Interrelationships between milk yield, anti-müllerian hormone levels, metabolic profile parameters, and first service conception rates in Simmental cows

Yavuz KASALAK[1,a], Muhammed Enes İNANÇ[2,b,✉]

[1]Burdur Mehmet Akif Ersoy University, Institue of Health Science, Department of Reproduction and Artificial Insemination, Burdur, Türkiye. [2]Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Burdur, Türkiye.

[a]ORCID: 0009-0005-1094-7438; [b]ORCID: 0000-0001-6954-6309.

ARTICLE INFO

Article History
Received : 14.02.2025
Accepted : 29.07.2025
DOI: 10.33988/auvfd.1640250

Keywords
AMH
Metabolic profile
Milk yield
Pregnancy
Simmental

✉**Corresponding author**
enesinanc@hotmail.com

How to cite this article: Kasalak Y, İnanç ME (XXXX): Interrelationships between milk yield, anti-müllerian hormone levels, metabolic profile parameters, and first service conception rates in Simmental cows. Ankara Univ Vet Fak Derg, XX (X), 000-000. DOI: 10.33988/auvfd.1640250.

ABSTRACT

This study aimed to evaluate the relationship between milk yield, anti-Müllerian hormone (AMH), metabolic profile test parameters (MPT), and first service conception (FSC) rate in Simmental cows on postpartum day 60. Sixty adult female Simmental cows, all of which were at least in their second lactation period, were selected. The recorded milk yields were categorized into two groups: low milk yield (LMY) (n = 30) and high milk yield (HMY) (n = 30). During milk assessment, the LMY group had a lower lactation number than the HMY group ($P<0.05$). Furthermore, the HMY group exhibited higher Ca/P ratios, glucose, and AMH levels. Phosphorus levels were significantly higher in the LMY group compared to the HMY group ($P < 0.05$). Animals with low AMH levels had lower glucose levels than those with medium and high AMH levels ($P<0.05$). Additionally, AMH levels were positively correlated with albumin (ALB)/ globulin (GLB) ($P<0.05$) and non-esterified fatty acids (NEFA) levels ($P<0.01$). There was a numerical difference for pregnant (818.24±307.54 pg/mL) and non-pregnant (743.96±197.70 pg/mL) animals. In conclusion, AMH levels were positively correlated with milk yield and some metabolic profile parameters in Simmental cows; however, no statistically significant association was found between AMH levels and pregnancy outcomes. Future research should include larger populations and consider additional factors, such as trace elements and reproductive status, to further enhance the understanding of these relationships.

Introduction

Optimal reproductive efficiency is a crucial factor influencing productivity in the dairy industry and an essential component of proficient dairy farm management (36). There is a pressing need for reliable biomarkers in cattle that are highly repeatable, variable, and heritable and that correlate with reproductive performance (15, 34). Field studies have explored various traits, including nutritional aspects, management practices, housing conditions, breeding strategies, and the health status of both the reproductive and overall physiological systems, elucidating the connection between metabolic status in cows and postpartum ovarian function and reproductive efficiency (3, 5). The metabolic profile test (MPT) serves as a diagnostic tool for metabolic diseases by providing insights into the metabolic status of dairy cattle (29, 46). Changes in MPT parameters typically begin during late pregnancy and continue into early lactation. This transition period is critical for reproductive performance, as metabolic imbalances can impair ovarian activity and conception rates (7, 29, 46). One of the key MPT indicators linked to reproduction is beta-hydroxybutyric acid (BHBA), an important marker of negative energy balance (NEB), which has been associated with delayed ovulation and reduced conception rates (18, 52). Furthermore, serum total protein (TP) and albumin (ALB) levels are essential for nutritional status and reproductive success, and their decline has been noted in animals with pyometra or during the postpartum period (4, 20). TP also contributes to nutrition, osmotic pressure, and acid-base balance (27). Elevated globulin (GLB) levels may reflect chronic inflammation, which can negatively affect fertility (29, 40). Serum aspartate aminotransferase (AST) is a sensitive and stable marker (25). Calcium (Ca), magnesium (Mg), and phosphorus (P) are crucial for reproductive hormone secretion, muscle function, and metabolic homeostasis (33, 45). An appropriate Ca-P-Mg balance supports estrus expression and ovulation. Glucose, the primary energy source, is also essential for ovarian function; low glucose levels have been linked to anovulation and subfertility. Blood urea nitrogen (BUN) reflects protein-energy balance, and extreme levels could potentially have adverse effects on fertility (28, 39).

Efficient reproductive management is crucial for ensuring successful herd renewal and achieving high milk yield in dairy cows. A recent study in postpartum dairy cows indicated that milk yield decreased in cows suffering from postpartum problems such as retained placenta (2). Improved milk production is commonly associated with advancements in breeding programs, animal husbandry technologies, animal welfare practices, and nutritional strategies (49). Fertility in dairy cows significantly influences herd economics and genetic progress (12). Among fertility indicators, the first-service conception (FSC) rate is a key measure of reproductive efficiency and overall herd profitability (22). FSC is defined as the proportion of cows that conceive from their first artificial insemination (AI) relative to the total number of inseminated cows (6). Reported FSC rates typically range from 26.7% to 50.7% (31, 43, 44). A low FSC rate can lead to extended open days, increased insemination and treatment costs, feeding inefficiencies, and early culling (9, 17). Therefore, identifying the biological and environmental factors that may limit FSC success is essential for improving the reproductive performance of dairy herds (31).

