



Investigation of Repeat Breeder Status, Fertility Success and Some Blood Parameters in Healthy, Follicular, and Luteal Cystic Cows

Serdal KURT^{1a}, Funda ESKİ^{2b}, Pinar AYVAZOĞLU DEMİR^{3c}

1. Kahramanmaraş İstiklal University, Elbistan Vocational School, Department of Veterinary, Kahramanmaraş, TURKEY.
 2. Cukurova University, Faculty of Ceyhan Veterinary Medicine, Department of Obstetrics and Gynecology, Adana, TURKEY.
 3. Kafkas University, Faculty of Veterinary Medicine, Department of Livestock Economic and Management, Kars, TURKEY.
- ORCID: 0000-0002-0191-3245^a, 0000-0002-9242-9271^b, 0000-0002-7010-0475^c

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
10.10.2021	29.11.2021	30.12.2021

Bu makaleye atıfta bulunmak için/To cite this article:

Kurt S, Eski F; Ayvazoglu Demir P: Investigation of repeat breeder status, fertility success and some blood parameters in healthy, follicular, and luteal cystic cows. *Atatürk University J. Vet. Sci.*, 16(3): 313-319, 2021. DOI: 10.17094/ataunivbd.1007865

Abstract: The present study investigated the relationship between treated follicular and luteal cysts and repeat breeder rate in dairy cows. In addition, it was aimed to determine the difference between the groups by measuring biochemical parameters and the effect of this on fertility success. The study was comprised of three groups; follicular cyst (Group 1; n = 30), luteal cyst (Group 2; n = 20), and control (Group 3; n = 20) groups. The E2 and AST levels increased in Group 3 compared to other groups (P<0.05; P<0.01). Cholesterol level was highest in Group 2 (P<0.01), and it is also increased in Group 1 compared to Group 3 (P<0.01). Glucose level decreased in Group 2 compared to Group 1 (P<0.05). ALP and GGT levels increased in Group 2 compared to other groups (P<0.01). ALT level increased in Group 3 compared to Group 2 (P<0.01). Compared to other groups, the artificial insemination rate per pregnancy decreased in Group 3 (P<0.01), and repeat breeder status significantly increased in Group 2 (P<0.01). In conclusion, both luteal and follicular cysts have devastating effects on fertility, but repeat breeder status increased in only cows with luteal cysts.

Keywords: Cow, Follicular cyst, Luteal cyst, Ovary, Repeat breeder.

Sağlıklı, Foliküler ve Luteal Kistli İneklerde Repeat Breeder Durumu, Fertilité Başarısı ve Bazı Kan Parametrelerinin Araştırılması

Öz: Bu çalışmada, sütçü ineklerde tedavi edilmiş foliküler ve luteal kistlerle repeat breeder oranı arasındaki ilişki araştırılmaktadır. Ayrıca bazı biyokimyasal parametreler ölçülerek gruplar arasındaki farkın belirlenmesi ve bunun fertilité başarısına etkisinin belirlenmesi amaçlanmaktadır. Çalışma foliküler kist (Grup 1; n = 30), luteal kist (Grup 2; n = 20) ve kontrol (Grup 3; n = 20) grupları olmak üzere üç grup üzerinde yürütüldü. E2 ve AST seviyeleri diğer gruplara göre Grup 3'te arttı (P<0.05; P<0.01). Kolesterol düzeyi Grup 2'de (P<0.01) en yüksek ve Grup 3'e kıyasla Grup 1'de arttı (P<0.01). Glikoz düzeyi Grup 1'e göre Grup 2'de azaldı (P<0.05). ALP ve GGT seviyeleri diğer gruplara kıyasla Grup 2'de arttı (P<0.01). ALT düzeyi Grup 2'ye kıyasla Grup 3'te yükseldi (P<0.01). Diğer gruplarla karşılaştırıldığında, gebelik başına suni tohumlama oranı Grup 3'te azalırken (P<0.01), Grup 2'de repeat breeder oranı önemli ölçüde arttı (P<0.01). Sonuç olarak, hem luteal hem de foliküler kistlerin fertilité üzerinde yıkıcı etkileri oldu, ancak repeat breeder oranı sadece luteal kistli ineklerde artış gösterdi.

