

Effect of synthetic GnRH analogues on ovarian follicular growth dynamic and oestrus

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Summary

The aim of the present study was to evaluate the stimulating effect of synthetic GnRH analogues without the use of prostaglandins on ovarian follicular growth dynamic and oestrus. The study was made on 30, 3 to 9-year-old Lithuanian Black and White (LBW) and German Black and White (GBW) breed cows. The study was conducted in the Lithuanian Veterinary Academy practical training and research farm. Oestrus in 23 cows was stimulated using synthetic GnRH analogues Dalmarelin (Lecirelin) (Fatro S. p. A., Italy) and in 14 cows with the use of Depherelin® (Gonavet® 50, Veyx-Pharma GmbH, Germany). Dalmarelin (Lecirelin) induced oestrus in 100% of the stimulated cows, and Depherelin® (Gonavet® 50) induced oestrus in 92.9 % of stimulated cows.

Following an injection of synthetic GnRH analogues, preparations Depherelin® and Dalmarelin, plasma progesterone concentration decreased, due to the ovulation of dominant follicle or onset of the new follicular wave.

Cows stimulated with Dalmarelin had a faster follicular growth rate and bigger size of follicles at ovulation compared to Depherelin® and control group cows ($p = 0.05$). In the pregnancy trial that followed 45 days after artificial insemination, the highest percentage (43.5 %) of pregnant cows was determined in the group of cows stimulated with Dalmarelin.

Keywords: ovaries, follicles, oestrus

GnRH-induced ovulation or atresia of the dominant follicle is followed by a new wave of follicular emergence within 3 to 4 days of treatment at any stage of oestrus cycle (21). Administration of GnRH also induces an FSH increase at any stage of the oestrous cycle (16). Thus, in cows treated with GnRH after the selection of a dominant follicle, gonadotropin surge is followed by a transient FSH increase, that is associated with the emergence of a new follicle wave. When GnRH treatment is applied before the selection of the dominant follicle, follicular growth is not affected (16). Treatment solely with GnRH preparations was used in the field to induce the ovulation before AI or to enhance conception rate (19), but there are no data concerning application of GnRH for oestrus and follicular growth stimulation. In contrast to some positive results, there was no favorable effect of similar treatments of GnRH or its analogues on pregnancy rates in other studies (1, 15). The aim of this study was to evaluate the stimulating GnRH effect on oestrus and influence on the changes of follicular growth dynamic, plasma progesterone (P_4) profile and pregnancy rate was evaluated.

Material and methods

In total, 40 experimental cows aged from 3 to 9 years and representing Lithuanian Black and White (LBW) and German Black and White (GBW) breeds were used in the present trial. All cows had disturbances in oestrus behavior and were inseminated previously for two or more oestrus cycles, but did not conceive. They were selected for oestrus stimulation, estimation of follicular growth dynamic and pregnancy detection with synthetic GnRH analogues: preparation Dalmarelin (Lecirelin, Fatro S. p. A., Italy) (Dalmarelin) and Depherelin® (Gonavet® 50, Veyx-Pharma GmbH, Germany) (Depherelin®). Experimental cows were divided into four groups depending on the size of the follicles determined at inspection, and based on the preparation used for oestrus stimulation. The control group ($n = 3$) consisted of cows that were previously inseminated two or more times, but still attain oestrus without stimulation, and were inseminated. In group I ($n = 7$) and in group III ($n = 6$) were cows having follicles of 16.5-17 mm diameter in the right or left ovary. In groups II ($n = 16$) and IV ($n = 8$) were cows with follicles smaller than 16.5-17 mm in diameter.

On first experimental day the size of follicles was estimated and blood samples were taken for P_4 analysis.

During the same day, 2 ml of Dalmarelin was intramuscularly injected to the cows of experimental groups I and II and 2 ml of Depherelin[®] was intramuscularly injected to the cows of groups III and IV. The cows that had oestrus were inseminated and 5-7 days after insemination their ovaries were scanned with ultrasound scanner to estimate the Corpus luteum (CL) formation. The cows that did not show oestrus received the second injection 10-12 days after the first injection and were inseminated 72 hours later.

