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Original article

EFFECT OF REPLACING THE SECOND GONADOTROPIN RELEASING HORMONE OF OVSYNCH AND CO-SYNCH PROTOCOLS WITH HUMAN CHORIONIC GONADOTROPIN ON THE REPRODUCTIVE PERFORMANCE OF PRIMIPAROUS LACTATING COWS DURING THE COOL MONTHS OF THE YEAR

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Summary

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The study objective was to evaluate the effect of replacing the second gonadotropin releasing hormone (GnRH2) of the Ovsynch and Co-Synch protocols with human chorionic gonadotropin (hCG) on ovulation, conception, pregnancy, and embryo loss rates in primiparous lactating dairy cows. Forty-nine primiparous Holstein cows 53 to 65 days in milk were allocated to the following groups: (1) OVS (Ovsynch; n=12): GnRH, 7 days, PGF2α, 56 hours, GnRH, 16-18 hours, timed artificial insemination (TAI); (2) COS (Co-Synch; n = 12): GnRH, 7 days, PGF2α, 72 hours, GnRH + TAI; (3) OVS-hCG (Ovsynch + hCG; n = 12): as OVS except that GnRH2 was replaced with 1500 IU hCG; (4) COS-hCG (Co-Synch + hCG; n = 13): as COS except that GnRH2 was replaced with 1500 IU hCG. Ovaries of all animals were scanned by transrectal ultrasonography to determine the incidence of ovulation on days 9, 10 and 11 at 24-hour intervals after the initiation of the treatments (day 0). Ovulation was defined as the disappearance from one scanning session to the next of a previously identified follicle ≥ 8 mm in diameter. Pregnancy diagnosis was performed by transrectal ultrasonography on days 30 ± 1 and 42 ± 1 post TAI to determine conception and pregnancy status, respectively. The results showed that the animals in OVS tended to have higher ovulation and conception rates (P=0.08). In addition, pregnancy rate was higher and embryo loss rate was lower for OVS than the other groups, but the differences were not significant (P>0.05). It was concluded that replacing GnRH2 with hCG in the Ovsynch and Co-Synch programmes has no beneficial effect on the reproductive performance of primiparous Holstein cows. However, conducting the experiment at a larger scale may provide more precise results.

Key words: Co-synch, human chorionic gonadotropin, Ovsynch, primiparous Holstein cow, reproductive performance

INTRODUCTION

Reproductive efficiency has major impacts on profitability of dairy producers and many methodologies have being used to improve it, including the use of reproductive hormones to regulate and control the estrus cycle (Keskin *et al*., 2010). According to Astiz & Fargas (2013), improvement of pregnancy rates obtained through timed artificial insemination (TAI) remains a high priority on most commercial dairy farms. However, in practice, TAI has been challenged due to unsatisfactory ovulation rates and low pregnancy rates (Zheng *et al.*, 2021). Hormonal protocols, most of which are based on the use of Ovsynch are used to synchronise ovulation before TAI (Astiz & Fargas, 2013). Based on experimental evidence (Keskin *et al*., 2010), the Ovsynch protocol was developed to synchronise ovulation in lactating dairy cows using gonadotropin-releasing hormone (GnRH) and prostaglandin F2α (PGF2α), by which ovulation is synchronised within an 8 h period from 24–32 h after the second GnRH (GnRH2) administration. This precise synchrony may allow for successful artificial insemination (AI) without estrus detection (Keskin *et al*., 2010).

