

Effects of noradrenaline or PGF₂ alpha for inducing estrus in dairy cattle

Noc Bautista VELASCO*, Kunitada SATO, Hitoshi ONO
and Masaru MIYAKE

*Department of Veterinary Obstetrics and Gynecology, Obihiro
University of Agriculture and Veterinary Medicine,
Obihiro, Hokkaido, Japan OSO*

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Abstract

Eleven heifers and six cows, all Holstein Friesian, were used in three trials to test for the presence of luteolytic effect of noradrenaline and PGF₂ alpha injected intravulva and to determine their ability to induce estrus.

Single injections of 2 mg noradrenaline or 5 mg PGF₂ alpha caused increased progesterone level on the first day after injection and decreased on the second day with heifers in Trial I while with cows in Trial II, progesterone levels decreased during the first two days. All the heifers and cows in the two trials returned to estrus within their normal estrous cycle. Two-time injections of the drugs in Trial III resulted to estrus occurring in all heifers within two to four days after the second injection.

Results of this study show that noradrenaline and PGF₂ alpha injected at 2 and 5 mg respectively, intravulva resulted in a decrease in peripheral blood progesterone within 2-4 days. The amount of the drugs seems to be not sufficient and a second injection is necessary to cause complete luteolysis. Estrus can be synchronized when they are injected twice at 10 days interval.

Introduction

The role of luteolysin in the termination of the functional corpus luteum is assessed by the decline in the concentration of progesterone in the peripheral blood and the occurrence of estrus. Previous studies have shown that PGF₂ alpha is an effective means of controlling estrus in cows without impairing fertility when injected. The administration of

PGF₂ alpha to the cow at any time between days 5 and 15 of the estrous cycle will initiate rapid luteolysis and cause most of the treated animals to return to estrus within 2 to 4 days (Lauderdale, 1975; Louis *et al.*, 1975). A two-injection schedule of 10 days apart can be used to minimize the problem of detecting estrus in randomly cycling herds (King and Robertson, 1974; Hafs *et al.*, 1975).

The study on the ovarian changes after the

* Former research exchange student, Department of Veterinary Obstetrics and Gynecology, Obihiro University of Agriculture and Veterinary Medicine. Present address: Dairy Training and Research Institute, University of the Philippines at Los Banos College, Laguna, Philippines, 3720.

treatment of noradrenaline and some clinical reports of estrus occurring after the injection of this sympathomimetic drug (Ono, 1977) led these workers to test for the presence of luteolytic property of this drug. Noradrenaline is considered to be a postganglionic adrenergic mediator present in the nerve fibers of the adrenal medulla and these neurons terminate in the hypothalamus (Hökfelt, 1973). This suggests that they may play a role in the regulation of the endocrine function and ultimately on the reproductive cycle of the animals (Cole and Cupps, 1977).

It is postulated that the luteolytic effect of prostaglandins is due to its vasoconstrictor properties. Prostaglandin can be and is normally transferred from the uterine vein to the ovarian artery (Land *et al.*, 1976) and the restriction of ovarian blood flow resulted in a marked fall in plasma progesterone concentration accompanied by morphological changes in the corpus luteum (Stacy and Gemmel, 1976). Auletta *et al.* (1972), in their study of oxytocin with noradrenaline suggested the possibility of uterine and ovarian blood flow being restricted by the vasoconstrictor properties of noradrenaline.

This study was conducted to test for the presence of luteolytic effect of noradrenaline and PGF₂ alpha injected intravulva and to determine their ability to induce estrus.

Materials and Methods

Eleven heifers and six cows, Holstein Friesian, were used in three trials. Trials I and II were conducted in the dairy farm of Obihiro University of Agriculture and Veterinary Medicine in Obihiro, Hokkaido, Japan. Trial III was conducted in the dairy farm of Hokkaido Livestock Development Corporation located Taiki, Hokkaido, Japan. All the animals used in this study were raised in a loose housing barn and fed with maintenance ration of corn silage, hay and grains. The design of the study is shown in Table 1.

