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SYNCHRONIZATION OF OESTRUS IN HEIFERS AND CYCLIC COWS WITH PROGESTAGENS AND PROSTAGLANDINS ANALOGUES ALONE OR IN COMBINATION

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SUMMARY

Several methods for synchronization of oestrus have been tested in groups of cyclic cows or heifers.

A) A progestagen treatment for a period equal to that of the luteal phase resulted in a high degree of synchronization : 90 p. 100 of treated females were in oestrus during a given 48 hour period. Artificial insemination without detection of oestrous, was feasible but fertility was generally low (34 p. 100).

A short progestagen treatment (9 to 10 days) in combination with a 5 mg oestradiol valerate injection on the first day resulted in only 68 p. 100 of females in oestrus within 5 days after the end of treatment and 58 p. 100 during a 48 hour period. Insemination without detection of oestrus was prohibited by the high proportion of females non synchronized, but fertility after AI at the induced oestrus (*i.e.* with detection of oestrus) was higher, 56 p. 100, than after a long progestagen treatment.

B) Two intramuscular injections of 500 µg of prostaglandin analogue (ICI-80996) at an interval of 10 days or a subcutaneous implant of 12 mg of SC 21009 (progestagen) for 10 days plus an intramuscular injection of the prostaglandin analogue at implant removal gave a similar degree of synchronization : 70 p. 100 of treated females were in oestrus during a given 48 hour period.

With the progestagen-prostaglandin analogue combination, the degree of synchronization was increased. The interval from the end of treatment to onset of oestrus was short (48 hours) when the level of endogenous progesterone was less than 0.5 ng/ml at the end of the progestagen treatment. This can be obtained in all treated females with a prostaglandin analogue injection 48 hours before SC 21009 implant removal. Thus, 92 p. 100 of treated females were in oestrus during a 48 hour period. Artificial insemination can be done without detection of oestrus.

A reduction in the length of progestagen treatment could obviate side effects of progestagens. A minimum 7 day progestagen treatment should be sufficient with the prostaglandin injection being given two days before the end of this period.

The control of sexual cycles is related to the control of the corpus luteum whose life-span and secretory activity result from trophic and lytic factors (DENAMUR, 1973 ; GODING, 1973). The aim is either to avoid the formation of a new corpus luteum or to induce luteolysis of an existing one (HANSEL and SCHECHTER, 1972).

Progestins block the ovulatory discharge of LH (PELLETIER and THIMONIER, 1969) and delay ovulation but do not alter, whithin limits, the secretory activity of a formed corpus luteum during its normal life span. Thus a progestagen treatment must be as long as the luteal phase, that is 18 to 20 days in cyclic cows and heifers.

On the other hand, in some cases, prostaglandins and oestrogens induce luteal regression (LAUDERDALE, 1972 ; ROWSON *et al.*, 1972 ; LEMON, 1975 ; THIMONIER *et al.*, 1974) but do not always inhibit ovulation.

Our purpose is to compare the efficiency of progestagens and luteolytic substances alone or in combination for the synchronization of oestrus in cyclic cows and heifers.

The efficiency of a synchronization treatment can be assessed by several criteria. The most commun is the percentage of females in oestrus within five days after the end of treatment. But maximum percentages of females in oestrus during a 24 or 48 hour period indicate the number of inseminations required. Finally, fertility is an important aspect of synchronization schemes.

I. — LONG PROGESTAGEN TREATMENT AND SHORT PROGESTAGEN TREATMENT ASSOCIATED WITH AN OESTRADIOL VALERATE ADMINISTRATION

A. — *Comparative efficiency of synchronization of oestrus*

Several progestagens can be used and the choice depends on the method of administration related to herd management (MAULEON, 1974).