Anti-Müllerian hormone (AMH), produced by granulosa cells of pre-antral and small antral follicles, is a key endocrine biomarker that reflects the antral follicle count and plays a crucial role in female reproductive physiology and the superovulation response (51). Its levels have been proposed as indicators of fertility, productive lifespan, and herd longevity in cattle. For instance, Jimenez-Krassel et al. (24) reported a positive correlation between AMH levels and herd longevity in heifers, whereas Cengiz et al. (8) observed higher AMH and lower insulin levels in pregnant cows than in non-pregnant cows. These findings support the potential of AMH as a predictive marker of reproductive success (1, 19). Elevated AMH levels in cows are generally associated with enhanced fertility and prolonged productive herd life; however, in older cows, higher AMH concentrations may correlate with a modest reduction in milk yield. In contrast, in young heifers, increased AMH levels have been shown to predict both improved fertility and greater milk production. Moreover, maternal milk yield and composition, particularly in primiparous cows, may influence AMH concentrations in their offspring, suggesting a potential intergenerational effect on reproductive capacity (24, 42). Despite growing interest, only a limited number of studies have examined the relationship between AMH and pregnancy outcomes, and even fewer have investigated the combined evaluation of AMH levels, MPT, and FSC in postpartum dairy cows (8, 16). This integrated approach remains especially in Simmental cattle, where most existing research tends to evaluate these parameters in isolation (21). Understanding the interplay among these factors may offer more comprehensive insights into the determinants of reproductive performance. The novelty of this study lies in its thorough and simultaneous assessment of AMH concentrations, milk yield, and an extensive panel of MPT parameters, all measured at a standardized postpartum time point (day 60) in a homogeneous population of multiparous Simmental cows maintained under uniform management conditions. This integrated approach provides a more precise understanding of the interrelationships between metabolic status, reproductive biomarkers, and lactational performance. Therefore, this study aimed to investigate the relationship between milk yield, AMH, MPT parameters, and FSC in Simmental cows on postpartum day 60.

Materials and Methods

Animals, Management, and Experimental Design: The study involved 60 clinically healthy Simmental cows, each in their second to fifth lactation, aged between 4 and 7 years, and weighing 500–550 kg. Before inclusion, all animals underwent a comprehensive health evaluation, including general and reproductive examinations. General health status was assessed through physical examination (e.g., temperature, heart rate, respiratory rate, rumen motility, and appetite), and reproductive health was evaluated via transrectal palpation and ultrasonography of the reproductive tract. Additionally, cows with a history of metabolic, infectious, or reproductive disorders (e.g., metritis, retained placenta, and ovarian cysts) were excluded from the study. This study was conducted at a private cattle farm located in Kurucay, Burdur, Turkiye (37°38'26' 'N, 30°09'59 '' E, 931 m altitude) during two distinct periods: June through September 2022, when the average temperature was approximately 23°C, and February through May 2023, when the average temperature was approximately 10°C. The GEA DairyPlan C21 farm monitoring program tracked the specified animals' milk output for 60 days following calving. AMH concentrations (51) and milk output were used to rank the animals in descending order. With a range of 19.5-29.8 kg/day (mean ± SEM, 26.84±0.5), the low milk yield (LMY) group (n=30) and the high milk yield (HMY) group (n=30) had the lowest and highest milk yields, respectively, with a range of 29.9-40.9 kg/day (mean ± SEM, 33.79 ± 0.59). Additionally, animals were separated into three groups: low, medium, and high levels of AMH (230.60 ± 12.11 pg/mL, 444.13 ± 18.31 pg/mL, and 1678.76 ± 461.25 pg/mL, respectively). In the study, both pregnant and non-pregnant animals' outcomes were assessed. In addition to being housed in an intensive raising facility, these cows were given unlimited access to drinking water and a total mixed ration (TMR) designed for nursing dairy cows, which produced 30 kg of 4% fat milk (33). The daily ration included 2.2 kg of barley flakes, 1.4 kg of cottonseed meal, 3.8 kg of corn flakes, 0.1 kg of vitamin mixture, 20 kg of corn silage, 1.7 kg of hay, 5.5 kg of alfalfa, 1.5 kg of sunflower seed meal, and 1 kg of soybean. In the morning and evening, TMR was provided twice daily. The TMR had 54% dry matter, 6.9% crude ash, 13.15% crude protein, and 2.4% crude fat, according to chemical analysis.

Determination of Body Condition Scores: Body Condition Score (BCS) of selected animals was recorded at the initiation of the synchronization protocol, using the evaluation method developed by Edmonson et al. (13). The scoring ranged from 1 (very lean) to 5 (very fat), with intervals of 0.25. Only animals with a BCS within the range of 2.75-3.75 were included in the study.

Cows Synchronization Protocol, AI, and Pregnancy Control: The study implemented the modified G6G protocol (Figure 1) with synchronization starting on postpartum day 42, followed by AI on day 60. The AI was performed via the rectovaginal method in the corpus uteri using thawed semen at 37°C for 30 s with the same proven fertile bull semen. The pregnancy rate on the 60th day after insemination was assessed using a Honda (HS-102V) ultrasound.

Blood Sample Collection, MPT, and AMH Analysis: For the MPT, a blood sample was collected from the vena coccygea immediately after AI into a gel-activator-containing vacutainer. The collected blood was centrifuged, and the resulting serum was removed and stored at -20°C until analysis. The serum content was analyzed using a DiaSys Response® 910VET autoanalyzer to determine BUN, AST, gamma-glutamyl transferase (GGT), glucose, TP, ALB, Ca, cholesterol, triglyceride, Mg, P, BHBA, and Non-esterified fatty acids (NEFA) levels. All the kits for all parameters were supplied by DiaSys (Germany). The GLB value was obtained by subtracting the TP value from the ALB value.

AMH concentration was assessed using a bovine ELISA kit (Cloud-Crone Corp., USA) (Catalog No: CEA228Bo) according to the manufacturer's protocol. Optical density was measured spectrophotometrically at 450 nm using a microplate reader (BioTek, 800 TS ELISA reader/USA). The concentration of serum samples was calculated in pg/mL by plotting a standard curve.

Statistical Analysis: Data were tested for normality using Shapiro–Wilk's test, followed by Levene's test for homogeneity of variances. Data were evaluated by grouping them as milk yield (low and high), AMH (low, medium, and high), and pregnancy rates (pregnant and non-pregnant). Data demonstrating normal distribution were analyzed by Student's t-test and one-way analysis of variance (ANOVA) to assess the differences between groups, while Mann-Whitney U and Kruskal-Wallis tests were used for non-normalized data. Post-hoc analysis was performed using Duncan's test for variables with significant differences between groups. All statistical analyses were conducted with a minimum margin of error of 5% using SPSS 22.0 software. Standard Error of the Mean (SEM) was used in the present study. Conception rate was assessed using the chi-square test. Correlation analyses were performed to determine the relationships between MPT parameters, AMH levels, and FSC rates.

