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SYNCHRONIZATION OF OESTRUS IN HEIFERS WITH NORETHISTERONE

By

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Successful attempts to bring about synchronization of oestrus in heifers have been achieved by means of both natural and artificial progesterons (*Lamond* 1964). Varying conception rates have been obtained following service during the synchronized heat periods.

By simultaneous use of progesterone and oestradiol *Willbank & Zimmerman* (1962) obtained better heat synchronization than with progesterone alone, and a conception rate among the experimental animals comparable with that of the controls. According to *Ulberg* (1955) and *Ulberg & Lindley* (1960) synchronization of oestrus is improved when oestradiol is given on the third day after termination of treatment with progesterone. The latter two workers reported that the conception rate was no worse than with progesterone treatment alone. *Wagner et al.* (1963) reported similar results after treatment with CAP, (6-chloro- Δ^6 -dehydro-17-acetoxypregesterone) and oestradiol.

Norethisterone (derived synthetically from 19-nortestosterone) is partly converted to oestrogen following oral administration in rats (*Edgren et al.* 1966, 1967), so considering the favourable effect of combined oestrogen and progesterone treatment mentioned above, an experiment which would test the value of norethisterone (17- α -ethynyl-19-nortestosterone) as a means of synchronization of oestrus in heifers was thought to be worthwhile. In this work special attention was given to the effect of the size of dose and to the stage of oestrous cycle when hormone treatment began.

MATERIALS AND METHODS

The experiment took place from January to April 1967. Fifty heifers from different herds were assembled on an experimental farm together with 10 of the farm's own heifers. All the experimental animals were Swedish Red and White Cattle between 13 and 23 months old. Their weights varied from 250 to 430 kg, and were confirmed by weighing.

The animals were kept in stalls and fed according to normal rearing standards. Two kg mixed concentrate (grain, oilcake and molasses) and 4 kg of silage (marrow-stem kale) were given every morning and 2—4 kg of hay together with 1—2 kg of straw in the afternoon. The animals' conformation was at first somewhat below average, but improved during the course of the experiment. Fifty g of mineral supplement and 50 g of calcium carbonate were given daily to each animal.

Apart from ringworm infection, the general health of the stock remained good during the whole experimental period.

During a pre-experimental period of at least five weeks and during the experiment itself, the animals' oestrous cycles were kept under observation by inspection twice daily. Clinical examinations including visual inspections of the external genitalia and rectal palpations of the cervix, uterus, and ovaries were carried out repeatedly during the pre-experimental period and during the course of the experiment. The state of the vaginal mucosa was studied by means of a glass speculum.

Guided by previous signs of oestrus and by the clinical examinations, the stage of cycle of individual heifers was confirmed when hormone administration began. Animals which showed two heat periods with a normal interval, or a single heat followed by rectal confirmation of ovulation, were included in the trials. The day of heat was taken as the first day of the cycle.

Doses of 0.2, 0.6 and 1.0 mg of norethisterone per kg body weight per day were tested. Because the body weight varied for different heifers the daily amount of hormone administered varied between 50 mg and 375 mg.

In all, the trials included 50 heifers in different stages of oestrous cycle. Ten heifers, which during the pre-experimental period had not demonstrated normal sexual activity, were excluded from the experiment. The animals were combined in trial groups so that for each dose a number of animals were in the luteal phase and the rest in the follicular phase of the cycle. The

dose consumed varied for individuals in this group between 0.75 and 1.15 mg (a mean of 1.0 mg) of hormone per kg body weight daily.

Trial II

This trial was designed to compare the effect of oral administration of 0.6 mg (group II A) and 1.0 mg (group II B) norethisterone per kg body weight given daily for 17 days.

Twenty-four heifers were divided into two groups of 12 animals according to the same conditions as in trial I (Table 1). The animals in group II A were fed 170 mg of norethisterone and in group II B 280 mg per head per day (Table 2). Individual heifers received amounts of hormone which varied in group II A between 0.50 and 0.66 mg (a mean of 0.6 mg) and in group II B between 0.84 and 1.04 mg (an average of 1 mg) per kg body weight daily.

At the first and, where necessary, the second heat after termination of hormone treatment the heifers were artificially inseminated with deep-frozen semen (in pellet form) which in all cases was a single ejaculate from a bull of proven fertility belonging to Södermanland county artificial insemination association. The inseminations were carried out by two of the association's experienced technicians. The fertility of the ejaculate in question was proved by inseminations during the A.I. centre's routine activity of 32 cows and heifers.

