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Association between metabolic parameters and embryo production in superovulated dairy cattle

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Abstract

This study aims to investigate the relationship between metabolic parameters and the number of embryos produced in superovulated cows with high genetic characteristics in milk yield. Eighteen Holstein donors were treated with classic superovulation protocols, AI and flushing. During superovulation, decreasing doses of FSH (follicle-stimulating hormone) were administered at 12-h intervals for 4 days. Plasma insulin-like growth factor (IGF1), glucose (GLU), beta-hydroxybutyric acid (BHB), non-esterified fatty acid (NEFA), blood urea nitrogen (BUN) and total protein (TP) levels were determined by using an autoanalyzer. The mixed model analysis of variance was used for statistical analysis. As a result, plasma IGF1, BHB and BUN had significant interactions with both groups and days ($p < .05$). Additionally, plasma TP–days interactions were significant ($p < .05$). Furthermore, there was a negative correlation between the number of embryos and plasma BHB levels ($p < .05$). In conclusion, under appropriate environmental conditions, metabolic profile control of donors can contribute to the embryo production process and to the studies on the metabolic infrastructure.

KEYWORDS
cattle, embryo, metabolic parameter, superovulation

1 | INTRODUCTION

The technology of producing and transferring embryos is a significant assisted reproduction technique utilized in the cattle industry (Snider et al., 2021). These techniques aim to improve the genetic characteristics of the herd by transferring embryos to a surrogate cow (Mapletoft & Bó, 2014). However, for obtaining embryos in an efficient embryo production programme, it is crucial to have a successful superovulation protocol and a high number of transferable embryos (Bó et al., 2010).

Superovulation in cattle aims to increase the number of fertilized and transferable embryos, providing a high chance of achieving pregnancy (Mapletoft & Bó, 2014). The most significant problem reported by researchers in embryo production and transfer studies is individual variability in superovulation success, environmental factors, feeding and stress factors. These factors also affect oocyte development and quality (Mapletoft & Bó, 2014; Sutton-McDowall et al., 2010).

Especially in cows with high milk yield, reproductive performance may be lower than the targeted level due to metabolism and negative energy balance. Negative energy balance, causing a decrease in fertility success (Souissi & Bouraoui, 2019), detrimentally affects embryo production success, animal breeding, animal production and costs. Nutritional factors alter metabolic function, leading to disruption of homeostasis, increased stress response and reduced reproductive function (Walsh et al., 2012). In addition, folliculogenesis, ovulation and early embryonic development are highly influenced by metabolic status, depending on nutrient requirements (Cardoso et al., 2020), that is mainly because metabolic parameters are directly related to hormonal response and follicular development. Therefore, metabolic parameter monitoring is a vital pathway for reproductive success (Leroy et al., 2008; Snider et al., 2021).

There are many markers of metabolic function. GLU, insulin and the IGF family are vital regulators of metabolic activities and affect reproductive function (Aziz et al., 2017; Snider et al., 2021; Velazquez et al., 2009). GLU is a crucial metabolite for cumulus–oocyte complex (COC) development. The majority of total glucose is metabolized via the glycolytic pathway to provide substrates such as pyruvate for energy production during oocyte maturation. The oocytes are sensitive to glucose concentration-dependent perturbations in nuclear and cytoplasmic maturation, leading to poor embryonic development after fertilization (Sutton-McDowall et al., 2010). In other words, dysregulation of glucose levels can cause changes in ovulation, fertilization and conception success (Walsh et al., 2012). Insulin-like growth factor plays a crucial role in the reproductive success of cattle by acting as a monitoring signal that allows reproductive functions to occur when nutritional conditions are appropriate for success in reproduction (Velazquez et al., 2009). Other metabolic markers are BUN, TP, NEFA and BHB. BUN is a product of indigestible protein breakdown in the rumen, and peripheral urea nitrogen concentration reflects protein metabolism in dairy cattle (Butler, 2000). Increased serum BUN concentrations adversely influence overall reproductive performance (Snider et al., 2021). NEFA concentrations are a metabolic marker for determining the mobilization of body fat stores and their concentration is highly correlated with reproductive success, as increased NEFA levels negatively affect ovulation and early embryonic development (Cardoso et al., 2020; Moussa et al., 2015). BHB is a ketone body involved in the mobilization of fat breakdown. Moreover, increased levels of BHB have a detrimental impact on reproductive performance (Beever, 2006). The relationship between lipid and protein mobilization markers may be a key element in maintaining the nutrient balance necessary for reproductive function while providing an ideal uterine environment during fertilization, embryo development and attachment (Snider et al., 2021).

