Acta Veterinaria-Beograd 2023, 73 (1), 87-101
UDK:636.2.082.1:612.63
636.2.084.52:546.23
636.2.084.52:577.161.3
DOI:10.2478/acve-2023-0007

Research article

PREVENTIVE SUPPLEMENTATION OF VITAMIN E AND SELENIUM AS A FACTOR IN IMPROVING THE SUCCESS RATE OF EMBRYO TRANSFER IN CATTLE

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(Received 20 July 2022, Accepted 13 January 2023)

The effects of stress on processes in the body are becoming an increasingly relevant research subject. The reproductive ability of bovine animals largely depends on these effects, whilst embryo transfer is increasingly being used as a reproduction method. In this study, we established the differences in the implantation ability of heifers that were treated (N=17) with selenium (Se) and vitamins AD_3E, and non-treated heifers. Upon transfer, we took blood samples from both groups and used the total antioxidant status (TAS) value to analyze the presence of reactive oxygen species (ROS), the levels of non-esterified fatty acids (NEFA) and the levels of vitamin E and Se in blood plasma. In the study, we were able to demonstrate that preventive measures in the form of supplementation of vitamin E and Se, mitigate the effects of oxidative stress, strengthen the ability of an organism to improve the dynamic relationship between free radicals and antioxidants, improve the energy status of cattle, positively impact reproductive parameters and increase the success rate of embryo transfer. The difference in the number of successful embryo implantations between the control and treated group was statistically significant, with 64.7% of treated heifers being pregnant after embryo transfer and giving birth to healthy calves. In the control group, the implantation success rate was 41.2%. The supplementation of antioxidants in the form of a combination of vitamin AD_3E and Se, proved to be a good method for strengthening the defense of an organism and an effective mean of preventive clinical approach for improving fertility parameters.

Keywords: oxidative stress, metabolic stress, antioxidants, vitamin E, selenium, embryo transfer

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INTRODUCTION

There is a noticeable deterioration in fertility of cows during the summer months. Therefore, research of performance factors in reproduction is all the more important. Consequently, the familiarization with environmental (high temperature) and dietary (composition of rations) factors is essential for the development of preventive and curative technologies and methods. One of the causes of the decrease in fertility in all economically significant farm animals over the summer months, is overheating of animals and heat stress [1,2]. High summer ambient temperature and high air humidity are the two factors which cause heat stress in cattle [3]. Heat stress affects reproduction and manifests itself in the form of poorly visible signs of conception, disturbed hormonal balance, decreased quality of oocytes, sperm and embryo, altered blood flow, reduced amount of feed consumed, and decreased success rate of insemination [4,5]. In particular, cows with a high production of colostrum and those in the middle of lactation, which are most metabolically stressed due to high production are particularly affected [6]. Hormonal disorders result in a reduction in the quantity of milk, and above all, reproductive issues occur. Heat stress negatively impacts the development of follicles in the ovaries and inhibits their growth and development [7]. The produced follicles are smaller, of poor quality and secrete smaller amounts of estradiol. Due to the low amount of estradiol, the duration of the estrous cycle and time spent in estrous is longer. Additionally, ovulation is also disrupted [8]. Ovulation may not occur at all and cysts remain on the ovaries, or ovulation gets delayed, and the success rate of insemination decreases [9]. Heat stress also affects the corpus luteum on the ovary, which produces progesterone. Progesterone is a gestation hormone and is essential to maintain gestation. If levels of this hormone decrease, the embryo cannot remain in the uterus and develop, so it gets miscarried (aborted). With prolonged heat stress, the decrease in progesterone in the blood is as high as 25% [9]. Due to the stress from overheating of an organism, the concentration of cortisol also increases. Cortisol is a stress hormone from the adrenal gland, which inhibits the implantation of the embryo into the uterine wall and causes early loss of embryos [10,11]. An ovulated oocyte from an inferior follicle shows signs of inferior quality; it has lower fertility; therefore, several inseminations are required for successful fertilization. In the case of fertilization, it miscarries more often [9]. Heat stress also indirectly affects embryos. Many embryos fail soon after fertilization, when they have only two or four cells. During the first few days after fertilization, the embryos are much more sensitive to heat stress than at a later stage, when their own ability to adapt has already developed [10,11].

