Livestock Studies, 2022; 62 (2): 74-80
http://doi.org/10.46897/livestockstudies.1173020

RESEARCH PAPER

LIVESTOCK STUDIES

The Effects of GnRH and hCG Administration on Pregnancy Rate in Postpartum Dairy Cows

Emre Karsavuranoğlu[1], Mustafa Kemal Sarıbay[1], Ece Koldaş Ürer[1,*]

[1] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology, 31060, Hatay, Türkiye

Article History
Received: 25 Apr. 2022
Accepted: 08 Aug. 2022
First Online: 09 Sep. 2022

Corresponding Author
Tel.: +905382394145
E-mail: ecekoldas@gmail.com
ecekoldas@mku.edu.tr

Keywords
Cow
Hormonal administration
Pregnancy rate
Postpartum

Abstract

This study was designed to determine the effects of GnRH administration alone at the time of artificial insemination (AI) or in combination with hCG 5 days after GnRH injection on conception rates in postpartum dairy cows. Cows in estrus, without any reproductive health problem, between days 70-120 postpartum were randomly assigned to 3 equal groups. Buserelin acetate (10 µg), was administered at the time of AI to Group I (n=40). Group II (n=40) was administered first with Buserelin acetate (10 µg) at the time of AI and then with 1500 IU of hCG on the 5th day after insemination. Group III was maintained for control purposes and did not receive any treatment. Pregnancy diagnosis was performed on day 30 post-AI by transrectal ultrasonography. Conception rates were 80% (32/40), 80% (32/40), and 57.5% (23/40) in Group I, II and III (P<0.05) respectively In conclusion, it was determined that the use of GnRH alone at the time of AI or additionally the use of hCG on the 5th day after AI increased the pregnancy rates in dairy cows between 70-120 days postpartum, however, there was no difference in pregnancy rates between these two treatments.

Introduction

One of the main goals of dairy farming is to ensure that dairy cows conceive in the optimal postpartum period, thus keeping the time between births within economically viable limits. The profitability of dairy production depends on the regular calving of dairy cows every 12 to 13 months. Therefore, the prolongation of this interval has adverse economic implications for dairy farmers (Boulton *et al*., 2017; De Vries, 2006; Jainudeen and Hafez, 2000).

The most common causes of infertility in cows are reported as delayed ovulation, anovulation and early embryonic death (Taponen, 2003). Ovulation induction protocols based on gonadotropin-releasing hormone (GnRH) and human chorionic gonadotropin (hCG) administration are widely used, which prevent ovulation delay and anovulation-related disorders and reduce hormone-related early embryonic deaths (Canadas *et al*., 2019; Kashyap *et al*., 2018; Taponen, 2003). Administration of GnRH analogs at artificial insemination (AI) induces ovulation by initiating a pre-ovulation LH peak and contributes to an increase in the secretory capacity of the corpus luteum (CL). Most previous studies indicate that GnRH analogues increase both the frequency and secretion of LH, increase the differentiation of theca-lutein and granulosa-lutein cells in the CL, can transform small luteal cells into large luteal cells, and thereby increase progesterone secretion and, chance of the survival of the embryo (Besbaci *et al*., 2020; Taponen *et al*., 1999; Willmore and Davis, 2019). It has been reported in some studies that GnRH administration at AI does not affect pregnancy rates (Gümen *et al*., 2011; Perry and Perry 2009). Inducing ovulation by injecting exogenous GnRH before the expected LH wave may leads to shortening of luteal function and adversely affects fertility (Taponen, 2003). Pursley *et al*., (1995) pointed out that induction of ovulation can causes smaller than expected follicles to ovulate. The effect of GnRH on ovulation depends on many variables such as the presence of the LH wave during administration, the dose of GnRH (Sertkol and Sarıbay, 2017) and the time of administration (Mee *et al.*, 1990). GnRH has been reported is capable of inducing the ovulation of follicles larger than 10 mm in diameter in cows (Valenza *et al.*, 2012).

