Effect of Restricted Suckling on the Superovulatory Response and Reproductive Performance in Postpartum Japanese Black Cows

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(Received 23 January 2012/Accepted 22 June 2012/Published online in J-STAGE 6 July 2012)

ABSTRACT. The reproductive performance of postpartum cows is affected by factors such as suckling and nutrition. We investigated the effect of a restricted suckling period on the superovulatory response and the fertility after flushing in postpartum Japanese Black cows. Forty-seven postpartum cows were used in this study. At 7 days postpartum, the cows were divided into 2 groups: (1) continuous access to calves from birth to weaning at 3 months postpartum (ad libitum suckling group; n=20); and (2) twice daily suckling to the calves penned adjacent to them (restricted suckling group; n=27). All cows were initiated a superstimulatory treatment with a controlled internal drug releasing device and follicle stimulating hormone at 40 days postpartum. Embryos were nonsurgically collected at 7 or 8 days after estrus. After uterine flushing, the cows were again used for reproduction. There were no significant differences between the ad libitum and restricted suckling groups in terms of the numbers of transferable (6.7 ± 5.4 versus 7.9 ± 7.0) and freezable embryos (5.5 ± 4.9 versus 6.2 ± 7.0). In contrast, the interval to the first estrus after flushing in the restricted suckling group was lower (<0.05) than that in the ad libitum suckling group (8.9 ± 5.7 days versus 27.9 ± 24.2 days). These results suggest that restricted suckling in postpartum Japanese Black cows does not affect the superovulatory response and embryo quality; however, it improves their fertility after flushing.

KEY WORDS: restricted suckling, suckling beef cow, superovulation.


In postpartum beef cows, suckling influences various aspects of reproductive performance such as the time required for the first ovulation [7, 14, 19] and estrus [13, 28, 29] to occur postpartum, and the number of days open [2, 18, 23]. The presence of a calf and the occurrence of suckling are related to the prolonged interval between parturition and the first ovulation and estrus [6, 7, 13, 19]. A prolonged anoestrus period in postpartum suckling beef cows appears to be a result of the lack of an ovulating dominant follicle rather than the delayed development of dominant follicles [15]. In postpartum beef cows in good physical condition, the ovarian follicle resumes development within several days postpartum. However, the frequency of the luteinizing hormone (LH) pulse in the pituitary body is low, and the dominant follicle cannot ovulate [31]. The first ovulation usually occurs approximately 30 days postpartum and is usually silent in suckling beef cows [3, 32]. The corpus luteum (CL) regresses prematurely at approximately 10 days of the first estrous cycle, with the second ovulation occurring at approximately 10 days after the first ovulation [3]. This second ovulation is generally associated with the estrus and a normal length luteal phase [3]. Early weaning and a restricted suckling period reduce the interval between parturition and the first postpartum ovulation and estrus. Short and temporary weaning and suckling that is restricted to once and twice per day reduces this interval [7, 28]. In postpartum Japanese Black cows, twice daily suckling reduces the intervals to first ovulation and estrus and to conception without hindering the growth of their calves [26]. In addition, suckling influences uterine involution. Izaike et al. [9] reported that an increase in milk yield at 10 days postpartum delays uterine involution, while an increase in the number of suckling events accelerates uterine involution in Japanese Black cows. Furthermore, uterine involution is usually completed at approximately 40 days postpartum in beef cows [8]. Based on previous knowledge, reproductive functions are believed to be recovered at approximately 40 days postpartum in beef cows. Furthermore, superstimulatory treatment and embryo recovery may be initiated during this period.

