Synchronization of Estrus with Prostaglandin $F_{2\alpha}$ in Cattle

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For more than ten years, grassland establishment has rapidly spread with the development of mass grazing of cattle in Japan. However, it has been pointed out that there are many problems related to management of feeding, health, breeding, etc., of cattle due to a short history of grazing.

The authors made investigations on the reproductive efficiency of cattle on grazing lands in the northern Tohoku region. It was recognized that it was exceedingly important for improving fertility to detect and capture estrous cows in order to perform artificial insemination at an optimum time[13] for insemination. However, it requires much labor and time to do this management of breeding in the open yard feeding system. Accordingly, it may be highly desirable to establish a technique of estrus synchronization of cows, such technique has attracted a keen interest in European and American countries for a long time. To find out a method to induce estrus and ovulation concentrated in a short period of time with a large number of cows showing different phases of the estrous cycle, many methods have been examined so far. A method using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is known to have a luteolytic effect, is regarded to be the most efficient, on the basis of the results of many studies[6,7].

For the purpose of searching for a technique of estrus synchronization, the authors carried out the studies on the time of $PGF_{2\alpha}$ administration, and effectiveness of $PGF_{2\alpha}$ administered singly or in combination with some hormones on the estrus synchronization. They also tried to clarify the fertility in the estrus period synchronized by the treatment. The results obtained are summarized in this paper.

Conditions of $PGF_{2\alpha}$ administration

Luteolytic effect of $PGF_{2\alpha}$ on cows varies with the phase of the estrous cycle when $PGF_{2\alpha}$ is administered[19,23]. Namely, when it is administered by 4 days after ovulation, the regression of the corpus luteum is incomplete, and ovulation occurs 8 to 22 days after the treatment. When it is administered 5 days after ovulation, the regression of the corpus luteum takes place rapidly, and is followed by the development of a follicle, and ovulation occurs 4 to 6 days after the treatment (Table 1)[19]. The changes in the progesterone level of peripheral blood plasma reflects the growing and regressive changes of the corpus luteum detected by rectal palpation[1]. In brief, $PGF_{2\alpha}$ exerts an apparent luteolytic effect only over a period from 5 to 16 days after the ovulation.

Administration of $PGF_{2\alpha}$ to cows is usually made by intrauterine infusion or intramuscular injection. To find an optimal site of infusion, the infusions into the uterine horn ipsilateral to the corpus luteum[6,15,19] and the uterine body[5] have been examined. The former was carried out, because a uterine

Table 1. Time interval in days between intrauterine infusion with $PGF_{2\alpha}$ and ovulation in the cow

	Number of cows	Day[a] of estrous cycle at treatment								
		2	3	4	5	9	10	11	15	16
Successive infusions	5		11	11	4 4		4			
Single infusion	14	22 14	19 18	13 8	4 4	4		4	6 4	5 4

a) Day 0 = Day of ovulation

luteolytic factor produced in the endometrium is translocated directly to the ipsilateral ovary by the counter-current mechanism[3]. Liehr et al.[51] and Nakahara et al.[19] indicated that the time interval between $PGF_{2\alpha}$ infusion into the uterine horn contralateral to the corpus luteum and ovulation was variable, and always longer than that between the infusion into the uterine horn ipsilateral to the corpus luteum and ovulation. In the intrauterine infusion of $PGF_{2\alpha}$, the time interval between the treatment and the appearance of estrus was a little longer when the volume of solvent of $PGF_{2\alpha}$ was 5.0 m*l* than when it was 2.5 m*l*[7].

Thus, it seemed that the intrauterine infusion of $PGF_{2\alpha}$ was the most effective when it was performed in the uterine horn ipsilateral to the corpus luteum. In this case, the desirable volume of $PGF_{2\alpha}$ solvent was less than 2.5–2.5 m*l* because it allowed the $PGF_{2\alpha}$ solution to stay in the treated uterine horn, and did not flow out into the opposite uterine horn. The dose of $PGF_{2\alpha}$ which can cause corpus luteum regression with certainty by a single intrauterine infusion has been reported to range from 1.5 to 2.0 mg[5,6].

On the other hand, the effective dose of $PGF_{2\alpha}$ for intramuscular injection has been reported to range from 20 to 30 mg[2,16,22], which was considerably larger than that for intrauterine infusion. Nakahara et al.[21] concluded, from their experiment on a housing cow, that the minimum effective dose of intramuscular injection of $PGF_{2\alpha}$ for inducing corpus luteum regression was 6 to 8 mg. For grazing cows, however, a dose of 10 mg was rather insufficient, and a satisfactory estrus synchronization could be obtained when the dose exceeded 15 mg[10].

