



# Relationship between the First Ovulation within Three Weeks Postpartum and Subsequent Ovarian Cycles and Fertility in High Producing Dairy Cows

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1        **Relationship between the First Ovulation within Three Weeks Postpartum and**  
2        **Subsequent Ovarian Cycles and Fertility in High Producing Dairy Cows**

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17  
18       Running head: First ovulation in postpartum dairy cows

## 1 **Abstract**

2

3 The aim of this study was to investigate the relationship between the first ovulation within 3 weeks  
4 postpartum and subsequent ovarian cycles and fertility in high producing dairy cattle in Hokkaido, Japan. In  
5 Experiment 1, 110 cows (44 primiparous and 66 multiparous) were used to determine the effects of the first  
6 ovulation within 3 weeks postpartum on subsequent ovarian cycles. Milk samples were collected twice weekly from  
7 7 to 100 days postpartum. The first ovulation was identified by an increase in milk progesterone (P4) to more than 1  
8 ng/ml within 3 weeks postpartum. The numbers of cows showing ovulation and anovulation within 3 weeks  
9 postpartum were 31 (70.5%) and 13 (29.5%) in the primiparous cows and 35 (53.0%) and 31 (47.0%) in the  
10 multiparous cows, respectively. The patterns of ovarian resumption after calving were classified into two types  
11 (normal ovarian cycles and abnormal ovarian cycles) on the basis of milk P4 concentrations. Initiation of normal  
12 ovarian function in cows ovulated within 3 weeks postpartum occurred earlier than in anovulated cows regardless of  
13 the number of calvings (primiparous, 27.8 days vs. 44.4 days; multiparous, 30.6 days vs. 55.7 days;  $P<0.01$ ). Out of  
14 the multiparous cows that ovulated within 3 weeks postpartum, initiation of normal ovarian function followed by a  
15 normal luteal phase was earlier than when it was followed by an abnormal luteal phase (25.5 days vs. 40.4 days;  
16  $P<0.05$ ). Milk P4 concentrations after the first ovulation were lower than those after the second ovulation in both the  
17 primiparous and multiparous cows ( $P<0.05$ ). In Experiment 2, 22 multiparous cows were used to determine the  
18 effects of the first ovulation within 3 weeks postpartum on subsequent fertility. Blood samples were collected once a  
19 week from 0 to 3 weeks postpartum. The interval from parturition to first service in ovulated cows was shorter than  
20 in anovulated cows (68.4 days vs. 94.8 days;  $P<0.05$ ). The conception rate by 100 days after calving tended to be  
21 higher in ovulated cows than in anovulated cows (50.0% vs. 16.7%,  $P=0.09$ ). In conclusion, our data strongly  
22 suggests that ovulation within 3 weeks postpartum is a crucial phenomenon for subsequent resumption of ovarian  
23 function and conception, and thus it can be used as an index of subsequent reproductive performance.

## 1 **Introduction**

2

3 During the last few decades, milk production per cow has drastically increased due to improvement in  
4 management, nutrition, and genetic selection [1, 2], but the reproductive performance of dairy cows has conversely  
5 declined [1-4]. Several studies suggest that the negative effects of milk production on reproductive function can only  
6 be observed in high producing dairy cattle [2, 4-7]. One of the main reasons for this declining reproductive  
7 performance is a lower rate of increase in feed intake compared to the energy necessary for milk production. Namely,  
8 cows experience a serious period of negative energy balance during early lactation that is characterized by loss of  
9 body weight and mobilization of body fat stores [7, 8].

10 The resumption of ovarian activity plays a crucial role in subsequent fertility [9-11]. Recent studies have  
11 shown that the interval from calving to the first ovulation has become longer in high producing dairy cows [1, 4], and  
12 this can be explained in part by a larger negative energy balance [1, 8]. In most dairy cows, medium-sized follicles  
13 appear within 5 days after calving and large follicles appear within 10 days postpartum [12, 13]. Approximately half  
14 of all cows ovulate within 3 weeks postpartum [9, 10, 14], but in the other half, the dominant follicle of the first  
15 follicular wave regresses and the first ovulation occurs about 4 follicular waves later after calving [15, 16]. This  
16 difference in timing of the first ovulation is attributed to suppression of the lutenizing hormone (LH) pulse frequency  
17 by a negative energy balance during this period [7, 17]. Therefore, cows with only a slight negative energy balance  
18 may show a shorter interval from calving to first ovulation than those with a severe one.

