INDUCTION OF MULTIPLE OVULATION IN THE COW WITH SINGLE INJECTIONS OF FSH AND HCG

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Summary. A gonadotrophin regimen was developed to induce a high incidence of double and triple ovulations in the beef cow using a single injection of FSH on Day 16 followed by a low dose of HCG on the day of oestrus. Conception rate was not affected by gonadotrophin treatment and 1/9 cows experiencing multiple ovulations gave birth to twin calves.

Administration of exogenous gonadotrophic hormones can result in enhanced follicular development, increased ovulation numbers and multiple births in the cow (Gordon *et al.*, 1962; Scanlon *et al.*, 1968; Turman *et al.*, 1971). Although favourable superovulatory responses have been achieved in cows with PMSG, the results have often been variable and ovarian refractoriness to repeated injections has occurred (Laster *et al.*, 1971). Recently a partly purified pituitary extract, FSH-P, has been used to stimulate follicles (Laster, 1972; Smith *et al.*, 1973; Bellows *et al.*, 1974). However, since FSH is rapidly removed from the peripheral plasma (Catchpole, 1963), it is generally accepted that continuous administration of this hormone for several days is a prerequisite for obtaining multiple ovulations.

It is of practical importance to limit the number of young to two or three per cow and the development of techniques for limiting the ovulation rate to result in a high incidence of twin or triplet births has been emphasized (Schilling & Holm, 1963; Laster *et al.*, 1971). Previous work at this laboratory resulted in a regimen for producing single or double ovulations in non-human primates (Dukelow, 1970). Applying these principles, it was the purpose of the present studies to determine the effects of single FSH injections and low levels of HCG on the incidence of double and triple ovulations and multiple births in cows.

All animals were Angus–Hereford or Angus–Holstein crosses and were maintained on grass pasture and supplemented with sorghum silage daily. Oestrus was determined by visual observations made twice daily.

Preliminary trials were conducted using seven mature nulliparous heifers that had been known to experience at least two oestrous cycles before the start of treatment. Ovarian activity was monitored by rectal palpation on Day 16 of the cycle, the day of oestrus and for 3 consecutive days after oestrus.

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Gonadotrophin injections were timed from a non-synchronized oestrus. The FSH-P (Armour-Baldwin Laboratories, Omaha) and HCG (A. P. L. Ayerst Laboratories, New York) were administered in a repetitive experimental design with both gonadotrophins administered to the same experimental group for three consecutive cycles (see Table 1). A preliminary report of these data has been published (Wildt & Dukelow, 1972).

Table 1. Numbers of ovulations achieved in cows after three gonadotrophin treatments

<i>Animal No.</i>	<i>Trial I</i> (25 mg FSH-P+ 2000 i.u. HCG)	<i>Trial II</i> (25 mg FSH-P+ 1000 i.u. HCG)	<i>Trial III</i> (12.5 mg FSH-P+ 1000 i.u. HCG)
1	4	4	1
2	2	1	1
3	2	2	0
4	3	1	2
5	2	0	2
6	1	0	2
7	5	—	—
Mean	2.57	1.33	1.33

Cow 7 showed intense standing oestrus and lengthened oestrous periods for as long as 6 days, as well as a decrease in the length of the subsequent oestrous cycles (11 and 7 days). This animal also exhibited the raised tailhead, lowered loin characteristic for about 6 weeks, after which ovarian cyclicity became normal although overt signs of oestrus were absent. Two of the heifers that ovulated two ova in Trial III were those that had failed to ovulate in Trial II and it was thus apparent that low levels of HCG and not antigonadotrophic activity were responsible for the reduced ovulation.

In comparing individual treatments, the high gonadotrophin dosages of Trial I, while producing the highest incidence of multiple ovulations, produced severe behavioural and reproductive abnormalities in one animal. The high FSH and low HCG dosage associated with Trial II gave variable results while the lower gonadotrophic treatment of Trial III resulted in 50% of the heifers producing double ovulations and no ovarian over-stimulation.

The dose of HCG of Trial I resulted in 86% ovulation of the follicles > 12 mm in diameter detected on the day of oestrus. This was higher than the ovulation rate (73%) observed in both Trials II and III. Standing oestrus was less apparent in the Trial II cycle and completely absent in the cycle of Trial III and detection of oestrus was therefore determined from the presence of vaginal discharge and by rectal palpation.

In a second experiment, oestrus was synchronized in six heifers before the gonadotrophin treatment. Progestins in oil were injected intramuscularly (i.m.) for 14 consecutive days. On Day 16 of the oestrous cycle after the synchronized cycle 25 or 12.5 mg FSH-P was injected (i.m.). On Day 18 and each day thereafter, the heifers were checked for standing oestrus by being exposed to a mature bull and observed for 1 hr. On the day of oestrus all heifers received

1000 i.u. HCG (i.m.), and then were killed 7 to 10 days later. Reproductive tracts were recovered and ovaries examined for follicles and CL.

Cows receiving 25 mg FSH ovulated two, two and five ova, while animals receiving 12.5 mg ovulated none, three and two ova. Thus, doses of FSH > 12.5 mg resulted in double ovulation, and also overstimulation of the ovaries.

A field trial was then conducted using 51 multiparous cows which had experienced at least two post-partum oestrous cycles before treatment. One group of cows received 12.5 mg FSH-P on Day 16 followed by an ovulatory dose of 1000 i.u. HCG on the day of oestrus. Control cows received i.m. injections of saline on Day 16 and the day of oestrus. Ovarian data were collected by rectal palpation on the day of oestrus and on the 7th day after oestrus in control cows, and on Day 16, the day of oestrus, and Days 3 and 7 after oestrus in treated cows. At the oestrus following gonadotrophin treatment, eighteen cows were artificially inseminated. The remaining sixteen treated cows were artificially inseminated in a subsequent untreated cycle. All seventeen control cows were inseminated at the first oestrus after the saline injection. Cows in all the groups were then exposed to five bulls of the same breed for 90 days. Because artificial insemination was performed with semen from a bull of different breed from that used for natural mating, fertility at first conception could be determined. All cows were rectally palpated at 50 days of gestation to determine pregnancy and then allowed to calve at term.

The mean ovulation rate and the % of cows ovulating more than one ovum was 1.00 and 0.0% for controls, 1.75 and 68.9% for cows receiving gonadotrophins but not inseminated, and 1.82 and 50.0% for cows receiving gonadotrophins and inseminated. Of the eighteen treated cows, ten (55.6%) conceived at first oestrus. There was one multiple birth, a set of twins (one male, one female). Ten of seventeen (58.8%) control cows conceived at first oestrus. There was no difference in the fertility at first breeding between the two groups.

Fertility was measured one to three cycles later, under different artificial insemination conditions, in the cows receiving gonadotrophins but not inseminated at first oestrus. Conception was 78.4% at first breeding. The interval from gonadotrophin administration to subsequent breeding was not related to the ability of the cow to conceive at first service.

The data indicated that a single injection of FSH-P followed by a low dosage of HCG was sufficient to induce multiple ovulation in the cow. The results agree with an earlier report (Bellows *et al.*, 1969) which indicated that dosage levels in excess of 12.5 mg FSH-P result in overstimulation of the ovary. The regimen of 12.5 mg FSH-P and 1000 i.u. HCG resulted in a high incidence of double and triple ovulations, but it was apparent that some of these ova were either not fertilized or the embryos degenerated during early pregnancy. Gordon *et al.* (1962) and Laster *et al.* (1971) have suggested that rectal palpation during early stages of gestation may contribute to abortion thus reducing pregnancy rates in gonadotrophin-treated cows.

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