Having mainly an LH-like effect, and to a very limited extent FSH-like effect, hCG is used to support CL formation and increase the circulating progesterone level (Schmitt *et al.*, 1996). Administration of hCG during early pregnancy enables both ovulation of the dominant ovarian follicle and formation of an accessory CL. Hence, the level of plasma progesterone increases. hCG has a longer half-life than GnRH and can even induce responsiveness of follicles less than 10 mm in diameter (De Rensis *et al.*, 2010; Rajamahendran and Sianangama, 1992, Santos *et al.*, 2001). In cows administered hCG on day 5 after estrus, plasma progesterone levels rise up to day 13, which in turn increases embryo survival (Rajamahendran and Sianangama 1992, Santos *et al.*, 2001).

In this study, it was aimed to demonstrate the efficacy of GnRH and hCG, which are widely used to increase pregnancy rates in problematic herds, in healthy animals without reproductive, infectious or metabolic abnormalities and in which estrus were successfully detected. For this purpose, the effectiveness of a single dose of GnRH administration at AI and, in addition to this protocol, hCG administration 5 days after AI on conception rate were investigated. Thus, it was aimed to demonstrate the evaluability of these hormones as part of the routine AI protocol in healthy cows.

Material and Method

Animals, Management and Feeding

This study was conducted on a commercial dairy farm that housed animals in semi-open free stall barns and adhered to regular record-keeping rules. From the animals housed in the farm, 120 healthy Holstein cows, aged 3-6 years, with a body condition score of 3-3.25 were included in the study. Prior to the study, it was confirmed by clinical examination that the cows in the study were reproductively and metabolically healthy. The animals included in this study were did not receive any hormonal therapy for estrus induction. Due to the management strategy of the farm unit, estruses were not detected in cows before 70th day pp, since pregnancy is not desired before this day.

Throughout the study, the farm's routine management and nutrition schedule were followed. The cows were housed in free-stall paddocks and fed a total mixed ration (TMR). The composition of TMR is listed in Table 1. The animals were given ad libitum access to water and mineral blocks. Milking was performed twice a day, in the morning and evening, by the farm staff at a fixed system milking parlor. The average milk yield, day in milk (DIM) and lactation number of groups was given in Table 2.

Table 1. Feedstuffs (kg) in the total mixed ration.

Ingredients	Quantity (kg)
Corn silage	21
Wheat straw	4.5
Barley, flaked	1.6
Sunflower meal (28% CP)	3
Cottonseed meal (30% CP)	3.5
Wheat bran	1.3
Limestone	0.25
Salt	0.05
Vitamin mix	0.02

Estrus detection

Visual detection of estrus was done by experienced staff three times a day for at least 20 minutes. Estrus was characterized by cows showed restlessness, attempt to mount other females/ permit them to do so, licking and sniffing of external genitals, or the presence of vulvar mucus (Palmer *et al.*, 2012). Cows showing signs of spontaneous estrus underwent ovarian examination by transrectal real-time ultrasonography using a 6–8 MHz linear array transducer (Falko, Pie Medical, Netherlands). Cows standing estrus with a follicle larger than 10 mm diameter which is known to have capacity of ovulate (Sartori *et al.*, 2001) were selected for AI.

Groups, Artificial Insemination and Hormonal Treatments

Cows standing estrus were randomly assigned to three equal groups. Group I (n=40) received 10 µg of GnRH alone (Receptal®, 0.004 mg buserelin/mL, Intervet, France, intramuscularly) in AI. Group II (n=40) received 10 µg GnRH (Receptal®, 0.004 mg buserelin/mL, Intervet, France, intramuscular) in AI and 1500 IU hCG (Pregnyl® 3x1500 IU, MSD, Belgium) five days later. Group III (n=40) was maintained for control and did not receive any treatment during or after AI. AI was performed by the same veterinarian approximately 12 hours after the onset of estrus. Estruses did not show seasonal distribution and were observed all year round. However, to minimize the potential impact of the season, the AI was completed each month with approximately equal numbers of animals from each group.

Table 2. Mean values of some milk-related parameters in the groups.