19 It is now accepted that if uterine involution is achieved after calving, early resumption of ovarian cycles is  
20 directly related to higher fertility [9, 18, 19]. On the other hand, other studies have demonstrated that early ovulation  
21 before 3 weeks postpartum is associated with reduced pregnancy rates and a prolonged calving to conception interval  
22 in multiparous dairy cows [20], suggesting that early luteal activity may have a negative impact on involution of the  
23 uterus after calving [21, 22]. Thus, it is still controversial whether early resumption of ovarian cycles improves the  
24 fertility of dairy cows.

25 The aim of our study therefore was to investigate the relationship between the first ovulation within 3 weeks  
26 postpartum and subsequent ovarian cycles and fertility in high producing dairy cattle in Hokkaido, Japan.

## 1 **Materials and Methods**

2  
3 All experimental procedures complied with the Guide for Care and Use of Agricultural Animals of Obihiro  
4 University.

### 6 **Experiment 1**

#### 7 *Animals*

8 Exp. 1 was carried out at the Field Center of Animal Science and Agriculture, Obihiro University of  
9 Agriculture and Veterinary Medicine (Obihiro, Japan), using 110 lactating Holstein cows (44 primiparous and 66  
10 multiparous) that calved between May 2001 and September 2003. The cows were housed in a free-stall barn  
11 throughout the year and offered a total mixed ration consisting of grass, corn silage, and concentrate. They also  
12 grazed during the period between May and October. Milking was performed twice daily (6:00 and 18:00). The  
13 average 305-day milk yield was approximately 8,600 kg, and the first month milk yield was  $27.5 \pm 0.7$  kg/day in  
14 primiparous cows and  $39.2 \pm 0.9$  kg/day in multiparous cows ( $P < 0.01$ ).

#### 16 *Milk sampling*

17 Milk samples were collected twice weekly (Tuesday and Friday) after milking from day 7 to day 100  
18 postpartum. After centrifugation at 3000 rpm for 45 min at 4C, the fat layer was removed, and the skim milk samples  
19 were kept at -30C until analysis for progesterone (P4) concentration.

#### 21 *Definition of ovarian activity after calving*

22 When the P4 concentration in the skim milk from a cow increased to more than 1 ng/ml, the cow was  
23 confirmed as having returned to luteal activity [23]. The first ovulation was identified by luteal activity within 3  
24 weeks after calving (Fig.1-i). The first day when the P4 concentration in the skim milk from cow increased to more  
25 than 1 ng/ml was regarded as the day of initiation of normal ovarian function (Fig.1-ii). A normal luteal phase was  
26 identified if the P4 concentrations in the skim milk samples retained levels of more than 1 ng/ml over a 7-day period

1 (Fig.1-iii). The patterns of ovarian resumption after calving were classified into two types (normal or abnormal  
2 cycle) on the basis of the P4 profiles in skim milk samples as follows:

3 Normal cycles: when cows had an ovarian cycle followed by a luteal phase of normal length and this was repeated  
4 more than two times.

5 Abnormal cycles: when cows had an abnormal ovarian cycle, such as a prolonged luteal phase (ovarian cycles after 3  
6 weeks postpartum with a long luteal phase  $\geq 22$  days), a short luteal phase (ovarian cycles after 3 weeks postpartum  
7 with a short luteal phase  $\leq 4$  days), and cessation of cyclicity (no luteal activity for at least 14 days between the first  
8 and second luteal phases).

## 9 10 **Experiment 2**

### 11 *Animals*

12 Exp. 2 was carried out using a commercial dairy herd in the Tokachi area of Hokkaido. Twenty-two lactating  
13 cows were used. They were housed in a tie-stall throughout the year and offered a total mixed ration consisting of  
14 grass silage. The average 305-day milk yield was 10,800 kg.