Anahtar Kelimeler: Foliküler kist, İnek, Luteal kist, Ovaryum, Repeat breeder.

[✉]Serdal Kurt

Kahramanmaraş İstiklal University, Elbistan Vocational School, Department of Veterinary, Kahramanmaraş, TURKEY.
e-mail: serdal.kurt@hotmail.com

INTRODUCTION

It is known that the most important purpose of dairy cattle is economic profitability and sustainability. The calving interval affects profitability in cattle breeding and must be within its normal limits for ideal profitability (1). However, reproductive problems that occur for any reason prolong the calving-to-conception interval in dairy cows. Ovarian cysts are the major cause of reproductive problems in dairy cattle (2-6). It is known that ovarian cysts may occur due to multifactorial reasons such as genetic structure, milk yield, nutrition, energy balance, postpartum diseases, and weather conditions (7). However, the mechanism of ovarian cysts is not completely understood yet (1). Ovarian cysts are defined as anovulatory ovarian structures that remain on the ovary for more than ten days without a functional corpus luteum and at least 20 or 25 mm in diameter (6); their incidence is known to be between 6-30% (1,6). They are divided into two as luteal and follicular (6-9) according to their wall thickness, fluid character, and histological structures (4,6). The clinical symptoms of ovarian cysts are variable, and cows with ovarian cysts may exhibit irregular estrus interval, nymphomania, masculine behavior, and anoestrus, depending on the character of the cyst (1). Therefore, they need to be treated to lessen their devastating effects on fertility. It is known that ovarian cysts are treated with various methods (4,10). It is informed that the most used method in the treatment of ovarian cysts is the ovsynch protocol. However, pregnancy rates are low in timed inseminations following treatment with ovsynch (1). This suggests that even if ovarian cysts are successfully treated, they result in poor fertility. On the other hand, although ovarian cysts can be treated, it is thought that they cause a decrease in the success of artificial insemination and may lead to repeat breeder. The repeat breeder cows are defined as cows that, although clinically normal, fail to

conceive after at least three consecutive inseminations (11-14). The incidence of repeat breeder cows can reach up to 24% (15), and it is known that this rate may vary depending on parameters such as management conditions, breeding methods, milk yield, and climate. On the other hand, it has been reported that repeat breeder increases the number of inseminations per pregnancy by reducing the success of artificial insemination, prolongs the calving-to-conception interval and increases the culling rate in dairy cows (16,17). So it results in significant economic losses. For these reasons, it is understood that repeat breeding is a condition that must be struggled to minimize in dairy farms. It is known that the main causes of repeat breeder are embryonic deaths and fertilization failure (18). On the other hand, this condition is closely related to deficiencies in ovarian cyclic activities without a pathology. Moreover, treated ovarian cysts are associated with later fertility failures.

The present study hypothesized that although cows with ovarian cysts are successfully treated and are clinically healthy, the fertility rate can decrease, and the repeat breeder rate can increase. Therefore, this study investigated the relationship between treated follicular and luteal cysts and repeat breeder rate in dairy cows. In addition, since it was thought that some hormones and biochemical parameters might have a role in fertility success, some biochemical parameters were measured on the first day of insemination, and the differences between the groups were investigated.

MATERIALS and METHODS

The present study was approved by the Ethics Committee of Ceyhan Veterinary Faculty, Cukurova University, Adana, Turkey (approval number 12/01 and 24.09.2021).

Animal and Management

This study was carried out on a total of 70 multiparous Holstein dairy cows. The dairy cows had similar body condition scores, lactation period, milk yield, age, and parity. The cows were milked twice a day, had free access to water, and were managed in free-stall barns. Cows were fed with a total mix ratio according to their individual needs and lactation periods.

