Asian Pacific Journal of Reproduction

Journal homepage: www.apjr.net

Document heading doi: 10.1016/S2305-0500(13)60183-9

Investigation on some biochemical parameters and effect of hormonal treatment in anoestrous dairy cows with cystic ovarian follicle

Stanimir A. Yotov[1*], Anatoli S. Atanasov[1], Georgi B. Georgiev[2], Julieta D. Dineva[3], Nadejda A. Palova[4]

[1]*Faculty of Veterinary Medicine, Department of Obstetrics, Reproduction and Reproductive Disorders, Trakia University, Malka Verea, Student campus, 6000 Stara Zagora, Bulgaria*
[2]*Faculty of Agriculture, Department of Genetics, Breeding and Reproduction, Trakia University, Malka Verea, Student campus, 6000 Stara Zagora, Bulgaria*
[3]*Institute of Biology and Immunology of Reproduction, Department of Immunobiology of Reproduction, Tzarigradsko shose 73, 1113 Sofia, Bulgaria*
[4]*Experimental Station of Agriculture, Bulgaria Square 1, 8300 Sredets, Bulgaria*

ARTICLE INFO

Article history:
Received 16 December 2013
Received in revised form 20 December 2013
Accepted 20 December 2013
Available online 20 January 2014

Keywords:
Cow
Postpartum
COF
Biochemistry
Treatment

ABSTRACT

Objective: To investigate some blood biochemical parameters in anoestrous dairy cows with cystic ovarian follicles (COF) during the first two months post partum and the effect of hCG-PGF2 α -GnRH treatment on their reproductive response. **Methods:** The investigation was performed on 20 lactating Holstein cows, divided into two groups: control group (n=8) and treated group (n=12). All animals had cystic ovarian follicles, detected during the two consecutive transrectal ultrasound examinations at 7-day interval. Blood samples from each animal were collected on the day of second ultrasonography (Day 0) and subjected to a hormonal and biochemical analysis. Treated cows were subjected to another ultrasound examination on day of prostaglandin injection and the presence of corpus luteum was recorded. The control animals were not treated, while the treated group received 1 500 IU hCG on day 0, PGF2 α 500 mg on day 7 and 100 μ g GnRH 48 hours latter. Eighteen hours after GnRH administration animals bearing corpus luteum on Day 7 were artificiallly inseminated. The last ultrasound examination was made forty days after start of the threapy. On the base of the obtained results, cumulative oestrous activity, cumulative ovulation rate, pregnancy rate and COF persistence were determined. **Results:** The blood analysis in the both groups on Day 0 showed significantly (P<0.05) lower mean concentration of progesterone, glucose and inorganic phosphate compared to the reference range values for cyclic dairy cows. Cumulative oestrous activity (91.7%) and cumulative ovulation rate (75.0%) were considerably higher (P<0.05) in treated group than control group, whereas the cystic follicle persistence (25.0%) was lower (P<0.05) in the treated group than control group. **Conclusions:** The present study confirmed the thesis for low blood levels of glucose and inorganic phosphate in cows presenting cystic ovarian follicles. It also indicated significant improvement of cumulative oestrous activity and ovulation rate in animals subjected to hCG-PGF2 α -GnRH administration than non-treated animals. The used hormonal protocol could decrease cystic ovarian follicle persistence in dairy cows.

1. Introduction

The etiology, pathogenesis and efficiency of the treatment of the cystic ovarian follicles (COF) in dairy cows have been studied extensively lately [1-5]. Cystic ovarian follicles are defined as follicular structure > 17 mm in diameter, persisting for more than 6 days with no corpus luteum (C.L) detectable by ultrasound and clearly interfering with the normal ovarian cyclicity [6,7]. The development of COF is associated with different predisposing factors, leading to negative energy balance (NEB) [6], but the exact mechanism is still not clear [8]. Numerous authors reported close relationships between blood levels of calcium, phosphate, total protein, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) and reproductive traits in dairy cows [9,10]. According to Cozzi *et al.* [11] parity, stage of lactation and season also influenced biochemical profile

