Applying New Concepts in Managing Fertility

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During the past few years, research in reproductive physiology and endocrinology has resulted in several new discoveries that have important implications in the management of fertility in dairy cattle. The purpose of this presentation will be to review some of these research findings and to discuss ways that this information might be used for improving reproduction in dairy herds now and in the future.

The Hypothalamic-Pituitary Axis

The hypothalamus at the base of the brain is the site of synthesis and secretion of many peptides that either act at the anterior pituitary to control release of pituitary hormones or that are transported to the posterior pituitary for storage and subsequent release. These peptides are synthesized in nerve cell bodies localized within specific regions of the hypothalamus referred to as nuclei. These peptides are transported through axons to terminals where they are released either into the portal vessels leading to the anterior pituitary or into the circulation leading from the posterior pituitary.

It is now recognized that these peptides are released in episodic pulses at various frequencies that are controlled by sensory input into the hypothalamus. The frequency of release of these peptides has profound effects on the reproductive system and other endocrine systems.

One peptide that is of central importance in regulation of reproduction is gonadotropin releasing hormone (referred to as GnRH or LHRH). The frequency at which GnRH is released affects the onset of estrous cycles in the postpartum cow and the regulation of follicular growth during subsequent estrous cycles. In the postpartum cow, the frequency of GnRH release increases from a low rate just after calving to about one pulse per hour prior to first ovulation. It is thought that this hourly frequency is necessary for initiation of sufficient LH release so that follicular growth can occur prior to first ovulation. After ovulation, the frequency of GnRH release decreases to about one pulse per four to six hours during the middle of the cycle. As the next estrus approaches, release of GnRH increases at a rapid rate and it is thought that the preovulatory surge of LH is caused by pulses of GnRH that occur at frequencies of more than one per hour.

Several factors affect the frequency of GnRH release in the postpartum cow. For example, it is thought that suckling by a calf slows the rate of release as does inadequate energy intake. These factors appear to be mediated within the central nervous system by the endogenous opioid peptides, endogenous hormones that act like morphine. These opioids apparently act on GnRH-secreting neurons to alter the rate of GnRH release from the terminals of the axons. Recent studies with several species have shown that the rate of GnRH release can be altered by using exogenous agonists or antagonists of these opioids. In the future, it is likely that some of these products will be available for stimulating earlier cycling in the postpartum cow. At present, these products are still considered as experimental compounds.

The anterior pituitary responds to the hypothalamic releasing peptides by secreting the appropriate pituitary hormone. In terms of reproduction, the key pituitary hormone are LH and FSH. It is now known that these two hormones are controlled by somewhat divergent mechanisms. For example, LH appears to be controlled almost exclusively by GnRH because pulses of LH in the peripheral circulation apparently are nearly always preceded by GnRH release from the hypothalamus. In contrast, FSH secretion is stimulated by GnRH and inhibited by inhibin, a peptide produced by developing follicles. Inhibin acts at the pituitary to reduce the amount of FSH released in response to GnRH. It is thought that inhibin plays an important role in selecting the follicle that will ovulate after onset of estrus. In the future, it may be possible to alter the number of follicles destined to ovulate by treating cows with inhibin or with antagonists of inhibin. Recent studies in sheep have shown that following treatment with inhibin, there is an increase in ovulation rate as the system overcompensates after the negative influence of inhibin is relieved. Whether such treatments may be useful for superovulation of donor cows remains to be determined.

The ability of the anterior pituitary to respond to GnRH seems to depend on the number of GnRH receptors that are located on the membranes of the pituitary cells. These receptors bind the GnRH and then release the gonadotropins. A relatively new concept about how these receptors function is that exposure to GnRH causes either an up-regulation or down-regulation in the number of receptors. For example, when the gonadotropin-secreting cells are first exposed to increasing output of GnRH from the hypothalamus, there is an increase in receptor numbers and this leads to increased output of LH in response to a fixed dose of GnRH. As output of GnRH continues, there is a down-regulation of receptors so the response to GnRH

decreases. Unfortunately, one seldom knows whether the pituitary is in an "up" or "down" mode, so it is difficult to predict the response to a therapeutic dose of GnRH. This helps explain the variable response that one sees when GnRH is given to induce ovulation in the postpartum cow or when it is given at insemination or to cows with cystic follicles. Estrogen also apparently plays a role in regulating these receptors because estrogen priming seems to promote more receptors. Thus a cow that is under the influence of estrogen at the time of treatment with GnRH will probably show a greater response than one which is under the influence of progesterone or one which has inactive ovaries.

Other factors may affect the pituitary's response to GnRH. It has been shown experimentally that administration of adrenocorticotropin (ACTH) or the adrenal steroids reduce the ability of GnRH to promote release of LH. Conditions that are stressful for cattle and that lead to an increase in secretion of ACTH and the adrenal steroids may dampen the cow's response to therapeutic doses of GnRH.