Groups and Study Design

The cows were divided into three groups as follicular cyst (Group 1; n = 30), luteal cyst (Group 2; n = 20) and healthy (Group 3; n = 20) cows. Only cows that were successfully treated in terms of follicular and luteal cysts and had no problems during the study were included in the study. Follicular and luteal cyst definitions were made according to previous studies (1,6). Cows with follicular and luteal cysts were treated with ovsynch protocols (1,6). After the treatment of follicular and luteal cysts, ovarian examinations were performed, and cows with successful treatment were included in the study. In addition, healthy cows were synchronized with ovsynch protocol. Then, cows showing signs of estrus were inseminated. If estrus was not detected, treated cows were not inseminated and were not included in the study. On the 30th day after artificial insemination, cows in all groups were examined for pregnancy with transrectal ultrasound (Honda Electronics®, HS-1500, 5–7.5 MHz, Turkey) administrations. However, non-pregnant and cows showing signs of re-estrus were inseminated again, and the same procedure was applied. The number of artificial inseminations performed until conception occurred was recorded for each cow; an artificial insemination success rate was created for each pregnancy. In addition, cows that were clinically normal but failed to conceive after at least three consecutive inseminations were defined as the repeat breeder (11,12), and they were recorded.

Blood Samples

In all groups, blood samples were collected from the jugular vein into vacutainer tubes (5 ml; Hema & Tube®) before morning milking on the day of the first insemination after synchronization (treatment). Insemination days in cows varied, but the average days of first insemination were between 90-100 days postpartum. These samples were centrifuged at $1.500 \times g$ for 10 min; serums were harvested and stored at -20°C until progesterone (P4), estradiol (E2), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), cholesterol, glucose, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gama glutamil transferaz (GGT) analyzes.

Biochemical Analysis

Serum P4, E2, LH, TSH, cholesterol, glucose, ALP, ALT, AST, and GGT levels were measured by electrochemiluminescence immunoassay (ECLIA) method using commercial kits (cobas®, Roche, Germany).

Statistical Analysis

The obtained data were analyzed with the SPSS 16.0 package program. Kolmogorov-Smirnov test was performed to determine whether the obtained data showed normal distribution. Since the Repeat breeding rate did not show a normal distribution, it was evaluated with the Non-parametric Kruskal Wallis test. All other parameters showed normal distribution, and they were analyzed with the parametric One-Way Anova test. The difference between the groups was analyzed using the two-factor analysis of variance (ANOVA test). Tukey test was used to determine the differences between groups when significant effects were found. In the study, $P < 0.05$ was accepted as the level of significance.

RESULTS

It was found that P4, LH, and TSH levels were similar between all groups ($P > 0.05$). E2 levels

significantly increased in Group 3 compared to other groups ($P<0.05$). It was determined that cholesterol level was highest in Group 2 ($P<0.01$), and also it increased in Group 1 compared to Group 3 ($P<0.01$). While the glucose level was similar in Group 3 according to other groups ($P>0.05$), its level decreased in Group 2 compared to Group 1 ($P<0.05$). The ALP and GGT levels were similar between Group 1 and Group 3 ($P>0.05$); however, their levels

increased in Group 2 than in other groups ($P<0.01$). ALT level was similar in Group 1 compared to other groups ($P>0.05$), but its level increased in Group 2 compared to Group 3 ($P<0.01$). While there was no difference between Group 1 and Group 2 in terms of AST ($P>0.05$), its level increased in Group 3 compared to other groups ($P<0.01$). The results of biochemical and hormonal parameters obtained from all groups are detailed in Table 1.

Table 1. Results of biochemical and hormonal parameters were obtained in all groups.

Tablo 1. Tüm gruplarda elde edilen biyokimyasal ve hormonal parametrelerin sonuçları.

