GnRH: From Physiology to 'Synch'-ology

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The Physiology of GnRH

Introduction

The identity and structure of the peptide, gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), was first described through the work of Schally and Guillemin [Schally et al., 1971; Amoss et al., 1971]. Later milestones describing the physiology of GnRH included the discovery of GnRH pulses in the portal circulation [Knobil, 1974] and the demonstration that this pulsatility was essential for preserving gonadotropin synthesis and secretion, hence reproductive function [Knobil 1980]. The synthesis and secretion of the neurohormone GnRH provides a critical interface between the nervous and endocrine systems that control reproduction. Understanding how these two systems communicate via GnRH allows greater understanding of the mechanisms of puberty [Foster, 1994], periods of anovulation related to suckling and nutrition [Wiltbank et al., 2002], seasonal reproduction [Karsch, 1990], and the estrous cycle [Goodman, 1994]. This review will examine the secretion patterns of GnRH regulating the ruminant estrous cycle and ultimately explain how veterinarians, researchers, and producers have been able to apply this knowledge for the improvement of reproductive management in farm animal species, specifically cattle.

Anatomy and Detection of GnRH Secretion

The primary site of endogenous GnRH protein synthesis is the cell body of the GnRH neuron. The GnRH neurons are scattered throughout the periventricular and medial preoptic areas of the hypothalamus. Axonal projections from the neurons extend from the upper regions of the hypothalamus and synapse directly onto a network of capillaries in the median eminence [Silverman et al., 1994]. GnRH is secreted when an action potential, originating in the body of the GnRH neuron, propagates through the axon and stimulates the mobilization and exocytosis of GnRH-containing secretory vesicles at the synaptic terminal. Once released from the neuron, GnRH enters directly into the blood of the portal vasculature. The portal vasculature is a relatively unique network of vessels in that it drains the blood from a capillary plexus in the median eminence and shunts the blood directly into a second capillary plexus perfusing the anterior pituitary [Page, 1994]. Once in the anterior pituitary, GnRH binds to a GnRH receptor that is expressed on the LH and FSH-containing cells, commonly referred to as the gonadotropes. Interaction of GnRH with it's receptor stimulates the free intracellular Ca2+/protein kinase C intracellular effector system in a biphasic manner. The first phase is an acute increase in free intracellular $Ca/^{2+}$ that induces exocytosis of secretory granules containing LH and/or FSH. These episodes of gonadotropin secretion are commonly referred to as gonadotropin pulses. In the longer term, GnRH binding and protein kinase C activation promotes expression of the genes encoding the LH and FSH-

beta subunits and hence supports a level of tonic (non-pulsatile) gonadotropin secretion. [Conn, 1995]. Following secretion from the gonadotropes, the LH and FSH proteins are dispersed into the general circulation where they will either be metabolized or bind to their respective receptors within the ovary.

The anatomy of the hypophyseal vasculature presented a rather daunting hurdle to early scientists interested in studying the secretion patterns of GnRH in ruminants. The direct shunting of the portal blood from the median eminence to the anterior pituitary meant that nearly all of the GnRH produced in the hypothalamus is metabolized within the cells of the pituitary before entering the systemic circulation. The resulting concentrations of GnRH protein in the peripheral circulation are so low that, for all practical purposes, they are immeasurable using standard radioimmunoassay procedures. The inability to measure GnRH directly forced early researchers to rely on information derived from patterns of LH to extrapolate an expected pattern for GnRH secretion [Goodman et al., 1994]. It wasn't until the groundbreaking work by Clarke et al., [1982] that the scientific community was able to observe firsthand the pattern of GnRH secretion in a ruminant species. The techniques described by Clarke, which were later adapted and refined by others [Caraty and Locatelli, 1988; Moenter et al., 1990], allowed for the continuous sampling of blood directly from the portal vessels that drain from the median eminence. The picogram concentrations of GnRH protein within the portal blood could then be evaluated by radioimmunoassay. Later groups [Gazal et al., 1998; Yoshioka et al., 2001] have described similar procedures for the direct determination of GnRH secretion in female cattle through sampling of cerebrospinal fluid near the median eminence.

Fortunately, the experiments employing these procedures to directly assess GnRH release have confirmed that there is a very close temporal and dose-response association between GnRH secretion from the hypothalamus and LH secretion from the pituitary. These observations implied that the many earlier experiments characterizing patterns of LH secretion provided a valid representation of GnRH secretion as well.