Synchronizing estrus by the use of $PGF_{2\alpha}$ alone

Grazing cows were treated by either of the two method; that is, an infusion of 4 to 6 mg of $PGF_{2\alpha}$ into the uterine horn ipsilateral to the ovary with a cropus luteum[20], and an intramuscular injection of 15 mg of $PGF_{2\alpha}$, during the luteal phase of the estrous cycle. In both groups, estrus was caused 2 to 5 days after the treatment at the rate of 86% (Fig. 1 and Table 2). Estrous signs were distinct in most of the cows. Most of the cows which had been recognized to show silent heat before the experiment manifest outstanding estrous signs. It is noteworthy that $PGF_{2\alpha}$ can be used effectively for the treatment of silent heat occurring at a high frequency in grazing cows. The conception rates obtained by artificial insemination in the synchronized estrous period was 73% of the cows treated by the intrauterine infusion and 71% of those treated by the intramuscular injection (Fig. 2 and Table 2). These conception rates were equal to or rather higher than that of untreated control cows. This result may be caused by the fact that it was possible to detect estrus which occurred concentratively within a limited period after the treatment, and then to determine an optimum time for insemination.

Table 2. Occurrence of estrus, and fertility after intramuscular injection of PGF (15 mg) in grazing cows

Herd	Number of cows						Conception rate at synchronized estrus
	Treated	Day between treatment and estrus					
		2	3	4	5	6≥	
A	15	1	10	2	1	1	53.9% (7/13)[a]
Y	15	1	7	3	1	3	75.0% (9/12)
T	15	2	8	3	1	1	85.7% (12/14)
O	20	3	8	5	0	4	70.6% (12/17)
Total	65	7	33	13	3	9	71.4% (40/56)
			56 (86.2%)				

a) Number of pregnant cows / Number of cows inseminated.

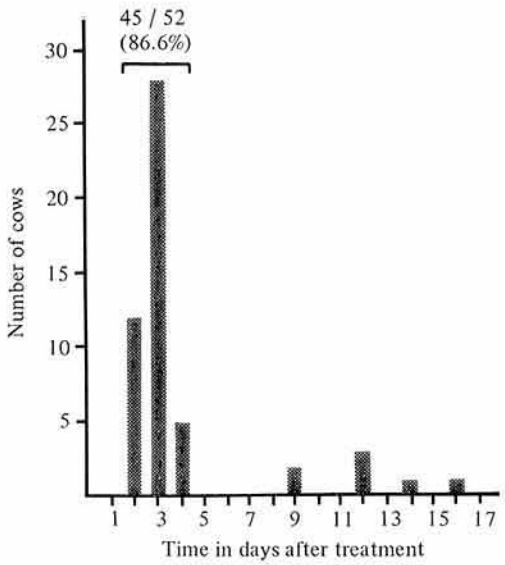


Fig. 1. Occurrence of estrus following intrauterine infusion with $PGF_{2\alpha}$ grazing cows

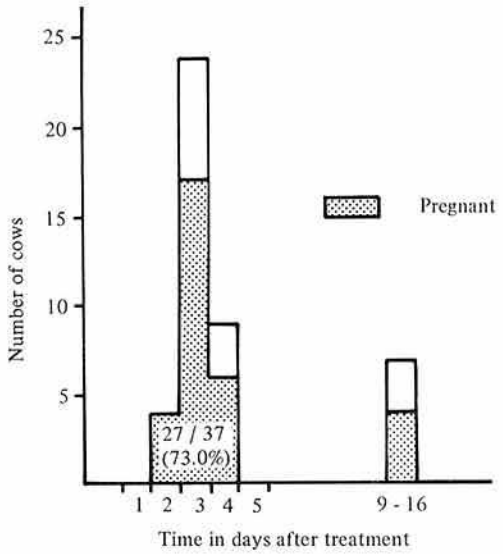


Fig. 2. Fertility in synchronized estrous period following intrauterine infusion with PGF_2 in grazing cows

Efficiency of synchronizing estrus and the resultant conception rate are markedly influenced by the health conditions of cows[10].