Parameters	Groups			P-value
	Group 1 (Mean \pm SEM)	Group 2 (Mean \pm SEM)	Group 3 (Mean \pm SEM)	
P4 (ng/mL)	0.15 \pm 0.015	0.24 \pm 0.034	0.20 \pm 0.018	$P>0.05$
E2 (pg/mL)	6.74 \pm 0.91 ^a	6.55 \pm 1.42 ^a	12.49 \pm 2.61 ^b	$P<0.05$
LH (mIU/mL)	0.10 \pm 0.00	0.10 \pm 0.00	0.10 \pm 0.00	$P>0.05$
TSH (uIU/mL)	0.007 \pm 0.001	0.005 \pm 0.00	0.005 \pm 0.00	$P>0.05$
Cholesterol (mg/dL)	284.83 \pm 9.25 ^a	329.95 \pm 2.74 ^b	229.56 \pm 8.49 ^c	$P<0.01$
Glucose (mg/dL)	99.42 \pm 25.90 ^a	31.20 \pm 1.43 ^b	42.03 \pm 1.90 ^{ab}	$P<0.05$
ALP (u/L)	38.70 \pm 2.76 ^a	64.45 \pm 2.38 ^b	37.40 \pm 2.61 ^a	$P<0.01$
ALT (u/L)	29.77 \pm 1.49 ^{ab}	34.05 \pm 0.25 ^a	26.96 \pm 1.68 ^b	$P<0.01$
AST (u/l)	101.45 \pm 3.84 ^a	109.38 \pm 7.39 ^a	82.54 \pm 4.75 ^b	$P<0.01$
GGT(u/L)	20.47 \pm 1.79 ^a	26.02 \pm 1.02 ^b	16.37 \pm 1.69 ^a	$P<0.01$

a,b,c: Different letters in the same line demonstrate the statistical difference between groups

P4: Progesterone, E2: estradiol, LH: Luteinizing hormone, TSH: Thyroid-stimulating hormone, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gama glutamyl transferase.

The artificial insemination rate per pregnancy was lower in Group 3 compared to Group 1 and Group 2 ($P<0.01$). However, no significant difference was found between Group 1 and Group 2 ($P>0.05$). While there was a similarity between Group 1 and

Group 3 in terms of repeat breeding rate ($P>0.05$), it increased significantly in Group 2 compared to the other groups ($P<0.01$). The rate of artificial insemination per pregnancy and repeat breeding status is given in detail in Table 2.

Table 2. Comparison of artificial insemination rate per pregnancy and repeat breeding status in all groups.

Tablo 2. Tüm gruplarda gebelik başına suni tohumlama oranı ve repeat breeder oranının karşılaştırılması.

Parameters	Groups			P-value
	Group 1 (Mean \pm SEM)	Group 2 (Mean \pm SEM)	Group 3 (Mean \pm SEM)	
AI per pregnancy	2.90 \pm 0.15 ^a	3.20 \pm 0.16 ^a	1.85 \pm 0.18 ^b	$P<0.01$
Repeat breeding rate	0.10 \pm 0.05 ^a	0.35 \pm 0.11 ^b	0.05 \pm 0.05 ^a	$P<0.01$

a,b: Different letters in the same line demonstrate the statistical difference between groups. AI: Artificial insemination.

DISCUSSION and CONCLUSION

In the present study, we suggested that there may be a predisposition to repeat breeder in clinically healthy cows that have been successfully treated for follicular and luteal cysts. We also thought that there might be differences in some hormones (P4, E2, LH, TSH) and biochemical parameters (cholesterol, glucose, ALP, ALT, AST, and GGT) in these cows may be reflected in fertility success. To clarify this assumption, we formed study groups from cows that

had previously been successfully treated for follicular and luteal cysts. In addition to comparing these two groups, a control group was created to demonstrate their differences from cows with no previous follicular and luteal cysts.