Results

The figure 2 presents the MPT profiles and other parameters of the animals categorized into two groups based on milk yield (LMY and HMY groups). A significantly lower lactation number was observed in the LMY group than in the HMY group (3.20 ± 0.21 vs. 4.00 ± 0.17) (P<0.05). Furthermore, the Ca/P ratio (1.74 ± 0.07 vs. 1.51±0.06), glucose levels (44.50 ± 2.58 vs. 36.05 ± 3.31 mg/dL), and AMH levels (1073.67 ± 318.21 vs. 461.29 ± 56.73 pg/mL) were higher, whereas P levels (4.99 ± 0.26 vs. 5.76 ± 0.25 mg/dL) were lower in the HMY group than in the LMY group (P<0.05).

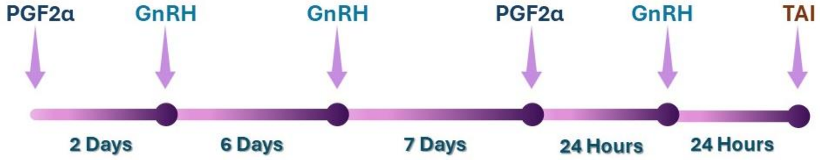

Figure 1. The modified G6G synchronization protocol
GnRH: Gonadotropin-releasing hormone
PGF2α: Prostaglandin F2 alpha
TAI: Timed artificial insemination

DOI: 10.33988/auvfd.1640250

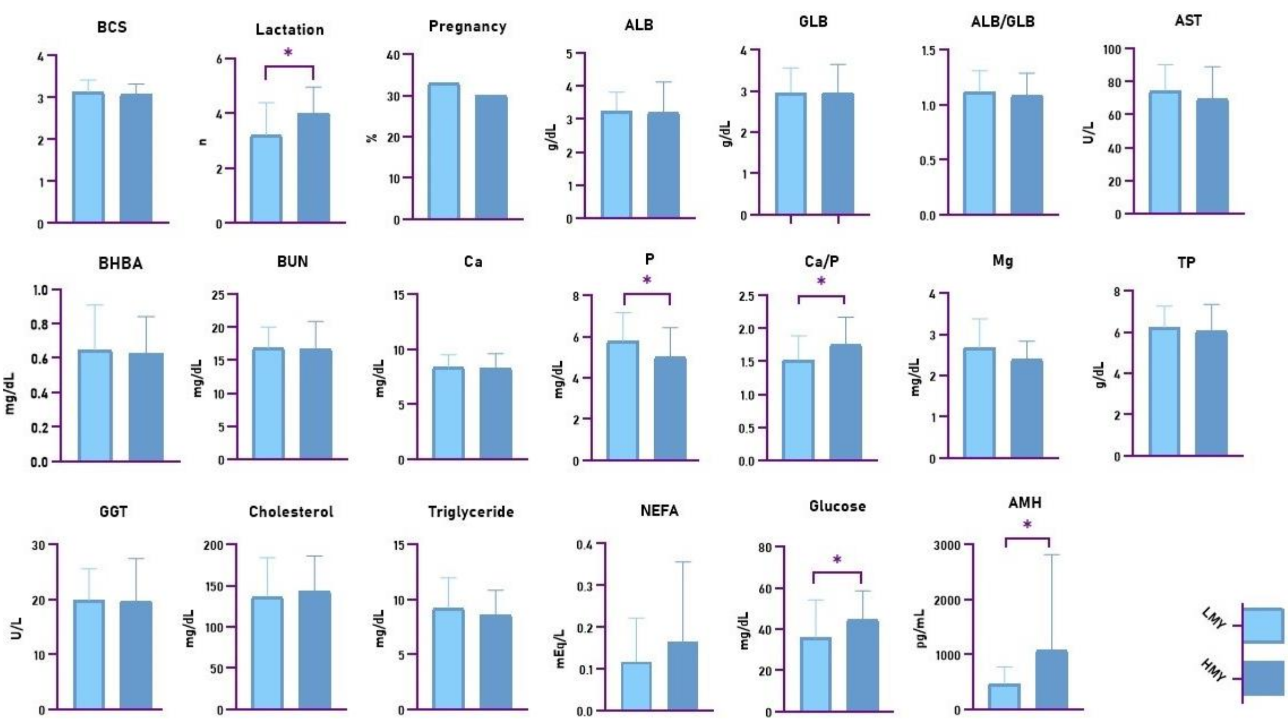

Figure 2. Metabolic profile and other parameter results according to milk yield

LMY: Low Milk Yield (19.5-29.8 [Mean: 26.84±0.5] kg), HMY: High Milk Yield (29.9-40.9 [33.79±0.59] kg), ALB: Albumin, AMH: Anti-Mullerian Hormone, AST: Aspartate aminotransferase, BCS: Body condition score, BHBA: Beta hydroxybutyric acid, BUN: Blood urea nitrogen, Ca: Calcium, GGT: Gama glutamyl transferase, GLB: Globulin, Mg: Magnesium, NEFA: Non-esterified fatty acids, P: Potassium, TP: Total protein
*P<0.05

