THE FERTILITY OF HEIFERS INSEMINATED AT PREDETERMINED INTERVALS FOLLOWING TREATMENT WITH MGA AND HCG TO CONTROL OVULATION

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Summary. Oestrus and the time of ovulation were controlled in eighty-seven mature non-pregnant heifers by using silastic implants of MGA followed by an injection of HCG 48 hr after removal of the implants. The heifers were allocated at random to three groups and inseminated with frozen semen before the estimated time of ovulation. Animals in Groups 1 and 2 were inseminated once at 14 and 24 hr, respectively, after HCG while animals in Group 3 were inseminated twice 14 and 24 hr after HCG. All heats observed after insemination were recorded until the animals were killed 55 days later, when 23% of them were found to be pregnant. The conception rate was not affected by changing the time or the frequency of insemination. Possible reasons for the low fertility are discussed.

INTRODUCTION

The oestrous cycle of cattle can be controlled by administering progesterone or one of its synthetic analogues (Lamond, 1964). The number of cows that show heat within a given period of time following treatment has, however, been inconsistent and fertility to natural and artificial matings at this heat is lower than normal (Hansel, 1967; Mauléon & Chupin, 1971). Both spermatozoa and eggs have a finite life-span in the reproductive tract of the female and their fertilizing ability declines with time. By allowing spermatozoa from different males to compete with each other at different times before ovulation, Dziuk (1970) found that there was a certain interval before ovulation when conception was most likely to take place. This optimum time interval between insemination and ovulation appears to be 12 hr in pigs, rabbits and ewes (Polge, Day & Groves, 1968; Miller, Roche & Dziuk, 1969; Dziuk, 1970; Roche & Crowley, 1971). Consequently, to ensure high fertility, animals should be inseminated in relation to the time of ovulation rather than in relation to the occurrence of heat. In the case of cattle, this necessitates precise control of the time of ovulation at the end of the progestagen synchronizing period.

Graves & Dziuk (1968) have reported that ovulation occurs about 40 hr

after an injection of HCG in cows fed 6-methyl-17-acetoxy-progesterone (MAP) for 18 days. The eggs shed as a result of this treatment were capable of normal fertilization. Roche & Crowley (1972) found that HCG given 48 hr after the removal of a silastic implant of melengestrol acetate (MGA) also controlled the time of ovulation in heifers, with 20% ovulating by 27 hr after the injection of HCG, 73% by 36 hr and 100% by 40 hr. The aim of the present experiment was to determine if inseminations carried out at prearranged times without reference to heat would give acceptable fertility levels in heifers in which the time of ovulation was controlled as described previously (Roche & Crowley, 1972).

MATERIALS AND METHODS

Mature cyclic Hereford X heifers housed in open cubicles and fed on autumn grass silage were used. Animals already pregnant were detected by rectal examination and discarded before the experiment. Silastic rubber implants, with a surface area of 1307 mm² and containing 495 mg MGA, were inserted under the skin in the dewlap of eighty-seven heifers for 21 days in December 1971 (Roche & Crowley, 1973); 48 hr after their removal, 1500 i.u. HCG were injected intramuscularly. The animals were then randomly assigned to three groups and were inseminated at a predetermined time before the estimated time of ovulation without reference to behavioural oestrus. Heifers in Groups 1 and 2 received one insemination; Group 1 at 14 hr and Group 2 at 24 hr after the injection of HCG. The heifers in Group 3 were inseminated twice, initially at 14 hr and again at 24 hr after HCG. The mean time of occurrence of ovulation in previous work with similar implants of MGA and HCG given at the same time was 34 hr after the injection of HCG (Roche & Crowley, 1972). Thus, inseminations carried out 14 and 24 hr after HCG were calculated to be 20 and 10 hr before the mean time of ovulation. Frozen semen was used and two artificial insemination (A.I.) technicians from a commercial A.I. station inseminated the animals. Following insemination, all subsequent heats were recorded. The animals were killed 55 days later and their reproductive tracts were examined to determine the number pregnant.

RESULTS

Occurrence of oestrus

Oestrus did not occur in any of the experimental heifers while MGA implants were *in situ*. Following the injection of HCG to induce ovulation, the heifers were observed twice daily for signs of oestrus but none of them was detected in heat. Thus, ovulation occurred unaccompanied by behavioural oestrus.

Fertility

The fertility of the heifers following all three treatments was very low as only 23% of the heifers were pregnant at slaughter 55 days after the fixed-time inseminations. The low conception rate was not affected by changing the timing or the number of inseminations.

Subsequent reproductive performance

The occurrence of the first recorded repeat heats after insemination showed a significant pattern with four definite peaks occurring. Thirteen heifers had repeat heats between Days 9 and 12 following A.I., eleven heifers repeated between Days 18 and 24, fifteen heifers repeated between Days 28 and 33 and seventeen repeated between Days 38 and 44. The high frequency of short return intervals suggests that a proportion of the heifers had oestrous cycles of

Table 1. The pregnancy rate in heifers following A.I. at fixed times relative to the estimated time of ovulation

Time of A.I. before ovulation (hr)	No. of heifers	Heifers pregnant	
		No.	%
20	24	6	25
10	23	5	22
20 and 10	40	9	23
Total	87	20	23

8 to 10 days' duration. The occurrence of these short cycles might also explain the third peak of returns to service at 28 to 33 days if it is assumed that these latter heifers underwent an unobserved or silent heat 9 to 10 days after insemination. In fact, the subsequent cycles of nine of the thirteen heifers with short return intervals were of the normal 18 to 23 days' duration and this suggests that the majority of these early returns to service were accompanied by ovulation.