Trial I

Three normally cycling heifers aged 17-22 months were used to test for the presence of luteolytic effect by determining the progesterone levels after the administration of noradrenaline or PGF₂ alpha. Each was injected intravulva with noradrenaline at a dose of 2 mg or PGF₂ alpha at a dose of 5 mg on the 10th day after the last estrus. Physiological saline served as control. Blood samples were collected via the jugular vein before injection and daily for 5 days thereafter. Serum was separated and stored in a freezer until analyzed. Visual observation of estrus was done daily during the postinjection period until the next estrus.

Trial II

Six cows aged 2-9 years were used in this trial.

Table 1. Experimental Design of the Study.

Trial	Number of Animals	Drug	Dosage	Site of Administration
I	1 heifer	Noradrenaline	2 mg	Intravulva
	1 heifer	PGF ₂ alpha	5 mg	Intravulva
	1 heifer	Physiological Saline	2 ml	Intravulva
II	4 cows	Noradrenaline	2 mg	Intravulva
	1 cows	PGF ₂ alpha	5 mg	Intravulva
	1 cows	PGF ₂ alpha	25 mg	Intramuscular
III ^a	2 heifers	Nordrenaline	2 mg	Intravulva
	2 heifers	PGF ₂ alpha	5 mg	Intravulva
	2 heifers	PGF ₂ alpha	25 mg	Intramuscular
	2 heifers	Physiological Saline	2 ml	Intravulva

^a All drugs were injected twice, 10 days apart.

They were selected based on observed estrus and on the plasma progesterone level on the 9th day after estrus or prior to the injection of the drugs. Cows with progesterone levels lower than 1 ng/ml were not included in this trial. Noradrenaline or PGF₂ alpha were injected on the 10th day of the estrous cycle. The presence of corpus luteum was confirmed by rectal palpation just prior to the treatment. Blood samples via the tail vein were collected using heparinized tubes before injection, daily for 5 days postinjection and on the 21st day after estrus. Plasma samples were separated immediately and stored in a freezer until analyzed. Vaginal as well as cervical dry smear examinations were done during the 2nd to 5th day postinjection in addition to the visual observation of estrus to ensure detection of silent heat.

Trial III

Eight heifers aged about 16 months from a randomly cycling herd were selected regardless of their stage in the estrous cycle and were assigned to their respective treatments. This trial was done to determine the effect of two-injection treatment in synchronizing estrus. The ovarian conditions were determined by rectal palpation before the first injection and later when the animals were in estrus and on the 5th day after the second injection. The heifers were injected simultaneously with their respective drugs twice, 10 days apart. A Kamar heat detection device was placed on the rump of each heifer after the second injection to aid in estrus

detection. Blood samples were collected before the first and second injections, when the heifers were in estrus and on the 5th day after the second injection. Serum samples were separated immediately and stored in a freezer until analyzed.

Progesterone level of the serum and plasma samples was measured using radioimmunoassay procedures by Makino (1973).

Results

Trial I

Based on the progesterone levels (Table 2), the heifers except the PGF₂ alpha-treated heifer had a functional corpus luteum before the injection as rectal palpation was not done to confirm it. There was an increase in progesterone level of 30, 34 and 5% in the noradrenaline, PGF₂ alpha and control heifers, respectively, one day after injection and a decrease on the 2nd day. However, only the noradrenaline-treated heifer had a substantial decrease which was lower (41%) than the preinjection level. For the PGF₂ alpha and control heifers, the decrease was minimal and was still higher than the preinjection levels. All the heifers did not show estrus within 2-4 days after injection. The progesterone concentration was back to the preinjection levels after 4 days in the noradrenaline and PGF₂ alpha-treated heifers. All the heifers came to estrus within their normal estrous cycle and rectal palpation on the day of estrus revealed a well-developed follicle.

