Effects of gonadotropin-releasing hormone administration or a controlled internal drug-releasing insert after timed artificial insemination on pregnancy rates of dairy cows

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This study investigated the effects of gonadotrophin-releasing hormone (GnRH) administration (Experiment 1) and a controlled internal drug-releasing (CIDR) insert (Experiment 2) after timed artificial insemination (TAI) on the pregnancy rates of dairy cows. In Experiment 1, 569 dairy cows that underwent TAI (day 0) following short-term synchronization with prostaglandin F₂α were randomly allocated into two groups: no further treatment (control, n = 307) or injection of 100 µg of gonadorelin on day 5 (GnRH, n = 262). In Experiment 2, 279 dairy cows that underwent TAI (day 0) following Ovsynch were randomly allocated into two groups: no further treatment (control, n = 140) or CIDR insert treatment from days 3.5 to 18 (CIDR, n = 139). The probability of pregnancy following TAI did not differ between the GnRH (34.4%) and control (31.6%, p > 0.05) groups. However, the probability of pregnancy following TAI was higher (odds ratio: 1.74, p < 0.05) in the CIDR group (51.1%) than in the control group (39.3%). Overall, CIDR insert treatment at days 3.5 to 18 increased pregnancy rates relative to non-treated controls, whereas a single GnRH administration on day 5 did not affect the pregnancy outcomes of dairy cows.

Keywords: dairy cow, post-insemination, pregnancy rate, progesterone concentration

Introduction

Increased milk production through improvement of genetics and nutritional management in herds is related to decreased reproductive performance [14]. Various biological (e.g., milk yield, energy balance, diseases, and other factors) and environmental factors may affect the reproductive performance of dairy cows [33]. Among them, progesterone insufficiency in the early luteal phase is associated with embryo mortality in cows [30]. The progesterone concentration post-insemination plays critical roles in stimulating production of a variety of endometrial secretions, embryo growth, and establishment and maintenance of pregnancy [5]. Similarly, progesterone inhibits luteolysis by decreasing sensitivity to oxytocin by binding to oxytocin receptors [6] and enhancing conceptus development, which stimulates secretion of interferon-τ [15].

Catabolism of steroids increases with increased feed intake in high-producing dairy cows and leads to inadequate progesterone concentrations during early embryonic development, resulting in reduced fertility [20]. In this regard, a number of studies were performed to increase the circulating progesterone concentration post-insemination, thereby increasing fertility in dairy cows [13,16,35]. An accessory corpus luteum (CL) is induced by treatment with gonadotrophin-releasing hormone (GnRH) or human chorionic gonadotrophin (hCG) at 5 or more days post-insemination [8,25,35]. Previous studies reported that treatment with GnRH or hCG increased the circulating progesterone concentration during the luteal phase [8,21,22]. Thus, some studies reported beneficial effects on pregnancy outcomes under limited conditions, i.e., for high-producing dairy cows during the warm season or in limited herds [13,21,28]; however, a number of studies reported no beneficial effects on pregnancy outcomes [22,31,32,37].

The circulating progesterone concentration post-insemination can also be increased through use of a controlled internal drug-releasing (CIDR) insert during the luteal phase. However, the timing and duration of such an insertion has been found to vary among studies [1,9,27,29]. Several studies demonstrated that a CIDR insert increased the circulating progesterone concentration [2,4,10]. However, many studies reported no...
improvement in pregnancy rates [2,12,18,34]. Moreover, only a few studies using a CIDR insert post-insemination comprising small numbers of experimental cows reported beneficial effects on pregnancy outcomes [9,16], indicating the need for more extended field trials.

Based on the characteristic functioning mechanisms, GnRH administration during the luteal phase results in ovulation, a dominant follicle and formation of an accessory CL, increasing the circulating progesterone concentration from 2 to 4 days after GnRH treatment [28,37]. In contrast, the circulating progesterone concentration may immediately (within 45 min) increase in response to a CIDR insert via the release of exogenous progesterone [19]. Therefore, the present study was conducted to compare the effects of a single GnRH administration at 5 days or CIDR insert treatment from 3.5 to 18 days after timed artificial insemination (TAI) on the pregnancy rates of dairy cows.

