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Development of Ovulation Synchronization and Fixed Time Artificial Insemination in Dairy Cows

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Abstract. Recently, reproductive management has become more difficult as a result of increased herd size. Problems with missing estrous signs and decrease in conception rate by artificial insemination (AI) performed at wrong timing have caused low AI conception rates. In 1995, ovulation synchronization and fixed-time AI (Ovsynch/TAI) was developed in the USA as a new reproductive technology, which was accepted as an useful reproductive management tool in many countries. However, no information on the use of Ovsynch/TAI was available in Japan. It was, therefore, warranted to show the ovulation rate and conception rate after Ovsynch/TAI using gonadotropin releasing hormone analogue (GnRH-A, fertirelin acetate) and prostaglandin F_{2α} (PGF_{2α})-THAM, both were commercially available in this country. The conception rate after Ovsynch/TAI has been known to vary among different herds and individuals. Investigation and analysis of factors affecting the conception rate was also warranted to improve the conception rate. A series of experiments were carried out to establish Ovsynch/TAI using domestically produced GnRH-A and PGF_{2α} and to study factors affecting conception rate after Ovsynch protocol. Ovsynch using 100 µg GnRH-A and 25 mg PGF_{2α} were observed using ultrasonography. As a result, a high synchronization rate of ovulation at 16 to 20 h after the second GnRH injection was confirmed. The conception rate after Ovsynch/TAI was compared in 87 cows with the conception rate after AI at estrus induced by PGF_{2α} (139 cows). Conception rate after Ovsynch/TAI was higher than the figure after AI at induced estrus (59.1% *vs* 20.9%, *P*<0.05). The dose of GnRH-A was also studied and a practical dose of GnRH-A was found to be 50 µg per cow. To clarify some factors affecting the conception rate after Ovsynch/TAI, 1,558 cows were investigated for the state of their ovaries, days after calving, parity, season, ovarian cyclicity postpartum and nutritional state at the day of Ovsynch. The overall conception rate after Ovsynch/TAI was 51.5%. Fifty-six cows (3.6%) showed estrus at 6 to 7 d after the first injection of GnRH-A. The conception rate after Ovsynch/TAI was low in cows that were 40 to 60 d postpartum, those in their 5th lactation or more, those bred in July to August, and those recovering ovarian cyclicity later than 56 d postpartum. The conception rate after Ovsynch/TAI was high in cows in which body condition score (BCS) was 3.75 at dry period and 3.0 at the day of Ovsynch. In conclusion, Ovsynch/TAI is an effective tool for the reproductive management of dairy cows. A steady and sufficient conception rate after Ovsynch/TAI could be expected by taking the factors affecting the conception rate into the consideration.

Key words: Artificial insemination, Dairy cow, Estrus, Ovulation synchronization

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There are about 1,730,000 dairy cattle in Japan and half of the cattle for breeding are in Hokkaido. Nakashunbetsu, a town in the Nemuro district of Hokkaido, is a dairy farming area and

there are about 24,000 cows. Recently, reproductive efficiency has been declining and

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infertility is a serious problem in the region. The estrous detection rate was 67% in 1992. It decreased to 55% in 2000. In addition, the conception rate was 56% in 1992, and then declined to 45% in 2000. As the consequence, the calving interval was prolonged from 398 d in 1992 to 416 d in 2000. The problem with reproductive efficiency is not a problem in Hokkaido alone. The conception rate of the country as a whole also decreased from 62.2% in 1991 to 54.9% in 2000. The declining conception rate of cows has been reported by the USA and Spain [1–3], too.

The reproductive efficiency of cows has been evaluated by the estrous detection rate, the conception rate and pregnancy rate (estrous detection rate $\times$ conception rate) [4, 5]. It is, therefore, necessary to increase both estrous detection rate and conception rate to improve the pregnancy rate for improving the reproductive performance in dairy herds [6].

The question remains as to why conception rates have decreased in Japan. Some possibilities include 1) the number of dairy cows per herd has increased as the dairy farming scale expands, 2) the number of free stall systems has increased with changes in reproductive management, and 3) the average milk yield per cow per lactation has increased by a genetic improvement of the cow. The average herd size in Nakashunbetsu was 103 cows in 1992, and increased to 122 cows in 2000. An increase in the number of cows in each farm results in difficulty in reproductive management. Moreover, the floor of the free stall is covered with concrete, which increases the occurrence of foot problem, and decreases the standing behavior of cows. In addition, the average milk yield per cow per lactation in Nakashunbetsu rose from 7,700 kg in 1992 to 8,100 kg in 2000. These changes might cause poor expression of estrous behavior and shortening of the duration of estrus [7–12]. As the consequence, the estrous detection has become more difficult than ever [13].