Table 2. Serum Progesterone Levels (ng/ml) in Heifers of Trial I Treated with Noradrenaline and PGF₂ alpha.

Time of Blood Sampling	Drug		Physiological Saline
	Noradrenaline	PGF ₂ alpha	
Before injection	1.21	0.63	1.22
After injection (days)			
1	1.74	0.96	1.49
2	0.71	0.91	1.24
3	2.18	0.79	0.66
4	1.22	0.64	2.12
5	1.15	0.92	0.86

Table 3. Plasma Progesterone Levels (ng/ml) in Cows of Trial II Treated with Noradrenaline and PGF₂ alpha.

Time of Blood Sampling	Noradrenaline ^a				PGF ₂ alpha	
	1	2	3	4	25 mg	5 mg
Screening	1.27	1.54	2.49	1.63	2.00	1.64
Before injection	1.53	1.20	2.10	3.31	1.10	1.36
After injection (days)						
1	0.89	1.14	1.78	3.28	0.51	0.80
2	0.77	0.95	3.70	1.20	0.90	0.82
3	0.82	1.34	2.93	1.94	0.65	2.00
4	0.99	1.73	1.74	2.63	0.50	1.49
5	0.95	0.55	1.62	1.61	0.82	1.47
21 days after last estrus	0.15	0.23	0.21	0.52	0.62	0.16

^a Four cows were used.

Table 4. Serum Progesterone Levels (ng/ml) in Heifers of Trial III with Two Injections of Noradrenaline and PGF₂ alpha.

Treatment	Heifer No.	Time of Blood Sampling			
		First Injection	Second Injection	In Estrus	Five days after 2nd Injection
Noradrenaline (2 mg)	9	1.49	0.41	0.45	0.84
	21	0.92	0.80	0.57	0.63
PGF ₂ alpha (25 mg)	1	0.89	1.62	0.43	0.57
	12	1.36	0.84	0.15	0.63
PGF ₂ alpha (5 mg)	3	1.13	2.37	0.17	0.36
	20	0.71	2.66	0.15	0.30
Physiological Saline (2 ml)	4 ^a	0.66	0.98	-	1.05
	26	1.09	0.47	0.13	0.68

^a In estrus on the 5th day after the second injection.

Trial II

All the cows used in this trial were confirmed to have a functional corpus luteum prior to the administration of the different drugs based on the record of their last estrus, progesterone levels at screening and with the aid of rectal palpation. The plasma progesterone levels of the cows are shown in Table 3. There was an average decrease from the preinjection levels of progesterone of 14% in the noradrenaline-treated cows one day after injection; 53 and 41% in 25 and 5 mg PGF₂ alpha-treated cows, respectively. The progesterone levels of the PGF₂ alpha- and in only one of the noradrenaline-treated cows again increased on the second day. Five days after injection, the progesterone

concentrations of all cows lower than the preinjection levels except one cow treated with 5 mg PGF₂ alpha. The progesterone concentration obtained 21 days after the last estrus was low which is characteristic of cows in pre-estrus or in estrus. Vaginal and cervical dry smear examinations during the 2nd to 5th day after injection revealed characteristics of animals in luteal phase.

Trial III

Rectal palpation before injection revealed differences in the ovarian conditions of the heifers used in this trial (Table 5). However, the progesterone levels do not conform with the ovarian conditions (Table 4). All the heifers were in estrus from 2 to 4 days after the second injection except in one

Table 5. Clinical Ovarian Findings in Heifers of Trial III Treated with Two Injections of Noradrenaline and PGF₂ alpha.