A negative energy balance (NEB) is a condition where cows are not able to consume enough energy to cover their needs. The energy status of cows is defined as the difference between the net energy intake and the net amount of energy required for maintenance and milk production [12]. When the energy intake is insufficient to cover the needs, animals use body reserves as a source of energy. In the state of

NEB, changes occur in the levels of various metabolites in the blood. NEFA and BHB values increase, whereas, the levels of glucose, cholesterol, urea [13], insulin and IGF-1 decrease [14]. The most significant changes in the NEFA values occur 2 weeks after calving [14]. These changes impact the functioning of the ovaries and consequently the fertility of animals [4]. The effect of heat stress on fertility is indirect, with deterioration of energy balance being the most common reason why cows fail to ovulate. In times of heat stress, the reduced consumption results in a drop in the levels of LH hormone and reduces the size of the dominant follicle. NEB is followed by a decrease in insulin, glucose and IGF-1 hormone levels and an increase in the growth hormone and NEFA blood levels. Insulin is required for follicle development and has a positive effect on oocyte quality, while IGF-1 and glucose stimulate follicle growth and embryo implantation [15]. By determining the BHB and NEFA values in the blood, we can estimate the level of NEB, which is a significant factor when seeking causes of fertility disorders in the early postpartum period [13]. Fat feeding increases dietary energy levels and thus potentially decreases the NEB [16]. If the added fat in the ration is more than 50-60 g/kg DM, the appetite of cows may decrease, which worsens the NEB state [17].

In cattle breeding, embryo transfer is becoming an increasingly popular method of reproduction. The method involves a transfer of embryos from one cow to another and allows for a higher number of fertilizations compared to natural fertilization. This allows us to get calves with superior genetics and production, from low producing cows with poor predispositions that pass their poor traits onto their offspring [18]. It is important that the receiving heifer or primiparous cow is perfectly healthy in reproductive terms, so that after ovulation, a quality corpus luteum develops on the spot of the ovulated follicle. Prior to embryo implantation, the recipient cow is treated using microelements and vitamins to increase the likelihood of a successful procedure [19]. The success rate of reproduction by means of embryo transfer depends on a number of factors: the quality of the embryo, the health and nutritional condition of the animals and various environmental factors [20]. Care should be taken to ensure that animals receive a proper nutritional supply of energy and protein, a sufficient amount of macroelements, in particular potassium, phosphorus and calcium; microelements: Se, manganese, copper, iodine and cobalt and a sufficient amount of vitamins A, D, E and carotenes, as this increases the chances of survival of embryos and the maintenance of pregnancy [18,21]. Environmental and climatic conditions, such as summer heat and heat stress already mentioned above [21,22], also have a major impact on the success rate of embryo transfer. Transfer rate of frozen-thawed embryos of 50% is considered successful [23]. Through analysis of experiments in which cows either received two embryos or received an embryo in general only 50-60% ET were successful and 50-60% of embryos and recipients are sufficiently competent to result in a calving [23-26].

Selenium is proven to be an essential nutrient; it is essential for the development, growth and health of animals. As an integral part, it is essential for the synthesis and normal functioning of more than 20 selenoproteins [27]. In particular, glutathione peroxidase and the deiodinase enzymes [28]. It plays a significant role in the anti-oxidative protection of an entire organism, as it participates in the defense against oxidative stress by scavenging free radicals. As a natural antioxidant, it impacts reproduction and growth, protects tissues and is vital for the immune system response. It is easily passed through the placenta, mammary glands and oocyte, to the fetus [29].

Research has shown a significantly reduced occurrence of placental and cystic ovarian disease in lactating cows which have received a supplement containing a combination of vitamin E and Se [30-35]. Vitamin E and Se play key roles in the protection of the body against ROS, improving immunity and reducing the incidence of mastitis [36-41].

In our study, heifers were preventively supplemented with antioxidants (Se, vitamins AD_3E) and analyses were done to determine whether this is an effective and sensible method for reducing oxidative stress, reducing negative energy balances and achieving greater embryo implantation ability. Additionally, differences were studied in the implantation ability between the heifers treated with Se and vitamins AD_3E, and the untreated heifers, as well as the effectiveness of reducing oxidative stress and, as a result, improving the transplantation success rate. For this purpose, we determined the concentration of vitamin E and Se and values of TAS and NEFA in the blood plasma and compared the values between the control and treated groups. Parameter TAS covers the combined action of all antioxidants in the plasma and body fluids and presents an integrated parameter. The TAS parameter provides biologically relevant oxidative stress information and reflects the dynamic relationship between free radicals and antioxidants in the blood plasma. Heat stress that causes oxidative stress, reduces TAS values due to a decrease in antioxidant levels.