Importance of Developing Practical Protocol for Ovsynch/TAI

The methods for improvement of the estrous detection rate include 1) the use of estrous detection devices, 2) synchronization of estrus, and 3) synchronization of ovulation.

1. Use of estrous detection devices

The heat mount detector (HMD), tail paint, and pedometer can be used for the estrous detection. In our area, problems with HMD being poked and destroyed by crows have occurred in the past. It is also time consuming for farmers to apply the devices regularly and to check the devices everyday. Therefore, the use of HMD and tail paint is not widely accepted here. The pedometer is not widely used either, because of its high cost. As a result, estrous detection devices are not widely used on dairy farms in our area. The synchronization of estrus is more commonly used in Japan.

2. Synchronization of estrus

As for the synchronization of estrus using progesterone alone, a long-term treatment is needed to expect a high estrous synchronization rate. This may often result in poor conception rate. Therefore, it is necessary to use the progesterone device in combination with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or estradiol [14–37]. Estrus can be synchronized by injecting $PGF_{2\alpha}$ during luteal phase [14, 38–43]. However, there is a variation in interval from $PGF_{2\alpha}$ injection to estrus, and the optimum time for insemination is difficult to be estimated. Some cows show poor estrous signs after $PGF_{2\alpha}$, and there are some cases where only post estral bleeding is observed. The interval from treatment to estrus varies depending on the stage of the follicular wave when $PGF_{2\alpha}$ is administered [14, 43–46]. It is necessary to control the follicular wave to reduce the variation of estrus after $PGF_{2\alpha}$ injection [47–51]. A synchronization rate of estrus can be improved by injecting gonadotropin releasing hormone (GnRH) 7 d before $PGF_{2\alpha}$ injection [52]. These protocols still require estrous detection and, therefore, are not useful in cows with poor expression of estrus.

3. Synchronization of ovulation and fixed-time AI

Synchronization of ovulation and fixed-time artificial insemination (Ovsynch/TAI) was first reported in 1995 [53]. Cows were first injected intramuscularly with 100 μ g of GnRH, which induced ovulation of the ovarian follicle in various stages and led to formation of the corpus luteum, and new follicular waves were synchronized. Seven days after GnRH injection, $PGF_{2\alpha}$ was administered. Two days after $PGF_{2\alpha}$

administration, GnRH was again injected. The corpus luteum regressed and the dominant follicle proceeded towards ovulation. Ovulation of this dominant follicle was synchronized 24 to 32 h later by the 2nd injection of GnRH. Therefore, artificial insemination (AI) at about 10 h before the ovulation, 16 to 20 h after the 2nd injection of GnRH, regardless of the presence of estrus, was considered to be the optimal time.

The major advantages of Ovsynch/TAI are as follows; 1) estrous detection rates can be increased to 100%, 2) sufficient conception rate is obtained after TAI, and 3) the pregnancy rate can be improved.

The author intended to introduce and develop the Ovsynch/TAI to solve some problems in reproductive management in dairy herds such as missing heat and poor conception rate due to AI at wrong timing, and resultant poor reproductive performance.

Development of Practical Protocol of Ovsynch/TAI in Japan

Effects of Ovsynch/TAI protocol using a domestic product of GnRH-analogue (GnRH-A, fertirelin acetate) and PGF_{2α}-THAM on ovulation synchronization were examined. Optimal doses of GnRH-A for the protocol were then investigated. And some factors influencing conception rate after

Ovsynch/TAI were described.

1. Ovulation synchronization rate after Ovsynch using GnRH-A and PGF_{2α}

The changes in the ovary following Ovsynch are shown in Table 1. Five cows were in the follicular phase and the other 5 were in the luteal phase at the time of first GnRH-A injection during the Ovsynch protocol. Ovulation was synchronized among all cows regardless of follicular phase or luteal phase. The conception rate after TAI was acceptable. Ovsynch using GnRH-A and PGF_{2α} marketed in Japan was confirmed as being practical [37].

2. Comparison in conception rate between cows inseminated estrus induced by PGF_{2α} injection and those bred at fixed-time after Ovsynch

Eighty-seven cows with corpus luteum were injected with PGF_{2α} and underwent AI. Another 139 cows were subjected to the Ovsynch in the luteal phase. The conception rates with these cows were compared. The conception rate in the Ovsynch group was significantly higher than in the PGF_{2α} group (59.1% vs 20.9%, $P < 0.05$) (Table 2). It was clarified that Ovsynch/TAI improved reproductive performance in dairy herds [37].