### 15 16 *Reproductive management*

17 The cows calved non-seasonally in both of the herds. Ovaries were scanned once a week by ultrasonography  
18 or rectal palpation beginning at 40 days postpartum. If there were apparent reproductive disorders (cystic follicles or  
19 anovulation), hormonal treatment using PGF2a and GnRH were applied after 60 days postpartum.

20 In the Exp. 2 herd, the cows were artificially inseminated at the observed estrus after 50 days postpartum. In  
21 addition to internal signs of estrus as detected by rectal palpation, cows with clear vaginal mucous discharge were  
22 considered to be in estrus. Conception was confirmed by ultrasonography or rectal palpation 40 and 60 days after  
23 each artificial insemination (AI). Cows that did not have a positive pregnancy diagnosis within the period of this  
24 study (180 days postpartum) were assumed to have conceived 21 days after their last unsuccessful service [20].  
25 Therefore, this adjusted days open was used as a measure of final fertility.

1 ***Blood sampling***

2 Blood samples were obtained by caudal venipuncture once a week between parturition and 3 weeks  
3 postpartum using sterile 10 ml tubes containing 200 ul of stabilizer solution (0.3 M EDTA, 1 % acetylsalicylic acid,  
4 pH 7.4). The tubes were immediately chilled in ice water for 10 minutes and centrifuged at 3000 rpm for 20 minutes  
5 at 4C. The plasma samples were kept at -30C until analysis for P4 concentration.

6  
7 ***Enzyme immunoassay for P4***

8 Determination of P4 in skim milk and plasma was performed by enzyme immunoassay after extraction by  
9 diethyl ether as described previously [24], and the extraction efficiencies for skim milk and plasma were 88% and  
10 93%, respectively.

11  
12 ***Statistical analyses***

13 Primiparous cows were analyzed separately from multiparous cows to avoid the potentially confounding  
14 influence of differences in nutrition and energy balance after parturition on reproductive performance in Exp. 1. The  
15 distribution of normal and abnormal ovarian cycles in primiparous and multiparous cows, ovulation within 3 weeks  
16 postpartum on repeated parturitions, and conception ratio were examined by chi-square test as an independency test.  
17 Other significant differences were analyzed by Student's t test. Results were expressed as means  $\pm$  standard error of  
18 the mean, with differences of  $P < 0.05$  considered significant.

19  
20  
21 **Results**

22  
23 **Experiment 1**

24 Initiation of normal ovarian function after calving in primiparous cows ( $33.0 \pm 2.6$  days) was earlier than in  
25 multiparous cows ( $42.6 \pm 2.9$  days,  $P < 0.05$ ). The numbers of cows with ovulation and anovulation within 3 weeks  
26 postpartum were 31 (70.5%) and 13 (29.5%) in primiparous cows and 35 (53.0%) and 31 (47.0%) in multiparous

1 cows, respectively (P=0.07, Table 1). In spite of ovulation or anovulation within 3 weeks postpartum, 59.1% of  
2 primiparous and 43.9% of multiparous cows had normal cycles. A few cows with their first ovulation within 3 weeks  
3 postpartum had a prolonged luteal phase, indicating a persistent corpus luteum, and the numbers of cows with a  
4 persistent corpus luteum in the ovulated and anovulated groups were identical (n=4 for both).

#### 6 ***Characteristics of the luteal phase after ovulation within 3 weeks postpartum***

7 In cows ovulated within 3 weeks postpartum, 16 out of 31 primiparous and 28 out of 35 multiparous cows had  
8 a short luteal phase ( $\leq 7$  days) after the first ovulation. Milk P4 concentrations after the first ovulation were lower  
9 than those after the second ovulation in both the primiparous and multiparous cows (P<0.05, Fig. 2).