	Group 1	Group 2	Group 3	P
Milk yield	20,31 ± 3,56	19,84 ± 4,11	21,03 ± 6,27	>0.05
Lactation number	2,64 ± 0,83	2,58 ± 0,92	2,61 ± 0,87	>0.05
DIM	93,49 ± 13,15	91,32 ± 11,58	92,63 ± 12,43	>0.05

Pregnancy Diagnosis

Pregnancy diagnosed 30 days after AI by transrectal ultrasonography. Pregnancy (conception) confirmed positive according to visualization of intact embryonic vesicle, embryo, and the heartbeat of embryo during ultrasound imaging. No other examination was done until the parturition. Pregnancy losses were determined by comparing conception and calving records.

Statistical Analysis

The average of the milk yield, lactation number and DIM data of the cows between the groups was determined by the one way ANOVA. The difference between the conception rates in the groups was made with the chi-square test. All statistical analyzes were performed using the SPSS 22.0 software package.

Results and Discussion

The pregnancy rates in groups are shown in Figure 1. Groups I and II significantly differed from the control group (Group III) for pregnancy rates ($P<0.05$), (Table 3). Single fetus abortion occurred at the second trimester of pregnancy in 2 cows, in Group 1 (1/40) and in Group 3 (1/40).

It was reported that the fertility of cows, which was around 90% in previous studies, decreased to approximately 70% on the 34th day of pregnancy due to embryonic deaths that may occur at a rate of 25% in the first three weeks of pregnancy (Fricke *et al.*, 1993, Mann and Lamming, 2001; Rosenberg *et al.*, 2003).

However, fertility rates have decreased as a result of breeding strategies that have increased milk yield over the years. To achieve the target of one calf per year from one cow, it is recommended to achieve conception within 83 days of postpartum. In this period shortened estrus and ovulation disorders may occur due to the high milk yield. Even if a successful conception is achieved, the pregnancy rate has decreased to 45% today with the effect of embryonic deaths (Walsh *et al.*, 2011). In a recent study, Rethmeier *et al.* (2019) suggested that the target of conception rate should be higher than 35% and the pregnancy rate should be higher than 24% in cow. In a study conducted in Holstein cows in Turkey, it was reported that the pregnancy rate at first insemination was 25.1% in Holstein cows. In the same study, it was reported that low fertility was caused by herd management and adaptation problems, therefore more attention should be paid to estrus detection and

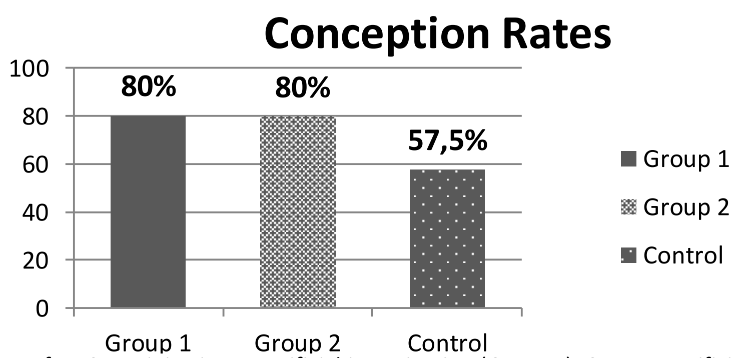

Figure 1. Conception rates after GnRH injection at artificial insemination (Group 1), GnRH at artificial insemination and hCG injection 5 days later (Group 2) and no treatment (Control).

Table 3. Conception numbers in groups.

	Group I	Group II	Group III	P
Conception (x/n)	32/40^{a}	32/40^{a}	23/40^{b}	0.034

a, b: Groups with different superscripts in the same line display statistically significant differences (*P*<0.05) insemination activities after the calving (Özdemir *et al.*, 2022). In addition to individual milk yield and number of days in milk, average herd milk yield was also shown to be effective on conception. Rearte *et al.*, (2018) reported that the pregnancy rate decreased by 1.3% in cows producing 8 kg/day more milk than the herd average in 63 DIM in high-yielding herds, and the pregnancy rate decreased by 14.8% in the same day in low-yielding herds. In the same study, it was reported that such a hazard of pregnancy effect was not observed on milk yield at 100 DIM in high milk yielding animals in herds with low milk yield. In this study, conception rates of all groups were found to be higher than in many other studies. First, the cows were medium to low milk yielding animals and are in the 70-120 DIM period (Table 2). We think that the harmful effects of lactation on conception was begin to decrease in this period. Secondly, the presence of dominant follicles was demonstrated by USG confirmation in cows whose estrus was detected by observation. We think that this double confirmation contributed to the increase in the pregnancy rate. In addition, the fact that the animals included in the study were completely healthy contributed to this result.

