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## **Relationships between hematological parameters and Cl and Na homeostasis in dairy herds and abortion**

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**Abstract**---Abortion is among reproductive parameters affecting the profitability of dairy herds. Abortion incidence is influenced and/or controlled by different infectious, environmental, and managerial factors and so forth. The effect of environmental and nutritional conditions on minerals, as well as interactions between minerals and physiological conditions, are leading parameters to consider during gestation. This study attempts to assess links between these factors and abortion by determining and measuring homeostasis-electrolyte factors, such as Na and Cl, and hematological parameters, such as complete blood count, in dairy herds with abortion. A total of 40 Holstein dairy herds were chosen and split into two 20-member control and aborted groups. Abortions were reported by ranchers, and blood samples were taken considering the history and current status of herds and clinical examinations. Blood samples were taken twice, once with EDTA to study hematological characteristics and once with no anticoagulant to measure hemostasis-electrolyte factors (Na, Cl) from the serum of herds with abortion. The relationships between these parameters and variations observed in dairy herds with abortion were compared with those in the control group. There was no significant difference in concentrations of sodium and chlorine between the two groups ( $p > 0.05$ ). There was also no significant difference between healthy herds with those with abortion in terms of hematological parameters ( $p > 0.05$ ).

**Keywords**---homeostasis factors, hematology, dairy herds, abortion.

## Introduction

Today, sustainable gestation and breeding healthy and strong calves are the main goals for many ranchers. However, abortion is a common problem in Iran and many other countries. In this context, reproductive performance is the foremost factor influencing the profitability of dairy herds (De Vries, C.A; 2005). Abortion affects reproduction and economic earning and, particularly in late gestation, leads to significant economic losses (Norman et al., 2012). Generally, the rate of abortion incidence has been reported to be from 7.6 to 21.6%. In Iran, the rate of abortion incidence is high (from 10 to 20% for infection-caused abortions in Tehran to up to 30% in Tabriz), compared to other nations such as the United States (9.8%) and New Zealand (1.2%). Abortion is a multifactorial phenomenon controlled by many factors, including infectious agents (bacterial, viral, fungal, and protozoan agents, etc.), and non-infectious factors such as nutritional and chemical agents, drugs, toxins, plant toxins, hormonal factors, and unknown causes (Alshawwa SZ, 2020; Al-Ghamdi M, et. al., 2020). Avoiding abortion in the herds is difficult, as many causes of abortion are unknown (Smith., 2015). Among the infectious agents, 50 to 65% are bacterial, followed by fungal (20 to 25%) and viral (15 to 25%) cases, as well as other agents (Gadicke et al., 2010) .

Additionally, reproductive performance can be influenced by a deficiency of minerals and vitamins. Many studies have reported the use of these substances, though results are different due to using various sources and their usability, and complex metabolic links between supplying such substances and reproductive performance, such as reproduction, lactation, and more (Mcdowell., 2002). In a study, concentrations of calcium, potassium, zinc, sodium, and magnesium were investigated in herds with abortion and placental retention. In herds with abortion, zinc concentrations were lower than in controls and those with placental retention, presumably due to elevated PGF2 $\alpha$  levels. However, the concentration of other minerals did not alter significantly. Interestingly, the concentration of calcium is increased in herds with brucellosis (Mcdowell, 2002). Fetal death and/or abortion have also been reported under selenium deficiency. Elevated concentrations of iodine are likewise risky for the fetus and reduce resistance to infections (Haj and Bahrami; 2019) .

Electrolytes are also vital because they are associated virtually with all metabolic processes in the body. Following increased evidence on electrolyte balance in various animal diseases, electrolyte therapy and replacement have become widespread in veterinary medicine (Ježek and Klinkon., 2010). To achieve an effective therapy, understanding the mechanisms involved in electrolyte changes and interpreting the laboratory results of electrolytes and acid-base balance is crucial. In animals, water accounts for about 45 to 70% of the body weight, in terms of the body fat percentage (BFP). Fat incorporates a little water content and thus more water is found in lean animals than fat counterparts (Seifi at al., 2017). Generally, body fluids include extracellular fluid (ECF) and intracellular fluid (ICF) compartments.