Thus, a daily i. m. injection of 6 or 7 mg of norethandrolone is sufficient to block oestrus and ovulation in cyclic cows and heifers (CHUPIN *et al.*, 1974 *a*). The same result can be obtained with 6 or 12 mg of SC 21009 implants, but, in this case, the treatment cannot be applied for a long period (CHUPIN *et al.*, 1974 *b*) because of the progressive decline of the progestagen in the implant.

The duration of treatment has an effect on the degree of synchronization of oestrus. All cows and heifers receiving norethandrolone for 18 days are in oestrus within the five days after the last injection (table 1). But, only 68 p. 100 of females receiving a short progestagen treatment (9 to 10 days) even in combination with an oestradiol valerate injection on the first day, are in oestrus during an equivalent five day period.

With a long progestagen treatment, 90 p. 100 of females are in oestrus during a 48 hour period. Two inseminations at an interval of 24 hours should result in normal conception rate for most females even if no detection of oestrus is done. But a single insemination does not appear to be feasible since only 58 p. 100 of cows are in oestrus during a given 24 hour period.

With a short progestagen treatment, insemination without detection of oestrus is prohibited by the high proportion of females not synchronized (table 1).

TABLE I

Synchronization of oestrus after a long (18 days) *or a short* (9 to 10 days) *Progestagen treatment in cyclic cows and heifers*

Treatment		No. of females	Percentage in oestrus after the end of treatment		Maximum in oestrus (%) in a period of	
Duration (days)	Nature		Within 5 days	During the first 60 hours	48 hours	24 hours
18	Norethandrolone 6 or 7 mg/day IM	78	100**	89.7**	89.7**	57.7
9 or 10	Norethandrolone 7 mg/day IM or SC 21009 implants (6 and 12 mg) + 5 mg oestradiol (1) valerate	88	68.2**	46.6**	57.9**	43.2

(1) On the first day of treatment.
** $P < 0.01$.

B. — *Corpus luteum secretory activity during a short progestagen treatment in combination with oestradiol valerate*

The secretory activity of the corpus luteum can be assessed by the level of endogenous progesterone since the progestagens we used did not cross react either in the competitive protein binding assay or in the radioimmunoassay for progesterone.

When the short progestagen treatment in combination with a 5 mg oestradiol valerate injection on the first day of implant insertion was started at the end of the luteal phase, normal corpus luteum regression (time and duration) occurred. But, oestrus and ovulation were delayed as shown by the long period with low endogenous levels of progesterone until the end of treatment (fig. 1). The interval between end of treatment and onset of oestrus was generally short (48 hours).

When the same treatment began just after oestrus and ovulation, the secretory activity of the corpus luteum was sometimes modified (fig. 1, cows A 140 and 996).

The interval from end of treatment to onset of oestrus was longer when the corpus luteum activity was not modified (5 days for example, cow A 58). It was short (48 hours, cows A 140 and 996) when the endogenous progesterone level at the end of the progestagen treatment was less than 0,5 ng/ml. Variations in the luteolytic action of oestradiol valerate from one female to an other can explain the low degree of synchronization in cows treated during the first days of oestrus cycle (table 2).