Ovarian cysts are among the important cause of reproductive disorder in high-producing dairy cows (1). Ovarian cysts result in economic loss in many ways because they cause infertility, and follicular cysts are more common than luteal cysts. However,

in both cases, fertility problems increase (6). It has been assumed that ovarian cysts arise as a result of abnormal LH secretion by the hypothalamic-pituitary-gonadal axis, and it was reported that up to 70% of ovarian cysts occur between 16 and 50 days postpartum (1). In the presented study, no distinction was made between the period when cysts appeared, but cows with both luteal and follicular cysts were included in the study if they were in the same lactation period. Cows in three groups were included in this study at the end of approximately two months postpartum. After detection of ovarian cysts in Group 1 and Group 2, they were treated with the ovsynch protocol (1). The cows in Group 3 were also synchronized with the same protocol. Then, cows showing signs of estrus were inseminated, and fertility parameters were compared between groups.

There was no difference between Group 1 and Group 2 in terms of artificial insemination rate per pregnancy. However, it was found that this rate was significantly lower in Group 3. According to these results, it is understood that even if luteal and follicular cysts are successfully treated, fertility is negatively affected in dairy cows. As the reason for this situation, it is thought that ovarian cysts cause permanent damages to the ovaries and dominant follicles, or embryonic deaths are high in these cows due to metabolic deficiencies. Although the treatment method may affect the reproductive performance of cows with ovarian cysts (16,19), a decrease in fertility success is inevitable. Similarly, many previous studies have reported that ovarian cysts reduce the success of artificial insemination (1,11,12,20). According to these results, it was expected that the repeat breeder rate would increase with the decrease in the success of artificial insemination in Group 1 and Group 2. However, it was determined that there was a similarity between Group 1 and Group 3 in terms of repeat breeding rate. On the other hand, the rate of repeat breeder increased only in Group 2 with luteal cysts. Therefore, it was revealed that luteal cysts have

more devastating effects on fertility than follicular cysts. However, this situation should not be attributed to only ovarian functions. It is known that differences in metabolic (7,21,22) and hormonal profiles can affect fertility success (23,24), and this situation may differ in cows with luteal and follicular cysts (7,25). In the present study, the levels of some hormones (P4, E2, LH, TSH) and biochemical parameters (cholesterol, glucose, ALP, ALT, AST, and GGT) were measured in all groups in order to reveal these differences and determine their effect on fertility success. We performed these measurements only at the first insemination time after cystic treatments because we thought that follicular and luteal cysts might be related to or affect these parameters. It has been reported that an abnormal steroid hormone profile may be associated with ovarian cysts in cattle (9). In the present study, no difference was found between all groups in terms of P4, LH, and TSH during insemination. However, we thought that this might be due to measurement time and that the treatment applied might have changed the hormone levels. On the other hand, while the E2 level was similar in Group 1 and Group 2, it was found to be significantly increased in Group 3. It is thought that the reason for this situation is that E2 is released at a higher level in Group 1 compared to other groups. According to this result, it is thought that serum E2 concentration is low during the first insemination in cows with cystic ovary and this may affect subsequent fertility success. In addition to hormonal factors, metabolic factors and negative energy balance may also be associated with ovarian cysts (9,25).

There is a strong relationship between ovarian metabolism and the use of glucose and cholesterol, and plasma cholesterol levels can be used to predict reproductive outcomes (26). Therefore, blood glucose and cholesterol levels reveal to be effective on ovarian functions. In the presented study, it was observed that Group 2 had the highest cholesterol level. Moreover, its glucose level is lower compared to Group 1. Considering the repeat breeder status

and the artificial insemination rate for pregnancy in Group 2, it is thought that this situation may be due to deficiencies in ovarian metabolism as a result of high cholesterol and low glucose levels. In addition, it is thought that the lower repeat breeder rate in Group 1 compared to Group 2 may be related to the cholesterol level. Also, considering the ALP, ALT, AST, and GGT parameters of the cows in Group 1 and Group 2, it is understood that these cows may have had liver dysfunction. Similarly, Puppel and Kuczyńska (27) reported that ALP, ALT, AST, and GGT activities could be used in the evaluation of liver function.