ECF is mainly made up of plasma, lymph, and transcellular fluid. ECF predominantly contains sodium as the cation (Na<sup>+</sup>) and chloride as an anion (Cl<sup>-</sup>), which are redistributed according to Gibbs-Donnan equilibrium. The

intravascular fluid of the ECF is structurally similar to the interstitial fluid, but it encloses more protein contents. Roughly, half of the body's total sodium is found in the ECF in which sodium functions predominantly. The remaining portion is mainly found in the bones and is not usable for the ECF. The concentration of sodium is controlled by intaking from dietary and excretion out of the body (Kirovski and Lazarević; 2011). In carnivores, sodium is usually abundantly found, but it may be deficient in herbivores if being not absorbed in sufficient quantities. Sodium, chlorine, and potassium are maintainers of osmotic pressure and regulate the acid-base balance for the rumen flora and cells (Probo and Giordano; 2012)

Hyponatremia (decreased plasma sodium) is often caused by an abnormal loss of large amounts of sodium in animals. For nutritional deficiency, compensatory renal toxins act and store sodium. Hyponatremia often occurs within the gastrointestinal tract during diarrhea or vomiting, or in renal diseases where the sodium storage mechanism is impaired due to tubular injury (Weston et al., 2012). Chlorine adjusts electrolyte balance in or out of cells. The lungs differentiate chlorine ions from bicarbonate ( $\text{HCO}_3^-$ ) when exchanging oxygen ( $\text{O}_2$ ) and carbon dioxide ( $\text{CO}_2$ ). Besides sodium, chlorine serves to maintain water balance and osmotic pressure. It also plays a critical role in adjusting the acid-base balance. Metabolically, chlorine is absorbed inactively and following the active transfer of sodium. Roughly, all the process takes place in the intestine (Weston et al., 2012). Chlorine is excreted parallel to sodium. Chlorine absorbed from food or stomach secretion eventually enters the bloodstream in the intestines. The surplus amounts of chlorine are excreted by the kidneys, with sodium in sweat, and feces. Elevated blood concentrations of chlorine are caused by metabolic acidosis due to prolonged diarrhea, respiratory alkalosis, drug overdose, water and salt retention, diabetes mellitus, and so on. By contrast, reduced blood concentrations of chlorine are due to prolonged vomiting or suction of gastric secretions, metabolic acidosis, chronic respiratory acidosis, salt restriction in CKD, burns, and more (Molefe et al., 2019).

### **Chlorine deficiency and toxicity**

Chlorine deficiency is rare and seldom occurs in gastrointestinal disorders such as vomiting, diarrhea, gastric suction, and excessive sweating, especially in infants, children, and athletes who are at risk. Hypochloremia has been reported in herds with chloride deficient formula. Complications include loss of appetite, impaired growth, muscle weakness, lethargy, severe metabolic alkalosis, and hypokalemia (Weston et al., 2012). According to Kohan Ghadr et al. (2008), to identify the genetic and epigenetic cause of abortion and stillbirth, cattle must be at least not infected with bovine viral diarrhea virus (BVDV) and leukemia. Therefore, the study of physiological and fundamental causes expands our knowledge about abortion and stillbirth and enables efficient breeding and management. However, a comprehensive study is costly and, thus, studies often focus on particular case indicators. Some attempts in this context include "risk factors for abortion in dairy cows from commercial Holstein dairy herds in the Tehran region" (Rafati and Mehrabani., 2010), "risk factors and rates of perinatal and postnatal mortality in cattle in Switzerland" (Bleul et al., 2011), "risk factors for perinatal mortality in dairy cattle: cow and fetal factors, calving process"

(Gundelach and Essmeyer., 2009), and "fetal death: comparative aspects in large domestic animals" (Jonker et al., 2004). Additionally, hormonal, biochemical, and hematological profiles in female camels (Ali and Tharwat and Al-Sobayil; 2010), hematology, and blood biochemistry in goats, as well as horses and their foals, before and after delivery, have been investigated. Given the various factors that influence abortion and stillbirth, the huge costs of such studies, and physiological factors contributing to these phenomena, we focus on electrolytes and other influential factors in Holstein dairy cows.

