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Effect of the administration route of gonadotropin releasing hormone on the reproductive performance of multiparous Holstein cows

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Abstract

We investigated the effect of epidural versus intramuscular administration of alarelin acetate (GnRHa; 25 μg) at artificial insemination (AI; day 0) and/or 7 days after AI on the pregnancy rate (PR) of Holstein cows that exhibited estrus spontaneously after their voluntary wait period. Cows (n = 117) were randomly allocated to treatment and control groups. In groups IM0, IM7, and IM07 (n = 12 per group), cows received GnRHa intramuscularly (IM) at 0 or 7 days, or both, respectively. In groups EP0 (n = 19), EP7 (n = 13) and EP07 (n = 11), cows received GnRHa at the sacrococcygeal epidural site (EP) at 0 or 7 days, or both, respectively. Animals in the control group (CON) received 5 mL of normal saline at 0 and 7 days. Pregnancy diagnosis was performed by rectal palpation of the uterine horns 60 days post AI. Results showed a significant difference in PR/first AI between IM0 and EP7 groups (16.67% vs. 61.54%; $P < 0.05$). Moreover, the results showed no significant differences in overall PR after two rounds of inseminations among all groups 60 days after the second AI. In conclusion, epidural injection of GnRHa 7 days after AI versus its intramuscular injection at AI improved PR/first AI significantly in Holstein lactating cows.

Keywords: artificial insemination; epidural administration; GnRH; Holstein cows; pregnancy rate; spontaneous estrus.

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Study contribution

Many methodologies are being used to improve reproductive efficiency, including the use of reproductive hormones. Gonadotropin releasing hormone plays a central role in mammalian reproduction. The induction of ovulation with exogenous gonadotropin releasing hormone could improve the reproductive efficiency by inducing ovulation or increasing circulating progesterone. In addition to the hypothalamus and pituitary gland, gonadotropin releasing hormone receptor have been shown to be expressed in extra-pituitary tissues including the spinal cord. Therefore, this experiment compared the effects of epidural versus intramuscular administration of a gonadotropin releasing hormone agonist at artificial insemination and/or on day 7 after artificial insemination on the reproductive performance of postpartum Holstein lactating cows. The results of this study showed that epidural injection of a gonadotropin releasing hormone agonist 7 days after AI versus its intramuscular injection at artificial insemination significantly improves pregnancy rate to the first artificial insemination in Holstein lactating cows.

Introduction

Reproductive efficiency is one of the key components of a profitable dairy system,[(1)] so is of great economic importance in the dairy industry.[(2)] It also has major impacts on the profitability of dairy producers and many methodologies are being used to improve it, including the use of reproductive hormones to regulate and control the estrus cycle.[(3)] Conception rates in dairy cows have decreased over the last three decades associated with physiological and endocrine changes.[(4)] The induction of ovulation with exogenous gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG) could improve the reproductive efficiency by increasing circulating progesterone (P4) leading to increased early embryo development or by changing timing of corpus luteum (CL) regression due to changes in follicular dynamics.[(5)] In the postpartum cow, failure to ovulate, failure of conception, or early embryonic loss have been mentioned as causes of sub-optimal reproductive performance.[(1)] Reduced pregnancies per artificial insemination (AI) in lactating dairy cows in confinement systems have led to dramatic reproductive inefficiency in the dairy industry.[(6)] Economic loss from reproductive inefficiency is associated with long calving intervals that result from low conception rates for the first breeding.[(2)] For dairy cows, a calving interval of 12 to 13 months is generally considered economically optimal, and detection of estrus and rate of conception are integral components in achieving this calving interval.[(7)]

Gonadotropin Releasing Hormone (GnRH) is a hypothalamic decapeptide that acts on gonadotropes in the anterior pituitary gland to release the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH),[(8,9)] therefore, it plays a central role in mammalian reproduction.[(10)] Approximately 25 % of bovine embryos are lost during the first 3 weeks of life. The maintenance of progesterone secretion by a viable corpus luteum is vital to early pregnancy and untimely luteolysis is probably a major cause of embryo loss. The use of GnRH as a 'holding' injection on the day of insemination to improve the chances of successful pregnancy, particularly in repeat breeder cows, has been a major indication for the commercial product for decades. The scientific rationale has been to induce ovulation at the