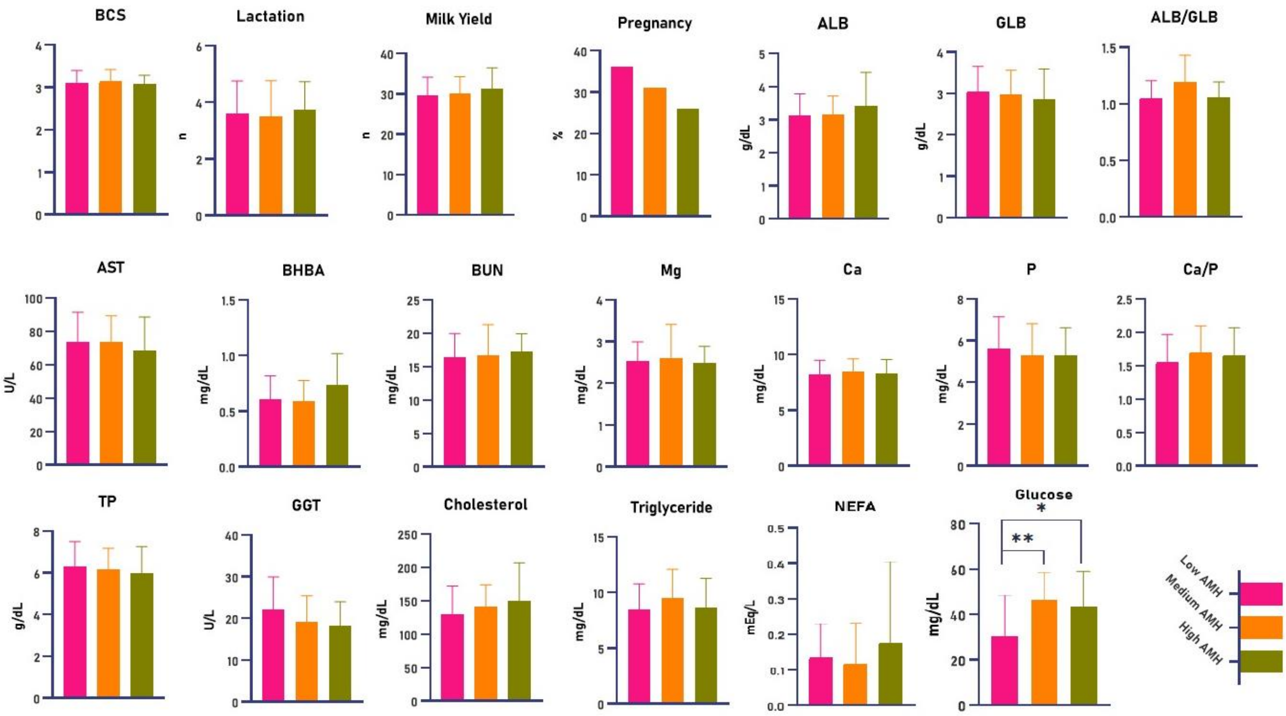

Figure 3. Metabolic profile and other parameter results according to AMH levels

Low AMH (230.60±12.11 pg/ml), Medium AMH (444.13±18.31 pg/ml), High AMH (1678.76±461.25 pg/ml), ALB: Albumin, AMH: Anti-Mullerian Hormone, AST: Aspartate aminotransferase, BCS: Body condition score, BHBA: Beta hydroxybutyric acid, BUN: Blood urea nitrogen, Ca: Calcium, GGT: Gama glutamyl transferase, GLB: Globulin, Mg: Magnesium, NEFA: Non-esterified fatty acids, P: Potassium, TP: Total protein. *P<0.05, **P<0.01

The MPT profile and other parameters of the animals were categorized into three groups (low [230.60 ± 12.11 pg/mL], medium [444.13 ± 18.31 pg/mL], and high [1678.76 ± 461.25 pg/mL]) based on AMH levels, as presented in Figure 3. Regarding AMH levels, animals with low AMH levels exhibited lower glucose levels (30.24 ± 4.16, 46.15 ± 2.53, 43.50 ± 3.70 mg/dL) than animals with medium and high AMH levels (P<0.05). The FSC rate is shown in Figure 4 and was determined to be 31.7% in the present study (P<0.05).

The MPT profiles and other parameters of the animals categorized into two groups based on pregnancy status (non-pregnant and pregnant) are shown in Figure 5. No statistically significant difference was observed between the groups according to pregnancy status (P>0.05). Although not statistically significant, the AMH level in pregnant animals (818.24 ± 307.54 pg/mL) was slightly higher than that in non-pregnant animals (743.96 ± 197.70 pg/mL).

The correlation results for the parameters used in this study are presented in Table 1. Milk yield was positively correlated with Ca/P and AMH. AMH levels positively correlated with ALB/GLB and NEFA levels. However, the pregnancy status showed no significant correlation with the parameters examined (P>0.05). Furthermore, the metabolic profile results demonstrated both positive and negative correlations (P<0.01, P<0.05).