Ovaries and follicular measurement were taken on a daily basis using an ultrasound scanner (Scanner 100 LC Vet, Maastricht, the Netherlands). The average diameter of ovary and follicle was assessed according to Ginther (8) and Sirois (17) and was comprised of the following measurements: the length and width of ovary or follicle. The arithmetical average of length and width is described as the average measure of the ovary and/or follicle.

Blood samples were collected from all experimental cows' jugular vein daily until the cow came to oestrus and was inseminated. The P₄ level was determined by radioimmunoassay using the commercial P₄ diagnostic kit PROG-RIA-CT (BioSource Europe S.A., Belgium) according to the method described by Duchens et al. (5). Statistical analysis was performed using the SPSS statistical package (SPSS Inc, 1989-2004) and Microsoft Excel spreadsheets. Data included in the model were analyzed using descriptive statistics, displayed in means and SD and one-way ANOVA analysis (post hoc tests). Probability values at $p < 0.05$ were considered as statistically significant.

Results and discussion

The GnRH-induced effects are indirect (2) as they act through induced release of luteinizing hormone and follicle stimulating hormone from the anterior pituitary gland (6). GnRH analogues and agonists are more potent than native GnRH (20). Synchronization of follicular waves and selection of new large follicle following GnRH injection at any stage of the oestrous cycle was used as a tool to further develop oestrous synchronization programs for a fixed timed AI (23). Many authors (3, 4, 9, 10, 13) suggest the use of GnRH with prostaglandins, but this oestrus or ovulation stimulation needs to be applied over an extended period of time. There is no data concerning the application of GnRH or its synthetic analogues for oestrus, follicular growth stimulation, and pregnancy rate.

We found that in cows stimulated with the Dalmarelin preparation follicular growth rate was faster ($p \leq 0.05$) compared with the cows stimulated with Depherelin[®]. On the first day of the experiment follicles in group I cows were 16.57 ± 2.59 mm in diameter and P₄ level at this time was 7.60 ± 10.46 nmol/l, in group III follicles were 15.95 ± 4.17 mm in diameter and P₄ level was 6.78 ± 9.91 nmol/l. Follicles in group II on the first day of the experiment were 11.64 ± 2.62 mm in diameter and P₄ level was 6.85 ± 11.51 nmol/l, and in group IV – 12.15 ± 2.28 mm in diameter and P₄ level in their blood plasma was 3.95 ± 5.31 nmol/l.

Normally during oestrus the P₄ level must be close to zero. If a cow is inseminated and conceived, the P₄ level increases and remains elevated during pregnancy. If no conception occurs, the P₄ level increases, reaches a peak level and then declines to near zero as the cow approaches a return to oestrus (7, 12). Some authors (18) found that following GnRH injection there is a rise in LH and FSH concentration in the blood, which raises estradiol-17 β and progesterone concentrations in the blood for up to 6 hours. We found that P₄ concentration after injection of Dalmarelin and Depherelin[®] decreased, then an increase was noted, but at the time of ovulation the P₄ concentration decrease was very dramatic ($p \leq 0.05$) (fig. 1). High variations in P₄ concentrations are expected because progesterone has a dominant role in regulating the oestrous cycle. High concentrations of progesterone inhibit the release of FSH and LH through its negative feedback control of the hypothalamus and anterior pituitary: progesterone also inhibits behavioral oestrus. The drop in progesterone removes the hypothalamus from its negative feedback inhibition. With removal of this inhibition pulses of GnRH, FSH, release of FSH, stimulate rapid follicular growth and increased secretion of estradiol. The sensitivity of the anterior pituitary to GnRH will increase through up-regulation of the GnRH receptors by the more frequent pulses of GnRH (7, 12).

Statistically significant changes in P₄ profiles among our experimental groups were not detected. The P₄ concentration in the control group at time of ovulation was 1.03 ± 0.58 nmol/l, in group I it averaged to 2.10 ± 1.20 nmol/l, in group II it averaged to 2.20 ± 1.10 nmol/l, in group III to 2.50 ± 2.20 nmol/l and in group IV to 1.90 ± 1.12 nmol/l ($p \leq 0.05$). Treatment with GnRH can increase progesterone secretion (14, 15, 18, 23) and decrease the function of the dominant follicle (14, 15, 20). The follicular growth rate and follicular size at ovulation was different in all groups tested (fig. 1).