Steroid hormones are broken down faster in high-yielding dairy cows due to their high metabolic rate. Thus, it takes time for estradiol and progesterone levels in the bloodstream to increase. A slow rise in blood estradiol level prolongs the induction of the preovulatory LH surge, which both delays ovulation and reduces the quality of the oocyte due to prolonged exposure to LH surges (Wiltbank *et al.*, 2012; Yeşilkaya and Erdem, 2021). When GnRH agonists are administered just before or during the LH surge, they can increase the pregnancy rate of cows by enhancing the spontaneous preovulatory LH surge (Morgan and Lean, 1993). GnRH has found wide use in cows due to its ovulation-stimulating effect and subsequent positive effect on luteinization (Rosenberg *et al.*, 2003; Wiltbank *et al.*, 2012). However, the effect of GnRH depends on the diameter of the preovulatory follicle present in the ovary during administration. It has been reported that the mean follicle diameter during ovulation in lactating cows is 17.2±0.5 (Sartori *et al.*, 2004). In beef cattle, the pregnancy rate was 53% in the presence of follicles with a diameter of 14.5 mm, and 35% if ovulation was induced in the presence of a follicle diameter of 10.3 mm or less (Perry *et al.*, 2005). When ovulation of a small follicle is induced, less estrogen is synthesized; CL volume and the ability to synthesize progesterone are insufficient (Vasconcelos *et al.*, 2001). Keskin et al., (2016) reported that cows with a preovulatory follicle diameter between 13.5 and 17.5 have a higher chance of pregnancy. Colazo et al. (2015) reported that an ovulatory follicle diameter of 14 mm and above does not change the predicted pregnancy probability, but a higher rate of late embryonic/early fetal mortality can be observed in cows with an ovulatory follicle diameter greater than 20 mm. In this study, the lowest follicle diameter that could respond to GnRH injection was accepted as 10 mm. Mean follicle diameters of the groups were not noted. We administered GnRH (Buserelin acetate, 10 µg) to group I and group II during AI, and obtained a higher pregnancy rate in these groups compared to the control group (P<0.05). The conception rate, which was particularly low in the control group, may be due to the 10 mm criterion we have chosen. GnRH administration shortens the estrus-LH peak and estrus-ovulation intervals and induces ovulation within 8-12 h post-insemination (Elmore, 1989; Jainudeen and Hafez, 2000; Morotti *et al.*, 2021). In this study, there may be animals whose follicle diameters have not yet reached the preovulatory size during AI. Ovulation can be induced in most of the cows in the GnRH administered groups, in accordance with the literature knowledge. However, the ovulation process would take longer time in animals in the same condition in the control group. This possibility may have had an effect on the conception process. However, in order to say that GnRH has an effect on conception, ovulation monitoring should be done, which was not done in this study.

Different pregnancy rates have been reported in studies examining the effects of GnRH analogues administration on pregnancy rates during AI. Shephard *et al.* (2014) reported that GnRH administration at AI increased the pregnancy rate by 11%. Shahneh *et al.* (2008) reported that administration of 15 µg of Gonadorelin during AI increased the pregnancy rate more than twofold compared to the non-administered control (55% vs 25%). Similarly, Iftikhar *et al.* (2009) found that administration of 50 µg Lecirelin acetate during AI increased the pregnancy rate 1.8 times (37.5% vs 68.75%). In the present study, we determined that the pregnancy rate of group I was 22.5% (or 1.39 times) higher than that of the control group ($P<0.05$). The pregnancy rate we obtained seems to be slightly higher than other studies. This is thought to be due to differences in the GnRH analogue administered, the dose of administration and the individual response of the animals. In this study, we selected healthy cows with moderate milk yield, in good condition, whose estruses were successfully detected, as we aimed to reveal the effects of GnRH and hCG in healthy cows. It can be said that the pregnancy rates obtained in this study reflect the pregnancy rates in optimal conditions.

