Praca oryginalna *Original paper*

Effects of asprosin levels and selected biochemical parameters on conception in repeat breeder cows

KUDRET YENILMEZ[1], SEZAI ARSLAN[2], SINAN VICIL[3], HASAN DOGAN[1]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Tekirdag Namik Kemal University, TR59030, Suleymanpasa, Tekirdag, Türkiye
[2]Department of Internal Medicine, Faculty of Veterinary Medicine, Tekirdag Namik Kemal University, TR59030, Suleymanpasa, Tekirdag, Türkiye
[3]Department of Biochemistry, Faculty of Veterinary Medicine, Tekirdag Namik Kemal University, TR59030, Suleymanpasa, Tekirdag, Türkiye

Received 14.02.2024 *Accepted 19.03.2024*

Yenilmez K., Arslan S., Vıcıl S., Dogan H.

Effects of asprosin levels and selected biochemical parameters on conception in repeat breeder cows

Summary

The purpose of this study was to investigate the effects of asprosin levels and various biochemical parameters (NEFA, BHBA, glucose, albumin, globulin, total protein, ALT, AST, and total bilirubin) on conception in repeat breeder (RB) cows. The study included sixty RB cows that had been inseminated at least three times, but had not been pregnant and had no gynecological abnormalities, as assessed by ultrasonography. From the sixty cows, two groups were selected at random: pregnant (Group I, n = 10) and non-pregnant (Group II, n = 10) animals. After estrus detection, a 10 mL blood sample was collected from each animal's tail vein. All animals were inseminated 12 hours after the start of estrus. The blood samples were centrifuged at 3000 rpm for 15 minutes, and the serum was extracted and stored at –80°C until analysis. On the 45th day after insemination, ultrasonography was used to examine the pregnancy. Asprosin levels were measured using ELISA, and biochemical tests (NEFA, BHBA, glucose, albumin, globulin, total protein, ALT, AST, total bilirubin) were performed with an autoanalyzer. Non-pregnant RB cows had considerably lower levels of asprosin ($p < 0.05$) than pregnant RB cows. When other biochemical parameters (BHBA, NEFA, glucose, albumin, ALT, AST, total bilirubin, total protein and globulin) were compared, there was no significant difference between the groups ($p > 0.05$). Thus, significantly elevated levels of asprosin in pregnant RB cows suggested that asprosin could play a role in RB cow conception. In addition, there was a positive correlation between asprosin levels and AST, total protein, and globulin levels in pregnant RB cows.

Keywords: repeat breeder, cow, asprosin, conception

Although the milk yield of dairy cows has recently greatly increased around the world, there has also been a decrease in fertility rates, and the number of inseminations per pregnancy has risen. One of the most crucial factors influencing cow fertility and the number of inseminations per pregnancy is the repeat breeder (RB) problem (7). Repeat breeders are cows that do not conceive after at least three inseminations and have no clinically identifiable anomalies in the genital organs or estrous cycle (39). Asprosin, first identified by Romere et al. in 2016 (34), is a protein hormone synthesized from white adipose tissue that regulates glucose homeostasis and hunger (9). According to Maylem et al. (25), asprosin may play a significant role in controlling ovarian follicle function and has direct effects on theca interna cells in the ovary. Batalha et al. (5) report that asprosin regulates estradiol and progesterone synthesis as well as cell proliferation in granulosa cells in the cow ovary.

It has been reported that high BHBA concentrations in cows increase the number of days open, prolong the calving to conception interval, and thus negatively affect fertility (6, 30). Various studies conducted on cows have reported that there is a relationship between fertility and NEFA levels, and that NEFA levels are high in cows with fertility problems (8, 10, 14). In dairy cows, NEFA and BHBA concentrations are inversely correlated with glucose concentrations, and as glucose concentrations decrease, NEFA and BHBA concentrations increase (27).

Optimal blood biochemical components are critical for maintaining physiological functions and are

required for the proper functioning of the reproductive system and other body systems. Consequently, it is crucial to elucidate the serum biochemical profile of dairy cows in various diseases and health stages (4). Although certain effects of asprosin on cow reproduction have been described, no studies on asprosin in RB cows have been found. As a result, the purpose of the current study was to evaluate the possible effect of asprosin on pregnancy in RB cows, as well as to establish the relationship between asprosin and several biochemical markers.