MATERIALS AND METHODS

Study concept and animal selection

The research was carried out at the Šmigoc farm on 34 heifers of the Holstein Friesian breed, which had been clinically inspected prior to being included in the study and were confirmed as healthy. All heifers were housed in a joint stable at the time of the study and thus exposed to the same conditions (climate, diet, etc.). We randomly assigned them into two groups, 17 in the treated (T) and 17 in the untreated, that is the control group (C), the average age of heifers in both groups was 18 months, generally, the age of all the heifers involved in the study varied between 17 and 20 months. For work performed on animals, the Administration of the Republic of Slovenia for Food Safety, Veterinary Medicine and Plant Protection, has issued a written permit (permit no. U34401-19/2016/8), and all of the work was performed in accordance with ethical standards and principles.

During the course of the study, all animals received the same basic rations, as well as concentrated feed in the same quantities. The basic ration consisted of hay, maize and grass silage, with the addition of concentrates. The content of Se was 0.235mg/kg of the basic ration and the vitamin E content was 36.2 mg/kg of the basic ration. They additionally received concentrated feed through a computer-controlled device, namely, 1.5 kg each, with a Se content of 0.355 mg/kg and 26.2 mg/kg of vitamin E. This covered the basic Se and vitamin E animal requirements.

The heifers were exposed to regular climate conditions. The study was carried out in the summer (August 2018), when the daily average temperatures ranged from 18 to 28 °C (maximum outdoor temperature was 37.7 °C and an average outdoor temperature of 23.4 °C).

Embryo transfer

The estrus of the recipient heifers was synchronized. For the transfer, deep frozen embryos of the Holstein Friesian breed were used. Twice before the transfer, namely 14 and 7 days prior, the treated group of heifers was administered Se (TOKOSELEN, quantified) and a combination of vitamins AD_3E (Vitamin AD_3E, Krka). In both treatments, the heifers received 35 ml vit. AD_3E (700 mg vit. E) i/m and 20 ml Tokoselen (3000 mg vit. E, 10 mg Se) i/m. The transfer of embryos was carried out in both groups of heifers on the same day. Prior to the start of the embryo transfer, we performed blood analysis and an ultrasound examination of the heifers, in order to exclude the potential presence of disease that could affect the success rate of transfers. Successful implantation was confirmed via ultrasound which confirmed gestation on the 30th and the 60th day.

Collection of blood samples, chemical and biochemical analysis

Prior to the transfer, blood was sampled from both groups of heifers to determine the levels of ROS, NEFA, Se and vitamin E in the blood plasma. The blood was collected into 5 ml test tubes containing an anticoagulant and was centrifuged immediately upon collection for 15 minutes at 1500 x g and at 4 °C. After centrifugation, the plasma was separated and immediately frozen at –196 °C until analysis. Se and vitamin E analyses in the blood plasma were performed at the Institute for Food, Feed and Environmental Safety, at the Veterinary Faculty in Ljubljana. Plasma Se levels were determined by the ICP-mass spectrometer (Varian, 820-MS, Mulgrave, Australia), and vitamin E concentrations with the HPLC system (Waters Alliance 2690, Milford, MA, USA). TAS was determined spectrophotometrically (600 nm) with the TAS reagent kit on the automated biochemical analyzer. The method of determination is indirect, as the free radicals created in the reaction (ABTS 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) are reduced by the antioxidants present in the sample which is measured by a decrease in absorbance at 600 nm after three minutes. The results are expressed as mmol/l Trolox equivalents (standard, 6-hydroxy-2,5,7,8-tetramethylcroman-2-carboxylic acid - vitamin E derivative). NEFA was defined by biochemical testing of the blood serum.

