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Induction of Estrus with PGF 2-Alpha and Associated Changes in Peripheral Blood Serum progesterone and Estrogen Concentrations in Dairy Cattle

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PGF 2α-Tham 塩による乳牛の発情誘発と末梢血中エストロンジェンならびにプロジェステロンの変化について

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Summary

Fourteen dairy cows and heifers were used to study the effect of PGF_2alpha in the induction of estrus and ovulation by intramuscular, intrauterine and intraovarian administration. Associated changes in blood serum progesterone and estrogen concentrations were also investigated by radioimmunoassay.

Within 3 days after intramuscular injection of 25 mg PGF_2alpha, estrus and ovulation were detected in two animals in Treatment I. Out of seven animals in Treatment II, four came into estrus and ovulated 3 to 5 days after 5 mg of PGF_2alpha was administered by intrauterine infusion. Of the 2 animals injected with 1 mg PGF_2alpha by intraovarian administration (Treatment III), one was in estrus 3 to 5 days after injection. The other animal did not show any sign of estrus but ovulation was detected more than 8 days after treatment. Of the three animals administered distilled water (Treatment IV, control), only one animal showed standing estrus 3 to 5 days after administration of 5 ml distilled water. Ovulation was also detected 3 to 5 days after administration.

In cows and heifers that showed remarkable responses to PGF_2alpha, there were rapid increases of estrogen and marked decreases of progesterone after PGF_2alpha administration.

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In coincidence with the growth or regression of the corpus luteum, progesterone rose and fell.

This study showed that PGF_2alpha is effective in inducing estrus and ovulation in cattle when administered during the luteal phase. The associated changes in progesterone and estrogen concentrations approximated that of the normal estrous cycle.

Introduction

The use of luteolysins in synchronizing estrus and ovulation owes much to their effect on the corpus luteum resulting in the decline and cessation of progesterone production and the start of another cycle.

Of materials possessing luteolytic activity, PGF_2alpha appears to be the most potent (INSKEEP, 1973; and HAFS, LOSIS, NODEN and OXENDER, 1974).

Several studies have been reported on the use of PGF_2alpha in cows, ewes and mares (ROWSON, TERVIT and BRAND, 1972; NAKAHARA, DOMEKI, KANEDA and YAMAUCHI, 1974; KANEDA, NAKAHARA and DOMEKI, 1975; UMO, 1975; and MIYAKE, SATO, YOSHIKAWA, TUTIDA and NAGASE, 1976) and different methods of administration of the drug have been tried by several workers (LIEHR, MARION and OLSON, 1972; LOUIS, HAFS and SEGUIN, 1973; DOMEKI, NAKAHARA, KANEDA and YAMAUCHI, 1975; and ROCHE, 1974).

Measurement of reproductive hormones such as progesterone and estrogen at different stages of the estrous cycle provided information on the sequences of changes in the reproductive tract and the hormonal interplay that took place (CHRISTENSEN, HOPWOOD and WILTBANK, 1974; HIXON, NADARAJA, SCHECHTER and HANSEL, 1975; and DOBSON, COOPER and FURR, 1975).

Several workers measured the concentration of a number of reproductive hormones found in the peripheral blood plasma of the cow around the time of estrus. STABENFELT, EWING and MCDONALD (1969) measured progestrone throughout the estrous cycle while HENRICKS, LONG, HILL and DICKEY (1974) measured estrogen and progesterone. The pattern of blood progesterone was investigated by DOMEKI, NAKAHARA, KANEDA and YAMAUCHI (1975) as reflected the changes of the corpus luteum examined by rectal palpation. RAJAMAHENDRAN, BEDIRIAN, LAGUE and BAKER (1976) on the other hand, reported that the decline in plasma progesterone after intrauterine injection of PGF_2alpha was delayed and variable compared to intramuscular injection.

The objectives of this study are: (1) to induce estrus and ovulation in dairy cattle using PGF_2alpha-Tham Salt (Prostin ® F_2 alpha Vet-Upjohn Limited) by different methods of administration, and (2) to determine the progesterone and estrogen concentrations of the peripheral blood serum of the animals as affected by different methods of PGF_2 alpha administration.