DISCUSSION

Following injection of HCG, the heifers did not show heat. This contrasts with the situation when MGA alone is used to synchronize heat (Zimbelman, Lauderdale, Sokolowski & Schalk, 1970). Others have also found that HCG reduces the incidence of heat in cattle when given in conjunction with progestagens (Mauléon, Rey, Mariana & Benoit, 1970; Roche & Crowley, 1972) or when given alone for treatment of ovarian hypoplasia (Nakahara, Domeki & Yamauchi, 1970).

The mechanism by which HCG suppresses oestrus in these circumstances is not well understood. It is well established that LH stimulates the synthesis of progesterone in luteal tissue both *in vivo* (Cook, Kaltenbach, Niswender, Norton & Nalbandov, 1969) and *in vitro* (Armstrong & Black, 1966). Zimbelman & Smith (1966) have shown that the ovaries of heifers treated with MGA contain one characteristic large follicle and preliminary histological evidence indicates that these follicles are highly luteinized (Guthrie, Lamond, Henricks & Dickey, 1970). These luteinized cells may be stimulated to produce increased amounts of progesterone following the injection of HCG. Increased amounts

of progesterone before ovulation may inhibit the expression of oestrus since Carrick & Shelton (1969) have shown that progesterone given in conjunction with oestradiol benzoate has an inhibiting effect on the occurrence of behavioural oestrus in spayed heifers. Failure to show oestrus after the injection of HCG may mean that the mechanisms for normal sperm transport were upset in these heifers.

The reasons for the very low conception rate obtained in all three groups in this experiment are not clear. Both Graves & Dziuk (1968) and Roche & Crowley (1972) have established that ovulation is complete in heifers 40 hr after an injection of HCG at the end of the MGA-synchronizing period. During the normal oestrous cycle, however, it has been reported that the interval between the transient LH surge and ovulation is about 24 hr (Schams & Karg, 1969; Henricks, Dickey & Niswender, 1970). Thus, the timing of ovulation in relation to the release of LH in animals treated with progestagen and HCG is different to that in normal cyclic animals. This indicates that normal physiological events are upset in synchronized animals receiving HCG.

The normal pattern of follicular growth and development in heifers receiving MGA is altered. The large follicle on the ovary of treated animals histologically appears atretic and contains increased numbers of luteinized cells (Guthrie *et al.*, 1970). There are also fewer small follicles present and high plasma progesterone levels up to 7 ng/ml have been reported in some treated heifers in which CL were absent. The levels of LH in serum also tended to be higher than in control heifers. The fertilization rate of eggs shed was low in heifers treated with MGA (Hill, Lamond, Henricks, Dickey & Niswender, 1971).

It is not clear whether HCG altered the population of follicles that would have ovulated spontaneously at the ensuing normal oestrous periods if no treatment was imposed. Follicles are in a continuous state of growth and atresia and it is only after Day 18 of the cycle that it is possible to identify the follicle that will ovulate at the next oestrous period (Dufour, Whitmore, Ginther & Casida, 1972). Thus, ovulation of immature or atretic follicles with HCG after the use of MGA could result in the formation of abnormal CL. Since HCG affects steroidogenesis, the pattern of ovarian and follicular steroid production before ovulation could have been altered. Ovarian steroids are known to affect capacitation (Soupart, 1967) and transport of spermatozoa (Quinlivan & Robinson, 1969) so that significant changes in the endogenous steroid pattern would affect fertilization. Thus, the lowered fertility found following the use of MGA alone was not changed by using HCG to control ovulation. In fact, the use of HCG seemed to have a further deleterious effect on fertility.

The physiological explanation for the occurrence of the short oestrous cycles found is not clear. It would seem that the injection of HCG following the removal of the implant did increase the incidence of such cycles, as earlier workers (Zimbelman *et al.*, 1970) who used MGA in feed for oestrus synchronization did not report their occurrence. Some of the CL formed from the characteristic MGA follicle may have a life-span shorter than normal. In a series of observations on the oestrous cycle of normal Friesian heifers for a period of 1 year, Wishart (1972) found only 1.4% of the cycles shorter than 18

days. Macmillan & Watson (1971) found that 10% of the cows returning to A.I. in New Zealand returned within the first 17 days and that a distinct peak representing 31.4% of all short return intervals occurred between 8 and 10 days after the first insemination. It is possible that these short cycles in cattle are more common following A.I. where the possible introduction of diluents or other foreign material into the uterus could cause premature regression of the CL.

In conclusion, this study has shown that the occurrence of oestrus in the heifer can be suppressed with implants of MGA. The results of this experiment also show that the low fertility which is characteristic of matings at the synchronized heat (Zimbelman *et al.*, 1970) is further reduced when HCG is used to control ovulation and insemination is carried out on a fixed-time basis. Though Boyd & Tasker (1971) did encounter low fertility at the first oestrus following MGA-HCG treatment for oestrus synchronization, they also noted that following the first and second services the fertility in the MGA-HCG-treated group was higher than in untreated or MGA-treated animals. They concluded that the use of MGA and HCG was worthy of further investigation. Earlier work, reviewed by Zimbelman *et al.* (1970), has revealed the limitations of using MGA alone for oestrus synchronization. The findings of the present study indicate that when MGA is used in combination with HCG for ovulation control, the subsequent fertility is so low that, in our view, such a treatment regimen has little prospect of affording a practical means of allowing all animals to be inseminated on the same day without reference to heat. Other hormones should be tried in an attempt to develop a technique that will give control of ovulation accompanied by normal fertility.

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