Nine—12 days after the first heat the ovaries were examined in order to ascertain the extent of corpus luteum development. Pregnancy was confirmed by rectal examination six weeks after the last insemination.

Trial III (field trial)

Fourteen Swedish Red and White heifers and one Hereford heifer, all between 17 and 25 months old (average age 21 months), and with an estimated average weight of 400 kg were included in the field trial.

The heifers were allowed to run free in a large loose box with adequate trough space for all to eat at the same time. Four—5 kg of mixed concentrate and about 8 kg of silage were given every morning, about 3 kg of hay and straw were fed in the afternoon.

The animals' conformation was generally above normal. All the animals underwent clinical examination before the trial's

commencement which revealed the presence of follicles and corpora lutea in the ovaries of all but two. No systematic observation of oestrus was carried out before the trial began.

Two-hundred-and-forty mg of norethisterone per animal (approx. 0.6 mg/kg body weight) was given daily for 17 days in the concentrate mixture.

Observation for heat signs was undertaken by the stockman and inseminations and pregnancy diagnosis by a trained inseminator. Deep-frozen semen in pellet form from one Hereford and two Friesian bulls of high fertility was used.

The semen was tested by insemination of 351 cows and heifers in routine insemination work.

RESULTS

Trials I and II

1. Observations during hormone treatment

Three heifers in the group receiving 0.2 mg norethisterone (I A) and one in the 1.0 mg group (II B) showed heat symptoms during hormone treatment occurring at the normal interval after the last heat before treatment began. On corresponding days several other heifers in the 0.2 mg group were seen to have a swollen vulva, a mucous discharge, somewhat open and reddened portio, a somewhat contracted uterus, and medium to large follicles in the ovaries, but no psychic signs of heat. The mucous discharge was usually copious, greyish and of varying viscosity. This symptom became more marked towards the last day of hormone treatment. In the group receiving the higher hormone level (0.6 mg and 1.0 mg/kg body weight) a mucous discharge and oedema of the vulva were observed in 65 % of the animals. However, almost all these animals possessed no palpable follicles, or only small ones, during the whole period of hormone treatment. In none of the groups was the occurrence of ovulation detected and no corpora lutea developed.

In 11 cases (22 % of all the experimental animals) a moderate to marked enlargement of the udder was observed during the period of treatment.

2. Extent of heat synchronization

Synchronization of oestrus after termination of treatment is shown in Figs. 1 and 2. The distribution of oestrus for repeating heifers is also shown.

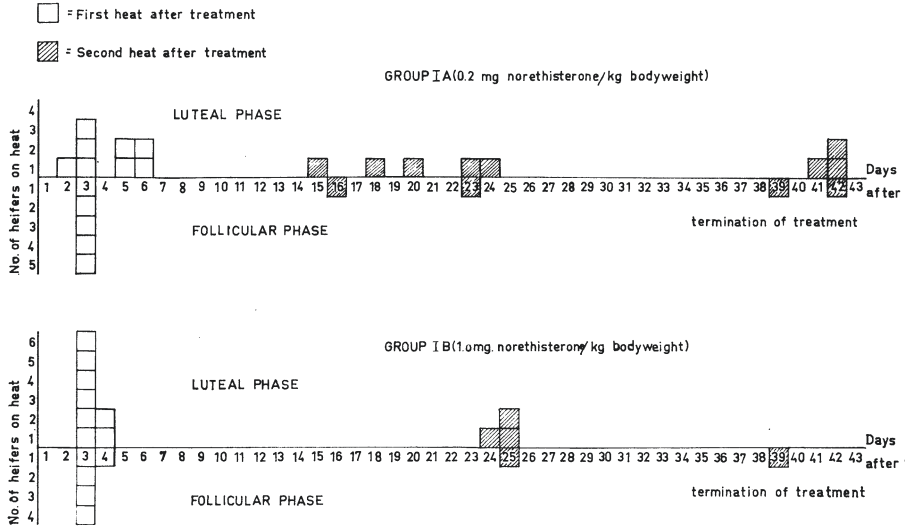


Figure 1. Distribution of first and second heats after treatment of heifers with norethisterone in trial I.

