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Possible causes and treatment strategies for the estrus and ovulation disorders in dairy cows

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Abstract. The reproductive performance of dairy cows has declined, along with an increase in their milk yield. First-service conception rates in lactating dairy cows are often lower than 50%. The precise detection of estrus is an important factor in the reproductive management of dairy cows for successful fertilization and pregnancy. However, estrus expression has been decreasing in modern dairy cows, affecting the detection rate of estrus. In addition to estrus, a high incidence of ovulation disorders affects the fertility of dairy cows. To address these problems, it is necessary to understand the changes in the endocrine functions that underlie estrus and ovulation disorders, and to develop effective treatment strategies. Recent studies have revealed that neurokinin B and neurokinin 3 receptor signaling play important roles in the regulation of the secretion of gonadotropin-releasing and luteinizing hormones, suggesting a potential clinical avenue for the stimulation of gonadal function. In this review, I have discussed the problems in estrus and ovulation disorders in modern dairy cows as well as the possible applications of neurokinin 3 receptor agonists in the treatment of these disorders.

Key words: Dairy cows, Estrus, Neurokin-3 receptor agonist, Ovulation, Stress

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Introduction

The milk productivity of dairy cows has increased because of genetic improvements. The milk yield per year per cow in Japan increased by 100 kg per year during the 20 years from 1995 to 2005, reaching 9,000 kg per year. Remarkably, high-yielding dairy cows have almost double the milk yield (> 10,000 kg per year) compared to those in the 1950s. In contrast, the reproductive performance of dairy cows has decreased worldwide [1–3]. First-service conception rates in US dairy herds decreased from approximately 65% in 1951 to 40% in 1996 [4]. Similarly, the average first-service conception rate of the Holstein dairy cows in Japan is often lower than 50%, according to a 2009 study [5]. Therefore, new strategies are required to overcome the low fertility of modern dairy cows.

The precise detection of estrus is an important factor in the reproductive management of dairy cows for successful fertilization and pregnancy. Artificial insemination (AI), in which cows are subjected to AI from mid-estrus to the end of standing estrus to maximize conception rates, has been widely practiced [6]. Estrus is generally detected by visual observation. The detection rate of standing estrus by visual observation twice a day was reported to be 89% in a 1968 study [7]. The duration of estrus was 17.8 h for dairy cows and 15.3 h for dairy heifers according to a study in 1948 [6]. However, since the 2000s, the percentage of animals expressing standing estrus has declined to less than 50%, with a duration of less than 10 h [8–10]. In addition to estrus, a high incidence of ovulation disorders (delayed ovulation, anovulation, and cyst formation) in modern dairy cows also affects the reproductive performance of dairy herds. To address these problems, it is necessary to understand the changes in endocrine function that underlie the estrus and ovulation disorders, and to develop effective treatment strategies.

Lactating dairy cows require greater nutrients for milk production than heifers and non-lactating dairy cows. Increased feed intake for lactation results in an increase in blood flow through the gastrointestinal organs and liver. The liver is a major site for steroid hormone metabolism [11, 12]. Increased steroid metabolism leads to a decrease in circulating concentrations of steroid hormones [11, 13, 14], which can negatively affect the fertility of lactating dairy cows. In addition, if cows cannot consume dietary energy to match their requirements for milk production, a negative energy balance status affects the normal development of follicles and corpus luteum and increases the risk of ovarian dysfunction by altering the secretion of gonadotrophin releasing hormone (GnRH) and other metabolic hormones, such as the insulin-like growth factor-1 [3, 15].

Kisspeptin plays a crucial role in the central regulation of GnRH secretion. Considerable evidence supports the hypothesis that a group of neurons in the arcuate nucleus contains kisspeptin, neurokinin B (NKB), and dynorphin (termed as KNDy neurons [16]) for the generation of GnRH pulses [17–19]. Loss-of-function mutations in *Tac3* and *Tacr3*, which encode NKB and its receptor, neurokinin3 receptor (NK3R), respectively, have been identified in patients with hypogonadotropic hypogonadism [20]. It was previously reported that administration of NKB stimulated the pulsatile release of LH in several animal models [18, 21, 22]. For example, intracerebroventricular administration of NKB induces an LH pulse and increases the expression of c-Fos, an index of neural activity, in ovine KNDy neurons [21]. In another study, intravenous administration of senktide, a selective NK3R agonist, increased GnRH pulse generator activity, as shown using the multiple-unit activity recording technique and LH secretion in goats [23]. Although more studies are needed to elucidate the mechanism of NKB action on gonadal function, it has been proposed that NKB or its analogs can be clinically applied