This study aims to investigate the relationship between some biochemical parameters and the number of embryos produced in cows with high genetic characteristics in terms of milk yield in the superovulation protocol. Moreover, by revealing the concentration of these parameters in the superovulation protocol, it aims to contribute to the studies on the metabolic infrastructure affecting embryo production.

2 | MATERIALS AND METHODS

2.1 | Materials

Eighteen Holstein donors between the ages of 3 and 5 years with similar milk yield (average 30/35 litre/day) and body condition scores (3–3.5) were selected from the International Livestock Research and Training Center with an ethical approvement number: (197/31.01.2022). The cows with no reproductive anomaly, reproductive diseases (endometritis, etc.), abortion or dystocia were selected. The feeding regime (Table 1) was prepared according to National Research Council, Committee on Animal Nutrition, and Subcommittee on Dairy Cattle Nutrition, 2001.

TABLE 1 The feeding regime.

Ratio (kg)	Morning	Evening
Corn silage (kg)	10	10
Wheat straw (kg)	1.50	1.50
Meadow grass (kg)	2.50	2.50
Alfalfa hay (kg)	1.5	1.5
Concentrate feed (kg)	6	6
Bypass-oil[a]	0.25	0.25

Note: The ratio was adjusted to meet nutrient requirements for the materials in the study (National Research Council, Committee on Animal Nutrition, and Subcommittee on Dairy Cattle Nutrition, 2001). Animals were fed by TMR (Whole Ration: Complete Feed) at 60/40 forage/concentrate level. Concentrate feed includes % 14.8 crude protein and 2.20 Mcal/kg DM energy. The cows were fed twice daily at 07:00 A.M and 07:00 P.M.

[a]Bypass-oil (Megalac, Volac International L.T.D).

Donors were divided into three groups according to the number of produced embryos. These groups consisted of donors with 1–2 embryos (Group A [$n=3$]), 3–4 embryos (Group B [$n=6$]) and 5–6 embryos (Group C [$n=9$]).

2.2 | Superovulation and flushing

For the study, progesterone (CIDR, Pfizer, Hamilton, New Zealand) was administered intra-vaginally to the donors regardless of the period of the sexual cycle (Day-0). Then, 10-cc intra-muscular FSH (Stimufol, Reprobiol®, Belgium) administration was applied at decreasing doses (2, 2; 1.5; 1.5; 1; 1; 1; 1; 0.5; 0.5 mL) at 12-h intervals for 4 days starting from the seventh day (Day-7). PGF2α (Dinolytic, Pfizer, Spain) was intra-muscularly administered on the ninth day (Day-9) morning, and CIDR was removed on the ninth day evening. On the eleventh day (Day-11) of the superovulation protocol, oestrus was monitored, and artificial insemination (AI) was performed in the morning and evening (Figure 1). The donors were between 70th and 90th day of lactation when AI performed.

On the eighteenth day (Day-18), epidural anaesthesia is performed by treating lidocaine to the site between the sacro-coccygeal intervertebral space and the first intercoccygeal intervertebral space. A double-way silicone balloon (Bioniche, Belleville, Canada) catheter, Lactated Ringer solution (Ringer VIP, Polifarma, Turkey) containing 1% foetal calf serum (Sigma-Aldrich, USA) and antibiotic 125 mg/L Kanamycin (Kanovet, Deva, Turkey), a 75-micron EmCon filter (Agtech, USA) and a 90-mm Petri including PBS + 20% FCS + 04% bovine serum albumin (BSA) solution were used during the flushing process. After the flushing, 50-cc intrauterine antibiotic (Gentamicin, Turkey) was administered. Finally, the flushing solutions were examined under a stereo microscope (Leica, S80AP0, Wetzlar, Germany), and embryos were detected.