Treatment	Heifer No.	Ovarian Condition ^a		
		Injection First	In Estrus	Five days after 2 nd Injection
Noradrenaline (2 mg)	9	R (Fol)	L (Ovul)	L (CL, Fol)
	21	L (CL)	R, L (Fol)	R (CL), L (Ovul)
PGF ₂ alpha (25 mg)	1	R (CL)	L (Fol)	L (CL)
	12	R (CL)	R (Fol)	R (CL)
PGF ₂ alpha (5 mg)	3	R (CL)	L (Fol)	L (CL)
	20	Ovul	R (Fol)	R (Ovul)
Physiological Saline (2 ml)	4	R (CL)	-	R (Fol)
	26	Estrus 4 days ago	L (Ovul)	L (CL)

^a By rectal palpation.

R=right ovary
L=left ovary
CL=corpus luteum
Fol=follicle
Ovul=ovulation

control heifer. Rectal palpation on the day of estrus revealed the presence of either a well-developed follicle or the occurrence of ovulation. The progesterone concentrations were lower compared to the levels before the second injection. Estrus was observed within 2-3 days after the second injection with noradrenaline; 2 days in 25 mg PGF₂ alpha and within 3-4 days in 5 mg PGF₂ alpha-treated heifers. One control heifer came to estrus 2 days after the second injection while the other was on the 5th day. Rectal palpation on the 5th day revealed the presence of developing corpus luteum in all heifers except one heifer which was in estrus on the 4th day.

Discussion

The injection of luteolytic drug on days 5 to 15 of the estrous cycle, which is characterized by the presence of a functional corpus luteum will result in the regression of corpus luteum and consequently the decrease in blood peripheral progesterone. Observation of the serum progesterone levels of the heifers in Trial I are comparatively low (Herriman *et al.*, 1979) and the animals used might have been in the early stage of corpus luteum development as the extent of growth was not known. In the study of

Bolt and Rollings (1976), they demonstrated that norepinephrine infused in diestrous ewes caused a rapid increase in the secretion rate of progesterone. This might explain the initial increase in the progesterone level which was followed by a decrease on the second day postinjection in the noradrenaline-treated heifer although the route of administration was different. However, the PGF₂ alpha-treated heifer initially had a low progesterone level and this might have an effect on its response to the drug. It seems that the decrease in the progesterone levels on the 2nd to 4th day in the noradrenaline- and PGF₂ alpha-treated heifers was not sufficient evidence for the presence of luteolytic effect as progesterone levels increased and were similar if not higher than the preinjection levels on the 5th day. Furthermore, it tends to support the report of Ono (1977) that for this drug to be highly effective, the progesterone levels should be high.

To eliminate the problem of using animals with low progesterone levels encountered in Trial I, screening was done using progesterone levels as one of the criteria in Trial II. The inclusion of 25 mg PGF₂ alpha in this trial served as a control as it has been the recommended dose by the makers of this drug. The results obtained from this trial suggest that

noradrenaline or the 5 mg PGF_2 alpha are effective only in decreasing the progesterone level but not to induce estrus during the first two days after injection. The results were almost similar to that obtained in Trial I except that there was no increase in progesterone level on the first day after injection. The control cow treated with 25 mg PGF_2 alpha had a 53% decrease in progesterone level on the first day but the rate of decrease was lower than those obtained by Rajamahendran *et al.* (1976) and Henricks *et al.* (1974) when they were able to induce estrus. However, in the study of Natural *et al.* (1977), heifers had a similar rate of decrease and yet were able to cause complete luteolysis and ultimately estrus resulted. It appears that the secretion of progesterone was decreased by the reduction in the number of progesterone-secreting granules of the corpus luteum as a result of incomplete luteolysis. The fact that the progesterone levels obtained on the 21st day after the last estrus was low which is characteristic of animals in pre- or in estrus (Lemon *et al.*, 1975; Stabenfeldt *et al.*, 1969) tends to show that the corpus luteum continued to exist and regressed within the normal cycle in all cows. It is speculated that the amount of the drugs might be too small to cause complete luteolysis (Rowson *et al.*, 1972). It is suggested that a second injection is necessary to attain complete luteolysis.