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appropriate time relative to insemination and to stimulate luteinization, thereby improving the chances of successful fertilization and embryo survival. However, its efficacy has been equivocal because individual studies have often lacked the statistical power to achieve significant results and therefore, outcomes have been very variable.(11) Treatment regimens for cattle also include early postpartum treatments with GnRH or agonists, relative to the first breeding, at the time of AI in repeat breeder cows, and in the luteal phase after AI.(2) The pharmacological basis for the therapeutic use of GnRH derives from its physiological effect of stimulating the release of LH and FSH from the anterior pituitary gland.(11)

In addition to the hypothalamus and pituitary gland, GnRH and its receptor (GnRHR) have been shown to be expressed in extra-pituitary tissues including the ovary,(12) testis, placenta, adrenal and mammary gland. Distribution and the localization of GnRH and GnRHR have been identified in several animals such as the chicken, horse, cattle, monkey and pig, using immunohistochemistry.(13) Particularly, Ramakrishnappa et al.(14) reported the presence of GnRH-receptor messenger RNA in bovine ovary, in both follicle and corpus luteum tissue. Moreover, GnRH receptors are also expressed at all levels of the mammalian spinal cord.(15) These premises highlight the likelihood that GnRH analogs may trigger their effects by action not only on pituitary receptors, but also on extrapituitary ones. This route can also be used for administering hormones and analog to achieve a selective local pharmacological response. As the bovine ovaries are innervated by sympathetic neurons derived from the ovarian plexus nerve and the hypogastric nerve, the epidural administration could indirectly influence ovaries.(16) It has been reported that administration of lecirelin, a GnRH analog, into the epidural space did not induce any side effect, such as meningitis, neuritis, or local inflammation.(17) Additionally, GnRH agonist may enhance its capacity of binding receptor by 100–200 times.(18)

The expected role of GnRH injected around the time of estrus is the induction of ovulation for the ovulatory follicles without stimulating the growth or the development of the subordinate follicles to be ovulatory ones.(19) In most of the studies on the effect of administration of GnRH on cows during estrus on their conception rate, the hormone was administered at the time of AI without consideration of the time elapsed since the onset of estrus. In a meta-analysis of 40 trials described in 27 papers, a significant effect of GnRH administration at the time of AI was found.(20)

If GnRH is injected during the luteal phase of the cycle, the resulting LH release stimulates either ovulation or atresia of the dominant follicle. Depending on follicular status at the time, treatment GnRH during the luteal phase likely advances either atresia, luteinization, or, ovulation followed by luteinization. Apart from stimulating progesterone secretion, these changes also decreased oestradiol-17β production. In turn, decreased oestradiol-17β production could inhibit up-regulation of oxytocin receptors and consequent inhibition of PGF2α secretion. Therefore, a reduction in estradiol secretion at this time might be expected to inhibit the luteolytic mechanism allowing some pregnancies to continue.(11) However, the presence of GnRH and GnRH receptors on the spinal cord and ovary in some species, and the kind of innervation of the ovary, let us hypothesize that GnRH and its analogs may also act when administered by epidural route, as happens for other drugs.(16) Therefore, this experiment compared the effects of epidural vs intramuscular administration of a GnRH agonist, Alarelin acetate, at AI and/or on Day 7 after AI on the reproductive performance of postpartum Holstein lactating cows (DIM: 76.0 $\pm$ 13.5 days).

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Materials and methods

Ethical statement

All experimental procedures were approved by the Research Ethics Committee of Razi University (No. 396-2-013), Kermanshah, Iran.

