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Effect of GnRH on ovulatory response after luteolysis induced by two low doses of PGF2α in lactating dairy cows

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ABSTRACT

This study investigated the effect of gonadotropin-releasing hormone (GnRH) on ovulatory response after complete luteolysis induced by two low doses of prostaglandin (PG)F2α in lactating dairy cows. Cows (n = 18) ranging between 45 and 65 days in milk were recruited for synchronization by a modified Ovsynch-48 protocol (GnRH-7 days-375 μg PGF2α-1 day-250 μg of PGF2α-1 day-GnRH) over a total of 23 estrous cycles. Synchronized cows (n = 16) were randomly assigned to GnRH and Saline groups in stage 1 of the experiment after 9–10 days of ovulation in synchronization. On days 0 and 1 (day 0 = first PGF2α administration), cows were treated with 375 and 250 μg PGF2α, respectively. On day 2, cows in the GnRH and Saline groups were administered 250 μg GnRH or 2.5 mL of 0.9% saline, respectively. Serum progesterone (P4) levels were measured and changes in the corpus luteum (CL) were ultrasonically monitored daily from day 0–3 to assess complete luteolysis. Preovulatory follicle diameter and ovulatory response were evaluated by ultrasonography. In stage 2, cows were treated in a manner converse to that in stage 1. The synchronization rate was 69.6% (16/23). In stages 1 and 2, cows showed complete luteolysis with P4 concentration <1 ng/mL or remaining CL area <50%. Average ovulation time was 29.3 ± 0.5 h, which mostly occurred between 28 and 30 h after GnRH injection. However, all cows in the Saline group ovulated later than 36 h post-injection, with an average time of 52.7 ± 8.6 h. There was no difference in preovulatory follicle diameter between the two groups (16.8 ± 0.5 and 17.3 ± 0.5 mm for GnRH and saline groups, respectively). Although ovulation rate was not correlated with treatment, the rate within 48 h of GnRH injection (93.3%) tended to be higher compared with that in the Saline group (60.0%). Thus, GnRH administration increased ovulation rate following complete luteolysis induced by two low doses of PGF2α. These results indicate that this simple protocol for dairy cows is an effective alternative to timed artificial insemination programs in the field.

1. Introduction

Over the last decade, there has been an increasing interest in developing new synchronization protocols with timed artificial insemination (TAI) to improve reproductive efficiency in the dairy industry [1,2]. Presynchronization strategies such as G6G [i.e., pre-prostaglandin (PG)F2α-2 days-pre-gonadotropin-releasing hormone (GnRH)-6 days] and Double-Ovsynch were developed to increase conception rates [3,4]. It is widely acknowledged that GnRH and PGF2α play a critical role in the induction of ovulation. Under heat stress, their combined administration can even induce three successive and continuous follicular waves that enhance fertility [5].

GnRH-induced ovulation is typically determined by ultrasonography 7 days after GnRH injection in cows [4,6,7]; however, it is triggered by a surge of luteinizing hormone (LH) that causes the rupture of dominant follicles within 24–32 h after the GnRH surge [8]. It was previously reported that cows ovulated between 26 and 32 h after the second GnRH administration in the Ovsynch protocol, and that the rate of ovulation was highest at 28 h [1]. Another study found that ovulation mostly occurred between 28 and 30 h after GnRH administration [9]. Even in cows with high progesterone (P4) concentration that received 100 μg GnRH, the ovulatory response was only 75.0%. In the combined presynchronization/Breeding-Ovsynch protocol, the first GnRH pulse in Breeding-Ovsynch induced ovulation and the formation of an accessory corpus luteum (CL); however, the incidence of partial luteolysis in cows treated with a standard dose of PGF2α was concurrently increased

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[10]. The rate of complete luteolysis may be increased by administering a second dose of PGF2α in Breeding-Ovsynch [11,12]. Two consecutive low doses of PGF2α were shown to efficiently enhance luteolysis and increase ovulation rate up to 90.0% [13], whereas 50% of cows ovulated between 96 and 120 h after the first PGF2a administration.

Previous reports described the ovulation time based on one standard dose of PGF2α followed by GnRH treatment in cows, in which ovulation occurred during a concentrated period [1,9]; however, details of the ovulation process post-GnRH administration after two consecutive injections of PGF2α has yet to be described in TAI protocols. The objective of the present study was to evaluate the effect of GnRH on the induced ovulation time and rate after complete luteolysis following treatment with two low doses of PGF2α in lactating dairy cows as a useful addition to the standard TAI protocol. We hypothesized that GnRH treatment would advance ovulation time in cows and increase the ovulation rate as compared to those in non-treated animals.

2. Materials and methods

2.1. Animals and management

This study was conducted on a dairy farm comprising about 50 Holstein cattle at the National Chung Hsing University in subtropical Taiwan from October 2016 to March 2017. Cows were housed in groups in free-stall barns with a slatted floor and sand bed and fed as described in a previous study [13]. During the experimental period, temperature and humidity were recorded at 6:00, 12:00, 18:00, and 24:00 h and the thermal-humidity index (THI) was calculated.