Since the advent of the synchronisation of ovulation programme (Ovsynch), which involves the administration of GnRH and PGF2α, various TAI programmes have been used for the efficient reproductive management of dairy herds (Kim *et al*., 2020). Many studies have been performed to improve the original Ovsynch program and maximise fertility of lactating dairy cows receiving TAI (Cunha *et al*., 2022). The GnRH-PGF2α-GnRH (GPG) protocol has two variants, Ovsynch and Co-Synch. The difference between these variants is that in the Ovsynch, TAI is 16–20 h after GnRH2, whereas in the Co-Synch, TAI is at the time of GnRH2 administration (Ahuja *et al*., 2005). These protocols synchronise the stage of follicular development and the time of luteal regression in a similar manner to the GnRH-PGF2α protocol, but there is also a pre-ovulatory follicle that is induced to develop and ovulate in response to a second luteinising hormone (LH) surge induced by GnRH2 administered 48 h after PGF2α (Ahuja *et al*., 2005). The second GnRH dose is designed to synchronise and induce ovulation but does not always achieve sufficient gonadotropic stimulation of the preprogrammed follicle, and the effectiveness of the protocol in some animals is compromised (De Rensis *et al*., 2010). Based on experimental evidence (De Rensis *et al*., 2008b), by administering the GPG programme, cows can be subjected to TAI without the need for estrus detection, however, 40–60% of treated animals do not conceive to TAI. Several factors may lead to the failure of TAI, of which, one could be related to the use of GnRH to induce ovulation (De Rensis *et al*., 2008b). One way to minimise this problem is to replace GnRH analogs by human chorionic gonadotropin (hCG) (Schmitz *et al*. 2017).

Human chorionic gonadotropin has been used to induce ovulation in several species because it binds to the LH receptor (Dias *et al*., 2020). Although hCG is chemically different from LH, its biological activity is primarily LH-like (Brito *et al*., 2017), therefore it has a potent LH-like effect in cattle, extends the life span of the corpus luteum (CL), increases progesterone synthesis, induces ovulation throughout the estrous cycle, promotes the formation of accessory corpora lutea when applied in the early luteal phase, and modifies follicular wave dynamics increasing the frequency of three-wave dominant follicular cycles (De Rensis *et al*., 2010). The effectiveness of hCG in inducing ovulation of the dominant follicle is similar to that observed in response to LH release after GnRH administration, however, as hCG has a longer half-life and produces a direct effect on the ovary independently of the pituitary gland, hCG instead of GnRH should be considered in TAI protocols (De Rensis *et al*., 2010). Human chorionic gonadotropin has also been advocated for use to increase fertility and early pregnancy rates (Davies Morel & Newcombe, 2008). Because hCG was more effective than GnRH at stimulating ovulation in dairy cattle, a proper dose of hCG might be a substitute for GnRH in various TAI protocols (Burns *et al*., 2008). Therefore, we hypothesised that replacing GnRH2 of the Ovsynch or Co-Synch protocols with hCG might improve the ovulation rate (OR), conception rate (CR), and pregnancy rate (PR) better than the standard Ovsynch and Co-Synch protocols in lactating dairy cows.

To the best of our knowledge, in the majority of studies, hCG has been administered to cows some days after AI (Tefera *et al*., 2001; Hanlon *et al*., 2005; Stevenson *et al*., 2007; Beltran & Vasconcelos, 2008; Burns *et al*., 2008; Stevenson *et al*., 2008; Buttrey *et al*., 2010; Khoramian *et al*., 2011; Rossetti *et al*., 2011; Bartolome *et al*., 2012; Giordano *et al*., 2012; Stevenson & Pulley, 2012; Nascimento *et al*. 2013; Alnimer & Shamoun, 2015; Akhtar *et al*., 2018; Kunde *et al*., 2018; Niles *et al*., 2019; Zolini *et al*., 2019; Agarwal *et al*., 2021; Zheng *et al*., 2021) or has been included instead of either the first GnRH of the GPG protocols (Keskin *et al*., 2010; Binversie *et al*., 2012; Schmitz *et al*. 2017; Cabrera *et al*., 2021), or both the first and the second GnRH of these protocols (Geary *et al.,* 2001; De Rensis *et al*., 2002; Schmitz *et al*. 2017). Only few studies have replaced GnRH2 of the GPG programmes with hCG (De Rensis *et al*., 2008a; De Rensis *et al*., 2008b; Schmitz *et al*. 2017; Garcia-Ispierto *et al*., 2018). Thus, the objective of the present study was to compare the effect of replacing GnRH2 of the Ovsynch and Co-Synch protocols with hCG on ovulation, conception, pregnancy, and embryo loss rates in primiparous lactating dairy cows during the cool months of the year.

MATERIALS AND METHODS

Ethical approval

All the procedures of the present study were carried out according to the recommendations and rules of the Research Ethics Committee of Razi University, Kermanshah Iran.