*Corresponding author: Stanimir A. Yotov, Faculty of Veterinary Medicine, Department of Obstetrics, Reproduction and Reproductive Disorders, Trakia University, Malka Verea, Student campus, 6000 Stara Zagora, Bulgaria.
Tel.: +359 42 699 513
Fax: +359 42 670 624;
E-mail: stanrad@abv.bg

of dairy cows. The most important role in the pathogenesis of cystic ovarian follicle have the lack of a pre–ovulatory LH–surge, insufficient in LH magnitude or LH surge at the wrong time during dominant follicle maturation [12]. Monniaux *et al.* [13] concluded that enhanced growth and steroidogenesis in antral follicles <10 mm preceded cyst formation in cow ovaries and low level of anti–Müllerian hormone in cysts was associated with luteinization. Alfaro *et al.* [14] registered that changes in the expression of ovarian steroid receptors could play a fundamental role in the pathogenesis of this disease.

The different hormonal treatments of follicular cysts in dairy cows are described by Brito and Palmer [15]. They showed that the most common option is GnRH administration, which results in a rapid increase in LH secretion and luteinization is the cysts. Resumption of normal cyclic ovarian activity of the treated cows was 72% –85% with interval from treatment to the first oestrus 19 to 23 days, and pregnancy rates at first oestrus from 46% to 58%. According to Probo *et al.* [3] recovery and conception rate after single GnRH application are 71.3% and 40%, respectively. The results of De Rensis *et al.* [16] indicated that during the cool period, there was a better effect of hCG than GnRH use. Other widespreaded options are Ovsynch and PRID or CIDR based protocols [17,18]. Reproductive response was comparable to that received one after GnRH or GnRH–PGF2α –GnRH treatments, but progesterone based protocols are labour–consuming. Moreover, according to Hernandez and Munoz [19] a combination of different hormonal treatment had a negative impact on the economic cost and it increased by 3.5%, 13.3% and 33.8% for cystic cows that received one, two, or three hormonal treatments, respectively. Irrespective of the obtained data, the etiology, pathogenesis and efficiency of cystic ovarian follicles treatment are still debatable and sometimes controversial. The information connected to the administration of hCG as a first option in the different treatment protocols of cystic ovarian follicles in dairy cows is rater limited.

The aim of the present study was to investigate some blood biochemical parameters in anestrous dairy cows with cystic ovarian follicles during the first two months post partum and the effect of hCG–PGF2α –GnRH treatment on their reproductive response.

2. Materials and methods

The study was performed on 20 lactating Holstein breed cows, 3 to 6 years of age, average daily milk yield (22.0± 2.8) kg, after a normal parturition, with no estrous activity between days 40th and 50th postpartum. Body condition score according to the system of Edmonson *et al.* [20] was 3.5, individual body weights ranged between 500 and 550 kg, the housing and feeding conditions were the same. The animals were reared at latitude of 42.183 N, longitude 25.567. The study was conducted between November and February.

All cows were with ovarian follicles (diameter >20 mm) in an absence of active luteal structure, lack of uterine tone and uterine disorders during the two consecutive transrectal ultrasound examinations at seven day interval. Ultrasonography was made by a SonoScape A5 Vet scanner (SonoScape Co. LTD, Shenzhen, China) and 7–12 MHz linear transducer. The first examination was accepted as start of the experiment.

Blood samples from each animal were collected by jugular venipuncture at day of a second ultrasound. After collection, blood serum was separated from coagulated blood by centrifugation and stored at –20 ℃ until analysis. Serum progesterone (P4) levels were measured by an enzyme immunoassay (EIA) using progesterone kit (Human, PROG ELISA, GmBh, Germany). The analytical sensitivity of progesterone ELISA test was 0.03–0.07 ng/mL (range of 0–40 ng/mL) with an intra– and inter assay coefficient of variation < 10%. Blood serum parameters such as glucose, calcium, inorganic phosphate, total cholesterol, AST, ALT and total protein were assayed on automated clinical chemistry analyzer BS–120 (Mindray MIL, Nanshan, Shenzhen, China). The obtained values were compared with the reference ranges for cyclic dairy cows, used from the Faculty of Veterinary Medicine (FVM) laboratory [21].