The objective of Trial III was to synchronize estrus in a randomly cycling herd using a two-injection regimen with the different drugs. The theory behind this method is that animals not responding to the initial injection will be re-treated 10 days later at which time they would be responsive to the drug (Roche, 1974). Based on the ovarian conditions before the first injection, two heifers, each from the noradrenaline and 5 mg PGF_2 alpha groups had a follicle or occurrence of ovulation and all remaining heifers belong to the 5 to 15 days group which are considered responsive to the drug (King, 1974). All the animals came to estrus within 2-4 days after the second injection except one cont-

rol animal which came to estrus on the 5th day. The presence of follicles on the day of estrus and subsequently a corpus luteum five days after the second injection shows there was a synchronized estrus in the animals. However, the progesterone levels do not conform with the result. The progesterone levels during the second injection should be high as they are assumed to be all in the luteal phase to be responsive to the drug. Only the 5 mg PGF_2 alpha and one in the 25 mg PGF_2 alpha-treated heifers had high progesterone levels which is evidence of a well-developed corpus luteum. There was on way to explain the behavior of progesterone during the second injection as rectal palpation was not done. With the results obtained, it appears that noradrenaline or 5 mg PGF_2 alpha can be used to synchronize estrus when injected twice at 10 days interval and are comparable to the injection of 25 mg PGF_2 alpha. However, because of the limited number of animals used, it is recommended to be tested further with sufficient number of animals to allow statistical analysis.

References

- 1) AULETTA, F. J., G. N. CURRIE and D. L. BLACK. (1972): Effect of oxytocin and adrenergic drugs on bovine reproduction. *Acta Endocrinol.*, 69 (2): 241-248.
- 2) BOLT, D. J. and R. ROLLINGS. (1976): Influence of catecholamines on secretion and metabolic clearance rate of progesterone in ewes. (Abst.) *J. Anim. Sci.*, 43 (1): 275.
- 3) COLE, H. H. and P. T. CUPPS. (1977): Reproduction in Domestic Animals. 3rd ed. Academic Press, New York. 665 pp.
- 4) HAFS, H. D. and J. G. MANNIS. (1975): Onset of oestrus after prostaglandin F_2 alpha in cattle. *Vet. Rec.*, 96 (6): 134-135.
- 5) HERRIMAN, I. D., D. J. HARWOOD, C. B. MALINSON and R. J. HEITZMAN. (1979): Plasma concentrations of ovarian hormones during the oestrous cycle of the sheep and cow. *J. Endocrinol.*, 81: 61-64.