It has been reported that administration of hCG to support luteal tissue after AI increases pregnancy rates (Ideta *et al.* 2003, Pandey *et al.* 2016). In contrast, some studies found no difference in pregnancy rates (Walton *et al.*, 1990, Niles *et al.*, 2019) despite marked increase in serum progesterone concentrations with the administration of hCG in early luteal phase (Shams-Esfandabadi *et al.*, 2007). Zheng *et al.*, (2021) reported that injection of hCG 5 days after AI was beneficial to the function luteal tissue and receptivity, although improvement in pregnancy rates was not-significant. In a study in which GnRH (Buserelin) was administered during ovulation in addition to hCG in the later period of the luteal phase (day 12), Paksoy and Kalkan, (2010) reported that the administration of hCG was ineffective. On the other hand, Zolini *et al*. (2019) applied hCG treatment 5 days after insemination and found that the increase in pregnancy rate was related to the genotype of individual cows. In our study, conception rates of Group I and Group II was the same (P>0,05). It is very difficult to say whether the result obtained here is due to the ineffectiveness of hCG or for other reasons that we did not reveal. In order to make an undisputable comment on this issue, it was necessary to evaluate another group that was not injected with GnRH during AI, but was injected only with hCG. Our results show that both protocols have a significant effect in increasing pregnancy rates in healthy animals.

Conclusion

We conclude that administration of GnRH alone during artificial insemination (AI) and in combination with hCG 5 days after GnRH injection increases conception rates in healthy pp lactating cows. Both protocols can be used to increase the conception rate. The use of hCG alone is recommended to reveal its efficacy on conception rates in future studies.

Author contributions: All authors contributed equally to the study.

Conflicts of interest: The author declare no conflicts of interest.

Ethical approval: This study was conducted according to the 19/02/2020 dated and 2020/02-7 numbered approval of the Local Ethics Board for Animal Experiments. In addition, the authors declared that Research and Publication Ethical rules were followed.

References

Besbaci, M., Abdelli, A., Minviel, J. J., Belabdi, I., Kaidi, R., & Raboisson, D. (2020). Association of pregnancy per artificial insemination with gonadotropin-releasing hormone and human chorionic gonadotropin administered during the luteal phase after artificial insemination in dairy cows; A meta-analysis. *Journal of Dairy Science*, 103(2), 2006-18. doi: 10.3168/jds.2019-16439

Boulton, A. C., Rushton, J., & Wathes, D. C. (2017). An empirical analysis of the cost of rearing dairy heifers from birth to first calving and the time taken to repay these costs. *Animal*, 11(8), 1372-80. doi: 10.1017/S1751731117000064.

Canadas, E. R., Gobikrushanth, M., Fernandez, P., Kenneally, J., Lonergan, P., & Butler, S. T. (2019). Evaluation of alternative strategies to treat anoestrous dairy cows and implications for reproductive performance in pasture-based seasonal calving herds: A pilot study. *Theriogenology*, 127, 130-36. doi: 10.1016/j.theriogenology.2019.01.008.

Colazo, M. G., Behrouzi, A., Ambrose, D. J., & Mapletoft, R. J. (2015). Diameter of the ovulatory follicle at timed artificial insemination as a predictor of pregnancy status in lactating dairy cows subjected to GnRH-based protocols. *Theriogenology*, 84(3), 377-83. doi: 10.1016/j.theriogenology.2015.03.034

De Rensis, F., Lopez-Gatius, F., Garcia-Ispierto, I., & Techakumpu, M. (2010). Clinical use of human chorionic gonadotropin in dairy cows: an update. *Theriogenology*, 73(8), 1001-08. doi: 10.1016/j.theriogenology.2009.11.027

De Vries, A. (2006). Economic value of pregnancy in dairy cattle. *Journal of Dairy Science*, 89, 3876-85. doi: 10.3168/jds.S0022-0302(06)72430-4

Elmore, R. G. (1989). Using GnRH, hCG, and anterior pituitary extracts in bovine hormonal therapy. *Veterinary Medicine*, 2, 214-16.

