

Influence of gonadotropin treatment after insemination on dairy cows to maintain pregnancy, and milk progesterone concentration

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Summary

The aim of the study is to evaluate the possibility of maintenance of cow pregnancy applying GnRH treatment after AI. The experiment was carried out on Lithuanian black-white lactating dairy cows on 90-100 DM. Were selected 45 cows on their 2nd and 3rd lactation with milk yield of 8.500-9.000 kg. The cows with 16 mm or greater ($n = 26$) CL in ovaries were selected for the experiment. All cows were given the injections of dinoprost solution 25 µg. AI was performed on the cows ($n = 20$) that showed oestrus. The first group (experimental) of cows ($n = 10$) were repeatedly given the injections of gonadorelin 100 µg im on the 7th day after AI. The second group (control) of cows ($n = 10$) were given the injections of 0.9% saline solution on the 7th day after AI. The ultrasound measurement was performed to determine the size of ovaries, and CL's. Concentration of P4 were detected in milk samples. Ultrasound testing as well as milk P4 determination were carried out 4 times in total, every 7 days starting on the day of AI. After 7 days following the injection of GnRH, and positive increase of the number of cows with accessory CL by 56% was determined, comparing with the second (control) group. The tendency of increasing size of CL in both groups after AI was noticed. The changes in the ovary size were similar in both groups. The average number of CL in the first group was by 36% ($P < 0.05$) higher than in the second group (1.5 and 1.1). The change of P4 was similar in both groups. The number of pregnant cows in the first group was by 10% higher than in the second group.

Keywords: cow pregnancy, gonadotropin, corpus luteum, insemination

The estimates of fertilization rates in dairy heifers fall within the range of 97-100%. However, in dairy cows they are more variable, falling within the range of 85-100%, not taking into account the milk yield (26). The estimate of the calving rate is the percentage of mated or AI cows that have been actually calved. The calving rate varies from 60% to 73.8% from AI cows (6, 25). Development during the embryonic period of pregnancy (until about the 42nd day of pregnancy, when the foetus can be delineated) is a complicated phenomenon involving, among other processes, embryonic genome activation, compaction, blastocyst formation, elongation of the trophoblast, secretion of interferon-tau (IFN τ) to maintain the corpus luteum (CL), differentiation of placental tissues, and development of the inner cell mass into the foetus (8). Any disorder of the

system may cause embryo mortality. Some embryos are lost in the days immediately after fertilization and up to the 10th-13th day of gestation. It is due to a failed embryo development. A significant increment in the total losses of embryos (20-25%) occurs before the 19th day after AI. It is caused by the failure of the embryo to prevent luteolysis (recognition of the embryo) and to maintain the secretion of progesterone (P4) necessary for its continuing development. Late embryo losses are around 5%-10% (16). This suggests that early embryonic mortality play a significant role in the establishment of cow pregnancy. The maternal body recognizes the embryo approximately on the 15th-20th day after the fertilisation. Placentation occurs during this period. The role of steroids is very important at this time. Progestins or human chorionic gonadotropin

injections increases pregnancy of cows after transplantation of embryos (10, 27). Early postovulatory uterine, luteal function and concentrations of hormones associated with trophoblastic and endometrial function during the maternal recognition of pregnancy are the factors that affect pregnancy (29). P4 supplementation (on the 5th-9th day after AI), increases embryo development and the production of IFN τ during the postovulatory rise (15). IFN τ of embryonic origin is as an agent of pregnancy recognition in ruminants (2). It is possible that P4 supplementation has a positive effect on the maintenance of pregnancy; however, the data are contradictory. According to Arndt et al. (1), P4 supplementation after AI had no effect on the pregnancy rate. In addition, it did not increase the circulating levels of P4 in lactating dairy cows in early pregnancy. It was observed that P4 supplementation during a timed AI increased the number of pregnancies per AI mostly in cows without CL, and reduced the risk of pregnancy loss in lactating dairy cows. However, AI of cows in oestrus during the synchronization protocol eliminated the benefit of supplemental P4 (4). Treatment with human chorionic gonadotropin had an impact on the follicular waves following AI and caused their ovulation. Accessory CL produces P4 and increases its concentration in blood (18). The administration of gonadotropin-releasing hormone (GnRH) post AI can improve the maintenance of pregnancy. GnRH induces the production of luteinizing hormone (LH) and influences follicle ovulation. It causes the formation of new CL and increases the level of P4. According to the results of Willard et al. (28), treatment with GnRH after AI increased serum concentrations of P4 and tended to increase pregnancy rates in cows exposed to mild summer heat stress. In general, to improve pregnancy rates in cows, in a number of studies GnRH was used between the 11th and 14th day after AI. However, the published results were not consistent (21). Nevertheless, treatment with GnRH between 11 and 13 days after AI depresses the luteolytic drive via reduced plasma oestradiol-17 β concentrations and the oestradiol-P4 ratio and improves the maintenance of pregnancy (19).

The aim of the study is to evaluate the possibility of maintenance of cow pregnancy by GnRH treatment after AI.