#### 11 ***Relationship between the first ovulation within 3 weeks postpartum and subsequent ovarian function***

12 In the multiparous cows, the number of animals with normal cycles followed by repetition of more than two  
13 luteal phases was larger in the group with ovulation within 3 weeks postpartum than that in the group without  
14 ovulation. On the other hand, the number of animals with abnormal cycles was larger in the group without ovulation  
15 than in the group with ovulation (P<0.01, Table 1). Initiation of normal ovarian function in cows ovulated within 3  
16 weeks postpartum was earlier than in anovulated cows despite the number of calvings (16.6 days earlier for the  
17 primiparous cows and 25.1 days earlier for the multiparous cows; P<0.01, Table 2). In the primiparous anovulated  
18 cows, initiation of normal ovarian function after calving followed by normal cycles was earlier than when followed  
19 by abnormal cycles (34.7 days vs. 56.5 days; P<0.05, Table 2), but there was no significant difference in the cows  
20 ovulated within 3 weeks postpartum. In the multiparous cows ovulated within 3 weeks postpartum, initiation of  
21 normal ovarian function occurred earlier in the cows with normal cycles than in those with abnormal cycles (25.5  
22 days vs. 40.4 days; P<0.05, Table 2).

#### 24 ***Ovulation within 3 weeks postpartum for two consecutive lactation cycles***

25 During the period of this study, 22 cows calved twice. In the two consecutive lactation cycles, 8 out of the 22  
26 cows repeated ovulation within 3 weeks postpartum and 4 of the cows showed signs of anovulation. Consequently,



1 there was no clear statistical tendency found in the distribution of occurrence for ovulation or anovulation within 3  
2 weeks postpartum in the same animals with two consecutive lactation periods after application of the chi-square test.

## 5 **Experiment 2**

6 Out of 22 cows examined, 10 cows (45.6%) ovulated within 3 weeks postpartum. The interval from  
7 parturition to first service in the ovulated cows was earlier than in the anovulated cows ( $P<0.05$ , Table 3). The  
8 100-day conception rate after calving in the ovulated cows tended to be higher than in the anovulated cows ( $P=0.09$ ).  
9 However, there was no significant difference between the 2 groups in the ratio of conception by 180 days after  
10 calving. The adjusted days open was longer in the ovulated cows than in the anovulated cows ( $P<0.05$ , Table 3).

## 13 **Discussion**

15 The present study clearly indicates that initiation of normal ovarian function in both primiparous and  
16 multiparous high producing dairy cows ovulated within 3 weeks postpartum is earlier than in anovulated cows. The  
17 results further show that this first ovulation relates to the shorter interval from parturition to first service compared  
18 with the long interval in anovulated cows, and consequently the ratio of conception by 100 days after calving tended  
19 to be higher in the ovulated cows.

20 The ratio of ovulation within 3 weeks postpartum in the primiparous cows tended to be higher than that of the  
21 multiparous cows (71% vs. 53%;  $P=0.07$ ), and 60% of the cows resumed normal ovarian cycles despite ovulation or  
22 anovulation within 3 weeks postpartum. On the other hand, ovulation within 3 weeks postpartum in the multiparous  
23 cows induced a high ratio of subsequent ovarian cycles followed by a normal luteal phase repeated more than two  
24 times. In the present study, a relatively high proportion of primiparous cows had ovulation within 3 weeks  
25 postpartum compared with a previous study, which found the rate to be 53% [20]. Recent studies indicate that the LH  
26 pulse frequency [8] and plasma insulin-like growth factor-1 (IGF-1) level [8, 25] are closely related to the first

1 ovulation during the postpartum period. Negative energy balance, which is caused by insufficient feed intake for  
2 milk production, reduces the LH pulse frequency [8, 17] and plasma IGF-1 level [26], results in delayed resumption  
3 of ovarian activity. The lower milk yield in the first month found in the primiparous cows in the present study may  
4 be one of the reasons for the high ratio of ovulation within 3 weeks postpartum and resumption of normal ovarian  
5 cycles. In addition, it is believed that the rate of dietary intake of the multiparous anovulated cows was too low for  
6 the energy necessary for milk production and that this affected ovulation within 3 weeks postpartum. Undoubtedly,  
7 further studies are needed to assess the relationships among milk production, metabolic and reproductive  
8 endocrinology, and resumption of ovarian function during the postpartum period.