GnRH Secretion During the Ruminant Estrous Cycle

The average concentration of GnRH during the luteal and follicular phases is not significantly different during the estrous cycle in the ewe [Goodman et al., 1994]. Nevertheless, a comparison of averages is rather deceiving in that it grossly misrepresents the underlying dynamic changes in the patterns of GnRH secretion throughout the different stages of the estrous cycle. GnRH is secreted in a pulsatile manner, simply implying that there are periods of little to no GnRH secretion intermittently disrupted by transient bursts, or pulses, of secretion. Regulating both the frequency and amplitude of GnRH pulses is critical to transmitting the appropriate signal for gonadotropin synthesis and secretion in the pituitary gland. Evidence for the relationship between GnRH pulses and gonadotropin synthesis has been shown both in vitro [Kaiser et al., 1995] and in vivo [Vizcarra et al.,1997; 1999]. In general, the administration of higher frequency, low amplitude GnRH pulses favors LH secretion, while low frequency, high amplitude pulses of GnRH favors FSH secretion. The key point here is that if GnRH pulsatility is changed, then the gonadotropin (LH and FSH) message being communicated to the ovary is also changed.

The next question obviously becomes, how are GnRH pulses regulated? There is no simple answer to this question and it remains an area of active research. The issue of GnRH pulse regulation is made more complex by data indicating that the GnRH neurons possess intrinsic pacemaker capabilities, that is, they have the ability to initiate and propagate action potentials, and thus secrete GnRH, in the absence of external synaptic inputs [Terasawa, 2001]. However, rather than implicating the GnRH neurons as the sole element responsible for regulating GnRH pulsatility, the general consensus is that there is a higher level control system, referred to commonly as the 'GnRH pulse generator', that is responsible for modulating GnRH secretion from the GnRH neurons [Lincoln et al., 1984].

Numerous factors influence the activity of the GnRH pulse generator, but the most relevant in terms of the estrous cycle are the gonadal steroids, estrogen and progesterone. Relying on the close association between GnRH and LH pulses, Goodman and Karsch [1980] examined the effects of luteal and follicular phase concentrations of progesterone and estradiol, respectively, on LH pulsatility in ovariectomized ewes. What they found was that when progesterone is the predominant hormone in the circulation, as during the luteal phase of the estrous cycle, LH secretion was characterized by very low frequency, high amplitude pulses. When follicular phase concentrations of estradiol were maintained, the LH pulses occurred at a higher frequency and significantly lower amplitude relative to the progesterone treatment. It was later confirmed [Moenter et al., 1991] that GnRH secretion matches the pattern predicted by the LH pulses. These profound effects of progesterone and estradiol on GnRH (and LH) pulsatility are critical for follicular development. It is only after a decline in progesterone, following regression of the corpus luteum, that the GnRH pulse generator is primed to produce the high frequency pulses of LH required to stimulate estradiol production that will result in ovulation of the dominant follicle [Wiltbank et al., 2002].

The GnRH surge and Ovulation

In addition to its effect on GnRH pulsatility, estradiol also has a positive feedback effect on GnRH secretion to induce the preovulatory GnRH and LH surge. Importantly, the presence of progesterone will block the positive feedback effects of estradiol on GnRH secretion and block the GnRH surge from occurring [Goodman et al., 1994]. In the absence of progesterone, an injection of estradiol, or estradiol from a growing dominant follicle, will elicit a surge of GnRH secretion that clearly departs from the typical pulsatile secretion of GnRH. Surge concentrations of GnRH, which can endure for ~20 hours in sheep, have been estimated to be 20 to 40 times greater than those observed during other periods of the estrous cycle. This magnitude of increase and duration suggests that a faculty, perhaps separate from the GnRH pulse generator, organizes and repeatedly stimulates the synchronous firing of a large proportion of the GnRH neurons [Goodman, 1994]. One of the more interesting features of the GnRH surge is that GnRH plays a determinisitic role in inducing the LH surge from the ruminant pituitary. Casting GnRH in a deterministic role simply implies that an abrupt increase in GnRH must be present to elicit the surge release of LH from the pituitary [Karsch et al., 1997]. This is in fact quite distinct from the GnRH requirements for an LH surge in primates and humans where GnRH plays only a permissive role and a surge of GnRH is not required to induce an LH surge [Hotchkiss and Knobil, 1994]. An

important consequence of the deterministic role of GnRH in ruminants is that the LH surge begins immediately after the beginning of the GnRH surge (or GnRH injection) and that a significant dose response relationship exists between the size of the GnRH surge and the magnitude of the LH surge [Karsch et al., 1997].