Animals

The experiment was conducted on 117 multiparous Holstein lactating cows with parity ranging from 2 to 4 on a Holstein dairy farm in Kermanshah (with a hot-summer Mediterranean climate (Csa); 34.4576 ° N, 46.6705 ° E, altitude: 1 350 m above sea level, average low and high temperature: –1.7 ºC and 37.8 ºC in December and July, average low and high relative humidity: 23 % and 75 % in January and July, respectively, and average annual precipitation: 478.7 mm), the capital city of Kermanshah province, west of Iran from June 2015 to February 2016. The cows (approximately 76.0 ± 13.5 days in milk) were housed in a free stall with open-air and sheltered areas, milked thrice daily throughout the experiment at approximately 8-h intervals, and average daily milk yield was 30.0 ± 5.0 kg/cow/d. The body condition score of the animals included in the study was ~ 3.0 (with a 1–5 scale) at AI. The animals were confirmed clinically not to have systemic or reproductive problems before being assigned to the study and fed a diet based on Sudan hay, alfalfa hay cubes, corn silage, chopped straw and concentrate supplements including minerals and vitamins after the milking time.

Study groups

The cows were inseminated approximately 12 h after being observed in standing estrus by an experienced inseminator with proven and qualified frozen-thawed semen provided by an approved center for collecting and preparing semen. Then the animals were assigned randomly to one of the study groups according to their parities, so that an approximately similar distribution of the parities could occur in the groups: EP0, the cows received 25 µg alarelin acetate (Alarelin acetate, Vetaroline, 10 mL vial, 5 µg/mL, Aburaihan pharmaceutical Co., Tehran, Iran) at the sacrococcygeal epidural site (EP) using a 21-gauge needle at AI (n = 19); EP7: the cows received 25 µg Alarelin acetate EP at Day 7 after AI (n = 13); EP07: the cows received 25 µg Alarelin acetate EP at AI and the same dose at Day 7 after AI (n = 11); IM0: the cows received 25 µg Alarelin acetate intramuscularly (IM) at AI (n = 12); IM7: the cows received 25 µg Alarelin acetate IM at Day 7 after AI (n = 12); IM07: the cows received 25 µg Alarelin acetate IM at AI and the same dose at Day 7 after AI (n = 12); CON: the cows received 5 mL sterile saline solution at AI and the same dose at Day 7 after AI (n = 38).

Pregnancy diagnosis

Pregnancy diagnosis was performed by rectal palpation of the uterine horns 60 days after AI. Cows returning to estrus before 60 days after the first AI in all treatment groups were re-inseminated 12 h after standing estrus and examined 60 days later for pregnancy status. Data including cow number, parity, date of the last calving, date of the first AI, result of the first pregnancy diagnosis, date of the second

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AI and result of the second pregnancy diagnosis were recorded for future analysis. The pregnancy rate to the first AI (PR/1st AI) was defined as the percentage of cows that were pregnant on day 60 after AI out of the total number of cows in the corresponding group. The cumulative pregnancy rate (CPR) was defined as the percentage of cows that were pregnant after two rounds of the AI (first AI plus return AI) on day 60 after the second AI out of the total number of cows in the corresponding group.

Statistical analysis

Data were analyzed using SAS® software (SAS System, Release 9.4. Cary, NC, USA: SAS Inst. Inc.). The analyses were performed in two steps including pregnancy rates to the first AI (PR/1st AI) and overall or cumulative pregnancy rates to the first and second AIs (CPR) by Logistic Regression method using Proc Genmod for determining the probability of significant differences among the treatment and control groups. χ^2 square statistics were used to determine the degree of difference between the experiment and control groups and the level of significance was set at $P < 0.05$.

Results

Pregnancy rate to the 1st AI

The numbers (%) of the cows in the experimental and control groups that were diagnosed to be pregnant to the first AI by rectal palpation 60 days after AI is shown in Table 1. Statistical analysis showed that the difference in PR/first AI was statistically significant only between the groups EP7 and IM0 ($P < 0.05$). The differences in PR/first AI between each of these two groups and the others and among the other groups were not statistically significant ($P \geq 0.05$, Table 1).

Cumulative pregnancy rate

Twelve of the 117 cows that were used in the experiment were culled from the herd after the second AI and before being examined via rectal palpation for pregnancy status because of different reasons such as low milk yield and lameness. Thus, they were excluded from the final analysis. Therefore, 105 cows were used for further analysis.

The numbers (%) of the cows in the experimental and control groups that were diagnosed to be pregnant after two rounds of AIs by rectal palpation 60 days after the second AI are presented in Table 1. Statistical analysis showed no significant difference among the study groups in CPR to the first and second AIs ($P \geq 0.05$, Table 1).

