

SYNCHRONIZED OVULATION WITH TIMED INSEMINATION VERSUS
EXOGENOUS PROGESTERONE WITH INSEMINATION AT AN INDUCED-
ESTRUS AS THERAPEUTIC STRATEGIES FOR OVARIAN CYSTS IN
LACTATING DAIRY COWS

By

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Abstract of Thesis Presented to the Graduate School
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CYSTS IN LACTATING DAIRY COWS

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Therapeutic strategies for bovine ovarian cysts could involve either the use of GnRH or exogenous progesterone; however there is no information available concerning the comparative efficacy of these two treatment strategies. The purpose of this study was to compare the clinical effectiveness of the Ovsynch and CIDR protocols under commercial conditions for the treatment of cystic ovarian disease in dairy cattle.

A total of 401 lactating dairy cows with ovarian cysts were enrolled in the study from October 13, 2003, to September 20, 2004. All cows diagnosed with ovarian cysts were alternatively allocated to 2 treatment groups on the day of diagnosis. Cows in the Ovsynch group were treated with GnRH on Day 0, PGF_{2α} on Day 7, GnRH on Day 9, and timed inseminated 16-20 h later. Cows in the CIDR group were treated with a CIDR Insert on Day 0 for 7 days. On Day 7, the CIDR was removed, and cows were treated with PGF_{2α}. All cows in the CIDR group were observed for estrus and cows exhibiting

estrus within 7 days following removal of the CIDR and PGF2 α administration were inseminated. The outcomes of interest for this experiment were the likelihood to be inseminated, the presence of a CL on Day 21, conception rate and pregnancy rate. Data for these variables were analyzed using logistic regression adjusting for parity, days in milk, body condition score, season, and milk production on the day of diagnosis.

The percentage of cows inseminated in the Ovsynch and CIDR groups were 82% and 44%, respectively. The odds for cows in the Ovsynch group to be inseminated were 5.6 times more than the odds for cows in the CIDR group. The percentage of cows with a CL on Day 21 for the Ovsynch and CIDR groups was 83% and 79%, respectively. The odds for cows in the Ovsynch group to have a CL on Day 21 were 2.2 times more than the odds for cows in the CIDR group. Despite the decreased likelihood for a CL, cows in the CIDR group had higher progesterone concentrations on Day 21. The conception and pregnancy rates for cows in the Ovsynch group were 18% and 14%, respectively. Conception and pregnancy rates for cows in the CIDR group were 23% and 9.5%, respectively. There was no significant effect of treatment on conception or pregnancy rate. The odds for primiparous cows to conceive were 4.1 times more than the odds for multiparous cows. The odds for cows in the 3rd and 4th quartiles of milk production on the day of diagnosis to conceive were more than the odds for cows in the lowest quartile of milk production.

The results of this study indicate that fertility is not different between cystic cows treated with either the Ovsynch or the CIDR protocol, although cows treated with the Ovsynch protocol were more likely to return to normal cyclicity.

INTRODUCTION

Bovine ovarian cysts are follicles that fail to ovulate at the time of estrus (Garverick, 1999). This condition presents an enormous economic problem because these cows are infertile as long as the condition persists (Kesler and Garverick, 1982). It has been reported that the interval from parturition to conception in cows with ovarian cysts is 64 days longer than that observed in cows without ovarian cysts (Bosberry and Dobson, 1989). Since the cost of each day a cow is not pregnant beyond the voluntary postpartum waiting period (usually 70 days post partum) is approximately \$2.50, a cow with ovarian cysts will incur an additional cost of \$55 to \$160 per lactation (Bartlett et al., 1986). Other costs of this condition include an increased culling rate, semen, and veterinary costs. This has been calculated to be approximately \$75 per case. Therefore, the total cost of this condition has been estimated to be \$130 to \$235 per cystic cow per lactation (Bartlett et al., 1986). The economic magnitude of this condition becomes more obvious in large (1,100-3,100 milking cows) dairy herds with an incidence of 9-25%.

The exact cause of ovarian cysts is not presently known, but it appears that an important component in the pathogenesis of this condition is the inappropriate, or lack of, release of gonadotropin-releasing hormone (GnRH) at the time of estrus. It has been suggested that an underlying mechanism in the development of ovarian cysts involves a hypothalamic lesion which causes follicular estrogen to be ineffective in inducing a GnRH/LH surge at the time of estrus (Gumen and Wiltbank, 2002), and that this hypothalamic lesion could involve the estrogen receptor α (ER α). Further, it has been

speculated that treatment with progesterone may induce the ER α in the mediobasal hypothalamus which will foster a GnRH/LH surge in response to follicular estrogen (Gumen and Wiltbank, 2002).

Collectively, this information suggests that therapeutic strategies for bovine ovarian cysts could involve either the use of GnRH or exogenous progesterone. In fact, several experimental protocols using these hormones have been shown to be effective in the treatment of bovine ovarian cysts. A protocol involving multiple injections of GnRH, prostaglandin F $_{2\alpha}$ (PGF $_{2\alpha}$), and timed insemination without detection of estrus has been shown to be effective in treating this condition (Bartolome et al., 2000). However, this protocol is labor-intensive and time-consuming since it involves handling of cows multiple times and a 10-day period of time. In the USA, use of exogenous progesterone for treating bovine ovarian cysts has been hampered by lack of FDA approval for the use of progesterone in lactating dairy cows.

However, an intravaginal device containing progesterone (EAZI-BREED™ CIDR®) was recently approved by the FDA for use in lactating dairy cows. This protocol is relatively simple, does not involve as much handling of cows, and is not as labor-intensive and time-consuming as the one using GnRH and PGF $_{2\alpha}$. Therefore, it could be a more acceptable clinical approach for the treatment of ovarian cysts in the lactating dairy cow. However, there is no information available concerning the comparative efficacy of these two treatment strategies in a single, large dairy herd.

The conceptual hypothesis of this study was that lactating dairy cows with ovarian cysts treated with exogenous progesterone and a luteolytic dosage of PGF $_{2\alpha}$ which are inseminated at an induced-estrus will have a different pregnancy rate compared to cows

with ovarian cysts which are subjected to synchronization of ovulation and timed insemination without detection of estrus.

The objective of this study was to compare the effectiveness of these two protocols in a single, large (1,500 milking cows) commercial dairy herd. By using a single, large herd dairy herd, we can obtain the large number of cows needed to show potential statistically significant differences, and be assured that all cows will be subjected to the same managerial and nutritional conditions during the period of the study. In this approach, the only experimental variable will be the treatment protocols.

LITERATURE REVIEW

Gonadotropin-Releasing Hormone

Introduction

Gonadotropin-releasing hormone (GnRH, also called luteinizing hormone-releasing hormone or LHRH) is a pivotal reproductive hormone in both the male and female. It is produced in the hypothalamus and released from the median eminence under the control of various neuronal inputs and gonadal steroids. Once released at the median eminence into the hypophyseal portal vessels, it traverses to the anterior pituitary gland where it binds to receptors on gonadotropes (Ojeda and McCann, 2000). Once bound to the gonadotrope, it stimulates the release of LH and FSH. These two glycoprotein hormones control folliculogenesis, ovulation, CL function and steroidogenesis. These functions provide steroidal feedback to the hypothalamus which ensures the continuation of normal ovarian cyclicity.

Chemical Nature of the Hormone

GnRH is a decapeptide consisting of pGlu-His-Trp-Ser-Trp-Gly-Leu-Arg-Pro-Gly, listed from the carboxyl to amino terminal. The highly conserved aspects of the peptide throughout evolution include the length (10 amino acids), the 1st four amino acids at the carboxyl-terminus, and the final two amino acids at the amino-terminus (Millar et al., 2004). The high degree of conservation indicates the importance of these characteristics for receptor binding and activation. The amino-terminus is essential for receptor binding and activation while the carboxyl-terminus is only essential for receptor binding.

Substitution of constituent amino acids at the amino-terminus can lead to a hormone with antagonistic properties (Millar et al., 2004).

In humans, GnRH originates from a 92 amino acid-precursor peptide and after proteolytic cleavage the decapeptide (GnRH) is associated with a 56 amino acid-peptide (Ojeda and McCann, 2000). A second variant of GnRH with unknown function has been found and differs by 3 amino acids. This new GnRH variant is called GnRH II and is mostly located in peripheral tissues such as the kidney, bone marrow and prostate (Millar et al., 2004). GnRH is packaged in storage granules which are transported down axons to the median eminence where their release is coordinated in a pulsatile manner.

Control of GnRH Secretion

GnRH is released in a pulsatile manner from the median eminence at low and infrequent levels throughout the luteal phase of the estrous cycle. During proestrus, the preovulatory estradiol surge causes an increase in the frequency of GnRH pulses, which result in an LH surge from the anterior pituitary gland. There is generally considered to be a negative correlation between GnRH/LH levels and progesterone concentration. This differential pattern of GnRH release during various stages of the estrous cycle is controlled by distinct GnRH neuronal populations and the different influences of gonadal steroids.

In order to characterize the involvement of ovarian steroids in the regulation of LH secretion, Karsch et al. (1980) performed a series of experiments in ewes which were intact, ovariectomized (OVX), OVX and progesterone supplemented, OVX and estrogen supplemented, or OVX and estrogen plus progesterone supplemented. Luteinizing hormone concentrations were monitored during the study period of one estrous cycle. A significant finding in that study was that ovariectomized ewes had elevated LH

concentrations which could be partially reduced by either estrogen or progesterone alone, but required the combination of both steroids to reduce LH to similar concentrations observed in intact animals.

Although both gonadal steroids have an inhibitory effect on LH (Karsch et al. 1980), their influence over many days on the pulsatile character of LH is another important component of normal ovarian cyclicity. During the luteal phase, LH pulses occurred approximately every 3.5 hours and each pulse was followed by an increase in estradiol (Baird, 1978). Similarly, OVX ewes treated with progesterone for 10 days had decreased LH pulse frequency and increased amplitude compared with OVX controls (Goodman and Karsch, 1980).

After removal of progesterone, there was an increase in both LH and estradiol concentrations, followed shortly by estrus (Karsch et al., 1979). In cyclic ewes, follicular phase LH pulses have an increased frequency and decreased amplitude, which results in an overall increase in basal LH concentration (Baird, 1978). This was verified experimentally when long-term subcutaneous estradiol implants in the absence of progesterone caused a decrease in LH pulse amplitude yet maintained a frequency similar to OVX controls (Karsch et al., 1980). This experiment also verified that the withdrawal of progesterone alone was not sufficient to induce the LH surge and estradiol alone was not sufficient for the full magnitude preovulatory LH surge. Despite the decreasing amplitude of LH pulses during the follicular phase, the estradiol produced by the follicle was increased in comparison to responses observed during the luteal phase (Baird, 1978). This suggests that, during the follicular phase, the follicle has an increased sensitivity to LH.

There is evidence that LH is responsible for the rising estradiol concentrations and the preovulatory increase in both of these hormones can be prevented by the administration of progesterone (Karsch et al., 1979). Similar results were also found in proestrus rats through measuring the concentration of LH releasing-hormone (LHRH) instead of LH. Animals which were ovariectomized and treated with estradiol had a similar LHRH surge to intact proestrus rats, while the two groups lacking estradiol (OVX controls and OVX progesterone treated rats) had significantly decreased LHRH surge (Sarkar and Fink, 1979). To further validate the involvement of estradiol in the GnRH surge, the expression of c-fos, a protooncogene used as a marker of cellular activation, was examined in GnRH cells during the different phases of an induced estrous cycle (Moenter et al., 1993). The results indicated that, while there was little c-fos activity during most of the estrous cycle, estradiol induced c-fos expression in 48% of GnRH neurons and many other non-GnRH hypothalamic neurons, indicating estradiol induced cellular activation. Collectively, these studies suggest that LH is involved in increasing estradiol concentrations and estradiol feeds forward to increase LH pulse frequency and ultimately triggers the GnRH/ LH surge.

Further characterization of the GnRH surge indicated that GnRH pulse frequency increased while amplitude decreased during the preovulatory phase and this cumulates into a GnRH surge with concentrations 40 times greater than basal (Moenter et al., 1991). The GnRH surge is characterized by an increase in pulse frequency which causes an LH surge and finally outlasts the LH surge (Moenter et al., 1991). During the GnRH surge there is an increase in the GnRH interpulse concentrations, which is built upon by the increased pulse frequency, until finally GnRH release switches from a pulsatile pattern

into a continuous pattern (Caraty et al., 1995). The entire GnRH surge is required for an LH surge of normal size and duration and if the surge is prevented during the ascending or descending phases, the LH surge will also abruptly stop (Evans et al., 1996).

Furthermore, once the surge has been triggered, the ovaries (or gonadal steroids) are not required for further hypothalamic stimulation to maintain the surge (Webb et al., 1981).

Many studies have focused on mapping the neuronal network involved in regulating GnRH secretion. This has involved examining the location and distribution of GnRH neurons, the distribution of neurons containing estrogen receptors (ERs) and the interneurons and neurotransmitters involved in relaying the signal between the estrogen responsive and GnRH containing cells. Other studies have focused on the variation in the activity of these neurons during different phases of the estrous cycle. In the ewe, GnRH neurons projecting to the median eminence were traced using fluorescent tracers and located to the diagonal band of the Broca/medial septal region, medial preoptic area, anterior hypothalamic area and the medial basal hypothalamus (Jansen et al., 1997). The percentages of the total GnRH population within each area did not differ significantly (Jansen et al., 1997). In an earlier study performed during the luteal phase, LHRH neurons were found throughout the medial basal hypothalamus with 5% interacting with other LHRH neurons (Leshin et al., 1988).

In the majority of studies, GnRH neurons have been found to lack estrogen receptors (Shivers et al., 1983; Lehman and Karsch, 1993). Then using reverse transcriptase- polymerase chain reaction (RT-PCR) technology in immortalized mouse GnRH neurons, Shen et al. (1998) found evidence for biologically active estrogen receptors. In the ewe, the ventromedial nucleus has estrogen receptors which are up-

regulated by progesterone and involved in transmission of the GnRH surge (Blache et al., 1994). These neurons, which are involved in estrus and the GnRH surge, secrete somatostatin (Herbison, 1995). Neurons containing estrogen receptors in different areas secrete different neurotransmitters. Specifically, 30% of ER neurons in the preoptic area secrete the inhibitory neurotransmitter γ -aminobutyric acid (GABA; Herbison, 1995), while in the arcuate nucleus only 3-5% secreted dopamine and 15-20% secrete β endorphin (β -END; Lehman and Karsch, 1993). Although only a small percentage of ER neurons exhibited co-expression of a neurotransmitter, many of them were in close contact with ER containing cells (Lehman and Karsch, 1993).

The neuronal path between the input of steroidal hormones and GnRH release is complicated and involves the interplay of many excitatory and inhibitory neurotransmitters. This area of research is rapidly evolving, while development of a reliable model is compounded by species differences in the control of GnRH release. The neurotransmitters generally considered excitatory on GnRH neurons (stimulate production and release of GnRH) include neurotensin (NT), norepinephrine, leptin and galanin; while neuropeptide Y (NPY), dopamine, GABA, and orexins can have a stimulatory or inhibitory effect depending on the steroidal milieu. Neurotransmitters with a consistent inhibitory effect include somatostatin, interleukin-1 β , neuropeptide K, and the endogenous opioid peptides: β -END and dynorphin.

Neurotensin is a neuropeptide possibly involved in transmission of the estradiol induced GnRH signal in rats. Smith and Wise (2001) found that there was an increase in NT biosynthesis on the morning of proestrus which was necessary for the preovulatory surge. In the same study, eighty percent of GnRH neurons expressed NT

immunoreactivity. The increase in NT biosynthesis observed during proestrus was paralleled by the estradiol surge, suggesting it may play a role in increasing NT expression (Smith and Wise, 2001).

The endogenous opioid peptides, such as β -END and dynorphin, are involved in suppression of LH. Although β -END has been colocalized with ERs (Lehman and Karsch, 1993), the role of steroids in mediating the release of β -END during the estrous cycle is not clearly defined. Neurons producing preproiomelanocortin, a precursor to β -END, were found interacting with 6% of LHRH neurons (Leshin et al., 1988). There was also extensive intermingling of these two neuron types at the zona externa of the median eminence, suggesting that β -END acts at both the GnRH cell bodies and axon terminals in mediating the release of GnRH (Leshin et al., 1988). This is supported by findings of increasing β -END concentrations in the median eminence from the luteal to follicular phase of the estrous cycle, then finally declining β -END concentrations during the LH surge (Herbison, 1995). It has been suggested that β -END may play a role in preventing the premature activation of GnRH neurons during the follicular phase (Herbison, 1995).

Opioid antagonists have been shown to stimulate LH secretion (Herbison, 1995). Recent data shows that estrogen and progesterone are likely both involved in the regulation of β -END activity at μ -receptors in the preoptic area and medial basal hypothalamus for the generation of the GnRH surge (Goodman et al., 2004). Furthermore, there is evidence to suggest that the role of progesterone in regulating LH pulse frequency is mediated through dynorphin activity at κ -receptors in the medial basal hypothalamus (Goodman et al., 2004). At these receptors, progesterone induced dynorphin acts to slow LH pulse frequency.

The inhibitory neurotransmitter, GABA, is released in synchrony with GnRH pulses indicating an involvement with LH pulsatility (Kalra et al., 1997). Although GABA is usually an inhibitory neurotransmitter, it can be stimulatory on adult GnRH neurons through the GABAA receptor (Sullivan and Moenter, 2004). Progesterone alters GABA secretion in the preoptic area (Scott et al., 2000) and a fall in GABA concentration is associated with the LH surge (Herbison, 1995). It was recently demonstrated that progesterone reduced both the frequency and size of GABAA receptor mediated presynaptic currents in mice, and this caused a reduction in pulsatile GnRH release (Sullivan and Moenter, 2005). In the same study, dihydrotestosterone had the opposite effect and increased GABAergic tone. It also caused an increase in the number of synaptic contacts between GABAergic and GnRH cells. An interaction between the metabolic hormones leptin, neuropeptide Y, and endogenous opioids with GABA indicates that GABA is involved in relaying information regarding metabolic status to the reproductive axis. Leptin increases GABAergic tone to GnRH neurons, providing stimulation for GnRH release in both fed and fasted animals, while NPY and endogenous opioids decreased GABAergic tone in fed animals only (Sullivan and Moenter, 2004).

Metabolic hormones with effects on the reproductive axis include insulin, leptin, orexins A and B, and NPY. Insulin is released in response to high blood glucose and acts to up-regulate cellular glucose intake but it is also a signal of satiety. Leptin is a satiety factor released by adipocytes. It increases with feeding and fat content and is generally considered to have a positive effect on reproduction (Houseknecht et al., 1998). Orexins A and B are orexogenic factors released from the lateral hypothalamic area and are involved in hunger regulation. Neuropeptide Y is an orexogenic neuropeptide released

during fasting and is generally considered a strong inhibitor of GnRH and LH release (Gazal et al., 1998). However, other evidence suggests that NPY may be involved in stimulating the GnRH surge in an estrogen dependent manner (Xu et al., 2000).

Neuropeptide Y can have a positive effect on GnRH secretion in the presence of steroids by increasing galanin concentration, but in the absence of steroids, it can increase β -END concentration and have a negative effect on GnRH release (Kalra et al., 1997). Galanin and NPY can also act at the level of the anterior pituitary gland to increase gonadotrope response to GnRH (Kalra et al., 1997). In contrast to these stimulatory effects, a dose of 500ug of NPY in cows caused immediate cessation of LH release for 4 hours and disruption of GnRH for 1.5 to 3 hours (Gazal et al, 1998).

The role of leptin in peripubertal animals was examined in a study using normal and nutritionally restricted heifers (Zieba et al., 2004). In these heifers, leptin caused a slight increase in mean LH concentrations and did not affect pulse frequency. The effects of insulin and leptin on GnRH secretion have been examined in vitro using cultured rat hypothalamic sections (Burcelin et al., 2003). In that study, addition of insulin resulted in a dose-dependent increase in GnRH secretion, with the first detectable significant difference occurring at insulin concentration 139% of baseline. In glucose controlled mice, hyperinsulinemia caused a 50-60% increase in LH concentrations (Burcelin et al., 2003). When leptin was added to the hypothalamic culture, there was no detectable increase in GnRH, but leptin did cause a significant increase in the GnRH response to insulin, suggesting a potentiation effect (Burcelin et al., 2003). In nutritionally stressed cows, leptin caused an increase in the mean size of GnRH pulses and increased LH pulse amplitude (Zieba et al., 2004). These results suggest that leptin can act at both the

hypothalamus to increase GnRH release and at the anterior pituitary to sensitize gonadotropin response to GnRH. It has also been suggested that fasting sensitizes the reproductive axis to the effects of leptin and this may occur through an up-regulation of leptin receptors in the hypothalamus (Zeiba et al., 2004).

Orexin-containing fibers have been co-localized with 75-85% of GnRH neurons (Campbell et al., 2003). Of these GnRH neurons co-localized with orexin containing fibers, 85% contained the orexin A specific receptor, OX-R1, which indicates a possible functional relationship. The OX-R1 receptor found on GnRH cells is generally a stimulatory receptor type suggesting that orexins may have a direct positive effect on GnRH secretion (Campbell et al., 2003). The effect of steroids on the orexin-induced stimulation or inhibition on GnRH cells has not been fully determined. It is possible that orexins may inhibit GnRH release through indirect pathways, while stimulating GnRH through a direct pathway, depending of the steroidal milieu (Campbell et al., 2003).

Another complicated explanation is the involvement of progesterone on both the tonic GnRH pulses and the initiation of the GnRH surge. MacLusky and McEwen (1978) identified two different populations of progesterone receptors (PR): 1) receptors in the cerebral cortex and midbrain, and 2) receptors in the hypothalamus, pituitary, preoptic area, and uterus. The latter group of progesterone receptors was induced and up-regulated by estrogen (MacLusky and McEwen, 1978). Furthermore, the progesterone receptors that were up-regulated by estrogen prior to the preovulatory LH surge seemed to be induced in the absence of ligand (Levine, 1997). Estrogen also enhances progesterone inhibition on tonic LH secretion by up-regulating PRs in the ventromedial nucleus and arcuate nucleus (Scott et al., 2000). There is evidence that the estradiol-induced

progesterone receptors and NPY work together to produce the GnRH surge (Xu et al., 2000). The NPY receptor, Y1r, stimulates GnRH release when bound to ligand. The estrogen-induced PRs in the arcuate nucleus can be activated either by progesterone or trans-activated by other neurotransmitters. Once activated these PRs act to increase the amount of Y1r mRNA and therefore amplify the signal created by NPY (Xu et al., 2000).

Function of GnRH

Activity on gonadotropes

Once GnRH is released at the median eminence, it enters the portal vessels and is carried to gonadotropes in the anterior pituitary gland. The gonadotropes synthesize and release LH and FSH in response to GnRH binding at its membrane receptor. The relative response of the gonadotropes to GnRH can be affected by many different hormones including estradiol, progesterone, inhibin, activin and follistatin (Caraty et al., 1995).

The GnRH receptor is a 7 transmembrane, G-protein coupled receptor. Once activated by binding with GnRH, guanine diphosphate (GDP) is exchanged for guanine triphosphate (GTP) on the associated G-protein complex (Anderson, 1996). This causes the $G\alpha$ subunit to dissociate from the $G\beta\gamma$ subunits and activate the intercellular messenger systems involved in the synthesis and release of LH and FSH. Although there is evidence for multiple intracellular messengers after receptor activation, the primary pathway is through activation of phospholipase C (PLC; Anderson, 1996). Activation of PLC increases intracellular inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG), IP3 then causes an increase in intracellular calcium and DAG activates protein kinase C (Anderson, 1996). Together these factors activate the calcium dependent exocytotic mechanisms leading to the release of LH and FSH, which are packaged into distinct secretory granules.