Fricke, P. M., Reynolds, L. P., & Redmer, D. A. (1993). Effect of human chorionic gonadotropin administered early in the estrous cycle on ovulation and subsequent luteal function in cows. *Journal of Animal Science*, 71(5), 1242-46. doi: 10.2527/1993.7151242x

Gümen A., Keskin A., Yilmazbas-Mecitoglu G., Karakaya E., Cevik S., Balci F. (2011). Effects of GnRH, PGF2α and oxytocin treatments on conception rate at the time of artificial insemination in lactating dairy cows. *Czech Journal of Animal Science*, 56, 279-283.

Ideta, A., Iwasa, S., Takedomi, T., Konishi, M., Sakai, S., Okamoto, Y., Urakawa, M., Miyamoto, A., & Aoyagi, Y. (2003). Pregnancy rates in recipient heifers after administration of human chorionic gonadotropin or a gonadotropin-releasing hormone agonist on day five of the estrous cycle. *Theriogenology*, 9, 359 (abstract).

Iftikhar, A., Anjum, R. H., Usmani, M. T., Tunio, A., & Abro, S. H. (2009). Improvement of conception rate in crossbred cattle using GnRH analogue therapy. *Pakistan Veterinary Journal*, 29, 93-4.

Jainudeen, M. R., & Hafez, E. S. E. (2000). Cattle and buffalo. In: B. Hafez, E. S. E. Hafez (Eds.,) Reproduction in Farm Animals (pp. 159-171). 7th Ed., Baltimore: Lippincott Williams & Wilkins.

Kashyap, A., Singh, M., Sharma, A., & Kumar, P. (2018). Comparison of conception rate following GnRH and hCG based synchronization protocols in functionally infertile cows. *The Indian Journal of Animal Science*, 88(11), 1266-68.

Keskin, A., Mecitoğlu, G., Bilen, E., Güner B., Orman, A., Okut, H., & Gümen, A. (2016). The effect of ovulatory follicle size at the time of insemination onpregnancy rate in lactating dairy cows. *Turkish Journal of Veterinary & Animal Sciences*, 40(1), 68-74. doi:10.3906/vet-1506-59

Mann, G. E., & Lamming, G. E. (2001). Relationship between the maternal endocrine environment, early embryo development and the inhibition of the luteolytic mechanism in the cow. *Reproduction*, 121, 175-80. doi: 10.1530/rep.0.1210175

Mee, M. O., Stevenson, J. S., Scoby, R. K., & Folman, Y. (1990). Influence of gonadotropin-releasing hormone and timing of insemination relative to estrus on pregnancy rates of dairy cattle at first service. *Journal of Dairy Science*, 73(6), 1500-1507. doi: 10.3168/jds.S0022-0302(90)78817-0

Morgan, W. F., & Lean, I. J. (1993). Gonadotrophin-releasing hormone treatment in cattle: a meta-analysis of the effects on conception at the time of insemination. *Australian Veterinary Journal*, 70(6),205-9. doi: 10.1111/j.1751-0813.1993.tb03304.x

Morotti, F., Lorenzetti, E., & Seneda, M. M. (2021). Artificial Insemination Program in Cattle. In: V. K. Yata, A. K. Mohanty, & E. Lichtfouse, (Eds.), Sustainable Agriculture Reviews 54. (pp. 1-53). Springer, Cham. Doi: 10.1007/978-3-030-76529-3

Niles, A. M., Fricke, H. P., Carvalho, P. D., Wiltbank, M. C., Hernandez, L. L., & Fricke, P. M. (2019). Effect of treatment with human chorionic gonadotropin 7 days after artificial insemination or at the time of embryo transfer on reproductive outcomes in nulliparous Holstein heifers. *Journal of Dairy Science*, 102, 2593-606. doi: 10.3168/jds.2018-15588

Özdemir, B. M., Koçak, Ö. & Özcan, M. (2022). Factors affecting fertility traits and milk yield of Holstein cattle with different origins raised in Trakya region. *Journal of Istanbul Veterinary Sciences*, 6 (1), 6-17 . doi: 10.30704/http-www-jivs-net.1066914

Paksoy, Z., & Kalkan, C. (2010). The effects of GnRH and hCG used during and after artificial insemination on blood serum progesterone levels and pregnancy rate in cows. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 16(3), 371-5.