The pattern of the LH surge resulting from an endogenous GnRH surge, or a single injection of 100 μg GnRH (the standard dosage used in cattle), appears to be quite distinct. Recent data from our laboratory suggest that when 100 μg of GnRH is administered as a single injection, the resulting maximal LH concentrations, as determined with hourly blood sampling, are greater after a GnRH injection (17.3 ±2.8 ng/ml) compared to an endogenous GnRH surge (8.8 ±7.1 ng/ml). In addition, the duration of the LH surge is shorter after a GnRH injection (6.8 ±0.3 h) compared to the endogenous GnRH surge (10.4 ±0.7 h) [Haughian and Wiltbank, unpublished observations]. Despite these differences in the pattern of the LH surge, both are sufficient to induce ovulation of a dominant follicle. A key word here is 'dominant' follicle, since follicles that are not dominant (pre-deviation follicles) do not express LH receptors and therefore will not ovulate in response to an LH surge [Sartori, Fricke et al., 2001]. If a GnRH injection is administered during a time that there are no dominant follicles present on the ovary, such as during follicular wave emergence [Ginther et al., 1996], the follicles will continue to develop. However, if a dominant follicle is present, the follicle will ovulate within 29 hours after the onset of the LH surge [Haughian and Wiltbank, unpublished observations].

Ovulation of the dominant follicle and emergence of a new follicular wave are intimately related. The LH surge causes a sharp reduction in estradiol secretion [Komar et al., 2001], and perhaps inhibin secretion [Bleach et al., 2001], from the preovulatory dominant follicle. Both estradiol and inhibin are potent repressors of FSH synthesis and secretion within the pituitary. As a result of ovulation, the inhibitory influences of estradiol and inhibin on FSH secretion are removed and thus there is a surge in FSH secretion and circulating FSH concentrations [Bergfelt et al., 1997]. This surge of FSH is responsible for the emergence and development of a new follicular wave [Ginther et al., 1996]. After about 2.5 to 3 days, a new dominant follicle(s) with ovulatory capacity is selected from this wave of developing follicles. The average functional lifespan of this dominant follicle, beginning from the time of follicle emergence at 4 mm, is approximately 8 to 11 days [Ginther et al., 1996]. Again, a critical point here is that a GnRH injection will produce the emergence of a follicular wave only if it first induces ovulation of a dominant follicle.

Lack of a GnRH surge in Cows with Follicular Cysts

Information regarding the general hormonal characteristics and etiology of follicular cysts and large follicle (≥ 15 mm) anovulatory conditions in cattle has been reviewed elsewhere [Garverick et al., 1997; Wiltbank et al., 2002]. In general, it appears that the underlying defect is lack of a GnRH surge in response to elevated estradiol concentrations. This was demonstrated in a recent report [Gumen et al., 2002] that used an injection of estradiol to induce a GnRH and LH surge in cows that had all large follicles ablated. Cows that did not luteinize any follicular tissue, and hence were not exposed to progesterone following the induced GnRH/LH surge, subsequently developed follicular cysts. This cystic condition was clearly related to a defect in the hypothalamus

because an injection of estradiol could not elicit an LH surge; however, treatment with GnRH (thus 'bypassing' the hypothalamus) induced a normal LH surge and ovulation. In addition, the ability of the hypothalamus to respond to the positive feedback effects of estradiol was restored following treatment with a CIDR (progesterone implant). These observations are consistent with other reports showing that treating cystic cattle with progesterone allowed for the reestablishment of estradiol positive feedback [Nanda et al., 1991] and a recovery from the cystic condition [Calder et al., 1999]. Taken together, these studies indicate that cystic follicles, and other related anovulatory conditions, appear to result from an anomaly in the hypothalamus that can be corrected by treatment regimens including progesterone and GnRH.

Use of GnRH in 'Synch'-ology

Introduction

GnRH was originally labeled for use in cystic cattle. Seguin et al. [1976] showed that treating cystic cows with various dosages of GnRH (0, 25, 50, 100, 150, and 250 μg) produced a dose dependent increase in LH concentrations and that the majority of the cows (18/20) treated with dosages ≥ 50 μg of GnRH had a subsequent increase in progesterone indicating luteinization or ovulation of a follicle. Encouraging experimental results such as this, and FDA approval of GnRH for cows with follicular cysts, have made GnRH an integral part of standard treatment protocols for cystic cattle.

More recently, GnRH has been found to be a valuable tool for controlling follicular and luteal dynamics in normally ovulating cows. One of the earliest reports suggesting that GnRH and PGF2α could be used in breeding synchronization programs was by Twagiramungu et al. [1992] who reported that an injection of the GnRH agonist buserelin, 6 days prior to PGF2α, increased synchronization rate in beef cattle. The implications of using GnRH in breeding programs were more fully realized after the development of the Ovsynch timed-insemination protocol [Pursley et al., 1995; Figure 2a]. The rationale behind the development of Ovsynch was to combine treatments aimed at controlling both follicular and luteal dynamics in a fashion such that the time of ovulation could be precisely synchronized.

Since the introduction of Ovsynch in 1995, this protocol has become widely utilized in the dairy industry. A number of other protocols have also been introduced that utilize GnRH as part of the protocol including: Co-synch, Pre-synch, Heat-synch, Select-synch, Modified Targeted Breeding, and most recently CIDR-synch (*see* Figure 2). An examination of the rationale behind the many adaptations that have been made to the original Ovsynch protocol provides an interesting perspective on how our knowledge and ability to control reproduction in cattle has evolved.