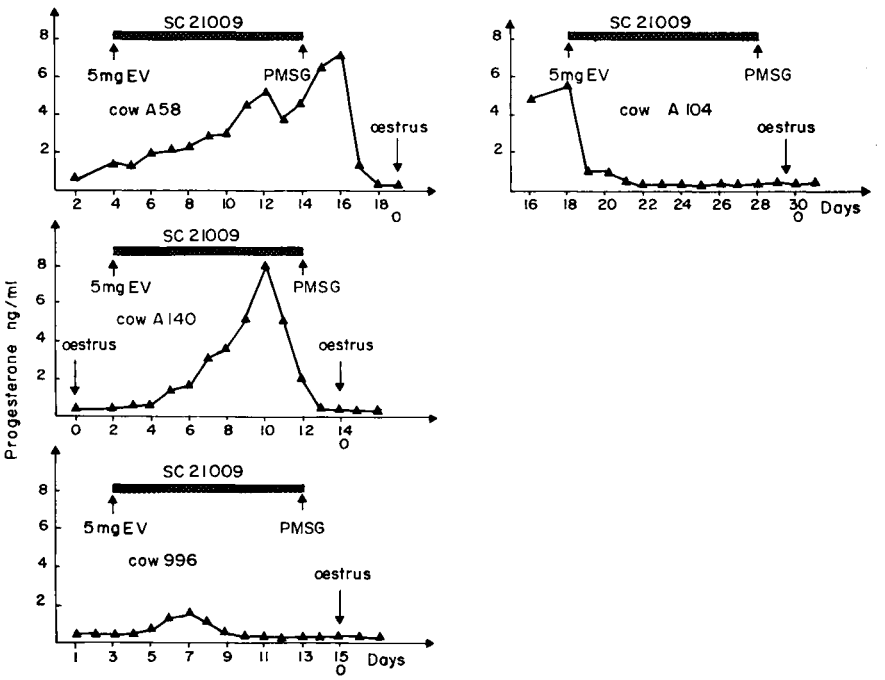

FIG. 1. — *Typical progesterone patterns during a 10 day progestagen treatment*
+ 5 mg of oestradiol valerate (EV)
+ PMSG

TABLE 2

Onset of oestrus after a short progestagen treatment in combination with an oestradiol valerate injection. Effect of stage of the cycle when treatment began

(from MAULEON, 1974)

Day of cycle when treatment began	No. of females	Percentage of females in oestrus on different days after the end of treatment					
		1-2-3	4-5-6	7-8-9	10-11-12	13-14-15	16-17-18-19
1- 4	25	16.0	20.0	16.0	16.0	16.0	16.0
5- 8	13	76.9	15.4	7.6	—	—	—
9-12	16	87.5	12.5	—	—	—	—
13-16	20	90.0	5.0	—	—	5.0	—
17-20	19	52.6	10.5	15.8	15.8	—	8.3

Thus, the degree of synchronization depends closely on the day of the cycle on which a short progestagen treatment commenced (MAULEON, 1974). Percentages of females in oestrus within 3 days after the end of treatment were respectively

89.8 and 29.6 ($P < 0.001$) when treatment began either between days 5 and 16 of the cycle or during the period near ovulation. In a group of cycling females receiving a short treatment (9 days) starting without regard to the day of cycle, 25 to 35 p. 100 of them are not synchronized.

C. — *Fertility*

Fertility after artificial insemination at the induced oestrus (*i.e.* with detection of oestrus) was low, 34 p. 100, after a long progestagen treatment and significantly different from that observed after a short one : 56 p. 100 (table 3). But, if artificial insemination was carried out without detection of oestrus, fertility was low with the short treatment because of the high proportion of non synchronized females (MAULÉON, 1974).

TABLE 3

Fertility (induced oestrus) *after progestagen treatment according to the duration of treatment*

Treatment		No. of females	Calving (%)
Duration (days)	Nature		
18	Norethandrolone 6 or 7 mg/day IM	59	33.9*
9 or 10	Norethandrolone 7 mg/day IM or SC 21009 implants (12 mg) + 5 mg oestradiol valerate	64	56.3*

Artificial insemination after detected oestrus.
* $P < 0.05$.

A high conception rate, thus, seems incompatible with a high degree of synchronization obtained by a long progestagen treatment.

A similar degree of synchronization was therefore attempted with a progestagen-prostaglandin combination or prostaglandin alone.

II. — PROGESTAGEN-PROSTAGLANDIN COMBINATION. TWO PROSTAGLANDIN INJECTIONS AT A 10 DAY INTERVAL. COMPARATIVE EFFICIENCY OF SYNCHRONIZATION OF OESTRUS

In the bovine, prostaglandin $F_{2\alpha}$ and its analogues are not luteolytic during the first five days of the cycle (ROWSON *et al.*, 1972 ; LAUDERDALE, 1972 ; THIMONIER *et al.*, 1974) and produce variable effects during the follicular phase. But

two injections at a 10 day interval may allow synchronization of oestrus in a group of cycling females. A prostaglandin injection at the end of a short progestagen treatment may result in luteal regression in those females with an active corpus luteum. We have compared the efficiency of these two treatments.

