Synchronization of Estrous Cycle in Cattle

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In recent years much interest has been centered on the synchronization of estrous cycle in farm animals, especially in beef cattle. The purpose of this attempt is to enable a large proportion of the breeding herd to be inseminated within a period of a few days.

The main physiological bases for synchronization of the estrous cycle include the regression of corpus luteum and the suppression of estrus or ovulation. Therefore, the suppressive effects of progesterone or its derivatives on the follicular development have been mainly investigated. Other approaches without using hormone have been also studied.

A number of experimental works of this sort, however, has shown that the conception rate to the insemination at the synchronized estrous period appeared to be reduced, although the time of estrus or ovulation can be effectively controlled.

Other important problems standing in the way of its practical application are the relatively high cost and the laborious process. The object of this paper is to review effectiveness of synchronization after various procedures and the fertility at the synchronized estrous period reported by many workers, including the research activities of this line in Japan.

Injection of progesterone

From the early stage of these studies, the suppressive effect of progesterone on the ovarian activities have been investigated. Progesterone alone, progesterone with or followed by estrogen or progesterone followed by gonadotrophin have been used. Ulberg *et al.* (1951) observed that progesterone ranging from 12.5 mg to 50 mg per day for 1 to 28 days caused the suppression of follicular development in dairy heifers. In their latter experiment (Ulberg *et al.* 1960), it was found that the estrus occurred in 80.7% of animals within 10 days after the end of the progesterone treatment at the same daily doses for 14 days.

Effective suppression of estrus and ovulation was also demonstrated by Avery *et al.* (1962) with 50 mg of progesterone daily and by Lamond (1962, 1964) with doses varying from 50 mg every 2 days to 20 mg daily. On the other hand, a single injection of a large dose of crystalline progesterone has been also performed. Nellor *et al.* (1956) observed that 89% of heifers receiving single injection of 540–560 mg of crystalline progesterone and 95% of animals receiving 700–1,120 mg of progesterone came in estrus 15–19 days and 15–23 days after the treatment, respectively.

Sakai, Ando and Yanai (1965) used saline suspension of crystalline progesterone in Japanese beef cattle and Holstein cows. Single intramuscular injection of 400 mg of progesterone on any one day between the fifth day and the 23rd day of the cycle induced estrus 12–17 days after the treatment in almost all treated animals.

Of these experiments, while it was discovered that effective synchronization can be ob-

tained by the progesterone treatment, marked reduction in the conception rate inseminated at the synchronized estrus has been noted. As the cause of low fertility, it seemed that the alteration in hormone balance produced by the introduction of large quantities of exogenous progesterone might be responsible so the effects of progesterone in combination with estrogen on the synchronization of estrus and the fertility have been investigated. Ulberg *et al.* (1960) injected 0.5–10.0 mg of estradiol benzoate 2.5–3.0 days after the last progesterone injection in beef cows and as many as 97% of cows came in estrus within 10 days after the treatment.

Wiltbank *et al.* (1965) conducted experiments by using progesterone and varying amount of estrogen in beef heifers. In 70 to 100% of animals which received daily injections of 20 mg or 40 mg of progesterone either alone or in combination with 10–160 mg of estradiol for 18–24 days, estrus occurred in a 4 day period after the end of these treatments, but fertility at the synchronized estrus was as low as 13–53 per cent. These results revealed that fertility could not be increased when estrogen was injected concurrently with progesterone.

Pregnant mare serum gonadotrophin (PMSG) has been also used following successive injections of progesterone in expectation of increasing the synchronization and fertility effectiveness (Nellor *et al.* 1956, Lamond *et al.* 1960, 1962 and Ray *et al.* 1961), but almost all experiments were unsuccessful.

Oral administration of progesterone derivatives

The major reasons why progesterone cannot be applied to pratical use for the estrus cycle synchronization are low fertility to insemination at the synchronized estrus and toilsome treatment of daily injections for 2 to 3 weeks in individual animals. Since 1961 oral administration of progesterone derivatives has been performed for synchronization of estrus. The use of progestational compounds in the ration could be expected to simplify the method.