## **Methods**

Blood samples were taken and complete blood count and differential cell count tests were performed. Then, clotted specimens were obtained and serum was isolated for testing homeostatic factors (Na and Cl) and hematologic parameters. All the samples were taken of Holstein cattle from farms around Tabriz (Iran). Holstein cattle were split into two healthy cows (control) and those with abortion (aborted) groups, each with 20 members. Blood samples were taken twice, once with EDTA to study hematological characteristics and once with no anticoagulant to measure hemostasis-electrolyte factors (Na, Cl) from the serum of herds with abortion. Samples were immediately transferred to the laboratory in ice-containing bags. The first series of samples were tested for hematological factors. Serum samples were stored at -20 °C for electrolyte tests. Blood samples containing EDTA were centrifuged at 2500 rpm for 15 to 20 min to isolate serum from samples. Samples with EDTA were tested for hematological factors and EDTA-free samples were evaluated for hemostasis-electrolyte factors (Na, Cl).

### **Hematocrit measurement**

Hematocrit (HCT) or packed cell volume (PCV) is measuring the volume percentage (vol%) of packed red blood cells (RBC) in blood. The test is carried out by centrifuging samples in a microhematocrit centrifuge intended by the ICSH Committee as the reference technique for measuring hematocrit and calibrating cell count devices. This step is similar to the microhematocrit method. WBCs were counted by the technique proposed by Feldman et al. (2006). Morphological investigation of WBCs and their differential diagnosis were according to Buret et al. (2004).

### **RBC count**

RBCs were counted using isotonic and diluent solutions that do not lead to lysis of blood cells. The number of RBCs was counted by a special pipette and RBC-diluting solutions (3% Hayem's isotonic saline). To count RBCs, EDTA-containing blood samples were taken up to a point (0.5). The solution was then sucked up to mark 101 of the RBC pipette cleaned by cotton or gauze to achieve a dilution of 1:200 with a diluting solution. Blood was thoroughly blended in the pipette on a shaker for a few minutes. When blood was thoroughly mixed with Hayem's solution, the first two drops were taken out, the grounded cover slide was placed on the Neubauer chamber, and the third drop of the suspension was poured between the slide and cover slide. The suspension was distributed between the two layers by the capillary. The suspension was allowed for a few minutes to

settle down the RBCs in the chamber. The chamber was filled once and suddenly while, simultaneously, overfilling and bubbles were avoided. Counting was started from the middle square with the best cell diffusion. Cells in the middle square and the four adjacent squares were counted, accounting for one-fifth of the total area of the middle square (80 squares out of 400 squares). The score obtained was multiplied by 5 and then considered as the number of cells in one square ( $1 \times 1 \times 0.1 \text{ mm}^3$ ). Considering the dilution ratio (1.200) and the depth of the counting chamber (0.1 mm), the final value was obtained by the equation below:

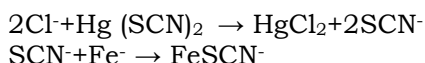
$$\frac{N}{\frac{1}{5} \times \frac{1}{10} \times \frac{1}{200}}$$

### Measurement of Na and Cl electrolytes

The concentration of Na was measured by the Caretium-921 electrolyte analyzer at Dr. Ali Armi's medical diagnostic laboratory to achieve accurate results. The device incorporates six electrodes, including sodium, potassium, chlorine, and calcium ions, each with a selective ion film. Concentrations of calcium and chlorine were measured by the HITACHI 912 biochemistry autoanalyzer.