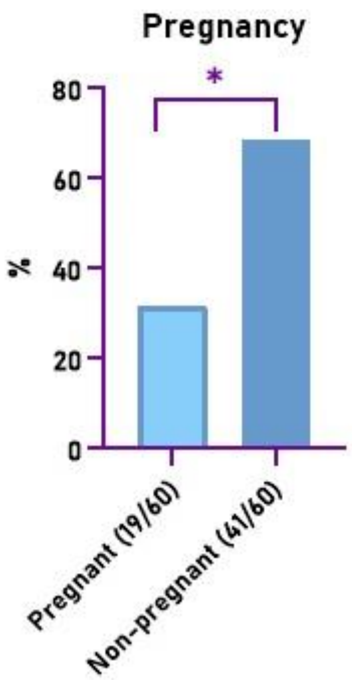

Figure 4. Total pregnancy rate as a result of synchronization in cows
*P<0.05

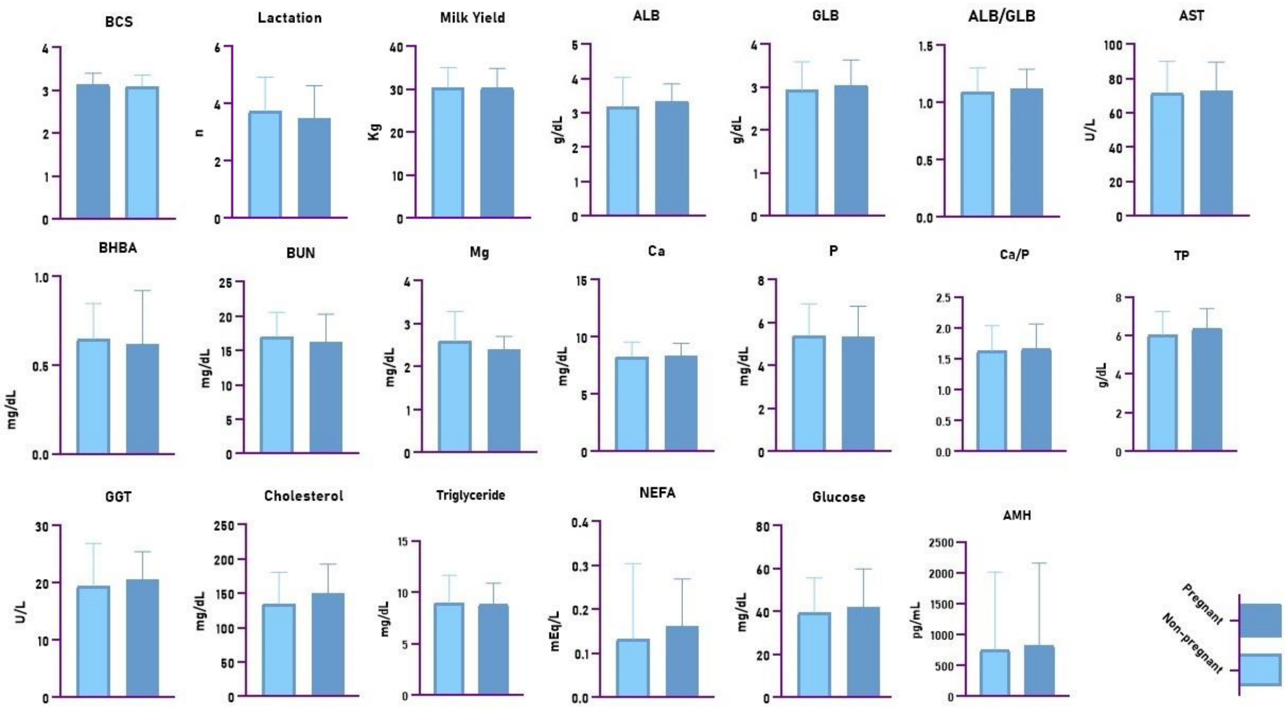

Figure 5. Metabolic profile and other parameter results according to pregnancy status

ALB: Albumin, AMH: Anti-Mullerian hormone, AST: Aspartate aminotransferase, BCS: Body condition score, BHBA: Beta hydroxybutyric acid, BUN: Blood urea nitrogen, Ca: Calcium, GGT: Gama glutamyl transferase, GLB: Globulin, Mg: Magnesium, NEFA: Non-esterified fatty acids, P: Potassium, TP: Total protein

DOI: 10.33988/auvfd.1640250

Table 1. Metabolic profile and correlations with other parameters

	BCS	Lactation	Milk yield	Pregnancy	ALB	ALB/GLB	AST	BHBA	BUN	Ca	Ca/P	Cholesterol	GGT	GLB	Glucose	Mg	NEFA	P	TP	Triglyceride
Lactation	0.205																			
Milk Yield (kg)	-0.128	.309*																		
Pregnancy	0.061	0.083	-0.016																	
ALB (g/dL)	-0.08	0.072	-0.017	0.101																
ALB/GLB	-0.206	-.345**	0.012	0.017	0.152															
AST (U/L)	0.008	-0.053	-0.201	0.032	.684**	0.074														
BHBA (mg/dL)	0.062	-0.075	0.012	-0.05	0.179	-0.012	0.14													
BUN (mg/dL)	-0.087	-0.087	0.1	-0.113	0.169	.338**	0.169	.339**												
Ca (mg/dL)	-0.082	-0.007	0.065	0.023	.707**	0.055	.707**	.274*	0.249											
Ca/P	-.347**	0.063	.273*	0.021	0.064	-0.088	0.028	-0.219	-.375**	0.179										
Cholesterol	-0.238	-0.023	0.181	0.151	.544**	0.224	.489**	0.201	0.227	.683**	0.236									
GGT (U/L)	0.053	0.067	-0.038	0.077	.482**	0.116	.342**	0.182	0.159	.266*	-.265*	.398**								
GLB (g/dL)	0.102	.264*	-0.066	0.088	.659**	-.399**	.592**	0.219	0.035	.679**	-0.039	.436**	.488**							
Glucose (mg/dL)	0.031	0.129	0.222	0.065	0.151	0.103	0.169	-0.1	0.185	0.245	.301*	.326*	-0.1	0.044						
Mg (mg/dL)	-0.161	-0.147	-0.172	-0.154	.368**	0.17	.481**	0.109	.329*	.436**	-0.093	.334**	0.103	.314*	0.02					
NEFA (meq/L)	-0.218	-0.061	0.198	0.088	-0.017	.375**	0.027	-0.144	-0.142	0.031	.346**	0.157	-0.023	-0.211	-0.031	-0.103				
P	.323*	-0.037	-0.233	-0.025	.327*	0.141	.344**	.337**	.496**	.324*	-.835**	0.099	.448**	.370**	-0.216	.316*	-0.241			
TP (g/dL)	0.022	0.136	-0.065	0.145	.816**	-0.111	.691**	0.24	0.171	.795**	-0.043	.580**	.584**	.933**	0.076	.406**	-0.12	.456**		
Triglyceride (mg/dL)	0.204	-0.127	-0.198	-0.03	.476**	0.156	.549**	0.162	.261*	.538**	-0.1	.491**	.382**	.425**	0.216	.375**	-0.119	.394**	.564**	
AMH (pg/mL)	-0.139	-0.057	.302*	0.027	-0.098	.266*	-0.061	-0.038	0.001	-0.001	0.22	0.079	-0.123	-0.235	0.208	-0.089	.592**	-0.174	-0.178	-0.108

ALB: Albumin, AMH: Anti-Mullerian hormone, AST: Aspartate aminotransferase, BCS: Body condition score, BHBA: Beta hydroxybutyric acid, BUN: Blood urea nitrogen, Ca: Calcium, GGT: Gama glutamyl transferase, GLB: Globulin, Mg: Magnesium, NEFA: Non-esterified fatty acids, P: Potassium, TP: Total protein. ** Correlation is significant at the 0.01 level., * Correlation is significant at the 0.05 level.