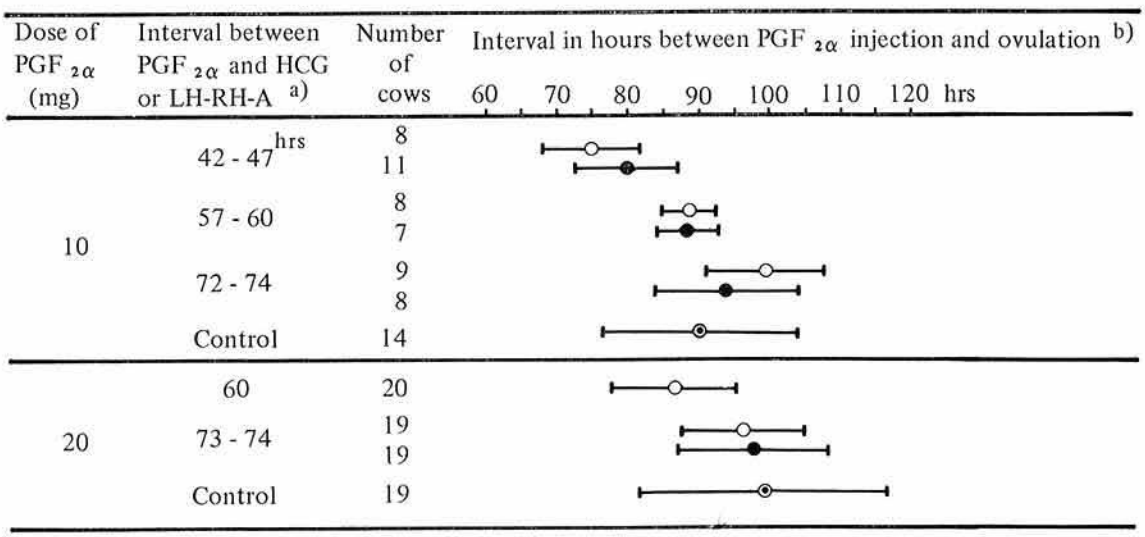
Synchronizing estrus by the use of $PGF_{2\alpha}$ in combination with hormones

In practical application of an estrus synchronizing technique by the use of $PGF_{2\alpha}$ to the breeding management of grazing cows, it is desired to make ovulation concentrated within a much limited period of time in order to facilitate insemination in a synchronized estrous period. For this purpose, $PGF_{2\alpha}$ was used in combination with various hormones, such as gonadotropin[7,18], luteinizing hormone-releasing hormone (LH-RH)[4] and estrogen[4,24].

1) Combined use of $PGF_{2\alpha}$ and ovulation-inducing hormones

Grazing cows were administered with 3 to 4 mg of $PGF_{2\alpha}$ by intrauterine infusion or with 10 to 20 mg of $PGF_{2\alpha}$ by intramuscular injection during the luteal phase of the estrous cycle. Then they were injected intramuscularly with 200 g of synthetic LH-RH[8], 200 μg of the synthetic analogue of LH-RH, [Des-Gly-NH_2[10], Pro-ethylamide[9]]—LH-RH (LH-RH-A)[8,9,12], or 1,000 to 2,000 IU[9,10] of HCG 42–47 hrs, 57–60 hrs, or 72–74 hrs after $PGF_{2\alpha}$ treatment. In two groups of cows treated with LH-RH-A and HCG, respectively, there was a tendency that the earlier cows were treated with these ovulation-inducing hormones, the less frequently they exhibited standing estrus, and the more frequently quiet ovulation occurred in them. In both groups, ovulation occurred concentratively within a short period of time in comparison with the control group and the earlier cows were treated with ovulation-inducing hormones, the shorter was the time interval between $PGF_{2\alpha}$ administration and ovulation (Fig. 3). In both groups the conception rate in the synchronized estrous period was essentially the same and it was 51%, which did not differ markedly from that of 61% in the control group, when the time interval between the administration of $PGF_{2\alpha}$ and that of ovulation-inducing hormone was 72–74 hrs. But, it was reduced to 46% and 25%, when this time interval was 56–60 hrs and 42–47 hrs, respectively. The conception rate in the cows which showed quiet ovulation was 36% which was lower than that of 53% in the cows exhibited standing estrus.

In another experiment, cows were injected intramuscularly with 10 mg or 20 mg of $PGF_{2\alpha}$ in the luteal phase of the estrus cycle, and then with 2,000 IU of HCG[11] or 200 μg of LH-RH-A[12] 42 or 72 hrs after $PGF_{2\alpha}$ injection. Irrespective of the dose of $PGF_{2\alpha}$, and the type and time of administration of ovulation-inducing hormone, the treated group showed a low frequency of appearance



a) [Des-Gly-NH_2[10], Pro-ethylamide[9]] - LH-RH.

b) ○ LH-RH-A (200 μg) - injected cows.

● HCG (1.000 - 2 000 IU) - injected cows.

⊢—○—⊣ Mean interval in hour ± S.D.

Fig. 3. Occurrence of ovulation after intramuscular injection with $PGF_{2\alpha}$ followed by additional HCG or LH-RH analogue in grazing cows

of standing estrus and a high incidence of quiet ovulation. However, ovulation occurred concentratively in a short period of time of about 32 hrs after the treatment, and the conception rate in the synchronized estrous period was normal.