2.3 | Blood samples and parameters

A total of 288 blood samples were taken from 18 donors, during FSH application days of the superovulation protocol, 4 times in a day (Figure 1). The blood samples were collected by using EDTA vacutainer tubes from each donor's tail vein. The samples in EDTA vacutainer tubes were centrifuged at 1107 *g* for 5 min, and plasmas were stored at −20°C. Plasma TP, BHB, BUN, NEFA and IGF1 levels were measured by Randox commercial kits using the autoanalyzer device.

2.4 | Statistical analysis

Descriptive statistics for each variable were calculated and presented as "mean ± standard error of mean (SEM)." The variables were examined as parametric test assumptions. To test the effects of groups, day of sampling and their interaction on IGF1, BHB, GLU, NEFA, TP and BUN, a linear mixed model for repeated measures was performed. Animals were included in the model as random effects, while groups, days of sampling and their interactions were included as fixed effects. Variance components were used as the covariance structure in the established model since it resulted in the lowest Akaike information criterion. When a significant difference was obtained, all significant terms were compared using a simple effect analysis with Bonferroni correction. The Spearman correlation coefficient was performed to assess the correlation between the number of embryos, IGF1, BHB, GLU, NEFA, TP and BUN levels. $p<0.05$ was considered statistically significant. All statistical analyses were performed using IBM SPSS-Version-23.0.

3 | RESULTS

Interactions of plasma IGF1, BHB, NEFA, GLU, TP and BUN levels with the groups and days are shown in Table 2 and Figure 2.

Table 2 and Figure 2 show that plasma IGF1, BHB and BUN had significant interactions with both groups and days ($p<.05$). Additionally, plasma TP–days interactions were significant ($p<.05$), whereas plasma TP–groups interactions were not significant ($p>.05$). Moreover NEFA–groups and NEFA–days interactions were not significant ($p>.05$), while the groups–days interaction in plasma NEFA was significant ($p<.05$).

Table 3 shows that there was a negative correlation between the number of embryos and plasma BHB levels ($p<.05$), while no significant correlation was found between plasma IGF1, GLU, NEFA, TP and BUN levels ($p>.05$).

4 | DISCUSSION

The most of IGF1 required for the development of bovine antral follicles is obtained from the peripheral circulation. However, granulosa cells in superovulated cattle can produce IGF1 (Velazquez et al., 2009). Kuehner et al. (1993) and O'callaghan et al. (2000) reported that IGF1 levels in plasma and follicular fluid increased during the superovulation period. On the other hand,

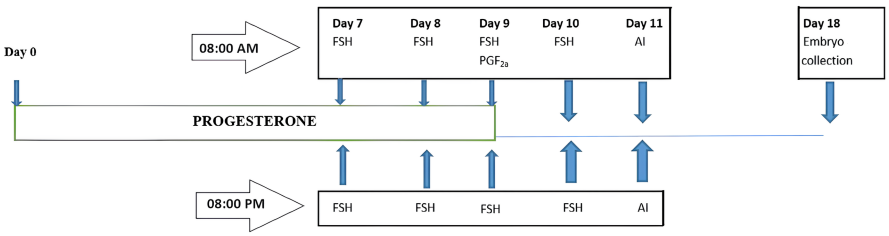

FIGURE 1 Superovulation protocol.

TABLE 2 Interactions of metabolic parameters with groups and days.

