Superovulation of Beef Heifers with Follicle Stimulating Hormone or Human Menopausal Gonadotropin: Acute Effects on Hormone Secretion

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Summary

The effects of superovulatory treatment (follicle stimulating hormone [FSH] versus human menopausal gonadotropin [HMG]) and of route of administration (intramuscular versus intravenous) of prostaglandin F_2a (PGF_2a) on hormonal profiles were determined in 32 Angus x Hereford heifers for breeding and subsequent embryo collection and transfer. Heifers were superstimulated either with FSH (total of 26 milligrams) or HMG (total of 1,050 international units) beginning on days 9 to 12 of an estrous cycle and PGF_2a (40 milligrams) was administered at 60 and 72 hours after the beginning of superovulatory treatments. Heifers were artificially inseminated three times at 12-hour intervals beginning 48 hours after PGF_2a treatment. Blood serum samples were collected immediately before treatments began and at frequent intervals until embryo collection 288 hours later. Concentrations of luteinizing hormone (LH) and FSH were not affected by hormone treatments, route of PGF_2a injection, or interactions between them. Estradiol-17ß (E_2-17ß) levels were higher in HMG- than in FSH-treated heifers 60 hours after gonadotropin treatment. Peak concentration of E_2-17ß occurred earlier in HMG- than in FSH-treated heifers and earlier in heifers injected with PGF_2a intramuscularly than those injected intravenously. Progesterone concentrations were not influenced by treatment or route of PGF_2a administration. The progesterone:E_2-17ß ratio was higher in FSH- than in HMG-treated heifers 24 hours after the LH peak. The high steroid hormone concentrations in superovulated beef heifers before and after ovulation may lead to asynchrony between stages of embryonic development, a situation that may interfere with the pregnancy outcome of superovulated embryos in recipient animals.

Introduction

The response in quantity and quality of embryos obtained after superovulation of cattle remains variable and continues to represent a limiting factor in the development of successful embryo transfer programs. This variability in ovarian response has been attributed to genetic factors, dynamics of follicular growth, and differences in luteinizing hormone (LH)- and follicle stimulating hormone (FSH)-specific activities of gonadotropin preparations.

Most of the available information on endocrine changes in superstimulated heifers has been obtained either with pregnant mare serum gonadotropin (PMSG) or pituitary extracts containing FSH. The effectiveness of human menopausal gonadotropin (HMG) as a superovulatory hormone treatment in cattle has been studied, and no differences in ovarian responses have been found when HMG has been compared to FSH or to PMSG. Prostaglandin F_2a (PGF_2a) commonly is administered to superstimulated donors to control the time of estrus and ovulation, and intravenous injection of PGF_2a or a prostaglandin analog seems to favor an endocrine balance and uterine environment compatible with the development of superovulated ova. The objectives of this study were to determine whether superovulatory treatment (FSH versus HMG) and route of administration of PGF_2a (intramuscular versus intravenous) influence the concentration of LH, FSH, estradiol-17ß (E_2-17ß), or progesterone in peripheral blood serum of embryo donor beef heifers.

Materials and Methods

Experimental Animals and Superovulatory Treatments

Thirty-two Angus x Hereford heifers (352 ± 8 kilograms body weight; mean ± standard error) were superstimulated beginning on day 9 to 12 of an estrous cycle. Heifers were divided into two groups of 16 each. Each group was superstimulated either with FSH (total dose of 26 milligrams per heifer; Burns Biotec Laboratories Inc., Omaha, Neb.) or HMG (Pergonal®; total dose of 1,050 international units heifer; Serono Laboratories Inc., Braintree, Mass.). The FSH was injected intramuscularly at a dosage of 4, 4; 3, 3; 3, 3; 2, 2; and 1, 1 milligram a.m. and p.m. per day for five days; HMG was injected intramuscularly at a dosage of 2, 2; 2, 2; 1, 1; 1, 1; and 1, 1 ampoules (1 ampoule = 75 international units FSH activity and 75 international units LH activity) a.m. and p.m. per day for five days. Sixty hours after gonadotropin treatment began, eight females from each group received 25 milligrams of PGF_2a (Lutalyse®; Upjohn Co., Kalamazoo, Mich.) either intramuscularly or intravenously, and 12 hours later, another 15 milligrams of the hormone was given by the same route. Heifers were checked for estrus twice daily, and 60 hours after first PGF_2a injection they were inseminated three times at 12-hour intervals. A blood sample from the jugular vein was

*Deceased.

collected immediately before treatment began, at 12-hour intervals during the first 60 hours, each 4 hours during the next 96 hours, and each 12 hours until the day (a.m.) of embryo collection (Figure 1).