Statistical analyses

The concentrations of TAS, Se, and vitamin E in blood plasma were compared between the treated and the control group. Using the Shapiro-Wilk test the regularity of the distribution of the obtained results was established, the average and mean values, the standard deviation (SD) and the 95% confidence interval per individual parameters were calculated. In the case of regular distribution, the parametric T-test was used to compare the statistically significant differences between individual groups. In cases where distribution was irregular, the non-parametric Mann-Whitney test was used. The p-values or the degree of a characteristic were determined, which were used when verifying presumptions or hypotheses on differences between the two models. The results were defined as statistically significant at $p \leq 0.05$. For the purpose of identifying a linear relationship between the measured parameters, the correlation coefficients were calculated.

When analyzing data, a personal computer and the statistical software package SPSS were used.

RESULTS

Table 1 shows the average values of vitamin E, Se, TAS parameters and NEFA levels in the blood plasma of heifers. As demonstrated in Table 1, there were statistically significant differences between the treated and the control group, as vitamin E and Se values in the plasma were significantly higher in the group of heifers which received two doses of the combination of vitamin AD_3E and Se. Additionally, NEFA values in this group of heifers were also significantly lower. The difference in TAS values was also statistically significant, and it is evident that the values were significantly higher in the treated group. The embryo transfer, or embryo implantation was successful in 7 heifers from the control group (41.2% success rate) and in 11 calves in the treated group (64.7% success rate), which were administered Se and a combination of AD_3E vitamins simultaneously to the synchronization of estrus. The difference in the success rate of implantations between the groups was statistically significant.

Table 1. Comparison of measured values of vitamin E, Se, TAS and NEFA between the control and treated group of heifers and the number of successful fertilizations of heifers within each particular group.

Group	Parameter	Vitamin E (µg/ml)	Se (µg/l)	TAS (mmol/l)	NEFA (mmol/l)	N in-calf heifers	% in-calf heifers
Control (N=17)	Mean value	2.76	104.65	1.29	0.63	7	41.2
	SD	0.41	16.82	0.09	0.47		
Treated (N=17)	Mean value	3.69	126.71	1.40	0.35	11	64.7
	SD	0.41	16.45	0.12	0.22		
	P	0.001	0.002	0.050	0.035	0.049	

Note: TAS - total antioxidant status, NEFA - non-esterified fatty acids.

As shown in Table 2, statistical analysis showed significantly higher levels of vitamin E and Se in blood plasma in the group of heifers where implantation was successful. The difference in the TAS and NEFA values among the heifers in which implantation was successful and the group in which the implantation was unsuccessful, was also statistically significant. The TAS values were significantly higher in the group of treated heifers, indicating a lower oxidative stress of this group. The values of the NEFA parameter were lower in the group of pregnant heifers, indicating a better energy balance of this group.

Table 2. Comparison of measured values of vitamin E, Se, TAS and NEFA between the successfully and unsuccessfully fertilised group of heifers.

Group	Parameter	Vitamin E (µg/ml)	Se (µg/l)	TAS (mmol/l)	NEFA
Unsuccessful implantation (N=16)	Mean value	2.89	108.53	1.32	0.61
	SD	0.55	19.94	0.11	0.48
Successful implantation - in-calf heifers (N=18)	Mean value	3.55	122.82	1.53	0.37
	SD	0.50	17.49	0.13	0.23
	p	0.001	0.033	0.044	0.049

Note: TAS - total antioxidant status, NEFA - non-esterified fatty acids.

In Table 3, we showed the calculated correlation coefficients and a linear relationship between the values of the parameters that we measured in the blood plasma. The results evidently show a highly positive correlation between the values of vitamin E and Se, which was expected, as they were administered simultaneously. There is also a highly positive correlation between the values of these two parameters and the success rate of embryo implantations.

Table 3. Correlation coefficients of the statistical relationship between vitamin E, Se, TAS and NEFA values in the blood plasma of heifers.

	TAS	Vitamin E	Se	NEFA
Vitamin E	0.42*			
Se	0.39*	0.67**		
NEFA	-0.24	-0.60**	-0.35*	
Successful implantation	0.48*	0.54**	0.37*	-0.32*

Note: TAS - total antioxidant status, NEFA - non-esterified fatty acids, * p-value (statistical significance) is less than or equal to 0.05 ($p \leq 0.05$), ** p-value (statistical significance) is less than or equal to 0.01 ($p \leq 0.01$).