			Days				*p*		
	Groups	*n*	7	8	9	10	G	D	G×D
IGF1 (ng/mL)	A	3	$55.09 \pm 5.56^{ab,y}$	$50.25 \pm 7.17^{b,y}$	$51.32 \pm 8.58^{b,y}$	$70.03 \pm 10.65^{a,y}$	.015	.002	.369
	B	6	$66.46 \pm 6.49^{ab,y}$	$55.64 \pm 4.92^{b,y}$	$40.65 \pm 3.01^{b,y}$	$67.52 \pm 8.31^{a,y}$			
	C	9	$66.48 \pm 4.2^{ab,x}$	$56.84 \pm 3.3^{b,x}$	$52.85 \pm 5.19^{b,x}$	$80.69 \pm 7.83^{a,x}$			
BHB (mmol/L)	A	3	$0.52 \pm 0.07^{b,x}$	$0.54 \pm 0.06^{b,x}$	$0.62 \pm 0.1^{b,x}$	$0.8 \pm 0.16^{a,x}$	.026	.001	.071
	B	6	$0.34 \pm 0.02^{b,y}$	$0.39 \pm 0.03^{b,y}$	$0.38 \pm 0.03^{b,y}$	$0.41 \pm 0.03^{a,y}$			
	C	9	$0.43 \pm 0.04^{b,y}$	$0.41 \pm 0.03^{b,y}$	$0.39 \pm 0.03^{b,y}$	$0.46 \pm 0.04^{a,y}$			
GLU (mg/dL)	A	3	72.66 ± 5.69	73.05 ± 4.74	77.26 ± 5.27	78.40 ± 7.98	.058	.790	.991
	B	6	83.26 ± 2.61	80.78 ± 5.44	85.32 ± 4.59	82.53 ± 3.44			
	C	9	82.25 ± 2.44	82.18 ± 3.46	82.57 ± 3.4	82.94 ± 2.71			
NEFA (mmol/L)	A	3	$0.44 \pm 0.3^{a,x}$	$0.10 \pm 0.03^{b,x}$	$0.44 \pm 0.27^{a,x}$	$0.22 \pm 0.11^{ab,x}$	.566	.105	.033
	B	6	$0.15 \pm 0.02^{a,x}$	$0.25 \pm 0.03^{a,x}$	$0.23 \pm 0.02^{a,x}$	$0.21 \pm 0.04^{a,x}$			
	C	9	$0.16 \pm 0.02^{a,x}$	$0.21 \pm 0.03^{a,x}$	$0.24 \pm 0.02^{a,x}$	$0.18 \pm 0.02^{a,x}$			
TP (g/dL)	A	3	8.03 ± 0.35^{ab}	8.23 ± 0.12^{ab}	8.50 ± 0.16^{a}	7.40 ± 0.58^{b}	.840	.002	.652
	B	6	7.74 ± 0.29^{ab}	8.18 ± 0.22^{ab}	8.65 ± 0.21^{a}	7.94 ± 0.22^{b}			
	C	9	7.83 ± 0.18^{ab}	8.12 ± 0.22^{ab}	8.08 ± 0.21^{a}	7.44 ± 0.38^{b}			
BUN (mmol/L)	A	3	$17.38 \pm 1.26^{b,x}$	$18.94 \pm 0.68^{a,x}$	$18.35 \pm 1.02^{a,x}$	$17.44 \pm 4.81^{b,x}$	.002	.001	.193
	B	6	$11.56 \pm 1.38^{b,y}$	$16.29 \pm 1.04^{a,y}$	$19.93 \pm 1.53^{a,y}$	$11.67 \pm 2.11^{b,y}$			
	C	9	$14.94 \pm 1.33^{b,x}$	$17.7 \pm 1.24^{a,x}$	$17.62 \pm 1.55^{a,x}$	$13.5 \pm 1.7^{b,x}$			

Note: ^{a,b}—in the same line represent the difference between days in each group ($p < .05$). ^{x,y}—in the same row represent the difference between groups in each day ($p < .05$). G—groups interactions (day Independent). D—days interactions (group Independent). G×D—groups–days interaction.
Abbreviations: BHB, beta-hydroxybutyric acid; BUN, blood urea nitrogen; IGF, insulin-like growth factor; NEFA, non-esterified fatty acids; TP, total protein.

Herrier et al. (1994) and Cushman et al. (2001) observed that plasma IGF1 level was not changed during the superovulation period. Furthermore, Simpson et al. (1994) determined an increase in plasma IGF1 concentrations on days 4 and 5 compared to days 1 and 2 during a 5-day superovulation treatment. Moreover, in the same study, a positive correlation was found in the level of IGF1 in plasma and follicular fluid only on Day 4. Aziz et al. (2017) reported that plasma IGF1 concentration before superstimulation was not significant for predicting the number and quality of embryos; however, there was a relationship between plasma IGF1 concentration at oestrus and on the day of embryo collection and total yield of grade A embryos. Additionally, Kasimanickam et al. (2020) reported that plasma IGF1 levels were positively correlated with fertility and embryo production, unless extreme levels were present. This study is in parallel with the studies conducted by Kasimanickam et al. (2020) and Aziz et al. (2017) in terms of plasma IGF1 level and interaction between groups. Moreover, in terms of the course of the plasma IGF1 level determined in the research during the FSH administration period, although it is not parallel with the results by Herrier et al. (1994) and Cushman et al. (2001), it is similar to the results of the study conducted by Simpson et al. (1994). In addition, considering the increase in IGF1 level detected on the 4th day of the application, the results of this research are similar to the study conducted by Kuehner et al. (1993) and O'callaghan et al. (2000). Considering that the main source of plasma IGF1 hormone is liver and indirectly granulosa cells, it can be said that the differences in IGF1 hormone levels detected during the application period are normal. Because, studies (Bilby et al., 2004, 2006; Yu et al., 2003) indicate that superovulation application mainly affects the IGF1 hormone level in follicular fluid, but plasma IGF1 level is less affected by the application.