DOI: 10.33988/auvfd.1640250

Discussion and Conclusion

This study aimed to assess the relationship between milk yield, AMH levels, MPT parameters, and the FSC rate in Simmental cows on postpartum day 60. The AMH level was significantly higher in the HMY group than in the LMY group. AMH is produced by ovarian follicles and released into the bloodstream (13). According to AMH level, Jimenez-Krassel et al. (24) separated heifers into four groups (the first group having the lowest AMH levels and the fourth group having the highest AMH levels) and kept track of them for at least two lactations. In this study, Group 1 had fewer lactation cycles and a shorter productive life. Group 1 also had a higher first-calving mortality rate than the intermediate group (Group 3) and lower milk production than the average of all groups. These results indicate that AMH may be a reliable tool for identifying heifers with longer lifespans.

The present data show that cows in the HMY group had higher AMH and lactation numbers, indicating that they were older, a finding that contrasts with the general understanding that AMH declines with age. These results can be attributed to several reasons (e.g., genetic merit, selection bias, and better-managed cows remaining longer in the herd). In addition, studies have found correlations between the number and quality of growing follicles and AMH levels (23). Upon comparing the results of these studies with those of the present study, lower AMH levels were obtained in the LMY group, whereas higher AMH levels were obtained in the HMY group, indicating compatibility. Correlation analysis revealed a positive correlation between milk yield and AMH levels, thereby supporting this outcome ($P<0.05$). Considering the recent finding that follicle number is a moderately heritable genetic trait in dairy cows (48), these results suggest that AMH could potentially serve as a marker for determining longevity in individuals. Average AMH levels in pregnant animals were found to be high, and AMH analysis offers valuable insights into the reproductive efficiency of herds (41). İleritürk and Kaynar (21) examined the relationship between Ca, β-hydroxybutyrate, P, Mg, TP, total cholesterol, free glycerol, triacylglycerol, serum protein, serum lipid profiles, and AMH in dairy cows during the three weeks prior to calving and three weeks following calving. These findings indicate that free glycerol and BHBA influence AMH concentration, whereas Mg levels and the proportion of cholesterol esters in total serum fat remain relatively stable throughout the transition period. AMH may also serve as a reliable biomarker for reduced follicular activity associated with negative NEB during the transition period. Additionally, it could be used as a criterion for culling animals that fail to conceive after multiple inseminations. While the findings were consistent with those obtained previously, the present study revealed only a numerical difference between AMH levels and pregnancy rates. Numerical differences may translate into statistical differences when a larger animal population is used. In this study, AMH levels were positively correlated with ALB/GLB and NEFA. Okawa et al. (36) categorized dairy cattle into four groups based on their prepartum day 14 (dry period) and postpartum (days 10 and 28) AMH levels. Mg, NEFA, and ALB/GLB were positively correlated with AMH levels on days 10 and 28. Acetone, acetoacetate, and 3-hydroxybutyric acid are ketones generated by the metabolism of volatile fatty acids (38). Depending on the severity of NEB, the production of ketone bodies may increase due to elevated levels of NEFA, oxidative stress, and reactive oxygen species, which are further exacerbated by stress factors in dairy cows during the perinatal period (10). In this study, NEFA levels were 0.13 ± 0.02, 0.11 ± 0.02, and 0.17 ± 0.05 mEq/L in animals with low, medium, and high AMH levels, respectively. The accepted optimum range of NEFA levels in cattle is 0.10-0.79 mEq/L (37). Additionally, ALB levels (g/dL) were determined as 3.40 ± 0.23, 3.15 ± 0.11, and 3.12 ± 0.14, and ALB/GLB ratios as 1.05 ± 0.03, 1.19 ± 0.05, and 1.05 ± 0.03 in animals with low, medium, and high AMH levels, respectively. The optimum ALB level in cattle is accepted within the 3.4-4.2 range (37). Based on these findings, we speculated that normal NEFA, ALB, and ALB/GLB values were positively correlated with AMH levels. Moreover, NEFA and acute-phase proteins within normal limits may be positively correlated with AMH levels. However, it is also necessary to continue working with animals with NEB and high NEFA levels.

The study revealed that animals with low AMH levels exhibited lower glucose levels than those with medium and high AMH levels ($P<0.05$). Furthermore, the high milk yield group demonstrated higher glucose levels and lactation numbers than the low milk yield group ($P <0.05$). AMH levels were associated with productive herd life and milk yield in cows and correlated with blood glucose levels and lactation number. Additionally, when analyzing the relationship between AMH and glucose levels, studies focusing on humans have revealed a potential association, particularly in patients with polycystic ovary syndrome (11, 47, 50). Based on the results obtained and the previous studies, it is suggested that blood glucose levels and milk production are correlated with AMH levels, and the variations observed may be attributed to differences in animal species, age, and animal. Furthermore, insights from human medical studies can be adapted and applied to veterinary medicine.

In the current study, all animals were monitored postpartum, and the modified G6G/Ovsynch ovulation synchronization protocol was administered on the 42nd day, followed by fixed-time AI on the 60th day. Kara et al. (24) examined the relationship between milk yield and

DOI: 10.33988/auvfd.1640250

the G6G protocol in Simmental cows after parturition. The pregnancy rate was 42.8% in animals 45-60 days after parturition. In another retrospective study conducted on Simmental cows with G6G synchronization, the pregnancy rate was 54.4% in cold weather, 36.6% in hot weather, and 48.9% overall (30). Kuru et al. (32) conducted a comparative study between the modified Ovsynch (double PGF2α with 24 h intervals) and Ovsynch protocols in Simmental cows, revealing pregnancy rates on the 60th day after insemination as 25.4% and 34.9%, respectively. In addition to the effect of many variables on the differences in the studies, it is interpreted that the differences in the studies vary depending on environmental factors, such as primiparous and multiparous animals, region, management, climate, care, feeding, success of the experience of the veterinarian performing AI, and modifications applied to the G6G synchronization protocol.