Radioimmunoassay of Gonadotropins and Steroid Hormones

Luteinizing hormone was measured by radioimmunoassay using highly purified bovine LH (bLH, NIH) for both radioiodination with ^{125}I and for standards (25 picograms to 20 nanograms). Assay sensitivity averaged .2 nanograms per milliliter. Intra- and interassay coefficient of variance were 8.0 and 4.8%, respectively. The FSH was measured using USDA FSH-BP1 as the standard. The coefficients of variance for samples containing 14 nanograms per milliliter within assay was 18% and between assay was 17.2%. Progesterone was solvent extracted and percent recovery averaged 90%; sensitivity of the assay was 100 picograms per milliliter. Intra- and interassay coefficients of variance were 9.0 and 11.0%, respectively. Estradiol-17ß was extracted and measured as we previously described. Sensitivity of the assay was 2 picograms. The intra- and interassay coefficients of variance were 12.8 and 12.6%, respectively.

Statistical Analysis

Data were analyzed by split-plot analysis of variance.

Results and Discussion

We focused on the endocrine changes that occurred in embryo donor beef heifers superovulated with FSH and HMG and injected intramuscularly or intravenously with PGF_2a. We observed no significant differences in the numbers of corpora lutea, oocytes, or embryos recovered nor of transferrable embryos after treatment either with FSH or HMG. Embryos obtained from HMG-treated beef heifers established fewer pregnancies, however, than did those from FSH-treated heifers. The circulating concentrations of LH, FSH, progesterone, and E_2-17ß in these heifers are presented in Figure 2.

Luteinizing Hormone

Concentrations of LH were not influenced by treatment, route of PGF_2a administration, or interactions between them. Interval from PGF_2a injection to LH peak was affected by gonadotropin treatment ($p < .05$) and route of PGF_2a administration ($p < .01$). Luteinizing hormone peaked earlier ($p < .05$) in HMG- (47 ± 3 hours) than in FSH- (54 ± 3 hours) treated animals, and those injected intramuscularly with PGF_2a had the LH peak earlier (45 ± 3 hours) than those injected intravenously (57 ± 3 hours; $p < .01$). Peak concentrations of serum LH were not affected by treatment or route of PGF_2a administration (Table 1). The peak LH concentration averaged 147 ± 16 nanograms per milliliters (range 28 to 430) in 31 of 32 heifers. Luteinizing hormone peak concentration, area under the LH peak, and interval from PGF_2a to LH peak were not correlated with the number of corpora lutea palpated, number of oocytes or embryos collected, or number of transferrable embryos.

Follicle-Stimulating Hormone

Concentrations of FSH were not affected by treatment, route of PGF_2a administration, nor the interaction between them (Figure 2); however, there was an interaction ($p < .01$) between time and treatment and between time and route of PGF_2a injection. Concentrations of FSH increased ($p < .01$) 24 hours after initial FSH-, but not after HMG-injection. The assay used here, however, could not differentiate endogenous from exogenous gonadotropins. Concentrations of FSH peaked near estrus and decreased abruptly afterward (Figure 2 and Table 1). Interval from PGF_2a to the FSH peak was affected by treatment ($p < .05$; 49 ± 2 hours in HMG versus 55 ± 3 hours in FSH) and route of PGF_2a administration ($p < .01$; 47 ± 2 hours for intramuscular versus 57 ± 3 hours for intravenous (Table 1). Peak concentrations of FSH were not affected by treatment, route of PGF_2a administration, or interactions between them (Table 1). In this study, 28 of 32 heifers had synchronous LH and FSH peaks. The LH:FSH peak height ratio was similar among treatments and route of PGF_2a administration.