3. Comparison of conception rate after Ovsynch/TAI using GnRH-A 100 µg and 50 µg

A group of cows subjected to Ovsynch/TAI using 100 µg GnRH-A was compared with the

Table 1. The changes in ovary and conception rate in cows following Ovsynch/TAI

	GnRH-A	PGF _{2α}			Ovulation (%)	Conception rate (%)
		F→CL	CL→CL	F→CL CL→CL		
Follicular phase	5	5	0	0	100	60
Luteal phase	5	2	1	2	100	80

F: ovarian follicle, CL: corpus luteum.

Table 2. Reproductive performance in cows following Ovsynch/TAI

	Luteal phase		Inactive ovaries		Follicular phase		Total	
	OV	CO	OV	CO	OV	CO	OV	CO
No. of cows	87	139	88	12	10	–	185	151
No. of cows inseminated	66	139	77	12	10	–	153	151
No. of cows conceived	39	29	37	4	6	–	82	33
Conception rate (%)	59.1 ^a	20.9 ^b	48.1	33.3	60.0	–	53.6 ^c	21.9 ^d

OV: Ovsynch, CO: Control. ab, cd: $P < 0.05$.

Table 3. Reproductive performance in cows following Ovsynch/TAI using 100 μ g or 50 μ g GnRH analogue

GnRH-A (μ g)	No. of cows	No. of cows inseminated	No. of cows conceived	Conception rate (%)
100	42	42	25	59.5
50	72	72	44	61.1

Table 4. Reproductive performance after Ovsynch/TAI of 1,558 lactating cows from 1996 to 2001

year	1996	1997	1998	1999	2000	2001	Total
No. of cows	358	167	247	337	314	135	1,558
No. of cows inseminated	319	149	227	315	312	135	1,457
No. of cows conceived	163	79	126	161	145	77	751
Conception rate (%)	51.1	53.0	55.5	51.1	46.5	57.0	51.5

Table 5. Influence of ovarian status at initiation of Ovsynch/TAI on conception rates

Ovarian status at day of Ovsynch	Presence CL	Absence F or CL	Presence F	Ovarian cyst	Unknown	Total
No. of cows	530	798	31	76	22	1,457
No. of cows conceived	285	420	15	19	12	751
Conception rate (%)	53.8 ^a	52.6 ^b	48.4	25.0 ^c	54.5	51.5

CL: corpus luteum, F: ovarian follicle. ac, bc: $P < 0.0001$.

conception rate of the 50 μ g group. No significant difference was observed in the conception rate between both groups (59.5% vs 61.1%) (Table 3). We conclude that using a dose of 50 μ g of GnRH-A reduced the drug costs of Ovsynch protocol, while maintaining the efficiency of the treatment [54].

4. Factors affecting the conception rate after Ovsynch/TAI

A total of 1,558 cows were investigated for the factors that affect the conception rate after Ovsynch/TAI, the state of the ovary, days after calving, parity, season, ovarian cyclicity postpartum and nutritional state at the day of Ovsynch. The conception rate in 1,558 cows after Ovsynch/TAI was 51.5% (Table 4).

(1) State of the ovary

The relationship between the state of the ovary at the day of Ovsynch and the conception rate after Ovsynch/TAI is shown in Table 5. The conception rate of cows with the presence of corpus luteum on the ovary at the day of Ovsynch was the highest in 53.8%, and it was the lowest in cows with an ovarian cyst. It was hypothesized that there were many cows with normal ovarian function in cows

that had corpus luteum. Moreover, it was previously reported that the conception rate was the highest when the Ovsynch protocol began from 5 to 11 d after estrus [55]. In the USA, it was reported that the conception rate of cows with ovarian cysts was low [56]. Cows with normal ovarian cyclicity at initiation of Ovsynch may show a high conception rate.

(2) Days after calving

Effect of the days after calving at the initiation of Ovsynch on the conception rate is shown in Table 6. The conception rate was the lowest at 40 to 60 d postpartum and highest at 101 to 120 d postpartum. It is assumed that a substantial number of cows in early lactation period had not recovered their ovarian cyclicity. This might have been one of the causes of the low conception rate. Previous studies also reported that the conception rate at 50 to 75 d postpartum at initiation of Ovsynch was lower than the figure at 76 d postpartum [57, 58].

(3) Parity

Table 7 shows the relationship between parity at the day of Ovsynch and the conception rate after Ovsynch/TAI. The conception rate with the

Table 7. Influence of parity on conception rates in cows treated with Ovsynch/TAI

Parity	1	2	3	4	5	6<	Total
No. of cows	402	323	256	210	143	123	1,457
No. of cows conceived	204	185	135	111	59	57	751
Conception rate (%)	50.7	57.3 ^a	52.7 ^d	52.9 ^d	41.3 ^b	46.3 ^c	51.5

ab: P<0.01; ac, bd: P<0.05.

