SYNCHRONIZATION OF OESTRUS
AND OVULATION IN THE MARE WITH
A TWO PG-HCG SEQUENCES TREATMENT

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SUMMARY

The use of PG and HCG was investigated to obtain oestrus synchronization in the mare. According to preliminary results, a treatment of two PG-HCG sequences begun at any stage of the oestrus cycle (PG on Day T_6 of treatment, HCG on Day T_6, PG on Day T_{14}, HCG on Day T_{20}) was tested in two groups of mares: 33 light mares treated in May and 15 Breton mares treated in September. This treatment induced synchronized ovulation in 75% of treated mares in a 4 days interval. The mechanism of action is described; the comparison of induced ovulation with spontaneous ovulation in non-treated mares showed no differences. This treatment is efficient from April to September, period during which mares do not present ovarian inactivity. If fertility is good, this treatment should allow to inseminate the mares on Day T_{19} and T_{21} of the treatment without oestrus detection.

The choice of the right time for mating or insemination of the mare is a great problem. Oestrus, the more classical criterion, is difficult to detect and does not allow a precise prediction of the time of ovulation. Two possibilities can be envisaged to make this choice easier: to improve this prediction or to induce oestrus and ovulation at predetermined times by a treatment. This second solution has the advantage of convenience and economy of labour. For this purpose we have used an analogue of PGF_2 alpha (Equimate ICI-81008) and HCG, two compounds which are known to be efficient in the mare.

The induction of luteolysis by prostaglandins (PG) or their analogues has been studied by several authors (DOUGLAS and GINTHER, 1972; ALLEN and ROWSON, 1973; NODEN, OKENBER and HAFS, 1974). When administered in a sufficient dose, the onset of oestrus occurs 48 to 72 hours later provided that the treatment is done during the luteal phase of the cycle at least four days after ovulation. But during
the induced oestrus, Allen and Rowson (1973) have shown that ovulation is not perfectly synchronized.

In cycling mares, intramuscular or intravenous injection of HCG on the second day of oestrus induces ovulation 24 to 48 hours later (Loy and Hughes, 1966; Sullivan, Parker and Larson, 1973).

We therefore used a PG-HCG sequence (HCG 6 days after PG) to reduce the variability of the follicular phase which follows the PG and allow the synchronization of ovulation that cannot be obtained with PG alone. But this treatment (one PG-HCG sequence) would be efficient in only a proportion of a group of mares (those treated after day 4 of the luteal phase of the cycle). Then we have used two successive PG-HCG sequences to synchronize all animals. The resulting treatment is described in table 1.

**TABLE I**

Synchronization treatment in the mare

| T₀   | Day of first injection, independent of the stage of the oestrous cycle |
| T₀   | Equinate*              | 250 µg | 1M injection |
| T₅   | HCG                   | 2500 IU | 1M injection |
| T₁₄  | Equinate              | 250 µg | 1M injection |
| T₂₀  | HCG                   | 2500 IU | 1M injection |

* A prostaglandin analogue ICI-81008

**MATERIALS AND METHODS**

Our treatment has been tested in the two following experiments.

*Experiment I*

Thirty three mares of different breeds (Thoroughbred, French saddle Horse, French Trotter) have been treated in spring, the first PG injection being done one April 30. The mares were observed for 13 days before treatment (Days T₋₁₃ to T₀), during the treatment (T₀ to T₂₀) and for 11 days post-treatment (T₂₁ to T₃₁).

- Oestrous detection was done daily by teasing the mares in a "breeding stall" with a stallion.
- Rectal examination of the ovaries was done at least every other day, but it was done every day if the mare was in oestrus or if a growing follicle was found.
- Daily blood samples were taken from the jugular vein. Plasma progesterone level was measured by a radioimmunological technique (Palmer and Jousset, 1974).

*Experiment II*

The same treatment (table 1) was used at the beginning of autumn (T₀ = Sept. 19 and 20) with 15 Breton mares (a draft horse breed) 2 1/2 years old. This trial was done to test the use of our technique with natural mating on Day 19 and 21 of the treatment, without oestrous detection.

In this case, the observations were simplified: only the progesterone assay was done, every other day from D T₋₁₈ to Day T₁₄, every day from Day T₁₄ to Day T₂₉, every other day from Day T₂₉ to Day T₄₇.