Disorders in liver metabolism are closely related to energy deficiency (28), and this situation has negative effects on fertility. It is thought that increased ALP, ALT, AST, and GGT levels in Group 2 compared to Group 3 reflect the problem in liver metabolism, and this situation is associated with ovarian functions and fertility success. On the other hand, the lower repeat breeder rate in Group 1 compared to Group 2 may be related to the level of these metabolic products. Because ALP, ALT, and GGT levels were similar in Group 1 compared to Group 3, only AST levels were higher. This can be considered as an indication that the liver function is better in Group 1 than in Group 2.

In conclusion, ovarian cysts are a complex condition, and both luteal and follicular cysts have devastating effects on fertility in dairy cows. While the repeat breeder status of cows with follicular cysts was similar to that of healthy animals, it was significantly higher in cows with luteal cysts. Therefore, it is understood that fertility problems have been more serious in cows with luteal cysts in the past. We also think that this situation may be related to the disorders in liver functions. Therefore, it is necessary to carry out more detailed studies by closely monitoring the nutritional status of the cows in further researches

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Borş Sİ., Borş A., 2020. Ovarian cysts, an anovulatory condition in dairy cattle. *J Vet Med Sci*, 82, 1515-1522.
2. Pesantez JL., Ortiz O., Hernandez-Ceron J., 2016. Incidence of ovarian follicular cysts and their effect on reproductive performance in dairy cows: A case study in Mexico. *Arch Med Vet*, 48, 289-291.
3. Yazlık MO., Çolakoğlu HE., Kaya U., 2017. İsviçre esmeri ineklerde kistik ve dominant folikül sıvılarının metabolik ve iyon kompozisyonlarının karşılaştırılması. *Vet Hekim Der Derg*, 88, 15-25.
4. Jeengar K., Chaudhary V., Kumar A., Raiya S., Gaur M., Purohit GN., 2018. Ovarian cysts in dairy cows: old and new concepts for definition, diagnosis and therapy. *Anim Reprod*, 11, 63-73.
5. Hombalegowda H., Ramakrishnappa N., Siddaraju NK., Sringshwaraiyah NC., Guelal S., 2019. Factors influencing cystic ovarian disease in Holstein Friesian (HF) crossbred cattle. *SSR Inst Int J Life Sci*, 5, 2317-2321.
6. Mimoune N., Azzouz MY., Khelef D., Kaidi R., 2021. Ovarian cysts in cattle: a review. *Vet Stanica*, 52, 587-603.
7. Mimoune N., Baazizi R., Azzouz MY., Benaissa MH., Kaidi R., 2019. Basic and new concepts of ovarian cyst pathogenesis in cattle. *Vet*, 68, 73-80.
8. Brodzki P., Brodzki A., Krakowski L., Dabrowski R., Szczubiał M., Bochniarz M., 2019. Levels of selected cytokines and acute-phase proteins in the serum of dairy cows with cystic ovarian disease and those in follicular and luteal phases of normal ovarian cycle. *Res Vet Sci*, 123, 20-25.
9. Nora M., Rachid K., Abdelmoumene G., Hocine BM., Yassine AM., 2018. Characterization of ovarian follicular and cystic fluids in cows. *Vet*, 67, 73-79
10. Bartolome JA., Thatcher WW., Melendez P., Risco CA., Archbald LF., 2005. Strategies for the diagnosis and treatment of ovarian cysts in dairy cattle. *J Am Vet Med Assoc*, 227, 1409-1414.