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Table 1. Comparison of the estradiol-17β (E_2) and leutinizing hormone (LH) profiles during the period from luteolysis to ovulation in lactating and non-lactating dairy cows

Item	Lactating (n = 5)	Non-lactating (n = 5)
Peak E_2 concentration (pg/ml)	13.3 ± 3.1	12.5 ± 3.1
Peak LH concentration (ng/ml)	11.7 ± 3.7	14.2 ± 2.8
Days from initiation of luteolysis to E_2 peak	3.2 ± 0.5	2.9 ± 0.8
Days from initiation of luteolysis to LH peak	3.5 ± 0.5	3.1 ± 0.7
Hours from E_2 peak to ovulation	34.2 ± 4.5	30.6 ± 3.9
Hours from LH peak to ovulation	27	27

Values are represented as the mean ± standard deviation (SD). E_2 peak: maximal E_2 concentration before ovulation. LH peak: maximal LH surge concentration. Modified from Endo *et al*. [27].

for the treatment of estrus and ovulation disorders by stimulating follicular growth and ovulation.

In this review, I will discuss the 1) problems with estrus detection and ovulation disorders in modern dairy cows, 2) the effects of stress on estrus and ovulation, and 3) strategies for the treatment of estrus and ovulation disorders, particularly the use of neurokinin 3 receptor agonists for clinical treatment.

Problems with Estrus Detection and Ovulation Disorders in Modern Dairy Cows

The high incidence of ovarian disturbances during the early postpartum period in modern dairy cows is often accompanied by nutritional and health problems [24]. Even when lactating cows have normal estrus cycles, their conception rate after breeding is markedly lower than that of heifers [1]. Previous studies have reported that plasma E_2 concentration around estrus is correlated with the intensity of estrus behavior and duration of estrus [25, 26]. To determine the characteristics of ovarian and endocrine profiles during the estrus cycle and periovulatory period in lactating dairy cows, the development of follicles and corpus luteum, plasma concentrations of estradiol-17β (E_2), progesterone (P_4), and LH, and timing of ovulation were compared with those of non-lactating dairy cows [27, 28]. In estrus cycles with two follicular waves, the maximal diameter of the ovulatory follicle is greater in lactating cows than in non-lactating cows [28]. An increase in the size of ovulatory follicles in lactating dairy cows has been reported previously [29, 30]. However, the profiles of E_2, P_4, and LH in plasma collected at 3-h intervals during the period from natural luteolysis to ovulation did not differ significantly between the lactating and non-lactating dairy cows [27] (Table 1). In this study, the lactating cows were in their mid-lactation period (> 80 days postpartum) and had a good nutritional status [27]. It is likely that lactation itself did not disturb the periovulatory endocrine events and that lactating cows could maintain normal estrus cyclicity.