The magnitude of the FSH release is less than that of LH and it appears that FSH release is constitutive while LH release is more highly regulated by GnRH (Anderson 1996). Estradiol and follistatin suppress FSH and are likely involved in its tonic secretion (Moss et al., 1981). In OVX and luteal phase ewes, LH and GnRH pulses were highly correlated while FSH pulses matched with only 50% and 25% of GnRH pulses (Padmanabhan et al., 2003). The proportion of FSH and GnRH pulses that were in concordance was considered a statistically random association in that study. Furthermore, the pulsatility of FSH was not affected by administration of a GnRH antagonist. These results suggest there is a component of the episodic FSH secretion that is independent of GnRH (Padmanabhan et al., 2003). These authors speculated that the continued episodic release of FSH may be due to any one of the following possibilities; incomplete receptor blockade, an intrinsic pituitary FSH rhythmicity, a 2nd class of GnRH receptors, the paracrine activity of activins, inhibins and follistatin, or an unknown hypothalamic FSH releasing factor.

The effect of estradiol on pituitary responsiveness to GnRH has been examined in many species. In a study using anestrous ewes pretreated with a bolus of differing doses of estradiol, Reeves et al. (1971) found that the higher dose (250ug) of estradiol increased pituitary responsiveness to exogenous LHRH. Despite the potential effect at the pituitary, these authors could not rule out that estradiol was also acting at the hypothalamus to increase endogenous GnRH. Adams et al., (1975) found that estrogen pretreatment during Days 10 and 11 of the estrous cycle followed by pulses of LHRH administered every 2 hours for 72 hours, caused an overall decreased pituitary response to the LHRH compared to oil treated controls. This was characterized by an initial increase in LH

which then diminished to near zero by one to two days. Later, using OVX ewes pretreated for 10 days with progesterone, estradiol or an empty implant, Goodman and Karsch (1980) found that ewes pretreated with estradiol had a slightly prolonged but significantly decreased LH surge in response to exogenous GnRH compared to the other two groups. These results lead to the question of whether or not prolonged administration of estradiol can be inhibitory at the pituitary. A recent study using OVX rats found that prolonged estradiol exposure caused an abolition of LH surges, but that this was due to a reduction in the percentage of activated GnRH neurons in the hypothalamus (Tsai and Legan, 2002).

The postulated effects of steroids on the GnRH induced release of LH and FSH included alterations in pituitary stores of these hormones and regulation of GnRH receptors (GnRH-R). The literature is not in complete agreement with respect to these mechanisms. One study found that pretreatment with progesterone had no effect on pituitary LH/FSH stores, but inhibited LH by decreasing hypothalamic release of GnRH (Moss et al., 1981). The same authors also found that estradiol decreased pituitary stores of LH and FSH, decreased FSH release and increased GnRH-R. Another study using hypothalamic-pituitary disconnected ewes pretreated with estradiol found the subsequent administration of progesterone directly inhibited pituitary LH response to exogenous GnRH (Grimus and Wise, 1992). This work suggests that estradiol sensitizes the pituitary to progesterone negative feedback, possibly through the estradiol dependent up-regulation of progesterone receptors.

Since gonadotrope response to GnRH is partially dependent on the number of GnRH receptors, numerous studies have worked to characterize changes in receptor

numbers as a result of gonadal steroid input. As previously mentioned, Moss et al., (1981) found that estradiol increased pituitary GnRH-R content. Kaiser et al. (1993) examined the effect of estradiol and testosterone replacement therapy in gonadectomized rats. They found that both steroids caused a reduction in the post-gonadectomy rise in GnRH-R, with estradiol resulting in a relatively greater reduction in receptor mRNA than testosterone. The role of progesterone in the regulation of GnRH-R was examined in the ewe after luteolysis. After the reduction in peripheral progesterone following luteolysis, there was an increase in GnRH-R mRNA by 12 hours and an increase in GnRH-R by 24 hours (Turzillo et al., 1994). During this study period estradiol and ER levels remained constant, suggesting the change in GnRH-R was due to decreased progesterone, or the removal of a negative feedback influence. These authors could not exclude the possibility that increased GnRH pulses may also be involved in up-regulating its own receptor.

The differential effects of pulsatile versus continuous GnRH administration on gonadotropin secretion is another area under investigation. Many studies have observed the phenomenon of declining LH release after frequent or continuous GnRH administration and have sought an explanation. Some of the original evidence for the need for GnRH pulsatility was found in monkeys with hypothalamic lesions given exogenous GnRH (Belchetz et al., 1978). In that study, continuous administration of GnRH caused a sustained decline in pituitary function, whereas intermittent administration restored normal pituitary function. Continuous GnRH infusion in ewes resulted in a peak LH response by 2 hours, which declined thereafter until reaching a

steady state at 20 hours (Nett et al., 1981). In the same study, FSH had a smaller peak by 2 hours and reached baseline by 5.5 hours.

One possible mechanism for the declining LH response observed following continuous GnRH administration is the depletion of pituitary gonadotropin stores. During the experiment by Nett et al. (1981), pituitary stores of LH/FSH were not affected and could not explain the declining release in response to continuous GnRH infusion. In contrast to this finding, Clarke et al. (1987) determined that LH pulse amplitude was correlated with pituitary stores, and that both amplitude and pituitary stores of LH were decreased when GnRH was given hourly compared to every 3 hours. In cattle, the continuous administration of GnRH caused a decrease in the amount of LH β mRNA but did not affect FSH synthesis (Vizcarra et al., 1997). Furthermore, hourly administration of GnRH improved luteal activity and resulted in an increased number of large follicles by days 7, 9 and 11 when compared to either GnRH administered continuously or once every four hours (Vizcarra et al., 1997).

Another possible mechanism for the declining pituitary response to continuous GnRH administration is the down-regulation of GnRH-R caused by its own ligand, GnRH. In ewes continuously infused with GnRH there was an increase in GnRH-R numbers between 0 and 4 hours of continuous administration, but thereafter receptors decreased until they were only half of their pre-infusion level by 24 hours (Nett et al., 1981). In vitro results, using cultured pituitary cells treated with continuous or hourly GnRH pulses, there was a 12.8 fold increase the GnRH-R mRNA in the hourly pulsed cells while there was no change in the continuously infused group (Kaiser et al., 1993). In cattle infused with continuous GnRH, there was a decrease in both the GnRH-R mRNA

and GnRH-R compared with cattle given pulsatile GnRH (Vizcarra et al., 1997).

Frequency of pulsatile GnRH administration, given hourly or once every 3 hours, did not affect GnRH –R numbers, even though LH pulse amplitude was decreased in the group receiving hourly GnRH (Clarke et al., 1987).

Extrapituitary functions of GnRH

GnRH receptors have been identified in various extrapituitary tissues including the ovary, placenta and prostate (Millar et al., 2004). Although many of the extrapituitary actions of GnRH are mediated indirectly by the gonadotropins, LH and FSH, some studies have suggested a direct action of GnRH. In females, long term administration of GnRH or a GnRH agonist has caused luteolysis, decreased fetal survival, decreased serum estrogen and progesterone concentrations, delayed puberty, delayed parturition, and inhibition of follicular maturation and ovulation (Hsueh and Jones, 1981). In males, long term administration of GnRH has caused decreased testicular androgen production, decreased LH and FSH receptor content, decreased testicular weight, inhibition of spermatogenesis, and decreased prostate and seminal vesicle growth (Hsueh and Jones, 1981).

Postulated mechanisms for the effects of GnRH agonist administration listed above include; desensitization of gonadotropes to GnRH, desensitization of gonadal cells to increased LH, or a direct extrapituitary action of GnRH (Hsueh and Jones, 1981). The latter possibility is supported by evidence indicating direct uptake of GnRH within the ovary (Hsueh and Jones, 1981). The luteolytic effect of GnRH agonist treatment seems to be species specific, such that in human and rats it is luteolytic (Hsueh and Jones, 1981) and in ruminants it is luteotropic (Davis et al., 2003). The positive effects of GnRH on

the ruminant CL are due to the luteotropic effects of LH and possibly, the formation of an accessory CL.

There is evidence that GnRH also has activity as a neurotransmitter at the hypothalamus and higher centers. GnRH neurons have synaptic contact with 5% of GnRH neurons projecting to the median eminence, indicating a role in regulating its own transmission (Leshin et al., 1988). Different studies have also suggested that GnRH plays a role at higher brain centers in the expression of sexual behavior and estrus (Hsueh and Jones, 1981).

Clinical Applications

GnRH has been widely used in the cattle industry because of its ability to induce an LH surge from the pituitary, its relatively small size, easy synthetic production, and lack of antigenicity compared to hCG. The GnRH induced LH surge causes ovulation of a dominant follicle (if present), CL formation, and the subsequent emergence of a new follicular wave. Clinical applications of GnRH include the treatment of cystic ovarian disease (Kittok et al., 1973), formation of an accessory CL as an aid for embryo survival (Schmitt et al., 1996), and synchronization of ovulation for timed insemination programs (Pursley et al., 1995; Momcilovic et al., 1998).

The underlying mechanism of cystic ovarian disease is a hypothalamic lesion involving the ER α (Gumen and Wiltbank, 2002). This lesion can be corrected by sufficient exposure to progesterone which causes the up-regulation of the ER (Gumen and Wiltbank, 2002). An injection of GnRH in cystic cows causes ovulation and CL formation in 75-80 % of cases (Archbald and Thatcher, 1992; Farin and Estill, 1993). The progesterone exposure which occurs after CL formation corrects the hypothalamic lesion and leads to resumption of normal ovarian function (Gumen and Wiltbank, 2002).

In programs designed to synchronize ovulation, GnRH is used to ovulate a dominant follicle from two separate waves. The first injection causes ovulation of a dominant follicle, CL formation and the emergence of a new follicular wave. An injection of prostaglandin can then be used to lyse the CL on day 7, while a second injection of GnRH on day 9 causes ovulation of the dominant follicle which arose after the 1st injection of GnRH. Using this protocol, the time of ovulation can be predicted and the cow can be inseminated without the detection of estrus (Pursley et al., 1995; Momcilovic et al., 1998).

Given that LH is the primary luteotropin supporting CL function in the cow, it has been speculated that exogenous supplementation of GnRH could improve fertility in cows by increasing LH and subsequently, progesterone production. Administration of GnRH every 2 hours for 72 hours caused an increase in luteal weight and progesterone content (Adams et al., 1975). A single injection of GnRH or agonist can create an accessory CL which produces additional progesterone but does not necessarily improve pregnancy rates (Schmitt et al., 1996). It was then speculated that longer acting implants of GnRH agonists may improve luteal function by providing a greater duration of LH support. Longer acting GnRH agonist implants increased mean LH concentrations, increased progesterone concentration, and prolonged luteal lifespan, even in the absence of an accessory CL and regardless of whether the implant was given on day 3 or day 12 of the estrous cycle (Davis et al., 2003).

The use of longer acting GnRH agonist within timed insemination programs has also been evaluated for its potential to increase CL function and inhibit follicular development. When a longer acting GnRH agonist implant, Deslorelin, was used to

replace the second GnRH injection in a timed insemination protocol it caused minimal and sporadic increases in plasma progesterone, decreased follicular growth and decreased expression of estrus in response to a prostaglandin injection 16 days later (Bartolome et al., 2004). Another study used two different doses of Deslorelin implants (450ug and 740ug) in place of the second GnRH of a timed insemination protocol, and compared pregnancy rates and progesterone concentration following treatment (Santos et al., 2004a). These results indicated that the pregnancy rate in response to the lower dose was comparable to the standard protocol, while the higher dose had a decrease pregnancy rate.

Folliculogenesis

Folliculogenesis is the process by which primordial follicles, defined as an oocyte surrounded by a single layer of squamous pregranulosa cells, are recruited and develop into antral follicles. They grow within a cohort of developing follicles until one arises as the dominant follicle, which eventually either ovulates or becomes atretic (Richards, 1980). These follicular cohorts are referred to as waves and usually occur two or three times during the estrous cycle. In slaughterhouse ovaries from heifers on known days of the estrous cycle, follicles >5 mm showed two waves of growth, between the 3rd and 4th days and the 12th and 14th days. Each wave resulted in a preovulatory size follicle, the follicle from the first wave underwent atresia while the follicle from the second wave ovulated (Rajakoski, 1960). Using ultrasonography, follicles can be mapped on the ovary and precisely followed throughout their growth. When ten nulliparous heifers were followed by ultrasound, seven had three, and three had two follicular waves (Pierson and Ginther, 1988).

In adult cows, there are generally two transient increases in the concentration of serum follicle-stimulating hormone (FSH): one coincides with the preovulatory LH surge

and one which occurs 12-24 hours after the luteinizing hormone (LH) peak (Dobson, 1977). At the emergence of each follicular wave there is a 50–75% increase in the concentration of serum FSH, and it has been suggested that this is the main initiating factor for a follicular wave (Adams et al., 1992). If the rise in FSH is delayed or inhibited, the follicular wave may be delayed or inhibited (Fortune, 1994). Exogenous FSH can recruit greater than normal numbers of follicles and support their growth beyond 5 mm. This leads to more follicles available for ovulation and is referred to as superovulation. These effects are dose-dependent and small amounts of exogenous FSH can produce co-dominant follicles (Rivera and Fortune, 2001).

Follicle-stimulating hormone may not be the only factor required for the initiation of a follicular wave since some hypophysectomized sheep and other laboratory animals also exhibit recruitment of primordial follicles and limited growth of a reduced number of preantral follicles (Roy and Greenwald, 1989). Follicles less than 4 mm are not dependent on the acute support of gonadotropins (Gong et al., 1996). While increasing concentration of FSH is the initiating cause of a follicular wave, it is the decline in FSH that signals the end of selection and development of a dominant follicle. All the growing follicles within a cohort equal to or greater than 5 mm contribute to the decline in concentrations of FSH (Gibbons et al., 1999) through the production of estradiol (Ginther et al., 2000a) and inhibin (Kaneko et al., 1995). The follicles in the developing cohort continue to require FSH for their growth and development even when the levels are declining. This was demonstrated by treating heifers with 6.0 mm growing follicles with estradiol (Ginther et al., 2000a). In this study, exogenous estradiol caused a decrease in FSH concentrations and delayed follicular development to the 10 mm stage by 48 hours

over controls. This study disproved the previous theory that the initial FSH surge was the major factor stimulating diameter growth in follicles from wave emergence to deviation. Evidence was also generated that the largest follicle during the growing phase was more sensitive to the decrease in FSH concentration. This was shown when the largest follicle lost its advantage and the second largest follicle overtook the largest to eventually become dominant in all treated animals compared to untreated controls (Ginther et al., 2000a).

The point at which the future dominant follicle begins to surpass the others in growth and estradiol production is known as deviation. Numerous studies are in agreement that morphologic deviation occurs when the largest follicle is on average 8.5 mm in diameter (Beg et al., 2002; Ginther et al., 1996; Rivera and Fortune, 2003). The proposed close two-way functional coupling that occurs between the dominant follicle and FSH concentrations states that when the follicular wave has reached the expected beginning of deviation, the low FSH concentrations are caused by the largest follicle, then are utilized and required by the largest follicle to establish dominance (Ginther et al., 2000b). This was demonstrated by treating heifers with estradiol to lower FSH levels when the largest follicle was 8.5 mm. The result was a transient decrease in FSH concentration and delayed growth of the follicle within 8 hours of the FSH nadir (Ginther et al., 2000b). The dominant follicle also has the greatest role in suppressing circulating FSH concentrations as shown in studies ablating the largest follicle at 8.5 mm and demonstrating an increase in FSH compared to controls (Ginther et al., 2000a). The increase in FSH concentrations caused by ablation occurs in less than 8 hours (Ginther et al., 1999).

After deviation, the low FSH concentrations are only required by the dominant follicle and are already too low to support development of the smaller follicles, which cease growing and eventually regress (Ginther et al., 2000b). In an in-vitro study using cultured granulosa cells, the lowest of 3 doses of FSH stimulated the greatest production of estradiol, inhibin A, activin A and follistatin, while the maximum progesterone output was achieved at the two higher doses of FSH (Glister et al., 2001). These dose-dependent effects of FSH on granulosa cell products seem to mimic in vivo actions. This occurs both at the time of deviation when FSH concentrations are low and the follicle is producing more estradiol, inhibin, activin and follistatin; and during the preovulatory gonadotropin surge when FSH is higher, granulosa cell luteinization occurs and progesterone output increases, while aromatase, inhibin/ activin and follistatin are very much decreased (Glister et al., 2001).

The dominant follicle switches from FSH to LH dependency just after the point of deviation or >8.5 mm. Follicle-stimulating hormone is needed for follicular growth up to 9 mm, beyond this point LH is required for growth up to ovulatory size (Gong et al., 1996). A small elevation in LH lasting for about 48 hours occurs around the time of deviation (Ginther et al., 2001a). The significance of the 'deviation LH surge' was examined by comparing the effects of progesterone treatment prior to and encompassing deviation, thereby inhibiting the normal increase in LH at this time (Ginther et al., 2001a). This resulted in no change in the timing of deviation, but caused a decrease in follicular fluid estradiol, oestrone, androstenedione, and free-IGF. However, there was an increase in follicular fluid IGFBP-2 with no change in follicular progesterone and immunoreactive inhibin. Changes in plasma were reflected as a decrease in estradiol

within 8 hours, an increase in FSH concentrations, and the elimination of the typical decrease in FSH concentrations which occurs at deviation and accompanies the transient increase in LH. The increase in synthesis and secretion of estradiol into circulation at the beginning of deviation is likely a function of the increase in LH. The reduced levels of oestrone and androstenedione in treated animals also indicated that LH stimulated increases in estradiol through the steroidogenic pathway (Ginther et al., 2001a).

The transient increase in LH around deviation is important for the function of the follicle, but its absence does not prevent diameter growth and morphologic deviation. Luteinizing hormone is only critical for continued growth in the days following deviation (Ginther et al., 2001b). The importance of LH for growth was demonstrated when progesterone was given to lower LH concentrations in pre- and post-deviation groups. There was no effect on the largest follicle in the pre-deviation group, while in the post deviation group the largest follicle was smaller than controls, had lower concentrations of estradiol, and had lower free IGF-1 concentration in follicular fluid (Ginther et al., 2001b).

The theca interna cells of antral follicles typically express LH receptor (LHr) mRNA, and this expression increases with follicular size (Xu et al., 1995). Despite this, there is no apparent relationship between LH binding to theca interna cells and follicle size. Expression of LHr mRNA is increased in the theca interna cells of dominant follicles (Xu et al., 1995). An important difference between the largest follicle (F1) and the second largest follicle (F2) after deviation is the expression of LHr mRNA in granulosa cells > 8mm (Xu et al., 1995). Granulosa cells had increased LHr activity on the day after deviation and an increase in the difference of LHr mRNA expression

between F1 and F2 8 hours prior to an increase in diameter and estradiol concentrations (Beg et al., 2002). Therefore, the elevation in LH concentration and expression of LHR in granulosa cells are some of the first events leading to deviation. Follicle stimulating hormone (FSH) has been shown to induce the expression of LHR mRNA in rat granulosa cells (Rani et al., 1981). Therefore, FSH responsiveness is crucial for the future responsiveness of the follicle to LH. The expression of LHR on granulosa cells may give the future dominant follicle an advantage over the others during the increase in LH accompanying deviation. The binding of LH to granulosa cells triggers the enzymatic processes involved in the conversion of androgens into estradiol.

Estrogen is a highly significant hormone during follicle growth and deviation. Some of its actions in the dominant follicle include; stimulating further follicular growth (Beg et al., 2003), stimulating proliferation of granulosa cells (Richards, 1980), a positive role in modulating the FSH dependent induction of LHR mRNA in granulosa cells (Rani et al., 1981), enhancing steroidogenesis, and increasing production of insulin-like growth factor 1 (IGF-I; Hsu and Hammond, 1987). At the point of deviation, there is a significant difference in follicular fluid estradiol concentrations, with the largest follicle having higher estradiol compared to the 2nd and 3rd largest follicles (Beg et al., 2002). The change in estradiol concentration and diameter between the two largest follicles is generally the point that defines deviation. As early as Day 2 of a follicular wave, the future dominant follicle had higher concentrations of estradiol in follicular fluid and granulosa cells secreted more estradiol in culture than future subordinate follicles (Evans et al., 1997).

Another area under investigation for its role in the deviation phenomenon is the IGF system. This system includes insulin, insulin-like growth factors I (IGF-I) and II (IGF-II), their binding proteins (IGFBP); specifically IGFBP-2, -3, -4 and -5, of which -2, -4 and -5 are most significant in the ovary; and the IGFBP proteases. Insulin, IGF-I and IGF-II share 45% of the same amino acid sequences (Spicer and Echterkamp, 1995) and have similar actions at many of the same receptors. IGF-I is produced in the liver and in the ovary by granulosa cells (Spicer et al., 1993), but the majority present in the ovary is derived from peripheral circulation. IGF-II is primarily produced by theca cells and is less potent than IGF-I (Lucy, 2000). IGF-I is a pleiotropic growth factor that stimulates growth and development in a variety of cell types. In the ovary, IGF has a mitogenic effect stimulating an increase in granulosa cell (Spicer et al., 1993) and theca cell (Stewart et al., 1995) numbers.

IGF has a significant role in steroidogenesis, which is the basis for its contribution to deviation. There are three mechanisms whereby IGF increases steroidogenesis. Firstly, IGF directly activates steroidogenic enzymes (Yang and Rajamahendran, 1998). Estradiol production increased when IGF was injected into follicular fluid (Ginther et al., 2004), or when pumped into the ovary (Spicer et al., 2000). Estradiol also increased when IGF was added to granulosa cell cultures (Spicer and Echterkamp, 1995). Secondly, IGF induces the expression of gonadotropin receptors. In cultured thecal cells, IGF increased the expression of LHr mRNA (Stewart et al., 1995). Thirdly, IGF has a synergistic effect with LH and FSH in the production of androgens and estrogens. In the presence of LH, IGF-I increased androstenedione and progesterone production in cultured thecal cells, whereas LH or IGF alone had little or no effect (Stewart et al., 1995). In granulosa cells,

the addition of IGF-I enhanced the effects of low doses of FSH on secretion of estradiol (Glister et al., 2001), and this effect was not dose dependent, where a lower dose of IGF (100ng/ml) increased estradiol more than a higher dose of IGF (200ng/ml; Spicer et al., 1993). In the same study, progesterone production was also greater in granulosa cells treated with IGF and FSH than with IGF alone.

Total IGF levels are often reported to be the same in the dominant and subordinate follicle. The primary difference is in the level of free vs. bound IGF. Dominant and future dominant follicles consistently have higher levels of free IGF and lower levels of the low molecular weight IGF binding proteins than the subordinate follicle (Lucy, 2000; Beg et al., 2002). After ablation of the dominant follicle, two of the earliest changes during the assumption of dominance for F2 were an increase in free IGF-I and a decrease in IGFBP-2 (Beg et al., 2002). This occurred when the follicle was in the diameter range of 8.4 - 8.7 mm. Another study found that the most consistent predictors of future dominance in 5 – 8 mm follicles were high follicular fluid concentrations of estradiol and low concentrations of IGFBP-4 (Mihm et al., 2000). In contrast to the previously mentioned studies, Mihm et al. (2000) found that IGFBP-2 was not different between future dominant and subordinate follicles. This could be due to the earlier developmental stage of the follicles in this study (5 – 8 mm vs. > 8 mm). Another possible explanation for the contrast between these studies could be accuracy of the tests used to measure and distinguish between the binding proteins. However, the important point is that the follicle destined for dominance has fewer low molecular weight IGF binding proteins; primarily IGFBP-4.

The mechanism through which the follicle develops a decreased number of low molecular weight IGFBP and increased concentrations of free IGF may be the critical

aspect to the establishment of dominance. There are three primary possibilities through which a decreased amount of IGFBP-4 may occur. These possibilities include 1) decreased genetic expression for IGFBP-4, 2) alterations in proteolytic degradation, and 3) decreased uptake from peripheral circulation. The majority of research has focused on the first two explanations, and the answer may be a combination of all three. In healthy follicles, theca cells continually express mRNA coding for IGFBP-4 while granulosa cells express mRNA for IGFBP-2, but only up to a diameter of 8 mm (Armstrong et al., 1998). Since expression of mRNA for IGFBP-4 did not change while its concentrations decreased, the authors postulated that its control is not through genetic expression but possibly by a protease. The change in expression for IGFBP-2 suggests that its control is genetic and was inhibited by increasing FSH (Armstrong et al., 1998). Interestingly, IGFBP-4 was found in granulosa cells after determining that these cells do not express its mRNA, and this led to the conclusion that IGFBP-4 was brought into the cells from peripheral circulation (Armstrong et al., 1998). It is likely that IGFBP-4 acts as a transport and storage unit of available IGF for granulosa cells. Free IGF can then be accessed by activation of the specific protease. Another study found that IGFBP-4 is degraded by a metallodependent protease (Mazerbourg et al., 2000) which was later identified as pregnancy associated plasma protein A (PAPP-A; Rivera and Fortune, 2003). Furthermore, IGFBP-2 is detrimental to development through sequestration of IGF which is needed for growth and development (Armstrong et al., 1998).