Study population

This study was conducted on a commercial Holstein dairy farm during the cool months of the year. Cows were housed in free-stall barns with shelters, had free access to fresh water, received a total mix ration that was adjusted to meet the requirements of the cows producing an average of 32 kg milk/day and milked three times daily with average intervals of 8 h. The reproductive systems of the cows were examined via rectal palpation on regular biweekly programmed visits to the farm for the presence of any clinical abnormalities first on day 30 and again immediately before the initiation of the synchronisation protocols on days 53 to 65 postpartum. Only those primiparous cows that were clinically normal were used in this experiment.

Study design

Cows (n=49) were randomly allocated to one of the following groups: (1) OVS (Ovsynch, n=12): GnRH (25 µg; Alarelin acetate, Vetaroline, 10 mL vial, 5 µg/mL, Abureyhan pharma Co., Veterinary Division, Tehran, Iran), 7 days, PGF2α (500 µg; D-Cloprostenol sodium, D-clo PG, 10-ml vial, 0.025 mg/mL, Royan darou Co., Veterinary Division, Semnan, Iran), 56 hours, GnRH, 16-18 hours, TAI; (2) COS (Co-Synch, n=12): GnRH, 7 days, PGF2α, 72 hours, GnRH + TAI; (3) OVS-hCG (Ovsynch + hCG, n=12): as OVS except that GnRH2 was replaced with 1500 IU hCG (Karma-HCG®, vial 5000 IU, BSV BioScience GmbH, Germany); and (4) COS-hCG (Co-Synch + hCG, n=13): as COS except that GnRH2 was replaced with 1500 IU hCG. All the treatments were administered intramuscularly. All the cows were inseminated by the same technician with frozen-thawed semen from bulls with approved good fertility.

Ovarian scanning

The ovaries of all animals in each group were scanned by transrectal ultrasonography (ultrasonic scanner, model: HS-1500V, SN: 70610484, Honda Electronic Co. LTD 20 Oyamazuka Oiwa-Cho, Toyohashi, Aichi, Japan) on days 9, 10, and 11 after the beginning of the protocols (day 0) in order to record the occurrence of ovulation. Ovulation was defined as the disappearance (from one scanning session to the next) of a previously identified follicle ≥ 8 mm in diameter (Pfeifer *et al*., 2018; Cabrera *et al.,* 2021). Ovulation rate was defined as the number of the cows in a group that ovulated until day 11, divided by the total number of the animals in the corresponding group × 100.

Pregnancy diagnosis

Pregnancy diagnosis was performed in all animals by transrectal ultrasound on days 30 ± 1 and 42 ± 1 post TAI. Those cows that returned to oestrus before the first or second pregnancy diagnosis were re-inseminated after being confirmed to be in estrus by rectal palpation of the uterine horns. Cows diagnosed as non-pregnant were resynchronised with the standard Ovsynch protocol and inseminated 16–18 h after GnRH2, but the subsequent data were not included in the study. In other words, each cow was used only once in the study. Conception rate was defined as the percentage of the cows in a group that were diagnosed pregnant on day 30 ± 1 post TAI divided by those in the corresponding group. Pregnancy rate was defined as the percentage of the cows in a group that were confirmed to be pregnant on day 42 ± 1 post TAI divided by those in the corresponding group. Embryo loss rate (ELR) was defined as the percentage of the pregnant cows that were diagnosed non-pregnant on day 42 ± 1 divided by those that were pregnant on day 30 ± 1.

Statistical analysis

Data were analysed using SAS® software (Statistical Analysis System, Release 9.4. Cary, NC, USA: SAS Inst. Inc.). The analyses were performed in three steps including OR, CR on day 30 ± 1, and PR and ELR on day 42 ± 1 by logistic regression method using Proc Genmod for determining the probability of significant differences among the groups. Chi-square analysis was used to determine the degree of difference between the groups. Differences at P≤0.05 were considered statistically significant.

RESULTS

Average milk production during the study period (from the beginning of the treatments to the second pregnancy diagnosis on day 42 ± 1) did not differ between groups (31.5 ± 0.5, 31.0 ± 0.7, 31.0 ± 0.4 and 32.0 ± 0.6 kg/d in OVS, COS, OVS-hCG and COS-hCG, respectively). Also, body condition scores were found to be similar among the study groups according to a 5-point scoring system (2.75, 2.72, 2.71 and 2.74 in OVS, COS, OVS-hCG and COS-hCG, respectively).