### Kit-based chlorine measurement

Concentrations of chlorine were measured manually and instrumentally by a kit obtained from Bionic Company.



Tests were performed at 480 nm (220 to 500 nm) with a 1 cm cuvette at 27 °C. The device's temperature was set at 15 to 20 °C and the device was blanked with distilled water. Standard reagents and samples were poured into the cuvette. After blending for 5 min at 37 °C, samples were red against the blank. The resulting color was stable for at least 30 min.

$$\text{Cl concentration} \left( \frac{\text{mMol}}{l} \right) = \frac{\text{observed absorption (A)}}{\text{standard (A) absorption}} \times \text{standard concentration}$$

### Results

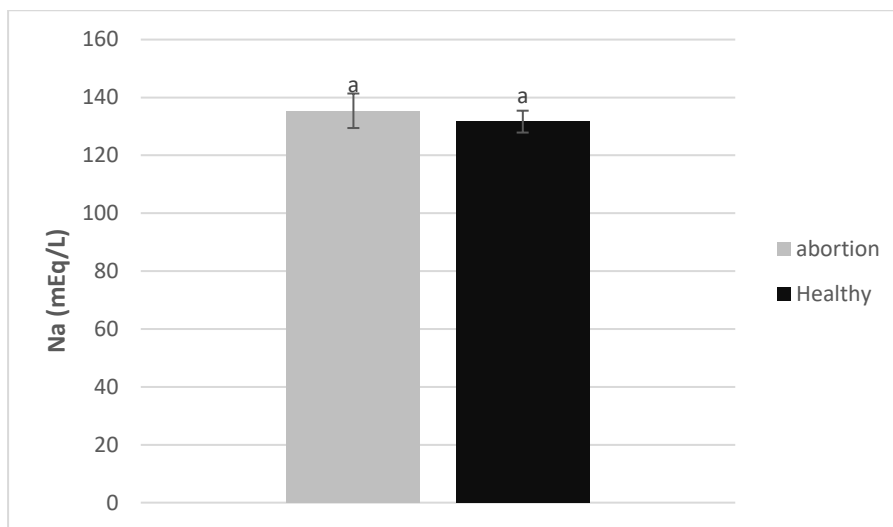
Means and standard deviation of electrolytes and hematological parameters were calculated in both groups. Electrolyte values and hematological parameters were then compared between the two groups using the independent samples t-test. Parameters with abnormal data distribution, such as counts of eosinophils, monocytes, and basophils, were investigated with the Mann-Whitney test. Data were analyzed in IBM SPSS Statistics at p-values less than 0.05 ( $\leq 0.05$ ), and graphs were drawn in Microsoft Excel.

Table 1  
Shows the mean and standard deviation of the electrolytes

| Table 1. The mean and standard deviation of electrolytes |                                |                               |         |         |
|----------------------------------------------------------|--------------------------------|-------------------------------|---------|---------|
| Parameters measured                                      | Infected herds (mean $\pm$ SD) | Healthy herds (mean $\pm$ SD) | t-value | p-value |
| Sodium (mEq/L)                                           | 135.4 $\pm$ 5.96               | 131.65 $\pm$ 3.78             | 1.736   | 0.099   |
| Chlorine (mMol/L)                                        | 110.67 $\pm$ 3.04              | 112.51 $\pm$ 4.37             | -1.109  | 0.281   |