Material and methods

The study was conducted on 60 repeat breeder cows kept on a private farm in Tekirdağ province, subjected to the same care, feeding, and breeding management. The cows were between the 120th and 150th day of lactation, with an average milk yield of 25 (25.29 ± 0.40) L/day. The cows had been inseminated at least three times, but had not become pregnant and did not show any gynecological abnormalities, as assessed by ultrasonography. Total mixed rations (TMR) were fed *ad libitum* twice daily to all animals. They always had access to clean drinking water. From these 60 cows, two groups were randomly selected: pregnant cows (Group I, n = 10) and non-pregnant cows (Group II, n = 10). The Animal Experiments Local Ethics Committee of Tekirdağ Namık Kemal University approved the study (T2023-1774).

Estrus in the cows was determined by measuring the increase in activity with a pedometer, assessing the rise in uterine tone during rectal examination, and identifying the presence of Graafian follicles in the ovaries. After estrus detection, a 10 mL blood sample was collected from each animal's tail vein. All animals in the study were inseminated with semen from the same bull with confirmed fertility 12 hours after the onset of estrus, and the procedure was performed by the same technician. The blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum, which was then refrigerated at –80 degrees until analysis. On the 45th day after insemination, ultrasonography was used to examine the pregnancy.

Asprosin serum levels in the serum samples were measured with an ELISA device (EPOCH, Bio Tek) using a bovine-specific ELISA kit (SinoGeneClon Biotech, Cat No: SG-60752) according to the manufacturer's instructions. An autoanalyzer (Gesan Chem 200) was used to analyze serum samples for biochemical markers (NEFA, BHBA, glucose, albumin, globulin, total protein, ALT, AST, and total bilirubin).

The number of samples was identified using a power analysis program (GPower 3.1). Based on the Wilcoxon-Mann-Whitney test, the sample size for the power analysis was determined to be 10, with a significance level of 0.05 and a 95% power. The Shapiro-Wilk test was employed to assess the normality of the data, revealing a normal distribution. In the pregnant and non-pregnant groups, those with normal distribution were identified using the parametric Unpaired t test, while those without normal distribution were determined using the nonparametric Mann-Whitney test, both from the GraphPad Prism 9.4.1 package program. The Spearman correlation test was employed for correlation analysis. The calculation used a statistical significance level of $P < 0.05$.

Results and discussion

A comparison of asprosin levels in the repeat breeder cows revealed that the non-pregnant RB cows had significantly lower asprosin levels (33.28 ng/mL) than the pregnant RB cows (Tab. 1).

There was no significant difference between the groups ($P > 0.05$) in terms of other biochemical parameters (BHBA, NEFA, glucose, albumin, ALT, AST, total bilirubin, total protein, and globulin) (Tab. 1).

In the pregnant RB cow group, Spearmen's correlation analysis showed a positive correlation between asprosin levels and AST, total protein, and globulin ($P < 0.05$) (Tab. 2).

Tab. 1. Comparison of biochemical parameters by groups

Parameter	Unit	Group I (n = 10) Mean ± SEM	Group II (n = 10) Mean ± SEM	P value
Asprosin	ng/mL	41.22 ± 1.92	33.28 ± 3.20	0.0455*
Glucose	mg/dL	42.02 ± 5.95	46.04 ± 8.12	0.6910
NEFA	mmol/L	0.094 ± 0.03	0.086 ± 0.016	0.9067
BHBA	mmol/L	0.346 ± 0.05	0.386 ± 0.04	0.5011
ALT	U/L	53.4 ± 5.37	56.38 ± 7.24	0.7425
AST	U/L	128.3 ± 10.47	130.5 ± 16.00	0.7430
T. Protein	g/dL	7.96 ± 0.22	7.965 ± 0.27	0.8879
Albumin	g/dL	3.417 ± 0.12	3.319 ± 0.13	0.5842
Globulin	g/dL	4.543 ± 0.23	4.646 ± 0.22	0.7491
T. Bilirubin	mg/dL	0.027 ± 0.01	0.034 ± 0.01	0.2601