The proteolytic activity involved in the degradation of IGFBP-4 was enhanced when IGF was added to follicular fluid, and decreased when IGFBP-2 and -5 were added (Mazerbourg et al., 2000). This agreed with Rivera and Fortune (2003) who demonstrated

a greater affinity of the proteolytic enzyme for IGFBP-4 when bound to IGF. Follicle stimulating hormone induced co-dominant follicles which showed similar proteolytic activity against IGFBP-4 as normal dominant follicles and greater than that of subordinate follicles (Rivera and Fortune, 2001). This suggested a potential role for FSH in inducing the protease, but did not rule out the simultaneous increase in estradiol as a factor. This was later addressed by the same authors who found that the increase in IGFBP-4 proteolytic activity and a decrease in IGFBP-4 were the earliest changes in normal growing follicles and FSH induced co-dominant follicles, occurring prior to significant changes in estradiol concentration or diameter (Rivera and Fortune, 2003).

Cystic Ovarian Disease in the Dairy Cow

Introduction

Cystic ovarian disease is a well-recognized condition in dairy and beef cattle. It occurs more commonly in cows than in heifers and is a significant source of economic loss for dairy farmers. It is a condition in which cows are anovular for an extended period of time, usually in the first 60 days postpartum period, even though a preovulatory follicle exists on the ovary. The exact underlying endocrinology is not completely understood, but it appears to be related to an unresponsiveness of the ER α in the hypothalamus to follicular estrogen at estrus (Gumen and Wiltbank, 2002).

Ovarian cysts can be classified in three ways: follicular cyst, luteal cyst or a cystic corpus luteum (Roberts, 1971). A follicular cyst is an anovulatory follicle, and the pathology and treatment will be the focus of this section. A luteal cyst is an anovulatory follicle that became partially luteinized. A cystic corpus luteum is a functional corpus luteum that developed a cavity, without disruption of the estrus cycle. The first two types

arise from the same pathology and disrupt normal cyclicity, while the third type is not considered abnormal (Roberts, 1971).

Definition and Diagnosis

The definition and diagnostic criteria for ovarian cysts are continually evolving as is evident by the inconsistency and changes over time in the literature. The classic definition for ovarian cysts are multiple or single follicle like structures, greater than 25 mm and persisting for 10 days or longer in the absence of a CL (Bierschwal et al., 1975; Kesler and Gaverick, 1982; Farin and Estill, 1993). In the more recent literature, they are defined as a follicle that persists for more than 6 days (Lopez-Gatius et al., 2002; Silvia et al., 2002) and has a diameter equal to or greater than ovulatory size (> 17 mm; Silvia et al., 2002; Halter et al., 2003). The other important criterion for a follicular cyst is the absence of luteal tissue (Silvia et al., 2002) or a corpus luteum (Lopez-Gatius et al., 2002).

Diagnosis of an ovarian cyst can be achieved by palpation per rectum, a history of anestrus or nymphomania, ultrasonographic examination and serum progesterone levels. Findings per rectum will include a flaccid uterus similar to diestrus, mucometra if the condition is chronic (Youngquist, 1986), single or multiple smooth, fluid-filled structures greater than 17 mm, and the absence of a CL. Differential diagnoses for ovarian cysts diagnosed by palpation per rectum includes a normal preovulatory follicle, a CL, a corpus hemorrhagicum, adhesions, salpingitis, hydrosalpinx, oophoritis, ovarian abscess, neoplasia, and cysts of the fimbria (Youngquist, 1986). Palpation of the uterus per rectum and ultrasonographic examination of the ovaries and uterus will aid in differentiating these structures. Ultrasonographic examination of the ovaries will reveal that the cyst is thin-walled and contains an anechoic antrum measuring greater than 17 mm. A luteinized

cyst will have increased wall thickness and feel firm on palpation per rectum, and on ultrasonographic examination will often have gray echogenic patches along the inner cyst wall or within the antrum (Farin et al., 1990) and a wall greater than 3 mm thick (Ribadu et al., 1994). A normal corpus luteum has an echogenicity different than the surrounding tissues and a well defined border (Pierson and Ginther, 1984).

Many normal corpora lutea contain an anechoic central cavity (cystic CL). These have been observed temporarily from day 5-7 of the estrous cycle in a proportion of heifers examined daily (Pierson and Ginther, 1984) and in approximately 30% of slaughterhouse ovaries examined (Peter, 1997). Ultrasonographic examination of the uterus would reveal signs typical of diestrus (Pierson and Ginther, 1988). If the uterus has tonicity, responds to palpation, and ultrasonography reveals a heterogenous texture, these are sign of impending estrus (Pierson and Ginther, 1988), and help differentiate between a follicular cyst and preovulatory follicle.

The accuracy of palpation per rectum, ultrasonography and peripheral progesterone levels to diagnose ovarian cysts has been debated in the literature. Palpation per rectum alone was reasonably accurate, but varies between studies and cyst type. One study found that when palpation per rectum was used to differentiate between cysts, whether follicular or luteal, and other ovarian structures the accuracy was 52% (Ribadu et al., 1994). Most luteal cysts can be successfully treated with prostaglandin. Therefore, it is generally beneficial to differentiate between follicular and luteal cysts, although this is where most of the inaccuracy occurs. Sprecher et al., (1988) found that palpation per rectum had a 75% positive predictive value (PPV) for follicular cysts, with a sensitivity and specificity of 61.9% and 50%; while for luteal cysts it had a PPV, sensitivity and specificity of 31%,

50.0% and 61.9%, respectively. In a different study, diagnosis of luteal cysts by palpation per rectum had a sensitivity, specificity and PPV of 43.4%, 64.7% and 68.4%, respectively; while ultrasonography had a sensitivity, specificity and PPV of 86.7%, 82.5% and 89.7%, respectively (Farin et al., 1992). For follicular and luteal cysts, one study found that the milk progesterone enzyme immunoassay had a PPV of 91.8% and 83.3% respectively (Sprecher et al., 1988). On average, the PPV for follicular cysts diagnosed by rectal palpation or ultrasonography are 66% and 74%, respectively; and the PPV for luteal cysts diagnosed by palpation per rectum or ultrasonography are 66% and 85%, respectively (Hanzen et al., 2000).

Overall, these results indicate that 1) palpation per rectum is more accurate for diagnosing follicular cysts over luteal cysts (Sprecher et al., 1988), 2) ultrasonography was better than palpation per rectum, especially for detecting luteal cysts (Farin et al., 1992) and 3) palpation per rectum combined with progesterone levels or ultrasound is generally more accurate than palpation per rectum alone (Sprecher et al., 1988, Farin et al., 1990).

The wide range of sensitivity, specificity and positive predictive values in the literature is partially due to the range in criteria used to define follicular and luteal cysts. After a cyst is diagnosed by palpation per rectum or ultrasonography, most studies use progesterone levels as the gold standard to differentiate between follicular and luteal cysts. Progesterone values used to determine a luteal cyst range from greater than 0.5ng/ml (Farin et al., 1990) to greater than 5.0ng/ml (Sprecher et al., 1988). Some studies have found intermediate to high progesterone levels when follicular cysts were diagnosed by ultrasound as having no luteal tissue (Jeffcoate and Ayliffe, 1995) or low

progesterone when luteal cysts are diagnosed as having wall thickness greater than 3mm (Douthwaite and Dobson, 2000). A possible explanation for the luteinized cyst with progesterone levels $<0.9\text{ng/ml}$ is that it has a function similar to a late CL, around day 19, when it can be observed but secretes very little progesterone and is unresponsive to PGF (Douthwaite and Dobson, 2000). There can be considerable variation in the density of the luteinization as measured by ultrasound (Farin et al., 1992) and occasionally histology is the only method that can accurately determine the presence of luteal tissue (Cook et al., 1991). A low level of peripheral progesterone does not distinguish between a preovulatory follicle and follicular cyst (Douthwaite and Dobson, 2000). There are numerous ways to accurately diagnose a follicular cyst. Probably the preferred approach is a combination of progesterone, ultrasonography and histology. It appears that the most practical method for field diagnosis relies on careful palpation per rectum of the ovaries and uterus, complimented by ultrasonography whenever possible or a follow-up examination in one to two weeks.

Incidence Rate and Risk Factors

The incidence of cystic ovarian disease is reported in the literature to be between 7.7 and 17% for dairy cows (Table 1) with an overall mean of 11.9%. The reported incidence on a given farm may depend on the frequency of examinations, where more frequent visits and a shorter interval (Erb and White, 1981) or a very long interval ($>30\text{d}$) where cows have time to spontaneously cure before being detected could both result in a lower incidence rate. Most investigations report a peak incidence rate during early lactation (Table 2) while a few have found an additional peak occurring between 150-220 days, giving a bimodal distribution. A possible explanation for the second peak is an

increased intensity in examinations of non-pregnant cows at this time (Bartlett et al., 1986).

Table 1. Reported incidence rates for cystic ovarian disease in the literature.

Literature cited	# Lactations	% Lactations
Morrow et al., 1966	357	12.3
Bierschwal, 1966	1 436	13.0 (11.1- Holstein, 17.0- Guernsey)
Whitmore et al., 1974	375	11.2
Erb and White, 1981	1 599	12.4
Bartlett et al., 1986	2 847	12.8
Gröhn et al., 1998	7 523	10.6
Fleischer et al., 2001	2 197	11.7
Hooijer et al., 2001	15 562	7.7
Lopez- Gatius et al., 2002	873	13.1 (43-49d) 11.2 (57-63d)

Table 2. Reported stages of lactation with an increased incidence of cystic ovarian disease.

Literature cited	# Lactations	First Peak	Second Peak
Morrow et al., 1966	357	1st post-partum ovulation	Not observed
Bierschwal, 1966	1 436	Days 21- 60	Not observed
Whitmore et al., 1974	375	Days 16 to 45	Not observed
Erb and White, 1981	1 599	Days 31-60	Days 151- 180
Bartlett et al., 1986	2 847	Days 31-40	Days 190-220

Many investigations have found varying trends in incidence, given certain characteristics or risk factors. These risk factors may provide useful insight into the underlying etiology of cystic ovarian disease. Two main risk factors that have been investigated are the effects of milk production and lactation number or parity. The incidence of cystic follicles was less during the first lactation compared with the second and third lactations (Whitmore et al., 1974; Hooijer et al., 2001). There is also evidence that there is an increasing incidence of cystic follicles with increasing age (Bartlett et al., 1986). Milk production and lactation number have been negatively correlated with spontaneous early cyst recovery (Lopez-Gatius et al., 2002). Lactations with cystic follicles were associated with 422 kg more 305 ME-D milk production than lactations

without cystic follicles (Bartlett et al., 1986), and a 1 kg increase in milk yield resulted in a 1.05 increased risk for cysts (Lopez-Gatius et al., 2002). Another study found that cows with follicular cysts produced an average of 379 kg more milk in the first 90 days post partum, and 438 kg more by 305 days than their non-cystic herd-mates (Johnson et al., 1966).

Cows experiencing abortion, dystocia, twins, retained fetal membranes, metritis, ketosis or other debilitating disease had a significantly higher incidence of cystic follicles compared to cows not experiencing these conditions (Morrow et al., 1966). Another study found that cows with an abnormal puerperium had a 1.9 times higher risk for developing cystic follicles than normal cows (Lopez-Gatius et al., 2002). Cows which became lame during the first 30 days postpartum had a significantly higher incidence of ovarian cysts compared to non-lame controls (25.0% vs.11.1%, respectively; Melendez et al., 2003). There is evidence for a seasonal increase in the incidence of cysts in some areas. In one study, cows calving in the summer months were 2.6 times more likely to develop cysts than those calving in the winter (Lopez-Gatius et al., 2002). However, other authors found no significant effect of season (Melendez et al., 2003; Bartlett et al., 1986).

Etiology

A genetic susceptibility to cystic ovarian disease has been suggested and researched by a number of authors. In one case study, a closed 300 cow dairy farm using natural service found that two of their eleven bulls produced a significantly higher proportion of daughters with cystic ovaries than expected, suggesting a heritable basis (Kirk et al., 1982). In recent studies using computerized records, the estimated heritability of cystic

ovarian disease has been determined to be 0.087- 0.102 (Hooijer et al., 2001) and 0.05-0.08 (Zwald et al., 2004a).

In a study using producer recorded data, the estimated genetic correlations between cysts and ketosis was 0.42, cysts and lameness was 0.16 and between cysts and displaced abomasum was 0.17 (Zwald et al., 2004b). The incidence of cystic ovarian disease also has a positive correlation with milk production traits, especially to protein levels (Hooijer et al., 2001). Therefore, selection pressure for milk production traits may inadvertently increase the incidence of COD over time. In one example, it was estimated that a 500 kg increase in 305-day milk yield will increase the incidence of COD by 1.5% (Hooijer et al., 2001). The possibility of increased susceptibility due to breed was suggested in a study which found an increased incidence in Guernseys, 18% vs 11% in Holstein–Friesians (Bierschwal, 1966).

Peripartuient disease has been linked to ovarian cysts, and several studies have found a positive relationship between the incidence of metritis and ovarian cysts (Fleischer et al., 2001; Morrow et al., 1966; Lopez-Gatius et al., 2002). A possible explanation for the basis of metritis leading to ovarian cysts is through the release of endotoxin by pathogenic gram-negative bacteria present in the uterus. Evidence for the role of bacteria and endotoxin was revealed during a comparison of postpartum cattle in which those who later developed cysts had increased cortisol and PGF metabolites (PGFM) concentrations and higher intrauterine culture scores prior to detection of the cysts (Bosu and Peter, 1987). It has been shown that intrauterine infusion of endotoxin in heifers caused suppression of the LH surge, and resulted in the formation of follicular cysts which persisted for 7 to 21 days (Peter et al., 1989).

The two primary sites where endotoxin is thought to exert its effects are the hypothalamus and the ovary. In the ewe, intravenous endotoxin infusion interrupted rising estradiol levels and delayed or inhibited the preovulatory LH surge. The interruption of the rising estradiol levels was due to an effect on follicular development (Battaglia et al., 2000). Furthermore, the effects of endotoxin on the estradiol-induced LH surge only occurred if the animal was exposed to endotoxin during the first 14 hours of the estradiol signal, or during the estradiol 'reading' phase (Battaglia et al., 1999).

Endotoxin also acts at the hypothalamus to stimulate ACTH release which resulted in increased adrenal cortisol production (Moberg, 1971). The intrauterine infusion of endotoxin in the experiment by Peter et al. (1989) caused a significant increase in cortisol levels. When ewes were infused with endotoxin, those responding with a greater increase in cortisol and progesterone levels were also more likely to have an inhibited or delayed LH surge (Battaglia et al., 2000).

Cortisol is a well-known inhibitor of the GnRH induced LH release at the level of the pituitary. Dairy heifers treated with ACTH had a significantly depressed LH response when injected with LHRH (Matteri and Moberg, 1982). Heifers continually infused with ACTH had significantly decreased LH concentrations which remained decreased throughout the cycle, and consequently, did not have an LH surge (Li and Wagner, 1983a). When repeated acute stress was applied to cattle, a proportion of the stressed animals did not have an LH surge while all of the control animals had an LH surge. These stressed animals also experienced increased cortisol which decreased with subsequent stress periods (Stoebel and Moberg, 1982). In vitro studies with bovine pituitary cells showed that cortisol pretreatment depressed the LH response to GnRH (Li and Wagner,

1983b) and the cortisol concentrations typical of postpartum suckled cattle are sufficient to inhibit the GnRH induced LH surge (Padmanabhan et al., 1983).

Given this information, the relationship between metritis and cysts is most likely due to direct actions of endotoxin on the ovary and, indirectly, through the actions of cortisol induced by endotoxin. It is also through cortisol that cattle under stress may be more likely to develop ovarian cysts, making stress another possible etiology for the disease.

Fate and Endocrinology

Bovine ovarian cysts are not static structures since they can persist for variable periods of time, or regress and be replaced by another cyst or an ovulatory follicle (Cook et al., 1990; Hamilton et al., 1995; Wiltbank et al., 2002). These three responses can be simplified into persistence, turnover or spontaneous recovery. A study which marked cysts with charcoal to follow their development found that in no case the cysts ovulated (Cook et al., 1990). In this study, cysts either persisted for the duration of the study period (40 days) or regressed and were replaced by an upcoming follicle. The interval between the detection of a cyst and the next follicular wave was longer by a difference of 4-5 days, and more variable, in cystic cows than in normal cows (Hamilton et al., 1995).

The endocrinology of cystic cows has been compared to normal cyclic cows by many authors (Zaied et al., 1981; Hamilton et al., 1995; Cook et al., 1991; Silvia et al., 2002; Wiltbank et al., 2002). There is no difference in FSH concentrations or profile between cystic and normal cows (Hamilton et al., 1995; Cook et al., 1991), and an increase in FSH precedes each new follicular cyst wave (Hamilton et al., 1995). Cows with cystic follicles have higher basal LH concentrations during the follicular phase than normal cows, primarily due to an increased pulse frequency and amplitude (Hamilton et

al., 1995; Cook et al., 1991). These cows fail to have an LH surge at the expected time of ovulation which results in a continuously growing or persistent, anovulatory follicle (Hamilton et al., 1995).

When GnRH is exogenously administered to cystic cows, the pituitary consistently responds with an LH surge, resulting in luteinization of the cyst and/or any other follicle present on the ovary with the ability to respond to an LH surge (Kittok et al., 1973; Cantley et al., 1975). In most cases, after luteinization occurs and progesterone levels rise, LH profiles return to normal and cyclicity resumes. Many cystic cows lack a GnRH/LH surge in response to elevated endogenous estradiol (Hamilton et al., 1995), or exogenously administered estradiol (Dobson and Alam, 1987; Refsal et al., 1988; Gumen et al., 2002; Gumen and Wiltbank, 2002). A comparison of the pituitary and hypothalamic concentrations of GnRH found that GnRH was lower in the combined preoptic area and hypothalamus proper in cystic cows (Cook et al., 1991). In the same study, anterior pituitary concentrations of LH, FSH, and receptors for GnRH did not differ between normal and cystic cows suggesting that the function of the hypothalamus may be altered in cystic cows.

Cows with cysts have variable serum and follicular fluid levels of progesterone and estradiol (Cook et al., 1990; Hamilton et al., 1995). These levels depend on the steroidogenic capacity and cell types present as well as the proportion of luteinization in the cyst (Cook et al., 1990). In general, estradiol concentrations were not different between cows with and without cysts (Hamilton et al., 1995; Cook, 1991). When estradiol levels were evaluated based on follicular stage, at the point where the follicle/cyst had reached ovulatory size, those which developed into follicular cysts had greater

estradiol concentrations than follicles which ovulated (Yoshioka et al., 1996; Hamilton et al., 1995). In ewes with induced persistent follicles, gonadotropin support in the form of LH pulses was required for follicular estrogen production for 10 days, at which point the follicle regressed and the ability to produce androstenedione and estradiol decreased despite continued LH pulses (Dobson et al., 1997). This may explain why cows with follicular cysts may initially have elevated estrogen levels, which then decreases despite high mean LH concentration.

Because they have no corpus luteum, cystic cows have lower progesterone concentrations than normal cyclic cows. Cystic cows frequently have an intermediate level of progesterone, defined as 0.1-1.0 ng/ml (Hamilton et al., 1995; Yoshioka et al., 1996; Cook et al., 1991). In one study, 66% of cows diagnosed with follicular cysts had intermediate levels of progesterone (Silvia et al., 2002). When monitoring cows with intermediate levels of progesterone, only 10% of developing follicles ovulated, and 66% resulted in cyst formation (Halter et al., 2003; Silvia et al., 2002). Meanwhile, cystic cows with low or high progesterone were more likely to ovulate or undergo follicular atresia than cows with intermediate progesterone (Halter et al., 2003).

Pathogenesis

The underlying pathogenesis involved in formation of follicular cysts has not been definitively ascertained. The prevailing hypothesis involves a lesion at the hypothalamus, in the area responsible for converting the positive feedback of estrogen into a GnRH surge and subsequent LH surge from the anterior pituitary.

In the normal sequence of events, rising levels of estradiol during the follicular phase, aided by declining levels of progesterone, result in the hypothalamic GnRH surge. Administration of exogenous estradiol to cows with naturally occurring cysts has resulted

in a variety of responses. In general, the normal and expected response of a timely estradiol induced GnRH and LH surge has been disrupted in cows with cysts with evidence for it being delayed or absent. One study recorded that cows with follicular cysts had a delayed GnRH/ LH response to exogenously administered estradiol benzoate by approximately 8 hours (Zaied et al., 1981). In contrast to the majority of studies, chronically cystic cows which were later ovariectomized responded to estrogen challenge 6 weeks after ovariectomy with higher peak LH levels than normal cows (De Silva and Reeves, 1988). The robust LH response in these cows may have been due to the time elapsed and/or the ovariectomy which may have allowed time for recovery of hypothalamic function. In another study of naturally occurring cysts, 47% of cows with luteal cysts (milk P4 >10 ng/ml) and 48% of cows with follicular cysts had a normal response to estradiol benzoate after treatment with cloprostenol (Nanda et al., 1991). The interesting aspect of this study was the similar response in cows diagnosed with both types of cysts, lending evidence to the hypothesis that both luteal and follicular cysts share the same underlying pathophysiology. Another study using 6 cows with low steroid concentrations and 6 under estrogenic influence found that administration of exogenous estradiol resulted in no LH response in 11/12 cows (Refsal et al., 1988).

Cystic cows typically have increased basal serum LH concentrations (Cook et al., 1991; Hamilton et al., 1995). Elevated LH levels are important for the maintenance of the cysts, but may or may not be involved in their development. In order to determine if elevated LH concentration can cause cysts, Hampton et al. (2003) administered high, frequent doses of LH to cows on Day 1 after emergence of a follicular wave and measured follicular response. Cows receiving exogenous LH were not at an increased

risk of developing cysts compared to saline infused cows. These cows still had an LH surge and ovulated, despite low progesterone and elevated LH levels. This study confirmed that although elevated basal LH levels may be involved in the maintenance of cysts, it is not likely a factor in the pathogenesis.

The potential role of steroidogenic enzymes, FSH and LH receptors has been examined for their role in the pathogenesis of cysts. The granulosa cells of chronic cysts have increased mRNA expression of LH receptors and 3β -hydroxysteroid dehydrogenase (3β -HSD) compared to normal dominant follicles (Calder et al., 2001). These findings were speculated to be secondary to the hypothalamic-pituitary dysfunction causing low progesterone and increased LH concentrations typically of cysts and not a direct cause of cyst development. A similar study comparing 3β HSD expression in granulosa and theca cells of normal, atretic and cystic follicles found that cysts had higher enzyme levels in granulosa cells and lower levels in theca cells compared to the normal follicles (Isobe et al., 2003). Compared to atretic follicles, cysts had much lower 3β HSD expression in theca cells which was speculated to be a critical enzymatic change necessary for atresia. This difference may be the cause of cyst persistence and altered steroid production (Isobe et al., 2003).

The lack of the positive feedback of estradiol on LH release can lead to ovarian cysts. This was shown in a study immunizing cattle against estradiol then monitoring LH levels and follicular development (Kaneko et al., 2002). All of the cattle immunized against estradiol developed follicular cysts. They had a mean LH concentration higher than non-immunized cows, and did not have a preovulatory LH surge.