The first successful synchronization of estrus in cattle by orally active progestins was reported by Hansel *et al.* (1961). Beef cows were given 968 mg and 500 mg of 6α-methyl-17 α-acetoxyprogesterone (MAP) daily for 20 days, and estrus occurred in 50% of animals 3–4 days after the end of the treatment. Thereafter, numerous orally active progesterone derivatives such as MAP, 6-chroro-dehydro-17-acetoxyprogesterone (CAP), 6α-methyl - 6 - dehydro-16-methylene-17-acetoxy-progesterone (Melengestrol acetate: MGA) and acetophenide-16α-17-dihydroxyprogesterone (DHPA) have been examined in cattle.

Effectiveness of synchronization and fertility at the synchronized estrus following the treatment with MAP and CAP which had been obtained by many workers are summarized in Tables 1 and 2 respectively. The results of these experiments indicated that synchronization seemed to be slightly better in animals treated with MAP than in those treated with CAP. Furthermore, although conception rate at the synchronized estrus in the MAP-fed cows was somewhat higher than that in the CAP-fed cows, it seemed to be 10–15% lower than that in untreated control animals. In Japan, Takeuchi, Shimizu, Toyoda, Kawai and Adachi (1966) obtained good results in Japanese beef cattle by feeding 0.05 mg per kg body weight of CAP daily for 15 days. However, fertility at the synchronized estrus appeared to be poor.

Zimbelman *et al.* (1966) reported that daily oral administration of Melengestrol acetate (MGA) caused inhibition of estrus in cattle and its potent was about 300 to 900 times as high as MAP. Daily doses of 0.2–2.0 mg of MGA inhibited estrus and the average interval from the last feeding to estrus ranged from 2.7 days at 0.2 mg to 6.3 days at 2.0 mg. In the animals inseminated at the synchronized estrus 42% were conceived.

Recently, Wiltbank *et al.* (1967, 1968) devised a technique involving feeding of 400–75 mg of DHPA daily for 9 days and injection

Table 1. Synchronization of estrus and fertility after feeding of MAP in cattle

Reported by	Animals**	Daily MAP dose (mg)	Days of MAP feeding	No. of animals treated	Percentage of synchronized animals (range in days)	Percentage of conception in synchronized animals
Hansel (1961)	B.C.	968 (0.97*) 500 (0.5*)	10 10	32	50 (3–4)	25
Collins (1961)	D.H.	0.5*	20	36	97 (2–5)	67
	B.H.	0.5*	20	15	93 (2–8)	—
Zimbelman (1961)	D.H.	0.5*	20	16	75 (2–4)	50
	B.C.	0.8*	15	10	90 (3–5)	60
Nelms (1961)	B.C.	220	15	33	100 (2–3)	67
Anderson (1962)	B.H.	150 & 210	20	20	100 (3–6)	55
Nestel (1963)	B.H.	187	18	38	76 (2–4)	—
Zimbelman (1963)	D.H.	135–400	20	57	88 (–6)	58
	B.H.	120–180	18	96	84 (–6)	26–60
Hansel (1966)	B.C.	240	18	96	76 (3–4) 84 (2–8)	65
	B.C.	240	18	136	75 (2–3) 86 (2–6)	49
Fahning (1966)	D.H.	0.4*	11–18	19	95 (2–4)	26
Dhindsa (1967)	B.H.	180	18	31	87 (–4)	39
	B.C.	180	18	99	55 (–4)	50

Note ** B: Beef cattle, D: Dairy cattle, H: Heifer, C: Cow
* mg/lb. body weight.

Table 2. Synchronization of estrus and fertility after feeding of CAP in cattle

Reported by	Animals**	Daily CAP dose (mg)	Days of CAP feeding	No. of animals treated	Percentage of synchronized animals (range in days)	Percentage of conception in synchronized animals
Van Blake (1963)	D.H.	0.02/lb.	20	20	95 (4–6)	53
	D.H.	12	18	25	92 (6–9)	70
	D.C.	12	18	8	88 (3–7)	57
Veenhnizen (1964)	B.C.	10	18	30	90 (–6)	50
	B.C.	10	18	37	84 (–4)	24
Hansel (1966)	B.C.	10	18	98	92 (3–8)	36
	B.C.	10*	19	97	82 (3–9)	41
	B.C.	10	18	138	55 (2–3) 83 (2–10)	35
Takeuchi (1966)	B.C.	0.05/kg.	15	7	86 (3–6)	33