In fact, superstimulatory treatment could be initiated at 40 days postpartum in suckling Japanese Black cows. We attempted to improve the reproductive efficiency of Japanese Black cows by implementing a satisfactory parturition interval (one parturition per year) and by embryo recovery within 60 days postpartum. On most farms that breed Japanese Black cattle, cows are suckled by their calves for 4 months or less after calving (Japanese Feeding Standard for Beef Cattle, 2008) [16]. In postpartum suckling cows, suckling affects the superovulatory response and reproductive performance after flushing. To popularize embryo recovery during the postpartum period, knowledge regarding the effects of suckling on the superovulatory response and reproductive performance after flushing should be increased. In this study, we investigated the effects of restricted suckling (twice daily suckling) on the superovulatory response and fertility after flushing in Japanese Black cows during the postpartum suckling period.
MATERIALS AND METHODS

Animals and feedings: Forty-seven postpartum Japanese Black cows aged 5.0 ± 1.7 years (mean ± SD) with parity 3.4 ± 1.5 (mean ± SD) and weighing 507.9 ± 47.2 kg (mean ± SD) at parturition were used in the present study. All cows were handled under the Guidelines for the Care and Use of Animals for Scientific Purposes of NARO Western Region Agricultural Research Center. During the postpartum period, the cows were fed 100% of the estimated daily nutrient requirements for postpartum suckling cows according to the Japanese Feeding Standard for Beef Cattle (2000) [1]. The cows were fed 100% of the estimated daily nutrient requirements to maintain the body weight at the time of parturition and for the daily production of 7 kg of milk for the first 30 days postpartum and 6 kg of milk from 30 days to 3 months postpartum. The calves were fed 100% of the estimated daily nutrient requirements of growing beef heifers with a daily weight gain of 0.8 kg. The calves were fed concentrate and hay to supply the requirements. The calves were weaned at 3 months after birth.

Experimental design: To investigate the effects of restricted suckling on the superovulatory response and the fertility after flushing, 47 postpartum suckling cows were classified into 2 groups at 7 days postpartum: (1) the first group had continuous access to the calves from birth until weaning at 3 months postpartum (ad libitum suckling group; n=20); and (2) the second group was restricted to twice daily suckling with calves that were penned adjacent to the nursing cows (restricted suckling group; n=27).

In light of previous studies [3, 8], it was speculated that cyclic ovarian activity and the normal estrous cycle were resumed and uterine involution was completed at 40 days postpartum in Japanese Black cows. Accordingly, we created, and implemented the protocol for superstimulatory treatment and embryo recovery for suckling cows at 40 days postpartum. Furthermore, we speculated that the ideal delivery interval (one calving per year) could be maintained by applying this protocol in superovulation and flushing during suckling period.

For the superovulatory response, the numbers of estimated CLs, remaining follicles, recovered ovaries and embryos, transferable embryos, and freezable embryos were recorded. The numbers of estimated CLs and remaining follicles were counted on uterine flushing by ultrasonography using an echo camera (SSD-900; Aloka, Tokyo, Japan).

After uterine flushing, the cows were reused for reproduction. They received artificial insemination (AI) based on the observation of estrous behavior and the a.m.–p.m. rule. Ultrasonography was performed 30–40 days after AI to determine the pregnancy status of the cows. The intervals to first estrus and conception after flushing and the number of days open were determined to assess the fertility after flushing.

Body weight and average daily gain (ADG) were used as indices of nutritional status. The body weights of the cows and calves were measured twice monthly following measurement at pre- and postpartum (cows) and at parturition (calves). The body weights of the cows and calves at 30, 60, and 90 days postpartum were estimated from the measured values. ADG was calculated for each month.

To determine the status of the ovaries and uterus before initiating the superstimulatory treatment, the ovaries and uterus of each cow were observed by ultrasonography at 40 days postpartum. Cows with ovaries including a CL at 40 days postpartum were defined as having recovered estrus before initiating the superstimulatory treatment. The uterine horn with a greater diameter compared with that of the contralateral horn was defined as the gravid horn at 40 days postpartum. The difference in the diameter between both uterine horns and the existence of an echogenic line in the uterine lumen were used as indices of uterine involution.