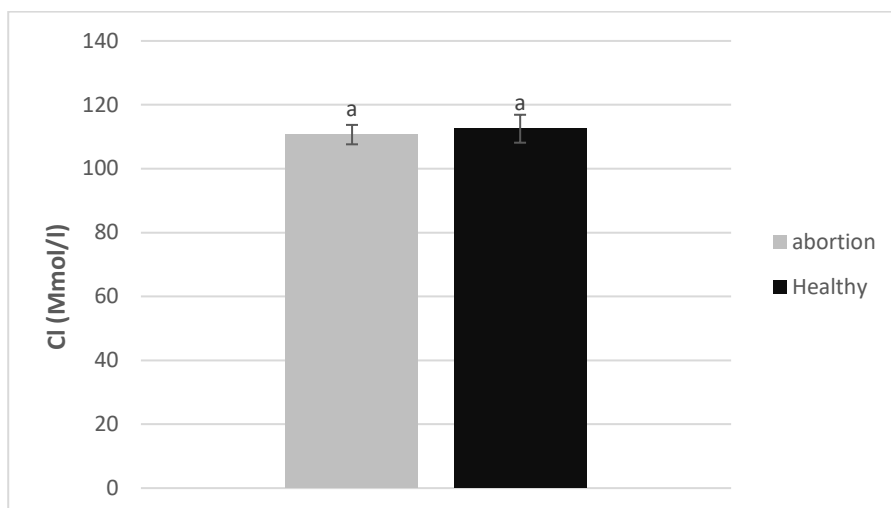
### Hemostasis factors

Graph 1 shows Na concentrations in both groups. There is no significant relationship between healthy herds and those with abortion in terms of Na concentrations ( $p = 0.099$ ).



Graph 1. Na concentrations in the study groups

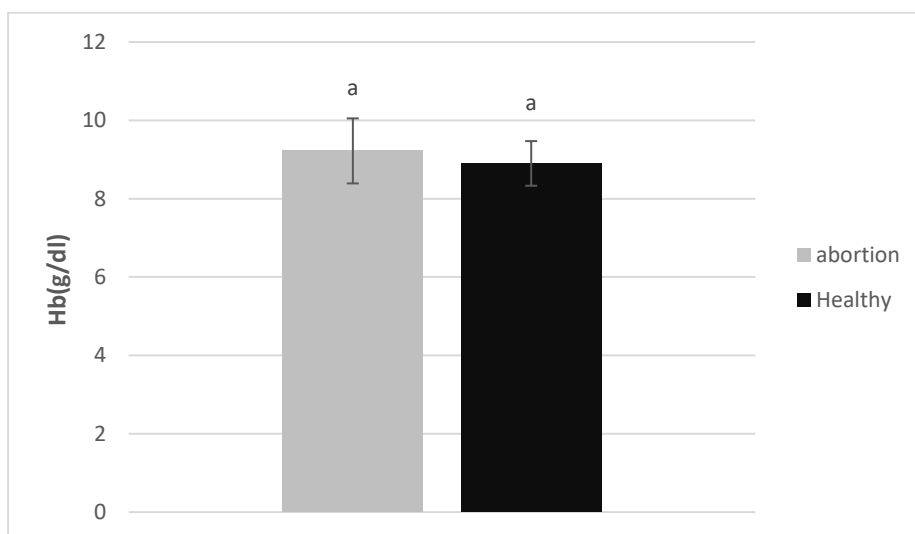
Graph 2 shows Cl concentrations in both groups, indicating no significant relationship between healthy herds and those with abortion ( $p = 0.281$ ).