Note ** B: Beef cattle, D: Dairy cattle, H: Heifer, C: Cow
* Fed in combination with 9mg of thyroprotein.

of 5 mg of estradiol valerate on the 2nd day of feeding. Physiological bases for this treatment involved an early regression of corpus luteum with exogenous estrogen (Wiltbank *et al.* 1961, Kaltenbach *et al.* 1964, Niswender *et al.* 1965) and a suppressive effect on ovulation with orally active progestins. By this treatment, estrus was synchronized in 86% of the treated animals in a 72-hour period and 54% of animals inseminated at that estrus became pregnant.

Intravaginal insertion of progestin impregnated sponge

In practical use of the progesterone derivatives, a long period of the hormone feeding and its relatively high cost preclude the field

applications of this technique. Robinson (1965) reported synchronization of estrus in sheep following removal of intravaginal sponges impregnated with 17α-acetoxy-9α-fluro 11β-hydroxyprogesterone (Flurogesterone acetate, Cronolone, SC-9880). Recently, Shimizu, Takeuchi, Kawai and Adachi (1967) showed that this technique may be applied for synchronization of estrus in cattle.

A cylindrical sponge impregnated with 100 mg or 200 mg of Cronolone was inserted into the vagina at the 5th, 10th or 15th day of the cycle and allowed to remain in it for 18 days. Estrus occurred in almost all cows within 2 days after the withdrawal of the sponge, and 50% of the animals inseminated at the estrous period conceived.

While an effective synchronization of estrus was also demonstrated by Wishart *et al.* (1968) with 200 mg of Cronolone, in combination with an injection of 750 iu of PMS 1–2 days before the removal of the sponge, Carrick *et al.* (1967) obtained an unsatisfactory synchronization and fertility with 100 mg or 200 mg of Cronolone.

By this intravaginal technique, the method for the hormone administration was simplified and its cost could be reduced considerably. However, the fertility at the synchronized estrus appeared to be somewhat poor. Futhermore, it should be noted that the sponge inserted into the vagina was expelled in 20–30% of animals during the treated period.

Injection of oxytocin

Armstrong *et al.* (1959) reported that daily injection of oxytocin during a critical period from the 2nd to 6th day of the cycle caused an early regression of corpus luteum and induced the precocious estrus in cattle. Hansel *et al.* (1961) used this mechanism in combination with progesterone injection to reduce the period of treatment.

Uterine stimulation

Yamauchi, Nakahara, Kaneda and Inui (1965, 1966, 1967) suggested that synchronization can be produced by uterine distention. This unique idea was based on the phenomenon that the estrous cycle length of the cow could be modified by uterine distention. Uterine distention was produced by injecting 42–137 gm of viscous gel-like substance "Gelceptor F" (Eisai Co., Ltd.) through the cervix into the uterus. "Gelceptor F" was prepared originally for treating endometritis in cows and contains 500 mg of Fradiomycin sulphate, 280 mg of Bithionol and 10,000 iu of Vitamin A palmitate per 100 mg of gel.

By the treatment on Day 2 (Day O referred to the day of ovulation) and on Day 6, the length of the cycle reduced to 9–10 days and 14–17 days, respectively. (Table 3) On the contrary, the treatment during the late luteal phase (Day 14 and 16, Day 17 or 18) lengthened the cycle, giving a mean of 25.8 days. However, cycles were of normal length in the animals treated at post-estrus, functional luteal phase or pro-estrus.

Consequently, in a large proportion of cows in which the treatment was performed between the early luteal and the late luteal phases, ovulations occurred 7–12 days (9.3 $\pm$1.6 days) after the treatment. It was also found that endometritis limited to the surface area of the endometrium was induced temporarily by this treatment. However, it was repaired by the time the animal returns to estrus, and fertility to insemination at that time appeared to be normal.

Based on these results, the suggestion was made that the treatment might be applied to the synchronization of estrus in animals at all stages of the cycle, except during the limited periods of pro-estrus, estrus and very early post-estrus. Nakahara, Domeki, Inui and Yamauchi (1967) developed the experimental works of this line, supposing that the inflammatory changes of the endometrium might be related to the life span of the corpus luteum.

