SYNCHRONIZATION OF OESTRUS WITH I.C.I. 79,939, AN ANALOGUE OF PGF_{2α}, AND ASSOCIATED CHANGES IN PLASMA PROGESTERONE, OESTRADIOL-17β AND LH IN HEIFERS

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(Received 14th June 1974)

Tervit, Rowson & Brand (1973) have described the induction of fertile oestrus in cattle by intramuscular injection of a low dose (1 mg) of I.C.I. 79,939, an analogue of prostaglandin F_{2α}, (Binder & co-authors, 1974; Dukes, Russell & Walpole, 1974) but neither this compound nor prostaglandin (PG) F_{2α}, given in a single dose at any one time, will induce oestrus in all cattle in a herd since animals treated before Day 5 of the oestrous cycle will not respond (Lauderdale, 1972; Liehr, Marion & Olsen, 1972; Rowson, Tervit & Brand, 1972a, b; Hill, Dickey & Henricks, 1973; Cooper & Furr, 1974). Cooper (1974) and Cooper & Furr (1974) have described a regimen which overcomes this difficulty and provides a potentially valuable aid to animal husbandry: two doses of a potent luteolytic PG analogue given 10 to 12 days apart will induce oestrus in all animals in a herd irrespective of the timing of their natural cycles.

The present work describes changes in plasma progesterone, oestradiol-17β and LH in heifers given two spaced doses of I.C.I. 79,939 and compares them with the changes seen around natural oestrus.

Six Friesian heifers, 1½ to 2 years of age, between Days 9 and 13 of their oestrous cycles, were given a single intramuscular injection of 750 μg I.C.I. 79,939 in 2 ml phosphate buffer, pH 7.4. This treatment was repeated 10 days later. Changes in ovarian and uterine morphology were monitored by palpation *per rectum* before both injections, daily or twice daily for 5 days thereafter and once 10 days after the second injection. Cervical mucus samples taken at the time of palpation were examined for 'Spinnbarkeit' (the capacity of semi-liquids to be drawn into threads) and for the presence of arborization in smears of dried mucus. Animals were judged to be in oestrus when the cervical mucus showed marked 'Spinnbarkeit' and 'fern' patterns after drying. In other experiments (Cooper, 1974), this has been shown to correlate closely with behavioural oestrus detected by vasectomized bulls.

Ovulation was detected by recording the disappearance of preovulatory follicles at rectal palpation, the appearance of a characteristic depression on the ovarian surface and the development of a well-formed corpus luteum in the same ovary.

Blood samples (20 ml) were collected from the jugular vein into heparinized syringes daily at 09.00 hours before the injections of I.C.I. 79,939, and at 09.00, 16.00 and 23.00 hours for 4 days thereafter; daily blood samples were also collected at 09.00 hours for 12 days after the second injection. The blood was centrifuged immediately at 800 g, 4°C for 15 min and the separated plasma was stored at -10°C to await assay. Plasma LH was measured by the double-antibody procedure of Niswender, Reichert, Midgley & Nalbandov (1969). Highly purified bovine LH (LER-1072-2) was used for iodination and NIH-LH-B7 was used as an assay standard; the first antibody was an equine antiserum prepared against bovine LH. Oestradiol-17 β was estimated by radioimmunoassay in ether extracts of plasma without chromatography as described

TEXT-FIG. 1. Plasma progesterone (histograms), oestradiol (▲) and LH (●) in a heifer given two injections of 750 µg I.C.I. 79,939 (vertical interrupted lines) 10 days apart.

by Dobson & Dean (1974); the specific rabbit antiserum, R₂B₂, used was prepared against oestradiol-17 β -6-carboxymethyloxime-bovine serum albumin (Dean, Exley & Johnson, 1971). Progesterone was estimated by radioimmunoassay in hexane extracts of plasma using a goat antiserum, 465/4, prepared against progesterone-11 α -succinyl-bovine serum albumin (Furr, 1973).

The spaced double-injection regimen produced precise synchronization of oestrus in all the heifers treated. After both treatments, the corpora lutea showed rapid morphological regression; this was associated with a significant fall in plasma progesterone concentration 6 hr after treatment, basal values being reached within 24 hr. Rapid follicular growth and secretion of oestradiol-

17 β ensued with a return to oestrus, as defined above, 48 to 96 hr after the first, and 48 to 55 hr after the second, treatment. A preovulatory surge of LH occurred 62 to 103 hr after the first, and 48 to 62 hr after the second, treatment and was followed by ovulation in all animals. The corpora lutea formed were all functional as judged by plasma progesterone levels and the cycles after the second treatment were of normal length (16 to 22 days). Text-figure 1 illustrates the typical changes which occurred in plasma hormone concentrations. These changes were similar to those occurring after the induction of oestrus with PGF_{2 α} (Louis, Hafs & Seguin, 1973; Nancarrow, Hearnshaw, Mattner, Connell & Restall, 1973; Stellflug, Louis, Seguin & Hafs, 1973; Louis, Hafs & Morrow, 1974).

In all animals, there was a small increase in LH 6 hr after treatment. This was similar to the increase seen after PGF_{2 α} (Louis, Stellflug, Tucker & Hafs, 1974) and is thought to be due to the rapid withdrawal, caused by luteolysis, of the negative feedback of progesterone, rather than to a direct action of I.C.I. 79,939 on the hypothalamic-pituitary axis. In all animals, there was also a small peak of oestradiol-17 β , 4 to 6 days after induced ovulation, similar to that reported in the cow after natural ovulation (Dobson, Hopkinson & Ward, 1973; Glencross, Munro, Senior & Pope, 1973). It has been suggested (Glencross *et al.*, 1973) that the oestradiol originates in the growing follicles which are known to be present at this time (Rajakoski, 1960). After luteolysis induced by the second injection of I.C.I. 79,939, these follicles may progress and ovulate rather than regress as they would normally do. This might account for the observation that the induction of oestrus was more rapid and precise after the second PG administration. Certainly, Tervit *et al.* (1973) obtained a more rapid return to oestrus after luteolysis with I.C.I. 79,939 when large follicles, induced by treatment with PMSG, were present. This speculation clearly warrants further investigation.

The sequence of changes in the reproductive tract and in plasma levels of LH, progesterone and oestradiol-17 β were essentially similar to those occurring around natural oestrus; induction of oestrus and ovulation was rapid and precise and the functional corpus luteum formed had a normal life-span. We believe, therefore, that this simple and effective treatment will not impair fertility and that it has the advantage of being applicable to large numbers of animals without reference to the timing of their natural cycles.

We wish to thank Dr R. B. Snook for the generous gift of an equine antiserum to bovine LH, Dr D. Exley for the oestradiol-17 β antiserum, Dr L. E. Reichert for highly purified bovine LH (LER-1072-2), and the National Institutes of Health, U.S.A., for the bovine LH standard (NIH-LH-B7). Excellent technical assistance was provided by Mr A. Norbury. One of us (H.D.) acknowledges the receipt of an Animal Health Trust Wooldridge Fellowship.

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