The animals were randomly divided into two groups: control group (n=8) and treated group (n=12). The control animals were not treated and a daily monitoring for oestrous behaviour during the experimental period was done. The cows that showed standing oestrus were artificially inseminated. Treated group received 1 500 IU hCG (Chorulon, MSD, Bulgaria) on day 0 (second ultrasound), PGF2α 500 mg (PGF Veyx forte, Veyx–Pharma GmbH, Schwarzenborn, Germany) on day 7 and 100 μg GnRH (Depherelin, Veyx–Pharma GmbH, Schwarzenborn, Germany) 48 hours latter. Moreover, treated cows were subjected to another ultrasound examination on the day of prostaglandin injection and the presence of corpus luteum was recorded. Eighteen hours after GnRH administration, a check–up for clinical oestrus signs by method of Loeffler *et al.* [22] was made.

Immediately after that, timed artificial insemination (TAI) was performed only in animals bearing corpus luteum (C.L.) on Day 7. The subsequent oestrous activity was detected by daily observations. The detection of corpus luteum during the ultrasound examinations was used as a criterion for successful ovulation. The last ultrasound of each cow from the control and the treated groups was performed forty days after Day 0. On the basis of the obtained results, reproductive response (cumulative oestrous activity, cumulative ovulation rate, pregnancy rate and cystic ovarian follicle persistence) was determined.

Statistical analysis was performed with Stat–Soft 1984–2000 Inc. statistical software (Copyright©1990–1995 Microsoft. Corp.), by means the option Other Significance Tests. This dialog is used to compute a variety of significance tests including the difference between two proportions and the difference between two means. Differences were considered significant in $P-$ values <0.05.

3. Results

The results about blood hormonal and biochemical parameters and reproductive response are presented in Table 1 and 2, respectively. At the start of the experiment, all cows had an ovarian cystic follicle without active luteal structure (Figure 1A). Predominantly, small or medium follicles, into opposite of the COF ovarium were observed. The initial diagnoses were confirmed during the second ultrasound examination. The mean progesterone concentration for control and treated group on Day 0 (0.24±0.14 ng/mL and 0.23±0.16 ng/mL), were significantly (P<0.05) lower than the reference range in cyclic dairy cows with corpus luteum. A similar result (P<0.05) was determined for blood glucose and phosphate, whereas the mean values of calcium, total cholesterol, AST, ALT and total protein were near the high limit of the reference range. Three cows from the control group, one on day 16th and two on day 42nd after the start of experiment, showed oestrous behaviour and were inseminated.

Table 1

Blood progesterone concentration and biochemical parameters in control and treated cows on the day of the second ultrasound examination (Mean±SD).

Blood serum parameters	Control group (n=8)	Treated group (n=12)	Reference*
P4 (ng/mL)	0.24±0.14[a]	0.23±0.16[a]	> 1[b**]
Glucose (mmol/L)	1.03±0.52[a]	1.01±0.54[a]	2.1–3.9[b]
Calcium (mmol/L)	2.59±0.19	2.61±0.13	1.98–2.5
Phosphate (mmol/L)	1.12±0.25[a]	1.13±0.27[a]	1.5–2.9[b]
Total cholesterol (mmol/L)	4.32±0.80	4.54±0.78	2.3–6.6
ALT (U/L)	27.00±10.00	28.00±9.00	17–37
AST (U/L)	92.00±14.00	91.00±12.00	48–100
Total protein (g/L)	84.00±4.50	85.00±3.90	65–85

*Reference values of cyclic dairy cows (Mayer and Harvey, 2004).
**Progesterone level in dairy cows with active corpus luteum (Vanholder *et al.*, 2006); Mean values at the same row with different superscripts differ at P<0.05.