Graph 2. Na concentrations in the study groups

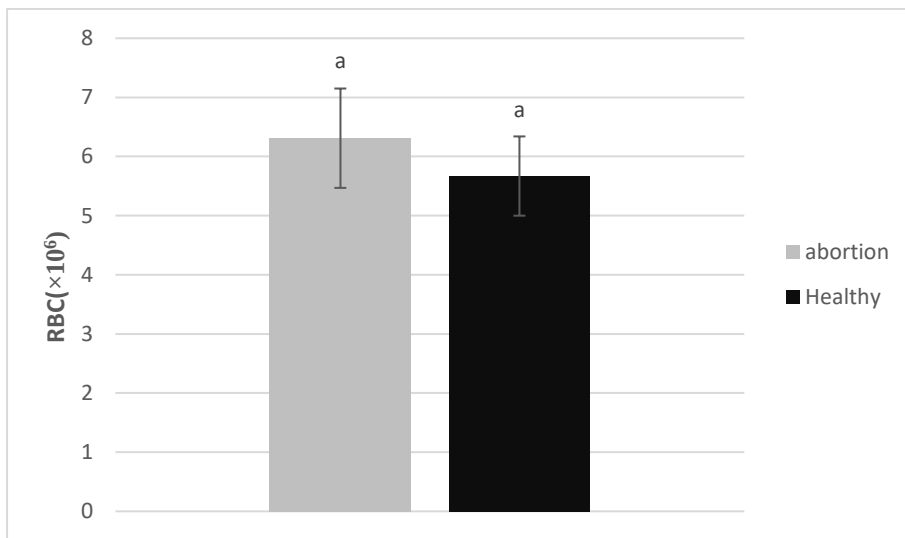
### Hematological parameters

Graph 3 shows Hb values in both study groups. There was no significant difference in Hb values between the groups ( $p = 0.334$ ).



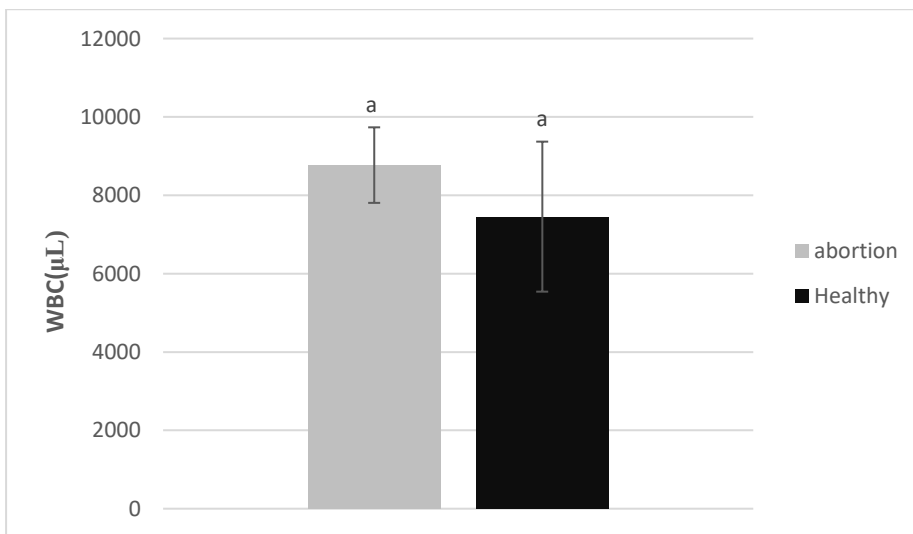
Graph 3. Hb values in the study groups

Graph 4 shows RBC counts in both study groups, indicating no significant difference in Hb values between the groups ( $p = 0.081$ ).



Graph 4. RBC values in the study groups

Graph 5 shows WBC counts in both study groups, indicating no significant difference in Hb values between the groups ( $p = 0.065$ ).



Graph 5. WBC values in the study groups

## Discussion

Abortion is a multifactorial phenomenon with considerable effect on production and economic yield by imposing significant economic losses, especially in late gestation. It is controlled by several factors, including infectious agents (bacterial, viral, fungal, and protozoan agents, etc.), and non-infectious factors such as nutritional and chemical agents, drugs, toxins, plant toxins, hormonal factors, and unknown causes. Both infectious and non-infectious factors are strongly involved in abortion pathogenesis. Studies have extensively reflected infectious



agents, while non-infectious agents are more common in endemic conditions. Infectious agents include bacteria, viruses, protozoa, and fungi. Non-infectious factors include management mistakes during breeding, nutritional and chemical factors, toxins, toxic plants, genetic factors, thermal stress, hormonal alterations, and variations in the concentration of blood metabolites. Ranchers often attribute abortion to diseases such as brucellosis, leptospirosis, and BVDV, while diseases and complications account for only a portion of affecting factors.