Ovulation rate

The ovaries of all cows were scanned by ultrasound on days 9, 10, and 11 after the beginning of the protocols (day 0) in order to record the occurrence of ovulation. The incidence of ovulation and the number of animals that ovulated between days 9 and 11 after the beginning of the protocols have been presented in Table 1. The greatest and least incidence of ovulations were observed in OVS and COS groups (91.7% and 58.3%, respectively). The difference tended to be significant (P=0.08).

Conception rate

As shown in Table 1, the highest and lowest conception rates confirmed by transrectal ultrasound on day 30 ± 1 post TAI were observed in OVS (91.7%) and COS groups (58.3%), respectively, and the difference tended to be statistically significant (P=0.08).

Pregnancy and embryo loss rates

Ultrasound scanning of the uterine horns was performed on day 42 ± 1 after TAI in order to determine PR and ELR. The highest and lowest PR were observed in OVS (58.3%) and COS-hCG groups (38.5%), respectively, but the differences were statistically insignificant (P >0.05). Moreover, the results showed that the highest and lowest ELR were recorded in COS-hCG (44.4%) and OVS (22.2%) groups, respectively, but the differences were insignificant (P>0.05) (Table 1).

DISCUSSION

Effects of hCG on fertility of lactating dairy cows have been variable. In the

Table 1. Ovulation, conception, pregnancy and embryo loss rates in primiparous lactating dairy cows synchronised with the Ovsynch or Co-Synch protocol with or without replacing the second gonadotropin releasing hormone (GnRH) with human chorionic gonadotropin (hCG)

Group	Ovulation rate % (number)	Embryo loss rate % (number) at day 42 ± 1	Pregnancy rate % (number) at day 42 ± 1	Conception rate % (number) at day 30 ± 1
OVS*	91.7 (11/12)	22.2 (2/9)	58.3 (7/12)	75.0 (9/12)
COS	58.3 (7/12)	25.0 (2/8)	50.0 (6/12)	66.7 (8/12)
OVS-hCG	75.0 (9/12)	28.6 (2/7)	41.7 (5/12)	58.3 (7/12)
COS-hCG	69.2 (9/13)	44.4 (4/9)	38.5 (5/13)	69.2 (9/13)

OVS (Ovsynch; n=12): GnRH, 7 days, PGF2α, 56 hours, GnRH, 16-18 hours, timed artificial insemination (TAI); COS (Co-Synch; n=12): GnRH, 7 days, PGF2α, 72 hours, GnRH + TAI; OVS-hCG (Ovsynch+hCG; n=12): as OVS except that GnRH2 was replaced with 1500 IU hCG; (4) COS-hCG (Co-Synch+hCG; n=13): as COS except that GnRH2 was replaced with 1500 IU hCG.

present study, the effect of replacing GnRH2 of the Ovsynch and Co-Synch protocols with hCG on the reproductive performance of primiparous lactating Holstein cows was investigated during the cool months of the year. The results regarding the ovulation rates on days 9, 10, and 11 (day 0: the day of initiating the protocols) and pregnancy rates on days 30 ± 1 and 42 ± 1 after TAI, and the embryo loss rates on day 42 ± 1 showed no significant differences between the groups, although the values in the OVS group were numerically better than those in the other groups and tended to be significant. Extensive information exists on the improvement of pregnancy rates by means of GnRH or GnRH analogs and hCG (Heuwieser *et al*., 1994). However, the effects of hCG administration on fertility in cattle have not been consistent among various studies (Hazano *et al*., 2020). It has been reported that the use of hCG in cattle improved pregnancy rates in some studies but not in others (Rajarnahendran *et al*., 1998). The review by De Rensis *et al*. (2010) documented that no differences have been reported in conception rates between control and treated dairy cows receiving hCG either during the luteal phase of the cycle or at AI, and that a better effect of hCG on fertility compared with GnRH during the warm but not the cold period of the year has been observed. The latter observation suggests that hCG is able to counteract the negative effect on fertility of heat stress during the warm season to some extent (De Rensis *et al*., 2010).