Table 2

Reproductive response of the control and treated cows.

Reproductive parameters	Control group (n=8)	Treated group (n=12)
Cumulative oestrous activity	37.5(3/8)[a]	91.7(11/12)[b]
Cumulative ovulation rate	25(2/8)[a]	75(9/12)[b]
Pregnancy rate	12.5(1/8)	33.3(4/12)
COF persistence	62.5(5/8)[a]	25(3/12)[b]

Percentages within the same row with different superscripts differ at P<0.05.

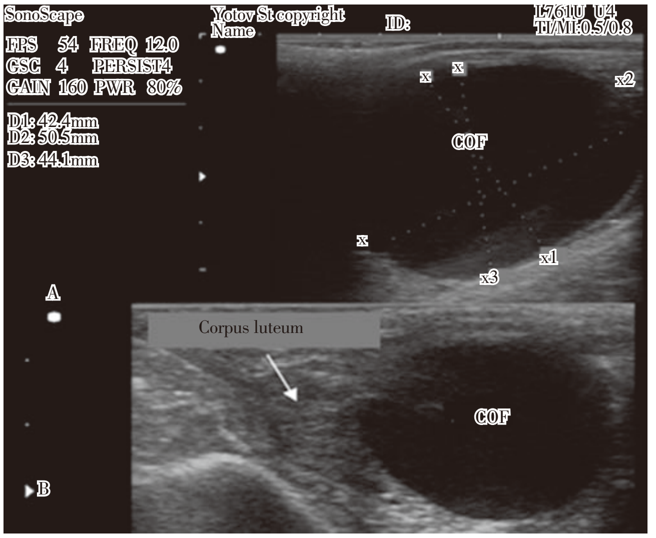

Figure 1. Ovarian ultrasonography at the start of the experiment (A) and seven days after hCG administration (B).

During the transrectal ultrasonography on Day 7, nine treated cows had a corpus luteum (Figure 1B) regardless of COF presence and were marked as animals with ovulation. Seven of them showed clinical estrus signs on the day of TAI. Two cows with non detectable estrus on the day of TAI and two animals without C.L on Day 7, expressed oestrous activity during the week before the last ultrasound examination. Only one cow did not show estrus until the end of the experiment. Cumulative oestrous activity in the treated group (91.7%) was significantly (P<0.05) higher than that (37.5%) of the control group.

The last ultrasound examination in the control group, showed that the cow in heat on day 16 was pregnant. Because of that she was included in the calculation of cumulative ovulation rate. The animals with registered oestrus on day 42 were with C.L and lacked an cystic ovarian follicle. In the remaining cases (62.5%), COF persisted during the entire period. At the same time, four out of seven treated cows with clinical oestrus signs on day of TAI were diagnosed as pregnant. Three animals, showing estrus clinical signs on the day of TAI were non pregnant, but showed no signs returning to estrus has not occured. They had only small and medium follicles into the ovaries. The cystic ovarian follicle persisted in one cow with a lack of C.L on Day 7 but no oestrous behaviour was observed during the entire experimental period. Irrespective of the hormonal treatment, both non inseminated cows with no C.L on Day 7 but, expressing oestrous behaviour later, presented cystic follicles other than previous ones. The cumulative ovulation rate in treated cows (75%) was higher (P<0.05), in comparison with 25% in untreated animals. The pregnancy rate in both groups (12.5% and 33.3%) did not differ significantly (P=0.16), but there was a positive trend for more pregnant animals after treatment. The incidence of COF persistence after hCG–PGF2α–GnRH administration (25%) was lower (P<0.05) than in cases with no treatment.

