ENDOCRINE CONSEQUENCES OF SUPEROVULATION IN RUMINANTS

INTRODUCTION

There are a number of ways to superovulate domestic animals. Each has its advantages and disadvantages. The major commercial products are equine chorionic gonadotrophin (eCG, previously called PMSG) and FSH. Commercial preparations are partly purified from mares serum and porcine pituitary glands, respectively. The basis of their action is to bind to and activate FSH receptors on granulosa cells of small and medium sized follicles, and to stimulate the continued growth of these follicles, likely by inhibiting apoptosis (see ref 1).

One of the undesirable side effects, particularly of eCG, is an inherent LH bioactivity in these preparations. Commercial FSH contains trace LH contamination, although newer formulations contain less than some older ones (e.g. Folltropin compared to FSH-P). ECG, due to its structure, reacts quite well with ruminant LH receptors, and thus has inherent FSH- and LH-bioactivity.

It is well known that ovaries are endocrine organs as well as reproductive organs. The corpus luteum (CL) synthesizes and secretes large quantities of progesterone. The CL also possesses large numbers of LH receptors. Follicles synthesize a number of steroids, and the major secretory product is estradiol. The secretion of estradiol is under the control of FSH, whereas the secretion of progesterone from the CL is under the control of LH. Thus it can be seen that superovulatory protocols are likely to alter ovarian steroid release. Here we will review the effects of superovulation on reproductive hormone secretion in ruminants.

EFFECTS OF SUPEROVULATION ON STEROID SECRETION

The most well-known effect of superovulation is the increase in plasma progesterone and estradiol concentrations during eCG-induced superovulation (e.g. ref 2). Progesterone concentrations increase during the luteal phase of the cycle, and remain higher in eCG-stimulated animals even after prostaglandin-induced luteolysis. Progesterone concentrations are not significantly affected by superovulation with FSH preparations containing little LH contamination (Folltropin, for example).

Superovulation also increases plasma estradiol concentrations, and generally the stimulation is greater with eCG than with FSH. As the ruminant CL does not secrete estradiol, this steroid is clearly of follicular origin. Few studies have been able to demonstrate any significant effect of superovulation on plasma testosterone or androstenedione concentrations. This is likely because the bulk of these steroids are not secreted into the blood, but directed to the granulosa cells for conversion into estradiol.

Superovulation increases the number of growing follicles, thus the increase in plasma estradiol concentrations could be the result of an increase in healthy estrogen-secreting follicles, or it could be due to a direct stimulation of steroidogenesis in the follicles present. Studies in sheep have shown that individual follicles from eCG-stimulated animals secrete more estradiol in culture that follicles from non-eCG treated sheep, suggesting increased steroidogenesis in follicles does contribute to the elevated plasma steroid concentrations (ref 3).

A series of studies at the molecular level in cattle investigated the mechanism of the increase in follicular steroidogenesis caused by eCG. It would seem that eCG stimulates expression of the genes encoding the enzyme cytochrome P450 17alpha-hydroxylase and the steroidogenic acute regulatory protein (StAR). These steps in the steroidogenic pathway are under the control of LH, and thus were stimulated by the LH-activity of eCG. Superovulation with FSH did not affect mRNA levels of any of the steroidogenic enzymes. Collectively, this suggests that eCG stimulates the conversion of cholesterol to progesterone (increase in StAR) and the conversion of progestins to androgens (increase in P450 17alpha-hydroxylase). The increased androgen production creates a larger reserve of precursor for estradiol synthesis, and hence

plasma estradiol concentrations are higher in eCG- compared to FSH-stimulated animals (ref 4). The increase in plasma estradiol observed in FSH-stimulated animals compared to non-stimulated animals is likely due to an increased number of follicles.

There are undesired consequences of increased estradiol production with eCG treatment. Owing to the long half-life of eCG, follicles are still being stimulated after ovulation, and estradiol levels remain high. High early luteal-phase concentrations of estradiol have deleterious effects on the oviduct, decreasing fertility. Thus the use of anti-eCG serum as part of the superovulatory protocol has become popular. The very high estradiol concentrations seen in FSH-stimulated buffalo may also be involved in the poor embryo recovery rates observed in this species. A further complication is seen in goats superovulated with eCG; in this species, luteal failure following ovulation is common, and has been linked to elevated estradiol concentrations in the early luteal phase. It is believed that high estradiol levels set off the prostaglandin-induced luteolytic cascade.

EFFECT OF SUPEROVULATION ON OTHER FOLLICULAR HORMONES

There are two other follicular hormones that have been measured following superovulation in cattle, and as is the case for steroids, secretion increased during superovulation. The better known of the two is inhibin. Inhibin is a dimeric protein that selectively inhibits FSH secretion. Very few reliable reports of inhibin secretion are available in ruminants, as current assays are not sensitive enough to measure dimeric (and biologically active) inhibin. However, it is known that inhibin bioactivity and dimeric inhibin concentrations are increased during superovulation.