Timing of the 1st GnRH injection

Modifications to the Ovsynch protocol have resulted from information suggesting that synchronization (i.e. ovulation within 48 hours after the 2nd GnRH injection) is dependent upon the stage of the estrous cycle at which the 1st injection of GnRH is administered. Vasconcelos et al., [1999] reported that lactating dairy cows were more likely to synchronize if they ovulated to the 1st injection of GnRH (92% if ovulated, 79% if no ovulation). Failure to ovulate to the 1st GnRH was most detrimental for cows that

were in the later stages (day 13-22) of the estrous cycle. More than 50% of the late-stage cows failing to ovulate to the 1st GnRH ovulated before the 2nd GnRH injection. Given the pattern of follicular dynamics in cattle, it makes sense that late-stage cows failing to ovulate to the 1st GnRH do not synchronize because they complete their natural cycle (i.e. regress the corpus luteum and ovulate) before PGF2α and GnRH intervention at the end of Ovsynch. Additionally, a percentage of cows (9%) that started the Ovsynch protocol in the early stages of the estrous cycle also failed to have a synchronous ovulation to the 2nd GnRH injection. The dominant follicle developing after the 1st GnRH injection may have become functionally non-dominant (turned over) prior to the 2nd GnRH. Moreira et al. [2000] has similarly concluded from work in heifers that optimal synchronization rates were realized when animals began the Ovsynch protocol from days 5 to 10 of the estrous cycle. In general, Ovsynch has not been very effective for synchronizing ovulation in heifers [Pursley et al., 1997]. This may stem from a shorter duration of follicular waves (more rapid turnover) in heifers than lactating cows. A reduced duration of follicular waves increases the odds of administering the 1st GnRH injection in the absence of a dominant follicle and/or turning over a follicular wave before ovulation can be induced with the 2nd GnRH injection.

Administering the 1st GnRH injection between days 5-13 of the estrous cycle resulted in an improvement in conception rates as compared to cows that received the 1st GnRH injection at Day 1-4 and 14-21 (42% vs. 32%; Vasconcelos et al., 1999). Synchronization protocols have been developed with the intent of pre-synchronizing cows so that they receive the 1st GnRH injection at the optimal mid-luteal phase. One such modification, commonly referred to as 'Pre-synch' (Figure 2e), involves two injections of PGF2α prior to the 1st GnRH injection of Ovsynch. The 1st PGF2α injection is given 14 days prior to the 2nd PGF2α injection, which is administered 12 days before the 1st GnRH injection in the Ovsynch protocol. Recent studies indicate that presynchronization improves conception rates when compared to the standard Ovsynch protocol [Moreira et al., 2001]. Other studies have evaluated the effect of a single PGF2α injection 12 days prior to Ovsynch and reported that pregnancy rates were either improved [Cartmill et al., 2001], or not affected [Cordoba and Fricke, 2001] relative to the standard Ovsynch protocol. Modified Targeted Breeding (Figure 2f) is similar to these protocols in that it involves an injection of PGF2α 14 days prior to Ovsynch; however, the insemination conducted after the second PGF2α is often conducted to an observed estrus rather than a timed insemination [Jordan et al., 2002].

GnRH and CIDR's for Anovulatory Cows

One of the more subtle advantages to using GnRH as the initial treatment in the Ovsynch protocol is the induction of ovulation or luteinization of a follicle in anovulatory cows. The resultant progesterone exposure in the anovulatory cows acts to prevent a short cycle following the 2nd GnRH injection. Moreover, it appears that a combination of GnRH and progesterone supplementation, which we have called 'CIDR-synch' (Figure 2b), also improves pregnancy rates in anovulatory dairy cattle [Pursley et al., 2001].

The CIDR was only recently approved for use in dairy cattle in the U.S and therefore the majority of research on CIDR's in synchronization programs has been conducted abroad. In an interesting set of experiments, Ryan et al. [1995] tested the effect of CIDRs in combination with either no treatment, GnRH, or an estradiol benzoate

injection administered at the time of CIDR insertion. Seven days after insertion, a $PGF_{2\alpha}$ injection was administered and the CIDR was removed 24 hours later. The group of cows that were treated with GnRH at the time of CIDR insertion had the highest estrus detection and pregnancy rates. Interestingly, cows treated with estradiol benzoate had comparable conception rates but a reduced estrous detection rate. Furthermore, cows treated with only a CIDR and PGF2α had comparable estrus detection rates, but the conception rate was decreased, probably as a result of ovulating a persistent follicle. A later report from this same group [Ryan et al., 1999] indicated that including an injection of estradiol benzoate 10 hours after the GnRH-CIDR-PGF2α protocol produced better estrus detection, estrus synchrony, and pregnancy rates compared to no estradiol treatment. These data clearly indicate that a synchronization protocol involving CIDRs should include a GnRH injection at CIDR insertion and that a timed-AI is a viable option following CIDR removal.