4. Discussion

The present study shows that suboptimal progesterone levels could be related with cystic ovarian follicles and corresponds with the research of Hatler *et al.* [23], reporting intermediate P4 concentrations (0.10-0.93 ng/mL) in 66% of investigated cows with follicular cysts. Similar suprabasal P4 levels (0.3-0.2 ng/mL) at the time of cyst formation have been found in 25% of the animals examined by Vanholder *et al.* [12]. The lower concentration of glucose (P<0.05) with respect to the reference range indicates energy deficiency, that is one of the factors predisposing to ovarian cysts [24]. The fluctuations of systemic glucose levels are related to cyclicity as the attainment of certain threshold concentrations determine the activation of glucose-sensitive GnRH neurons into the hypothalamus [25]. The similar trend in inorganic phosphate concentrations suggested its influence on the rise of COF. We suggest that this macroelement has an indirect impact on COF formation through alteration of Ca:P ratio. In agreement with this hypothesis, Sefi *et al.* [9] stated lower conception rates when blood concentrations of calcium and inorganic phosphate were in a low limit of the reference. The study of Mohebbi-Fani *et al.* [10] showed that dietary deficiency of one or more trace minerals may induce mixed mineral deficiencies and cause long standing problems in the performance of the herd with no clinical signs of deficiency diseases.

The main reason for COF is altered feedback mechanism of oestrogens on the hypothalamus-pituitary can result in an aberrant GnRH/LH release and hence in cyst formation [26]. This is in agreement with reproductive response of the animals from the control group showed spontaneous regression of cystic ovarian follicles with expression of an oestrous activity. We suggest that these structures have become non-steroidal producing and that a new follicular wave, leading to adequte estrogen production has started. According to Monniaux *et al.* [13] in ovaries with follicular cysts, the presence of some large follicles with steroid concentrations similar to those of large follicles in normal ovaries suggests that a "normal" follicular growth is possible in ovaries with cysts. Additionally, increased food intake and gradually decreasing milk production in a late postpartum period resulted in elimination of a negative energy balance effects. This assumption is confirmed from obtained spontaneous ovulation and pregnancy rate in part of the cows. Similar information for spontaneous regression of cystic follicles has been reported by other research [27], but sometimes anovulatory condition continued because the chance for small antral follicle to become a cyst would be increased when a cyst was already presented on ovaries [28]. Regardless of the above, the significantly (P<0.05) higher cumulative estrous activity (91.7%) and ovulation rate (75.0%) in the treated group indicated an improvement of reproductive performance after the hormonal treatment. The results for cumulative estrous activity are supported by Brito and Palmer [15], who determined resumption of normal cyclic activity after treatment between 72% and 85%. In our opinion, hCG as a first option ensures successful ovulation when ovulatory follicle and PGF2α sensitive corpus luteum on Day 7th are available. These result is contrary to the data of Štastná and Štastný [5], showing only 10.99% C.L after the first gonadotropin administration, but their investigations have been performed in significantly higher number of cows with average productivity 9580 kg milk in hotter time (from April to August) after GnRH-PGF2α-GnRH treatment. In our study we used hCG as a first treatment because of its direct luteinizing effect on the ovarian cells and expanded lifespan [28]. Unsuccessful response of three cows without C.L on Day 7th, could be explained with a lack of follicles in ovulation phase during the hCG treatment. The significantly (P<0.05) higher cumulative ovulation rate and the low cyst persistence in treated than untreated cows provide additional evidences for benefits of used protocol.

According to López-Gaitus and López-Béjar [29], COF persisted in 47% of GnRH-PGF2α-GnRH treated animals. The current investigation presents lower rate (25%) of cyst persistence in treated cows than reported by the abovementioned authors and significant (P<0.05) higher COF persistence compared to the control group. Probably, hCG administration as a first option has a good suppressing effect on the cysts growth and it is associated with more ovulations and successful COF regression latter. The detected oestrous activity in non-inseminated animals and the results from the last ultrasonography support this speculation.