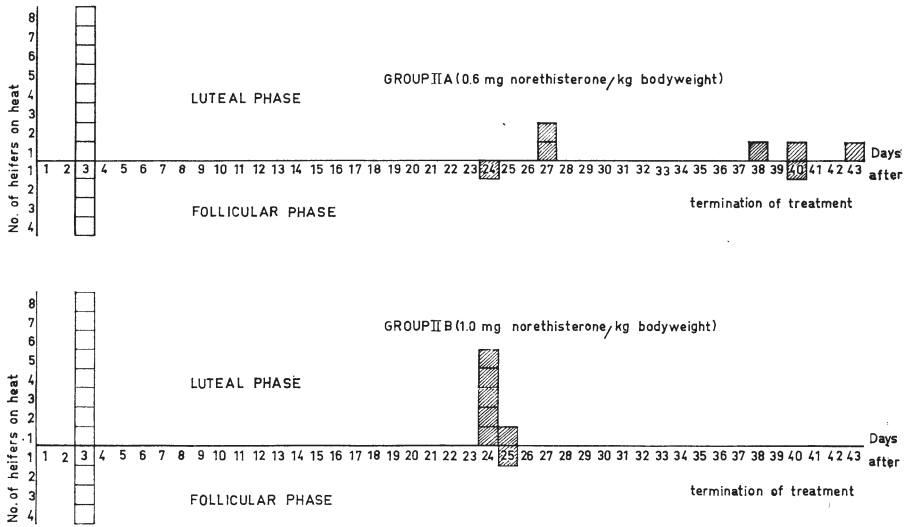


Figure 2. Distribution of first and second heats after treatment of heifers with norethisterone in trial II.

The synchronized heats were distinct and easy to confirm. Approximately half of the animals demonstrated heat symptoms during a period of 24—36 hrs. and the others during a shorter period.

a. Effect of dose size

The lowest level of hormone (0.2 mg) produced poor heat synchronization (Fig. 1). Oestrus appeared from the second to the sixth day and eight of 13 heifers (62 %) were on heat on the third day after withdrawal of treatment.

With the other doses heat synchronization was very good and no difference in effect was demonstrated between the two doses 0.6 mg and 1.0 mg norethisterone per kg body weight (Fig. 2). In all 34 of 37 heifers (92 %) which had received 0.6 mg or 1.0 mg norethisterone came on heat on the third day after termination of treatment and three one day later.

Three of 13 heifers (23 %) on 0.2 mg, six of 12 (50 %) on 0.6 mg and 19 of 25 (76 %) on 1.0 mg showed metoestral bleeding after heat. The bleeding was more pronounced than usual.

Palpation of the ovaries on the 9th—12th day after the appearance of synchronized oestrus showed that corpora lutea had developed in eight of 13 heifers which had received the 0.2 mg level of hormone. In two of the others were found large ovarian cysts of lutein type. Two heifers at the 0.6 mg level very probably had cystic corpora lutea while all other heifers at the 0.6 mg and 1.0 mg dose level had normal corpora lutea.

b. Effect of cycle stage

Only with the lowest dose of hormone did there appear any connection between stage of cycle and heat synchronization. Heifers receiving this dose which were in the luteal phase when treatment began, came on heat between the second and sixth day after withdrawal of the hormone, while heat in all heifers in the follicular phase appeared on the third day (Fig. 1). On the other hand neither ovulation failure nor the development of ovarian cysts and cystic corpora lutea that occurred could be attributed to the stage of the heifers' cycle when hormone treatment began.

3. *Fertility*

a. Effect of dose size

Conception rates for the different groups are evident from Table 2. Conception rates for the 0.2 mg group at the first two inseminations were 30.8 %, for the 0.6 mg group 58.3 % and for the two 1.0 mg groups together it was 76.0 %.

(18—25 days) and three out of seven heifers (42.9 %) in the 0.6 mg group. In the 1.0 mg group 11 out of 12 heifers (92 %) returned after a normal interval.

Trial III

No heifer was observed on heat during the period of hormone administration. However, several demonstrated swelling of the external genitalia and a somewhat grey, viscous discharge from the vulva. Enlarged udders were remarked in two cases. The extent of heat synchronization is evident from Fig. 3, which shows that 14 out of 15 heifers (91 %) came on heat on the third and fourth days after termination of hormone treatment. One heifer came on heat on the second day. All the heifers had normal corpora lutea 12—13 days after the synchronized oestrus.



Figure 3. Distribution of heat and first returns after heat synchronization treatment of heifers with norethisterone in trial III.

From Table 2 it is clear that 10 out of 14 (71.4 %) heifers inseminated at the first heat became pregnant, and that a further three became pregnant following insemination at the second heat period after termination of hormone.

Three out of five heifers (60 %) returned on the 23rd day after termination of treatment. One of these, for technical reasons, had not been inseminated at the first heat. One heifer returned on the 27th day and another did not show any heat within the normal oestrus interval although she later proved to be non-pregnant.