NEFA and BHB parameters are known as the indicators of negative energy balance in cattle (Kang et al., 2020; Van der Drift et al., 2012). Furthermore, NEFA and BHB levels are highly affected by feeding regimens and metabolic factors (Xu et al., 2010). Many authors consider BHB concentrations between 1.2 and 2.9 mmol/L as subclinical ketosis and ≥3.0 mmol/L as clinical ketosis Benedet et al. (2019). Studies have shown that high concentrations of BHB have detrimental effects on bovine granulosa cell viability and cell growth (Gareis et al., 2018; Jorritsma et al., 2004). Chaput and Sirard (2020) reported that high BHB levels (>0.9 mmol/L) can have a negative effect on in vivo embryo production. In this research, results were parallel to those reports and it was observed that the group with the highest BHB value was Group A, in which 1–2 embryos were obtained. Moreover, a negative correlation was found between plasma BHB level and number of embryos. Considering that the plasma BHB levels of the groups were within the reference value range and less than 0.9 mmol/L, it is reasonable to find a weak negative correlation. In addition, although it was within the reference value range, the

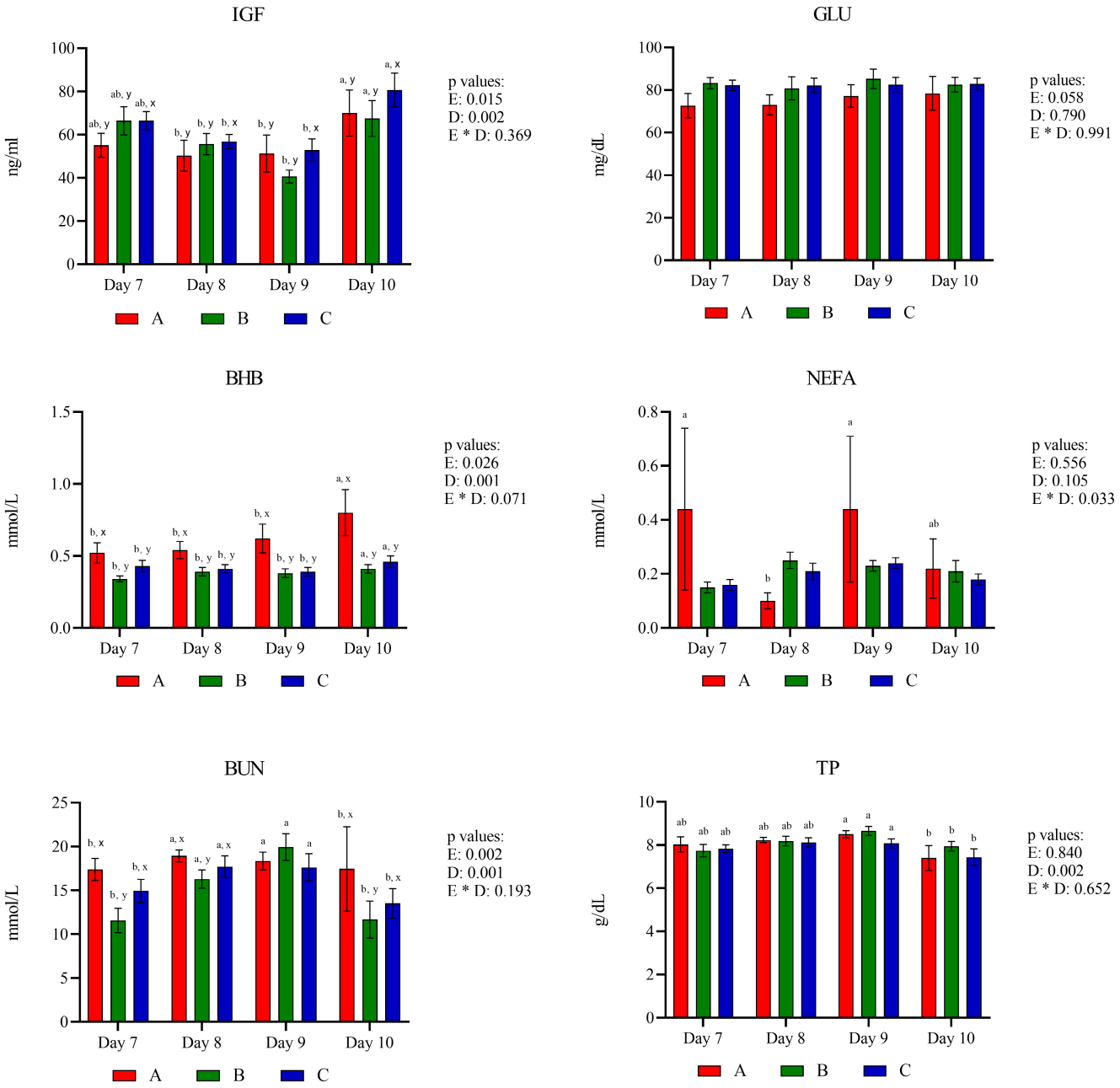

FIGURE 2 Plasma IGF, BHB, glucose, NEFA, TP and BUN values by days and groups. [a,b]—superscripts represent the difference between days in groups ($p < .05$). [x,y]—superscripts represent the difference between groups in days ($p < .05$).

plasma BHB level was significant in terms of the relationship between groups and days, which is thought to be due to the fact that serum BHB levels come from two different sources. In other words, it is not known which of the VFAs formed in the rumen comes from adipose tissue, and therefore, it can be explained by the fact that these values may also be negative in the studies of obtaining embryos by superovulation application.

High NEFA concentrations negatively affect energy metabolism and the endocrine system, resulting in lower fertility rates (Bionaz et al., 2020; Lomander et al., 2012; Moussa et al., 2015). In addition, Snider et al. (2021) reported that FSH hormone treatment had no effect on the change in plasma NEFA values, but NEFA concentrations varied according to days. This research is in parallel with the study conducted by Snider et al., 2021 in terms of plasma NEFA levels. Djallel et al. (2021) did not observe an alteration in plasma NEFA values during the superovulation period. However, in this study, plasma NEFA levels of Group A differed on the 9th and 10th days of superovulation. This result differs from the research conducted by Djallel et al. (2021) in terms of daily variation of plasma NEFA levels. This difference in Group A is thought to be due to the fact that the sample size of Group A is less than the other groups. Nevertheless, the plasma NEFA levels determined during all treatment days in the study did not exceed the reference values (Animal Health Diagnostic Center, 2023).