The other hormone measured is gonadotropin surge attenuating factor (GnSAF), a protein that attenuates the magnitude of the preovulatory LH surge. Although this hormone has not yet been isolated and characterized, bioactivity in blood of humans and cattle has been shown to

increase during FSH-induced superovulation. It is unknown at present if the increases in these hormones is due to an increased number of follicles, or also to an increase in their secretion.

EFFECTS OF SUPEROVULATION ON LH SECRETION

The most obvious and reported effect of superovulation on LH secretion is an inhibition or complete absence of the preovulatory LH surge (ref 6). The reason for this is unknown, but may be related to an observed down-regulation of follicular LH receptors, to the increased release of GnSAF, or potentially to a down-regulation of pituitary GnRH receptors. It is also well-known that the interval between prostaglandin-induced luteolysis and the LH surge is shorter in superovulated compared to control cattle, and may be shorter in eCG- compared to FSH-stimulated animals. This information is of interest, as animals with unusually early or late surges, or absent surges, produce significantly fewer embryos.

Mean LH concentrations measured before the preovulatory surge were reported to be affected by superovulation in some but not all studies. Mean LH concentrations, however, are not always an accurate measure of endogenous LH secretion, as LH is secreted in discrete pulses. LH is necessary for follicle development, but too much stimulation by LH bioactivity can be deleterious, as observed above for eCG. Thus the effects of superovulation on LH secretion and consequences for embryo development are of practical as well as academic interest.

Only a few reports have examined the effects of superovulation on pulsatile LH secretion (reviewed in ref 7). One study of LH pulse secretion during FSH-P or eCG superovulation in lactating cows showed no significant differences between control and stimulated animals, and no change in LH pulse frequency between luteal and follicular phases, which is unusual.

The other reports in the literature all support an effect of eCG or FSH treatment on pulsatile LH secretion. LH pulse frequency is significantly reduced by either superovulatory

protocol, to the point where pulse frequency was completely abolished in some animals. Although most studies examined animals during the induced follicular phase of the stimulatory cycle, it has also been shown that LH pulse frequency is severely dampened within 8 hours of starting superovulation, thus during the luteal phase of the cycle.

What could cause such a rapid decrease in LH pulse frequency? It is not a direct effect of eCG or FSH on the pituitary gland, as these preparations do not affect LH secretion in short-term ovariectomized animals. Indirect evidence also suggests that ovarian follicles may mediate the effect, as hyper-stimulation immediately following ablation of visible antral follicles does not result in suppressed LH pulse frequency. The major follicular candidates are estradiol and progesterone, which are stimulated by superovulation and which are classical regulators of LH pulse secretion. However, theweight of evidence is against progesterone: first, LH pulse frequency is suppressed by FSH, even though progesterone is not stimulated by this treatment; second, LH pulse frequency is equally suppressed in animals for which progesterone concentrations were increased or decreased (use of PRID/CIDR). A detailed examination of the time-course of changes in steroid secretion and LH pulses failed to demonstrate that plasma estradiol concentrations increased before LH pulse frequency decreased, so there is no hard evidence to suggest that estradiol is a major mediator. It is likely to be a combination of steroids and maybe undefined factors such as GnSAF.

What is the relevance of changes in endogenous LH secretion to the embryo transfer industry? It is known that higher levels of LH-activity in superovulatory drugs have a deleterious effect on the number of oocytes or embryos produced, and yet a minimum amount is required for optimal follicle maturation. So does the suppression of endogenous LH pulses act to prevent the accumulation of too much circulating LH bioactivity, or does it reduce LH activity to below a minimum required for follicle/oocyte development?

Alas, the answer is not clear at this time. An attempt to increase LH pulse frequency during superovulation by injecting small 'pulses' of GnRH did not affect the number of growing follicles or plasma estradiol concentrations, however these animals failed to ovulate and thus no embryos were recovered. It is likely that this treatment down-regulated pituitary GnRH receptors, and thus the animals were unable to generate preovulatory LH surges.

CONCLUSIONS

The data summarized above lead us to conclude that, during ovarian hyperstimulation in cattle, plasma progesterone, estradiol, inhibin and GnSAF concentrations are increased, and there is a concomitant reduction in endogenous pulsatile secretion of LH. The exact cause of the suppression of LH remains unknown. The effects on the reproductive system of these various endocrine changes should not be ignored, as they may contribute to the great variation encountered in standard superovulatory protocols.

KEY REFERENCES

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