In group I the left ovary follicular growth rate was fastest, as compared to the other experimental groups and had a faster growth rate in the right ovary than that in control group. In group I follicular growth rate in the right ovary was on the average 1.13 ± 1.01 mm per day, in group II cows – 0.82 ± 0.53 mm per day, whereas in group III the follicular growth rate was 0.62 ± 0.25 mm per day, and in group IV – 0.77 ± 0.6 mm per day ($p \leq 0.05$). In the left ovary of group I cows follicular growth rate was 0.85 ± 0.43 mm per day, in group II – 0.73 ± 0.48 mm, in group III – 0.71 ± 0.52 mm and in group IV – 0.83 ± 0.47 mm per day. In the control group follicular growth rate in the left ovary was 0.87 ± 0.18 mm per day ($p \leq 0.05$). In group I cows' ovaries, follicles at the oestrus day were the biggest compared with other experimental groups and bigger by 9.77% than in control group cows. The average size of ovulatory follicle in group I on oestrus

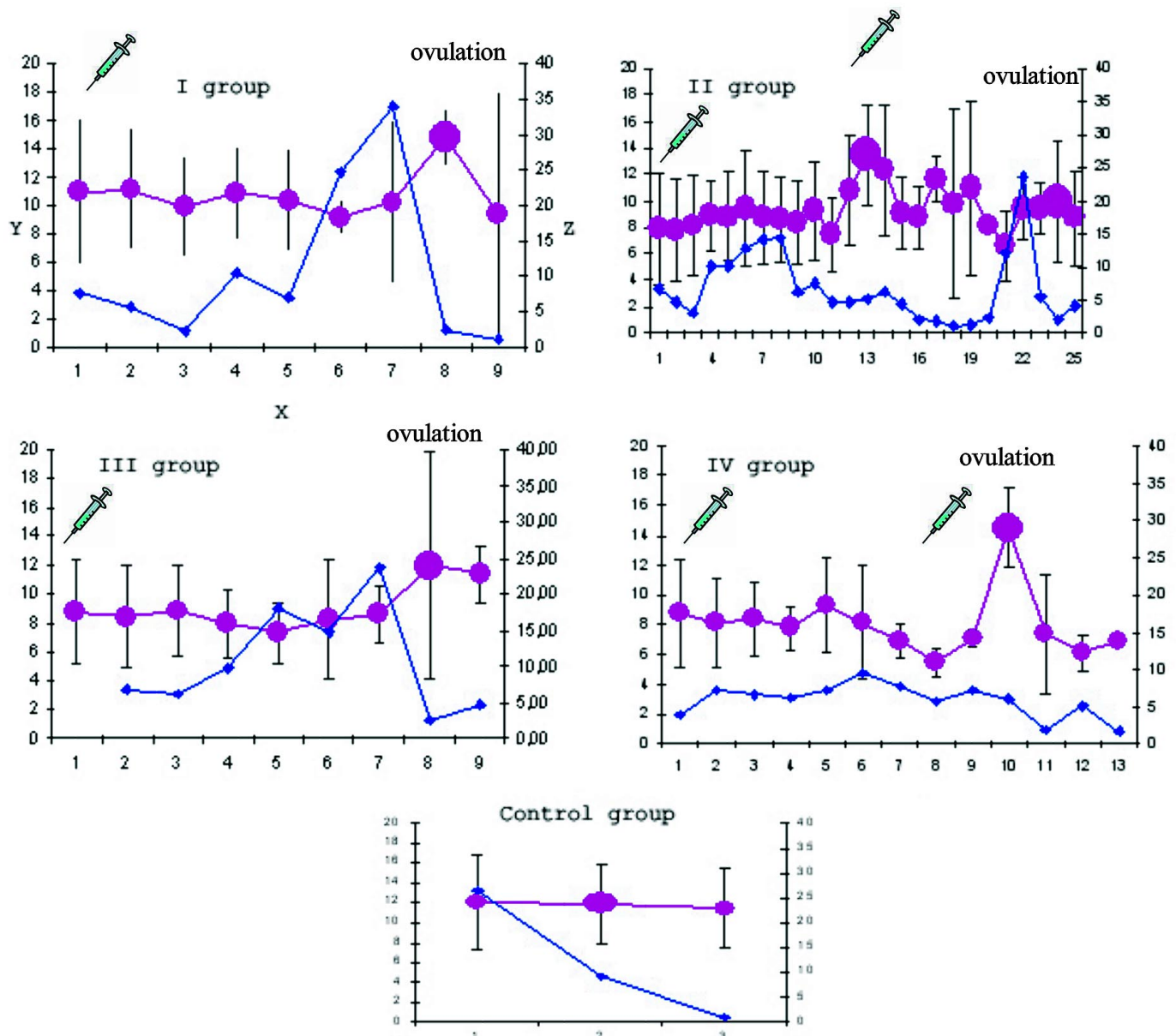


Fig. 1. Follicular growth and progesterone profile changes following administration of synthetic GnRH analogues

Explanations: mean \pm SD – follicular size and statistical deviation. In the X axis is shown times of assessment in days, in Y – size of follicles in the ovaries in mm, in the Z axis – progesterone level in nmol/l. The follicle and progesterone profile changes in cows: stimulated with Dalmarelin, as described in group I and group II; stimulated with Depherelin[®] as described in group III and group IV and in the control group. The syringe shows the day of application of preparations

day was 14.80 ± 2.01 mm, in group II – 13.47 ± 3.83 mm, in group III – 13.85 ± 4.45 mm, in group IV – 14.42 ± 2.72 mm, whereas in the control group cows did not have matured follicles capable of ovulating in the left ovary ($p \leq 0.05$). The right ovary follicular growth was slowest in the II experimental group compared to the control and other experimental groups.

The GnRH stimulates ovulation and rebuilds disturbed oestrus cycles (21). It was found that after injection of synthetic GnRH analogues they terminate the current follicular wave and start the new wave after injection independently of the stage of oestrus cycle. This occurred because following GnRH administration, there is a rise in LH, which influences ovulation