Similar endocrinological states and ovarian pathologies to cows with cystic ovarian disease have been induced in genetically modified mice with specific estrogen and progesterone receptor genes knocked out. Estrogen receptor alpha knock out (α ERKO) mice typically have hemorrhagic polycystic ovaries, elevated LH concentrations due to the lack of α ER/ estrogen mediated negative feedback on the hypothalamic-pituitary axis, and an amplified steroidogenic pathway similar to the follicular stage (Couse et al., 2004). The α ER is the primary estrogen receptor present in the hypothalamus while the β ER is the primary estrogen receptor present in the ovary and uterus (Couse and Korach, 1999). Estrogen receptor beta knock out (β ERKO) mice do not have hemorrhagic cystic ovaries, even in the presence of abnormally high pulsatile LH. During periods of high pulsatile LH, β ERKO mice have a steroidogenic profile similar to the luteal phase (Couse et al., 2004). These results indicate that α ER is needed for the negative feedback of estradiol on the hypothalamus and that the β ER is needed for the phenotypical manifestation of cystic ovaries.

Progesterone receptor knock out (PRKO) mice have a similar endocrinological and physical profile to the α ERKO mice, with high basal LH levels and the lack of a preovulatory LH surge (Chappell et al., 1997). Although, one difference is that follicles of the PRKO mice grow to ovulatory size, but not beyond as in the α ERKO mice. PRKO mice lack the ability to produce an estrogen induced LH surge (Chappell et al., 1999). This indicates that the progesterone receptor (PR) is required for transmission of the estrogen-induced signals leading to the LH surge. In rats, a PR antagonist effectively blocked the estrogen-induced GnRH surge (Chappell and Levine, 2000). In the ewe,

progesterone priming increased the amplitude of the GnRH surge in response to estradiol (Caraty and Skinner, 1999)

Localization of the specific area involved in transmitting the estradiol signal determined that the PRs located in the anteroventral periventricular nucleus are critical to transmission of the signal in the rat (Chappell and Levine, 2000). Progesterone receptors are up-regulated by estrogen (Chabert-Buffet et al, 2000). This was shown in the rat when estrogen induced PR expression within 2 hours in a dose-dependent manner (Shughrue et al., 1997). The progesterone receptors induced by estrogen may even be transactivated in the absence of ligand (Levine, 1997). In this way, PR expression is induced by estrogen and then used as a transcriptional regulator in transmission of the estrogen signal to the GnRH releasing cells in the hypothalamus. The ligand independent activation of the PR likely mediates the neurosecretory estrogen feedback signals that result in an increased pituitary response to GnRH (Levine, 1997).

Other aspects of uncovering the details of the estradiol induced LH surge involved determining the specific location of these receptors in the hypothalamus in various species and the factors controlling their expression. Early studies in the rat localized ER mRNA expression in the arcuate nucleus and the ventrolateral portion of the ventromedial hypothalamus as areas under hormonal regulation (Simerly and Young, 1991). In this study, administration of estradiol to female rats for 24 hours caused a 40% down-regulation of ER mRNA with slightly more of a decrease occurring in the arcuate nucleus than in the ventromedial hypothalamus. In the ewe, ER staining localized expression to the preoptico-hypothalamic continuum with areas of greatest density at the arcuate nucleus and the ventromedial hypothalamus (Blache et al., 1994).

Hormonal regulation of ER expression was examined through artificially-induced stages of the estrous cycle which demonstrated that, similar to the rat, estrogen caused down-regulation of ER expression, but also that mid-luteal phase or elevated progesterone concentrations caused an increase in ER expression in the ventromedial hypothalamus (Blache et al., 1994). In this study, ER levels did not change significantly in the arcuate nucleus with various hormonal treatments.

Since it is known that GnRH neurons contain few or no estrogen receptors, estrogen must activate ER-containing interneurons to transmit the signal to GnRH containing neurons (Caraty et al., 1998; Peterson et al., 2003). In the ewe, the hypothalamic areas involved in estrogen feedback regulation on the LH pulse were determined in a study using estradiol microimplants (Caraty et al., 1998). These authors found that estrogen exerted strong negative feedback effect on the medial preoptic area and caudal medial basal hypothalamus while it exerted a strong positive effect in the ventromedial nucleus. Collectively, these results lend evidence to the hypothesis that estrogen transmits its signal to the GnRH containing neurons through ER-containing interneurons located in the ventromedial nucleus and possibly through progesterone receptors expressed in the medial preoptic area and anterior ventral periventricular nucleus. Estrogen is responsible for the down-regulation of its own receptor while progesterone is involved in up-regulating ER expression.

It is postulated that dairy cattle with ovarian cysts have a compromised expression of ER α in the hypothalamus and are unable to produce an LH surge in response to estrogen. Although ER levels in the hypothalamus of cows with cysts have not been directly measured, there has been research on receptor expression in the anterior pituitary

and ovary. The abnormality identified between the ER and PR in the ovary and the ER and PR in the pituitary of cystic cows is that they are not positively correlated, as would be expected in normal cows (Odore et al., 1999). Cows with cysts had similar expression of ER in the pituitary when compared to cows with normal dominant follicles, but the ovarian ER levels were much lower than in cows with normal dominant follicle and similar to levels expected during the luteal phase. In general, pituitary and ovarian receptor concentrations are correlated with each other and match with estrogen concentrations. Therefore, when estrogen levels are high, ovarian and pituitary receptor levels will also be elevated. Although cows with cysts had higher estrogen levels than normal cows, they had decreased expression of ER in the ovary (Odore et al., 1999). In a study examining the ER and PR expression in anestrus ewes, GnRH administration with and without progesterone caused an increase in pituitary ER and PR levels (Tasende et al., 2002). They also found that progesterone treatment decreased ER and PR levels in the uterus and that, in general, the receptor expression at the pituitary and uterus are correlated.

Treatment with progesterone has been shown to correct the hypothalamic lesion that causes cows and ewes with cysts to be unable to produce an LH surge in response to estrogen. This likely occurs through the progesterone mediated up-regulation of ER α expression in the ventromedial hypothalamus, and possibly through activation of PR containing interneurons involved in the transmission of the estrogen signal to GnRH neurons. Support for this hypothesis is evident in studies where cows with cysts were unresponsive to estrogen treatment until exposure to progesterone occurred for a period of 7 days (Gumen and Wiltbank, 2002; Nanda et al., 1991). The same response was seen

in ewes in which cysts were induced using constant estrogen administration (Ozturk et al., 1998). Following this period, progesterone administration resulted in a normal response to subsequent estradiol challenge.

According to the literature on mice, rats and ewes, abnormally high or abnormally low levels of estrogen preceding the expected time of ovulation could lead to the down-regulation of its own receptor, as well as possibly the progesterone receptor, which are both involved in the transmission of the estrogen signal. A GnRH/ LH surge in the absence of an ovulatory follicle and without subsequent progesterone exposure will result in the development of a follicular cyst (Gumen et al., 2002). This is likely because the hypothalamus requires progesterone exposure to up-regulate the ER and reinitiate the next signal. Any condition which may disrupt the timely GnRH/ LH surge could potentially lead to the development of ovarian cysts.

Research examining the endocrinology of subfertile cattle has led to the hypothesis that these cattle, which are subfertile for various reasons, may share a similar lesion to cows with spontaneously occurring cysts. Early investigations comparing cows with cysts, lame cows, and thin, non-cyclic cows revealed that all three groups had the same lack of an LH response to exogenously administered estradiol (Dobson and Alam, 1987). As an explanation for the thin, non-cyclic cows, estradiol may have an especially strong negative feedback effect on the hypothalamus. It is postulated that in thin, postpartum cows experiencing negative energy balance, the hypothalamus is extremely sensitive to the inhibitory effects of estradiol causing decreased LH secretion (Wiltbank et al., 2002). Cattle in a negative energy balance will, therefore, have follicular growth to the point of deviation, but as the follicle produces more estradiol, the hypothalamus

responds by decreasing GnRH and LH levels which causes the follicle to be unable to continue growth beyond this point.

Lame cattle lacked an LH surge in response to exogenously administered estradiol (Dobson and Alam, 1987). In another study, lame cattle were more likely to develop follicular cysts than non-lame cohorts (Melendez et al, 2003). A possible explanation for the underlying mechanism is through stress causing a disruption in the GnRH/ LH surge.

The role of stress in the pathogenesis of ovarian cysts has been partially elucidated through studies examining the endocrinology of cows with ACTH-induced cysts. The hormonal profile in cows with cysts induced by estradiol compared to ACTH is different yet their response to subsequent estradiol administration is similar. Estradiol induced cysts occurred as a result of an estradiol induced LH surge in the absence of a preovulatory size follicle and prior to complete luteal regression, while ACTH induced cysts were the result of the absence of an LH surge (Refsal et al., 1987). Similar to spontaneously occurring and estradiol-induced cysts, cows with ACTH-induced follicular cysts did not have an LH surge in response to exogenously administered estradiol (Ribadu et al., 1999). This suggested that cows with ACTH-induced cysts share a similar hypothalamo-pituitary lesion to cows with spontaneous ovarian cysts.

A proposed mechanism for stress/ ACTH induction of cysts involves suppression of the LH/FSH surge and decreased follicular steroidogenesis. This is through the inhibitory effects of increased cortisol and progesterone production by the adrenals. Progesterone can block the GnRH/ LH surge at the hypothalamus while cortisol suppresses estradiol secretion and LH receptor content in granulosa cells (Kawate, 2004). The combination of these effects cause the follicle to produce an ineffective amount of

estrogen for a GnRH/LH surge, compounded by suppression of the GnRH surge at the hypothalamus, resulting in continued follicular growth and cyst formation.

The source of elevated ACTH is another area under investigation for its role in the pathogenesis of cysts. One hypothesis involves a lack of cortisol-mediated negative feedback causing increased ACTH levels. Decreased circulating cortisol may be caused by the dysfunction of enzymes responsible for oxidizing cortisol to cortisone. Thurston et al., (2003) found that cysts contained more inhibitors of the Type 1 11β HSD, a ketosteroid reductase enzyme involved in conversion of cortisone to cortisol, compared to follicular fluid from large antral follicles. These inhibitors could potentially also effect enzyme activity in the liver and other locations to cause an overall decrease in circulating cortisol, therefore altering negative feedback of ACTH (Thurston et al., 2003).

Another possible role of stress in the pathogenesis of cysts was examined in a study performing immunohistochemistry on pituitary cells of normal cyclic cows and cows with cysts (Busato et al., 1995). There was a deficiency in cell number and staining intensity of gonadotropes immunoreactive for LH and evidence of hyperactivity for corticotropes in cystic cows. It is, therefore, possible that the increase in ACTH secretion and the subnormal activity of LH cells may be secondary to the activation of the ACTH cells, although it is possible that the same mechanism may alter both cell populations.

Treatment

Ovarian cysts which occur prior to the first postpartum ovulation are more likely to undergo spontaneous recovery, with a resolution rate of 50- 60%, than those occurring after the 1st postpartum ovulation with a resolution rate of 20% (Youngquist, 1986; Peter, 1997). Morrow et al., (1966) reported a spontaneous recovery rate of 48% and a higher recovery rate among cysts diagnosed prior to the first postpartum ovulations than those

developing after the first postpartum ovulation, although this was not statistically significant. In a study following 42 cows with cysts identified in the first 90 days post partum, 12 spontaneously recovered within 30 days of diagnosis, another 12 recovered between 31-90 days, 5 recovered between 91-168 and 13 had not recovered by 300 days (Whitmore et al., 1974). The overall recovery rate by 300 days in this study was 69%. Another study reported the mean cyst duration following detection to be 31.0 ± 4.3 days (Carroll et al., 1990). Of those cows undergoing spontaneous recovery, approximately 35-45% will have a repeat incidence (Peter, 1997).

Manual rupture of the cyst has been recorded with various recovery rates. This treatment can cause the undesirable effects of hemorrhage and adhesions on the ovary and ovarian bursa (Roberts, 1971; Younquist, 1986). Manual rupture did not restore hypothalamic responsiveness to subsequent estradiol administration in all of nine cows while pretreatment with progesterone restored responsiveness in all of seven cows (Nanda et al., 1991). This indicates that although the cyst may not be physically present on the ovary, as in the case of manual rupture, a lesion still persists at the hypothalamus.

Hormonal treatments for ovarian cysts have included human chorionic gonadotropin (hCG), gonadotropin releasing hormone (GnRH), progesterone and prostaglandin. Hormonal treatment is preferred over the uncertainty of spontaneous recovery and the damage caused by manual rupture. The general purpose of GnRH treatment is to induce an LH surge, causing further luteinization of the cyst or ovulation of a responsive growing follicle within a developing wave. Luteinization or the presence of a CL will increase circulating progesterone concentrations, decrease basal LH concentrations and potentially restore hypothalamic responsiveness to estradiol and

normal cyclicity. Treatment with hCG simulates the LH surge and acts directly on the ovary to induce further luteinization of the cyst. Administration of 10,000 units of hCG to 21 cows resulted in a 85.7% (18/21) recovery rate with cows returning to estrus within mean interval of 20.5 days (Morrow et al., 1966). Despite the effectiveness of hCG as a treatment, and due to its large molecular size and immune interactions, it has the negative side-effects of potential severe reactions and a loss of effectiveness over time. It is also a more expensive and less stable compound than GnRH (Archbald and Thatcher, 1992).

Administration of GnRH to cystic cows results in return to cyclicity in approximately 75-80 % of cases (Archbald and Thatcher, 1992; Farin and Estill, 1993). The first study using GnRH to treat cysts involved 5 cows treated with 100ug of GnRH IV, three times 120 minutes apart and found that all cows responded with an increase in LH (Kittok et al., 1973). The greatest increment in LH levels occurred after the 2nd injection and estrus was observed in all cows within 20-24 days. Biershwal et al., (1975) evaluated the effectiveness of a single IM injection of GnRH at doses of 0, 50, 100 and 250ug. They found that 50, 100 and 250ug doses were statistically equal although there was a slightly better response to the 100ug dose and all were better than 0ug (Bierschwal et al, 1975). In a companion paper, of 18 cows treated with 50-250ug of GnRH, 72% were observed in estrus within 20 ± 1.5 days. Cows responding positively had an increase in LH and progesterone on day 0, and increased estrogen levels from days 1-13 as well as an increase in firmness of the cyst with a decrease in ovarian size (Cantley et al., 1975). Whitmore et al., (1979) observed a 76% recovery rate in 225 cows treated with 100ug GnRH, with estrus occurring in 15-30 days. They also examined the effect of repeated

injections and found no decrease in effectiveness with subsequent injections at 2 to 4 week intervals as may be expected with hCG treatment.

A study examining the preventative use of GnRH for ovarian cysts compared the frequency of cysts and reasons for culling in a group of 204 cows treated with GnRH or saline at 14 days post partum (Britt et al., 1977). These authors found that fewer GnRH treated cows developed cysts and were culled for infertility reasons compared to saline treated cows.

Although GnRH was determined to be an effective treatment of ovarian cysts, the interval from treatment to subsequent estrus detection was approximately 15-30 days. Induction of luteolysis using prostaglandin F₂ α (PGF₂ α) could potentially shorten the time to estrus after GnRH caused luteinization of the cyst or another follicle. Kesler et al. (1978) compared the treatments of GnRH only, PGF₂ α only and GnRH followed by PGF₂ α in 9 days in cystic cows. The treatment of GnRH followed by PGF₂ α in 9 days resulted in the most consistent return to estrus while PGF₂ α only resulted in inconsistent and variable intervals of return to estrus (Kesler et al., 1978). Another study comparing GnRH followed by PGF₂ α in 14 days to GnRH alone and insemination at observed estrus in both groups found no difference in the conception and pregnancy rates of the two groups (Archbald et al., 1991). Since the success of treatment in this study depended on expression of estrus, an important and limiting factor to the treatment success, when defined as conception or pregnancy, is the estrus detection rate.

The use of protocols using GnRH and PGF₂ α to synchronize ovulation, allowing for timed insemination without the need for detection of estrus, have proven to be effective in lactating dairy cows (Pursley et al., 1995; Momcilovic et al., 1998). The

effectiveness of such protocols in dairy cows with cysts has been evaluated and compared to other traditional methods. Normal cows subjected to a protocol using 100ug of GnRH on day 0, 25 mg PGF2 α on day 7, 100ug GnRH on day 9 and timed insemination 16 hours later (Ovsynch) have typically achieved conception rates of approximately 31-32% (Bartolome et al., 2000; Gumen et al., 2003). It was speculated that this protocol would work well in cystic cows as well as eliminate the need for estrus detection which has been an inhibitory factor in the success of some treatments. Bartolome et al. (2000) compared the Ovsynch protocol to GnRH on day 0 followed by PGF2 α on day 7 and insemination at detected estrus as treatments for cystic ovarian disease. Although the conception rate of cows in the Ovsynch protocol was significantly lower than those inseminated at detected estrus (23.6% v. 51.7%), the overall pregnancy rate was not different between the two groups (23.6% vs. 18%). This study also found a significantly lower pregnancy rate among all the cystic cows compared to normal cows.

In a similar study comparing two protocols relying on insemination at detected estrus, one group received both GnRH and cloprosternol on day 14 and were observed for estrus while a second group received GnRH and cloprostenol on day 0, followed by cloprostenol on day 14 and then were observed for estrus (Lopez-Gatuis and Lopez-Bejar, 2002). These authors found the second group receiving the GnRH and PGF2 α on day 0 and cloprostenol on day 14 had a lower cyst persistence rate, better estrus detection rate and a higher ovulation rate. In part two of the same study, a protocol using Ovsynch with an additional cloprostenol injection on day 0 along with the GnRH on day 0 was compared to the standard Ovsynch protocol. The best results were obtained from the protocol using cloprostenol and GnRH at the same time on day 0 for ovulation rate,

pregnancy rate and less cyst persistence. The major benefit of this protocol was that it allowed for detection of estrus in the week after the first cloprostenol injection and prior to the timed insemination.

When Ovsynch and estrus detection were compared for a 21-day period in both ovular and anovular cows with varying follicular sizes, there was no significant difference in the conception rate for the two protocols (Gumen et al., 2003). Ovular cows had a significantly better conception rate than anovular cows (32 vs 9%). Anovular cows in the estrus detection group had a 42% spontaneous recovery rate. Although not all the anovular cows in this study were cystic, approximately 20% of those cows would be considered cystic by standard definitions and a further 58% had follicles greater than 15 mm persisting for two weeks prior to diagnosis.

The regular use of bST (bovine somatotropin) to increase milk production in dairy cows throughout their lactation has led to investigations on its reproductive effects. In normal cows, bST resulted in a significantly lower conception rate when administered 4-6 days prior to initiation of the Ovsynch protocol but not if administered 1-3 days prior to Ovsynch (Bartolome et al., 2002). In that study, the interval from the bST injection to initiation of the Ovsynch protocol had no effect on conception rate in cystic cows. In another study, pretreatment of cystic cows with bST and GnRH, GnRH, bST or no treatment prior to Ovsynch found the best conception rate in the group receiving no pretreatment (Bartolome et al., 2003). The group receiving bST only had a significantly lower conception rate than the group receiving no pretreatment.

Pretreatment with GnRH is thought to increase the chance of the presence of luteal tissue or a CL at the time of the PGF 2α injection and to synchronize follicular wave

growth. A comparison of Ovsynch and a GnRH + Ovsynch protocol in cystic cows resulted in a significantly higher conception rate for the GnRH + Ovsynch group over the Ovsynch group (30.0% vs. 20.2%, respectively; Bartolome et al., 2005a). This study also found there was a significant interaction between type of cyst and treatment, where cows with a diagnosis of follicular cyst had a better pregnancy rate in the GnRH+ Ovsynch protocol over the Ovsynch protocol. There was no significant difference in pregnancy rate for cows with luteal cysts for either protocol.

Progesterone as a treatment for cystic ovarian disease has been used both for its ability to lower LH concentrations and to reset the hypothalamus' responsiveness to the positive feedback effects of estrogen. A shift in serum progesterone from low to high (0.5-5.0 ng/ml) concentrations caused LH pulse frequency and amplitude as well as estradiol concentration to decrease significantly within 6 hours (Bergfeld et al., 1996). In cows treated with estradiol and progesterone together, the suppressive effect on LH was greater than with either steroid alone (Stumpf et al., 1993). A significant regression in the size of the cyst occurred following treatment with progesterone when compared to cystic cows with no treatment during the same time period (Todorki et al., 2001; Calder et al., 1999).

Some evidence for the role of progesterone in restoring hypothalamic responsiveness to estradiol came from a study by Nanda et al. (1991). These authors examined the results of estradiol treatment in cystic cows before and after administration of progesterone and found that only a proportion responded to estradiol given before progesterone whereas all responded with a GnRH/ LH surge after progesterone exposure for 7 days (Nanda et al. 1991). Similar results were found in a study in which ewes were

exposed to high levels of estradiol for 12 days and became unresponsive to further estradiol injections (Ozturk et al., 1998). In this study, administration of progesterone restored the ability of the hypothalamus to respond to estradiol. In two studies by Gumen et al. (2002), a large follicle anovulatory condition similar to cysts was induced in cows, and in every case these cows were not responsive to exogenous estradiol until treated with progesterone. Treatment with GnRH continued to produce an LH surge in all cows but estradiol did not, unless the cow had received progesterone (Gumen and Wiltbank, 2002; Gumen et al., 2002). Gumen and Wiltbank (2005) performed two further experiments to define the effect of CIDR inserts on progesterone levels and the length of exposure needed to restore hypothalamic responsiveness to estradiol. In the first experiment, cows with induced cysts were treated with a CIDR (1.9g progesterone) for 0, 1, 3 and 7 days. The results indicated that the CIDR caused serum progesterone to reach a steady state concentration of 1.3ng/ml within 3 hours, and the minimum exposure time required to restore hypothalamic responsiveness to estradiol was 3 days. In the second experiment using anovulatory cows, some of which would be considered cystic, treatment with a CIDR (1.9g progesterone) for 0, 1 or 3 days indicated the best response was achieved in cows treated for 3 days. All of the cows treated for 3 days ovulated within 1 week after CIDR removal.

The efficacy of progesterone as a treatment for spontaneous cysts has been studied alone or in combination with GnRH and/or PGF2 α . In a trial of cystic cows treated with 2 progesterone-releasing intravaginal devices (PRID, 1.55g progesterone each; total of 3.10g) for 9 days, treatment resulted in decreased circulating LH within 1 day and induced a new follicular wave (Calder et al., 1999). All PRID cows ovulated within 3 to 4

days after PRID removal. This treatment also resulted in a significant decrease in cyst size compared to untreated controls. Another study used beef donor cows with ovarian cysts and treated them with a CIDR (1.9g progesterone) for 14 days (Todorki et al., 2001). During the CIDR treatment, 2 or 3 follicular waves emerged and regressed, with an average of 7 days between each waves. The ovulatory follicle had an average period of 7.6 days from emergence to ovulation. Serum progesterone levels increased from 0.6ng/ml on day 0 to 2.6ng/ml by day 1. Serum progesterone then decreased from 2.6ng/ml on day 1 to 1.3ng/ml on day 14. All the CIDR-treated cows ovulated after CIDR removal and continued to display normal estrous cycles for the next two months, while control cows did not. The fact that normal estrous cycles continued for a period of two months is a good indication that the underlying hypothalamic defect was corrected by the treatment. In a recent study, 8 cows with cysts, 3 of which also had a CL, were treated with a CIDR for 9 days, and also given GnRH on day 0 and PGF2 α on day 7 to synchronize follicular wave development. Of these cows, 7 out of 8 ovulated following CIDR removal (Ambrose et al., 2004). Collectively, these studies provide evidence that progesterone should be an equivalent or better treatment than GnRH alone or the Ovsynch protocol for cows with cystic ovarian disease.

Economics of Cystic Ovarian Disease

Cystic ovarian disease represents a considerable economic concern for dairy managers. The high lactational incidence rate of 7-25% makes this disease a major cause of reproductive insufficiency in dairy cows (Peter, 1997). The rate of spontaneous recovery is low and variable, ranging from 20% (Youngquist, 1986; Peter, 1997) to 48% (Morrow et al., 1966) and with an average duration of 31.0 ± 4.3 days (Carroll et al., 1990). The time from diagnosis to spontaneous recovery is unpredictable with

approximately 58% recovering within 90 days and 30% still unresolved by 300 days (Whitmore et al., 1974). Among cows undergoing spontaneous recovery, approximately 35-45% will have a repeat incidence (Peter, 1997). Even with treatment, cystic lactations have a longer interval from calving to conception when compared to non cystic herd-mates (+33.5 days- Bartlett et al., 1986; +64 days- Bosberry and Dobson, 1989). Therefore it is generally more economical to treat the condition whenever it is identified.