Variable results have been reported on the effect of administering hCG at breeding on conception rates in dairy and beef cows. It has been evidenced that injection of hCG at breeding or immediately around breeding increased conception rate in beef heifers and dairy cows (De Rensis *et al*., 2010). In contrast, others have reported no effect or reduced conception rates when hCG was given at the time of breeding or a few days after AI (Bartolome *et al.*, 2012; Stevenson & Pulley, 2012; Sánchez *et al.*, 2018; Schmitz *et al*. 2017; Niles *et al*., 2019; Agarwal *et al*., 2021). These differences could be related to the period of the year in which the treatment has been performed (De Rensis *et al*., 2010), the parity of the dam (primiparous vs. multiparous) (Nascimento *et al*., 2013; Zolini *et al*., 2019), the genotype of the animals (Zolini *et al*., 2019), and the dose and the day of administration of hCG (Besbaci et al., 2020). For example, De Rensis *et al*. (2008b) reported a positive effect on fertility of replacing hCG instead of GnRH2 of the Ovsynch program in dairy cows only during the warm period of the year. In addition, De Rensis *et al.* (2002) reported that using hCG instead of the first and second GnRH of the Ovsynch program was equally effective to significantly improve fertility in postpartum dairy cows under heat stress, but this effect was not significant during the cool season, which is consistent with the results of the present study that was conducted during the cool months of the year. Therefore, the authors suggested that they would favor the use of the GnRH based protocol because of its lower cost and other benefits. According to Nascimento *et al*., (2013), fertility-enhancing effect of administering hCG to lactating dairy cows on day 5 after TAI was very large in first-lactation cows but not observed in older cows in their field study. Zolini *et al*. (2019) showed that pregnancy per AI was improved by hCG administration 5 days after AI in primiparous lactating Holstein cows but not in multiparous cows. The authors also demonstrated an interaction between hCG treatment and the animal genotype regarding fertility. Inconsistent with these data, the results of the present study did not show improvement in pregnancy per AI in primiparous dairy cows. One reason for this discrepancy between the results of the present study and those of Zolini *et al*. (2019) may be the time of administering hCG. Another reason may be related to the difference in the dose of hCG used in the experiment of Zolini *et al*. (2019) with that of the present study (3,300 IU vs. 1,500 IU, respectively). In addition, in a meta-analysis, Besbaci *et al.* (2020) showed that the use of GnRH and hCG after AI should be focused on cows expected to have low or moderate fertility; moreover, day and dose of treatment have to be considered as well. In the present study, effect on ovulation, conception, pregnancy and embryo loss rates of replacing GnRH2 of the Osynch and Co-Synch protocols with hCG was evaluated in primiparous lactating Holstein cows during the cool months of the year. Cows were selected regardless of being cyclic or not at the initiation of the protocols. However, fertility after GnRH or hCG administration has been reported to be related to the stage of the estrous cycle at which the treatment has been performed, the type of animals (anestrus or cyclic), and the season of the treatment (De Rensis *et al*., 2010). In the present study, the protocols were intiated irrespective of the estrus cycle stage. It has been shown that lactating dairy cows that started on Ovsynch at day 5 to 9 of the estrous cycle had a greater chance of becoming pregnant to TAI (Colazo *et al*., 2013).