9         There is adequate evidence indicating that first ovulation closely relates to earlier resumption of ovarian  
10 function, earlier first service, and conception in high producing dairy cows [9, 19]. On the other hand, other  
11 researchers have shown in multiparous cows that the ratio of animals having normal ovarian cycles among cows  
12 anovulated within 3 weeks postpartum (85%) was higher than in ovulated cows (68%) [20]. Our results in high  
13 producing dairy cows in Hokkaido confirmed the above results and showed that initiation of normal ovarian function  
14 in ovulated cows within 3 weeks postpartum is earlier than that in anovulated cows despite the number of calvings  
15 ( $P<0.01$ ). In previous studies, it has been shown that earlier resumption of normal ovarian cycles is induced by  
16 reducing loss of body weight, increasing dry matter intake [18], and improving body condition score [27]. Therefore,  
17 it may be possible to use identification of ovulation within 3 weeks postpartum as an index for evaluation of  
18 nutritional status in relation to resumption of normal ovarian function.

19         In previous studies, it has been shown that the luteal phase after the first ovulation postpartum is abnormal  
20 and shorter than normal luteal phases [28, 29]. Similarly, our data showed that a short luteal phase ( $\leq 7$  days) after  
21 the first ovulation within 3 weeks postpartum occurred in a half of the primiparous cows and 80% of the multiparous  
22 cows. In addition, it was also clear that the plasma P4 concentration after the first ovulation was lower compared to  
23 that after the second ovulation despite the number of calvings ( $P<0.05$ ). It has been suggested that such a short  
24 period of elevated P4 concentrations during the postpartum period induces expression of a normal ovarian cycle [30,  
25 31]. Therefore, the present study indicated that a short luteal phase with low P4 level after the first ovulation was  
26 sufficient for resumption of ovarian activity.

1 It has been shown that the plasma concentration of P4 after early first ovulation increases the occurrence of  
2 uterine infection and endometritis [21]. It is also known that endometritis prevents release of luteolytic PGF2a from  
3 the uterus [21] and often induces a persistent corpus luteum [32]. Our data showed that the number of cows with a  
4 persistent corpus luteum in the ovulated and anovulated groups were identical. Thus, the increase in P4 concentration  
5 after ovulation within 3 weeks postpartum had no negative effect on recovery of the uterus after calving in this herd.

6 During the period of this study, 22 cows calved twice and one cow showed no significant repeatability in  
7 ovulation or anovulation within 3 weeks postpartum over the course of two consecutive lactation cycles. Therefore, it  
8 is likely that the occurrence of ovulation or anovulation within 3 weeks postpartum does not depend on genetic  
9 factors.

10 The present results from the herd in Exp. 2 clearly showed that the occurrence of ovulation within 3 weeks  
11 postpartum positively affected the interval from parturition to first service, the ratio of conception by 100 days after  
12 calvings, and the adjusted days open. Resumption of ovarian activity has been shown to play an important role in  
13 subsequent fertility in dairy cows [9, 11], and abnormal ovarian cycles before service have been shown to have  
14 negative effects on reproductive performance, including interval to first AI and pregnancy rate [33]. In addition,  
15 early estrous activity and higher numbers of estrous cycles before service may be indicators of greater restoration of  
16 reproductive efficiency [34]. Thus, first ovulation within 3 weeks postpartum is considered to have an impact on the  
17 recovery of ovarian function and high fertility, and this well supports the results of a previous study by other  
18 researchers [10]. In this context, monitoring of the first ovulation within 3 weeks postpartum using plasma or milk  
19 P4 could be used to estimate nutritional and reproductive status after calving and may be useful for management of  
20 nutrition and voluntary waiting period.

21 In conclusion, ovulation within 3 weeks postpartum could be an early index of recovery of normal ovarian  
22 function and subsequent reproductive performance in high producing dairy cows. The exact relationship between the  
23 occurrence of first ovulation, nutritional status, and fertility should be clarified by large-scale field and experimental  
24 studies in Hokkaido.

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Table 1. Number of cows ovulated or anovulated within 3 weeks postpartum and resumption of normal or abnormal ovarian cycles in primiparous and multiparous cows

	Within 3 weeks postpartum		Significance of differences
	Ovulation	Anovulation	
<b>Primiparous cows</b>			
Total	31	13	-
Normal cycles followed by repetition of more than two luteal phases	19 (61.3%)	7 (53.8%)	ns
Abnormal cycles †	12 (38.7%)	6 (46.2%)	
<b>Multiparous cows</b>			
Total	35	31	-
Normal cycles followed by repetition of more than two luteal phases	23 (65.7%)	6 (19.4%)	**
Abnormal cycles †	12 (34.3%)	25 (80.6%)	

Values are mean±SEM.