Materials and Methods

Animals·Seven multiparous Holstein-Friesian cows with average age of 69 months were selected from the herd at the Dairy Farm of Obihiro University. The animals averaged 35 days post-partum at the start of the experiment and the normal estrus was observed in all animals. They were housed in a dry lot and fed a maintenance ration of grains and grass silage. At the latter part of the experiment, they were released into the pasture every morning and driven back to the dry lot in the afternoon where they stayed overnight.

Seven nulliparous heifers were randomly selected from a herd of several heifers grazing on several paddocks on a cooperative farm at Sarabetsu-mura, Hokkaido. The average age of the heifers was 20 months. They were confirmed to be normally cycling at least once by visual observation of the animals.

Synchronization Procedures·At the start of the experiment, all animals were

Table 1. Clinical ovarian findings of experimental animals before the administration of PGF_2alpha and distilled water

Treatment	Animal Number	Age (Months)	Last Parturition	Ovarian Condition
I	1	19	Heifer	CL*
	2	19	Heifer	CL
II	3	46	April 5, 1976	CL
	4	70	April 30, 1976	CL
	5	18	Heifer	CL
	6	17	Heifer	CL
	7	20	Heifer	CL
	8	17	Heifer	CL
	9	30	Heifer	CL
III	10	73	April 9, 1976	CL
	11	60	April 30, 1976	CL
IV	12	104	May 13, 1976	CL
	13	68	April 7, 1976	CL
	14	61	April 20, 1976	CL

*CL=palpable functional corpus luteum

Table 2. The experimental design of the study

Treatment	Number of Animals	Method of Administration	Dosage
I	2	Intramuscular	25 mg PGF_2alpha
II	7	Intrauterine	5 mg PGF_2alpha
III	2	Intraovarian	1 mg PGF_2alpha
IV	3* (1)	Intraovarian	Distilled water
	(2)	Intrauterine	Distilled water

*Control group

palpated per rectum (Table 1). Rectal palpation revealed no abnormalities of the ovaries and the genital tract. Once a functional corpus luteum was detected, the animal was administered PGF_2alpha or distilled water.

The animals were assigned to four treatments (Table 2). Two animals were injected with 25 mg of PGF_2alpha dissolved in 5 ml distilled water, intramuscularly. Five mg of PGF_2alpha dissolved in 1 ml of distilled water was infused into the uterus of seven animals with the use of a metal catheter. Two animals were injected with 1 mg each of PGF_2alpha into the ovary with the use of YAMAUCHI's Ovarian Injector.

Three animals were used as control. In one animal, 1 ml distilled water was injected into the corpus luteum of the ovary while in the other two animals, 5 ml distilled water was introduced into the uterine horn ipsilateral to the corpus luteum with the use of a metal catheter.

After administration of PGF_2alpha or distilled water, KaMarr heat detecting devices were placed on the back of the animals to aid in heat detection. The animals were observed every day until standing estrus was detected. Insemination was done right after the animals were observed in estrus. Changes in ovarian and uterine morphology were monitored by rectal palpation.

Hormone assay·In five cows, about 20 ml of blood from the jugular vein was collected at the normal estrus, ten days after the end of estrus, at induced estrus and wthin ten days after the induced estrus. In the remaining animals, blood samples were collected at the time of PGF_2alpha or distilled water administration (luteal phase, Day 10) and at induced estrus. After blood collection, the test tube was allowed to stand at room temperature for about 24 hours and then the serum was separated and stored at $-20°C$ until assayed.

Serum progesterone and estrogen concentrations were determined by radioimmunoassay as described by MAKINO (1973) with some modifications.

Results

I. Clinical Findings Following PGF_2alpha Treatment

Clinical ovarian findings·Intramuscular administration of 25 mg PGF_2alpha induced regression of the functional corpus luteum and the development of normal follicle below 3 days after treatment. Standing estrus was observed in all animals in this group.

On the other hand, infusion of 5 mg PGF_2alpha into the uterus induced the degeneration of the corpus luteum and the normal development of follicles in five out of seven animls. The remaining two animals (numbers 5 & 6) were found to retain the same corpus luteum without any signs of regression even after 3 to 5 days after infusion of PGF_2alpha. These two animals did not show any sign of estrus despite one animal developing a normal follicle 3 to 5 days after administration. The animal was inseminated at this time.