In conclusion, AMH levels were positively correlated with milk yield and certain MPT in Simmental cows on postpartum day 60. Although pregnant animals had numerically higher AMH levels than non-pregnant animals, this difference was not statistically significant. Therefore, no definitive conclusion can be drawn regarding the association between AMH levels and pregnancy outcomes. Further studies with larger sample sizes are needed to clarify this potential relationship. These results should be evaluated with various trace elements in addition to MPT in large animal populations, including heifers and primiparous cows, and studies should be continued.

Acknowledgements

This study was derived from the master's thesis of the first author.

Financial Support

This research was supported by Project No. 0914-YL-23/2017K12-41003 by Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordination.

Ethical Statement

The animal use protocol for this study was approved by the Local Ethics Committee on Animal Research of Burdur Mehmet Akif Ersoy University (17.11.2021/821).

Conflicts of Interest

The authors declared that there is no conflict of interest.

Author Contributions

YK: Data collection, measurement of MPT and ELISA parameters, and artificial insemination in cows. MEİ: Study conception and design, drafting of the manuscript, and critical revision of the content.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

References

1. **Alward KJ, Graves WM, Palomares RA, et al** (2021): *Characterizing Anti-Müllerian Hormone (AMH) concentration and change over time in Holstein dairy cattle*. Theriogenology, **168**, 83-89.
2. **Amin Y, Zakaria A, Hasan A** (2021): *The efficacy of treatment of retained placenta with chlortetracycline and oxytetracycline through local intrauterine route in dairy cows*. J Anim Health Prod, **9**, 100-106.
3. **Amin YA, Abu El-Naga EM, Noseer EA, et al** (2019): *Synchronization with controlled internal drug release (CIDR) and prostaglandin F2α (PGF2α) influences oxidant/antioxidant biomarkers and mineral profile in summer-stressed anoestrous buffalo (Bubalus bubalis)*. Theriogenology, **134**, 34-41.
4. **Amin YA, Ali RA, Fouad SS, et al** (2021): *The deleterious effect of postpartum pyometra on the reproductive indices, the metabolic profile, and oxidant/antioxidant parameters of dairy cows*. Vet World, **14**, 329-38.
5. **Amin YA, Mahmoud AEZ, Ali RA, et al** (2023): *Treatment of inactive ovaries of Holstein dairy cows by epidural injection of GnRH analogue (receptal) and its impact on the reproductive hormones, oxidant/antioxidant profile and micro and macro-elements profile*. Animals (Basel), **13**, 653.
6. **Ata A** (2013): *Sütçü siğirlarda döl verimi ölçütlerinin güncel yorumu*. MAKU J Health Sci Inst, **1**, 30-41.
7. **Başoğlu A, Sevinç M** (2004): Evcil Hayvanlarda Metabolik ve Endokrin Hastalıklar. Pozitif Matbaacılık, Konya.
8. **Cengiz M, Tohumcu V, Furqan Asghar Chacher M, et al** (2023): Endocrinological and metabolic profile in relation to pregnancy at the first insemination in cows housed under cold conditions. Vet Sci Pract, **17**, 71-5.
9. **Chang YM, Andersen-Ranberg IM, Heringstad B, et al** (2006): *Bivariate analysis of number of services to conception and days open in Norwegian red using a censored threshold-linear model*. J Dairy Sci, **89**, 772-8.
10. **Chastant S, Saint-Dizier M** (2019): *Inflammation: friend or foe of bovine reproduction?* Anim Reprod, **16**, 539-547.
11. **Chun S** (2015): *1-h Postprandial glucose level is related to the serum anti-Müllerian hormone level in women with polycystic ovary syndrome*. J Gynaecol Endocrinol, **31**, 815-818.
12. **Drackley JK, Cardoso FC** (2014): *Prepartum and postpartum nutritional management to optimize fertility in*

high-yielding dairy cows in confined TMR systems. Animal, **8,** 5-14.