Table 6. Influence of day of initiation of Ovsynch/TAI on conception rates

Days of postpartum	40–60	61–90	91–120	121–150	151–220	221<	Total
No. of cows	159	478	225	176	264	155	1,457
No. of cows conceived	76	240	123	93	137	82	751
Conception rate (%)	47.8	50.2	54.7	52.8	51.9	52.9	51.5

Table 8. Influence of month of Ovsynch/TAI on conception rates

Month	1	2	3	4	5	6	7	8	9	10	11	12	Total
No. of cows	169	122	130	130	142	126	109	106	87	120	95	121	1,457
No. of cows conceived	94	70	68	58	71	77	38	41	47	71	52	64	751
Conception rate (%)	55.6 ^b	57.4 ^{bg}	52.3 ^c	44.6 ^f	50.0 ^d	61.1 ^{bh}	34.9 ^a	38.7 ^e	54.0 ^c	59.2 ^{bg}	54.7 ^c	52.9 ^c	51.5

ab: P<0.001; ac, be, fh: P<0.01; ad, ce, fg: P<0.05.

Table 9. Relationship between interval from calving to recovery of ovarian cyclicity and conception rates after Ovsynch/TAI in cows

Days postpartum until ovarian recovery	<34	35–55	56<
No. of cows	11	6	22
No. of cows conceived	8	3	6
Conception rate (%)	72.7 ^a	50	27.3 ^b

ab: P<0.05.

groups of 5 and 6th lactation cows were lower than 2nd lactation cows. Old cows, in general, have a higher incidence of ovarian dysfunction [59] and uterine diseases. Pursuley *et al.* [60] previously reported that the conception rate in 2nd lactation cows were the highest and that parity influenced the conception rate after Ovsynch/TAI.

(4) Season of the year

The influence of the season at the initiation of Ovsynch on the conception rate after Ovsynch/TAI is shown in Table 8. The conception rate was the lowest during July and August. No difference in conception rate after Ovsynch/TAI was observed during the 5 months from January to May [61]. Arechiga *et al.* [62] reported that the conception rate after Ovsynch/TAI decreased in the summer. These results are similar to the finding in this study.

The estrous detection rate of cows with silent estrus in the summer can be improved by Ovsynch/TAI. Therefore, it is considered that Ovsynch/TAI is an effective tool for the improvement of estrous detection rate and pregnancy rate in the summer.

(5) Ovarian cyclicity postpartum

Table 9 shows the relationship between the recovery of ovarian cyclicity and the conception rate after Ovsynch/TAI. The conception rate was lower in cows with delayed recovery of ovarian cyclicity beyond 56 d postpartum [63]. It has been reported that the conception rate was low when Ovsynch/TAI was initiated early postpartum [57, 60]. It is likely that there are a substantial number of cows with delayed recovery of ovarian cyclicity during early lactation period, and these cows also show delayed uterine involution. As the

Table 10. Relationship between the BCS at days -10, 30 and 40 and day of Ovsynch and conception rates after Ovsynch/TAI in cows

BCS	Days after calving			
	-10	30	40	56<*
2.25			11.1 ^c	0 (0/2)
2.5		11.1 (1/9)	(%)	33.3 (3/9)
2.75		33.3 (1/3)		(%)
3.0	0 (0/1)	52.5 (10/19)	53.8 ^d	14.3 (1/7)
3.25	0 (0/4)	75 (3/4)	(%)	66.7 (12/18)
3.5	22.2 (2/9)	50 (2/4)	50 (1/2)	51.9
3.75	46.7 (7/15)		0 (0/1)	(%)
4.0	77.8 (7/9)			85.7 (12/14)
4.25	100 (1/1)			50 (1/2)
				0 (0/1)

Conception rate (No. of cows conceived/No. of cows examined)

* The day when Ovsynch was conducted.

ab : P<0.01, cd : P<0.05.

Table 11. The incidence and distribution of estrus after 1st GnRH analogue injection and conception rate

Days after 1st GnRH-A injection	1	2	3	4	5	6	7	8	9	Total
No. of cows	1	6	2	3	3	28	28	3	1	75
No. of cows inseminated	1	4	1	0	1	9	18	2	1	37
No. of cows conceived	0	2	0	0	0	2	8	1	0	13
Conception rate (%)	0	50	0	0	0	22	44	50	0	35

consequence, the conception rate after Ovsynch/TAI in these cows may be low.