From these results it was confirmed that it was possible to induce ovulation concentrated within a short period of time by the combined use of $PGF_{2\alpha}$ and ovulation-inducing hormones. Moreover, a time interval of 60 hrs between the injection of $PGF_{2\alpha}$ and the subsequent injection of ovulation-inducing hormone was considered to be appropriate for an efficient synchronization of ovulation.

2) Combined use of $PGF_{2\alpha}$ and estrogen

Two groups of grazing heifers were administered intramuscularly with 15 mg of $PGF_{2\alpha}$ in the luteal phase of the estrus cycle, with and without a simultaneous intramuscular injection of 0.5 mg of estradiol benzoate (E_2B)[14]. In the group treated with E_2B, estrus and ovulation occurred concentratively within a shorter period of time sooner after the treatment than in the control group without E_2B treatment. The conception rate in the synchronized estrous period was similar between the two groups (Table 3). A subnormal formation of the corpus luteum after ovulation was noticed in the group treated with E_2B, though in only a few cows.

Subsequently, grazing heifers as well as housing heifers kept under mass feeding were injected simultaneously with 15 mg of $PGF_{2\alpha}$ and 0.5 mg of E_2B by the intramuscular route, during the luteal phase of the estrous cycle, and then with 1,500 IU of HCG by the same route either at the time of insemination in the synchronized estrous period or 2 to 3 days after ovulation. In the two groups, treated with HCG at the time of insemination and 2 to 3 days after ovulation, respectively, the number of cows with a hypoplastic corpus

Table 3. Occurrence of estrus and ovulation, and fertility after intramuscular injection with $PGF_{2\alpha}$ plus E_2B in grazing cows

Treatment[a]	Number of cows			Conception rate at synchronized estrus
	Treated	In estrus within 60 hrs[b]	Ovulating within 96 hrs[b]	
		%	%	[c]
$PGF_{2\alpha}$	15	8 (53)	10 (67)	54% (7/13)
$PGF_{2\alpha}+E_2B$	16	14 (88)	14 (88)	56% (9/16)

a) $PGF_{2\alpha}$, 15 mg; E_2B: estradiol benzoate, 0.5 mg.
b) Hours after treatment.
c) Number of pregnant cows / Number of cows inseminated.

Table 4. Findings of the corpus luteum 10–12 days after ovulation in heifers treated with simultaneous injection with $PGF_{2\alpha}$ and E_2B[a] followed by additional HCG, and fertility at synchronized estrus after treatment

Exp. group	Time of administration of HCG[b]	No. of heifers	No. of heifers with small corpus luteum[c]	No. of heifers with additional corpus luteum	No. of heifers pregnant
			%	%	%
A	at the time of A.I.	40	2 (5.0)	0	23 (57.5)
B	2–3 day after ovulation	40	0	33 (82.5)	22 (55.0)
C	—	39	7 (18.0)	0	16 (41.0)

a) $PGF_{2\alpha}$, 15 mg; E_2B: estradiol benzoate, 0.5 mg.
b) HCG, 1,500 IU.
c) Corpus luteum less than 1.6 cm in length.

luteum was smaller than in the group not treated with HCG. In the group treated with HCG 2–3 days after ovulation, 1 to 2 additional corpora lutea were formed in a majority of the cows. The conception rate in the synchronized estrous period was a little higher in the two HCG treated groups than in the untreated control group (Table 4). There was no difference in it between two treated groups.

On the basis of these results, it was concluded that the simultaneous intramuscular injection of $PGF_{2\alpha}$ and E_2B was effective for synchronizing estrus and ovulation. It was also clarified that an additional administration of HCG at the time of insemination in the synchronized estrous period or 2–3 days after ovulation was effective for the development of a corpus luteum and the improvement of fertility.

Conclusions

From all the results so far obtained, it is quite likely that the technique of estrus synchronization by the use of $PGF_{2\alpha}$, either singly or in combination with ovulation-inducing hormone or estrogen, may be practically applicable to grazing cows as a means of saving labor required for the management of breeding, as well as of increasing the reproductive efficiency of a herd as a whole, including cows suffering from breeding disorders, such as silent heat and persistent corpus luteum. For an efficient application of this technique to the field management of breeding, it is an essential prerequisite that a cow to be treated should have a normal estrous cycle. A problem on the management of breeding which will attract a serious attention in future is an ovarian quiescence occurring at a high frequency in the field. Therefore, the first consideration should be given to an appropriate feeding management to promote a normal sexual maturation of heifers after birth, and a normal recovery of the ovarian function in cows after calving.

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