Anestrus is a major component of postpartum infertility in domestic animals, including dairy cows, extending the calving interval and resulting in significant economic losses for farmers [31]. The condition of anestrus is characterized by a lack of ovarian cycles or gonadal activity, with repeated turnover of follicles without ovulation [32]. The existing follicles are incapable of producing sufficient E_2 to induce estrus owing to the decreased pulsatile secretion of GnRH and LH [33]. The causes of the poor expression of estrus and estrus signs in modern dairy cows have been investigated in relation to endocrine factors, herd management, and estrus detection methods. Previous studies on endocrine events during estrus and ovulation have revealed that alterations in the concentrations of ovarian steroids and gonadotropins may influence the expression of estrus and estrus signs. For example, Lyimo *et al*. [25] found a high correlation between E_2 concentrations and visual estrus symptoms. Another study suggested that high milk production decreases the duration of estrus, probably due to decreased circulating concentrations of E_2 [26]. Suprabasal progesterone concentrations during the periestrus period, which may be caused by incomplete luteolysis and acute stress, may inhibit the expression of estrus behavior [34] and lead to delayed ovulation [35]. Some of these alterations have been reported to be associated with high milk production in modern dairy cows [34]. As management factors, the expression of estrus behavior can be influenced by housing and flooring type. Concrete is the most commonly used material for confined dairy herds. However, cows prefer to walk and stand on rubber rather than concrete flooring [36]. Studies on the expression of estrus behavior showed that cows express more mounting activity in barns than in free-stall flooring or cubicle-housing systems [37], and cows showed less mounting and standing activity on concrete floors than on dirt floors [38, 39]. The hardness and slipperiness of the concrete floor can contribute to the development of foot lesions and lameness [40]. Lame cows have longer lying times and spend less time standing, walking, and expressing estrus behavior [41]. Another study reported that the frequency of standing to be mounted was lower in lame cows than in normal cows, although the duration of estrus was similar between them [42].

Effects of Stress on Estrus and the Ovulation Process

Stress can suppress the immune and reproductive functions via hypothalamic-pituitary-adrenal (HPA) axis activation, contributing to the development of clinical disorders and reduced fertility in dairy cows [43]. Stress is thus considered a possible cause of estrus and ovulation disorders in dairy cows; however, this is difficult to evaluate.

Cortisol concentration in hair has been used as an index of chronic stress in several species, including humans, wildlife, and domestic animals [44–46]. I previously examined the effect of repeated adrenocorticotropic hormone (ACTH) administration on the expression of estrus, follicular growth, and ovulation in goats with normal estrus cyclicity and measured the hair cortisol concentrations at 0, 1, and 2 months after ACTH administration to confirm the accuracy with which the cortisol concentration in hair reflects the circulating cortisol concentrations [47]. In Experiment 1, goats were administered ACTH (0.625 µg/kg body weight, n = 6) or saline (n = 6) intramuscularly once a day for seven days on days 11–17 of the estrus cycle (day 0 was the day of ovulation). In Experiment 2, goats were administered the same dose twice daily on days 11–24 of the estrus cycle. In neither experiment, ACTH administration affected the estrus expression or ovulation timing. However, the number and maximal diameter of ovulatory follicles increased after administration of ACTH twice a day for two weeks, that is, in Experiment 2. In previous studies in cattle [48, 49] and goats [50], experimentally induced stress by administration of high doses of ACTH caused the formation of follicular cysts. Indeed, the dose of ACTH used to induce follicular cysts (2.5 µg/kg body weight) [48] was much higher than that in the above study (0.625 µg/kg body weight) [47]. Exposure to more intensive stress, rather than the duration of exposure, can cause the formation of follicular cysts.

To determine the effects of chronic stress on the health and reproductive functions of dairy cows on commercial dairy farms, hair cortisol concentrations during the pre- and postpartum periods were investigated in association with the animals' body condition, hock health status, and reproductive parameters (e.g., timing of first AI and days open) [51]. In this experiment, 33 Holstein dairy cows that were delivered in July or August 2016 at 13 commercial dairy farms were examined, and hair was collected at –19.2 ± 11.4, 44.8 ± 11.9, 103.0 ± 9.9, and 168.0 ± 9.7 days postpartum. Body condition scores were negatively correlated with hair cortisol concentrations (r = –0.255), and hock health scores (3-point scale, 1 = healthy to 3 = evident swelling and/or severe injury) were positively correlated with hair cortisol concentrations ($r = 0.236$, $P < 0.05$). Furthermore, subfertile cows had increased hair cortisol concentrations during their early to mid-lactation periods compared with the dry period, while fertile cows did not show any difference in hair cortisol concentrations among the sampling times. These results suggest that cows with health and nutritional problems experience greater chronic stress, which may impair their fertility. It has been observed that lame cows spend more time lying and less time standing, walking, and expressing estrus behavior [41]. In addition, administration of ACTH or cortisol to mimic chronic stress inhibited the preovulatory LH surge, estrus, and ovulation [52], and induced persistent cystic follicles in cattle [48, 49].