Furthermore, cows with ovarian cysts have an increased likelihood to be culled. The percentage of cystic cows culled during the affected lactation, or the disease specific culling risk, was 20.9 % (Gröhn et al., 1998). In a slaughter house survey, 85% of cows culled for infertility had cysts (Kubar and Jalakas, 2002). In another study, cows with cystic lactations had a 1.23 relative risk for being culled than cows with non cystic lactations and the average cost attributable to culling alone was \$43/ case of cystic ovarian disease (Bartlett et al., 1986).

Previous reports analyzing the economic aspects of cystic ovarian disease have focused on the total economic impact of the condition per lactation (Bartlett et al, 1986) or used the technique of decision tree analysis to determine whether it is beneficial to treat cystic ovarian disease or wait for spontaneous recovery (White and Erb, 1980) and at what day postpartum should cows be screened for the condition (White and Erb, 1982). In the study by Bartlett et al. (1986), costs considered for the economic analysis included the average additional non-pregnant days, additional cows culled, additional semen costs, additional reproductive exams, drugs and labor costs. Estimates for the cost of semen, labor, drugs, reproductive exams, replacement and salvage costs were obtained from a producer survey. The cost for an additional day open was determined using estimates

from the literature. The incidence rate, culling risk and average non-pregnant days for cows with cystic ovarian disease were obtained from available herd data. The total costs were added together to obtain the average costs associated with cystic ovarian disease and the result was \$137/ cystic lactation. Although this study provides a rough estimate of the total cost associated with cystic ovarian disease, it does not take into account the future loss in production in subsequent lactations or other probabilities, such as pregnancy loss and involuntary culling. It also does not consider that these cows would be subject to the herd culling policy and culled once they met any of the culling criteria. It is also likely that conditions on a modern dairy farm are very different from the farms used in that study. Based on this analysis, it is obvious there is a considerable economic impact of cystic ovarian disease. Therefore, future emphasis should focus on optimizing treatment decisions from both a clinical and economical aspect, since the condition can not be prevented.

One method of determining the economic impact of a treatment choice is the decision tree technique (White and Erb, 1980). This method considers the chances of different outcomes due to a decision and multiplies the probability of an outcome by the total cost associated with it. These are summed for all the possible outcomes based on a decision and provide an estimate for the cost of that decision. When there are two possible decisions, the decision with the least associated costs is chosen and the other is excluded. Each possible decision is analyzed in this way progressing from the future to the present. Finally a value can be applied to decision choices in the present that represents all the future probabilities and costs based on that decision.

In the early 1980s, two studies were published using the technique of decision tree analysis for decisions involving cystic ovarian disease. The objective of the 1st study was to determine which day postpartum it would be cheaper to wait for spontaneous recovery than to treat cows with cystic ovarian disease (White and Erb, 1980). The 2nd study was a follow up to the first and determined the ideal day postpartum to screen all cows for cystic ovarian disease (White and Erb, 1982). Using this technique, it was determined that it was always more advantageous to treat cows for cystic ovarian disease than to wait for spontaneous recovery (White and Erb, 1980). The only costs considered in this analysis were the additional days open, the additional reproductive exams and drug costs. In the follow up study, it was determined that the ideal day postpartum to screen cows for cystic ovarian disease was at day 45 (White and Erb, 1982). These studies did not consider many factors in their analysis but utilized a sound approach for estimating the costs associated with a decision based on future probabilities.

Computer models using dynamic programming to simulate dairy herds over time provide a realistic method of determining the future profit of a cow (de Vries, 2004). This type of economic analysis takes into consideration more factors and follows cows for a longer period of time, or for as long as a profit is affected, and considers the realistic probability that pregnancy may be lost at any time and the risk of involuntary culling. Dairy herd simulation models can be used to measure the economic impact of decisions or events such as changes in heifer replacement policy (de Vries, 2004), conception rate and estrus detection efficiency (de Vries and Conlin, 2004). There have not been any reports comparing the economics of two different treatment protocols for cystic ovarian disease using conditions representative of modern dairy farms. An economical analysis

using this type of dairy herd simulation model may prove to be an effective method for the comparison of two potential treatment choices for cows with cystic ovarian disease.

Timed Insemination Programs in the Dairy Cow

Timed insemination programs eliminate the need for estrus detection, decrease the number of days open beyond the voluntary waiting period and decrease the variation in monthly pregnancy rates. Heat detection rate, or estrus detection efficiency, can be defined as the number of cows observed in estrus divided by the number of estruses which should have occurred in a 21 day period (Heersche and Nebel, 1994). Heat detection rate has a greater impact on the number of days to 1st service, number of days open, and the length of the calving interval than either the conception rate or reproductive culling policy (Rounsaville et al., 1979). Furthermore, an increase in the heat detection rate from 35%-55% decreases the average days to 1st service by 1 day (Rounsaville et al., 1979). The economic significance of a day open is that for each additional day open beyond the voluntary waiting period the total milk production is decreased by 2.4kg of milk and 0.112kg of fat (Louca and Legates, 1967). In another study, each day open beyond the voluntary waiting period decreased the annual milk production by 4.5 kg in 1st lactation cows and by 8.6 kg in 2nd+ lactation cows (Olds et al., 1979). Furthermore, each additional day open between 40 and 140 DIM decreased the income over feed costs by \$0.71 for 1st lactation cows and by \$1.18 for 2nd+ lactation cows.

Estrus detection is an even greater challenge in large dairies where labor may be limited and other factors limit estrus behavior expressed by the cows. An average cow has an estrus period of 5.8 hours in which she displays an average of 6.7 mounts lasting for 3.2 seconds each (At-Taras and Spahr, 2001). This indicates that there is an average of 22 seconds over a 5.8 hour period where the cow could be observed in estrous. In hot

weather, total duration in which estrus behavior is displayed decreases to an average of 2.9 hours (At-Taras and Spahr, 2001). The use of bST also tends to decrease estrus detection rates, particularly in multiparous cows (Santos et al., 2004b). When cows were observed for 30 minutes twice a day, greater than 50% of ovulating cows did not display mounting behavior and instead displayed other secondary signs of estrus such as chin resting, restlessness or mucous vulvar discharge (Van Eerdenburg et al., 2002). These cows which showed secondary behavioral signs of estrus were more likely to ovulate greater than 24 hours from the time first observed. Estrus detection accuracy, or the proportion of cows observed in estrus that are truly in estrus, is another potential source of error and cause of decreased conception rates. The error rate for estrus detection is estimated to be greater than 20% in approximately 30% of dairy herds and a significant cause of decreased conception in these herds (Nebel et al., 1987).

Programs using Prostaglandin F2 Alpha

In 1972, Rowson et al. determined that intrauterine administration of $\text{PGF}_{2\alpha}$ into the horn ipsilateral the CL between days 5 and 16 of the estrous cycle resulted in estrus by the 3rd morning following administration. These authors also determined that the same treatment between days 1 and 4 of the estrous cycle was ineffective. This study opened the door to the use of prostaglandin as a potential tool for controlling the estrous cycle of dairy cattle. It would therefore be possible to synchronize cows so that estrus detection could be condensed into a shortened period of time and decrease the labor required.

When $\text{PGF}_{2\alpha}$ was administered to lactating dairy cows between days 6 and 17 of the estrous cycle, 85% displayed estrus within 144 hours (Macmillan et al., 1978). If $\text{PGF}_{2\alpha}$ is administered to random cows without knowledge of the stage of the estrous cycle, any cows which by chance are between days 1 and 5 will not display estrus.

Whereas any cows which are at day 17 or greater will naturally display estrus during the expected time period. It was then hypothesized that the administration of two doses of $\text{PGF}_{2\alpha}$ at an interval of 11 to 14 days would improve estrus synchrony. The rationale behind this protocol was that cows which were unresponsive to the first injection because they were either in proestrus or metestrus at that time would be between days 6 and 17 of the estrous cycle by the time of the second injection, or 11-14 days later. This theory was tested by Cooper in 1974, who gave 2 doses of $\text{PGF}_{2\alpha}$ 11 days apart to heifers and observed that 90% displayed estrus within 48-72 hours after the 2nd injection and another 6% displayed estrus between 72 and 96 hours. Jackson et al. (1979) also gave two injections of $\text{PGF}_{2\alpha}$ 11 days apart and found that estrus occurred earlier and in a more narrow range than with only 1 injection. Another study determined that luteolysis occurred after in the 1st injection in 60% of cows and after the 2nd injection in 72% of cows (Stevenson et al., 1987).

The use of two doses of $\text{PGF}_{2\alpha}$ at an interval of 14 days is widely applied in modern dairy herd management as a means of setting up cows for other timed insemination programs and as prevention for endometritis and pyometra. Pankowski et al. (1995) administered sequential injections of $\text{PGF}_{2\alpha}$ 14 days apart and compared the degree of synchronization and resulting fertility for 3 sequential injections of $\text{PGF}_{2\alpha}$, 2 sequential injections of $\text{PGF}_{2\alpha}$, and traditional methods of managing postpartum cows involving palpation per rectum and intrauterine infusion. In that study, the protocol involving three sequential injections of $\text{PGF}_{2\alpha}$ was the most cost effective method and resulted in the greatest synchrony of estrus following the third injection. Despite these advantages, there was no significant effect of treatment on fertility. Lopez-Gatius et al. (2003) administered

2 doses of $\text{PGF}_{2\alpha}$ at days 22 and 36 postpartum then subjected cows to a standard reproductive examination on days 50 and 71 postpartum. In that study, cows in the treated groups had fewer cases of cystic ovarian disease and pyometra, an increased proportion with luteal activity, a higher ovulation rate, a greater number in estrus, and a higher pregnancy rate.

The interval between $\text{PGF}_{2\alpha}$ administration and estrus is highly variable among cows when it is given at random stages of the estrous cycle. The protocol using 2 doses 11-14 days apart decreases the variability in the interval between administration and estrus, therefore increasing the synchrony. The time period between luteolysis and estrus is thought to be related to the maturity of the follicle at the time of $\text{PGF}_{2\alpha}$ administration. If the follicle is large, then time to estrus is decreased whereas if it is small, more time is required for the follicle to reach ovulatory size. The underlying follicular waves play a significant role in determining the time to estrus when $\text{PGF}_{2\alpha}$ is administered in the early to mid-luteal phase. Jackson et al. (1979) recognized that the time to estrus was shorter in cows treated with $\text{PGF}_{2\alpha}$ on days 7-8 and days 15-16 than on days 12-14. These authors also suggested that the presence of a large follicle was the factor involved in shortening the time to estrus and decreasing the variability.

Similar to the above findings, when $\text{PGF}_{2\alpha}$ was administered early in the estrous cycle (days 5-9) the interval to estrus was shorter than when it was administered later in the estrous cycle (King et al., 1982). When heifers were given $\text{PGF}_{2\alpha}$ on day 7 or 15 of the estrous cycle, 88% and 73% respectively, were in estrus between 32 and 56 hours post $\text{PGF}_{2\alpha}$; whereas when it was given on day 11, only 13% were in estrus between 32 and 56 hours post $\text{PGF}_{2\alpha}$ (Tanabe and Hann, 1984). Later using ultrasound, Savio et al.

(1990) determined that the 1st wave dominant follicle of the estrous cycle will ovulate following PGF_{2α} administration on day 7. That study confirmed, through the use of ultrasound, the hypothesis that the 1st wave dominant follicle has ovulatory capacity if luteolysis occurs and that the presence of a dominant follicle increases the degree of estrus synchronization following PGF_{2α}. Another interesting finding was that the interval from PGF_{2α} to estrus was generally shorter in heifers than in cows (King et al., 1982).

Although PGF_{2α} was useful for synchronization of estrus, as long as estrus detection was still required it continued to be a limiting factor in the overall reproductive efficiency. Programs where insemination could be performed at a predetermined time and completely eliminate the need for estrus detection would be ideal, but only if acceptable fertility could be achieved. In an attempt to develop this type of program, Lauderdale et al. (1974) compared three groups of cows: 1) cows observed for estrus and inseminated, 2) cows treated with PGF_{2α} and inseminated at detected estrus, and 3) cows treated with PGF_{2α} and timed inseminated at 72 and 96 hours. In that study, conception rates (52.2-55.8%) and overall pregnancy rates (30-42%) were not significantly different between treatment groups which indicated that acceptable fertility could be achieved using timed insemination.

Macmillan (1978) examined a protocol where cows were given PGF_{2α} between days 6 and 17 of the estrous cycle and were timed inseminated 72 hours after PGF_{2α} and cows which were observed in estrus after the first insemination received a second timed insemination at 96 hours post PGF_{2α}. This protocol resulted in a 57% conception rate but did not entirely eliminate the need for estrus detection. When 2 doses PGF_{2α} were given 11 days apart and insemination was timed at 72 and 96 hours post PGF_{2α}, the conception

rate was 39% in heifers (Macmillan et al., 1978) and 30% in lactating dairy cows (Stevenson et al., 1987). A single timed insemination at 80 hours post PGF_{2α} resulted in a 23% (Stevenson et al., 1987) to 46.2% (King et al., 1982) conception rate in lactating dairy cows and a 46.7% conception rate in heifers (King et al., 1982). When a single timed insemination was used at 80 hours post PGF_{2α}, the stage of the estrous cycle at which PGF_{2α} was given significantly influenced the conception rate in heifers. Heifers given PGF_{2α} early in the estrous cycle (days 5-9) had a significantly decreased conception rate, likely due to the fact that the majority were in estrus 30 hours prior to insemination (King et al., 1982).

There are a proportion of cows with mature corpora lutea which do not respond to an intramuscular injection of PGF_{2α}. It is possible that in these cases, repeated administration of PGF_{2α} may be needed to achieve luteolysis. Archbald et al. (1993a) tested protocols administering two doses of PGF_{2α} 8 hours apart and 24 hours apart and compared these to the standard single dose. The results of that study indicated that 2 doses of PGF_{2α} 8 hours apart increased the number of cows in estrus compared to the other two protocols and did not affect fertility.

Programs using Prostaglandin F2 Alpha and Gonadotropin- Releasing Hormone

In 1974, Kaltenbach et al. determined that GnRH given intramuscularly resulted in an LH and FSH surge, with peak levels occurring within 100 minutes after the injection. In that study, estrus and ovulation occurred in most cows within 24 hours. This opened up possibilities for the use of GnRH in the synchronization ovulation following an injection of PGF_{2α}. In the same study, Kaltenbach et al. (1974) also attempted to synchronize ovulation after PGF injection but failed due to failure of the PGF_{2α} in causing luteolysis. Fernandez-Lima et al. (1977) gave PGF_{2α} followed by treatment with

a GnRH agonist (D-ala-GnRH) 64 hours later, this treatment either induced an LH surge or potentiated a natural LH surge. These results agree with those of Kaltenbach et al. (1974) and indicate that GnRH has the potential to improve synchronization of ovulation when used in combination with PGF_{2α}.

The next challenge was to determine the exact combination and timing of GnRH and PGF_{2α} administration that would produce an acceptable conception rate, eliminate the need for estrus detection and decrease and reduce the variability around the calving interval. Rodriguez et al. (1975) compared three different protocols; a control group with standard estrus detection, a group receiving PGF_{2α} followed by estrus detection, and a group receiving PGF_{2α} + GnRH in 48 hours + timed insemination 15 hours later. The timed insemination protocol using GnRH resulted in a conception/ pregnancy rate of 22% which was significantly lower than that observed in the control group (36%). In a comparison of three different protocols using sequential injections of PGF_{2α} and timed insemination with or without GnRH to a standard protocol of estrus detection, conception rates were lower in the timed insemination groups and the calving interval was not reduced, although the variability in the interval to 1st service was significantly reduced (Lucy et al., 1986).

In a study where GnRH was given at the time of insemination and 72-80 hours after PGF_{2α}, GnRH treatment improved fertility compared to no treatment or PGF_{2α} given concurrently with timed insemination (Archbald et al., 1992). In that study, the best pregnancy rate was still obtained with insemination at detected estrus, but there was an effect of season where cows treated with GnRH in the spring but not in the summer had higher conception rates. When GnRH was given at the time of estrus and insemination to

repeat breeder cows in an attempt to ensure ovulation and improve conception rates, there was no significant effect on fertility when compared to no treatment (Archbald et al., 1993b).

Administration of GnRH causes ovulation of a dominant follicle, if present, and removes the estradiol inhibition on the anterior pituitary resulting in an FSH surge and the emergence of a new follicular wave. It was therefore speculated that GnRH could also be used to control follicular wave development and ensure that a dominant follicle would be present on the ovary at the time $\text{PGF}_{2\alpha}$ treatment. Furthermore, it was speculated that treatment with GnRH 6 or 7 days prior to $\text{PGF}_{2\alpha}$ would also decrease the interval between $\text{PGF}_{2\alpha}$ and estrus and increase estrus synchrony. In a study where cows were inseminated at detected estrus following $\text{PGF}_{2\alpha}$, GnRH pretreatment 6 days prior to $\text{PGF}_{2\alpha}$ increased the synchrony of estrus from 50% to 83.3% and decreased the variability in time to estrus while having no effect on fertility or the average time to estrus (Twagiramungu et al., 1992). A similar study in which cows were treated with either $\text{PGF}_{2\alpha}$ or GnRH followed by $\text{PGF}_{2\alpha}$ in 7 days and monitored for estrus, cows in the GnRH pretreatment group had a decreased interval to estrus and improved synchrony (Wolfenson et al., 1994). Results from these two studies indicate that GnRH administered 6 or 7 days prior to $\text{PGF}_{2\alpha}$ can alter follicle development and improve the synchrony following $\text{PGF}_{2\alpha}$.

A protocol (Ovsynch®) was then developed in which cows were given GnRH, $\text{PGF}_{2\alpha}$ 7 days later, followed by GnRH in 48 hours. The first GnRH would either cause ovulation of a dominant follicle and emergence of a new follicular wave or coincide with the emergence of a natural follicular wave (Pursley et al., 1995). The $\text{PGF}_{2\alpha}$ would cause

regression of the corpus luteum and the 2nd GnRH would cause ovulation of the newly developed follicle, usually within 24-32 hours. Using this protocol, ovulation could be synchronized to an 8 hour period (Pursley et al., 1995). Furthermore, this protocol resulted in acceptable conception rates (50%- Pursley et al., 1995; 33%- Momcilovic et al., 1998; 35.3%- Stevenson et al., 1996).

Many researchers have compared the Ovsynch protocol to other accepted methods of estrus synchronization and timed insemination. Often the conception rate to the Ovsynch protocol was less than observed with insemination at detected estrus (Stevenson et al., 1996; Stevenson et al., 1999). Despite a lower conception rate, pregnancy rates with the Ovsynch protocol were frequently better than pregnancy rates following insemination at detected estrus (Stevenson et al., 1996; Momcilovic et al., 1998; Stevenson et al., 1999). This is because there is no effect of heat detection in the Ovsynch protocol as all cows are inseminated. Others have found similar conception and pregnancy rates between cows receiving the Ovsynch protocol or inseminated after detected estrus, either natural or induced by PGF_{2α} (Momcilovic et al., 1998; Burke et al., 1996; Pursley et al., 1997).

Other major benefits to the Ovsynch protocol, besides potentially increasing the pregnancy rate, is a significantly decreased interval from calving to conception (Momcilovic et al., 1998), decreased median days to 1st service and more cows pregnant by 60 and 100 days post partum (Pursley et al., 1997). In disagreement with these findings, Burke et al. (1996) found there was no significant effect of Ovsynch on the interval from calving to conception or the overall pregnancy rate by 120 days postpartum, although they noted that the monthly conception and pregnancy rates were more

consistent in cows receiving the Ovsynch protocol than in cows inseminated at detected estrus.

Other research has focused on determining the ideal stage of the estrous cycle to initiate the Ovsynch protocol. Previous observations have suggested that the Ovsynch protocol does not work as well if the 1st GnRH fails to cause ovulation of a dominant follicle, as may occur when a the largest follicle has not yet acquired ovulatory capacity. In general, follicles acquire ovulatory capacity just after deviation or when they are greater than 10 mm in diameter (Sartori et al., 2001). If the follicle is at a point just prior to deviation at the time of the 1st GnRH, luteal regression and ovulation may occur prior to the 2nd GnRH or the follicle will be undergoing atresia at the time of the 2nd GnRH. Vasconcelos et al. (1999) determined that the best response to the 1st GnRH injection was obtained when cows were between days 5-9 of the estrous cycle and the most significant factor influencing response to the 2nd GnRH was the response to the 1st GnRH. Furthermore, initiation of the Ovsynch protocol in the 1st half (days 1-12) of the estrous cycle resulted in a better synchronization rate than when the protocol was initiated in the 2nd half of the estrous cycle (91% vs. 80%; Vasconcelos et al., 1999). In heifers, the ideal stage to initiate the Ovsynch protocol is between days 5 and 10 of the estrous cycle or in the early luteal phase, with the worst results occurring when protocol was started beginning on days 2, 15 or 18 (Moreira et al., 2000b).

The amount of flexibility that may exist for the timing of the 2nd GnRH and the timed insemination has been another area of research. It would be a major benefit to herd managers if acceptable fertility could be achieved without as much cow handling as required in the original Ovsynch protocol. Pursley et al. (1998) found that acceptable

fertility was achieved if the timed insemination occurred anywhere between 0 and 24 hours after the 2nd GnRH, but not at 32 hours after. Furthermore, the group inseminated at the same time as the 2nd GnRH (0h) had decreased pregnancy losses and an increased female to male ratio. The flexibility in the timing of the 2nd GnRH in relation to the PGF_{2α} injection has also been studied. Peters and Pursley (2003) found that fertility and follicle size at ovulation increased linearly with increasing time from the PGF_{2α} when they compared different protocols where the 2nd GnRH was given at 0, 12, 24, or 36 hours after the PGF_{2α}. There was also a tendency for an increased incidence of short luteal phases when the 2nd GnRH was given at 0 hours compared to at 36 hours. This study showed that although ovulation was still synchronized, fertility was compromised when the GnRH was given too soon after the PGF_{2α} and that this may possibly be due to a shortened luteal phase. In partial agreement with this finding, Moreira et al. (2000b) found that decreased follicle size at ovulation was associated with decreased serum progesterone during the luteal phase. It was also determined that the fertility of cows observed in estrus between the PGF_{2α} and 2nd GnRH injection was normal if they were inseminated at that time (Kasimanickam et al., 2004).

Body condition score has been found to significantly affect conception rates to the Ovsynch protocol. Stevenson et al. (1999) found that for each 1 unit increase in BCS, conception rate increased by 10%, and cows with higher BCS also had higher progesterone concentrations. Burke et al. (1996) also found a positive correlation between BCS and pregnancy/ conception rates as well as between progesterone and conception/ pregnancy rates. When cows were separated in two groups based on their BCS, cows with a low BCS (<2.5) had a significantly decreased conception rate to the 1st

service using the Ovsynch protocol than cows with higher BCS (Moreira et al., 2000a). In the same study, an economical analysis determined that if the percentage of low BCS cows in a herd was decreased from 30% to 10%, the economic benefit would be \$10.33/cow, or \$10 330 in a 1000 cow herd.

The economic benefit of applying the Ovsynch protocol to the standard reproductive management of an entire herd is another important area of concern. The Ovsynch protocol has a higher insemination submission rate than protocols relying on estrus detection and therefore has more cost associated with semen. There are also more injections required and more labor involved in cow handling. On the other hand, programs relying on estrus detection require more labor for the observation of cows. Ovsynch can potentially increase the overall pregnancy rate, decrease the interval from calving to conception, and decrease proportion of cows open for long periods. These benefits may be limited in herds with excellent estrus detection efficiency. In an economic comparison between the Ovsynch protocol and a protocol using PGF with insemination at detected estrus, the Ovsynch protocol resulted in a better pregnancy rate and was \$29.44/ pregnancy cheaper (Britt and Gaska, 1998). In another study comparing insemination at detected estrus and the Ovsynch protocol in 2 herds with different reproductive management, the Ovsynch protocol was better in the herd with lower estrus detection efficiency while the estrus detection protocol was better in the herd with better estrus detection efficiency (Tenhagen et al., 2004).