Explanations: $P < 0.05$*; NEFA – non-esterified fatty acid BHBA – beta-hydroxybutyric acid; ALT – alanine aminotransferase; AST – aspartate aminotransferase; T. Protein – total protein; T. Bilirubin – total bilirubin

Tab. 2. Biochemical parameters correlated with asprosin and their coefficients by groups

		r	P value	P value summary
Group I	Asprosin vs. ALT	0.1597	0.3419	ns
	Asprosin vs. AST	0.7333	0.0156	*
	Asprosin vs. T. Protein	0.7500	0.0127	*
	Asprosin vs. Globulin	0.8833	0.0015	**
Group II	Asprosin vs. ALT	0.7143	0.0288	*
	Asprosin vs. AST	0.3095	0.2309	ns
	Asprosin vs. T. Protein	–0.1677	0.3471	ns
	Asprosin vs. Globulin	–0.3810	0.1799	ns

Explanations: $P < 0.05$*; $P < 0.01$**; vs. – versus; ns – not significant; ALT – alanine aminotransferase; AST – aspartate aminotransferase; T. Protein – total protein

With the exception of ALT ($P > 0.05$), no statistically significant correlation between asprosin levels and the other biochemical parameters was seen in the non-pregnant RB cow group (Tab. 2).

Discovered in the last decade, asprosin is a protein hormone produced by white adipose tissue that stimulates hunger by causing the liver to release glucose (35). Recent studies have shown that asprosin treatment improves ovarian steroidogenesis, folliculogenesis, and fertility in female rats (19). Literature regarding asprosin levels in cows is limited. Asprosin is a regulator of granulosa cell functions in the ovary of cows, according to research by Batalha et al. (5). Kılınç and Rişvanlı (20) found that asprosin levels in cows were quantifiable and linked to ketosis. Kılınç et al. (21) found that pregnant cows have higher asprosin levels compared to non-pregnant cows. In this study, the pregnant RB cows had considerably higher asprosin levels than the non-pregnant RB cows. In the pregnant RB cows, asprosin levels correlated positively with AST, total protein, and globulin levels.

The serum glucose level in RB cows, at 43.00 mg/dl, was significantly ($P < 0.05$) lower than that in normal healthy cows (53.98 mg/dl) (4). In RB cows, hypoglycemia can have three causes: increased peripheral glucose absorption, lack of gluconeogenesis or glycogenolysis, and endogenous hyperinsulinemia. Surprisingly, the association between hyperglycemia and fertility is still disputed. The present study found no difference in glucose levels between pregnant and non-pregnant RB cows. These findings are consistent with those of Jung et al. (18).

Lin et al. (24) found that in cows in the postpartum period, negative energy balance (NED) occurred, plasma glucose levels declined, and asprosin levels increased. Plasma NEFA and BHBA levels increase in cows with NED. Several studies on cows have found that high levels of NEFA and BHBA had a negative effect on fertility (6, 10, 14, 30). The effects of NEFA and BHBA on the fertility of RB cows were investigated in the current study, but no difference in NEFA or BHBA levels was found between pregnant and non-pregnant RB cows. Furthermore, no significant association was found between asprosin, NEFA, and BHBA. Jung et al. (18) concluded that NEFA levels in RB cows do not affect fertility, which is consistent with our findings, and no studies on BHBA levels in RB cows were found.

Liver metabolism disorders are strongly associated with energy shortage, which negatively impacts fertility (17). Kurt et al. (22) found that cows with luteal cysts had higher ALT levels and a lower repeat breeder rate than healthy cows. According to Pandey et al. (31), fertile cows and repeat breeder cows had the same ALT levels. Jung et al. (18) found no difference in ALT levels between RB cows which did and did not become pregnant. In the present study, no difference was identified between the ALT levels of the two groups. There was no difference in liver enzyme levels between the groups, indicating that there was no liver damage in repeat breeder cows. The association between AST levels and fertility in cows is debatable: some authors (37) claim that higher AST activity in the postpartum period slows ovarian cyclic activities, while others (14) report that it has no effect. Kurykin et al. (23) found no difference in AST between RB and early lactation cows, and Jung et al. (18) observed no difference in AST between RB cows. Our study found no difference in AST values between pregnant and non-pregnant RB cows, which is consistent with the findings of Kurykin et al. (23) and Jung et al. (18).