	IGF1	BHB	GLUCOSE	NEFA	TP	BUN
Embryo number	.046	−.182*	.063	−.123	−.029	−.071

Note: Values indicated with * in the same line are statistically significant (*$p < .05$).

TABLE 3 Correlation analysis results of the number of the embryos and parameters.

Also, it is thought that small changes in daily NEFA values may be due to metabolic functions.

Glucose serves as the primary nutrient to maintain the energy balance of the cow and is closely related to feeding strategies. Providing adequate amounts of glucose is crucial for the promotion of cumulus expansion and nuclear maturation (Dupont et al., 2014; Sutton-McDowall et al., 2010). In this context, Omari et al. (2020) reported that low glucose levels affect gonadotropin levels, leading to anoestrus and negative effects on reproduction. On the other hand, Snider et al. (2021) determined that serum glucose levels did not change during superovulation. Interestingly, Rasolomboahanginjatovo et al. (2014) compared serum and uterine fluid glucose levels on different days with samples taken from donors treated with and without superovulation. There was no difference between the superovulated and non-superovulated groups. In addition, there was no differences in serum glucose concentrations on different days. However, a significant difference was found in terms of uterine glucose levels. Leroy et al. (2008) reported that follicular fluid may contain higher glucose than serum. In this study, plasma glucose levels were found to be within or very close to the values accepted as normal for cattle (Animal Health Diagnostic Center, 2023). Furthermore, this study is in parallel with Snider et al. (2021) and Rasolomboahanginjatovo et al. (2014) in terms of plasma glucose levels and daily changes of plasma glucose levels. In the research, the interaction of plasma glucose level with groups and days was not found statistically significant, and no significant correlation was found with the number of embryos. This result could be explained by the fact that the donors belonging to the groups had similar yield characteristics and were fed with the same feeding regime. Moreover, as different glucose levels can be detected in follicular fluid and uterine fluid, the location of the sample is also considered to be an influential factor.