Geary *et al*. (2001) reported that regardless of parity (primiparous or multiparous) the overall pregnancy rate of suckled beef cows synchronised with the Co-Synch protocol was higher in cows treated with GnRH than that in cows treated with hCG instead of the first and second GnRH. In addition, hormone treatment (GnRH or hCG) had no effect on pregnancy rates of cyclic or anestrous cows. The authors concluded that hCG was not a suitable replacement for GnRH to synchronise ovulation with the Co-Synch protocol in multiparous cows, although further studies with hCG in primiparous cows are needed to confirm that it is better than GnRH among cows of this age group (Geary *et al*., 2001). Schmitz *et al*. (2017) reported similar pregnancy rates in suckled beef cows when GnRH was administered at the beginning and at the end of the Co-Synch protocol or when hCG was substituted for GnRH at the end of the Co-Synch protocol, and concluded that substitution of hCG for GnRH proved to be of no advantage, which is in agreement with the results of the current study in which administering hCG instead of GnRH2 in the Co-Synch programme had no advantage over GnRH in improving fertility in lactating dairy cows. De Rensis *et al*. (2008a) found that during the warm period, the pregnancy rates of the cystic dairy cows were similar no matter if they received hCG or not for the second GnRH of the Ovsynch program, however, during the cool period, there was a beneficial effect to use hCG at day 9 of the Ovsynch protocol compared to GnRH on cumulative pregnancy rate after two rounds of inseminations. In the meta-analysis by Besbaci *et al*., (2020), it was recommended that the use of GnRH and hCG after AI should be focused on cows with low or moderate fertility. The primiparous cows that were used in the current study, had no history of compromised fertility. Therefore, one reason that replacing GnRH2 of the GPG programmes with hCG in the current study had no benefit on fertility may be the good status of fertility in the herd from which the cows of the present experiment were provided. Studies that used large numbers of animals have generally reported a modest improvement on pregnancy rates of lactating cows, irrespective of whether AI or embryo transfer was used. However, such positive effects on pregnancy rate are not equivocal, with others failing to demonstrate an effect of hCG administration on pregnancy rate (Maillo *et al*., 2014). In the study by Garcia-Ispierto *et al*. (2021), hCG treatment given at the end of a 5-day P4-based protocol for estrus synchronisation improved the pregnancy rate in lactating dairy cows receiving an *in vitro*-produced embryo. According to De Rensis *et al*. (2008b) the use of hCG instead of GnRH2 in the Ovsynch protocol in a TAI programme improved fertility in dairy cows during the time of the year when reproductive performance was compromised by heat stress. However, Burns *et al*. (2008) suggested that in beef cows hCG might not be a suitable replacement for GnRH in a CO-Synch + progesterone insert protocol. In contrast, in the study by Garcia-Ispierto *et al*. (2018) the results indicated the efficacy of hCG treatment to induce ovulation at the end of a 5-day P4-based protocol for FTAI in lactating dairy cows.

Geary *et al*. (2001) demonstrated that ovulation to GnRH2 or hCG instead of GnRH2 did not differ between GnRH-treated and hCG-treated suckled beef cows synchronised with the Co-Synch protocol. Similarly, Burns *et al*. (2008) demonstrated that treatment of cows with 500 IU of hCG ovulated follicles as effectively as GnRH treatment. In the present study, although ovulation rate was numerically the greatest and tended to be greater in cows synchronised with the standard Ovsynch, it did not differ significantly among cows that received or not hCG instead of GnRH2 of the Ovsynch or the Co-Synch protocols. However, although the responses vary greatly between studies, hCG has been shown to promote primary luteal function, induce formation of accessory corpora lutea, and improve conception rates (Beltran & Vasconcelos, 2008; Nascimento *et al*. 2013). The variability may be partly due to different hCG doses or days of administration used in different studies and partly due to breed differences and other factors including parity, days in milk, and total milk production (Agarwal *et al*., 2021). It has been reported (Geary *et al*., 2001) that hCG-treated 2-year-old cows tended to have higher pregnancy rates than GnRH-treated 2-year-old ones regardless of calf presence, and that multiparous cows had higher pregnancy rates to the GnRH treatments than to hCG treatments independent of calf removal. The authors concluded that among all cows, replacing GnRH with hCG resulted in numerically lower ovulation and pregnancy rates (Geary *et al*., 2001). As documented by De Rensis *et al*. (2010), administration of hCG at AI can reduce embryo losses especially during the warm season. Geary *et al*. (2001) suggested that further studies with hCG in primiparous cows are needed to confirm that it is better than GnRH among cows of this age group. In the current study conducted during the cool months of the year, hCG was used instead of GnRH2 in the Ovsynch and Co-Synch protocols and although the ovulation, conception and pregnancy rates were higher and the embryo loss rate was lower in cows treated with the Ovsynch program than those in the other groups, the differences between the groups were insignificant.

CONCLUSION

In conclusion, the results of the present study indicated that replacing the second GnRH of the Ovsynch and Co-Synch programmes with hCG has no significant advantage in improving the ovulation, conception, pregnancy and embryo loss rates in primiparous lactating Holstein cows during the cool months of the year. Therefore, due to the lower cost of GnRH compared to hCG, it is not rational to replace the second GnRH with hCG in the Ovsynch and Co-Synch protocols. However, further studies involving larger sample sizes are needed to determine if administration of hCG instead of GnRH2 of the GPG protocols has any beneficial effect on the reproductive performance of primiparous lactating cows.

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