Low plasma protein levels in cows cause a deficiency of the amino acids needed to synthesize gonadotropins and gonadal hormones, which in turn leads to anestrus and impairs fertility (16). Previous research (12, 34) found no difference in total protein levels between repeat breeder cows and typical cyclic cows, which supports our findings. In contrast to these findings, some studies report lower TP levels in RB cows than in normal cycling cows (2, 36), while others report higher levels (1, 26). The present study found no difference in TP levels between pregnant and non-pregnant RB cows.

Plasma albumin and globulin levels have been found to be low in RB cows (38), and albumin has been shown to be effective in the production of gonadotropins required to initiate ovulation, while globulin is important in steroidogenesis (28). Asrar et al. (3) reported that there was no difference in albumin and globulin levels between RB cows and normal cycling cows, but albumin and globulin levels in RB cows were below normal values. The current study found no difference in albumin and globulin levels between pregnant and non-pregnant RB cows, which is consistent with the findings of Asrar et al. (3).

Pariza et al. (32) found that total bilirubin levels were higher in RB cows than in the control group. The current study, as well as a study by Jung et al (18), found no difference in total bilirubin levels between pregnant and non-pregnant RB cows. This finding suggests that RB has no effect on the functions of organs such as the liver and gall bladder.

In conclusion, the fact that pregnant RB cows have significantly increased asprosin levels suggests that asprosin may be related to the pregnancy of RB cows. Furthermore, the positive correlations observed between asprosin levels and AST, total protein, and globulin levels in pregnant RB cows indicate that asprosin levels may be influenced by AST, total protein, and globulin levels. However, it should be noted that more research on a larger number of animals is needed to fully understand the effects of asprosin on conception in RB cows.