11. Moss N., Lean IJ., Reid SWJ., Hodgson DR., 2002. Risk factors for repeat-breeder syndrome in New South Wales dairy cows. *Prev Vet Med*, 54, 91-103.
12. Lopez-Gatius F., Garcia-Ispuerto I., 2020. Treatment with an elevated dose of the GnRH analogue dephereline in the early luteal phase improves pregnancy rates in repeat-breeder dairy cows. *Theriogenology*, 155, 12-16.
13. Funeshima N., Miura R., Katoh T., Yaginuma H., Kitou T., Yoshimura I., Konda K., Hamano S., Shirasuna K., 2021. Metabolomic profiles of plasma and uterine luminal fluids from healthy and repeat breeder Holstein cows. *BMC Vet Res*, 17, 1-10.
14. Nowicki A., 2021. Embryo transfer as an option to improve fertility in repeat breeder dairy cows. *J Vet Sci*, 65, 231-237.
15. Salasel B., Mokhtari A., Taktaz T., 2010. Prevalence, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. *Theriogenology*, 74, 1271-1278.
16. Lopez-Gatius F., Lopez-Bejar M., 2002. Reproductive performance of dairy cows with ovarian cysts after different GnRH and cloprostenol treatments. *Theriogenology*, 58, 1337-1348.
17. Khosa BK., 2020. Repeat Breeding: A Dent to Dairy Farm Economy. *Int J Curr Microbiol App Sci*, 9, 4085-4091.
18. Amiridis GS., Tsiglianni TH., Dovolou E., Rekkas C., Vouzaras D., Menegato I., 2009. Combined administration of gonadotropin-releasing hormone, progesterone, and meloxicam is an effective treatment for the repeat-breeder cow. *Theriogenology*, 72, 542-548.
19. Abdalla H., de Mestre AM., Salem SE., 2020. Efficacy of ovulation synchronization with timed artificial insemination in treatment of follicular cysts in dairy cows. *Theriogenology*, 154, 171-180.
20. Sakaguchi M., Sasamoto Y., Suzuki T., Takahashi Y., Yamada Y., 2006. Fate of cystic ovarian follicles and the subsequent fertility of early postpartum dairy cows. *Vet Rec*, 159, 197-201.
21. Çolakoğlu HE., Yazlık MO., Pekcan M., Kaya U., Kaçar C., Vural MR., Kurt S., Yildirim MM., Bas A., Küplülü Ş., 2019. Impact of Prepartum body condition score loss on metabolic status during the transition period and subsequent fertility in Brown Swiss dairy cows. *J Vet Res*, 63, 375-382.
22. Macmillan K., Gobikrushanth M., Helguera IL., Behrouzi A., Colazo MG., 2020. Relationships between early postpartum nutritional and metabolic profiles and subsequent reproductive performance of lactating dairy cows. *Theriogenology*, 151, 52-57.
23. Kendall NR., Flint APF., Mann GE., 2009. Incidence and treatment of inadequate postovulatory progesterone concentrations in repeat breeder cows. *Vet J*, 181, 158-162.
24. Ueno D., Goto A., Hazano K., Haneda S., Matsui M., 2021. Sustained high progesterone concentrations during estradiol-progesterone based estrus synchronization protocol in Japanese Black cows affects fertility by influencing preovulatory follicle size and its ovulation. *Jpn J Vet Res*, 69, 151-161.
25. Vanholder T., Leroy JL., Dewulf J., Duchateau L., Coryn M., de Kruif A., Opsomer G., 2005. Hormonal and metabolic profiles of high-yielding dairy cows prior to ovarian cyst formation or first ovulation post partum. *Reprod Domest Anim*, 40, 460-467.
26. Westwood CT., Lean IJ., Garvin JK., 2002. Factors influencing fertility of Holstein dairy cows: a multivariate description. *J Dairy Sci*, 85, 3225-3237.
27. Puppel K., Kuczynska B., 2016. Metabolic profiles of cow's blood; a review. *J Sci Food Agric*, 96, 4321-4328.
28. Jorritsma R., Wensing T., Krui, TA., Vos PL., Noordhuizen JP., 2003. Metabolic changes in early lactation and impaired reproductive performance in dairy cows. *Vet Res*, 34, 11-26.