The TAS values show a high correlation with vitamin E and Se values and also highly correlate with successful transfer. The values of the NEFA parameter show a significantly high correlation with Se and vitamin E values, additionally, the NEFA values have also been shown to be statistically significantly lower in those heifers in which the implantation was successful. The interdependencies between the NEFA and TAS parameters were not proven as statistically significant, although they do indicate a negative correlation, namely, that the TAS was higher in heifers with better energy balance (lower NEFA).

DISCUSSION

Heat stress has a negative effect on the reproductive parameters of cattle, therefore, there is an increase in the number of failed inseminations during the summer months. Preventive supplementation of Se and vitamin E has already proved to be successful in reducing these issues and has shown to positively impact cattle health [36-39]. When heat stress occurs, it depends mainly on the ambient temperature and air humidity. With high humidity, heat stress can already occur at temperatures above 20 ºC, and a temperature of 25 ºC is often mentioned as the critical temperature above which it is necessary to start implementing measures to mitigate or prevent heat stress [2]. Comparing the data above, this study was carried out in the summer, when the daily average temperatures ranged from 18 to 28 °C (maximum outdoor temperature was 37.7 °C and an average outdoor temperature of 23.4 °C). Therefore, based on temperature data, it can be assumed that high environmental temperature would expose heifers to heat stress. Our study aimed to establish whether the preventive supplementation of vitamin E and Se could also be associated with the success rate of embryo transfers in cows, namely, we tried to find the differences in implantation abilities among the heifers we treated with Se and vitamins AD_3E, and the untreated heifers. Upon transfer, we took blood samples from both groups, treated and untreated heifers, and analyzed the values of vitamin E, Se, TAS and NEFA in blood plasma.

Positive effects of treatment, supplementation of vitamin E and Se proved to be effective, since the levels of vitamin E and Se in the blood plasma were significantly and substantially higher in the treated heifers (Table 1, p. Lt; 0.001). The results show that the control group of heifers had relatively low levels, almost insufficient levels of vitamin E (2.76 μg/ml) and Se (104.65 μg/ml) in the blood plasma. Levels of Vitamin E that are higher than 3.5 μg/ml are sufficient, between 2.5 and 3.5 μg/ml are within the limits and levels lower than 2.5 μg/ml insufficient [42]. Levels of Se under 120 μg/ml are considered insufficient, whereas optimal levels are considered above 200 μg/ml [43]. A highly positive response to the treatment is likely to be related to the shortage of these elements in the diet, therefore the values rapidly increased upon their inclusion. Vitamin E has a significant effect on metabolism and acts as a stabilizer on other vitamins. In animal organisms it is associated with metabolism of Se and acts as an antioxidant. Vitamin E cooperates with Se and protects the cells from oxidative damage caused by free radicals formed as a consequence of heat stress. The addition of vitamin E in combination with vitamin C has an antioxidant effect, as it protects cells from oxidative damage caused by free radicals, decreases levels of the stress hormone cortisol in the blood and helps in coping with heat stress. An organism applies two means of protection using vitamin E against oxidative damage. It was demonstrated in humans that vitamin E, being a potent antioxidant, participates in a number of physiological processes and that its deficiency can be associated with an increase of ROS levels, cell damage and, consequently, damage of different tissues, including inflammation, thus leading to different ailments [44-47].

Se levels in blood plasma below 120 µg/ml are considered as insufficient and the optimal values are above 200 µg/ml [43]. Insufficient supply of Se in pregnant lactating cows may lead to heart muscle disease in newborn calves. Severe Se deficiency leads to a premature birth (abortion) or loss of a calf upon parturition. Muscle dystrophy in adult animals is not as frequent and as intense as in the offspring. Feed can directly impact the levels of Se in animal products and thus their nutritional value and technological quality. There are two main sources of Se, namely: inorganic Se (e.g., selenite) and organic Se (e.g., selenomethionine) [35]. Se bound in organic form - chelate is better absorbed. The difference between the efficacy of organic and inorganic Se forms is in the efficacy of incorporation into the selenoenzymes (in the functional form of selenocysteine). Inorganic salts are almost always absorbed more quickly than organic molecules, but their biochemical transformation paths towards Se-cysteine are complicated and time-consuming [48]. Organic Se is equally absorbed in all parts of the digestive tract, whereas the inorganic Se is better absorbed in the small intestine. By supplementing Se in the inorganic form, we can increase the concentration of Se in milk and colostrum from cattle. This is even more effective in the case of organic Se, e.g., Se-methionine. When Se is added to the feed ration in the organic form, an increase of up to eight times greater can occur, compared to the feed ration with supplemented with the inorganic form of Se (selenite) [29].