A. — *Experimental procedure*

158 females were divided into 2 groups and received :

— either two intramuscular injections of 500 µg of ICI-80996, a prostaglandin analogue (WALPOLE, 1975) at an interval of 10 days (Group A),

— or a subcutaneous implant of 12 mg of SC 21009 for 10 days plus an intramuscular injection of the prostaglandin analogue at implant removal (Group B).

As shown in table 4, the distribution of females in the two groups was similar.

Detection of oestrus was carried out twice a day with vasectomized bulls and androgenized cows (SIGNORET, 1975).

TABLE 4

Experimental design.
Number of females in each group

Treatment	Day of the cycle when treatment began																			Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
A	7	6	5	6	7	4	4	4	1	3	2	s2	5			7	7	4	5	79
B	7	6	5	6	7	4	4	4	1	3	2	2	5			7	7	4	5	79

A : 2 injections (500 µg) of a prostaglandin analogue (ICI-80996) at a 10 day interval.
B : Progestagen implants (12 mg SC 21009) for 10 days + 1 injection of 500 µg ICI-80996 at implant removal.

B. — *Onset of oestrus at the end of the two treatments*

The onset of oestrus was similar in the two groups of females (table 5). The degree of synchronization obtained was lower than that observed after a long progestagen treatment. Indeed, only 40 p. 100 of cows were in oestrus during a given 24 hour period and 70 p. 100 during a given period of 48 hours. In the two groups, there was a small number of non synchronized females. Ovulation without oestrus, long follicular phase, failure of luteal regression after the second PG injection were the reasons for non synchronization.

The study of the levels of progesterone allows a better understanding of the reasons for the relative low degree of synchronization after the two treatments.

TABLE 5

Synchronization of oestrus after the second prostaglandin analogue injection (A) *or removal of implants* (B)

	Treatment	No. of females	Percentage in oestrus after the end of treatment		Maximum in oestrus (%) in a period of	
			within 5 days	during the first 60 hours	48 hours	24 hours
A	2 × 500 µg ICI 80996	79	82.3	46.8	68.4	43.0
B	12 mg SC 21009 + 500 µg ICI 80996	79	93.7	48.1	73.4	40.5

1° *In group A*, when the first prostaglandin analogue injection did not induce luteolysis, the second was generally given after the 10th day of the same cycle. The interval from second prostaglandin injection to onset of oestrus was long (72 to 96 hours, fig. 2).