13. **Durlinger AL, Visser JA, Themmen AP** (2002): *Regulation of ovarian function: the role of anti-Müllerian hormone*. Reproduction, **124**, 601-609.
14. **Edmonson AJ, Lean IJ, Weaver LD, et al** (1989): *A body condition scoring chart for Holstein dairy cows*. J Dairy Sci, **72**, 68-78.
15. **Gobikrushanth M, Purfield DC, Colazo MG, et al** (2018): *The relationship between serum anti-Müllerian hormone concentrations and fertility, and genome-wide associations for anti-müllerian hormone in Holstein cows*. J Dairy Sci, **101**, 7563-7574.
16. **Gobikrushanth M, Dutra PA, Felton CA, et al** (2016): *The association between anti-mullerian hormone concentrations, antral follicle count and fertility measures in dairy cows.* J Anim Sci, **94**, 506-507.
17. **González-Recio O, Pérez-Cabal MA, Alenda R** (2004): *Economic value of female fertility and its relationship with profit in Spanish dairy cattle*. J Dairy Sci, **87**, 3053-61.
18. **Güngör Ş, Özkara H, İnanç M, et al** (2022): *The effect of blood BHBA level on fertility in sheep*. J VetBio Sci Tech, **7**, 220-227.
19. **Hamed MA, Amin YA, Mohamed RH, et al** (2023): *Evaluation of chemical castration using intra-testicular injection of zinc gluconate into the testis of the male donkey versus surgical castration: antimullerian hormone as an endpoint marker*. BMC Vet Res, **19**, 140.
20. **Ibrahim RM, Amin YA, Rehan IF, et al** (2024): *Laboratory investigation of haematological and biochemical parameters throughout the periparturient phase in fat-tailed ewes*. SVU Int J Vet Sci, **7**, 39-55.
21. **Ileritürk M, Kaynar Ö** (2023): *Effect of anti-mullerian hormone, metabolic profile and mineral levels at transition period on the calving – conception interval in cows*. Kocatepe Vet J, **16**, 143-59.
22. **Inchaisri C, Hogeveen H, Vos PLAM, et al** (2010): *Effect of milk yield characteristics, breed, and parity on success of the first insemination in Dutch dairy cows*. J Dairy Sci, **93**, 5179-5187.
23. **Ireland JJ, Zielak-Steciwko AE, Jimenez-Krassel F, et al** (2009): *Variation in the ovarian reserve is linked to alterations in intrafollicular estradiol production and ovarian biomarkers of follicular differentiation and oocyte quality in cattle*. Biol Reprod, **80**, 954-64.
24. **Jimenez-Krassel F, Scheetz D, Neuder L, et al** (2015): *Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life*. J Dairy Sci, **98**, 3036-3045.
25. **Kaneko JJ, Harvey JW, Bruss ML** (2008): Clinical Biochemistry of Domestic Animals. Academic Press, California.
26. **Kara U, Düzer M, Hızlı H, et al** (2023): *Determining the optimum voluntary waiting period and synchronization protocol in Simmental cows*. Kocatepe Vet J, **16**, 401-409.
27. **Karagül H, Fidancı UR, Altıtaş A, et al** (2000): Clinic Biochemistry. Medisan, Ankara.
28. **Kennerman E** (2000): *Serum lipoprotein levels in cows with subclinical ketosis*. Turk J Vet Surg, **6**, 52-55.
29. **Kennerman E** (2011): *Metabolic profile test in dairy cows*. Türkiye Klinikleri J Vet Sci, **2**, 96-110.
30. **Khalil AAY** (2019): *Fertility response of lactating dairy cows subjected to three different breeding programs under subtropical conditions*. Beni-Suef Univ J Basic Appl Sci, **8**, 6.
31. **Kim IH, Jeong JK** (2019): *Risk factors limiting first service conception rate in dairy cows and their economic impact*. Asian-Australas J Anim Sci, **32**, 519-526.
32. **Kuru M, Kaçar C, Oral H, et al** (2020): *Effect of two prostaglandin F2α injections administered 24 hours apart on the pregnancy rate of Simmental cows subjected to the Ovsynch or Ovsynch + Controlled internal drug release (CIDR) protocols*, Med Weter, **76**, 660-665.
33. **Meyer D, Harvey J** (1998): Laboratory Medicine Testing: Specimen Interferences and Clinical Enzymology. 3-22 p. In: Veterinary laboratory Medicine. Interpretation and Diagnosis. WB Saunders Company, Philadelphia.
34. **Nawaz M, Jimenez-Krassel F, Steibel J, et al** (2018): *Genomic heritability and genome-wide association analysis of anti-Müllerian hormone in Holstein dairy heifers*. J Dairy Sci, **101**, 8063-75.
35. **NRC** (2001): Nutrient requirements of dairy cattle. National Academies Press. Nutrient requirements of dairy cattle. National Academy Press, Washington DC.
36. **Okawa H, Monniaux D, Mizokami C, et al** (2021): *Association between anti-Müllerian hormone concentration and inflammation markers in serum during the peripartum period in dairy cows*. Animals, **11**,1241.
37. **Plumb DC** (2018): Plumb's Veterinary Drug Handbook. John Wiley & Sons, Stockholm, Wisconsin.
38. **Puppel K, Kuczyńska B** (2016): *Metabolic profiles of cow's blood; a review*. J Sci Food Agric, **96**, 4321-8.
39. **Quiroz-Rocha GF, LeBlanc SJ, Duffield TF, et al** (2009): *Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition*. Can Vet J, **50**, 383.
40. **Radostits OM, Gay CC, Hinchcliff KW, et al** (2006): Diseases associated with viruses and diseases associated with bacteria. 96-101. In: Veterinary Medicine. Philadelphia.
41. **Sabuncu A, Dal GE, Enginler SO, et al** (2019): *Association of Anti-Müllerian Hormone concentrations between the pregnancy rates and pregnancy continuity of cows in different age groups*. Vet Med, **64**, 302-308.
42. **Schwarzmann L, Marchand A, Knutti B, et al** (2023): Effects of postpartum diseases on antral follicle count and serum concentration of anti-müllerian hormone in dairy cows. Anim Rep Sci, **255**, 107291.
43. **Siddiqui M, Das Z, Bhattacharjee J, et al** (2013): *Factors affecting the first service conception rate of cows in smallholder dairy farms in Bangladesh*. Reprod Domest Anim, **48**, 500-505.
44. **Tillard E, Humblot P, Faye B, et al** (2008): *Postcalving factors affecting conception risk in Holstein dairy cows in tropical and sub-tropical conditions*. Theriogenology, **69**, 443-57.
45. **Turgut K** (2000): Veteriner Klinik Laboratuvar Teşhis. Bahçıvanlar Baskı, Konya.
46. **Van Saun RJ (2007):** Application of a pooled sample metabolic profile for use as a herd screening tool. 24-25 In: Danish Bovine Practitioner, Middelfart.

47. **Verdiesen RM, Onland-Moret NC, van Gils CH, et al** (2021): *Anti-Müllerian hormone levels and risk of type 2 diabetes in women*. Diabetologia, **64**, 375-84.
48. **Walsh S, Mossa F, Butler ST, et al** (2014): *Heritability and impact of environmental effects during pregnancy on antral follicle count in cattle*. J Dairy Sci, **97**, 4503-11.
49. **Whitfield L** (2020): *Milk production, fertility and the modern dairy cow*. Livestock, **25**, 72-75.
50. **Yetim Sahin A, Bas F, Yetim Ç, et al** (2019): *Determination of insulin resistance and its relationship with hyperandrogenemia, anti -müllerian hormone, inhibin A, inhibin B and insulin-like peptide-3 levels in adolescent girls with polycystic ovary syndrome*. Turk J Med Sci, **49**, 1117-1125.
51. **Yildiz M, Bastan I, Guler S, et al** (2024): *Effect of serum anti-Müllerian hormone levels on the superovulation response in Holstein heifers*. Vet Med Sci, **10**, e1507.
52. **Yildiz R, İder M, Ok M** (2019): *Beta hidroksi bütirik asit düzeyinin diğer metabolik test parametreleri üzerine etkisi*. Vet Hekim Der Derg, **90**, 15-21.

Publisher's Note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

DOI: 10.33988/auvfd.1640250