The study was carried out during the summer months (the daily average temperatures ranged from 18 to 28 °C), we therefore assumed that high environmental temperature would expose heifers to heat stress, and that due to the created imbalance between the production and safe removal of free radicals, oxidative stress would also occur. Based on temperature data it can be assumed that heifers were exposed to heat stress. By supplementing vitamin E and Se, the aim was to compensate for the consequences of the existing imbalance between oxidizing agents and antioxidants. The results showed that, in combination, the two proved to be good antioxidants that improve overall health of cows and their reproductive abilities. Oxidative stress was determined indirectly through the TAS parameter. The results presented significant differences in the values of this parameter between the control group and the group of heifers that was supplemented with vitamin AD_3E and Se, which is consistent with previous research [36,37,39] in which they demonstrated that the two supplements play a crucial role in removing reactive oxygen compounds from the body. Vitamin E is included as a potent antioxidant in several physiological processes, and its deficiency is associated with increased levels of ROS, cell damage and, consequently, damage of various tissues [44-46], Se is also involved in the antioxidant defense of the entire organism, since it participates in the defense against oxidative stress by scavenging for free radicals. The addition of antioxidants has a positive effect on the ability of cells to neutralize and detoxify the damaging effects of free radicals and supplementation of vitamin E and Se had a positive effect on the change in the activity of antioxidant enzymes.

Another factor that indicates that treatment managed to mitigate the effects of heat stress on the heifers, was a significantly improved energy balance of the group of treated heifers. In the blood plasma of untreated heifers, statistically higher NEFA levels were found, which indicates an inferior energy status. This result can be attributed to a combination of vitamin E and Se, given that all heifers were otherwise exposed to identical conditions (same feed mix, same breed). By supplementing vitamin E and Se, the negative effect of lower energy balance on animal fertility was reduced.

The comparison of the number of successful embryo implantations between the control and treatment group appears to be in favor of the latter, as in this group 64.7% of heifers were pregnant after embryo transfer and gave birth to healthy calves. In the control group, the implantation success rate was at 41.2%. As the transfer rate of frozen-thawed embryos of 50% is considered successful [23-26], the higher success rate of ET in the treated group can be attributed to the effect of adding the combination of vitamin AD_3E ad and Se.

In our study, it was demonstrated that preventive supplementation of antioxidants can reduce the effects of heat stress, exert a positive impact on reproductive parameters and increase the success rate of embryo transfers. Heat stress disrupts the hormonal balance, as it affects the production of progesterone, which is crucial in maintaining gestation. Therefore, it can be concluded, that the treatment of heifers has a positive effect on the levels of this hormone and consequently helps to maintain an embryo in the uterus of the receiving heifer. Hormone cortisol is largely responsible for the fact that the transplanted embryos failed to implant. Reducing the effects of heat stress, it can be assumed, that the levels of the stress hormone cortisol were also reduced. Cortisol levels were not included in this study therefore further investigations should be performed. Numerous studies [33,34,49] have already shown the positive effects of supplementation of different vitamins and microelements in the animal diet and its positive effects on fertility parameters.

In this study a comparison of vitamin E and Se and TAS and NEFA values between the group of heifers, which remained pregnant following the transfer and the group of heifers in which the transfer was unsuccessful was done. The differences were statistically significant for all parameters. The levels of vitamin E and Se in the blood plasma of pregnant heifers was significantly higher. The heifers which remained pregnant after the transfer had a significantly higher TAS value and a better energy balance. This information serves as further evidence that supplementation of vitamin E and Se had a positive effect on the fertility, or the ability of heifers to successfully undergo embryo implantation. Based on our study, we can conclude that supplementation of antioxidants is significant in improving fertility parameters. The results of our study confirmed the results of some prior studies [30-32], in which they concluded that vitamin E and Se significantly improve the energy balance of bovine animals, which positively impacts fertility parameters. Feeding with protective microelements increases energy levels and thus reduces the value of NEB [16].