Superstimulatory treatment and embryo recovery: All cows received a controlled internal drug releasing device (CIDR; Eazi-Breed; Livestock Improvement Association of Japan, Tokyo, Japan) at 40 days postpartum (day 0) and were administered injections of 2 mg of estradiol benzoate (EB; Kawasaki Seiyaku K.K., Kawasaki, Japan). All cows were subsequently superstimulated with a total dose of 20 Armour units of follicle stimulating hormone (FSH; Antrin 40; Kawasaki Seiyaku K.K.) twice daily with gradually decreasing doses from days 5 to 7. CIDR was withdrawn, and 0.75 mg of cloprostenol (PGF2α; Estrumate; Nagase Medicals Co., Ltd., Itami, Japan) was injected on day 7. AI was performed twice on the basis of a daily observation of estrus behavior. Embryos were nonsurgically recovered 7 or 8 days after estrus by flushing with lactated Ringer’s solution (Haruzen-V injection; Nippon Zenyaku Kogyo Co., Ltd., Koriyama, Japan) supplemented with 1% calf serum (Invitrogen Japan K.K., Tokyo, Japan). The developmental stage and quality of the recovered embryos were evaluated.
according to the Manual of International Embryo Transfer Society [20]. Transferable embryos included morulae and blastocysts rated as quality 1 (excellent and good) or 2 (fair), and freezable embryos included morulae and blastocysts rated as quality 1. The ovaries were examined by ultrasonography, and the numbers of CL and remaining follicles (RF) were counted. After uterine flushing, the cows were injected with 0.75 mg of PGF2α, and 50 ml of 2% povidone-iodine (Meiji Seika, Tokyo, Japan) were injected into the uterus. The superstimulatory treatment protocol used in the present study is shown in Fig. 1.

Statistical analysis: All quantitative data were analyzed using a one-way analysis of variance (ANOVA) with R software (R Foundation for Statistical Computing, Vienna, Austria), and the ratio of pregnant cows was analyzed using Fisher’s exact test. \( P<0.05 \) was considered significant.

RESULTS

Changes in the body weights of the cows and calves are shown in Fig. 2. The body weight of the cows in both groups decreased by approximately 40 kg for calving. No significant differences were observed in the pre- and postparturition body weight of the cows between the 2 groups; however, the decrease in the body weight of the cows caused by parturition was the same in both the groups. The postpartum body weight of the cows in the \( \text{ad libitum} \) suckling group showed a downward trend, whereas that in the restricted suckling group tended to increase slightly.
The group showed a slightly upward trend. The body weights of the cows at 30, 60, and 90 days postpartum in the *ad libitum* suckling group were significantly lower than those in the restricted suckling group (*P*<0.05). Furthermore, the ADGs of the cows in the *ad libitum* suckling group during the first and second months postpartum were significantly lower than those of the cows in the restricted suckling group (Table 1; *P*<0.05). No significant differences were observed in the body weight of calves in between the 2 groups at birth and in the postpartum period (Fig. 2). However, the ADG of calves at 3 months postpartum was significantly lower in the *ad libitum* suckling group than in the restricted suckling group (Table 2; *P*<0.05).

The status of the ovaries and uterus at 40 days postpartum is shown in Table 3. The diameter of each uterine horn was approximately 20–30 mm at 40 days postpartum, and ultrasonographic measurements did not reveal significant differences between the uterine diameters of the gravid and non-gravid uterine horns of the cows in either group. Uterine involution was considered complete at 40 days postpartum in most cows. In total, 20 of the 27 cows in the restricted suckling group, and 13 of the 20 cows in the *ad libitum* suckling group had CL at 40 days postpartum. More than 2/3rds of the cows in both groups were considered to have satisfactorily resumed estrous cycling at 40 days postpartum.

The superovulatory responses are shown in Table 4. No significant differences were observed between the groups in terms of the numbers of CLs, RFs, recovered ova or embryos, and transferable and freezable embryos. Furthermore, the status of the postpartum recurrence of estrus, which defined the ovarian status at 40 days postpartum, did not significantly affect the superovulatory responses (Table 5).

