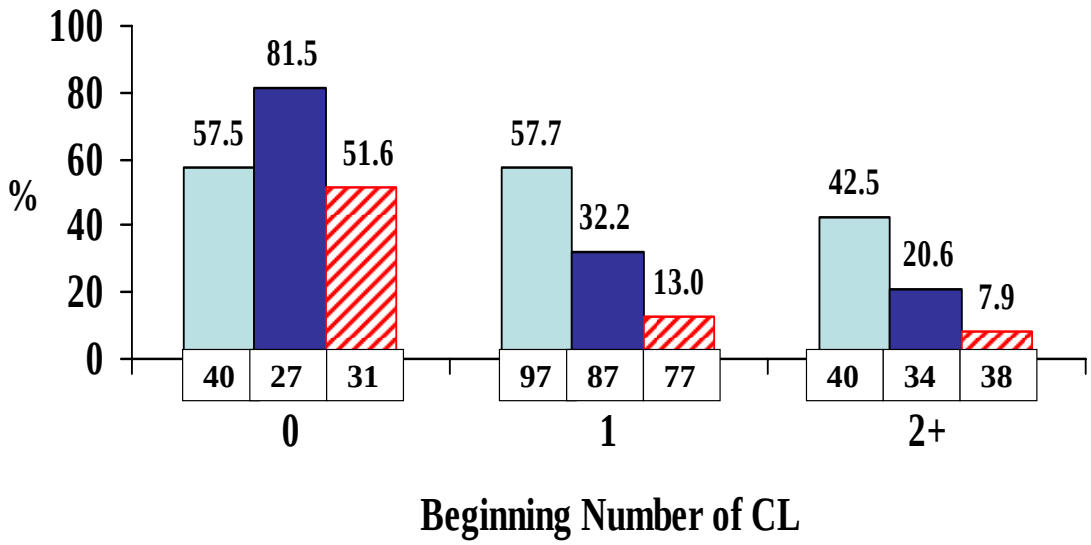

Figure 5. Incidence of ovulation in cows having 0, 1, or ≥ 2 corpora lutea (CL) that ovulated in response to treatment with hCG (grey bars), GnRH (solid bars), or untreated control (cross-hatched bars). A tendency ($P = 0.08$) was detected for a treatment × beginning number of CL interaction (Exp. 3).