Recent Concepts of Ovarian Hormone Secretion

In recent years it has become obvious that the number of hormones produced by the ovary is much greater than previously thought. The roles of these hormones are just beginning to be understood. The ovary is the primary source of estrogens and progesterone, but it also produces an array of proteins and peptides that apparently are involved in controlling both the development of follicles and the function of the corpus luteum (CL).

Ovarian follicles begin to secrete significant quantities of hormones when they reach the early antral stage. In the cow, the number of follicles at this stage at any point in time exceeds 20. The developing follicle is divided into two cellular compartments, the granulosal cells and the thecal cells. As the follicle grows, the thecal cells bind LH from the anterior pituitary and this stimulates the secretion of androgens. In contrast, the granulosal cells bind FSH, which promotes the conversion of androgens to estrogens. The granulosal cell also appear to be the site of synthesis of inhibin, but it is unknown how inhibin secretion is controlled. Just prior to ovulation, the granulosal cells develop the capacity to bind LH, and this is apparently involved in the process of ovulation.

Studies in several species have shown that the follicles also produce an FSH binding inhibitor that blocks binding of FSH to the ovarian cells, a GnRH-like peptide that may be involved in regulating the number of follicles that are allowed to develop, an oocyte maturation inhibitor that may control development of the oocyte, and a follicle regulatory protein that may affect the conversion of androgen to estrogen. It is obvious that control of hormone secretion by the developing follicles is quite complex, and it is likely that we will not be able to control follicular development with precision until we understand how all of these hormones interact in controlling follicular development.

In cows with cystic follicles, it is clear that the function of the primary developing follicle has been altered. Some recent studies suggest that cystic follicles have degenerating granulosal cells that do not respond to the appropriate pituitary hormones. In such cows, it seems that promotion of the development of a new follicle or ovulation of another smaller developing follicle by treatment with GnRH or chorionic gonadotropin is necessary to resolve the condition. It is unclear why some cows with cystic follicles fail to respond to appropriate therapeutic treatment.

The corpus luteum of the ovary forms after ovulation and begins to secrete appreciable amounts of progesterone within two to four days. It is now known that the corpus luteum also produces at least two other hormones, oxytocin and relaxin.

The CL is formed from both the granulosal and thecal cells of the ovulated follicle. The increase in size of the CL during the early part of the estrous cycle is primarily the result of an increase in the size of these cells rather than in the number of cells. These cells are referred to as luteal cells once the CL is formed. It is now thought that the granulosal cells form what are known as large luteal cells and the thecal cells form small luteal cells.

The large and small luteal cells differ markedly in how they respond to hormones. For example, the small luteal cells will respond to LH by increasing their output of progesterone, but the large luteal cells are unresponsive to LH. In contrast, the large luteal cells will respond to prostaglandin F (PGF) by decreasing progesterone output, but the luteal cells are unresponsive to PGF. The large luteal cells, but not the small cells, respond to prostaglandin Es (PGEs) by increasing output of progesterone. There is also some research which suggests that the large cells may produce a cytotoxic substance that destroys small cells during luteolysis. Finally, there is evidence that some small cells may develop into large cells as the estrous cycle progresses and this process apparently continues during gestation.

These recent findings about the different responses of large and small luteal cells to various hormones have important implications in terms of therapy and in terms of recognition of pregnancy (to be discussed later). As we increase our understanding of how these cell types interact, we should be able to improve methods for synchronizing estrus and inducing luteolysis precisely. In this regard, it is now known that the CL (apparently the large luteal cells) produces oxytocin in appreciable amounts during luteolysis. Current theories suggest that the luteal oxytocin travels to the uterus to cause release of PGF at the appropriate time of the estrous cycle.

In addition to oxytocin, the CL produces relaxin, a protein hormone, that plays an important role in dilation of the cervix and relaxation of the pelvic ligaments at parturition. In the future it is likely that relaxin may be useful for reducing the incidence of difficult deliveries, especially in smaller-sized cows.

Role of the Uterus in Luteolysis

For several years it has been known that the uterus produces a luteolytic hormone that causes the regression of the CL at the end of the estrous cycle. It is now generally accepted that this factor is PGF. It is now believed that PGF from the uterus is secreted in a pulsatile fashion and that the pulses of PGF are driven by pulses of oxytocin from the ipsilateral (adjacent) ovary. The ability of oxytocin to stimulate PGF secretion is dependent on oxytocin receptors in the uterus. Estrogen from midcycle follicles is thought to induce the formation of oxytocin receptors, but this is apparently affected by the midcycle levels of progesterone. If progesterone is too high, then the number of receptors is apparently lower, but as progesterone begins to decline, the number of receptors increases, leading to an increase in secretion of PGF. The PGF apparently stimulates more oxytocin release from the CL and this cascade continues until luteolysis is complete. Several factors, including uterine infections, apparently interfere with this process and lead to prolonged or irregular estrous cycles.