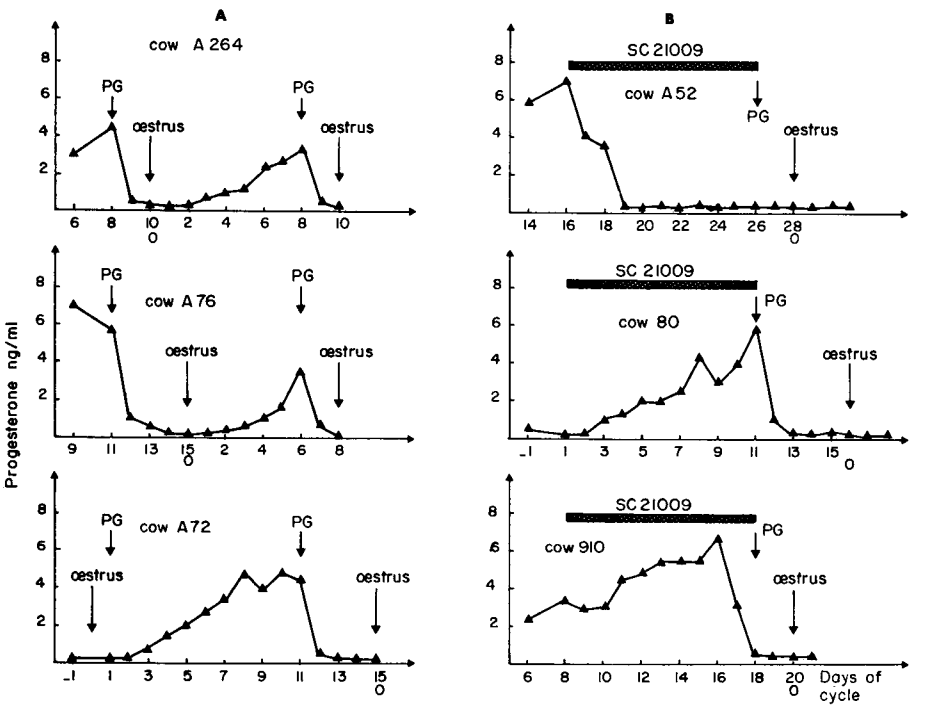

FIG. 2. — *Evolution of progesterone levels in females receiving either 2 injections of a prostaglandin analogue* (ICI-80996) *or a progestagen-treatment* (SC 21009) *associated with an injection of the same analogue*

But, when the first prostaglandin administration induced luteal regression, the second was made between days 6 and 8 of a new cycle and the onset of oestrus after the second administration was short : 48 hours. However, in two cases, the second injection was given before the 5th day of the induced cycle and no luteolysis took place.

Effectively, in the first case, only 16 p. 100 of cows and heifers were in oestrus within 60 hours after the second injection compared to 67 p. 100 ($P < 0,01$) in the second case (table 6).

TABLE 6

Onset of oestrus after the second prostaglandin analogue injection (A) *or removal of implants* (B)

Treatment			No. of females	Percentage in oestrus after the end of treatment		Maximum in oestrus (%) in a period of	
				within 5 days	during the first 60 hours	48 hours	24 hours
A	First injection between	days 1 and 5	31	90.3	16.1**	71.0	48.4
		days 6 and 19	48	77.1	66.6**	77.1	64.6
B	Implants inserted between	days 1 and 9	44	90.9	27.7**	65.9	50.0
		days 10 and 19	35	97.1	80.0**	82.9	71.4

** $P < 0.01$.

The relatively low degree of synchronization observed with this treatment can be explained by variations in the interval from second injection to onset of oestrus depending on the time at which treatment was applied. Follicular growth waves (MARIANA and NGUYEN HUY, 1973) during the cycle are probably responsible for the variations in the onset of oestrus.

2° *In group B*, when treatment began during the first half of the cycle, a functionnal corpus luteum was present when the prostaglandin analogue was injected. Luteolysis was then observed but the interval from end of treatment to onset of oestrus was long (72 to 96 hours : fig. 2). It was shorter (48 hours) when the level of endogenous progesterone at the end of the SC 21009 treatment was less than 0.5 ng/ml, that is when the corpus luteum had already regressed.

Thus, when the treatment began during the first half of the cycle, only 28 p. 100 of cows and heifers were in oestrus within 60 hours after removal of SC 21009 implant, compared to 80 p. 100 ($P < 0.01$) when the treatment commenced during the second half of the cycle (table 6).

These variations in the interval from end of treatment to onset of oestrus explain the relatively low degree of synchronization observed in a group of females receiving this treatment.

III. — ATTEMPTS TO IMPROVE THE DEGREE OF SYNCHRONIZATION

In groups A and B of the preceeding experiment, the synchronization was higher than that after a short progestagen treatment in combination with oestradiol valerate but the relatively low percentage of females in oestrus during a 48 hour period (70 p. 100) did not allow an artificial insemination without detection of oestrus.

We have tried, therefore, to increase the degree of synchronization. This is possible by the use of a combined progestagen-prostaglandin treatment.