Embryo transfer is a relatively new biotechnological method of reproduction in cattle rearing, that is increasingly being used as an alternative means of reproduction, which can ensure the transfer of the best or selected genetic material to the progeny. Since this is a relatively new area, there is very little existing research available, therefore all findings that contribute to the success rate of embryo transfer, are all the more important. The results of our research are significant in the field of prevention, they indicate how to prepare the recipient heifers/cows for the transfer of an embryo, in a way that allows a more successful implantation of the embryo. We have demonstrated the existing differences in implantation abilities between the control and treatment group, we can therefore conclude that this can be attributed to the effects of supplemented antioxidants. This method of reproduction will become even more important, especially because it allows the use of heifers for breeding which do not carry the best genetic material but are otherwise in good physical condition and healthy in reproductive terms. Further studies, progress and wider use of embryo transfer will enable us to ensure generations of animals with the best productive properties, to increase the production of milk, meat and other products. Moreover, the second significant added value of the use of the embryo transfer in the animal world, is the fact that this reproduction method will allow the conservation of rare and indigenous breeds of animals.

CONCLUSION

In our study preventive measures were demonstrated, such as supplementation of vitamins and microelements, that can hinder the effects of heat stress and improve the dynamic relationship between free radicals and antioxidants in blood plasma (TAS) and improve the energy status of cattle. A combination of vitamin AD_3E and Se proved to be a good source for strengthening the antioxidant protection of an organism.

There is plenty of literature describing the positive effects of vitamin E, however, it should not be overlooked the positive and beneficial properties on health and reproductive parameters that vitamin A and D provide.

Author's contributions

JŠ, MS and JM carried out the field work and studies, conceived the study, and participated in its design and coordination and helped to draft the manuscript. KPV and BJS carried out the laboratory work. All authors read and approved the final manuscript.

Conflict of interest statements

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

The study was carried out on 34 calves of the Holstein Friesian breed at the Šmigoc farm. For work that was performed on animals, the Administration of the Republic of Slovenia for Food Safety, Veterinary Medicine and Plant Protection, has issued a written permit (permit no. U34401-19/2016/8), and all of the work was performed in accordance with ethical standards and principles.

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PREVENTIVNA SUPLEMENTACIJA VITAMINOM E I SELENOM U CILJU USPEŠNIJEG EMBRIOTRANSFERA KOD KRAVA

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Uticaj stresa na procese u organizmu postaje sve značajniji element istraživanja. Reproduktivne karakteristike kod goveda većim delom zavise od ovih efekata pri čemu se embriotransfer sve više koristi kao reproduktivna metoda. U ovoj studiji ispitivane su razlike u sposobnostima implantacije kod junica koje su tretirane (N=17) selenom (Se) i vitaminima AD3E kao i junica koje nisu tretirane. Posle transfera, uzimana je krv iz krava obe grupe pri čemu je vrednost ukupnog antioksidativnog statusa (TAS) upotrebljena za analizu prisustva reaktivnih kiseoničnih molekula (ROS), nivoa ne-esterifikovanih masnih kiselina (NEFA) i nivoa vitamina E i selena u krvnoj plazmi. U studiji, moguće je bilo pokazati da preventivne mere u smislu suplementacije vitaminom E i Se, smanjuju efekte oksidativnog stresa, pojačavaju sposobnost organizma da poboljša dinamički odnos između slobodnih radikala i antioksidanasa, poboljšavaju energetski status goveda, pozitivno utiču na reproduktivne parametre i poboljšavaju uspeh stepena embriotransfera. Razlika u broju uspešnih embriotransfera između kontrolnih i tretiranih grupa životinja, bila je statistički značajna pri čemu je 64,7% tretiranih junica ostajalo steono posle embriotransfera. Sve tretirane junice su dale zdravu telad. U kontrolnoj grupi, uspeh implantacije je bio 41,2%. Suplementacija antioksidansima u obliku kombinacije vitamina AD3E i selena, pokazala se kao dobra metoda kojom se obezbeđuje jačanje odbrambenih mehanizama organizma i efikasan način preventivnog kliničkog prilaza poboljšavanja parametara fertiliteta.