Progesterone

In this study, progesterone concentrations were not affected by treatment or route of PGF_2a administration. A slightly greater increase in progesterone concentrations was seen in the HMG- (twofold) than in the FSH- (1.2-fold) treated heifers 48 hours after gonadotropin treatment, reflecting a possible stimulatory effect of HMG on development of the corpora lutea, perhaps due to its LH-like activity.

Progesterone concentrations dropped markedly after PGF_2a administration and remained low throughout estrus (Figure 2). Progesterone increased steadily after ovulation until the day of embryo collection in most of the heifers. When progesterone concentrations were adjusted to coincide with the LH peak and compared 24 hours before and after it, there was an interaction between treatment and time of LH peak on progesterone concentrations. The FSH-treated heifers injected intravenously with PGF_2a reached basal progesterone levels earlier ($p < .05$) and were lower (.2 nanograms per milliliters) at 12 hours after the LH peak than heifers from the other treatment groups (.6 nanograms per milliliter). Twenty-four hours after the LH peak, progesterone levels began to increase in FSH-treated heifers but they remained at basal concentrations in HMG-treated animals. Progesterone concentrations in superstimulated beef heifers were positively correlated ($r = .46$; $p < .01$) with the number of corpora lutea 64 hours after a synchronous LH and FSH peak and were maintained until the day of embryo collection ($r = .59$; $p < .01$). We collected 56 embryos (22% of the total 262) from five of six animals, most of which (52/56) were

morphologically normal morula and blastocysts. Sixteen of these 52 embryos were transferred to recipients (17% of the total number of embryos transferred, 16/93), and 14 of the 16 transferred embryos did not develop into fetuses.

Estradiol-17ß

Treatment of heifers with FSH- or HMG influences ($p < .05$) E_2-17ß concentrations in peripheral serum, but concentrations were not influenced by route of PGF_2a administration. Concentrations of E_2-17ß were affected by time ($p < .01$), interaction between time and treatment ($p < .01$), and interaction between time and route of PGF_2a administration ($p < .01$). Preovulatory E_2-17ß levels began to increase steadily ($p < .05$) after 24 hours from initial injection of HMG and 48 to 60 hours after initial injection of FSH. Sixty hours after initial superovulatory treatment, E_2-17ß levels had increased seven-fold in the HMG group and had doubled in the FSH group. It is likely that this increase in estrogen concentration results from increased follicular growth stimulated by the gonadotropins. Concentrations of E_2-17ß decreased markedly at time of the LH peak and remained low until the time of embryo collection (Figure 2). The interval from PGF_2a to E_2-17ß peak was less ($p < .05$) in HMG- (44 ± 3 hours than in FSH (52 ± 2 hours) treated heifers after initial injection of PGF_2a. Animals injected with PGF_2a intramuscularly reached ($p < .01$) peak concentrations of E_2-17ß 43 ± 3 hours after PGF_2a injection; those injected intravenously reached peak concentrations 53 ± 2 hours after PGF_2a treatment. The earlier estradiol increase in HMG-treated heifers may have contributed to the earlier ($p < .05$) estrus seen in the HMG-treated animals (Table 1). Peak concentrations of $E_2$17-ß were not different between treatments or route of PGF_2a (Table 1). Estradiol-17ß peak concentrations were correlated with the number or corpora lutea ($r = .47$; $p < .01$) and with the number of embryos or oocytes recovered ($r = .61$; $p < .01$). The preovulatory estrogen concentrations have been suggested to be more useful in predicting follicular response of individual donors than postovulatory progesterone concentrations. The delay in the initial preovulatory increase of E_2-17ß that we observed in the FSH-treated heifers but not in the HMG-treated heifers suggests that a lag period for follicular maturation is needed during FSH stimulation of the ovaries. This could translate into higher pregnancy rates in recipients of embryos obtained from FSH-injected heifers. Moreover, the relative concentrations of both progesterone and E_2-17ß after the LH peak may also explain the reduced pregnancy rates with embryos derived from HMG-treated heifers.