It is suggested that recovery of the ovarian cyclicity after calving be facilitated to increase the conception rate after Ovsynch/TAI.

(6) Nutrition

The relationships between body condition score (BCS) at 10 d prepartum, 30 and 40 d postpartum and the day of Ovsynch and the conception rate after Ovsynch/TAI are shown in Table 10. It was indicated that the conception rate of cows with BCS of ≤ 3.5 at 10 d prepartum and ≤ 2.5 at the day of Ovsynch was low [63]. Burke *et al.* [61] also pointed out the relationship between an increase of BCS and improvement of the conception rate after Ovsynch/TAI. Likewise, Moreira *et al.* [64] reported that the conception rate of the cows with low BCS at the day of Ovsynch was low. Results of the present study and previous studies show that BCS of cows influences the conception rate after Ovsynch/TAI.

Constraints of Ovsynch/TAI and Some Solution

It has been demonstrated that Ovsynch/TAI is effective for the improvement of the reproductive performance in dairy herds in Japan. However, some constraints for Ovsynch/TAI remain; 1) some cows came into estrus before the day of TAI, and the ovulation was not synchronized [61], and 2) cows in negative energy balance postpartum had lower conception rates [63, 64].

Table 11 shows distribution of the days from the 1st GnRH injection until estrus in cows showing estrous sign during Ovsynch protocol. Many cows showed estrus at 6 to 7 d after the 1st GnRH injection. If Ovsynch protocol was initiated at 11 to 14 d after estrus, the cows had no corpus luteum around 7 d after the 1st GnRH and could express estrous signs [43].

BCS at the initiation of Ovsynch influences the conception rate after Ovsynch/TAI [61, 64]. Cows that had a severe negative energy balance and decreased BCS during early lactation period show ovarian quiescence. Therefore, even if Ovsynch is performed, the conception rate after Ovsynch/TAI will be low in these cows.

Fig. 1. Development of practical protocol using palpation per rectum and Ovsynch/TAI in combination.

More recently, Presynch-Ovsynch protocol, which administers PGF_{2α} twice at a 14 d interval before Ovsynch/TAI, was developed [65]. Occurrence of estrus and ovulation before TAI during Ovsynch protocol can also be eliminated by using CIDR inserted from the day of Ovsynch to the PGF_{2α} administration (CIDR-Ovsynch) [66]. Heatsynch is basically similar to Ovsynch [67]. However, estradiol is used instead of the 2nd GnRH. The estrous signs appear after Heatsynch. The cost of the hormone treatment for Heatsynch is cheaper than the cost for Ovsynch.

Development of Practical Protocol Using Palpation Per Rectum and Ovsynch/TAI

Using the rectal palpation together with the Ovsynch/TAI may be practical in Japan where the cost of the hormone is high (Fig. 1). Cows are palpated per rectum at the initial day of Ovsynch. Ovsynch/TAI should be implemented only in cows with normal uterus. Then, the cows are palpated again after 7 d; 1)Ovsynch/TAI is continued in cows with corpus luteum at the 2nd palpation (Type I & II). 2)If no corpus luteum is present at the 2nd palpation, cows are injected with estradiol and are withdrawn from the protocol (Type III). 3)Cows with ovarian quiescence are treated with GnRH-A again at Day 7 and are palpated later at Day 14. Cows still with ovarian quiescence at Day 14 are excluded from the protocol (Type IV-a).

4) Cows with corpus luteum at Day 14 are subjected to Ovsynch/TAI protocol (Type IV-b). This makes Ovsynch/TAI protocol more reliable and efficient.

Current Status and Future Perspective of Ovsynch/TAI

Dairy farmers under the author's service evaluated the Ovsynch/TAI as follows; 1)time for estrous detection and AI is saved. 2)number of AIs reduced due to decreased double AIs. 3)less variation in monthly milk production of the herd. 4)Ovsynch/TAI is a necessary and indispensable reproductive management tool.

In 1996 when Ovsynch/TAI was first introduced, only 1.8% of dairy herds in Nakashunbetsu were using Ovsynch/TAI. At present, 12% of the dairy herds in the area are utilizing the protocol. Some dairy farmers have been using Ovsynch/TAI for all cows after the voluntary waiting period in the herd. Other farmers are utilizing Ovsynch/TAI as a treatment of postpartum anestrous cows, post service anestrous cows and cows with ovarian cysts. The protocol is also being used for synchronization of ovulation in embryo transfer recipient cattle.

Modern dairy industry requires more efficient and reliable way of reproductive management of cows. Further development of ovulation synchronization protocols based upon Ovsynch/TAI may greatly contribute to the advance of

reproductive technology and to improve dairy industry.

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