Materials and Methods

Animals
Experiments were carried out at 10 dairy farms located in Chungcheong Province, Korea. Each farm had 50 to 200 milking cows that were maintained in a loose housing system. The cows were fed a total mixed ration and milked twice daily. The mean milk yield was approximately 9,500 to 11,000 kilogram per year per cow.

A total of 569 Holstein dairy cows (176 primiparous and 393 multiparous; mean parity, 2.6 ± 1.1) in seven farms were enrolled in Experiment 1, while 279 dairy cows in three farms (88 primiparous and 191 multiparous; mean parity, 2.4 ± 1.3) were enrolled in Experiment 2. The voluntary waiting period from parturition to the first artificial insemination (AI) was 60 days in both experiments. All experiments were carried out with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University, Korea.

Experimental design

Experiment 1: A total of 569 dairy cows with a functional CL > 20 mm in diameter as confirmed by ultrasonography using a Tringa Linear Veterinary Ultrasound scanner fitted with a 5.0 MHz array transducer (Esaote, The Netherlands) received 500 μg of the prostaglandin F2α (PGF2α) analogue, cloprostenol (Estrumate; MSD Animal Health, Korea) and another injection of 1 mg of estradiol benzoate (SY Esrone; Samyang, Korea) 24 h after PGF2α treatment. Cows underwent TAI (day 0) 36 h after estradiol benzoate injection. Following TAI, cows were randomly allocated into two groups: no further treatment (control, n = 307) or 100 μg injection of the GnRH analogue, gonadorelin (Godorel; Uni-Biotech, Korea) on day 5 (GnRH, n = 262; Fig. 1).

Experiment 2: A total of 279 dairy cows received 100 μg of gonadorelin, and a second dose of 100 μg of gonadorelin 56 h after injection of cloprostenol. These cows then underwent TAI (day 0) 16 h after the second injection of gonadorelin. Following TAI, cows were randomly allocated into two groups: no further treatment (control, n = 140) or placement of a CIDR insert containing 1.9 g of progesterone (CIDR; InterAg, New Zealand) into the vagina from days 3.5 to 18 (n = 139, Fig. 1).

Ultrasonography and pregnancy diagnosis
In Experiment 1, a subset of cows from the GnRH (n = 44) and control (n = 39) groups underwent ultrasonographic examinations to assess CL numbers on days 5 and 13 (9 days after GnRH injection, Fig. 1). These examinations were performed to determine if an accessory CL formed following GnRH administration. Pregnancy was diagnosed in both experiments on day 35 using ultrasonography and trans-rectal palpation.

Measurement of progesterone levels in serum samples
In Experiment 2, blood samples of subsets of cows from the CIDR (n = 17) and control (n = 16) groups were collected via the tail vein on days 3.5, 5, and 13 to determine serum progesterone concentrations (Fig. 1). Samples were then centrifuged at 2,000 × g for 10 min at 4°C, after which the serum was harvested and frozen at −80°C until assayed. The serum progesterone level was determined using the Immulite 1000 Immunoassay System (DPC Cirrus, USA) according to the manufacturer’s guidelines. The intra- and inter-assay coefficients of variation were 6.4 and 8.0%, respectively.

Statistical analyses
For statistical analyses, cow parity was categorized into primiparous or multiparous. The calving season was categorized into spring (March to May), summer (June to August), autumn

Fig. 1. A diagram of the experimental design for the timed artificial insemination (TAI) regimens, and treatment with gonadotrophin-releasing hormone (GnRH) (Experiment 1) or a controlled internal drug-releasing (CIDR) insert (Experiment 2) post-insemination. PG, 500 μg of cloprostenol; EB, 1 mg of estradiol benzoate; Ultras, ultrasonography to examine corpus luteum(s); GnRH, 100 μg of gonadorelin; Preg Diag, pregnancy diagnosis using ultrasonography; CIDR, CIDR insert containing 1.9 g of progesterone.
Effects of GnRH or CIDR insert on pregnancy rates