Four Breton stallions of normal fertility were used to cover the mares on the chosen days, each stallion doing 2 services per day during four successive days.
RESULTS

The ovarian activity of the mares during our synchronization treatment is shown in table 2 (Experiment I) and in table 3 (Experiment II). As the results are very similar, they were pooled for analysis.

TABLE 2

Individual ovarian activity of the 33 mares during experiment I

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I. Effect of the treatment in cyclic mares

Among the 48 treated mares, 41 responded in the expected way. Three types of response must be considered according to the period of the cycle on day T0.

1. Beginning of the treatment on one of the first four days of the luteal phase (Class A).

The first PG injection is not efficient (fig. 1). The luteal phase goes on. The first HCG has no visible effect. By contrast the second PG injection (T14) done near the end of the luteal phase is efficient. A rapid luteolysis is induced. A follicular phase begins around day T18 and ends around day T22 after an ovulation induced by the
TABLE 3
Individual ovarian activity of the 15 Breton mares during the experiment II

(period of high plasma progesterone level)

(Class A)
B1
B2
B3
B4
(Class B)
B5
B6
B7
B8
B9
B10
(Class C)
B11
B12
B13
B14
B15

abnormal pattern

Oestrus
(Day 0 = beginning of treatment)

Mare N°5
PGF  HCG  PGF  HCG

Fig. 1. — Plasma progesterone pattern of a mare treated on one of the first four days of the luteal phase
day T₂₀ HCG injection. Ten mares (7 in Experiment I and 3 in Experiment II) have followed this pattern.

2. **Beginning of treatment later in the luteal phase** (Class B).

The first PG injection is then efficient (fig. 2). A follicular phase begins around day T₂ and HCG induces an ovulation on day T₈. The second PG injection is given on day six of a new luteal phase, late enough to induce luteolysis around day T₁₆. The induced follicular phase begins at the same time as in Class A; likewise the HCG induced ovulation occurs around day T₂₂. Mares in persistent luteal activity respond in the same way. This type of response was obtained in 21 mares (14 + 7).

![Fig. 2. — Plasma progesterone pattern of a mare treated during luteal phase, more than four days after ovulation](image)

3. **Beginning of treatment during the follicular phase of cycle** (Class C).

The first PG injection does not disturb the cyclic pattern (fig. 3). The mare ovulates spontaneously before HCG injection, and is ready to respond to the second PG injection. Induced follicular phase and ovulation are synchronized with previous classes. Ten mares (8 + 2) were in this case.

![Fig. 3. — Plasma progesterone pattern in a mare treated during follicular phase](image)

II. **Effect of treatment in non-cyclic mares**

Seven mares did not follow the expected pattern. These animals were not cyclic, either at the beginning or at the end of the treatment.

— Four mares (Class I) showed a low progesterone level (< 1 ng/ml plasma) during the whole pretreatment period (fig. 4). These mares which did not show pattern of cyclic mares before treatment, however ovulated between day T₀ and day T₈.
either before or after the first HCG injection. They responded normally to the remainder of the treatment, and their induced follicular phase had a normal duration. This pattern was found only during the first experiment which took place at the end of the winter period of ovarian inactivity.

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Two mares (Class E) treated during luteal phase did not ovulate after the luteolysis induced by the first PG injection. None of the two HCG injections induced ovulation and the progesterone level remained low from day T₂ up to the end of the experiment (day T₄₇) (fig. 5). These two cases were found during the second experiment done at the end of September, the beginning of the winter period of ovarian inactivity.

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Finally, one mare in Experiment II had a non-cyclic abnormal ovarian activity. Its progesterone level fluctuated around 1 ng/ml plasma. In this case, the treatment did not induce a cycle.