A recent study by the NC-113 multi-state research project evaluated the effect of CIDR combined with the Ovsynch protocol (CIDR-synch) in lactating dairy cows (Pursley et al., 2001). Overall, there was an improvement in conception rates in cows treated with CIDR-synch as compared to Ovsynch (41% vs. 51%; n = 634). Interestingly, there was no difference in conception rates between Ovsynch and CIDR-synch in cycling cows; however, the CIDR dramatically increased conception rates in non-cycling cows (34.7% vs. 55.2%; n = 182). Treatment with CIDR had no effect on percentage of cows that had a synchronous ovulation after the 2nd GnRH injection. Thus, the CIDR can improve fertility in non-cycling lactating dairy cows. Although there is substantial variability between commercial dairies, the average percentage of high-producing dairy cows not cycling by 60 days post-partum appears to range from 20 to 25%.

Timing of PGF2α, the 2nd GnRH injection, and insemination

Seven days has typically been the standard duration of time between the 1st GnRH and PGF2α injection. Nevertheless, Vasconcelos et al., 2000 showed that shortening the time interval from 7 to 6 days resulted in similar pregnancy rates to a timed artificial insemination. In beef cattle this shorter interval may be beneficial (Twagiramungu et al., 1992). The feasibility of shortening the 1st GnRH to PGF2α interval for use in dairy heifers requires further investigation.

Attempts to reduce the frequency of animal handling while maintaining acceptable conception rates has led researchers to examine various time intervals between both the PGF2α and 2nd GnRH injection and the 2nd GnRH injection and timed-insemination. It appears that there is a progressive increase in conception rates as the time interval between PGF2α and the 2nd GnRH is increased from 0 h, to 12 h, to 24 h, to 36 h (Peters and Pursley, personal communication). The effect of increasing the time interval between PGF2α and 2nd GnRH from 36 h to 48 h or to greater time intervals (60h, 72h) has not yet been tested.

Pursley et al. (1998) used the Ovsynch protocol to synchronize ovulation in 732 lactating dairy cows to test the hypothesis that there was an optimal time for AI in relation to a synchronized ovulation. To assure validity of results, the times for the hormonal injections were varied throughout the day so that all cows could be bred at the same time with the technician blind to the treatment group for each cow. As shown in

Table 1 the greatest conception rate was found at 16 h after the second GnRH but AI at any time between 0 and 24 h after GnRH gave fairly similar pregnancy rates per AI. However, AI after the time of expected ovulation (32 h) had lower pregnancy rates. The pregnancy loss also tended to be greater than at other times and therefore the calving rate at 32 h was substantially lower than in cows bred at the other times (i.e. prior to ovulation). There was also a difference in the gender ratio of calves due to time of AI.

Table 1. Reproductive measures in lactating Holstein cows inseminated at specific times in relation to ovulation synchronized by an injection of GnRH.

	Time from second GnRH until AI				
	0 h	8 h	16 h	24 h	32 h
Cows, no.	149	148	149	143	143
PR/AI, %[a]	37	41	45	41	32*
Pregnancy loss, %[b]	9*	21	21	21	32**
Calving rate %	32	34	36	32	23*
Female:male ratio (%)	61:39[c]	45:55	54:46	54:46	65:35[c]

* - Different from other groups within row ($P < 0.05$).
** - Different from other groups within row ($P < 0.10$).
a - Quadratic effect of treatment on PR/AI ($P < 0.01$).
b - Linear effect of treatment on pregnancy loss ($P < 0.05$).
c - Different ($P < 0.05$) when compared to expected female:male ratio of 46:54.

We have now performed another study on time of AI (Vasconcelos et al., unpublished) by evaluating 1,601 breedings that were done either 0 or 24 h after the second GnRH treatment. We chose these 2 times because they are the most practical times for dairy producers to give injections and do AI because many dairies only lock up cows one time per day. In addition, this study was designed to evaluate whether the lower pregnancy loss and greater female calf ratio that we found at the 0 h time would be replicated in another study. We found that conception rates were greater when cows were bred 24 h (35.1%) as compared to 0 h (29.2%) after the second GnRH injection. This difference was not large but it was statistically significant ($P < 0.05$) due to the large number of breedings that were evaluated. The lower pregnancy loss in the 0 h group was not replicated in this experiment with both groups having substantial pregnancy loss from day 28 until calving (average of 24.7% pregnancy loss). There were 54% female calves in the 0 h group and 46% females in the 24 h group. This result approached statistical significance ($P < 0.1$) but was not entirely convincing. The scientific literature is not clear on whether there is an effect of time of AI on gender ratio (for review see Rorie, 1999) with some studies showing no effect (for example Foote, 1977) and others showing a significant effect (for example Pursley et al., 1998). It is hoped that future studies examining the optimal time of AI will critically evaluate the pregnancy loss and gender ratio in order to provide additional information on this topic.