References

1. *Ahmed M. E., Ahmed F. O., Frah E. A., Elfaki I.*: Blood biochemical profile of Sudanese crossbred repeat breeder cows. African J. of Biotec. 2017, 16, 366-370.
2. *Amle M., Patodkar V., Shelar R., Birade H.*: Serum biochemical levels of repeat breeder cross bred cows under rural condition of Satara District of Maharashtra. International Journal of Advan. Vet. Sci. Techn. 2014, 3, 109-113.
3. *Asrar S. I. N., Rimayanti R., Ismudiono I., Mafruchati M., Yuliani M. G. A., Riady G.*: Total protein, albumin, and globulin levels of blood serum in repeat breeder Holstein Friesian cows. J. Anim. Reprod. 2023, 12, 85-92.
4. *Barson R. K., Padder S., Sayam A. S. M., Rahman M. M., Bhuiyan M. M. U., Bhattacharjee J.*: Serum glucose, urea nitrogen, cholesterol, and total proteins in crossbred repeat breeder and normally cyclic cows. J. Adv. Vet. Anim. Res. 2019, 6, 82-85.
5. *Batalha I. M., Maylem E. R. S., Spicer L. J., Bello C. A. P., Archilia E. C., Schütz L. F.*: Effects of asprosin on estradiol and progesterone secretion and proliferation of bovine granulosa cells. Mol. Cel. Endocrin. 2023, 565, 1-10.
6. *Dechow C. D., Baumrucker C. R., Bruckmaier R. M., Blum J. W.*: Blood plasma traits associated with genetic merit for feed utilization in Holstein cows. J. Dairy Sci. 2017, 100, 8232-8238.
7. *Dochi O., Takahashi K., Hirai T., Hayakawa H., Tanisawa M., Yamamoto Y., Koyama H.*: The use of embryo transfer to produce pregnancies in repeat-breeding dairy cattle. Theriogenology 2008, 69, 124-128.
8. *Dubuc J., Duffield T., Leslie K., Walton J., LeBlanc S.*: Risk factors and effects of postpartum anovulation in dairy cows. J. Dairy Sci. 2012, 95, 1845-1854.
9. *Duerrschmid C., He Y., Wang C., Li C., Bournat J. C., Romere C., Chopra A. R.*: Asprosin is a centrally acting orexigenic hormone. Nature Med. 2017, 23, 1444-1453.
10. *Garverick H., Harris M., Vogel-Bluel R., Sampson J., Bader J., Lamberson W., Spain J., Lucy M., Youngquist R.*: Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. J. Dairy Sci. 2013, 96, 181-188.
11. *Gobikrushanth M., Purfield D. C., Canadas E. R., Herlihy M. M., Kenneally J., Murray M., Butler S. T.*: Anti-Müllerian hormone in grazing dairy cows: Identification of factors affecting plasma concentration, relationship with phenotypic fertility, and genome-wide associations. J. Dairy Sci. 2019, 102, 11622-11635.
12. *Guzel S., Tanriverdi M.*: Comparison of serum leptin, glucose, total cholesterol and total protein levels in fertile and repeat breeder cows. Rev. Brasil. Zoo. 2014, 43, 643-647.
13. *Ireland J. J., Smith G. W., Scheetz D., Jimenez-Krassel F., Folger J. K., Ireland J. L., Mossa F., Lonergan P., Evans A. C.*: Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. Reprod. Fertil. Dev. 2011, 23, 1-14.
14. *Jeong J., Choi I., Kang H., Hur T., Jung Y., Kim I.*: Relationship between serum metabolites, body condition, peri- and postpartum health and resumption of postpartum cyclicity in dairy cows. Livest. Sci. 20145, 181, 31-37.
15. *Jimenez-Krassel F., Folger J., Ireland J. H., Smith G. W., Hou X., Davis J. S., Lonergan P., Evans A. C., Ireland J.*: Evidence that high variation in ovarian reserves of healthy young adults has a negative impact on the corpus luteum and endometrium during reproductive cycles of single-ovulating species. Biol. Reprod. 2009, 80, 1272-1281.
16. *Joe Arosh A., Kathiresan D., Devanathan T. G., Rajasundaram R. C., Rajasekaran J.*: Blood biochemical profile in normal cyclical and anoestrous cows. Indian. J. Anim. Sci. 1998, 68, 1154-1156.
17. *Jorritsma R., Wensing T., Krui T. A., Vos P. L., Noordhuizen J. P.*: Metabolic changes in early lactation and impaired reproductive performance in dairy cows. Vet. Res. 2003, 34, 11-26.
18. *Jung M. Y., Kang S., Lim D. H., Kim T. I., Lee K., Ha S.*: Serum biochemical profiles of repeat breeder Holstein Friesian cows. Korean J. Vet. Ser. 2021, 44, 239-246.
19. *Kelestimur H., Oz Z. D., Ozdede M. R., Serhatlioglu I., Tektemur N. K., Kacar E.*: FRI390 The effects of chronic asprosin administration on ovarian tissue. J. Endoc. Soci. 2023, 7, 114-1584.