The two animals in Treatment III both had functional corpus luteum which regressed 3 to 5 days after injection of 1 mg PGF_2alpha directly into the ovary. Developing follicles

were also detected. However, only one animal showed sign of standing estrus.

In the control group (Treatment IV), there was no response from the animal injected with 1 ml of distilled water directly into the ovary where the corpus luteum was located. No sign of standing estrus was detected, but ovulation occurred 12 days after. However, this was within the normal estrous cycle.

The corpus luteum of the two animals infused with 5 ml of distilled water into the uterus, regressed more than 5 days after. Sign of standing estrus was detected in one animal (No. 14). Despite the presence of a follicle in another, standing estrus did not occur. At this time, however, a regressing corpus luteum was observed by rectal palpation and ten days after, ovulation was detected indicating that it occurred within the normal estrous cycle.

Induction of estrus and ovulation·As was shown in Table 3, estrus and ovulation were detected on the two animals within 3 days after PGF_2alpha treatment (Treatment I). Out of seven animals in Treatment II, four came into estrus 3 to 5 days after treatment and ovulation was also detected 3 to 5 days later.

In Treatment III, one animal was in estrus 3 to 5 days after PGF_2alpha administration and ovulation was detected more than 5 days after treatment. The other animal did not show any sign of estrus but ovulation was detected more than 8 days after treatment. In Treatment IV, only one animal (No 14.) showed standing estrus in 3 to 5 days and ovulation was detected more than 8 days after distilled water administration.

Non-returns at 90 days·Of the 11 animals examined for pregnancy after treatment, 3 were found to conceive to services during the induced estrus. The results on the basis of non-returns at 90 days are shown in Table 3; 2/2, 1/7, 0/2, and 1/3 for Treatments I, II, III and IV, respectively.

Table 3. Induction of estrus and ovulation and 90-day non-returns of dairy cows and heifers after administration of PGF_2alpha and distilled water.

Treatment	Number of Animals	Days After Administration								90-day NR*
		Estrus				Ovulation				
		0-3	3-5	5-8	Total	0-3	3-5	5-8	Total	
I	2	2	–	–	2	2	–	–	2	2/2
II	7	–	4	–	4	–	4	–	4	1/7
III	2	–	1	–	1	–	–	1	1	0/2
IV	3	–	1	–	1	–	–	1	1	1/3

*The first number represents the number of animals pregnant after 90 days while the second number represents the total number of animals in each treatment.

II. Blood Serum Progesterone and Estrogen Concentrations

Serum progesterone and estrogen concentrations of animals in all treatments are shown in Figs. 1, 2, 3 and 4 for Treatments I, II, III and IV, respectively. Blood serum progesterone concentrations in all treatments were plotted against the blood serum estrogen

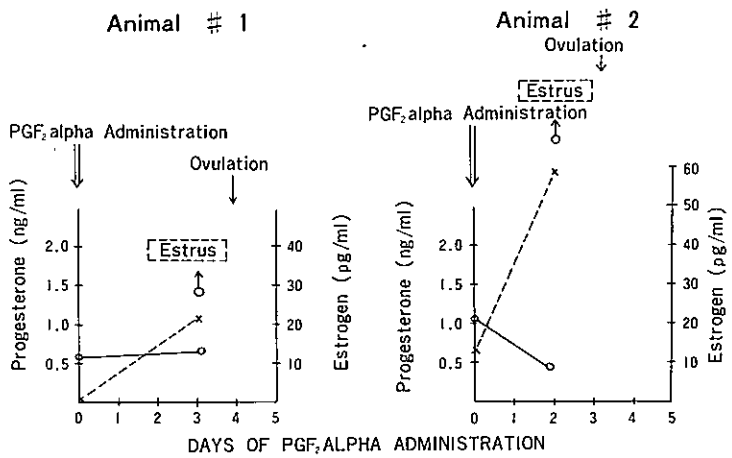

Figure 1. Progesterone and estrogen concentrations of animals treated with PGF_2alpha intramuscularly, 25 mg (Treatment I); solid line=progesterone and broken line =estrogen