In fact, the percentage of females in oestrus within 60 hours after the end of a progestagen treatment was 86 when the level of endogenous progesterone was less than 0,5 ng/ml at implant removal, but only 36, when this level was greater than 0,5 ng/ml (table 7). The percentages of females in oestrus during a 24 hour period were respectively, 80 and 45 for these two classes of animals ($P < 0.01$). With the prostaglandin analogue, it is easy to obtain endogenous level of progesterone less than 0.5 ng/ml at the end of a short progestagen treatment beginning without regard to day of cycle. Prostaglandin analogue must be injected before the end of the progestagen treatment.

TABLE 7

Onset of oestrus in cyclic cows and heifers after removal of SC 21009 implants
(12 mg for 10 days)

Level of endogenous progesterone at implant removal	No. of females	Percentage in oestrus during the first 60 hours after the end of treatment	Maximum in oestrus (%) in a period of	
			48 hours	24 hours
> 0,5 ng/ml	55	36.3**	81.8	45.4**
≤ 0,5 ng/ml	59	86.4**	94.9	79.6**

** $P < 0.01$.

A. — *Onset of oestrus in cyclic heifers receiving a prostaglandin analogue injection 2 days before or at the end of a 9 day progestagen treatment*

Thirty-five cyclic heifers received a progestagen treatment (12 mg SC 21009, subcutaneous implant) for 9 days commencing between days 1 and 7 of the cycle (D_0 = day of observed oestrus).

Seventeen females (Group *a*) received an intramuscular injection of 500 µg of the ICI-80996 compound 2 days before implant removal (R_{-2}), and eighteen (Group *b*) at implant removal (R_0).

Levels of progesterone were estimated in all females on days R_{-2} and R_0.

All treated heifers had an endogenous level of progesterone higher than 0.5 ng/ml at R_{-2}, but only heifers of group *a* had an endogenous level of progesterone less than 0.5 ng/ml at R_0. 76 p. 100 of them were in oestrus within 60 hours after the end of treatment compared to 28 p. 100 for heifers of group *b* ($P < 0.05$) (table 8).

It seems clear that the time of prostaglandin analogue injection modified the onset of oestrus after the end of the short progestagen treatment.

TABLE 8

Onset of oestrus in cyclic heifers receiving a prostaglandin analogue injection (ICI-80996) *2 days before or at the end of a 9 day progestagen treatment* (SC 21009)

(Beginning of progestagen treatment : between days 1 and 7 of the oestrous cycle)

Time of prostaglandin analogue injection	No. of females	Percentage in oestrus after the end of treatment		Maximum in oestrus (%) in a period of	
		within 5 days	during the first 60 hours	48 hours	24 hours
R_{-2} Group *a*	17	100	76.4*	88.2	64.7
R_0 Group *b*	18	100	27.8*	94.4	66.7

* $P < 0.05$.

B. — *Onset of oestrus in cows receiving a prostaglandin analogue injection 2 days before or at the end of a 9 day progestagen treatment*

The same procedure was used in a herd of 102 milking cows of the Friesian breed. However, the progestagen treatment started regardless of the day of the cycle between 20 and 93 days after parturition. Fifty-one cows were injected on R_{-2} (Group *a*) and 51 on R_0 (Group *b*).

Endogenous levels of progesterone were not measured.

Synchronization of oestrus for the two groups was different : 86 p. 100 of group *a* and 59 p. 100 of group *b* were in oestrus within 60 hours after implant removal (table 9) and the percentages of females in oestrus during a 24 hour period were respectively 71 p. 100 (group *a*) and 51 p. 100 (group *b*) ($P < 0.05$).

Thus, a prostaglandin analogue injection before the end of the progestagen treatment increases the degree of synchronization.

In the two groups (*a* and *b*), fertility of cows inseminated after detection of oestrus was similar and approximated 50 p. 100.