Palmer, M. A., Olmos, G., Boyle, L. A., & Mee, J. F. (2012). A comparison of the estrous behavior of Holstein-Friesian cows when cubicle-housed and at pasture. *Theriogenology*, 77(2), 382-388. doi: 10.1016/j.theriogenology.2011.08.010

Pandey, N. K. J., Gupta, H. P., Prasad, S., & Sheetal, S. K. (2016). Plasma progesterone profile and conception rate following exogenous supplementation of gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone releasing intra-vajinal device in repeat-breeder crossbred cows. *Veterinary World*, 9(6), 559-62. doi: 10.14202/vetworld.2016.559-562

Perry G. A., & Perry B. L. (2009). GnRH treatment at artificial insemination in beef cattle fails to increase plasma progesterone concentrations or pregnancy rates. *Theriogenology*, 71, 775-9. doi: 10.1016/j.theriogenology.2008.09.050

Perry, G. A., Smith, M. F., Lucy, M. C., Green, J. A., Parks, T. E., MacNeil, M. D., Andrew, J. R., & Thomas, W. G. (2005). Relationship between follicle size at insemination and pregnancy success. *Proceedings of the National Academy of Sciences of the United States of America*, 102(14), 5268-73. doi: 10.1073/pnas.0501700102

Pursley, J. R., Mee, M. O., & Wiltbank, M. C. (1995). Synchronization of ovulation in dairy cows using PGF2α and GnRH. *Theriogenology*, 44(7), 915-23. doi: 10.1016/0093-691x(95)00279-h

Rajamahendran, R., & Sianangama, P. C. (1992). Effect of human chorionic gonadotrophin on dominant follicles in cows: formation of accessory corpora lutea, progesterone production and pregnancy rates. *Journal of Reproduction and Fertility*, 95(2), 577-84. doi: 10.1530/jrf.0.0950577

Rearte, R., LeBlanc, S. J., Corva, S. G., de la Sota, R. L., Lacau-Mengido, I. M., & Giuliodori, M. J. (2018). Effect of milk production on reproductive performance in dairy herds. *Journal of Dairy Science*, 101(8), 7575-84. doi: 10.3168/jds.2017-13796

Rethmeier, J., Wenzlau, M., Wagner, M., Wiedemann, S., & Bachmann, L. (2019). Fertility parameters in German dairy herds: Associations with milk yield and herd size. *Czech Journal of Animal Science*, 64(11), 459-464. doi: 10.17221/206/2019-CJAS

Rosenberg, R. M., Wolfenson, D., & Bloch, A. (2003). Effects of GnRH administered to cause at the onset of estrus on timing of ovulation and conception. *Journal of Dairy Science*, 86, 2012-21. doi: 10.3168/jds.S0022-0302(03)73790-4

Santos, J. E. B., Thatcher, W. W., Pool, L., & Overton, M. W. (2001). Effect of human chorionic gonadotropin on luteal function and reproducive performance of high-producing lactating Holstein dairy cows. *Journal of Animal Science*, 79(11), 2881-94. doi: 10.2527/2001.79112881x

Sartori, R., Haughian, J. M., Shaver, R. D., Rosa, G. J. M., & Wiltbank, M. C. (2004). Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. *Journal of Dairy Science*, 87(4), 905-20. doi: 10.3168/jds.S0022-0302(04)73235-X

Sartori, R., Fricke, P. M., Ferreira, J. C. P., Ginther, O. J., Wiltbank, M. C. (2001). Follicular deviation and acquisition of ovulatory capacity in bovine follicles. *Biology of Reproduction*, 65, 1403-09. doi: 10.1095/biolreprod65.5.1403

Schmitt, E. J. P., Diaz, T., Barros, C. M., De la Sota, R. L., Drost, M., Fredriksson, E. W., Staples, C. R., Thorner, R., & Thatcher, W. W. (1996). Differential response of the luteal phase and fertility in cattle following ovulation of the first-wave follicle, with human chorionic gonadotropin or an agonist of gonadotropin-releasing hormone. *Journal of Animal Science*, 74(5), 1074-83. doi: 10.2527/1996.7451074x

Sertkol, R. & Sarıbay, M. K. (2017). Laktasyondaki İneklerde Tohumlama Anında Azaltılan GnRH Dozunun Gebelik Oranı Üzerine Etkisi. *Harran Üniversitesi Veteriner Fakültesi Dergisi*, 6 (1) , 46-50.