Abortion can happen at any point of gestation but the embryo is at the highest risk during the first trimester. Risks of abortion are decreased during the second trimester and are increased slightly in late gestation. Abortion can cause placental retention, endometritis, and diminished reproductive performance. Days open, i.e., days between calving and conception, can further facilitate abortion in herds. Abortion is not simply losing the calf, but also the loss of a genetic pool for breeding, and an increase in calving distance. Such an increase expands the generation gap that is inversely correlated with genetic progression in which more expanded gaps slow genetic progression. Therefore, ranchers will considerably suffer from both breeding and economic sides.

In this research, 40 Holstein dairy cattle were chosen and split into two control and aborted groups, each with 20 members. It was attempted to avoid bias by choosing cattle in pairs from each farm. Therefore, cattle were chosen of the same gender and gestational age. In Iran, Holstein cattle account for about 71% of dairy herds and thus contribute to the large portion of milk produced. Sodium is an extracellular electrolyte acting to maintain osmotic pressure. Sodium is absorbed via the gastrointestinal tract and is mainly excreted in the urine (Kupczynski et al., 2002). Any decrease in the total amount of sodium in the body will increase the production of aldosterone, culminating in the reabsorption of nearly all of the sodium by the kidneys. Importantly, sodium reabsorption is associated with hydrogen or potassium transfer in the opposite direction. Sodium retention by the kidneys is decreased in CKD with polyuria.

In a study on healthy and aborted Holstein cows, the Na content in healthy cows and those with abortion was 136.6 mEq/L and 139.6 mEq/L, indicating no significant difference between the two groups ( $P > 0.05$ ) (Jarosz., 2013). Chlorine is an extracellular electrolyte. In a study on healthy cattle and those with abortions, the chlorine concentration in the healthy and aborted groups was 99.20 mEq/L and 96.90 mEq/L, with no significant difference between the two groups ( $p > 0.05$ ) (Kurpanska., 2013). The concentration of electrolytes is a vital factor from late gestation to early lactation, in terms of sufficient milk production and supplying nutrients and energy (Piccione et al., 2012). Ulutas et al. (2003) reported considerable variations in iron, sodium, calcium, potassium, and magnesium before delivery and during lactation. Such variations can critically contribute to assessing renal function and electrolyte balance.

In this study, the content of electrolytes measured was compared between the two groups. For data evaluation, research variables were investigated by descriptive statistics (mean and standard deviation). There was a significant difference in electrolyte indices (e.g., potassium levels), where the content of potassium was higher in the aborted group ( $p = 0.022$ ). For hematological parameters, RBCs, Hb,

and WBCs were more abundant in the aborted group than the control group ( $p < 0.05$ ).

## Conclusion

In general, the results of this study indicate that homeostasis and hematological indices are not significantly different. Variations in the content of main electrolytes during gestation and after delivery are critical for maternal health and proper fetal and calf growth (Skrzypczak et al., 2014).

## Future prospective

- Assessment of relationships between homeostasis factors (Ca, K, Na, and Cl) and hematology in other races, besides Holstein
- Investigation of other factors such as Mg, Fe, PO<sub>4</sub>, and HCO<sub>3</sub><sup>-</sup> in Holstein cattle for abortion
- Assessment of important early diagnosis indicators such as clinical symptoms (e.g., difficulty walking and lameness in all four legs, salivation, anorexia, ulcers on the tongue and gums)
- Study of listeria and bacilli bacteria transmitting to cattle and inducing abortions
- Evaluation of chemicals such as nitrate, arsenic, and oxalate as effective factors in abortion
- The study of the concentration of thyroid hormones and activities of liver enzymes for early diagnosis of abortion in cattle.

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