BUN value is a parameter used to indicate the protein/energy ratio in the diets of healthy dairy cows (Webb & De Bruyn, 2021). Many researchers have shown that high blood urea nitrogen (BUN) concentrations in cows have a negative effect on oocyte quality, embryo development, pregnancy and fertility (De Wit et al., 2001; Ocon & Hansen, 2003; Rhoads et al., 2006). Firstly, in this research, plasma BUN levels of all groups were within the reference range. However, in this study, interestingly, plasma BUN values of Group A and Group C were similar, while plasma BUN level of Group B was found to be lower than Group A and Group C. Although a research parallel to these results was not found, the fact that plasma BUN values were similar in Group A and Group C can be accepted as normal considering the studies (Yawongsa, 2006) which reported that plasma BUN levels are not expected to have a negative effect on reproduction. It can also be explained by the fact that the values were within the reference value range.

Plasma TP level is a parameter influenced by key environmental factors such as nutrition, milk yield, age and somatic cell count in milk (Bobbo et al., 2017). Moreover, blood serum proteins affect fertility and cycle in dairy cattle (Bobbo et al., 2017; Park et al., 2010). However, in a study conducted by Guzel and Tanriverdi (2014) involving both fertile and repeat breeder cows, it was reported that there was no statistical difference in TP levels between donors. Similarly, in the research by Takahashi et al. (2013), different numbers of embryos were obtained using different feeding protocols, but there was no significant difference in TP levels. Moreover, in a similar study by Agrawal and Maurya (2002), no statistical difference was found in TP levels ($p > .05$), as well. In this research, the results of the interaction of plasma TP level with group and day and its correlation with embryo number were in parallel with those studies. Although a similar statistical difference was detected only between the 9th and 10th days in terms of plasma TP levels in all groups, plasma TP levels of all FSH administration days were close and within the reference value range (Animal Health Diagnostic Center, 2023). It is thought that the main reason for obtaining parallel results in terms of plasma TP levels between the groups is the similarity of effective environmental factors such as age, lactation, feeding programme and ration contents of the animal materials. In addition, the fact that the plasma TP levels of the groups were within the range of reference values (Animal Health Diagnostic Center, 2023) is thought to be effective in the absence of a significant result in terms of plasma TP level and group interaction.

5 | CONCLUSION

In conclusion, it was determined that the interaction of plasma IGF1 and BHB values with the groups was significant. According to this, it was revealed that increasing plasma IGF1 levels had a positive effect, but increasing plasma BHB levels had a negative effect on in vivo embryo production success. Besides that, the interaction of FSH application days and plasma IGF1, BHB, TP and BUN values was found to be significant. However, it was obtained that this interaction between FSH application days had no effect on the number of produced embryos. On the other hand, considering the negative correlation of plasma BHB level with the number of embryos, it has been observed that increasing plasma BHB level negatively affects the number of produced embryos. Consequently, it has been concluded that, under appropriate environmental conditions, metabolic profile control of donors undergoing superovulation in cattle can contribute to the embryo production process. Further studies are needed to determine the metabolic profile of the donors, with more animals, samples and parameters.

AUTHOR CONTRIBUTIONS

Engin Ünay: Design, drafting the manuscript and critical revisions. **Alaeddin Okuroğlu:** Design, interpretation, drafting the manuscript, critical revisions and data collection. **Mehmet Borga Tirpan:** Drafting the manuscript, critical revisions and data collection. **Muhammed İkbal Coşkun:** Nutritional design. **Ramazan Sevgi:** Data collection. **Mehmet Ali Yilmaz:** Data collection. **İlker Ünal:** Data collection. **Abdülkadir Erişek:** Analysis. **Erkan Say:** Data collection. **Muharrem Satilmiş:** Data collection. **Ufuk Kaya:** Analysis and interpretation.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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