20. *Kilinc M. A., Risvanli A.*: The relationship of asprosin with β-hydroxybutyric acid and postpartum disorders in cows. Acta Vet. Hung. 2022, 70, 58-63.
21. *Kılınç M. A., Rişvanlı A., Şafak T., Yılmaz Ö., Yüksel B. F., Şeker İ.*: Gebe ineklerde kan asprosin düzeylerinin biyokimyasal parametrelerle ilişkisi. Tr. J. Nature. Sci. 2022, 11, 111-116.
22. *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. 2021, 16, 313-319.
23. *Kurykin J., Waldmann A., Tiirats T., Kaart T., Jaakma Ü.*: Morphological quality of oocytes and blood plasma metabolites in repeat breeding and early lactation dairy cows. Reprod. Domest. Anim. 2011, 46, 253-260.
24. *Lin M., Jiang M., Yang T., Ma X., Zhao G.*: Effect of bovine asprosin on gluconeogenic gene expression and glucose productionin primary bovine hepatocytes, doi: 10.21203/rs.3.rs-880144/v1.
25. *Maylem E. R. S., Spicer L. J., Batalha I., Schutz L. F.*: Discovery of a possible role of asprosin in ovarian follicular function. J. Mol. Endocrin. 2021, 66, 35-44.
26. *Mimoune N., Messai C. R., Khelef D., Salhi O., Azzouz M. Y., Kaidi R.*: Reproductive parameters and metabolic profile of repeat breeder cows. Livestock Res. Rural Res. Rural Develop. 2017, 29.
27. *Mohebbi-Fani M., Omidi A., Mirzaei A., Nazifi S., Nowroozi K. A.*: Field study on glucose, non-esterified fatty acids, beta-hydroxybutyrate and thyroid hormones in dairy cows during the breeding period in Fars province, Iran. Iranian J. Vet. Res. 2019, 20, 55.
28. *Mondal M. K., Paul S. K.*: Haemato-biochemical profile in repeat breeding cross-bred cows. Explor. Anim. Med. Res. 2012, 2, 60-65.
29. *Mukherjee E., Carroll R., Matfin G.*: Endocrine and metabolic emergencies: hypoglycaemia. Therapeutic Advan. Endocrin. Met. 2011, 2, 81-93.
30. *Ospina P. A., Nydam D. V., Stokol T., Overton T. R.*: Associations of elevated nonesterified fatty acids and β-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 2010, 93, 1596-1603.
31. *Pandey V., Singh A. K., Sharma N.*: Blood biochemical profile in fertile and repeat breeding crossbred cows under field conditions. Vet. Pract. 2009, 10, 45-48.
32. *Pariza K. F., Hossain M. M., Awal M. A., Islam J., Islam M. A.*: Comparative study of hematological and biochemical profiles of repeat breeding Zebu cows. J. Sher-e-Bangla. Agric. Univ. 2011, 6, 8-12.
33. *Ribeiro E. S., Bisinotto R. S., Lima F. S., Greco L. F., Morrison A., Kumar A., Santos J. E. P.*: Plasma anti-Müllerian hormone in adult dairy cows and associations with fertility. J. Dairy Sci. 2014, 97, 6888-6900.
34. *Regmi G., Dhakal I. P.*: Systemic levels of iron, phosphorus, and total protein in normocyclic versus repeat breeder Holstein Friesian crossbred cows of Kesharbag, Chitwan, Nepal. Vet. World 2020, 13, 2353-2357.
35. *Romere C., Duerrschmid C., Bournat J., Constable P., Jain M., Xia F., Chopra A. R.*: Asprosin, a fasting-induced glucogenic protein hormone. Cell. 2016, 165, 566-579.
36. *Sabasthin A., Kumar V. G., Nandi S., Murthy V. C.*: Blood haematological and biochemical parameters in normal cycling, pregnant and repeat breeding buffaloes (Bubalus bubalis) maintained in isothermic and isonutritional conditions. Asian Pacific J. Reprod. 2012, 1, 117-119.
37. *Samarütel J., Waldmann A., Ling K., Jaakson H., Kaart T., Leesmäe A., Kärt O.*: Relationships between luteal activity, fertility, blood metabolites and body condition score in multiparous Estonian Holstein dairy cows under different management. J. Dairy Res. 2008, 75, 485-490.
38. *Widayati D. T., Bintara S., Natawihardja I., Maharani D.*: Blood biochemical profile in fertile and repeat breeder Ongole Cross Breed Cows. Pakistan J. Biol. Sci. 2018, 21, 166-170.
39. *Yusuf M., Nakao T., Ranasinghe R. B., Gautam G., Long S. T., Yoshida C., Koike K., Hayashi A.*: Reproductive performance of repeat breeders in Dairy Herds. Theriogenology 2010, 73, 1220-1229.

Corresponding author: Asist. Prof. Kudret Yenilmez, Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Tekirdağ Namık Kemal University, Tekirdağ, Turkey; e-mail: kyenilmez@nku.edu.tr