### Table 1. Average daily gain (ADG) of cows in the *ad libitum* and restricted suckling groups

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>ADG in 0–30 days postpartum (kg)</th>
<th>ADG in 30–60 days postpartum (kg)</th>
<th>ADG in 60–90 days postpartum (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>20</td>
<td>36.3 ± 16.9</td>
<td>−0.2 ± 0.6</td>
<td>0.1 ± 0.4</td>
</tr>
<tr>
<td>Restricted</td>
<td>27</td>
<td>39.4 ± 21.6</td>
<td>0.4 ± 0.6</td>
<td>0.2 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. Values with different superscripts (a and b) within the same column are significantly different (*P*<0.05).

### Table 2. Average daily gain (ADG) of calves in the *ad libitum* and restricted suckling groups

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>ADG in 0–30 days postpartum (kg)</th>
<th>ADG in 30–60 days postpartum (kg)</th>
<th>ADG in 60–90 days postpartum (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>20</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>Restricted</td>
<td>27</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. Values with different superscripts (a and b) within the same column are significantly different (*P*<0.05).

### Table 3. The status of the ovaries and uterus on 40 days postpartum in the *ad libitum* and restricted suckling groups

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>No. of cows having CL in ovary (%)</th>
<th>Diameter of gravid uterine horn (mm)</th>
<th>Diameter of non-gravid uterine horn (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>20</td>
<td>13 (65.0)</td>
<td>24.6 ± 2.8</td>
<td>23.3 ± 2.6</td>
</tr>
<tr>
<td>Restricted</td>
<td>27</td>
<td>20 (74.1)</td>
<td>23.5 ± 2.5</td>
<td>22.4 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. No significant differences were observed between the 2 groups. The uterine horn having a wider diameter was defined as the gravid horn.

### Table 4. Superovulatory responses in the *ad libitum* and restricted suckling groups

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>No. of estimated CLs</th>
<th>No. of remaining follicles</th>
<th>No. of recovered ova &amp; embryos</th>
<th>No. of transferable embryos</th>
<th>No. of freezable embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>20</td>
<td>20.3 ± 10.0</td>
<td>4.9 ± 3.6</td>
<td>10.1 ± 7.2</td>
<td>6.7 ± 5.4</td>
<td>5.5 ± 4.9</td>
</tr>
<tr>
<td>Restricted</td>
<td>27</td>
<td>17.6 ± 9.2</td>
<td>5.4 ± 6.2</td>
<td>11.6 ± 8.9</td>
<td>7.9 ± 7.0</td>
<td>6.2 ± 7.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. No significant differences were observed between the 2 groups.