Shahneh, A. Z., Mohammadi, Z., Fazeli, H., Babak, M. M. S., & Dirandeh, E. (2008). The effect of GnRH injection on plasma progesterone concentrations, conception rate and ovulation rate in Iranian Holstein cows. *Journal of Animal and Veterinary Advances*, 7(9), 1137-41. doi: 10.1017/S1752756200027095

Shams-Esfandabadi, N., Shirazi, A., Mirshokrai, P., & Bonyadian, M. (2007). Influence of hCG administration after AI on conception rates and serum progesterone concentration in cattle. *Pakistan Journal of Biological Sciences*, 10(16), 2709-13. doi: 10.3923/pjbs.2007.2709.2713

Taponen, J. (2003). Ovarian function in dairy cattle after gonadotropin-releasing hormone treatments during perioestrus [Doctoral Thesis, University of Helsinki]. Auditorium Maximum, Hämeentie 57, Helsinki.

Taponen, J., Katila, T., & Rodríguez-Martínez, H. (1999). Induction of ovulation with gonadotropin releasing hormone during proestrus in cattle: influence on subsequent follicular growth and luteal function.

Animal Reproduction Science, 55(2), 91-105. doi: 10.1016/s0378-4320(99)00011-1

Valenza, A., Giordano, J. O., Lopes Jr, G., Vincenti, L., Amundson, M. C., & Fricke, P. M. (2012). Assessment of an accelerometer system for detection of estrus and treatment with gonadotropin-releasing hormone at the time of insemination in lactating dairy cows. *Journal of Dairy Science*, 95(12), 7115-27. doi: 10.3168/jds.2012-5639

Vasconcelos, J. L. M., Sartori, R., Oliveira, H. N., Guenther, J. G., & Wiltbank, M. C. (2001). Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology*, 56(2), 307-14. doi: 10.1016/s0093-691x(01)00565-9

Walsh, S. W., Williams, E. J., & Evans, A. C. O. (2011). A review of the causes of poor fertility in high milk producing dairy cows. *Animal Reproduction Science*, 123(3-4), 127-138. doi: 10.1016/j.anireprosci.2010.12.001

Walton, J. S., Halbert, G. W., Robinson, N. A., & Leslie, K. E. (1990). Effects of progesterone and human chorionic gonadotrophin administration five days postinsemination on plasma and milk concentrations of progesterone and pregnancy rates of normal and repeat breeder dairy cows. *Canadian Journal of Veterinary Research*, 54(3), 305-8.

Willmore, A., & Davis, T. L. (2019). Effect of chronic administration of a gonadotropin-releasing agonist on luteal function and pregnancy rates in dairy cattle. *Animal Science Journal,* 90(11), 1432-43. doi: 10.1111/asj.13284

Wiltbank, M. C., Souza, A. H., Carvalho, P. D., Bender, R. W., & Nascimento, A. B. (2012). Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. *Reproduction, Fertility and Development*. 24(1), 238-43. doi: 10.1071/RD11913

Yeşilkaya, Ö. F., & Erdem, H. (2021). Sütçü İneklerde Döl Veriminin Artırılmasına Yönelik Tohumlama Sırası/Sonrası GnRH Uygulamaları. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, 16(1):101-9. doi: 10.17094/ataunivbd.761446

Zheng, P., Feng, R., Huang, F., Hou, L., Wang, M., Adeniran, S.O. & Huang, H. (2021). GnRH and hCG improve the function of corpus luteum and uterine receptivity at timed artificial insemination in postpartum dairy cows. *Pakistan Veterinary Journal*, 41(3): 347-52. doi: 10.29261/pakvetj/2021.027

Zolini, A. M., Ortiz, W. G., Estrada-Cortes, E., Ortega, M. S., Dikmen, S., Sosa, F., Giordano, J. O. & Hansen, P. J. (2019). Interactions of human chorionic gonadotropin with genotype and parity on fertility responses of lactating dairy cows. *Journal of Dairy Science*, 102(1), 846-56. doi: 10.3168/jds.2018-15358