In order to induce inflammation of the endometrium, two preparations of iodine solution; i.e. Lugol's solution at various concentration (I : IK : distilled water=1 : 2 : 75–6,000)

Table 3. Modification of estrous cycle of the cow by single or double intrauterine injections of Gelceptor at various stages of cycle

	Time of treatment						
	Post-estrus	Early luteal phase		Functional luteal phase	Late luteal phase		Pro-estrus
	Day 1	Day 2	Day 6	Day 12	Days 14 and 16	Days 17 or 18	Days 17 or 18
Length of cycles (days)	20–*20* 22– *8*–23 23–*25*–22 20–*21*	21–*22*–20 22– *9*–23 21–*10*–27 24–*10*–21 22– *9*–21	23–*14*–27 22–*17*–19 26–*17*–25 20–*14*–21	20–*20*–20 22–*21*–20 22–*22*–22 22–*22*–21	20–*24*–22 21–*25*–22 21–*25*–23	22–*33*–23* 22–*27*–23 23–*28*–22	21–*20*–21 19–*19*–21
Mean cycle length (days)	21·2– *18·5*– 22·5	22·0– *12·0*– 22·4	22.8– *15.5*– 23.0	21.5– *21.3*– 20.8	21.4–*25.8*–22.4 (except*)		20.0– *19.5*– 21.0
Treatment cycle compared to previous and following cycles untreated	Various	Shortened ($P<0.05$)	Shortened ($P<0.01$)	Not different	Lengthened ($P<0.01$)		Not different

(Yamauchi et al. 1967)

Figures show the lengths of successive cycles, the italics indicating the cycle in which treatment was given.

* Treatment cycle was abnormally long.

and solution of Polyvinyl pyrrolidone iodine (PVP-I, Iodine solution, Meiji Seika, Kaisha, Ltd.) at the concentration of 10–100 mg of povidone iodine per ml were used. These were injected through the cervix into the uterus.

By the treatment with 5–40 ml of Lugol's solution at early luteal phase (Day 3), an early regression of corpus luteum was induced as expected and resulted in a shortening of the estrous cycle. (Table 4) The ovulation occurred 6–13 days (8.6 ±1.9 days) after the treatment and the mean length of the treated cycle was reduced to 11.6 ±1.9 days.

On the contrary, the treatment at the late luteal phase (day 16) resulted in a somewhat lengthening of the estrous cycle and the ovulation occurred 9–11 days (10.0 ±1.0 days) after the treatment. Similar results were obtained by the treatment with PVP-I solution. Nakahara, Domeki and Yamauchi (1966) also investigated the effectiveness of synchronization in Japanese beef cattle by injecting the iodine solution into the uterus. (Table 5) A total of 291 animals at various stages of the estrous cycle, including 8 in normal reproductive conditions and 283 with breeding difficulties such as silent heat (259), endometritis (4) and repeat breeding cows (20) were used in this experiment, and 20 ml of PVP-I solution at the concentration of 20–22 mg of povidone iodine per ml or Lugol's solution (I : IK : distilled water= 1 : 2 : 150) were utilized.

In 67.7% of the 247 animals treated during the luteal phase, estrus occurred 6–11 days after the treatment. Eighteen animals which did not show estrus during this period came in estrus approximately one cycle later, indicating that they had silent ovulation around this period. The treatment during the luteal phase might occur during this period in approximately 75% of the animals treated.

Whereas in almost all animals (42 out of 44) treated at pro-estrus, estrus and post-estrus, cycles were of normal length and estrus occurred around the expected day of the cycle. A total of 192 animals treated during the luteal phase were inseminated 3–21 days after the treatment, of which 89 (51.7%) conceived. Particularly, 67 (50.4%) out of the 133 animals which came in estrus 6–11 days after

Table 4. Effect of intrauterine infusion of Lugol's solution on estrous cycle of cow