### Table 5. Superovulatory responses in the status of the postpartum recurrence of estrus

<table>
<thead>
<tr>
<th>The status of the postpartum recurrence of estrus</th>
<th>n</th>
<th>No. of estimated CLs</th>
<th>No. of remaining follicles</th>
<th>No. of recovered ova &amp; embryos</th>
<th>No. of transferable embryos</th>
<th>No. of freezable embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence</td>
<td>33</td>
<td>17.6 ± 8.2</td>
<td>5.5 ± 5.9</td>
<td>11.3 ± 7.8</td>
<td>8.3 ± 6.3</td>
<td>6.6 ± 4.9</td>
</tr>
<tr>
<td>Non-recurrence</td>
<td>14</td>
<td>21.1 ± 12.1</td>
<td>4.2 ± 3.1</td>
<td>10.2 ± 9.3</td>
<td>5.3 ± 6.0</td>
<td>6.4 ± 5.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. No significant differences were observed between the 2 groups.
The suckling stimulus is known to suppress postpartum ovarian activities and prolong the interval between calving and the first postpartum ovulation [2, 7, 10, 13, 14]. Therefore, restricted suckling promotes postpartum ovarian activity [13, 14, 21, 25, 26, 29]. Recently, the mechanism by which the suckling stimulus suppresses gonadotropin releasing hormone (GnRH) secretion has been clarified. The neuropeptide kisspeptin, which is produced by the kiss-1 gene, plays an important role in the regulation of GnRH secretion [24]. The administration of kisspeptin-10, a shorter variant of kisspeptin that retains the complete biological properties of kisspeptin, stimulates GnRH secretion in cows [4]. Yamaeda et al. [30] demonstrated that the gene expression of kisspeptin in the arcuate nucleus–median eminence region in lactating rats is lower than that in nonlactating rats. They concluded that suckling stimulation inhibits the expression of kisspeptin and kiss-1 in the hypothalamus, and this inhibition leads to the suppression of follicular development and ovulation through the inhibition of GnRH and LH secretion. It is conceivable that a similar mechanism operates in the anovulation of the dominant follicle in postpartum suckling cows. Based on previous findings, suckling appears to affect embryo recovery during the suckling period, and this effect can be prevented by restricted suckling. However, no significant differences were observed in the superovulatory responses between the 2 groups in the present study. The effect of suckling did not appear to be sufficient to influence the superovulatory response. The second ovulation with the estrus and a normal length luteal phase usually occurs approximately 40 days postpartum in suckling beef cows [3]. Indeed, the cows in both groups had an equal ovarian status at 40 days postpartum and 2-thirds of the cows in both groups had resumed estrous cycling at that point in the present study. It is speculated that the equal ovarian status at time of initiating the superstimulatory treatment is associated with an equal superovulatory response of the cows in both groups.

In mice, blood estrogen regulates kiss-1 expression [27]. Estrogen from the mature follicle induces recruitment of estrogen receptor α and histone acetylation in the kiss-1 promoter region of the anteroventral periventricular nucleus (AVPV). Consequently, estrogen enhances chromatin loop formation of kiss-1 promoter and kiss-1 gene enhancer, resulting in an increase in AVPV-specific kiss-1 gene expression. The increase of kiss-1 gene expression in the AVPV that induced by estrogen may be closely associated with the induction of GnRH and LH surges and ovulation of the mature follicles. Exogenous injections of FSH for superstimulatory treatment and cloprostenol injections for luteolysis treatment increase the concentration of estrogen in the blood following the LH surge [11]. In the present study, porcine FSH and cloprostenol, when used for superstimulatory treatments, may have strongly increased the estrogen concentration in the blood; this high concentration may affect the pituitary gland via the feedback mechanism. Consequently, the feedback effect of estrogen on the pituitary gland leads to a surge in LH throughout kisspeptin and eventually ovulation of the superstimulated follicles. Therefore, the influence of suckling stimulus on LH and GnRH levels appears to be weak. It may be necessary to measure the concentration of LH and kisspeptin in further studies.

In the present study, the body weight of the cows in the ad libitum suckling group was higher than that in the restricted suckling group from 30 to 90 days postpartum; however, the cows in both the groups were fed the same nutrient requirements for maintenance of body weight and milk production. The cows in the ad libitum suckling group were believed to be incapable of absorbing nutritional requirements as efficiently as those in the restricted suckling group. This decrease in the body weight of the cows in the ad libitum suckling group appears to compensate for the deficiency in the energy expenditure required for milk production. Nolan et al. [17] investigated the results of ovum pick up and flushing following superstimulatory treatment using FSH in beef heifers with high and low levels of energy intake. They concluded that low energy intake, both before and during superstimulatory treatment, results in an increased number of follicles and improved embryo quality, thereby resulting in a higher yield of transferable embryos. High concentration intake has a negative effect on embryo yield and quality following superstimulatory treatment. In contrast, Gong et

<table>
<thead>
<tr>
<th>n</th>
<th>Interval to 1st ovulation (days)</th>
<th>Interval to conception (days)</th>
<th>Days open (days)</th>
<th>No. of pregnant cows after flushing</th>
<th>No. of pregnant cows within 80 days postpartum (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>20</td>
<td>27.9 ± 24.2a</td>
<td>44.2 ± 30.1</td>
<td>101.0 ± 30.2</td>
<td>20</td>
</tr>
<tr>
<td>Restricted</td>
<td>27</td>
<td>8.9 ± 5.7b</td>
<td>27.8 ± 28.2</td>
<td>84.3 ± 28.2</td>
<td>27</td>
</tr>
</tbody>
</table>