Material and methods

The experiment was carried out on Lithuanian black-white lactating dairy cows on 90-100 DM. Were selected 45 cows on their 2nd and 3rd lactation with milk yield of 8.500-9.000 kg. The cows were kept in loose housing system and fed according to their physiological needs and milk yield. Their daily ration was as follows: maize silage – 24 kg, haulage – 11 kg, barley straw – 0.8 kg, molasses – 0.5 kg, and composite feed – 8.74 kg with the composition of dried sugar-beet slices (20.59%), soybean meal (with 49% protein content) (17.16%), triticale (34.32%), rape

cake (22.88%), Bergophor CaProVit H mineral (2.29%), fodder limescale (0.8%), salt (NaCl) (0.34%), monocalcium phosphate (0.69%), and sodium bicarbonate (0.93%). The change of ovaries and pregnancy were examined with Digital Diagnostic Ultrasound Devices (HG 9300 series EV/14090030/type B/model HG-9300) ultrasound scanner from Caresono Technology Co., Ltd at the frequency of 6.5 MHz, using a linear rectal transducer. The size of ovary was calculated as the product of two perpendicular diameters. The cows with 16 mm or greater ($n = 26$) CL in ovaries were selected for the experiment. All cows were given the injections of dinoprost solution 25 μ g. AI was performed on the cows ($n = 20$) that showed oestrus. To determine the presence of oestrus, the cows were inspected before the morning milking and an hour after the evening milking. The cows that showed oestrus were determined by the presence of standing reflex. Having observed the first signs of oestrus (60 hours after the injection of dinoprost), all cows were given the injections of gonadorelin 100 μ g *im*. AI was performed six hours following the injections. All AI cows were divided into two groups. The first group (experimental) of cows ($n = 10$) were repeatedly given the injections of gonadorelin 100 μ g *im* on the 7th day after AI. The second group (control) of cows ($n = 10$) were given the injections of 0.9% saline solution on the 7th day after AI. Milk samples were taken from all cows (30 ml, after milking) and ultrasound measurements were performed to determine the size of ovaries. The ultrasound measurement was performed to determine the size of ovaries, and CL. The size of ovaries and CL were calculated 4 times in total every 7 d starting on the day of the AI. Cow pregnancies were determined on the 28th day after AI. Concentrations of P4 were detected in milk samples. Concentrations of P4 were estimated with Hormonost Microlab (Germany), using the reagents of BioLab GmbH (Germany).

Statistical analysis was performed using SPSS for Windows 15 (SPSS Inc., Chicago, IL, USA). The analysis was performed using descriptive (ANOVA) model and Pearson correlation coefficient. The differences were determined by Student's t-test. The data was statistically significant when $P \leq 0.05$.

Results and discussion

The main hormone controlling the uterine environment is P4, and circulating plasma concentrations of P4 were closely correlated with early embryo development (15). The earliest day on which a dominant follicle is large enough to respond consistently to GnRH is day 5 (5). GnRH injections are applied from day 4 to day 15 after AI to prevent early embryonic death. The aim of these applications is to stimulate secondary CL formation and P4 secretion (11, 20). A significant maintenance of pregnancy in dairy cattle after administration of GnRH is not evident on the 5th day after AI. A similar study reported that the administration of GnRH on day 5 or day 15 after AI did not increase pregnancy rate but reduced pregnancy losses on days 27-55 (3). Howard et al. (9) indicated that GnRH administered on day 5 after AI did not significantly improve conception rates (CR) in dairy cattle (CR by

2% higher after treatment). Kaygusuzoglu et al. (11) proved that pregnancy rates of heifers were higher in the group treated with GnRH than those in the group treated with saline. But by ours data, treatment with GnRH after AI increased the number of pregnant cows by 10%. It was observed that the injection of GnRH on the 6th day after the ovulation increased the total area of luteal tissue. It was generated as a result of formation of an accessory CL (7). The changes in the ovary size were similar in both groups. Maximum average size was determined on the 14th day after AI. The tendency of the size of CL changing similarly as the ovary size was noticed. According to the provided data, no evident effect of GnRH on the luteal function by size of ovary was identified (Fig. 1).

Average number of CL in the first group was by 36% ($P < 0.05$) higher than in the second group (1.5 and 1.1). A higher number of CL is related to a higher number of pregnant cows in the second group (Fig. 2). One matured CL is sign of one ovulation. In the first group, seven days after the second GnRH injection, the number of cows with accessory CL increased by 56%, comparing with the second group. In the second group, no increase in accessory CL was observed. On the 21st day after AI, in both groups, the number of cows with single CL decreased, which was caused by oestrus. The number of cows without CL were also similar in both groups. Following our data, the tendency of increasing number of accessory CL and CL size on the 14th day after AI was determined. The treatment with GnRH had an impact on the total count of CL. The total count of CL on the 14th day after AI or on the 7th day after treatment was significantly higher in the first group. The results were confirmed by another study, where GnRH was injected on the 5th-7th d and 11th-13th d after AI. A higher occurrence of accessory CL was noticed on 5th-7th day (43.9% vs 33.5%) (17). Increase in CL tissue after administration of GnRH after AI was also observed in another study (28).