Strategies for the Treatment of Estrus and Ovulation Disorders

Estrus detection

Accurate and efficient detection of estrus is important for determining the appropriate timing of AI in a herd of cows and to detect cows with estrus and ovulation disorders. Visual observation is the most common method for detecting estrus. However, visual methods rely on observations of cows standing to be mounted, and the detection of standing estrus is becoming more difficult in modern dairy cows because of their weak expression of estrus behavior. Therefore, alternative or supplemental methods are necessary. Cows become more active during estrus than at any other time during the estrus cycle. Based on this observation, devices that monitor the daily physical activity of cows, such as pedometer- and accelerometer-based systems, have been developed for the detection of estrus [53, 54]. While the efficacy of these estrus detection devices has been confirmed in large herds, it remains unclear whether they can be applied to smaller herds, because the number of cows in estrus at the same time can influence the duration and intensity of estrus [55]. The efficacy of estrus detection using an accelerometer attached to the neck (Hatsuhatsu; P.A. Technology, Gunma, Japan) among small herds (10 to 15 animals) of lactating dairy cows housed in free-stall barns was investigated [56]. A clear increase in the activity of cows at estrus was observed, with a peak value 9.2 ± 3.3 (5.2–11.0) times higher than the mean value recorded during the luteal phase. In addition, the accelerometer system could detect estrus correctly for 85.2% of the estrus cycles examined, which was higher than that of visual observation (51.6%). Hojo *et al*. [57] also reported that activity monitoring using a pedometer-based system can be effectively used for the detection of silent estrus without standing events. Michaelis *et al*. [58] reported that estrus detection rates were higher when combining visual observation and automated activity monitoring than when either method was used alone. The ability to detect estrus mainly depends on the experience of the farmer and the time invested in estrus detection [59]. Using devices to automatically detect estrus signs can improve estrus detection rates.

Clinical applications of NK3R agonists

Senktide is a highly selective agonist of NK3R and has much higher metabolic stability than native tachykinins [60]. Although the precise mechanism underlying the action of senktide is not fully understood, its stimulatory effect on pulsatile LH/GnRH secretion [22, 61] is an attractive feature for its clinical application in several reproductive disorders associated with insufficient GnRH/LH secretion. As a new clinical approach for estrus and ovulation disorders, a pilot study was performed to examine the effect of intravenous treatment with senktide on LH secretion and follicular development in female goats clinically diagnosed with anestrus [62]. Six anestrus goats were administered 200 nmol senktide at 4-h intervals for 24 h. Almost all goats (5/6) showed a pulsatile increase in LH secretion after each injection of senktide, while the remaining goats showed a surge-like increase in LH secretion immediately after the first administration. Thereafter, estrus and ovulation were observed in 2/6 and 5/6 goats, respectively. This suggests that stimulation of LH release by senktide can enhance the development and production of E_2 from the follicles, and this increased E_2 concentration further induces estrus and endogenous LH surge for subsequent ovulation in some cases. Due to the limitations of clinical trials, further studies were performed to determine the effect of senktide on LH secretion, follicular development, estrus, and ovulation in feed-restricted goats [63]. Feed restriction was applied to mimic the nutritional status of anestrus patients. In this study, feed was restricted to 50% maintenance for 15 days, which reduced the number and diameter of follicles compared with goats on 100% maintenance. Under these experimental conditions, feed restriction did not affect estrus expression. However, intravenous administration of 200 IU senktide at 4-h intervals for 24 h to the feed-restricted goats caused a rapid increase in LH after each injection, followed by an increase in plasma E_2 concentration. These results suggest that senktide can be applied for the treatment of anestrus and ovulation failure induced by nutritional deficiency and stress in dairy cows.