Insemination of cows at the same time as the 2nd GnRH treatment has become known as the Co-synch protocol. Data from beef cattle suggest that conception rates following Co-synch are similar to that of Ovsynch [Geary et al., 2001]. Thus, the optimal duration of time between the 2nd GnRH injection and the timed-insemination appears to

be 16 h but very good results appear to be obtained with insemination anytime from 0 to 24 h after the 2nd GnRH injection.

Cost-saving Measures

The high cost of GnRH used in the Ovsynch protocol has led researchers to look for alternatives to GnRH. The Heat-synch protocol (Figure 2d), which substitutes an injection of ECP (1 mg) for the 2nd GnRH injection, has become quite popular in the dairy industry because ECP is relatively inexpensive. Along with the reduced cost, some feel that an additional advantage of using ECP is that many cows display a standing estrus following treatment. The sequence of injections in the Heat-synch protocol is similar to that of Ovsynch except that the ECP injection is administered closer to the time of PGF2α (24 hours after) and the timed-AI is performed 48 hours after ECP. Pancarci et al. [2002] has reported that the pregnancy rates following a combination of the Pre-synch and Heat-synch protocols were not different from those following only the Pre-synch protocol. In addition, this group reported that approximately 75% of cows display estrus following ECP treatment. These data suggest that ECP may be a viable alternative to GnRH as the final injection of the Ovsynch protocol; however, this is said with some reservation as the potential consequences of estrogen treatments (such as induced follicular atresia) merit further investigation.

Select-synch (GnRH-7 d-PGF2α-AI at detected estrus) is perhaps the simplest and least expensive protocol (in terms of hormone expenses). The Select-synch protocol forgoes the 2nd GnRH injection and relies instead on the detection of estrus following PGF2α. This protocol is not technically considered to be a timed-insemination protocol because of the dependency on estrus detection. In an experiment comparing the standard Ovsynch protocol with Select-synch, Stevenson et al. [1999] reported that despite slightly better conception rates in cows bred to a natural estrus following Select-synch, pregnancy rates were numerically greater following Ovsynch due to the low AI submission rate in Select-synch cows (68.2% for Select-synch vs. 100.0 % for Ovsynch).

An additional modification aiming to reduce the cost of the Ovsynch protocol is the use of a 'half-dose'(50 μg) instead of the full-dose (100 μg) of GnRH at the 1st and 2nd GnRH injections. Fricke et al., [1998] reported that there were no differences in pregnancy rates following the Ovsynch protocol using the full or half-dose of GnRH at each injection. Furthermore, it was estimated that the money saved from this simple alteration amounted to approximately $20.27 per pregnancy. The data from this study suggest that the half-dose of GnRH is adequate to induce ovulation in lactating dairy cattle; however, the use of the half-dose was reported to have reduced conception rates to the fixed-time insemination in a study involving beef cattle [Funk and Anderson, 2000]. The reasons for this disparity are not clear given the lack of information regarding the profile of LH release following administration of the full and half-dose in dairy and beef cattle.

References:

1. Schally AV, Arimura A, Kastin AJ, Matsuo H, Baba Y, Redding TW, Nair RM, Debeljuk L, White WF. Gonadotropin-releasing hormone: One polypeptide