2.2. Experimental design

A total of 18 cows ranging between 45 and 65 days in milk (DIM) with normal uterus involution were subjected to synchronization of the estrous cycle via a modified Ovsynch-48 protocol, and then assigned to the following experiment after successful synchronization (Fig. 1). The parity of the cows mostly ranged from 1 to 3, except for two cows that gave birth four and six times, respectively. For synchronization, cows received 250 μg GnRH [100 μg/mL Fertagyl/gonadorelin by intramuscular injection (i.m.); Intervet Deutschland, Unterschleiβheim, Germany]; 7 days later, they were treated with 375 μg PGF2α (250 μg/mL Estrumate/cloprostenol sodium, i. m.; Intervet Deutschland; SynPG). A second dose of PGF2α (250 μg) was then administered after 24 h, and another 250-μg dose of GnRH was administered 48 h after the first PGF2α administration (SynG2). If the cows were not synchronized with complete luteolysis and ovulation and still remained between 45 and 65 DIM, they were re-treated using the modified Ovsynch-48 protocol.

Between 9 and 10 days after ovulation of synchronization, cows were enrolled in stage 1 of the experiment, and randomly assigned to the GnRH and Saline groups by parity. The time of first PGF2α administration was defined as day 0 of stage 1. On days 0 and 1, cows were treated with 375 and 250 μg PGF2α, respectively. On day 2, cows in the GnRH and Saline groups were administered 250 μg GnRH or 2.5 mL of 0.9% saline, respectively. If ovulation occurred within 240 h, the cows were enrolled in stage 2 of the experiment; non-ovulated cows were not used further. Between 9 and 10 days after ovulation of stage 1, cows in stage 2 were treated in a manner converse to that in stage 1—i.e., cows treated with GnRH in stage 1 were administered saline in stage 2 and vice versa.

2.3. Measurement of CL area, follicle diameter, and ovulation

Transrectal B-mode ultrasonography of the ovaries was performed using a portable scanner equipped with a 7.5-MHz linear-array transducer (SonoSite Ultrasound System; SonoSite, Bothell, WA, USA) at SynPG, 24 h after the second pulse GnRH of synchronization (SynG2a24), and once daily on days 0 through 3 of stages 1 and 2 (Fig. 1). The longitudinal and transverse axes of the CL were measured at right angles with electronic calipers when the maximum CL area was observed in images [14–16]. The CL area was calculated using the formula for an oval area and the area on day 0 in each cow was defined as 100%, with the remaining CL area on days 1 through 3 expressed as the percentage of this value [13].

Follicle diameter was determined by averaging the longitudinal and transverse axes at SynPG, SynG2, SynG2a24, and once daily on days 0 through 3 of stages 1 and 2 (Fig. 1). The maximum follicle diameter at SynG2a24 and on day 3 of stages 1 and 2 were defined as the preovulatory diameter. However, if a cow ovulated before SynG2a24, the preovulatory follicle diameter was recorded as that at SynG2. Ovulation was identified by the disappearance of preovulatory follicles followed by development of a new CL at the same site in the ovary. Upon synchronization, ovulation was detected at SynG2a24 and 32 h after the second GnRH pulse. In stages 1 and 2, ovulation was verified every 2 h from 24 to 36 h after GnRH or saline injection, then every 4 h up to 72 h, every 12 h up to 120 h, and every 24 h up to 240 h until ovulation occurred. The time of

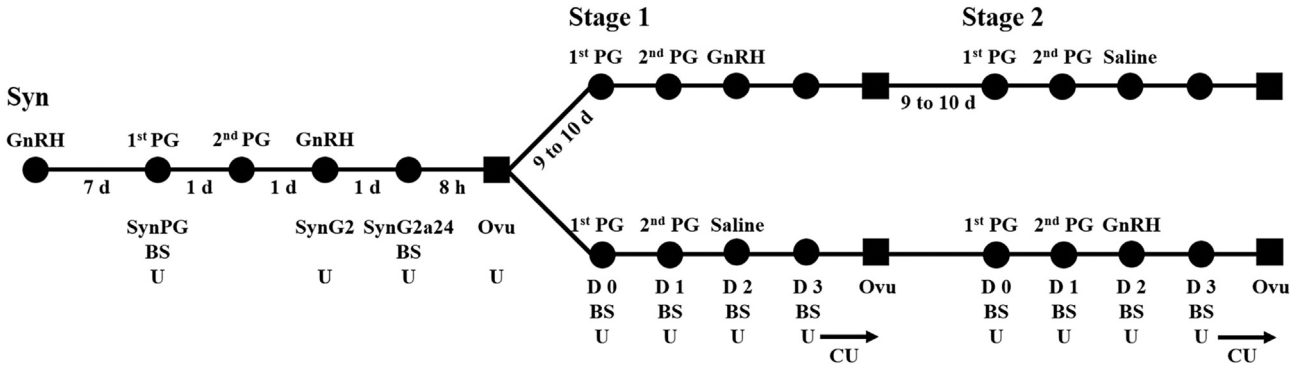

Fig. 1. Diagram of experimental procedures. BS: blood sampling for progesterone; CU: consecutive ultrasonography to detect ovulation every 2 h from 24 to 36 h after gonadotropin-releasing hormone (GnRH) or saline injection, then every 4 h up to 72 h, every 12 h up to 120 h, and every 24 h up to 240 h; D 0 to D 3; 1st and 2nd PG: cows were received 375 μg of cloprostenol, and 24 h later a second cloprostenol treatment (250 μg) was performed; GnRH: 250 μg of gonadorelin; Ovu: the time of ovulation; Syn: synchronization; SynG2: the time of GnRH administration in synchronization; SynG2a24: 24 h after SynG2; SynPG: the time of first prostaglandin (PG)F2α administration in synchronization; U: ultrasonography of the ovaries.