Considering the clinical applications of NK3R agonists, less frequent dosing would be more convenient for veterinarians and farmers. Another experiment was performed to compare the effects of continuous administration versus a single injection of senktide on LH secretion, estrus, follicular development, and ovulation in follicular-phase goats [64]. While a single injection of senktide caused a pulsatile increase in LH secretion, continuous intravenous administration caused a sustained increase (Fig. 1) that advanced the timing of ovulation in more than half of the cases [64]. However, 4 of the 6 goats receiving senktide infusion did not show estrus. It appears that the sustained increase in LH secretion acted as a trigger for ovulation. The ovulatory LH surge induces a follicular/luteal phase shift in hormone production, whereby a decline in enzyme levels for estradiol production occurs shortly after the LH surge [65]. The absence of estrus in senktide-treated goats may be

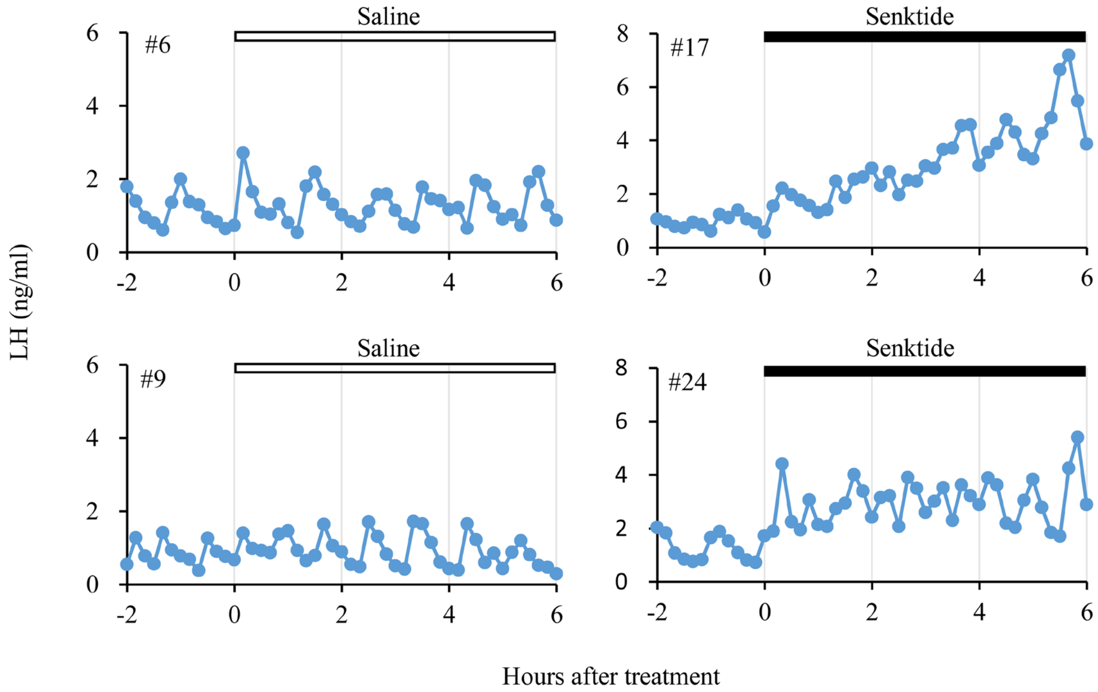

Fig. 1. Representative profiles of the luteinizing hormone (LH) concentrations in goats treated with the continuous infusion of senktide (right panels) or saline (left panels). Senktide was infused at a rate of 20 nmol/200 µl/min for 6 h (solid bar). Open bar indicates the infusion of saline. Modified from Endo *et al*. [64].

due to an earlier decline in estradiol concentration. A recent study on lactating cows [66] confirmed that the intravenous infusion of senktide elicited an increase in LH pulse frequency and shortened the interval from calving to first postpartum ovulation, although it was not mentioned whether estrus was observed. Further studies are warranted to optimize the administration protocol, including the drug dose, route of administration, and timing of treatment.

Conclusion

I found that lactating dairy cows developed larger ovulatory follicles than non-lactating cows, although the E_2, P_4, and LH profiles during the periovulatory period did not differ between the lactating and non-lactating cows. Cows with health and nutritional problems appeared to experience greater chronic stress, as reflected by the higher cortisol concentrations in their hair, which may impair their fertility. To improve the detection rate of estrus, automated estrus detection systems, such as accelerometers, can be used to augment visual observation by farmers. Furthermore, the administration of NKB to cycling goats stimulated LH secretion and enhanced the ovulatory process. Although further research is needed, the efficacy of this drug in stimulating LH secretion suggests that it has great potential for the treatment of estrus and ovulation disorders in dairy cows.

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