regulates secretion of luteinizing and follicle-stimulating hormones. Science 1971; 173:1036-1038.
2. Amoss M, Burgus R, Blackwell R, Vale W, Fellows R, Guillemin R. Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) or ovine origin. Biochem Biophys Res Commun 1971; 44: 205-210.
3. Knobil E. On the control of gonadotropin secretion in the rhesus monkey. Rec Prog Horm Res 1974; 36:1-46.
4. Knobil E. The neuroendocrine control of the menstrual cycle. Rec Prog Horm Res 1980; 36: 53-88.
5. Foster DL. Puberty in the sheep. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction, 2nd Edition, Vol. 2. New York: Raven Press; 1994:411-452.
6. Wiltbank MC, Gumen A, Sartori R. Physiological classifications of anovulatory conditions in cattle. Theriogenology 2002; 57(1):21-53.
7. Karsch FJ, Moenter SM. Neuroendocrine regulation of seasonal breeding cycles in the ewe. J Exp Zool 1990; 4(suppl): 17-21.
8. Goodman RL. Neuroendocrine control of the ovine estrous cycle. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction, 2nd Edition, Vol. 2. New York: Raven Press; 1994:659-709.
9. Silverman AJ, Livine I, Witkin JW. The gonadotropin-releasing hormone (GnRH) neuronal systems: Immunocytochemistry and in situ hybridization. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction, 2nd Edition, Vol. 1. New York: Raven Press; 1994:1683-1711.
10. Page RB. The anatomy of the hypothalamo-hypophyseal complex. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction, 2nd Edition, Vol. 1. New York: Raven Press; 1994:1527-1621.
11. Conn PM, Janovick JA, Stanislaus D, Kuphal D, Jennes L. Molecular and cellular bases of gonadotropin-releasing hormone action in the pituitary and central nervous system. Vitam Horm 1995; 50:151-214.
12. Clarke IJ, Cummins JT. The temporal relationship between gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology, 1982; 111:1737-1739.
13. Caraty A, Locatelli A. Effect of time after castration on secretion of LHRH and LH in the ram. J Reprod Fertil 1988; 82:263-269.
14. Moenter SE, Caraty A, Karsch FJ. The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. Endocrinology 1990; 127:1375-1384.
15. Gazal OS, Leshin LS, Stanko RL, Thomas MG, Keisler DH, Anderson LL, Williams GL. Gonadotropin-releasing hormone secretion into the third-ventricle cerebrospinal fluid of cattle: Correspondence with the tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptide Y. Biol Reprod 1998; 59:676-683.
16. Yoshioka K, Suzuki C, Arai S, Iwamura S, Hirose H. Gonadotropin-releasing hormone in third ventricular cerebrospinal fluid of the heifer during the estrous cycle. Biol Repod 2001; 64:563-570.
17. Kaiser UB, Sarbagh E, Katzenellenbogen RA, Conn MP, Chin WW. A mechanism for the differential regulation of gonadotropin gene expression by

gonadotropin-releasing hormone. Proc Nat Acad Sci USA 1995; 92:12280-12284.
18. Vizcarra JA, Wetteman RP, Braden TD, Turzillo AM, Nett TM. Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotropin subunits in cows. Endocrinology 1997; 138:594-601.
19. Vizcarra JA, Wetteman RP, Morgan GL. Influence of dose, frequency, and duration of infused gonadotropin-releasing hormone on secretion of luteinizing hormone and follicle-stimulating hormone in nutritionally anestrous beef cows. Dom Anim Endo 1999; 16(3):171-181.
20. Terasawa E. Luteinizing hormone-releasing hormone (LHRH) neurons: Mechanism of pulsatile LHRH release. Vitam Horm 2001; 63:91-129.
21. Lincoln DW, Fraser HM, Lincoln GA, Martin GB, McNeilly AS. Hypothalamic pulse generators. Rec Prog Horm Res 1984; 41:369-411.
22. Goodman RL, Karsch FJ. Pulsatile secretion of luteininzing hormone: Differential suppression by ovarian steroids. Endocrinology 1980; 107:1286-1290.
23. Moenter SM, Caraty A, Locatelli A, Karsch FJ. Pattern of gonadotropin-releasing hormone (GnRH) secretion leading up to ovulation in the ewe: existence of a preovulatory GnRH surge. Endocrinology 1991; 129:1175-1182.
24. Karsch FJ, Bowen JM, Caraty A, Evans NP, Moenter SM. Gonadotropin-releasing hormone requirements for ovulation. Biol Reprod 1997; 56:303-309.
25. Hotchkiss J, Knobil E. The menstrual cycle and its neuroendocrine control. In: Knobil E, Neill JD (eds.), The Physiology of Reproduction, 2nd Edition, Vol. 2. New York: Raven Press; 1994:711-749.
26. Sartori R, Fricke PM, Ferreiria JCP, Ginther OJ, Wiltbank MC. 2001. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biol. Reprod. 65: 1383-1391.
27. Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K. Selection of the dominant follicle in cattle. Biol Reprod 1996; 55:1187-1194.
28. Komar CM, Berndtson AK, Evans ACO, Fortune JE. Decline in circulating estradiol during the periovulatory period is correlated with decreases in estradiol and androgen, and in messenger RNA for P450 aromatase and P450 17a-hydroxylase, in bovine preovulatory follicles. Biol Reprod 2001; 64:1797-1805.
29. Bleach ECL, Glencross RG, Feist SA, Groome NP, Knight PG. Plasma inhibin A in heifers: Relationship with follicular dynamics, gonadotropins, and steroids during the estrous cycle and after treatment with bovine follicular fluid. Biol Reprod 2001; 64:743-752.
30. Bergfelt DR, Smith CA, Adams GP, Ginther OJ. Surges of FSH during the follicular and early luteal phases of the estrous cylce in heifers. Theriogenology 1997; 48(5):757-768.
31. Garverick HA. Ovarian follicular cysts in dairy cows. J Dairy Sci 1997; 80(5):995-1004.