After AI, the concentration of P4 increases along the maturation of CL. The change of P4 was similar in both groups: no significant differences were found. No apparent effect of GnRH on P4 was identified (Fig. 3).

Correlation between the size of ovaries was identified on the 7th and 21st day after AI $r = 0.8$, ($P < 0.05$) and on the 14th and 21th day ($r = 0.7$), ($P < 0.05$) No significant correlation between the sizes of ovaries, those of CL, and the concentration of P4 was identified. Correlation between the size of ovaries on 7th day after AI and pregnancy was determined. After AI, the level of P4 was in correlation with the number of pregnant cows as follows: $r = 0.5$, $P < 0.05$ on the 14th day and $r = 0.7$, $P < 0.05$ on the 21th day. Although no higher P4 concentrations and higher number of accessory CL was identified, the number of pregnant cows in the first group was by 10% higher than in the second group.

It was demonstrated that P4 supplementation during the postovulatory rise increases embryo development

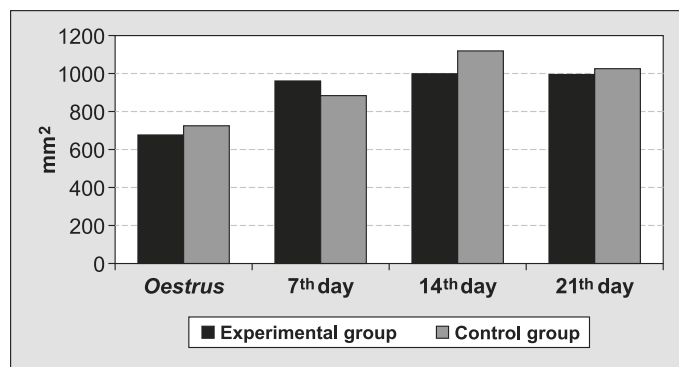


Fig. 1. Average ovary size in mm² after AI. Ovaries changed in size during the oestrous cycle. No significant difference between the control and the experimental group.

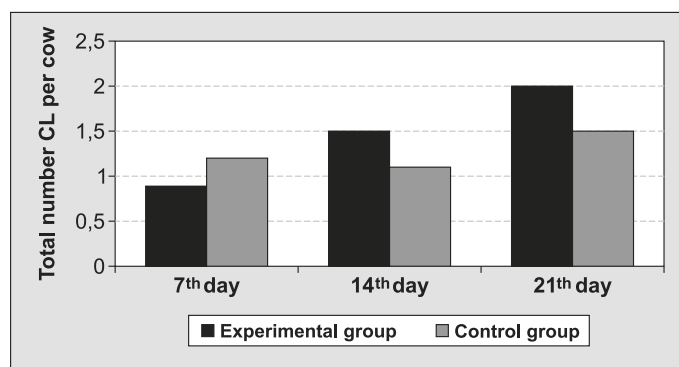


Fig. 2. Total number of CL per cow. The number of CL per cow in the experimental group was higher than in the control group. The data presented in the diagram: total number of CL per cow.

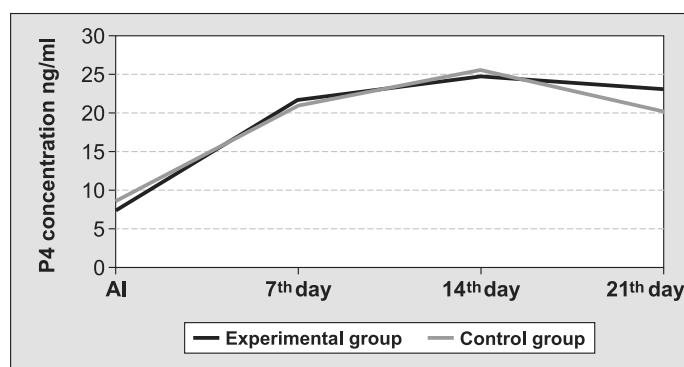


Fig. 3. P4 concentration in milk after AI. It was estimated that the maximum level of P4 occurred in the middle of the estrous cycle. The change of P4 similar in both groups.

and IFN τ production (15). It substantiated the use of P4 to maintain pregnancy. Pregnancy losses were not observed in females in which the injection of hCG or GnRH induced the establishment of two new luteal structures (30). The development of accessory CL led to a higher P4 concentration. Correlation ($r = 0.85$) between the diameter of CL and P4 concentration in plasma was high (22). Several authors reported that positive associations between CL size and circulating P4 concentrations depended on the stage of the oestrous cycle (12, 13, 24). The benefit of P4 in reducing pregnancy loss, however, was observed regardless the

presence of CL (23). This study supports the outcomes showing that administration of GnRH after AI does not result in the increase of P4 concentration.

GnRH increased the number of accessory CL, but did not facilitate the increase of P4 concentration and did not support the maintenance of pregnancy.

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