- 6) HOKFELT, T., K. FUXE, M. GOLDSTEIN and O. JOHANSSON. (1973): Evidence for adrenaline neurons in the rat brain. *Acta Physiol. Scand.*, 89; 286-288.
- 7) KING, G. J. and H. A. ROBERTSON. (1974): A two injection schedule with prostaglandin F₂ alpha for the regulation of the ovulatory cycle of cattle. *Theriogenology*, 1: 123-128.
- 8) LAND, R. B., D. T. BAIRD and R. J. SCARAMUZZI. (1976): Dynamic studies of prostaglandin F₂ alpha in the utero-ovarian circulation of the sheep. *J. Reprod. Fertil.*, 47: 209-214.
- 9) LAUDERDALE, J. W. (1975): The use of prostaglandins in cattle. *Ann. Biol. Anim. Bioch. Biophys.*, 15 (2): 419-425.
- 10) LEMON, M., J. PELLETIER, J. SAUMANDE and J. P. SIGNORET. (1975): Peripheral plasma concentrations of progesterone, oestradiol-17 β and luteinizing hormone around oestrus in the cow. *J. Reprod. Fertil.*, 42: 137-140.
- 11) LOUIS, T. M., H. D. HAFS and J. N. STELLEFLUG. (1975): Control of ovulation, fertility and endocrine response after prostaglandin F₂ alpha in cattle. *Ann. Biol. Anim. Bioch. Biophys.*, 15 (2): 407-417.
- 12) MAKINO, T. (1973): Radioimmunoassay of plasma sex steroid. *Folia Endocrinol. Japonica*, 49: 629-645.
- 13) NATURAL, N. G., Y. SAWAMUKAI, K. SATO, H. ONO and M. MIYAKE. (1977): Induction of estrus with PGF₂ alpha and associated changes in peripheral blood serum progesterone and estrogen concentrations in dairy cattle. *Res. Bull. Obihiro Univ.*, 10: 643-653.
- 14) ONO, H. (1977): Changes in the ovaries of dairy cows treated with noradrenaline. Seminar paper presented at the Dept. Ani. Sci. U. P. College of Agriculture, College, Laguna, Philippines. Aug. 1977.
- 15) RAJAMAHENDRAN, R., K. N. BEDIRAN, P. C. LAGUE and R. D. BAKER. (1976): Luteolytic activity of a synthetic prostaglandin and PGF₂ alpha in heifers. *Prostaglandins*, 11 (1): 143-153.
- 16) ROCHE, J. F. (1974): Synchronization of oestrus and fertility following artificial insemination in heifers given prostaglandin F₂ alpha. *J. Reprod. Fertil.*, 37: 135-138.
- 17) ROWSON, L. E., A. R. TERVIT and A. BRAND. (1972): Synchronization of estrus in cattle by means of PGF₂ alpha. *Proc. Seventh Inter. Congr. Anim. Reprod. Artif. Insem.*, II p. 865.
- 18) STABENFELDT, G. H., L. L. EWING and L. E. McDONALD. (1969): Peripheral plasma progesterone levels during the bovine oestrous cycle. *J. Reprod. Fertil.*, 19: 433-442.
- 19) STACY, B. D. and R. T. GEMMEL. (1976): Morphological and functional changes in the corpus luteum after restriction of ovarian blood flow. (Abst.) *J. Reprod. Fertil.*, 46: 526.

乳牛に対するノルアドレナリン
または PGF₂ alpha の発情
誘起効果について

ノエ バウチスタ ベラスコ*, 佐藤
邦忠, 小野齊, 三宅勝

帯広畜産大学獣医学科家畜臨床繁殖学教室,
帯広市 080

(*現 ヒリッピン大学農学部附属酪農技術研究
所, ロスベニオス, ラグナ, ヒリッピン)

摘 要

11頭のホルスタイン種未経産牛と6頭の経産牛を3群に分け、ノルアドレナリンと PGF_{2 α} を陰唇粘膜内に注射し、発情誘起効果があるかどうかを検討した。

2 mg のノルアドレナリンまたは 5 mg の PGF_{2 α} の1回注射では、第1群の未経産牛では、注射翌日血中黄体ホルモンは増加し、2日目に減少した。これに対し第2群の経産牛では、注射翌日および2日目の2日間、血中黄体ホルモンは減少した。しかし第1群、第2群の試験牛とも正常性周期の日数で発情が回帰した。

未経産牛 2 頭ずつに、10 日間隔で 2 回、ノルアドレナリン 2 mg または $\text{PGF}_{2\alpha}$ 5 mg を陰唇粘膜内、あるいは $\text{PGF}_{2\alpha}$ 25 mg を筋肉内に投与したところ、すべての牛は第 2 回目注射後 2~4 日以内に発情した。

以上の結果から、ノルアドレナリンを 2 mg, $\text{PGF}_{2\alpha}$

を 5 mg それぞれ陰唇粘膜内に投与すると、血中黄体ホルモンは 2~4 日以内に減少するが、これだけでは発情を誘起するには不十分で、第 1 回目注射後 10 日目に同量のノルアドレナリンまたは $\text{PGF}_{2\alpha}$ を追加注射すれば発情誘起は可能であることが分った。