32. Seguin BE, Convey EM, Oxender WD. Effect of gonadotropin-releasing hormone and human chorionic gonadotropin on cows with ovarian follicular cysts. Am J Vet Res 1976; 37:153-157.
33. Gumen A, Sartori R, Costa F, Wiltbank MC. A GnRH/LH surge without subsequent progesterone exposure can induce development of follicular cysts. J Dairy Sci 2002; 85:43-50.
34. Nanda AS, Ward WR, Dobson H. Lack of LH response to oestradiol treatment in cows with cystic ovarian disease and effect of progesterone treatment of manual rupture. Res Vet Sci 1991; 51:180-184.
35. Calder MD, Salfen BE, Bao B, Youngquist RS, Garverick HA. Administration of progesterone to cows with ovarian follicular cysts results in a reduction in mean LH and LH pulse frequency and initiates ovulatory follicular growth. J Anim Sci 1999; 77(11):3037-3042.
36. Twagimiringu H, Guibault LA, Proulx J, Dufour JJ. Synchronization of estrus and fertility in beef cattle with two injections of buserelin and prostaglandin. Theriogenology 1992; 38(6):1131-1144.
37. Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 1995; 44:915-923.
38. Vasconcelos JLM, Silcox RW, Rosa GJM, Pursley JR, Wiltbank MC. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 1999; 52:1067-1078.
39. Moreira F, de la Sota RL, Diaz T, Thatcher WW. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J Anim Sci 2000; 78:1568-1576.
40. Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation of synchronized estrus. J Dairy Sci 1997; 80:295-300.
41. Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 2001; 84:1646-1659.
42. Cartmill JA, El-Zarkouny SZ, Hensley BA, Lamb GC, Stevenson JS. Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J Dairy Sci 2001; 84:1051-1059.
43. Cordoba MC, Fricke PM. Evaluation of two hormonal protocols for synchronization of ovulation and timed artificial insemination in dairy cows managed in grazing-based dairies. J Dairy Sci 2001; 84:2700-2708.
44. Jordan ER, Schouten MJ, Quast JW, Belschner AP, Tomaszewski MA. Comparison of two timed artificial insemination (TAI) Protocols for management of First Insemination Postpartum. J Dairy Sci 2002; 85:1002-1008.
45. Pursley JR, Fricke PM, Garverick HA, Kesler DJ, Ottobre JS, Stevenson JS, Wiltbank MC, NC-113 Regional Research Project. Improved fertility in noncycling lactating dairy cows treated with exogenous progesterone during Ovsynch. Midwest Branch ADSA 2001 Meeting, Des Moines, IA; 63 abstr.

46. Ryan DP, Snijders S, Yaakub H, Ofarrell KJ. An evaluation of estrus synchronization programs in reproductive management of dairy herds. J Anim Sci 1995; 73(12):3687-3695.
47. Ryan DP, Galvin JA, O'Farrell KJ. Comparison of oestrous synchronization regimens for lactating dairy cows. Anim Reprod Sci 1999; 56(3-4):153-168.
48. Roy GL, Twagiramungu H, 1996. A fixed time AI program using the GnRH-PGF-GnRH method for beef females. J Anim Sci 74 (Suppl 1):462.
49. Geary TW, Whittier JC, Hallford DM, MacNeil MD. Calf removal improves conception rates to the Ovsynch and CO-synch protocols. J Anim Sci 2001; 79(1):1-4.
50. Pancarci SM, Jordan ER, Risco CA, Schouten MJ, Lopes FL, Moreira F, Thatcher WW. Use of estradiol cypionate in a presynchronized timed artificial insemination program for lactating dairy cattle. J Dairy Sci 2002; 85:122-131.
51. Stevenson JS, Kobayashi Y, Thompson KE. Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combinations of gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$. J Dairy Sci 1999; 82:506-515.
52. Fricke PM, Guenther JN, Wiltbank MC. Efficacy of decreasing the dose of GnRH used in a protocol for synchronization of ovulation and timed AI in lactating dairy cows. Theriogenology 1998; 50(8):1275-1284.
53. Funk DL, Anderson LH. Effect of dosage of gonadotropin-releasing hormone (GnRH) in an estrus synchronization protocol on conception rates to a fixed-time insemination in postpartum beef cows. J Dairy Sci 2000; 83(suppl.1):217-218 (abstr.)

Figure 1. Amino acid sequence of the GnRH (gonadotropin-releasing hormone) decapeptide (Schally et al., 1971; Amoss et al., 1971).

Gonadotropin-Releasing Hormone									
1	2	3	4	5	6	7	8	9	10
pyro-Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly-NH2

Figure 2. Schematic summary of synchronization protocols combining GnRH and other hormones approved for use in dairy cattle.