Programs using Progesterone

Progesterone is naturally released by the corpus luteum in a normal estrous cycle and while progesterone is high, ovulation does not occur even though a dominant follicle may be present on the ovary. Exogenous progesterone causes suppression of both

ovulation and estrus (Christian and Casida, 1948). Progesterone can be administered by daily feeding of oral progestins or subcutaneous progesterone implants. Stainless steel coils coated with silastic rubber impregnated with progesterone and inserted into the vaginal results in adequate and more consistent plasma P4 levels than the method of daily feeding (Roche, 1976).

Progesterone treatment has been used together with PGF_{2α} in different protocols for the synchronization of ovulation and estrus. When a PRID was inserted for 7 days and PGF_{2α} was given on day 6 of PRID treatment, or 1 day before removal (PRID7-PGF6), estrus synchronization was improved compared to untreated controls and protocol using a PRID for 6 days with PGF_{2α} at the time of removal (Smith et al., 1984). In the same study, conception rate to timed insemination was better in cows treated with PRID7-PGF6 over cows treated with two injections of PGF_{2α} 14 days apart. A comparison of two treatment protocols, both involving two injections of PGF_{2α} 14 days apart but with or without insertion of a PRID for the final 7 days, found that the insertion of a PRID improved conception rates especially in cows with low progesterone at the beginning of the protocol (Folman et al., 1990). Similarly, addition of progesterone for the final 5 days between 2 injections of PGF_{2α} 14 days apart improved conception rates when compared to cows not receiving progesterone but not compared to untreated controls (Xu et al., 1997).

Turnover of the dominant follicle is caused by the negative feedback effect of progesterone on LH and the removal of progesterone allows LH to rise and ovulation to occur. It has been discovered that when the first wave dominant follicle develops in a milieu of low progesterone (2ng/ml), LH concentration is higher and the first wave

dominant follicle persists for many days than ovulates quickly upon removal of progesterone (Savio et al., 1993). Conversely, high progesterone concentrations (3-5ng/ml) suppress LH and cause turnover of the first wave dominant follicle and the emergence of a new follicular wave (Savio et al., 1993). Further evidence which supports this theory comes from a study comparing cows treated with either high or low progesterone for 10 days (Wehrman et al., 1993). Cows in the low progesterone group had increasing concentrations of estradiol and a shorter interval to estrus upon removal of progesterone while cows in the high progesterone group had decreasing concentrations of estradiol, a longer interval to estrus, and an improved conception rate.

Elevated progesterone, either in the form of an additional injection or as endogenous progesterone from a CL, can also alter follicular development. An injection of both estradiol and progesterone at the insertion of a progesterone-releasing device in heifers, improved estrus response rates and increased synchrony (Roche et al., 1974). The underlying mechanism for this was partially elucidated in a trial where heifers were fed melengestrol acetate (MGA) for 11 days, with an injection of PGF_{2α} on day 2 of treatment and with or without an injection of progesterone on day 9 (Anderson and Day, 1994). The additional progesterone injection on day 9 caused atresia of the first wave dominant follicle and the emergence of a new follicular wave. Heifers in the progesterone group also had a higher conception rate while those not receiving the additional progesterone, and without a CL, developed persistent follicles. Furthermore, the type of progesterone administered is important when no CL is present. In the absence of a CL, protocols using either a PRID or norgestomet both resulted in the development of persistent follicles, yet cows in the PRID group had a significantly higher conception rate

(Smith and Stevenson, 1995). These results emphasize the importance of both the type of progesterone and the presence of a CL.

Progesterone protocols frequently also involve the simultaneous use of estrogens. In cows given Syncro-Mate-B implants (ear implant containing norgestomet), estradiol valerate (estra-1,3,5(10)-triene-3, 17-diol(17 β)-, 17-pentanoate) causes regression of the CL and atresia of the 1st and 2nd largest follicles with the subsequent emergence of a new follicular wave (Bo et al., 1991). A protocol using an oral progestin treatment for 9 days with an injection of estradiol valerate on day 2 resulted in effective estrus synchronization and a similar conception rate to untreated controls (Wiltbank and Kasson, 1968). In the conditions of New Zealand, administration of a CIDR with an estradiol benzoate capsule for 8 days and PGF on day 7 resulted in 89% of cows exhibiting estrus within 5 days, but a lower overall conception rate than untreated controls (Xu et al., 1996).

Follicle wave emergence can also be controlled by the addition of GnRH to a progesterone protocol. Through ovulation of a responsive follicle, GnRH can be used to synchronize follicular wave development prior to progesterone removal or to synchronize ovulation after progesterone withdrawal. In a comparison of 3 groups given either GnRH, estradiol benzoate, or no treatment coincident with CIDR insertion, GnRH treatment caused progesterone to rise rather than decrease as it did in the other two groups (Kim et al., 2005). In the same study, the GnRH group also had earlier follicular wave emergence, a larger ovulatory follicle and an increased pregnancy rate. Administration of GnRH at the same time as CIDR insertion caused ovulation of a dominant follicle and subsequently increasing progesterone concentrations throughout the treatment period (Ando et al., 2005). In that study, estrus synchronization was also improved in cows

receiving GnRH compared to cows not receiving GnRH. Administration of GnRH 30 hours after the removal of a progesterone-releasing device improved both the synchronization of ovulation compared to controls (Roche, 1975; doValle et al., 1997) and the final calving rate after a timed insemination (doValle et al., 1997).

When GnRH is used to synchronize follicular wave emergence at the beginning of progesterone treatment and to synchronize ovulation after progesterone withdrawal, with $\text{PGF}_{2\alpha}$ given on day 7 to cause luteolysis; the protocol becomes synonymous to the Ovsynch protocol just with the addition of progesterone. Comparisons of Ovsynch to an Ovsynch + CIDR protocol indicate that the addition of the CIDR significantly increases serum progesterone concentration over the Ovsynch protocol alone (Kawate et al., 2004; El-Zarkount et al., 2004). The Ovsynch + CIDR protocol also improved conception rate compared to Ovsynch alone (72% vs. 47%; Kawate et al., 2004). Another study found that the Ovsynch +CIDR protocol significantly improved conception rate but only when the majority of cows were anestrous and the positive effect was lost when the majority of cows were diestrous (El-Zarkouny et al., 2004).

In some regions where dairy cattle are grazed, calving is seasonal and estrus detection efficiency is good; the ideal estrus synchronization protocol would result in tight synchronization of ovulation and estrus with good fertility, but does not necessarily require timed insemination. Studies in Ireland have examined many different protocols for their ability to synchronize estrus and improve overall fertility. In one study, the protocol with the highest estrus detection rate and pregnancy rate was one in which cows received a GnRH injection on the day of CIDR insertion, $\text{PGF}_{2\alpha}$ 7 days later and removal of the CIDR 8 days later (GnRH+CIDR8-PGF7), this protocol was better than $\text{PGF}_{2\alpha}$ +/-

estradiol benzoate, and CIDR +/- estradiol benzoate on the day of CIDR insertion (Ryan et al., 1995). In a subsequent study, the interval from CIDR withdrawal to estrus was shortened and the synchronization of estrus was improved when cows were treated with the same protocol (GnRH+CIDR8-PGF7) but with an injection of estradiol benzoate 10 hours after CIDR removal (Ryan et al., 1999). Another study in Australia compared two protocols, both using two injections of estradiol benzoate 9 days apart, with PGF_{2α} on day 7, but with the insertion of an intravaginal progesterone releasing device (IVD) for either 7 or 8 days (Cavalieri et al., 2004). The protocol where the IVD remained in place for 8 days decreased the interval to estrus and improved synchronization compared to the 7 day protocol with no significant effect on fertility. These results indicate that the synchronization of estrus through the use of progesterone can be improved by controlling follicular wave emergence and ovulation, using either GnRH or estrogens, in conditions where breeding is seasonal and estrus detection efficiency is good. Furthermore, a protocol where the cow is exposed to progesterone for 8 days, instead of 7 days, may improve synchronization.

MATERIALS AND METHODS

This study was conducted in a large dairy herd (approximately 1,500 milking cows) in north east Florida (Baldwin, FL). These cows were milked 3 times per day and had a rolling herd average 305-day milk production of 9000 kg. They were kept in open barns with dry manure bedding between milking. Between 60-63 days post partum, all cows were given bovine somatotropin every 14 days (bST; 500 mg, intradermally; Posilac; Monsanto Co, St. Louis, MO). Cows were fed a total mixed ration formulated to meet or exceed the requirements of the National Research Council (2001).

The period of study was approximately one year, from October 13, 2003 to September 20, 2004. During this time, the farm was visited once a week. This dairy herd is on a reproductive herd health program administered by veterinarians of the Veterinary Medical Teaching Hospital (College of Veterinary Medicine, University of Florida), and all reproductive, health, and management records were computerized. Cows were routinely vaccinated against bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), parainfluenza virus (PI-3), leptospirosis, campylobacteriosis, clostridiosis, and gram-negative organisms (J-5 vaccine) according to recommendations by attending veterinarians.

Using a protocol for synchronization of ovulation and timed insemination in this dairy herd, the conception rate of cows without ovarian cysts was 30% while that of cows with ovarian cysts was 17%. It was anticipated that a protocol involving the use of a controlled internal drug releasing (CIDR) device with 1.38g progesterone will increase

the conception rate of cows with ovarian cysts to 30%. Therefore, a sample size of 130 cows per group was needed to demonstrate that this difference will be significantly different at a level of $P < 0.05$ (95% confidence intervals and 80% power).

A total of 401 lactating dairy cows with ovarian cysts were used in this study. This number was used to allow for the loss of cows from the herd for various reasons. On the day of each visit (Day 0), cows diagnosed with ovarian cysts were alternatively allocated to the 2 groups. The diagnosis of ovarian cysts was based on per rectum palpation of the ovaries and uterus, and by ultrasonographic examination of the ovaries. The criteria used on rectal palpation were the presence of multiple follicles on the ovary with at least one follicle being ≥ 17 mm diameter (Halter et al., 2003), the absence of a corpus luteum (CL) on either ovary, and the lack of tonicity of the uterus (Archbald et al., 1991; Bartolome et al., 2002; Bartolome et al., 2003; Zemjanis, 1970). On ultrasonographic examination of the ovaries, ovarian cysts were recognized by the hypoechogenicity of the structure (Pierson and Ginther, 1984), and the absence of a CL on either ovary.

Cows in the Ovsynch group ($n = 201$) were treated with GnRH (100 μg , im; Cystorelin®; Merial Limited, Iselin, NJ, USA) on Day 0, PGF_{2 α} (25 mg, im; Lutalyse® Sterile Solution; Pfizer Animal Health, New York, NY, USA) on Day 7, GnRH (100 μg , im; Cystorelin®; Merial Limited, Iselin, NJ, USA) on Day 9, and timed inseminated 16-20 h later, without detection of estrus. Cows in the CIDR group ($n = 200$) were treated with a CIDR (1.38g progesterone, intravaginal insert; EAZI-BREED™ CIDR® Pfizer Animal Health, New York, NY, USA) on Day 0 for 7 days. On Day 7, the CIDR was removed, and cows were treated with PGF_{2 α} (25 mg, im; Lutalyse® Sterile Solution; Pfizer Animal Health, New York, NY, USA). All cows in the CIDR group were observed

for estrus at least twice daily, and cows exhibiting estrus following removal of the CIDR were inseminated according to the am-pm rule. Therefore, cows observed in estrus in the morning were inseminated in the afternoon, and cows observed in estrus in the afternoon were inseminated late that night. In either instance, cows were bred within 8-12 hours after the first sign of estrus.

On Day 0, baseline data for parity (1st lactation= primiparous, 2nd + lactation= multiparous), days in milk (DIM), milk production (kgs/day) on the day of diagnosis, time of year of cyst diagnosis and body condition score (BCS; scale of 1 to 5; Ferguson et al., 1994) were recorded. Time of year was recorded to include the possible seasonal effects on reproduction in the analysis. Based on previous research (Al-Katanani et al., 1999), the cooler and more favorable time of year for pregnancy is October to February, while the warmer and less favorable time of year is March to September. Therefore, cows enrolled from October to February and March to September were recorded as receiving treatment in the cool and warm seasons, respectively.

Pregnancy was determined in all cows between Days 30-31 after insemination using ultrasonography, and reconfirmed using per rectum palpation of the uterus and previously described techniques (Zemjanis, 1970) between 42-45 days.

On Day 21, all cows were subjected to ovarian ultrasonography and per rectum palpation to determine the presence of a CL. The presence of a CL at this time was used to indicate that ovulation was induced, and that the protocols resulted in resumption of normal ovarian cyclicity.

Since production of progesterone by the CL is best determined by analysis of the peripheral plasma of the cow (MacDonald, 1980), a blood sample for progesterone

determination was obtained on Day 21 to verify the presence/absence of a functional CL. Blood was obtained from the coccygeal vein into evacuated tubes (Vacutainer®; BD, Franklin Lakes, NJ, USA) and immediately placed on ice. Samples were centrifuged at 3000 x g for 30 min, and serum was stored at -20 °C until assayed for progesterone. Serum progesterone concentrations were determined using a sequential competitive chemiluminescent enzyme immunoassay (DPC Immulite® Progesterone; Diagnostic Products Corporation, Los Angeles, CA, USA). A serum progesterone concentration ≥ 1 ng/ml was used to verify the presence of a functional CL and to determine the sensitivity, specificity, positive predictive value and negative predictive value of palpation per rectum for the diagnosis of the presence of a CL.

Baseline data for parity, DIM, time of year, BCS and milk production on the day of diagnosis were compared using Chi-square and ANOVA ($P < 0.05$), respectively. Least squares means and ANOVA were used to determine the variables significantly associated with Day 21 serum progesterone concentration. The outcomes of interest for this experiment were the likelihood to be inseminated, presence of a CL on Day 21, conception and pregnancy rates for cows in each group. Conception rate was defined as the number of cows diagnosed pregnant divided by the total number inseminated. Pregnancy rate was defined as the number of cows diagnosed pregnant divided by the original number enrolled in each group. Data for these variables were analyzed using logistic regression adjusting for parity, DIM, BCS, milk production at diagnosis, and time of year. The explanatory variables were evaluated using the backward elimination procedure and variables that significantly affected the outcome remained in the model (Agresti, 1996). Treatment effect was forced to remain in the models. All possible

interactions between treatment and explanatory variables were tested before the final model was chosen. Statistical significance was declared if $P < 0.05$ or the 95% confidence interval (95% CI) for the odds ratio did not include 1. A 95% CI which does not include 1 indicates with 95% certainty that the true odds ratio is within the range of the confidence interval and represents a true association.

RESULTS

Baseline Data Comparisons

A total of 401 cows was enrolled in the study of which 201 cows were enrolled in the Ovsynch group and 200 cows were enrolled in the CIDR group. Baseline data recorded included cyst diagnosis time of year (warm/cool), days in milk (DIM), parity [primiparous (PP)/ multiparous (MP)], body condition score (BCS), and milk production on the day of diagnosis (kg/day). The median DIM were 132 days. The median parity was 1.0, the median body condition score was 2.75, and the median time of year for cyst diagnosis was the warm time of year (March to September). The mean milk production was 29.5 kg/ day. Baseline data for cows in all groups are presented in Table 3. A P-value was determined based on ANOVA for continuous variables or χ^2 statistic for discrete variables. Baseline data for body condition score, milk production, parity, DIM, and season of cyst diagnosis were not significantly different between treatment groups.

Table 3. Baseline data comparisons for cows in the Ovsynch and CIDR groups.

Baseline Variable	Ovsynch n=201	CIDR n=200	P Value
Average BCS	2.75	2.75	0.7977
Range	2.0-3.75	1.75-4.0	
Milk Production, kg	29.5	28.3	0.3975
Range	9.9-52.2	12.6-45.0	
Parity- % Primiparous	59%	61%	0.8538
1 st lactation / total	119/201	121/200	
Average DIM	158	169	0.2821
Range	61-510	59-521	
Season of diagnosis, % Cool	42%	40%	0.6275
# cool/ total	85/201	79/200	

Prior to further analysis, the continuous variables of DIM and BCS were dichotomized into binomial variables based on median values, while milk production on the day of diagnosis was divided in 4 categories based on quartiles. The division into binomial and categorical variables was done to prevent loss of power. Days in milk were categorized into early and late lactation (≤ 132 days and > 132 days, respectively). Body condition score was categorized into low and high based on a score of ≤ 2.75 or > 2.75 . There was an even distribution for the range of milk production on the day of diagnosis, which is presented in Figure 1. The four categories of milk production on the day of diagnosis were as follows; cows in Category 1 producing < 22.5 kg, cows in Category 2 producing ≥ 22.5 and < 28.8 kg, cows in Category 3 producing ≥ 28.8 and < 35.6 kg, and the top producers in Category 4 which were producing ≥ 35.6 kg on the day of diagnosis.

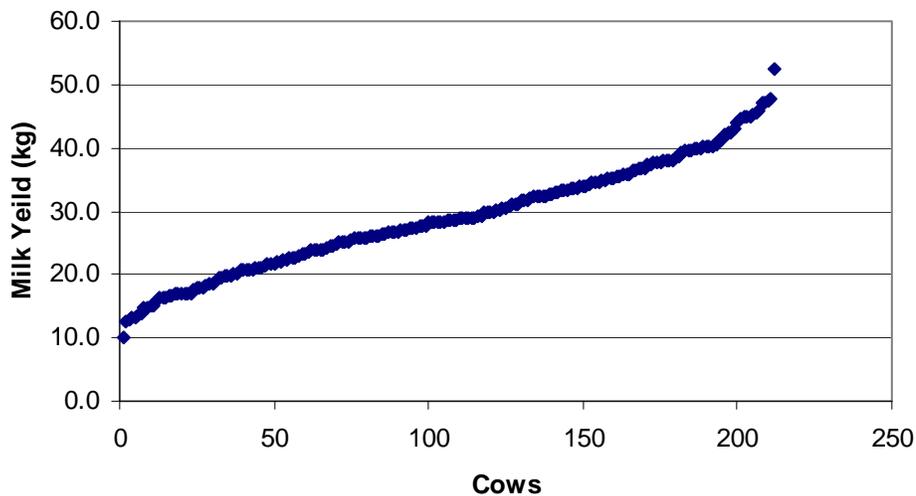


Figure 1. The distribution of milk production on the day of diagnosis (Kg) for cows in both groups combined.

Likelihood to be Inseminated, Return to Cyclicity, Conception and Pregnancy Rates

Overall, the percentage of cows bred in the Ovsynch and CIDR groups was 82% and 44%, respectively. The percentage of cows returning to cyclicity in Ovsynch and CIDR was 83% and 79%, respectively. The conception rate for cows in Ovsynch and CIDR were 18% and 23%, respectively. Pregnancy rate for cows in Ovsynch and CIDR groups were 14% and 9.5%, respectively. All of these results are presented in Table 4 and Figure 2.

Table 4. Summary of heat detection rate, conception rate, pregnancy rate and missing values/ lost cows by group.

Items	Ovsynch n=201	CIDR n= 200
% Inseminated	82% (164/ 201)	44% (87/200)
% Return to cyclicity	83% (136/164)	79% (137/174)
Conception rate	18% (29/158)	23% (19/82)
Pregnancy rate	14% (29/201)	9.5% (19/200)
Number of cows lost:		
AI but no pregnancy diagnosis	6	5
Number missing CL data	38	26

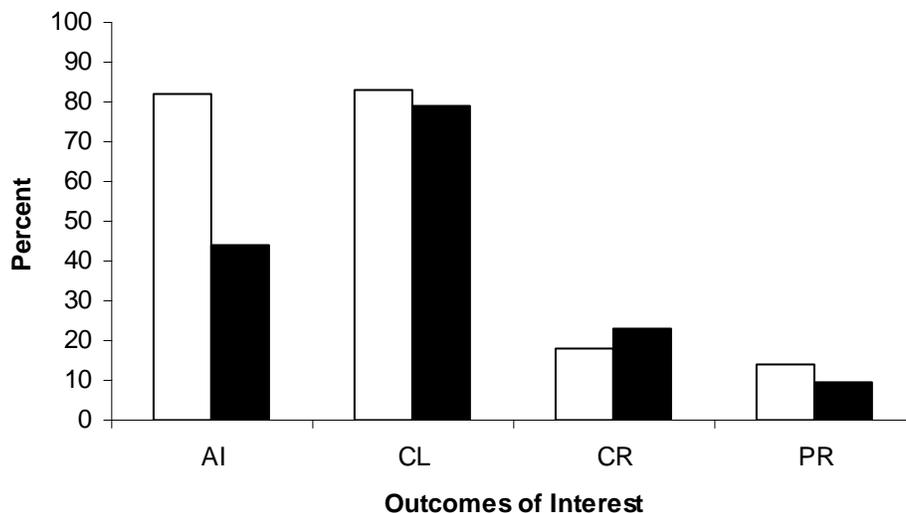


Figure 2. The proportion of cows per group that were inseminated (AI), returning to cyclicity, conception rate (CR), and pregnancy rate (PR) for the Ovsynch group (white bars) and the CIDR group (black bars).

Cows in the Ovsynch group were enrolled in a timed insemination program and should ideally have had a 100% insemination rate, but due to different reasons beyond the control of this study, a proportion of these cows were not inseminated (82% inseminated; Table 4). Cows in the Ovsynch group that were not inseminated were not included in the denominator used for conception rate, but these cows were included in the denominator used for pregnancy rate. The percentage of cows inseminated in the CIDR group is representative of the estrus detection rate for this herd. After cows were inseminated, a small number in each group were either sold from the herd or not available for pregnancy determination. These numbers are presented as ‘Cows lost’ in Table 4 and were subtracted from the total (denominator) used to calculate conception rate.

Data for cows in the Ovsynch and CIDR groups were analyzed to determine the association between the explanatory variables (treatment, DIM, BCS, parity, milk production, and time of year) and the outcome variables (likelihood to be inseminated, presence of a CL on Day 21, conception rate and pregnancy rate). Logistic regression of the full model was used to determine explanatory variables which were significantly associated with the outcome. Interactions between significant variables and treatment were tested for and included in the model, if found to be significant. The final model was chosen using backward elimination, forcing the effect of treatment to remain in the model and including all significant variables.

Likelihood to be Inseminated

In the CIDR group, 44% of cows were inseminated while 82 % of cows were inseminated in the Ovsynch group. The mean number of days between removal of the CIDR/PGF injection (Day 7) to observation of estrus and insemination was 3.1 and ranged from 1 to 5 days.

The likelihood for a cow to be inseminated was significantly associated with the effect of treatment (Table 5; Figure 3). The odds for cows in the Ovsynch group to be inseminated were 5.6 times more than the odds for cows in the CIDR group (95% CI= 3.5-8.8; Table 5).

Table 5. The risk of insemination for cows in the Ovsynch and CIDR groups adjusted for parity, DIM, BCS and milk production on the day of diagnosis.

Explanatory variables	Odds Ratio	95% CI
Treatment- Ovsynch vs. CIDR	5.6	3.5 – 8.8
Season- Cool vs. Warm	1.4	0.8 – 2.3
Parity- PP vs. MP	0.6	0.3 – 1.2
DIM- Early vs. Late	1.3	0.7 – 2.5
BCS- Low vs. High	0.6	0.3 – 1.2
Milk production on the day of diagnosis		
Category 2 vs. 1	0.6	0.3 – 1.4
Category 3 vs. 1	0.8	0.3 – 2.1
Category 4 vs. 1	1.1	0.4 – 2.7

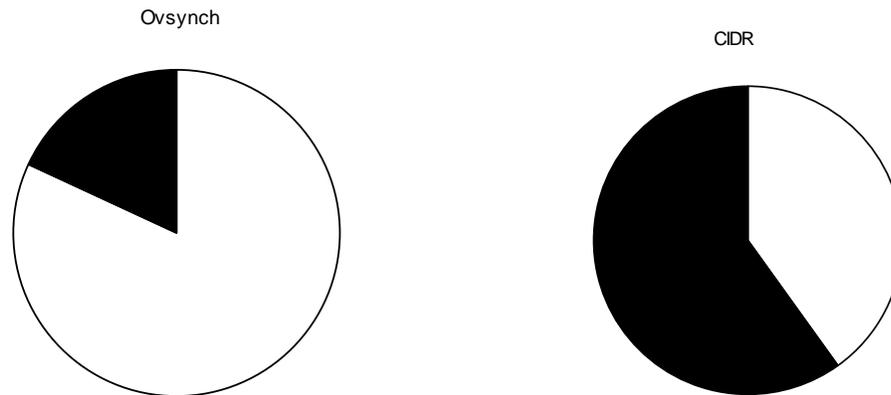


Figure 3. The proportion of cows inseminated (white) compared to cows not inseminated (black) for the Ovsynch and CIDR groups.