DISCUSSION

These experiments demonstrate that norethisterone at levels of 0.6 and 1.0 mg/kg body weight per day effectively inhibits heat and ovulation in heifers. They further show that 0.2 mg/kg is

too low a dose, since three out of 13 heifers came on heat during the period of treatment.

Several animals displayed a swollen vulva, mucus discharge, an open and reddened portio, and a contracted uterus during norethisterone treatment. Similar changes were observed when cattle were treated with progesterone by *Ulberg et al.* (1951), with MAP by *Zimbelman* (1963) and with MGA (melengestrol acetate) by *Zimbelman & Smith* (1966). These were thought by the two latter workers to indicate that follicles which developed during treatment were oestrogen-producing. The changes in this investigation were most marked in the group receiving 0.2 mg/kg norethisterone, where follicle development was greatest.

Contrary to the results of experiments with progesterone (*Ulberg & Patterson* 1954), MAP (*Nellor et al.* 1960) and CAP (*Van Blake et al.* 1963), an increased dose of norethisterone was not followed by a longer interval between the last day of treatment and the inception of oestrus. An explanation for this can be that norethisterone, apart from the fact that it is quickly metabolized in the animal body, has an oestrogenic effect which contributes to the appearance of heat symptoms.

A total of 51 out of 52 heifers (98 %) came on heat the third and fourth days after the termination of treatment with 0.6 and 1.0 mg/kg norethisterone.

The results can be compared with the best which have been obtained with other synthetic progesterones. The conception rate after first insemination varied between the groups from 41.7 % to 71.4 %. The mean of 55 % of the heifers pregnant after the first insemination is comparable with results from routine insemination of non-synchronized heifers.

Employing the low dose, 0.2 mg/kg resulted in not only poor oestrus synchronization, but also a low conception rate from the first heat after treatment. The result of palpation on the 9th—12th day after oestrus (no corpus luteum in three heifers and ovarian cysts in two) as well as the fact that 75 % of the heifers repeated after abnormal intervals, suggests that follicle development after the termination of treatment was poorly controlled.

No relation between cycle phase and the interval between last day of treatment and the beginning of oestrus was seen in the 0.6—1.0 mg/kg groups. Similar observations were made regarding progesterone by *Ulberg* (1955) and *Donker et al.* (1958). Following treatment with the low dose of norethisterone (0.2

mg/kg), oestrus synchronization appears to be poorer if the administration of hormone began when the animals were in the luteal phase.

Whether the cycle phase had any effect on the conception rate could not be ascertained in this investigation. *Collins et al.* (1961) found no difference in fertility between heifers in different stages of the oestrus cycles when treatment with MAP began.

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SUMMARY

Sixty-five heifers in different stages of the oestrus cycle were fed norethisterone once daily for 17 days at doses of 0.2, 0.6 and 1.0 mg/kg body weight.

During treatment swollen vulva, mucus discharge, open and reddened portio and udder enlargement were noted.

Norethisterone was effective in suppressing oestrus and ovulation at doses of 0.6 and 1.0 mg/kg. Extremely good heat-synchronization followed the treatment and 98 % of the heifers came on heat within a two-day period. Fifty-five % conceived after the first insemination. In the group receiving 0.2 mg norethisterone per kg, three out of 13 heifers showed psychic heat during treatment. Poor heat synchronization and low conception rate were obtained in this group.

A possible effect of cycle stage on heat synchronization was noted only in the 0.2 mg group where a better result was obtained when treatment began in the follicular phase.

SAMMANFATTNING

Brunstsynkronisering hos kvigor med hjälp av noretisteron.

Sextiofem kvigor i olika stadier av brunstcykeln utfodrades med noretisteron — 0,2, 0,6 och 1,0 mg per kg kroppsvidt, en gång dagligen i 17 dagar.

Under behandlingen iaktogs svullen vulva, slemflytning, öppen och rodnad portio samt juverförstoring.

Noretisteron, 0,6 och 1,0 mg per kg, hämmade effektivt brunst och ovulation. Mycket god brunstsynkronisering följde på behandlingen och 98 % av kvigorna blev brunstiga inom en tvådagars period. Femtiofem procent blev dräktiga efter första inseminationen. I gruppen som fick 0,2 mg noretisteron per kg visade 3 av 13 kvigor psykisk brunst under behandlingen, som åtföljdes av dålig brunstsynkronisering och låg fertilitet.

En möjlig effekt av cyklusstadiet på brunstsynkroniseringen framkom endast i 0,2 mg gruppen där bättre resultat erhöles när behandlingen började i follikelfas.

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