Values are means ± SD. Values with different superscripts (a and b) within the same column are significantly different (P<0.05).
al. [5] reported that a short-term increase in dietary intake can enhance the recruitment of ovarian follicles in heifers. Additional feeding accelerates follicle development, increases fertility, and decreases early embryonic death [2, 12]. However, no significant difference in terms of the superovulatory response was observed between the 2 groups in this study, and the results appear to be consistent with those of previous studies. Suitable feeding without an excessive decrease in body weight may be important for promoting the superovulatory response in the postpartum suckling cows.

Completion of postpartum uterine involution was indicated by the equivalent diameters of both uterine horns, and the disappearance of the echogenic line in the uterine cavity, both of which can be determined by ultrasonography [8]. The uterus of postpartum Japanese Black cows usually involutes until 40 days postpartum [8]. In the present study, super-stimulatory treatment was initiated at 40 days postpartum. Uterine involution appeared to be completed by that time, as indicated by the equivalent diameters of the left and right uterine horns in both groups and the disappearance of the echogenic line in the uterine cavity, as determined by ultrasonography. Based on these findings, the difference between the groups, with regard to the effect of uterine involution on the superovulatory response, appears to be insignificant in the present study.

Interestingly, fertility after flushing was better in the restricted suckling group than in the ad libitum group, although the results of flushing were similar in both groups. Fertility after flushing in postpartum suckling cows appears to be strongly affected by nutritional status. The cyclic ovarian activities are suppressed during the early postpartum period in suckling beef cows, and the suppression is exacerbated by dietary restriction and poor physical condition [29]. The interval to first postpartum ovulation is prolonged in the suckling beef cows with poor physical condition [3]. In the present study, the postpartum body weight of the cows in the restricted suckling group tended to increase slightly, whereas the body weight of the cows in the ad libitum suckling group tended to decrease until 60 days postpartum. Furthermore, the body weights of the cows in the ad libitum suckling group at 30–90 days postpartum were significantly lower than those in the restricted suckling group. From these results, providing additional feed for milk production appears suitable for the restricted suckling cows; the postpartum nutritional status of these cows remained at a satisfactory level until 90 days postpartum. In contrast, providing additional feed for milk production is not sufficient for the ad libitum suckling cows to remain at a constant body weight, because the nutrients that these cows receive are used for milk production at the expense of the restoration of body weight. Consequently, the fertility of the ad libitum suckling group after flushing may worsen compared with that of the restricted suckling group.

The body weight of calves from birth to 90 days postpartum was similar in both groups, because the age and parity of the cows were similar. The ADG of Japanese Black calves is affected by the use of dry feed as a calf starter at 30 days postpartum [22]. Calves in the restricted suckling group appeared to become accustomed to dry feed sooner than those in the ad libitum suckling group, with subsequent differences reflected between the 2 groups in terms of their body weight and ADG. From the viewpoint of calf growth, restricted suckling appears to be advantageous compared with ad libitum suckling.

In conclusion, twice daily restricted suckling improves reproductive performance after flushing within 60 days postpartum in suckling Japanese Black cows. No significant differences were observed in the results of flushing between the ad libitum and restricted suckling groups. Furthermore, the growth of calves born to cows in the restricted suckling group was similar to that of calves born to cows in the ad libitum group. In the restricted suckling group, approximately 48% of cows conceived within 80 days postpartum. Thus, restricted suckling may be effective in advancing the next reproductive cycle, when calving and flushing occur within 1 year in postpartum Japanese Black cows.

REFERENCES

ling stimulation on uterine involution in beef cows. Jpn. J. Anim. Reprod. 35: 45–49. [CrossRef]