Follicular Development

Follicular development in cattle is a dynamic process that is characterized by continual recruitment of small follicles and continual atresia of larger follicles. At any point in time, the ovaries of the cow contains numerous small follicles undergoing initial development, a moderate population of medium-sized follicles in various stages of development, and one or two large follicles referred to as the dominant follicles.

It is unknown why inactive primary follicles are recruited to develop. The ovaries of cattle contain thousands of these inactive follicles, and some begin development at regular intervals beginning during fetal development. In fact the number of small inactive follicles in the ovaries is highest about midgestation in the fetus and there is a continual decline in the number of these follicles throughout the life of the cow.

During the estrous cycle, there appear to be three waves of follicular development, only one of which results in the formation of a dominant follicle that will ovulate following the next heat. The first wave of follicular development begins after estrus and one or two dominant follicles will appear near the end of the first week after heat. The role of these follicles is not clear, but they produce appreciable amounts of estrogen that may be involved in transport of gametes. These dominant follicles undergo atresia without ovulating. Another wave of follicles appears about midcycle, and estrogen secretion from these may be involved in inducing oxytocin receptors on the uterus for subsequent luteolysis. We have demonstrated recently that one of these follicles can be ovulated if a cow is given 5,000-10,000 units of chorionic gonadotropin on day 10 of a cycle. The last wave of follicle development occurs near the end of a cycle and one dominant follicle from this wave will mature and ovulate following the next heat.

The mechanism whereby dominant follicles suppress the development of other follicles is unknown, but dominant follicles have some unique characteristics. For example, the dominant follicles secrete the majority of estrogen that is produced by both ovaries and they bind both LH and FSH in greater amounts than smaller follicles. Apparently local regulatory mechanisms within the ovary give certain follicles an advantage during development so that they become the dominant follicles and suppress the development of less competitive follicles.

Since follicular development is a dynamic process, it is clear that one cannot predict accurately by palpation which follicle will ovulate. In studies which utilized injections of charcoal into the stroma of developing follicles, it has been observed that the follicle which will ultimately ovulate can only be identified within about 48 hours prior to estrus. Thus it is doubtful that palpation can be used to predict time of estrus and ovulation unless the cow is palpated at least once daily. Follicles that are palpated in the presence of a functional corpus luteum are not the ones which will ovulate after estrus. The largest follicle present on the ovaries at the onset of estrus is the one which will ovulate in most cases.

Prediction of the Time of Ovulation

It is now clear that ovulation occurs about 25 to 30 hours after the preovulatory LH surge. It is not possible under practical conditions to determine when LH levels increase in the blood, but the occurrence of the LH surge is closely linked to the onset of estrus. This link is caused by the fact that increasing levels of estrogen that induce estrus also induce the LH surge through the positive feedback mechanism. In general the LH surge occurs from a few hours before to a few hours after onset of estrus, so the best way to know when the LH surge occurs is to know as accurately as possible when heat begins. Since the LH surge is temporally related closely to onset of heat, one can use onset of heat to predict time of ovulation. It should be pointed out that the end of estrus is not strongly related to the time of ovulation because the duration of estrus varies considerably among cows. There is little information available on the incidence of delayed ovulation or what might cause delayed ovulation. The data that are available suggest that delayed ovulation is of minor importance in terms of fertility.

Sperm Transport

Recent studies on sperm transport in several species suggest that some of our old concepts of sperm transport may be incorrect. It now appears that it takes several hours after insemination for the sperm that will ultimately fertilize the egg to reach the ampulla of the oviduct, where fertilization occurs. In those studies, the oviduct was ligated at various times after mating to determine how long it took for a sufficient number of sperm to reach the uterine end of the isthmus of the oviduct, the temporary storage site for

sperm. In general it took 6 to 8 hours for sperm to accumulate in this site in sufficient numbers to give normal fertility. These findings have practical importance in terms of timing of inseminations. Inseminations should be given about 6 to 12 hours after onset of heat for sperm to reach the target site and undergo appropriate changes (capacitation) before being able to fertilize the egg.

Maternal Recognition of Pregnancy

The process whereby the conceptus exerts an effect on the dam to prevent regression of the CL is beginning to be understood in cattle. It appears that several mechanisms may be involved. Around day 15 to 16 after mating, the conceptus apparently produces proteins which exert local effects within the uterus to reduce the amount of PGF released by the uterus. These proteins may block oxytocin receptors on the uterine lining and thereby block the ability of oxytocin to cause release of PGF. The conceptus may also secrete PGEs that travel to the ovary to stimulate progesterone secretion by small luteal cells, thus maintaining the function of the CL. As these mechanisms become clearer, it might be possible in the future to improve embryo survival by altering some of these processes.

Paper presented at the Dairy Herd Health Programming Conference (1986), University of Minnesota, St. Paul, MN, Dr. James Hanson, Coordinator.