The lack of pregnancy in three animals with oestrous behaviour on the day of TAI that did not express estrus later, regardless of COF absence, indicates an inadequate follicular development. Non-detected corpora lutea during the last ultrasonography point to lack of ovulation during the previous oestrus. We consider that the follicles in the two treated animals, expressing clinical oestrus signs during the week before the end of experiment had not also ovulated, because they presented COF during the last ultrasound examination. The same phenomenon to repeated cysts formation was reported by other studies [50,52]. Nevertheless, the used protocol could contribute for reduction of the cystic ovarian follicles in dairy cows and decreasing of economical losses.

In conclusion, the present study confirmed the thesis for low blood levels of glucose and inorganic phosphate in cows presenting cystic ovarian follicles. It also indicated significant improvement of cumulative oestrous activity and ovulation rate in animals subjected to hCG-PGF2α-GnRH administration than non-treated animals. The used hormonal protocol could decrease cystic ovarian follicle persistence in dairy cows.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors gratefully acknowledge to Mr. Ivan Ivanov and all staff from Glufishevo farm, District Sliven, Bulgaria to their support during the experiment.

References

[1] Elisabeth PM, Natascha G, Martina H. Retrospective analysis of ovarian cysts in dairy cows. Part 1: Risk factors. *Tierärztl Umschau* 2010; **65**: 208–213.

[2] Grado-Ahuir JA, Aad PY, Spicer LJ. New insights into the pathogenesis of cystic follicles in cattle: Microarray analysis of gene expression in granulosa cells. *J Anim Sci* 2011; **89**: 1769–1786.

[3] Probo M, Comin A, Mollo A, Cairoli F, Stradaioli G, Veronesi MC. Reproductive performance of dairy cows with luteal or follicular ovarian cysts after treatment with buserelin. *Anim Reprod Sci* 2011; **127**: 135–139.

[4] Roth Z, Biran D, Lavon Y, Dafni I, Yakobi S, Brawtal R. Endocrine milieu and developmental dynamics of ovarian cysts and persistent follicles in postpartum dairy cows. *J Dairy Sci* 2012; **95**: 1729–1737.

[5] štastná V, štastný P. Efficiency of treatment of follicular cysts in cows. *Slovak J Anim Sci* 2012; **45**: 118–122.

[6] Silvia WJ, Hatler TB, Nugent AM, da Fonseca LFL. Ovarian follicular cysts in dairy cows: An abnormality in folliculogenesis. *Dom Anim Endocrinol* 2002; **23**: 167–177.

[7] Wiltbank MC, Gümen A, Sartori R. Physiological classification of anovulatory conditions in cattle. *Theriogenology* 2002; **57**: 21–52.

[8] Peter AT, Levine H, Drost M, Bergfelt DR. Compilation of classical and contemporary terminology used to describe morphological aspects of ovarian dynamics in cattle. *Teriogenhology* 2009; **71**: 1343–1357.

[9] Sefi HA, Farzaneh N, Mohri M. Relationship between fertility, serum calcium and inorganic phosphorus in dairy cows. *Iranian J Vet Res* 2005; **6**: 76–83.

[10] Mohebbi-Fani M, Nazifi S, Ansari-Lari M, Namazi F. Mixed mineral deficiencies in a dairy herd with subclinical production disorders. *Comp Clin Path* 2010; **19**, 37–41.

[11] Cozzi G, Ravarotto L, Gottardo F, Stefani AL, Contiero B, Moro L, et al. Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation and season of production (Short communication). *J Dairy Sci* 2011; **94**: 3895–3901.

[12] Vanholder T, Opsomer G, De Kruif A. Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: A review. *Reprod Nutr Dev* 2006; **46**: 105–119.

[13] Monniaux D, Di Clemente N, Touze J-L, Belville C, Rico C, Bontoux M, et al. Intrafollicular steroids and anti-Müllerian hormone during normal and cystic ovarian follicular development in the cow. *Biol Reprod* 2008; **79**; 387–396.