Discussion

Conception rates in dairy cows have decreased over the last three decades associated with physiological and endocrine changes.[4] The induction of ovulation with

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Table 1. Pregnancy rate to the first AI (PR/first AI; pregnant cows/total cows in the corresponding group) and cumulative pregnancy rate (CPR; pregnant cows/total cows in the corresponding group) to the first and second AI in Holstein lactating dairy cows received alarelin acetate at the sacrococcygeal epidural site (EP) using a 21-gauge needle and/or 7 days after AI

Group*	EP0	EP7	EP07	IM0	IM7	IM07	CON	Total
†PR/1st AI (%)	7/19[ab] (36.84)	8/13[a] (61.54)	3/11[ab] (27.30)	2/12[b] (16.67)	4/12[ab] (33.33)	6/12[ab] (50.00)	16/38[ab] (42.10)	46/117 (39.32)
‡CPR (%)	12/19[a] (63.16)	9/11[a] (81.82)	5/11[a] (45.45)	6/11[a] (54.55)	7/10[a] (70.00)	7/10[a] (70.00)	23/33[a] (69.70)	69/105 (65.71)

*Cows received 25 μg alarelin acetate at the sacrococcygeal epidural site (EP) using a 21-gauge needle at AI (EP0), at Day 7 after AI (EP7), at AI and at Day 7 after AI (EP07), or intramuscularly at AI (IM0), at Day 7 after AI (IM7), and at AI and at Day 7 after AI (IM07); †PR/1st AI: The percentage of cows pregnant at day 60 after AI out of the total number of cows in the corresponding group. ‡CPR: The percentage of cows pregnant after two rounds of AI (first AI plus return AI) at day 60 after the second AI out of the total number of cows in the corresponding group.[ab]Different superscript letters within a row denote significant difference ($P < 0.05$).

exogenous GnRH could improve the reproductive efficiency by increasing circulating P4 leading to increased early embryo development or by changing timing of CL regression due to changes in follicular dynamics.(5) Several studies have tested the effect of GnRH given at the time of AI(4,20–22) and at different times before(16) or after AI,(21,23–25) with conflicting results. Equivocal effects on fertility may have been due to different reproductive management systems used in the various experiments.(26) In this study, an investigation was conducted into the effects of epidural versus intramuscular injection of an analog of GnRH, Alarelin acetate, at and/or 7 days after AI on pregnancy rates in multiparous Holstein lactating cows exhibited estrus spontaneously after the voluntary wait period approximately 76.0 ± 13.5 days postpartum. Analyses of the data showed a significant difference in the PRs between the cows in EP7 and IM0 groups on Day 60 after the first AI, but no significant difference in PRs to the first AI was found between each of these two groups with EP0, EP07, IM7, IM07, and CON groups and among the latter groups themselves. Perry and Perry(22) reported no improvement in first-service conception rates by administration of GnRH at the time of AI in beef cattle. In this study although numerically more PRs to the first AI were observed in the cows in EP7 and IM07 groups vs CON group, the differences were not significant. In contrast, although numerically fewer PRs to the first AI were found in the cows in EP0, EP07, IM0, and IM7, the differences were not significant. Also, the results showed no significant differences in the CPRs 60 days after the second AI among the study groups. Most CPR to the first and second AIs 60 days after the second AI was found in the cows in the EP7 group (81.82 %), but the difference between EP7 and the other groups was not significant.

Inconsistent results have been reported about the effects of GnRH and its agonists administered near or at the breading on the reproductive performance in cattle. Some studies found significant or insignificant improvements in conception rates.(4, 11, 27-31) whereas others reported no benefit from GnRH treatment at the first breeding.(21,22,32) Of the research reporting the significant advantage, the increase in conception rate varied from 4.6 to 13.3 %.(2) Heuwieser et al.(2) reported 6.4 and 6.9 % improvements in conception rate for cows in second or greater lactation, respectively. The authors also stated that cows in the first lactation will not