Table 1. Odds ratio (OR) of variables included in the logistic regression model of the probability of a pregnancy following TAI in Experiment 1

| Variable level | Pregnancy rate, % (number of cows) | OR    | 95% CI          | p value |
|----------------|------------------------------------|-------|-----------------|---------|
| AI season      |                                    |       |                 |         |
| Spring         | 41.7 (53/127)                      | 1.00  | 0.60–1.71       | >0.05   |
| Summer         | 27.6 (48/174)                      | 0.51  | 0.30–0.85       | <0.01   |
| Autumn         | 30.8 (53/172)                      | 0.62  | 0.37–1.00       | <0.1    |
| Winter         | 34.4 (33/96)                       | 0.68  | 0.38–1.21       | <0.05   |
| Cow parity     |                                    |       |                 |         |
| Primiparous    | 39.2 (69/176)                      | 1.00  | 0.60–1.71       | >0.05   |
| Multiparous    | 30.0 (118/393)                     | 0.71  | 0.48–1.05       | <0.1    |

Results

Experiment 1

The percentage of cows with an accessory CL identified by ultrasonography on day 14 was higher in the GnRH group (68.2%, 30/44) than in the control group (0%, 0/39) (p < 0.01). Logistic analysis revealed that treatment group, farm, postpartum interval, and BCS did not affect the probability of pregnancy following TAI, and the pregnancy rate in the GnRH and control groups was 34.4% and 31.6%, respectively (p > 0.05). However, cow parity and AI season affected the probability of pregnancy following TAI (Table 1).

Experiment 2

Fig. 2 shows the serum progesterone concentrations on days 3.5, 5, and 13. Treatment group had no effect (p > 0.05), but sampling time and interaction between treatment group and sampling time had significant effects (p < 0.0001 and p < 0.05, respectively). The serum progesterone concentration was higher in the CIDR group (2.5 ± 0.2 ng/mL) than in the control group (1.6 ± 0.2 ng/mL) on day 5 (p < 0.005, Fig. 2). There was

Table 2. Odds ratio of variables included in the logistic regression model of the probability of a pregnancy following TAI in Experiment 2

| Variable level | Pregnancy rate, % (number of cows) | OR    | 95% CI          | p value |
|----------------|------------------------------------|-------|-----------------|---------|
| Treatment group|                                    |       |                 |         |
| Control        | 39.3 (55/140)                      | 1.00  | 0.60–1.71       | >0.05   |
| CIDR           | 51.1 (71/139)                      | 1.74  | 1.03–2.92       | <0.05   |
clarify the beneficial effects of GnRH administration or treatment with a CIDR insert after TAI on the pregnancy rates of dairy cows. Cows treated with a CIDR insert at days 3.5 to 18 had a higher probability of pregnancy after TAI following an increased circulating progesterone concentration during the early luteal phase than non-treated control cows (Experiment 2). However, GnRH treatment had no beneficial effects on pregnancy outcomes, although the percentage of cows with an accessory CL after TAI was higher among GnRH-treated cows than non-treated cows (Experiment 1).

Injection of GnRH on day 5 induced ovulation and formation of an accessory CL in 68.2% of cows in Experiment 1, which is slightly higher than the previously reported rate of 60% [29]. Other studies reported accessory CL induction rates of 93% and 100% in dairy heifers and cows injected with GnRH at 5 days post-insemination, in which GnRH administration increased the circulating progesterone concentration [8,24]. However, neither of these studies reported an increase in subsequent pregnancy rates. Moreover, several studies reported that GnRH injection at 4 to 5 days post-insemination had no beneficial effects on the pregnancy outcomes of cows [11,32,37]. Similarly, the present study revealed no beneficial effects of GnRH injection at 5 days post-insemination on pregnancy outcomes. However, a few studies reported beneficial effects of GnRH treatment on pregnancy outcomes in limited conditions. For example, Sterry et al. [27] showed that GnRH treatment at 5 days after TAI improved pregnancy rate per AI for noncycling, but not cycling dairy cows. Additionally, the beneficial effects of GnRH treatment post-insemination on fertility vary among studies. Therefore, further studies should be conducted to clarify the beneficial effects of GnRH administration post-insemination on pregnancy outcomes.