III. — Synchronization of ovulation

Time of ovulation can be estimated by the progesterone pattern (Palmer and Jousset, 1974). Indeed the plasma progesterone level exceeds 1 ng/ml 24 to 48 hours after ovulation. The distribution of progesterone rises is shown in figure 6. A maximum (50% of the cases) occurs on day T₂₄ consequent to ovulations occurring on day T₀₂ or T₂₃. 75% of the mares ovulated in a 4 days period during which two systematic inseminations could be done. The other observations done during the first experiment (table 4) confirmed this good synchronization of oestrus (73% beginning in a 4 days interval) and ovulation (85% detected in a 4 days interval).
DISCUSSION

Up to the present time, no technique has been proposed to synchronize ovulations in order to cover or inseminate mares without any oestrous detection. Therefore, no comparisons are possible. However our results are encouraging as they resulted in 75 p. 100 of mares ovulating in a 96 hours period with two different breeds and in two different seasons.
The presence of mares in ovarian inactivity at the beginning of Experiment I and at the end of Experiment II shows that we have worked at the limits of the favourable period. These limits are the same as those of the occurrence of ovarian inactivity in non treated mares (PALMER and JOUSSET, 1974). The existence of some periods of persistent luteal activity during summer is not an objection to the efficiency of our treatment as it includes a luteolytic effect. By contrast, any technique for the winter period should include stimulation of follicular activity.

Any change in the intervals between injections would probably have a negative effect on the synchronization rate: on the one hand induction of ovulation by HCG needs sufficient follicular growth and induction of luteolysis by PG a four-days-old corpus luteum; therefore the intervals between injections must not be shortened. On the other hand, day $T_{14}$ is the moment of spontaneous luteolysis of mares of Class A and day $T_{21}$ the time of spontaneous ovulation of these mares. With longer intervals these mares would not be synchronized.

The observations made during the first experiment (table 4) give us some indication of the quality of the induced ovulation. Oestrus was present in 78 p. 100 of the mares, but it was sometimes difficult to detect. Only 60 p. 100 induced ovulations were found by rectal palpation. This percentage is lower than normal as we usually detect 80 p. 100 of ovulations. We think that it is the consequence of ovulation of smaller follicles. In spite of this small size, the formed corpus luteum has a normal progesterone secretion (fig. 7). In addition the quality of ovulation can be

![Graph showing progesterone levels over time.](image)

**Fig. 7.** — *Comparison between progesterone increase after*  
I: natural ovulations  
II: induced ovulations  

All differences are not significant.
tested by fertility. At the present time, it is too early to give a definitive fertility
rate. Among the 15 mares covered in October (Experiment II) 3 had no induced
ovulation and one ovulated spontaneously before the first mating. Seven of the eleven
remaining mares had still a high plasma progesterone level up to day T17 (25 days
after ovulation); so they are probably pregnant (PALMER, THIMONIER, LEMON, 1974).
This result is encouraging enough to go on testing fertility in a larger number of
animals.

CONCLUSION

The synchronization of oestrus and ovulation should eliminate many problems
in horse breeding: choice of the right time for mating, better distribution of the work
of the stallion, better management, possibility of use of artificial insemination.

Our technique (PG on day To, HCG on day T6, PG on day T14, HCG on day T20)
is a first solution for non-lactating mares during the summer season. Other treatments
must be found for post partum mares and for the winter period.

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RÉSUMÉ

SYNCHRONISATION DE L'ŒSTRUS ET DE L'OVULATION CHEZ LA JUMENT
AVEC UN TRAITEMENT DE DEUX SÉQUENCES PG-HCG

L'utilisation de PG et HCG a été testée pour synchroniser les chaleurs chez la Jument. D'après
des résultats préliminaires, un traitement de deux séquences PG-HCG, commencé à n'importe
quel moment du cycle (PG au jour To du traitement, HCG au jour T6, PG au jour T14, HCG au
jour T20) a été testé sur deux lots de juments : 33 juments de sang traitées en mai et 15 juments
Bretonnes traitées en septembre. Ce traitement a induit la synchronisation des ovulations chez
75 p. 100 des juments traitées, dans un intervalle de 4 jours. Le mécanisme d'action est décrit ;
la comparaison des ovulations induites avec les ovulations spontanées chez des juments non trai-
tées ne montre aucune différence. Ce traitement est efficace d'avril à septembre, période pendant
laquelle la Jument ne présente pas d'inactivité ovarienne. Si la fertilité est bonne, ce traitement
devrait permettre d'inséminer les juments les jours T19 et T21 du traitement sans détection
d'œstrus.

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