TABLE 9

Onset of oestrus in cows receiving a prostaglandin analogue injection (ICI-80996) *2 days before or at the end of a 9 day progestagen treatment* (SC 21009)

Time of prostaglandin analogue injection	No. of females	Percentage in oestrus after the end of treatment		Maximum in oestrus (%) in a period of	
		within 5 days	during the first 60 hours	48 hours	24 hours
R_{-2} Group *a*	51	96.0	86.3**	92.2*	70.6
R_0 Group *b*	51	92.1	58.8**	72.5*	51.0

* $P < 0.05$.
** $P < 0.01$.

CONCLUSION

Use of prostaglandin $F_{2\alpha}$ and its analogues during the last two years represents an important advance in the synchronization of oestrus in cyclic cows and heifers. A two « prostaglandin injection system », with an interval of 10 to 12 days, gives synchronized oestrus in groups of cyclic females. However, the degree of synchronization is still relatively low and does not yet allow a single artificial insemination during a restricted period without detection of oestrus.

The progestagen-prostaglandin combination can probably improve the precision of synchronization allowing a single insemination.

A reduction in the length of progestagen treatment could obviate side effects of progestagens. A minimum of 7 day progestagen treatment should be sufficient with the prostaglandin injection being given two days before the end of this period.

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RÉSUMÉ

SYNCHRONISATION DE L'ŒSTRUS CHEZ LES GÉNISSES ET LES VACHES CYCLIQUES PAR LES PROGESTAGÈNES ET LES ANALOGUES DES PROSTAGLANDINES SEULS OU EN COMBINAISON

Plusieurs méthodes pour la synchronisation de l'œstrus dans des groupes de vaches et génisses cycliques ont été essayées :

A) Un traitement progestagène long, d'une durée égale à celle de la phase lutéale (18 jours) donne un degré de synchronisation élevé puisque 90 p. 100 des femelles sont en œstrus sur une période de 48 heures. Des inséminations artificielles peuvent être réalisées sans détection préalable de l'œstrus, mais la fertilité est faible (34 p. 100).

Un traitement progestagène court (9 à 10 jours) associé à une injection de 5 mg de valérate d'œstradiol le premier jour, ne permet pas la synchronisation de l'œstrus chez l'ensemble des femelles traitées : 68 p. 100 seulement viennent en œstrus dans les 5 jours qui suivent l'arrêt du traitement et 58 p. 100 sur une période de 48 heures. L'insémination artificielle sans détection préalable de l'œstrus est donc impossible. En revanche, la fertilité est plus élevée (56 p. 100) lorsque les inséminations sont faites après détection de l'œstrus qu'après un traitement long.

B) Deux injections d'un analogue de la prostaglandine $F_{2\alpha}$ (ICI-80996 séparées par un intervalle de 10 jours ou l'injection de ce même analogue à la fin d'un traitement progestagène court (implants de SC 21009 mis en place pour une durée de 10 jours) donnent un degré de synchronisation identique : 70 p. 100 des femelles traitées sont en œstrus sur une période de 48 heures

Dans le cas particulier de l'association progestagène (SC 21009) — analogue de la prostaglandine $F_{2\alpha}$ (ICI-80996), il est possible d'augmenter le degré de synchronisation. En effet, l'intervalle fin du traitement — début de l'œstrus est court (48 h) lorsque le niveau de progestérone endogène est inférieur à 0,5 ng/ml à la fin du traitement. Ceci peut être obtenu chez la totalité des femelles traitées par injection de l'analogue 48 heures avant le retrait des implants SC 21009. Dans ces conditions, 90 p. 100 des femelles traitées sont en œstrus sur une période de 48 heures. L'insémination artificielle sans détection préalable de l'œstrus est possible.

La fertilité est alors voisine de 50 p. 100.

Une réduction de la durée du traitement progestagène peut être envisagée. La durée minimale serait de 7 jours, l'agent lutéolytique étant injecté 48 heures avant l'arrêt du traitement.

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