[14] Alfaro N, Salvetti N, Velazquez M, Stangaferro M, Rey F, Ortega H. Steroid receptor mRNA expression in the ovarian follicles of cows with cystic ovarian disease. *Res Vet Sci* 2012; **92**: 478–485.

[15] Brito LFC, Palmer CW. Cystic ovarian disease in cattle. *Large Anim Vet Rounds* 2004; **4**: 1–6.

[16] De Rensis F, Bottarelli E, Battioni F, Capelli T, Techakumphu TI, Garciispierto I, et al. Reproductive performance of dairy cows with ovarian cysts after synchronizing ovulation using GnRH or hCG during the warm or cool period of the year. *Theriogenology* 2008; **69**: 481–484.

[17] Todoroki J, Kaneko H. Formation of follicular cysts in cattle and therapeutic effects of controlled internal drug release. *J Reprod Dev* 2006; **52**: 1–11.

[18] Ergene O. Comparison of PRID+PG+GnRH and GnRH+PG+GnRH protocols in the treatment of postpartum anestrus cows. *J Anim Vet Adv* 2012; **11**: 211–213.

[19] Hernandez DB, Munoz LD. Economic cost of follicular cysts in postpartum dairy cows. *Rev Colomb Cienc Pec* 2012; **25**: 252–257.

[20] Edmonson AJ, Lean IJ, Weaver LD, Farver T, Webster G. A body condition scoring chart of Holstein dairy cows. *J Dairy Sci* 1989; **72**: 68–78.

[21] Mayer DJ, Harvey JW. *Veterinary laboratory medicine. interpritation and diagnosis*. 3rd ed. Philadelphia: WB Saunders Co.; 2004, p. 311.

[22] Loeffler H, De Vries MJ, Schukken YH, De Zeeuw AC, Dijkhuizen AA, De Graaf FM, et al. Use of technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. *Theriogenology* 1999; **51**: 1267–1284.

[23] Hatler TB, Hayes SH, Da Fonseca LF, Silvia WJ. Relationship between endogenous progesterone and follicular dynamics in lactating dairy cows with ovarian follicular cysts. *Biol Reprod* 2003; **69**: 218–223.

[24] Nelson ST, Martin AD, Østerås O. Risk factors associated with cystic ovarian disease in Norwegian dairy cattle. *Acta Vet Scand* 2010; **1**: 52–60.

[25] Senger PL. *Pathways to pregnancy and parturition*. 2nd ed. Pullman: Current Conceptions Inc.; 2003, p. 138.

[26] Opsomer G. Cystic ovarian follicles in dairy cattle. *Reprod Manage Bull of Partners & Reprod* 2009; **6**(8): 1–6.

[27] Peter AT. An update on cystic ovarian degeneration in cattle. *Reprod Dom Anim* 2004; **39**: 1–7.

[28] Rajamahendran R, Sianangama PC. Effect of human chorionic gonadotropin on dominant follicles in cows: formation of accessory corpora lutea, progesterone production and pregnancy rates. *J Reprod Fert* 1992; **95**: 577–584.

[29] Lopez-Gatius F, Lopez-Bejar M. Reproductive performance of dairy cows with ovarian cysts after different GnRH and cloprostenol treatments. *Theriogenology* 2002: **58**; 1337–1348.

[30] Kengaku K, Tanaka T, Kamomae H. Changes in the peripheral concentrations of inhibin, follicle-stim-ulating hormone, luteinizing hormone, progesterone and estradiol-17 beta during turnover of cystic follicles in dairy cows with spontaneous follicular cysts. *J Reprod Dev* 2007; **53**: 987–993.

[31] Rajmon R, šichtař J, Vostrý L, Řehák D. Ovarian follicle growth dynamics during the postpartum period in Holstein cows and effects of contemporary cyst occurrence. *Czech J Anim Sci* 2012; **57**: 562–572.