In summary, we report here that superstimulation of heifers with FSH or HMG influences circulating concentrations of E_2-17ß but not of LH, FSH, or progesterone. Route of PGF_2a administration did not significantly affect these hormone secretion patterns, although significant interactions between treatment and route of PGF_2a injection were observed on progesterone serum concentrations when coincident with the time of the LH peak.

Implications

The high steroid hormone concentrations in superovulated beef heifers before and after ovulation may lead to asynchrony between stages of embryonic development, a situation that may interfere with the pregnancy outcome of superovulated embryos in recipient animals.

Table 1. Hours (± SEM) from prostaglandin $F_2\alpha$ ($PGF_2\alpha$) injection to estrus, and to luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol 17-β (E_2-17β) peaks and preovulatory peak concentrations.

Treatment			Hours from first $PGF_2\alpha$ to				Peak serum concentration		
Gonadotropin	$PGF_2\alpha$	No. of heifers	Estrus	LH peak*	FSH peak*	E_2-17ß*	LH ng/mL	FSH ng/mL	E_2-17ß pg/mL
FSH	i.m.	8	59[b]	47	48	46	162	38	42
FSH	i.v.	7	60[b]	61	61	58	164	39	33
HMG	i.m.	8	46[c]	42	45	40	111	32	42
HMG	i.v.	8	54[c]	52	52	48	181	37	46

[a] Main effect of gonadotropin, $p < .05$; route of $PGF_2\alpha$) $p < .01$>
[b,c] Numbers within columns with different superscripts are different at $p < .05$ for gonadotropin treatments.*

Figure 1. Experimental design for induction of superovulation, breeding, and embryo collection in beef heifers. Blood collection intervals are described in the Materials and Methods section. AI = artificial insemination; PGF_2a = prostaglandin F_2a; FSH = follicle-stimulating hormone.

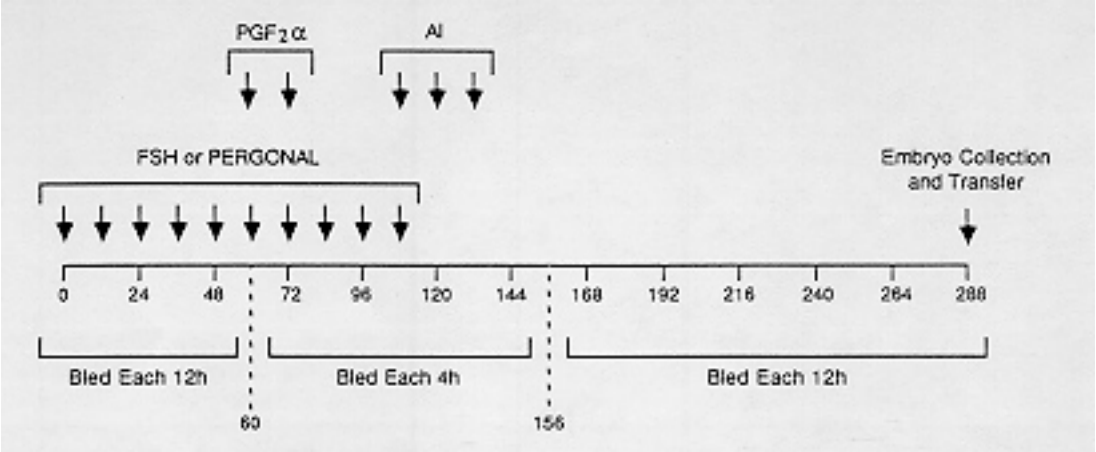

Figure 2. Mean concentrations of progesterone (o), follicle-stimulating hormone (FSH, _), and estradiol-17ß (l) in peripheral serum of beef heifers superovulated either with FSH-P or human menopausal gonadotropin and then injected intravenously or intramuscularly with PGF_2a, as described in the Materials and Methods section. The pooled SE for progesterone is 1.8 nanograms per milliliters, for estradiol-17ß is 2.1 picograms per milliliters, and for FSH is 2.2 nanograms per milliliters.