\*\*P<0.01; ns, not significant by chi-square test.

† Cows having an abnormal cycle, such as a prolonged luteal phase (ovarian cycles after 3 weeks postpartum with a long luteal phase  $\geq 22$  days), short luteal phase (ovarian cycles after 3 weeks postpartum with a short luteal phase  $\leq 4$  days), or cessation of cyclicity (no luteal activity for at least 14 days between the first and second luteal phases).



Table 2. Initiation of normal ovarian function in primiparous and multiparous cows showing ovulation or anovulation within 3 weeks postpartum

	Within 3 weeks postpartum		Significance of differences
	Ovulation	Anovulation	
<b>Primiparous cows</b>			
Initiation of normal ovarian cycles after calving (days)	27.8±2.7 (n=31)	44.4±4.5 (n=13)	**
Normal cycles followed by repetition of more than two luteal phases	24.6±2.2 (n=19)	34.7±2.8 <sup>a</sup> (n=7)	*
Abnormal cycles †	32.3±5.6 (n=12)	56.5±7.8 <sup>b</sup> (n=6)	*
<b>Multiparous cows</b>			
Initiation of normal ovarian cycles after calving (days)	30.6±3.1 (n=35)	55.7±3.8 (n=31)	**
Normal cycles followed by repetition of more than two luteal phases	25.5±1.6 <sup>x</sup> (n=23)	47.2±8.8 (n=6)	**
Abnormal cycles †	40.4±7.9 <sup>y</sup> (n=12)	57.2±4.4 (n=25)	ns

Values are mean±SEM.

\*\*P<0.01; \*P<0.05; ns, not significant.

<sup>a,b</sup> Indicates differences of P<0.05 within anovulated cows.

<sup>x,y</sup> Indicates differences of P<0.05 within ovulated cows.

† Cows having an abnormal cycle, such as a prolonged luteal phase (ovarian cycles after 3 weeks postpartum with a long luteal phase ≥ 22 days), short luteal phase (ovarian cycles after 3 weeks postpartum with a short luteal phase ≤ 4 days), or cessation of cyclicity (no luteal activity for at least 14 days between the first and second luteal phases).

Table 3. Reproductive performance of multiparous cows

	Within 3 weeks postpartum		Significance of differences
	Ovulation (n=10)	Anovulation (n=12)	
Days to first service	68.4±8.2	94.8±7.9	*
Conception by 100d postpartum (%)	50.0	16.7	P=0.09
Conception by 180d postpartum (%)	71.0	58.3	ns
Adjusted days open <sup>1</sup>	110.0±14.7	149.5±11.7	*

Values are mean±SEM.

\*P<0.05; ns, not significant.

<sup>1</sup>Cows that did not have a positive pregnancy diagnosis within the period of this study (180d postpartum) were assumed to have conceived 21 days after their last unsuccessful service.

1 Figure Legends

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3 Figure 1. Ovarian cycles monitored using skim milk progesterone profiling. (i) Ovulation within 3 weeks  
4 postpartum, (ii) initiation of normal ovarian function after calving, and (iii) luteal phase.

5 Figure 2. The highest skim milk progesterone concentrations during the luteal phase after 1st ovulation  
6 (i.e, ovulation within 3 weeks postpartum; solid column) and 2nd ovulation (open column). \*P<0.05.

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1 Figure 1

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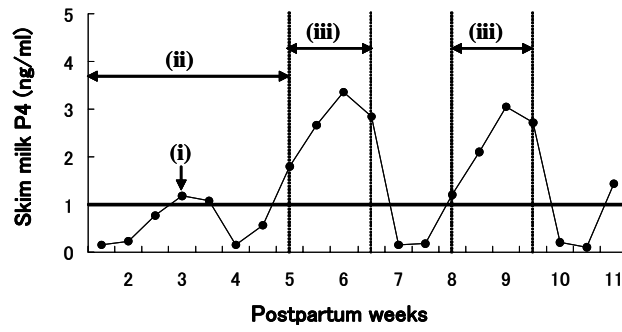
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1 Figure 2

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