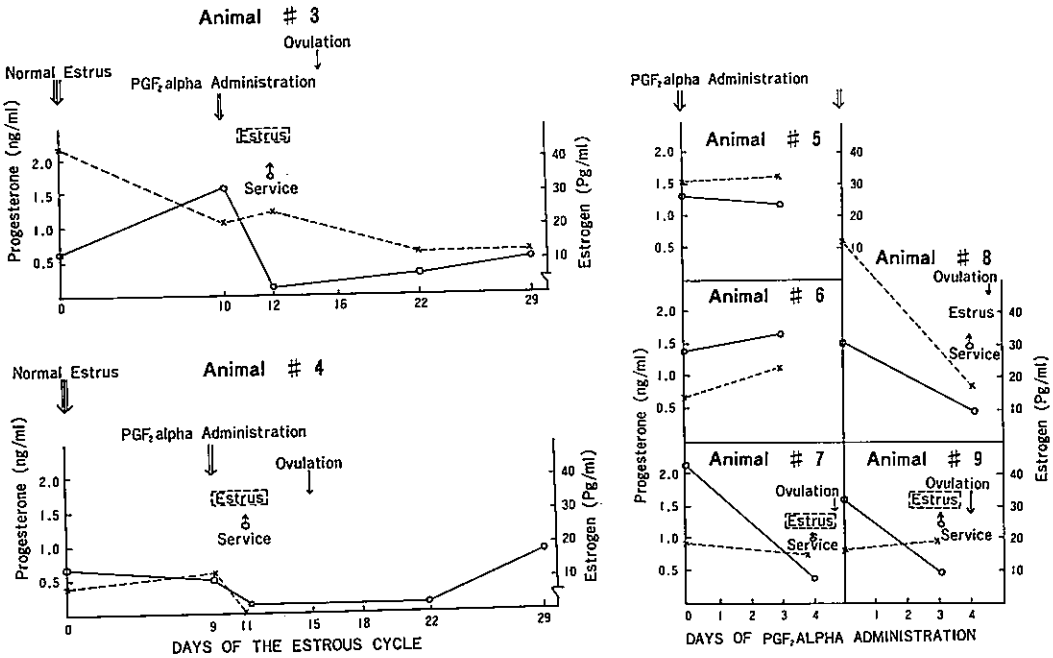

Figure 2. Peripheral blood serum progesterone and estrogen concentrations of animals treated with PGF_2alpha by intrauterine administration (Treatment II); solid line=progesterone and broken line=estrogen

concentrations on the same figures.

At the time of PGF_2alpha or distilled water administration, progesterone concentrations ranged from 0.51 to 1.00 ng/ml; from 0.55 to 2.31 ng/ml; from 0.51 to 3.17 ng/ml; and

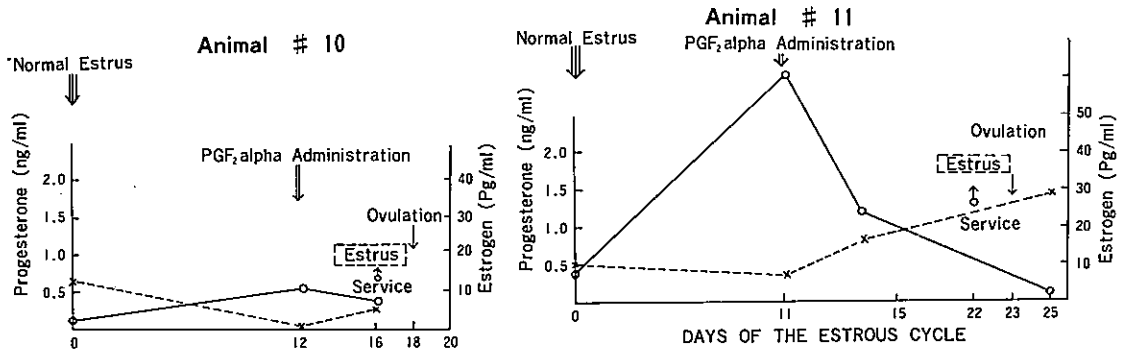

Figure 3. Peripheral blood serum progesterone and estrogen concentration of animals treated with PGF_2alpha by intraovarian administration (Treatment III); solid line=progesterone and broken line=estrogen