CIDR, as an exogenous supplement of progesterone, immediately (within 45 min) increases the circulating progesterone concentration in dairy cows [19]. Thus, various investigations of the effects of CIDR treatment post-insemination on subsequent fertility were performed [9,12,16]. In the present study, the serum progesterone concentration was higher in CIDR-treated cows than non-treated control cows on day 5, reaching 2.5 ng/mL (vs. 1.6 ng/mL in control cows); however, the concentrations at days 3.5 and 13 were similar between groups. These findings are similar to those of a previous study, in which dairy cows were treated with a CIDR insert from 3 to 20 days after TAI. In this previous study, the circulating progesterone concentrations were higher in cows with a CIDR insert than in those without one at 4 and 7 days post-insemination, whereas there was no difference in concentration between groups on other days [17]. Our results that the serum progesterone concentration was higher in CIDR-treated cows than non-treated control cows on day 5 are supported by those of other studies [2,4,10]. However, a previous study reported that use of a CIDR insert from 4 to 18 days post-insemination did not increase the circulating progesterone concentration, which was regarded as the result of increased metabolism of progesterone by the liver [1]. Importantly, our logistic regression analysis in Experiment 2 revealed that only the treatment group (CIDR treatment) affected the pregnancy rate following TAI, no other factors (farm, cow parity, AI season, postpartum interval, and BCS) had any effects. Our results are supported by those of a previous study [16] in which use of a CIDR insert from 5 to 19 days post-insemination increased the serum progesterone concentration and consequently the pregnancy rate. In addition, another study showed that use of a CIDR insert from 3.5 to 10 days post-insemination increased the circulating progesterone concentration and tended to increase the pregnancy rate [10]. However, a number of studies using CIDR insert treatment post-insemination, in which the timing and duration of CIDR insert use varied, reported no beneficial effects on pregnancy outcomes [18,27,34]. It is unclear why only a few studies have reported beneficial effects on pregnancy outcomes. However, it is postulated that initiating the use of a CIDR insert as early as 3.5 to 5 days post-insemination might be associated with improved pregnancy outcomes. Although the circulating progesterone concentration was higher in CIDR insert-treated cows than in non-treated cows on day 5, it was similar between the two groups on day 13, suggesting the importance of progesterone concentration during the early luteal phase to pregnancy outcomes. Stronge et al. [30] also demonstrated a positive relationship between milk progesterone concentration on days 5, 6, and 7 and embryo survival, implying that the critical window for progesterone supplementation is between the morula and hatching blastocyst stages. Moreover, the progesterone concentration during early and mid-diestrus is associated with changes in endometrial gland ducts [36], which likely increase the supply of nutrients to the developing conceptus and accommodate changes in subsequent placentation [26]. A previous study also reported a positive relationship between increased circulating progesterone concentration from days 3 to 6.5 and embryo survival rate in CIDR insert-treated heifers [2]. Green et al. [7] reported that production of interferon-τ at 16 days post-insemination was positively correlated with the progesterone concentration on days 4 and 5. Similarly, it was reported that progesterone administration from 5 to 9 days after AI enhanced both embryo development and secretion of anti-leuteolytic interferon-τ [15]. Dairy heifers, which have high
fertility, exhibit a steeper rise in the post-ovulation progesterone concentration than lactating dairy cows starting 4 days after insemination [23]. Taken together, these findings may help explain the importance of the timing of progesterone supplementation using a CIDR insert post-insemination [10].

In summary, treatment with a CIDR insert from days 3.5 to 18 increased pregnancy rates following an increase in the circulating progesterone concentration during the early luteal phase relative to non-treated control dairy cows. However, a single GnRH injection had no beneficial effects on pregnancy outcomes, although the percentage of cows with an accessory CL after TAI was higher among GnRH-treated cows than non-treated control cows. Thus, the beneficial effect of CIDR treatment on pregnancy outcomes in cows might be associated with the earlier increase in the circulating progesterone concentration during the luteal phase (metestrous stage).

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Conflict of Interest

There is no conflict of interest.

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