Return to Cyclicity

The presence of a CL on Day 21 (10 days after expected ovulation) was used to determine the number of cows returning to normal cyclicity. The percentage of cows in the Ovsynch and CIDR groups with a CL on Day 21 was 83% and 79%, respectively.

The proportion of cows that were unavailable on Day 21 for determination of a CL is

presented in Table 4 (# missing CL data). Cows unavailable for CL diagnosis were not included in the denominator used for calculating percent returning to cyclicity. The presence of a CL on Day 21 was significantly associated with the effect of treatment (Table 6). Adjusting for milk production, DIM, parity, season, and BCS, the odds for cows in the Ovsynch group to be diagnosed with the presence of a CL on Day 21 were 2.2 times more than the odds for cows in the CIDR group (95% CI= 1.04- 4.81; Table 6). No other explanatory variables were significantly associated with the presence of a CL on Day 21.

Table 6. The risk for the presence of a CL on Day 21 for cows in the Ovsynch and CIDR groups adjusted for parity, DIM, BCS and milk production on the day of diagnosis.

Explanatory variables	Odds Ratio	95% CI
Treatment- Ovsynch vs. CIDR	2.2	1.04 – 4.81
Season- Cool vs. Warm	1.1	0.50 – 1.90
Parity- PP vs. MP	0.5	0.26 – 1.30
DIM- Early vs. Late	0.6	0.27 – 1.34
BCS- Low vs. High	0.6	0.27 – 1.34
Milk production on the day of diagnosis		
Category 2 vs. 1	1.2	0.41 – 3.56
Category 3 vs. 1	0.6	0.23 – 1.73
Category 4 vs. 1	1.3	0.41 – 4.20

The presence of a CL on Day 21 was significantly associated with pregnancy ($P=0.05$). The odds for cows with a CL on day 21 to become pregnant were 7.7 times more than for cows without a CL (95% CI= 0.97-58.8).

Serum progesterone concentrations obtained on day 21 at the time of CL diagnosis in a subset of cows were used to confirm results obtained by palpation per rectum and ultrasonography, and to determine the accuracy of palpation per rectum in the diagnosis of a CL. In general, when cows were diagnosed as not having a CL, serum progesterone concentrations were low (mean= 0.64ng/ml; Table 7) with the exception of 3 outliers

(Figure 4). The majority of cows diagnosed with a CL had progesterone concentrations greater 3.0ng/ml, although values ranged from 0.02- 7.7 (Table 7; Figure 4).

Table 7. Descriptive statistics for progesterone values (ng/ml) based on the presence or absence of a CL

	CL	No CL
Median	3.09	0.02
Mean	2.87	0.64
Standard error	0.17	0.27
Standard deviation	2.00	1.38
Range	0.02-7.74	0.02-6.09

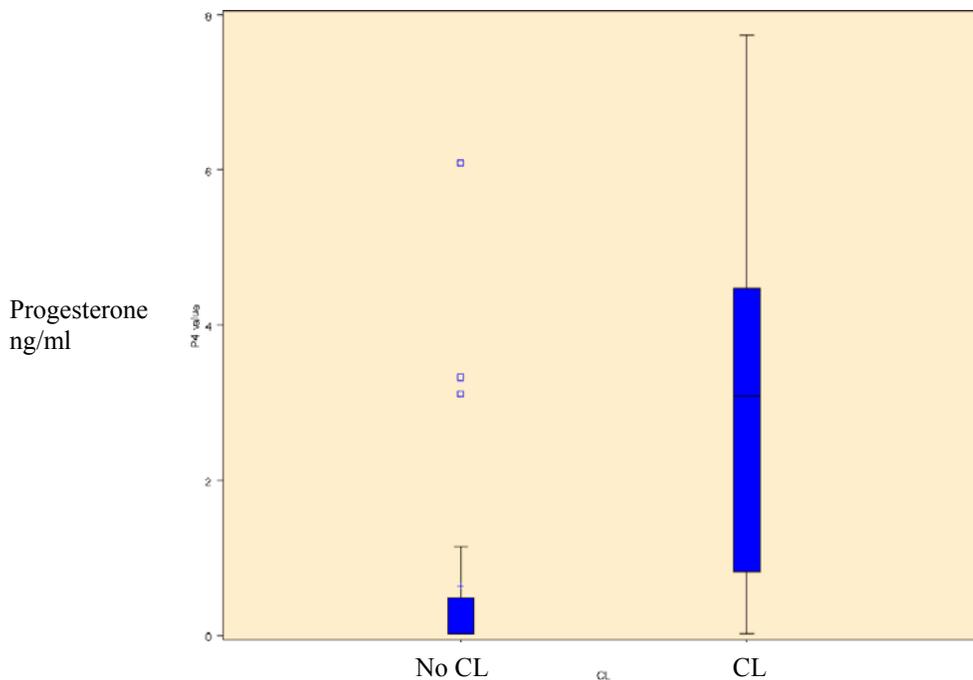


Figure 4. Box and whisker plots of progesterone values for cows with (CL) or cows without (No CL) a CL.

A contingency table was used to evaluate the accuracy of palpation per rectum and ultrasound for the diagnosis of a CL (Table 8). In this table, the true presence of a CL was based on a progesterone value of ≥ 1.0 ng/ml while the absence of a CL was based on a progesterone value < 1.0 ng/ml. Given this data and using established calculations (Slennig, 2001), the true prevalence of a CL was 63%, while the test prevalence (palpation per rectum) was 83%. The sensitivity and specificity of palpation per rectum

and ultrasonography in the diagnosis of a CL were 97% and 39.7%, respectively. The positive and negative predictive values of palpation per rectum and ultrasonography in the diagnosis of a CL were 73.3% and 88.5 %, respectively.

Table 8. Contingency table for the diagnosis of a CL based on palpation per rectum and U/S (RP+U/S) or progesterone value greater than or equal to 1.0ng/ml.

Presence of a CL based on RP+U/S	Progesterone Value		Total
	> 1.0ng/ml	< 1.0ng/ml	
CL	96	35	131
No CL	3	23	26
Total	99	58	Overall Total= 157

None of the cows with a clinical diagnosis of a CL and serum progesterone concentration < 1.0 ng/ml were pregnant at the following pregnancy diagnosis (Table 9). Of the remaining 95 cows with a CL and serum progesterone concentration > 1.0 ng/ml, 14 (15%) were pregnant (Table 9).

Table 9. Progesterone level as it relates to pregnancy in cows diagnosed with a CL on day 21.

Serum Progesterone	Not Pregnant	Pregnant	Total
Low P4 (\leq 1.0ng/ml)	35	0	35
High P4 (>1.0ng/ml)	82	14	96
Total	117	14	131

Using logistic regression for only those cows with a CL, the association between serum progesterone concentration and pregnancy was further defined. This analysis indicated that for each 1.0ng/ml increase in serum progesterone, the odds for a cow to be diagnosed pregnant increased by 1.6 times (95% CI= 1.01-2.47).

The general linear model procedure and least squares means were performed to determine which variables significantly influenced progesterone concentration on Day 21. Explanatory variables with a significant influence on progesterone concentration were: the presence of a CL on Day 21, BCS, milk production on the day of diagnosis, and

treatment (Table 10). As expected, cows diagnosed with the presence of a CL on Day 21 had significantly higher progesterone concentrations ($P < 0.0001$; Table 10). Cows with a body condition score > 2.75 had significantly higher progesterone concentrations ($P = 0.047$; Table 10). Cows with milk production ≥ 28.8 kg had significantly higher progesterone concentrations ($P = 0.017$; Table 10). Also, cows in the CIDR group had higher progesterone concentrations than cows in the Ovsynch group ($P = 0.0452$; Table 10), despite a decreased risk for the presence of a CL on Day 21 ($P = 0.039$; Table 6). The distribution of progesterone concentrations in the Ovsynch and CIDR groups is presented in Figure 5. The least squares means for cows without a CL was similar to cows in the Ovsynch and CIDR groups at 0.81 ng/ml and 0.56 ng/ml, respectively, however, the least squares means were considerably different for cows these groups diagnosed with the presence of a CL at 2.63 ng/ml and 3.32 ng/ml, respectively.

Table 10. Least squares means and standard error of the mean for progesterone concentrations and associations with the explanatory variables.

Variable	Progesterone LSM +/- SEM(ng/ml)	P value
Treatment		
Ovsynch	1.64 +/- 0.24	0.045
CIDR	2.25 +/- 0.24	
CL on Day 21		
Presence	3.18 +/- 0.18	<0.0001
Absence	0.71 +/- 0.28	
Milk Production on Day 0		
< 28.8 kg	1.55 +/- 0.22	0.017
≥ 28.8 kg	2.34 +/- 0.26	
BCS		
≤ 2.75	1.60 +/- 0.20	0.047
> 2.75	2.29 +/- 0.32	
Parity		
1 st Lactation	2.19 +/- 0.26	0.125
$> 2^{\text{nd}}$ Lactation	1.69 +/- 0.22	
DIM		
<132 days	2.16 +/- 0.26	0.203
>132 days	1.73 +/- 0.23	

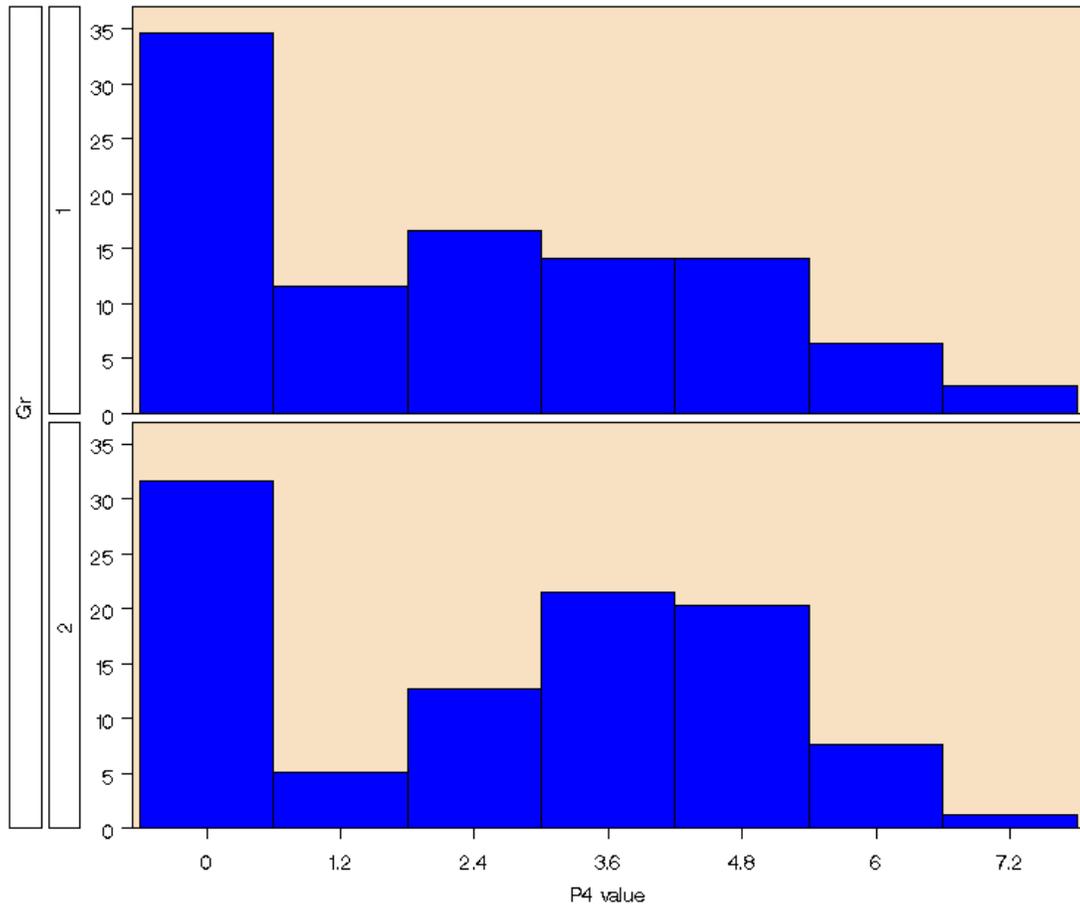


Figure 5. Distribution of progesterone concentrations (labeled 'P4 value'; ng/ml) on Day 21 for cows in the Ovsynch (labeled 'Gr 1') and CIDR (labeled 'Gr 2') groups.

Conception and Pregnancy Rates

Conception and pregnancy rates for cows in the Ovsynch group were 18 and 14%, respectively, while conception and pregnancy rates for cows in the CIDR group were 23 and 9.5%, respectively (Table 4; Figure 2). Conception and pregnancy rates for cows in the Ovsynch group were not equal due to the difference caused by the 18% of cows that were lost between enrollment and pregnancy determination. All cows were included in the denominator for pregnancy rate while only those with an examination at 45 days were included in the denominator for conception rate.

With conception as the dependent variable, logistic regression of the full model and backward elimination showed no significant effect of treatment. The significant variables associated with conception rate were parity and milk production on the day of diagnosis. The odds for primiparous cows to conceive after treatment were 4.7 times more than the odds for multiparous cows (95% CI= 1.52-11.9; Table 11; Figure 6). The odds for cows with milk production in the 3rd quartile on the day of diagnosis (Category 3) to conceive were 12.78 times more than the odds for cows with milk production in Category 1 (95% CI= 1.42-114.9; Table 11; Figure 7). The odds for cows with milk production in Category 4 to conceive were 9.4 times more than the odds for cows with milk production in Category 1 (95% CI= 1.004-88.4; Table 11; Figure 7).

Table 11. The risk of conception for cows in the Ovsynch and CIDR groups adjusted for parity, DIM, BCS and milk production on the day of diagnosis.

Explanatory variables	Odds Ratio	95% CI
Treatment- Ovsynch vs. CIDR	0.8	0.27 – 2.30
Season- Cool vs. Warm	1.4	0.72 – 2.92
Parity- PP vs. MP	4.1	1.52 – 11.9
DIM- Early vs. Late	2.2	0.76 – 6.3
BCS- Low vs. High	0.8	0.27 – 2.24
Milk production on the day of diagnosis		
Category 2 vs. 1	6.3	0.66 – 59.9
Category 3 vs. 1	12.8	1.42 – 114.9
Category 4 vs. 1	9.4	1.004 – 88.44

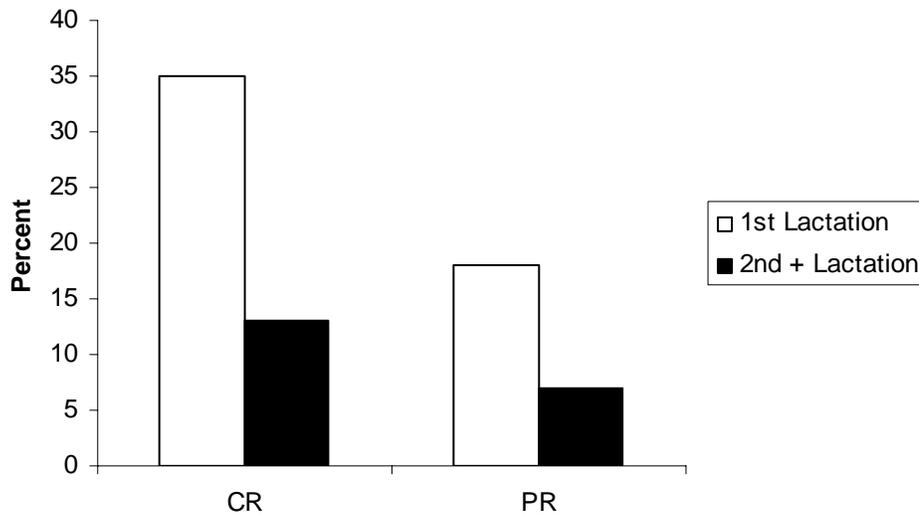


Figure 6. Conception rate (CR) and pregnancy rate (PR) divided by primiparous (1st Lactation; white) and multiparous (2nd + Lactation; black) for cows in the Ovsynch and CIDR groups.

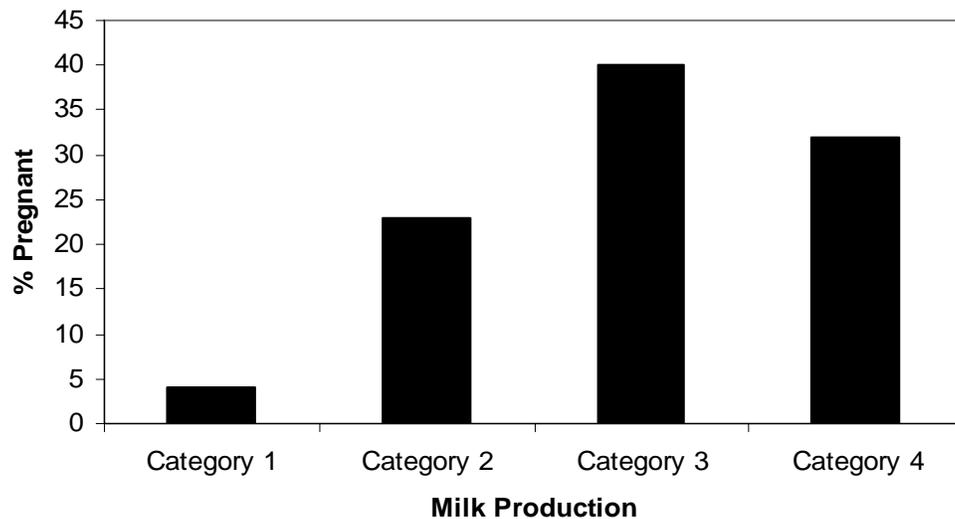


Figure 7. Conception rate by milk production category for cows in the Ovsynch and CIDR group.

The interactions of treatment x parity and treatment x milk production were not significant. Body condition score (BCS) and DIM were forced into the model, and milk production was removed to determine any associations between BCS, DIM and milk production. These variables still had no significant effect on conception rate.

With pregnancy rate as the dependent variable, logistic regression of the full model and backward elimination showed no significant effect of treatment. Similar to the results for conception rate, the variables significantly associated with pregnancy rate were parity and milk production. The odds for primiparous cows to become pregnant after treatment were 3.7 times more than the odds for multiparous cows (95% CI= 1.25-10.6; Table 12; Figure 6). The odds for cows with milk production in the 3rd quartile on the day of diagnosis (Category 3) to become pregnant after treatment were 10.9 times more than the odds for cows with milk production in Category 1 (95% CI= 1.28-93.4; Table 12). The odds for cows with milk production in Category 4 to conceive after treatment were 9.3 times more than the odds for cows with milk production in Category 1 (95% CI= 1.04-82.6; Table 12).

Table 12. The risk factors associated with pregnancy rate for cows in the Ovsynch and CIDR groups adjusted for parity, DIM, BCS and milk production on the day of diagnosis.

Explanatory variables	Odds Ratio	95% CI
Treatment- Ovsynch vs. CIDR	2.1	0.80 – 5.78
Season- Cool vs. Warm	1.6	0.84 – 3.10
Parity- PP vs. MP	3.7	1.25 – 10.6
DIM- Early vs. Late	2.4	0.87 – 6.99
BCS- Low vs. High	0.8	0.28 – 2.05
Milk production on the day of diagnosis		
Category 2 vs. 1	5.3	0.58 – 47.7
Category 3 vs. 1	10.9	1.28 – 93.4
Category 4 vs. 1	9.3	1.04 – 82.6

DISCUSSION

The hypothesis of this study was that exogenous progesterone with insemination at detected estrus would be a better therapeutic strategy for dairy cows with cystic ovarian disease than the Ovsynch protocol. The rationale behind this hypothesis was based on the current theory that cystic cows are progesterone deficient, and require progesterone exposure to correct the hypothalamic defect predisposing to this condition (Gumen and Wiltbank, 2002). Based on this theory, it was expected that exposure to exogenous progesterone would up-regulate the number of estrogen receptors in hypothalamic neurons involved in transmission of the GnRH surge, thus correcting the underlying hypothalamic defect.

Further support for this hypothesis comes from recent work which has demonstrated that the Ovsynch protocol causes CL formation in only 50.9-70.9% of cows following the first GnRH injection (Hendricks, 2004). This variable percentage occurs because the developmental stages of the underlying follicular waves in cystic cattle are unknown at the time of the 1st GnRH injection. The importance of the underlying follicle wave has been demonstrated in previous work which indicates that the ideal time to initiate the Ovsynch protocol is during the early luteal phase of the estrous cycle, when a dominant follicle with ovulatory capacity is present on the ovary (Vasconcelos et al., 1999; Moreira et al., 2000b). The 2nd GnRH injection of the Ovsynch protocol causes ovulation of the dominant follicle from the wave initiated after the first GnRH injection. Therefore, the success the 2nd GnRH injection is partially dependent on the success of 1st

GnRH injection in turning over the follicular wave. The Ovsynch protocol may not be the ideal treatment for cystic ovarian disease considering its variability in the proportion of cows that ovulate following the 1st GnRH injection. This potential lack of ovulation limits the success of the 2nd injection and prolongs the period of progesterone deficiency for a proportion of these cows. However, the presence of a CL on Day 7 of the Ovsynch protocol increases the risk for pregnancy in cystic cows (Hendricks, 2004).

Given this information, it was speculated that a protocol in which 100% of cows were exposed to progesterone, prior to a natural ovulation, would correct the underlying hypothalamic defect better than the Ovsynch protocol and improve the pregnancy rate. Contrary to our expectations, the results of the present study indicate that the CIDR protocol and the Ovsynch protocol were just as effective as therapeutic strategies for ovarian cysts with respect to conception and pregnancy rates, while the Ovsynch protocol was more effective than the CIDR protocol with respect to return to normal ovarian function.

The likelihood for cows to be inseminated in the CIDR and Ovsynch groups were 44% and 82%, respectively. The percentage of cows inseminated in the CIDR group (44%) is representative of the estrus detection rate for this farm. Ideally, the Ovsynch group should have had 100% insemination rate but due to various reasons beyond the control of this study, 18% of cows in the Ovsynch group were not inseminated according to the protocol. This lack of compliance was considered part of the reality of performing a large field study in a privately owned herd and possibly reflects the true circumstances of many herds. Some reasons for the lack of insemination at the appropriate time may have included cows that were unavailable due to movement from their respective herd

(i.e. moved into the hospital barn) and cows not inseminated due to labor availability or management reasons. Considering this information, it is also possible that a comparable proportion of cows in the CIDR group were either not observed for estrus or may have been observed in estrus but not bred for similar reasons to those listed above. If this is true it would be fair to speculate that the potential estrus detection rate for the CIDR group, under optimal conditions, could have been higher. Assuming there was some degree of lack of compliance in both groups, and to be fair to the CIDR group where this lack of compliance is hidden within the estrus detection rate, the total number of cows enrolled in each group was used as the denominator for pregnancy rate calculations. This was done even though it is common practice to include only cows inseminated when evaluating results for an Ovsynch protocol.

Despite the proportion of cows not inseminated in the Ovsynch group, the odds for cows to be inseminated in this group were 5.6 times more than the odds for cows in the CIDR group. Although this outcome was not unexpected when comparing a timed-insemination protocol to one relying on estrus detection, it emphasizes the major benefit of a timed-insemination protocol in a herd with less than optimal estrus detection. Furthermore, in a herd where the compliance for the Ovsynch protocol may be higher, and with a similar estrus detection rate to the present herd, the Ovsynch protocol could result in an even greater likelihood for cows to be inseminated. Conversely, a higher estrus detection rate in the CIDR group would decrease the difference in the likelihood to be inseminated between the two groups.