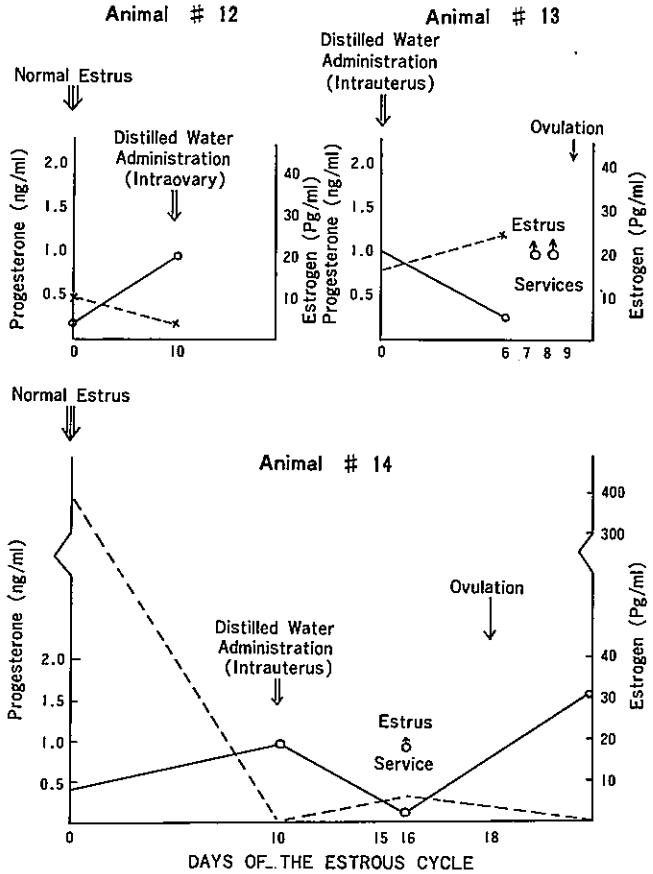

Figure 4. Peripheral blood serum progesterone and estrogen concentration of animals treated with distilled water by intraovarian and intrauterine administration (Treatment IV); solid line=progesterone and broken line=estrogen

from 0.95 to 1.08 ng/ml in Treatments I, II, III and IV, respectively.

At induced estrus, progesterone levels ranged from 0.36 to 0.68 ng/ml; from 0.31 to 1.69 ng/ml; from 0.23 to 1.29 ng/ml; and from 0 to 0.25 ng/ml in Treatments I, II, III and IV, respectively.

Estrogen concentrations at PGF_2alpha or distilled water administration ranged from 0 to 13.5 pg/ml in Treatment I; from 3.97 to 62.6 pg/ml in Treatment II; from 0 to 6.9 pg/ml in Treatment III; and from 0 to 17.36 pg/ml in Treatment IV.

At induced estrus, the estrogen levels ranged from 21.5 to 62.2 pg/ml in Treatment I; from 0 to 32.2 pg/ml in Treatment II; from 7.23 to 16.3 pg/ml in Treatment III; and from 0 to 25.9 pg/ml in Treatment IV.

From animals wherein blood samples were collected at day 0 (day of ovulation), at day 10 (luteal phase), at induced estrus and within 10 days after induced estrus, it can be observed that a clear pattern emerged.

Progesterone concentration was low at normal estrus, reaching a peak at the time of PGF_2alpha or distilled water administration at day 10 of the luteal phase, after administration up to 5 days progesterone concentration decreased very rapidly and was lowest at the time of induced estrus and then slowly increased within 10 days after induced estrus.

On the other hand, the reverse is true of estrogen concentrations. At the time of normal estrus, it was very high gradually decreasing until PGF_2alpha or distilled water administration at day 10 (luteal phase) and then increased very suddenly until the time of induced estrus.

It was noted that 3 to 5 days after PGF_2alpha or distilled water administration, progesterone consentration of animals in Treatment I markedly decreased by 31.15%, 50% in Treatment II, 58.7% in Treatment III and 78.64% in Treatment IV, respectively.