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benefit from a GnRH injection at 25 to 35 d after calving, but cows in second or greater lactation will benefit from a GnRH injection at 25 to 35 d after calving.(2) Kharche and Srivastava(31) indicated that the pregnancy rate in crossbred cows could be improved by the GnRH treatment, Buserelin-acetate at the time of AI. Madureira et al.(33) stated that treatment with GnRH at the time of AI increased fertility sometimes in *Bos indicus* cows but not in heifers. In an experiment carried out in winter, it was shown that GnRH or its analogs increased conception rate when administered at the onset of estrus but had no effect when administered later in the estrous period.(34) In another experiment, Aboul-Ela and El-Keraby(35) found that treatment with GnRH at the time of AI resulted in a significant increase in the conception rate from the first insemination over that of the control group in Friesian cows. Drew and Peters(36) carried out a study where GnRH was administered to dairy cows on the day of the first insemination, and the overall response was a non-significant increase in PR. In contrast, the results of a study showed that treatment with GnRH at AI following detection of cattle in standing estrus did not influence conception rates or overall pregnancy rates among beef cows and heifers. In dairy cows, there have been conflicting results regarding the benefit of GnRH treatment at AI, but it appears most beneficial in cows with decreased fertility.(22)

The use of a single GnRH injection before or after AI to increase pregnancy rates has been described in both dairy and beef cattle.(21) Some studies have reported significant improvements of 10–12 % in pregnancy rates(36,37) while others did not report.(25,38,39) Szenci et al.(21) reported that intramuscular administration of 50 µg of a GnRH agonist on Day 12 after AI did not improve reproductive performance in Holstein–Friesian dairy cows, so a single injection of GnRH at Day 12 post-AI cannot be recommended as a general method for improving reproductive performance in dairy cows. López-Gatius and Garcia-Ispierto(25) stated that the treatment of Holstein-Friesian dairy cows with a GnRH analog intramuscularly 5–7 days after AI had no effects on non-repeat breeders, but improved fertility in repeat-breeder cows. Rizzo et al.(16) reported that epidural administration of lecirelin (a GnRH analogue) significantly improved PRs in pluriparous Friesian cows affected by follicular cysts compared to intramuscular administration. Chebel et al.(23) reported that resynchronization with GnRH given on Day 21 after AI for initiation of a timed AI protocol before pregnancy diagnosis does not affect pregnancy rate and pregnancy loss in lactating dairy cows. Another study reported that treatments with GnRH increased conception in primiparous cows during summer, and in cows with lower body condition.(20) Equivocal effects on fertility may have be due to different reproductive management systems used in the various experiments.(40–42) Therefore, the decision for routine use of GnRH should consider that efficacy depends upon body condition, parity, and time of administration.(2)

Conclusions

It was concluded that epidural and/or intramuscular administration of an analog of gonadotropin releasing hormone, Alarelin acetate, at AI and/or at Day 7 after AI in multiparous Holstein lactating dairy cows that exhibited estrus spontaneously after their voluntary wait period improved PRs significantly only in the cows that received GnRHa at the sacrococcygeal epidural site 7 days after AI (EP7) compared to the

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cows that received the hormone intramuscularly at AI (IM0). However, there were no significant differences between each of these two groups and the EP0, EP07, IM7, IM07, and CON groups. Also, cows in EP7 and IM07 groups showed numerically greater PRs to the first AI compared to CON, but the differences were not statistically significant. Moreover, the results revealed no significant differences in overall PRs to the first and second insemination among all treatment and control groups 60 days after the second AI.

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Data availability

All relevant data are within the manuscript. The datasets generated and/or analyzed during this research are available from the corresponding author on reasonable request.

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The research was conducted as a DVM thesis at Razi University, Faculty of Veterinary Medicine and Sirous Mozaffari, was a DVM student in the faculty. This research was conducted with support from a local dairy farm in Kermanshah, Iran by providing the animals, labor and necessary records, but the farm owner played no role in study design, data collection and analysis, decision to publish, preparation of the manuscript, and financial funding.

Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

Author contributions

Conceptualization: M Goli.
Investigation: M Goli, S Mozaffari.
Methodology: M Goli, S Mozaffari.
Supervision: M Goli, S Mozaffari.
Validation: M Goli, S Mozaffari.
Writing – original draft: S Mozaffari.
Writing – review and editing: M Goli, S Mozaffari.

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