Cow No. — Order of cycles	Infusion of Lugol's solution			Modification of cycle length (days)					Days from infusion to ovulation (c)		
	Day of cycle (a)	Conc. (b)	Doses (ml)	Previous cycle	Treated cycle (c)			Following cycle			
					S.	U.	L.		S.	U.	L.
11 — 2	3	4	40	23	16			20	13		
12 — 4	3	2	40	22	10			21	7		
13 — 3	3	2	40	21	12			23	9		
13 — 5	3	2	40	23	12			22	9		
15 — 4	3	1	40	22	12			23	9		
18 — 2	3	1	40	23	13			20	10		
13 — 19	3	1	5	25	9			26	6		
14 — 20	3	1	5	24	10			21	7		
21 — 5	3	1/2	5	24	11			21	8		
15 — 13	3	1/5	12.5	20	10			32	7		
14 — 22	3	1/10	25	21	13			50	10		
20 — 8	3	1/10	25	24	10			22	7		
12 — 22	3	1/10	25	25			29	23			26
21 — 7	3	1/20	25	21	14			46	11		
19 — 14	3	1/20	25	26	10			26	7		
11 — 18	3	1/20	25	23		21		23		18	
20 — 10	3	1/30	25	22		21		—		18	
13 — 22	3	1/40	25	25		21		—		18	
Average ± S.D.				11.6±1.9					8.6±1.9		
	(e)										
10 — 8	16°	1	40	22			27	23			11
12 — 11	16°	1	40	22			26	23			10
13 — 13	16°	1	40	22			25	24			9
14 — 12	16*	1	40	20		18		18		2	
Average ± S.D.				26.0±1.0					10.0±1.0		

(Nakahara et al. 1967)

a): The day on which ovulation occurred was considered as 0 of the cycle.
b): Conc. 4, 2 and 1 mean the concentrations of Lugol's solution in which 1 part of I_2 and 2 parts of IK are dissolved in 75, 150 and 300 parts of distilled water (d.w.) respectively. Conc. 1/2–1/40 means 2–40 fold dilution of the solution of Conc. 1 with d.w.
c): S.……Shortened, U.……Unchanged, L.……Lengthened
e): °……Late luteal phase, *……Proestrus

the treatment became pregnant.

Fertility to the first estrus within 21 days after the treatment appeared to be rather good because almost all animals used in this experiment had breeding difficulties such as silent heat, endometritis or repeat breeding cows. Conception rate at the second estrus after the treatment seemed to be fairly higher than that at the first estrus. Of the animals treated during the luteal phase, 67.7% (44 out of 65) conceived at the second estrus.

From these results, it was concluded that the intrauterine treatment with the iodine solutions could be applied for the control of the estrous cycle, although effectiveness of synchronization and fertility was not satisfactory and the treatment was limited to animals at the luteal phase. Future development and application of this unique idea might be made available to the field of bovine reproduction

Table 5. Occurrence of estrus following intrauterine treatment with iodine solution in cattle

Stages of treatment	No. of animals treated	Days from the treatment to occurrence of estrus																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27≦
Pro-estrus	25	3	6	9	2	1	1															1					2	
Estrus	8																					2	3	1		1	1	
Post-estrus	11							1		1									1	2	2	2	1			1		
Early luteal phase	93				1	8	6	14	19	10	10	8	1	1	1		1		2				1				2	8
								75 (80.7%)																				
Functional luteal phase	154					1	4	9	17	22	25	18	14	6	5	5	3		2							1		22
										105 (68.2%)																		
Total	291	3	6	10	11	11	25	36	33	35	26	15	7	6	5	4		4	1	2	2	5	5	1		3	5	30

(Nakahara et al. 1966)

Note 1. Animals treated include 8 with normal reproductive condition and 283 with breeding difficulties such as silent heat (259), endometritis (4) and repeat breeding cow (20).

2. Twenty ml. of iodine solution, either polyvinyl pyrrolidone iodine (20–22 mg/ml) of Lugol's solution (I : IK : distilled water = 1 : 2 : 150) were injected through the cervix into the uterus.

although the mechanisms involved in it are not known.

A considerable number of investigations have been conducted on the estrous cycle synchronization in cattle with or without using steroid hormone. However, many practical problems such as laborious process, relatively high cost, low synchronizating effect and low fertility have prevented a widespread application of this technique. Further investigations are necessary to accomplish the simple and precise technique available for practical use.

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