Estrogen concentration on the other hand, increased by 83.78% in Treatment I, decreased to 20.98% in Treatment II, increased by 70.68% in Treatment III and increased by 25.33% in Treatment IV.

The decrease in estrogen concentration in Treatment II did not prevent four of the animals from showing signs of estrus. From out of seven animals, five showed a developing follicle as detected by rectal palpation. The marked decrease in progesterone concentration in this group resulted in estrus of all but two animals which retained their corpus luteum despite the injection of PGF_2alpha.

Discussion

Results from this study showed that regardless of method of administration, PGF_2alpha was effective in inducing estrus and ovulation in cattle. The intramuscular injection of 25 mg PGF_2alpha resulted in maximum responsiveness to estrus and ovulation in the animals, although KANEDA, NAKAHARA and DOMEKI (1975) concluded that 10 mg was

enough to effectively synchronize estrus and ovulation in cattle. HAFS, MANNS and DREW (1975) reported on the other hand, that 20 mg for heifers and 30 mg for cows were needed to synchronize estrus and ovulation.

PGF_2alpha deposition into the uterus at lower dosages were reported to be ideal in inducing estrus and ovulation in cattle (ROWSON, TERVIT and BRAND, 1972; and NAKAHARA, DOMEKI, KANEDA and YAMAUCEI, 1974). Results of this study confirmed the results of these workers.

Likewise, the deposition of PGF_2alpha at other sites of the reproductive tract (LAMOND, TOMLINSON, DROST, HENRICKS and JOCHLE, 1973; and OHTA, UMEZU and TAKEUCHI, 1974) or by subcutaneous injection (LAUDERDALE, 1972) was effective in causing luteolysis in the cow.

The effects of PGF_2alpha on the concentrations of steroid hormones indicated a marked increase in estrogen and dramatic decrease of progesterone right after PGF_2alpha administration. It was observed that progesterone rose and fell in coincidence with the growth or regression of the corpus luteum.

As would be expected, after a rapid decrease in progesterone and a rise in estrogen, estrus soon followed. Progesterone and estrogen levels in this study are comparable with those reported by LOUIS, HAFS and SEGUIN (1973); HENRICKS, LONG, HILL and DICKEY (1974); CHRISTENSEN, HOPWOOD and WILTBANK (1974); and LEMON, PELLETIER, SAUMANDE and SIGNORET (1975).

It may be speculated that as a result of the regression of the corpus luteum and the absence or very low concentration of progesterone, estrogen levels rose. It may be possible, however, that PGF_2alpha had a direct effect on the estrogen concentrations. However, until now the mechanism is not yet known despite the conclusion of HIXON, NADARAJA, SCHECHTER and HANSEL (1973) that PGF_2alpha stimulates estrogen secretion, presumably by follicular elements of the ovary.

This study showed that administration of PGF_2alpha-Tham Salt during the luteal phase of the estrous cycle caused luteolysis of the corpus luteum in cattle and PGF_2alpha was observed to be effective in inducing estrus and ovulation in cattle. The associated changes in progesterone and estrogen concentrations approximated that of the normal estrous cycle.

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要　　約

14頭の経産ならびに未経産乳牛の黄体期に $PGF_2\alpha$-Tham 塩を筋肉内，子宮内ならびに卵巣実質内に投与し，発情，排卵が誘発されるか否かを検討した。一部のウシについては，末梢血中のプロジェステロンとエストロジェンを RIA 法で測定した。

1. PG 25 mg を筋注した2頭では3日後に発情，排卵があった。

2. PG 5 mg を子宮内に投与した7頭のうち4頭は，3～5日後に発情，排卵した。

3. PG 1 mg を卵巣実質内に注射した2頭のうち1頭は3～5日後に発情したが，他の1頭は8日以降になっても発情，排卵は見られなかった。

4. 対照として蒸溜水のみを子宮内注入および卵巣実質内に投与した3頭中，子宮内注入の1頭のみ3～5日後に発情，排卵が認められた。

5. PG に反応を示したウシでは，急激な卵巣黄体の退行に一致して血中エストロジェンの増加とプロジェステロンの減少が見られた。

以上のことから，ウシにあっては黄体期に PG を投与すると，性周期が短縮し，発情，排卵の起こることがわかった。