Cows in the Ovsynch group were at an increased risk for the presence of a CL following treatment compared to cows in the CIDR group (OR= 2.2; 95% CI=1.04 –

4.81). This indicates that more cows with cystic ovarian disease will return to normal ovarian function following treatment with the Ovsynch protocol than with the CIDR protocol. Although the percentage of cows in each group with a CL on Day 21 appeared similar (CIDR= 79%; Ovsynch =83%), the effect of treatment was significant when the model was adjusted for the influence of other variables (DIM, BCS, parity, season and milk production).

The increased risk for cows in the Ovsynch group to have a CL on Day 21 may be partially explained by the action of the 2nd GnRH injection in causing a dominant follicle to ovulate. The CIDR protocol relies on a natural GnRH/LH surge and ovulation, which may or may not occur. When exogenous GnRH is administered, as in the Ovsynch protocol, an LH surge occurs in almost every case and when a dominant follicle is present, ovulation will occur in the majority of cases (Kaltenbach et al., 1974). In a study examining the ovarian response of cystic cows to both a CIDR and Ovsynch protocol, one cow which did not ovulate following CIDR removal was successfully induced to ovulate by administration of GnRH, thus emphasizing the value of a second GnRH in ensuring that ovulation occurs (Ambrose et al., 2004).

Another possibility is the GnRH given on Day 0 may have increased the risk for the presence of a CL on Day 21. Bartolome et al. (2005b) treated cystic cows with 4 protocols. All of those protocols included a PGF injection on Day 7 and a GnRH injection on Day 9, 16h prior to timed insemination, but with or without a GnRH injection on Day 0 and with or without the insertion of a CIDR for 7 days beginning on Day 0. In that study, cystic cows which did not receive a GnRH injection on Day 0 tended to be at a decreased risk for the presence of a CL on Day 17 compared to cows

which received GnRH (AOR=0.1; P=0.06). Although the present study used different protocols than those used by Bartolome et al. (2005b), it is possible that similar to those findings, the 1st GnRH given on Day 0 could have been the difference between the two protocols which led to more cows with a CL on Day 21.

The percentage of cows returning to cyclicity in the Ovsynch group in this study (83%) was similar to that found by Ambrose et al (83% or 15/18; 2004) and slightly greater than that found by Fricke and Wiltbank (73.1%; 1999). Return to cyclicity rates in cystic cows treated with various progesterone protocols have been reported to be between 61.5- 100% (Johnson and Ulberg, 1966; Zulu et al. 2003; Todorki et al., 2001; Calder et al., 1999). This variation is likely due to the variable number of cows used, and the application of different protocols. The present study included more cows and used a progesterone protocol slightly different than previous reports, yet achieved similar results (79%- return to cyclicity).

Return to cyclicity in the present study was determined by the presence of a CL on Day 21, or 11 days after expected ovulation. Serum progesterone values were obtained at the time of diagnosis to evaluate the accuracy of palpation per rectum and ultrasonography in determining the presence of a CL. Based on a serum progesterone value ≥ 1 ng/ml for the true presence of a CL, palpation per rectum and ultrasonography had a sensitivity, specificity, and positive predictive value (PPV) of 97%, 39.7%, and 73.3%, respectively. This indicated that although palpation per rectum resulted in very few cows diagnosed with the absence of a CL while having high serum progesterone (false negatives); there were many more cows diagnosed with the presence of a CL while having low serum progesterone (false positives). The accuracy of palpation per rectum

for the diagnosis of a CL in the present study (PPV= 73.3%) is slightly less than previous reports (87%- Archbald et al., 1992; 79%- Archbald et al., 1993a). A possible explanation for this is that by day 11 or 12 of the estrous cycle, when morphological evidence of a CL exists, a proportion of these may be undergoing functional regression. Although a normal CL usually begins regression around day 14 of the estrous cycle (Stevenson, 1997), it may occur early in a proportion of cows with shorter estrous cycles. Furthermore, a significantly higher proportion of anovular cows (23%) have short luteal phases (<11 days) following the Ovsynch protocol than ovular cows (6%; Gumen et al., 2003). Thus, it is possible that because of their progesterone deficient state, more cows in the Ovsynch group were experiencing short luteal phases and regression by 11 days after ovulation. It was interesting that in the present study, 75% (6/8) of cows with progesterone between 0.5 and 1.0ng/ml were diagnosed with a CL by palpation per rectum. This suggests there may have been a proportion of cows with morphological features of a CL, yet these corpora lutea were producing less progesterone than expected.

Treatment significantly influenced progesterone concentrations obtained on Day 21 at the time of CL diagnosis. Despite the fact that cows in the Ovsynch group were more likely to be diagnosed with the presence of a CL (OR=2.2; 95% CI=1.04 – 4.81), cows in the CIDR group had a significantly higher mean progesterone concentration (2.25 vs. 1.64ng/ml; P=0.045). There are two possible explanations for this occurrence. Firstly, more cows in the CIDR group may have had intermediate levels of progesterone as a result of increased luteinization of the cyst, thus artificially raising progesterone concentrations in the absence of a CL. If this was the case, mean progesterone concentration for cows diagnosed with the presence of a CL would be similar in both

groups, while mean progesterone concentration for cows diagnosed with the absence of a CL would be higher in the CIDR group reflecting luteinization of the cyst. Results of the least squares means for the interaction of treatment and CL indicate the opposite effect. Cows in the Ovsynch and CIDR groups had similar progesterone values when diagnosed with the absence of a CL (0.8 and 0.5ng/ml, respectively), yet when cows were diagnosed with the presence of a CL, the progesterone values were considerably more influenced by treatment (2.6 vs. 3.3ng/ml, respectively). Secondly, it is possible that corpora lutea induced by the Ovsynch protocol secrete less progesterone than corpora lutea resulting from a natural GnRH/LH surge following the withdrawal of exogenous progesterone.

The finding of the present study that corpora lutea induced by the Ovsynch protocol secrete less progesterone than corpora lutea induced by the CIDR protocol can be explained by, but is not limited to, two possible physiological mechanisms. One possibility is that progesterone pretreatment in cystic cows may have a priming effect on the hypothalamus to improve the GnRH/LH surge, tonic LH support for the CL, and/or alter the ability of the CL to respond to LH through changes in receptor dynamics. This theory is supported by work completed in the postpartum cow, which is also in a progesterone deficient state prior to ovulation and similar to cows with cystic ovarian disease. Postpartum cows pretreated with progesterone were less likely to experience a short luteal phase after GnRH induced ovulation (Rutter et al., 1985), or a natural ovulation (Ramirez-Godinez et al., 1981), than cows without progesterone pretreatment. Similarly, postpartum beef cows which received a progesterone implant prior to an induced ovulation had a profile of progesterone concentration that was higher over the following 18 days than those not receiving a progesterone implant. Gumen et al. (2003)

found that anovular cows (cows without a CL) were more likely to have short luteal phases following treatment with the Ovsynch protocol than ovular cows. These results suggest the possibility that cows in the CIDR group, where a greater proportion were under the influence of progesterone prior to ovulation, may have had improved CL function and a greater serum progesterone concentration by day 11 after ovulation.

The other possibility is that there was a difference between the ovulatory follicle of the Ovsynch and CIDR groups at the time of ovulation, perhaps in size or developmental stage, which altered the functionality of the future CL as it matured. The Ovsynch protocol results in ovulation of follicles which can be a variety of sizes and with a significant proportion ≤ 11 mm (26%) compared to the other sizes (Perry et al, 2005). In the same study, the majority of spontaneously ovulating follicles were > 13 mm. Furthermore, follicles induced to ovulate which were less than 12.8 mm had significantly lower serum estradiol on the day of insemination, a slower rise in serum progesterone, and tended to have lower progesterone concentrations and increased embryonic mortality (Perry et al., 2005). In cows that ovulated spontaneously, follicle size had no effect on progesterone or fertility. Another study determined that the post-ovulatory progesterone rise can be delayed by inducing luteolysis in the presence of a pre-ovulatory follicle < 10 mm (Robinson et al., 2005). This resulted in a longer follicular phase prior to ovulation and a smaller ovulatory follicle than when luteolysis was induced in the presence of a follicle > 10 mm. This smaller ovulatory follicle then formed a CL with decreased serum progesterone production, even though ovulation occurred spontaneously. These results suggest that the physiological maturity of the preovulatory follicle (its steroidogenic capacity, number of granulosa cells, LH receptors) can alter subsequent CL development

and progesterone production. It is possible that in the present study, the Ovsynch protocol induced the ovulation of less mature follicles compared to those which ovulated spontaneously in the CIDR protocol. This lack of maturity in a proportion of follicles may have resulted in corpora lutea with decreased progesterone production, and a significant difference in the serum progesterone concentrations by day 11 after ovulation between the Ovsynch and CIDR groups.

Although the finding that cows in the CIDR group had higher progesterone on Day 21 is intriguing, it was not the intent of this study to evaluate the factors influencing this outcome. Regardless, and perhaps more importantly, the influence of treatment on Day 21 progesterone concentration did not translate into an effect on fertility in the present study. This was despite the finding that for cows diagnosed with the presence of a CL, each 1ng/ml increase in serum progesterone increased the odds for pregnancy by 1.6 times. It is possible that the difference in progesterone concentration would have also resulted in a significant effect of treatment on fertility if more cows had been used in this study or other factors influencing conception and pregnancy rates, such as compliance and heat detection efficiency, were improved. Using the conception rates obtained in this study, 805 cows per group would have been needed to demonstrate a significant difference. The general view that increased CL progesterone production increases the risk for pregnancy is supported by other studies which have shown a positive association (Moore et al., 2005; Perry et al., 2005).

The positive association between BCS and milk production with Day 21 progesterone concentration was not unexpected. It is possible that cows with a low body condition score or low milk production were in a state of negative energy balance at the

time of ovulation. Energy balance influences the amount of cholesterol available for steroidogenesis as well as the enzymatic activity. Thus, it follows that a negative energy balance could result in decreased progesterone production by the CL.

There was no significant association between treatment and conception rate in the present study. The conception rate in the Ovsynch group (18%) was similar to that observed by Bartolome et al. (23.6%; 2000 and 16.7%; 2005b) and Hendricks (19.5%; 2004), but less than that observed by Fricke and Wiltbank (36.8%; 1999) using the same protocol in cows with cystic ovarian disease. The conception rates obtained in response to the Ovsynch protocol in cows with cystic ovarian disease, in this and other studies, continues to be lower than that observed in cyclic cows (33%-Momcilovic et al., 1998; and 46-55%- Pursley et al., 1995).

The conception rate for cows in the CIDR group in the present study was 23%. This result is in agreement with the conception rates found by Zulu et al. (20%; 2003), using a PRID containing 1.55g of progesterone inserted for 12 days, and by Bartolome et al. (27.3%; 2005b), using a CIDR containing 1.38 g progesterone followed by timed insemination. Yet these conception rates are all considerably lower than that observed by Johnson and Ulberg (48.7-52.5%; 1966) using 14 daily injections of 50 or 100 mg of progesterone. This difference is likely due to the fact that cows in that study were observed for estrus over a prolonged period (>45 days), therefore allowing more time for recovery. Bartolome et al. (2005b) achieved a pregnancy rate of 37.5 % in cystic cows by inserting a CIDR for the first 7 days of the Ovsynch protocol. More studies are needed to determine if a CIDRsynch protocol improves conception rate and is more cost effective than the standard Ovsynch protocol in cows with ovarian cysts.

Many studies have reported a 100% ovulation rate following progesterone treatment in cystic cows (Nanda et al., 1991; Todorki et al., 2001; Calder et al., 1999), yet there are few who have reported conception and pregnancy rates, and none of which have used a CIDR protocol similar to the present study. In studies where fertility results have been reported using various progesterone protocols (Johnson and Ulberg, 1966; Zulu et al., 2003; Bartolome et al., 2005b), there was not an obvious improvement compared to results reported using other treatment protocols, such as Ovsynch. The present study provided a direct comparison between a progesterone based protocol and the Ovsynch protocol for the treatment of cystic ovarian disease in one large herd. The findings of the present study indicate that there was not a significant difference in the fertility of cystic cows treated with either the Ovsynch protocol or the CIDR protocol.

The pregnancy rates in the Ovsynch and CIDR groups were 9.5% and 14 %, respectively. Contrary to the expectations of this study, the effect of treatment was not significantly associated with pregnancy rate. The pregnancy rate was defined as the number of cows pregnant divided by the total number enrolled in each group. As previously mentioned, the denominator in the Ovsynch group included cows that were enrolled but not inseminated which is why; contrary to common approaches, the conception and pregnancy rates differ for this group.

Success of treatment, with respect to pregnancy rate, in the CIDR group depended on detection of estrus, which was 44% in this farm. If the insemination rate had been higher in the CIDR group, this may have translated into a higher pregnancy rate. Therefore in a herd with a higher rate of estrus detection, the CIDR protocol may result in a higher pregnancy rate with potentially less labor and cow handling than the Ovsynch

protocol. However, in a herd with a lower rate of estrus detection, the Ovsynch protocol is more likely to be a better treatment for ovarian cysts.

Cows with in the top two categories of milk production on the day of diagnosis (Category 3 and 4) were more likely to conceive following insemination than cows with the lowest level of milk production (Table 11). Typically, higher milk production is associated with an increased incidence of cystic ovarian disease (Johnson et al., 1966; Lopez-Gatius et al., 2002; Bartlett et al., 1986). Yet, the association of milk production with response to treatment or spontaneous recovery has seldom been evaluated. The present results suggest that when cystic ovarian disease occurs in higher producing cows, they have a better response to treatment and improved fertility compared to cows with lower milk production. In partial disagreement with this finding, Lopez-Gatius et al. (2002) found that cows diagnosed with cystic ovarian disease between days 43-49 postpartum with high milk production were less likely to spontaneously recover than cows with low milk production.

Many studies have examined the relationship between milk yield and fertility in normally cyclic cows and, in contrast to the present study, found an antagonistic relationship. Despite this, there are still a handful of studies that disagree with the hypothesis that milk production and fertility are negatively correlated. These studies suggest there are other factors with a greater negative influence on fertility than milk production. Peters and Pursley (2002) found that cows with above average milk production had a greater pregnancy rate in response to the Ovsynch protocol than cows with lower than average milk production (45.8 vs. 33.8%). Similar to the results of the present study, Buckley et al. (2003) found that cows with the 3rd highest estimated 305-d

cumulative solids corrected milk yield were 1.4 times more likely to become pregnant to the 1st service compared to cows with the lowest 305-d cumulative solids corrected milk yield. High milk production does not necessarily imply that the animal is also in a negative energy balance. Low producing cows often have lower dry matter intake and are at a greater risk for anestrus and low fertility than high producing cows (Staples and Thatcher, 1990). It is possible that cows with lower milk production are also experiencing a higher incidence of lameness, mastitis, and periparturient disorders. These other conditions may be affecting their fertility as well as their milk production. It has been speculated that improved reproduction in high-producing cows probably reflects better feeding, healthier cows and improved reproductive management (Lucy, 2001).

A direct comparison between this and other studies with respect to milk production should be done with caution as many studies have analyzed milk production over a longer period, such as the month of diagnosis or 305-day milk yield. The values obtained in the present study reflect the milk production of one day only, the day of diagnosis, and do not indicate how milk production was changing at the time of diagnosis or the potential 305-d yield for the cow. Despite this, the variable used for milk production in the present study had a significant influence on progesterone concentration, conception and pregnancy rates. In the present study, it was a very important explanatory variable for the response to treatment and subsequent fertility of cows with cystic ovarian disease.

An interesting finding in the present study was that primiparous cows were more likely to be diagnosed pregnant after treatment than multiparous cows. Primiparous cows are generally considered to be at a lower risk for cystic ovarian disease than multiparous cows (Whitmore et al., 1974; Bartlett et al., 1986; Hoojer et al., 2001). It is important to

remember that relative risk for the condition does not necessarily relate to how well they will respond to treatment. In partial agreement with the present study, Lopez-Gatius et al. (2002) found that primiparous cows diagnosed with cystic ovarian disease between days 43-49 postpartum were more likely to spontaneously recover than multiparous cows, such that a 1 unit drop in lactation number was associated with a 1.4 increased probability of recovery. Similarly, of cows without ovarian cysts that were treated with the Ovsynch protocol, primiparous cows were more likely to conceive than cows with ≥ 3 lactations (Peters and Pursley, 2002).

In contrast to these findings and to those of the present study, Bartolome et al. (2003) found that in cystic cows pretreated with or without bST and GnRH prior to the Ovsynch protocol, primiparous cows were 0.4 times less likely to conceive than multiparous cows. It is possible that differences in response to treatment between primiparous and multiparous cows in this and other studies may be partially explained by management practices involving primiparous cows. Age at calving, nutrition, stress and housing environment differences between herds may partially explain the variable response to treatment and fertility among primiparous cows in different studies. Again, comparisons and contrasts between this and other studies should be done with caution as many other studies divided cows into 1st, 2nd, and 3rd+ lactations while the present study used only 1st and 2nd+ lactations. In studies that have divided cows by 1st, 2nd, and 3rd+ lactations, many have observed that 1st lactation cows have different results than cows in the 3rd+ lactation, but were not significantly different from 2nd lactation cows.

In the present study, season and BSC were not significantly associated with the response to treatment. As with milk production and parity, there is evidence for an

association between season, body condition score and the incidence of cystic ovarian disease. In one study, cows calving in the summer months were 2.6 times more likely to develop cysts than those calving in the winter (Lopez-Gatus et al., 2002). However, other authors have found no significant effect of season on the incidence of cysts (Bartlett et al., 1986). The effect of season on response to treatment and fertility is less often reported in cystic cattle than in normal cattle. Using the periods of October to December and January to May, Bartolome et al. (2000) did not find an effect of season on the fertility of cystic cows following different treatment protocols in a Florida dairy herd. Typically in subtropical environments such as Florida, fertility is significantly decreased during the summer months (Al-Katanani et al., 1999; Donovan et al., 2003; De Renesis and Scaramuzzi, 2003). The lack of seasonal effect on response to treatment in the present study may be due to successful heat stress management within the study herd. It also may be that cystic cattle are at a decreased risk for the negative effects of heat stress on fertility

A low body condition score, or a change in body condition score over time, has been associated with an increased risk for cystic ovarian disease as well as other metabolic diseases. A 1 unit change in BCS in cows between day 60 prepartum and parturition resulted in an 8.4 times increased risk for developing cystic ovarian disease between days 43 and 49 postpartum (Lopez-Gatus et al., 2002). In another study, there was a significant negative linear relationship between cows with low BCS (<3.25) and the percentage of anovular cows, such that the percentage of anovular cows decreased as the BCS increased (Gumen et al., 2003). Furthermore, in the same study anovular cows with smaller follicles ($<14\text{mm}$) also tended to have the lowest BCS (≤ 2.5). The

association of BCS with fertility in cows without ovarian cysts has been evaluated more frequently than its association with fertility in cows with ovarian cysts. In the present study, the only significant relationship involving BCS was a positive association with serum progesterone on Day 21 ($P=0.047$; Table 10).

In a study combining the results of cows with ovarian cysts and cow in proestrus, cows with a BCS >3.0 had an increased risk for the presence of a CL 7 days after timed insemination compared to cows with a BCS <3.0 (Bartolome et al., 2005b). In the same study, cows with a BCS > 3.0 also tended to have an increased risk for pregnancy on days 30 and 55 after insemination. In another study using normally cyclic cows, cows with low BCS (≤ 2.5) were less likely to be inseminated during the first 3 weeks after the voluntary waiting period than cows with higher BCS, and cows that reached a low nadir BCS (≤ 2.5) had a reduced likelihood for pregnancy to the first service (Buckley et al., 2003). It is possible that the lack of association between BCS and fertility in the present study was because BCS was only recorded on one occasion, on the day of diagnosis, which does not indicate the direction of change or the energy balance of the cow at that time. If detected, a declining BCS would indicate a negative energy balance and would be more likely to negatively affect fertility.

After enrollment, more cows were lost than expected throughout the study period. These losses were the result of death, disease and culling decisions. A total of 11 cows were inseminated but lost prior to pregnancy diagnosis and a total of 64 cows were unavailable on Day 21 for the diagnosis of a CL (Table 4). Many of the cows unavailable for CL diagnosis were in the hospital barn or missed being separated on the day of the reproductive examination. It was anticipated that a small percentage of cows would be

lost after enrollment. It was for this reason that more cows were originally enrolled (n=401) than required to show a significant difference based on sample size calculations (n=260). Due to the additional cows enrolled, it is unlikely that the cow losses experienced were detrimental to the results.

Compliance within the Ovsynch protocol (18% were not inseminated) was less than expected. Some potential reasons for the lack of insemination have been previously mentioned. If compliance had been better within the Ovsynch protocol, the results would favor the Ovsynch protocol. For example, if compliance was 100% and conception rate was unchanged, pregnancy rates for the Ovsynch and CIDR groups would have been 18 and 9.5%, respectively. There would be a statistically significant difference between these two pregnancy rates and cows in the Ovsynch group would have had an increased likelihood to be diagnosed pregnant once enrolled in the study.

In conclusion, fertility was not statistically different between cystic cows treated with either the Ovsynch protocol or exogenous progesterone and inseminated at an induced estrus. Cows treated with the Ovsynch protocol were more likely to return to normal cyclicity than cows treated with the CIDR protocol. Despite a decreased likelihood for cows in the CIDR group to be diagnosed with the presence of a CL, cows in this group had higher average progesterone concentrations 11 days after ovulation, and corpora lutea induced by the CIDR protocol secreted more progesterone than those induced by the Ovsynch protocol. Primiparous cows had an increased likelihood for conception than multiparous cows, regardless of treatment. Cows in the 3rd and 4th quartiles of milk production on the day of diagnosis were more likely to conceive than those in the 1st quartile, regardless of treatment.

SUMMARY AND CONSLUSIONS

A total of 401 lactating dairy cows with ovarian cysts were treated with either the Ovsynch protocol or a protocol using a CIDR. Cows in the Ovsynch group were timed-inseminated while cows in the CIDR groups were inseminated at an induced estrus. The proportion of cows in each group that were inseminated, returned to normal cyclicity, and became pregnant was compared using logistic regression. Serum progesterone concentration was determined 11 days after expected ovulation (at the time of CL diagnosis). Cows in the Ovsynch group were more likely to be inseminated and more likely to return to normal cyclicity compared to cows in the CIDR group. However, cows in the CIDR group had higher progesterone concentrations 11 days after expected ovulation compared to cows in the Ovsynch group. There was no difference between the two treatment protocols in either the conception or pregnancy rates. Factors which were significantly associated with fertility were parity and milk production on the day of diagnosis. Primiparous cows and cows with higher milk production were more likely to become pregnant than multiparous cows or cows with lower milk production on the day of diagnosis.

In conclusion, there was no difference in fertility between cows with ovarian cysts treated with either the Ovsynch or CIDR protocols. However, cows treated with the Ovsynch protocol were more likely to return to normal cyclicity.

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BIOGRAPHICAL SKETCH

Mary Bronwyn Crane was born on August 3, 1978, in Prince Edward Island, Canada. She grew up in the small town of Kensington, P.E.I., where her family had a small farm and raised purebred beef cattle. She had a strong inclination towards animals and enjoyed working with her father who was a rural veterinarian. After completing high school in Kensington, she attended Acadia University in Wolfville, Nova Scotia, enrolled in a Bachelor of Science program. Within two years she completed the pre-veterinary curriculum at Acadia University, then was accepted at the Atlantic Veterinary College in Charlottetown, P.E.I. Upon graduation from veterinary college in 2002, she moved to Gainesville, Florida, where she joined a small animal practice as an associate veterinarian. After one year of small animal practice in 2003 she decided to pursue her long time interest in food animal practice and applied to the Farm Animal Reproduction and Medicine Service Internship program at the College of Veterinary Medicine, University of Florida, and was accepted. After completing the one year internship in 2004, she enrolled in the graduate program at the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, to obtain the degree of Master of Science.