or regression of the dominant follicle and formation of CL (3, 4, 9, 10, 13), which also explains changes in progesterone profile. While in our experimental group II were cows, with a dominant follicle which was smaller than physiologically necessary to enable ovulation, the GnRH administration induced regression of this follicle and started a new follicular wave and established a new, undisturbed oestrus cycle, during that were two clear follicular waves. In other studies improvement of fertility has been observed by the administration of GnRH or its analogues in lactating dairy cows (11, 23).

In contrast to these positive results, there was no favorable effect of similar treatments of GnRH or

GnRH analogues on pregnancy rates in other studies (1, 15). It was found that synthetic GnRH analogues significantly affected oestrus stimulation. In the Dalmarelin treated group all 23 cows (100%) came to oestrus, of which 7 (30.4%) came to oestrus only after the second injection, which was carried out 12 days after the first injection. It was also found that following injection of Depherelin[®], 13 of 14 cows (92.9%) came to the oestrus. Out of these 13 cows, 2 (15.3%) showed oestrus after a complementary injection. One 7-year-old LBW breed cow did not show any oestrus signs after secondary Depherelin[®] injection; although the follicular growth rate was not irregular, the dominant follicle remained incapable of ovulation. Ullah et al. (22) observed that GnRH treatment at oestrus in dairy cows improved pregnancy rates and increased peripheral progesterone concentration. Conversely, some authors (1, 15) found, that GnRH administered to lactating dairy cows at the time of AI did not affect pregnancy rates.

Cows in the experimental groups were examined with an ultrasound scanner for pregnancy detection 45 days after AI. Only 1 pregnant cow (33.3%) was found in the control group. After stimulation with Depherelin[®], and Dalmarelin, 5 cows (35.7%) in groups III and IV and 10 cows (43.5%) in groups I and II became pregnant, respectively.

In conclusion, of the 2 synthetic GnRH analogues Dalmarelin can be slightly better than Depherelin[®] in induction of follicular growth and oestrus in infertile cows.

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