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Investigation of Hypothermia and Hypoglycemia Complex in Newborn Lambs*

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The aim of this study was to investigate the complex of hypothermia and hypoglycemia occurring in the first 3 days (especially in the first 5-12 hours) of the life of lambs.

The material of the study consisted of twenty healthy (control group), twenty mildly hypothermic and ten severe hypothermic lambs. After the general clinical examination of all the lambs included in the study (heart and respiratory rate, body temperature), blood samples were taken from *V. Jugularis* according to the procedure and serums were separated. The blood glucose level was determined by a glucometer from the whole blood samples whereas total protein and albumin were determined in the serum by a refractometer.

It was determined that the mean values of body temperature, respiration and heart frequencies were 39.36±0.22°C, 55±5.79 pcs/min and 146.9±9.37 pcs/min in healthy lambs, 37.66±0.48°C, 34.90±5.48 pcs/min and 72.40±10.15 pcs/min in mildly hypothermic lambs, and 36.49±0.34°C, 20.00±4.52 pcs/min and 51.00±4.35 pcs/min in severely hypothermic lambs, respectively. It was detected that the mean values of glucose, albumin and total protein of the blood were 103.20±30.07 mg/dL, 3.22±0.38 g/dL and 6.18±1.17 g/dL in healthy lambs, 55.25±13.29 mg/dL, 3.02±0.36 g/dL and 4.91±1.11 g/dL in mildly hypothermic lambs, and 20.20±4.83 mg/dL, 2.53±0.42 g/dL and 3.90±0.55 g/dL in severely hypothermic lambs, respectively.

With regard to the mean values of the clinical (body temperature, respiration and heart frequencies) and biochemical (glucose, albumin, and total protein) parameters, the differences between all groups were found to be significant (P<0.05).

It was attempted to replace the glucose reserves and adjust the body temperature to the normal level by administering colostrum via the feeding bottle or stomach tube or intraperitoneal glucose injection for the treatment of animals with neonatal hypothermia and hypoglycemia (mild hypothermia and severe hypothermia).

All the lambs in the control group were alive while 25% of the lambs with mild hypothermia and 70% of the lambs with severe hypothermia died.

In conclusion, the early diagnosis and timely treatment of mild cases are important because of the high level of mortality in severely hypothermic-hypoglycemic cases.

Key Words: *Hypothermia, hypoglycemia, newborn, lamb*

Yeni Doğan Kuzularda Hipotermi ve Hipoglisemi Kompleksi Üzerine Araştırmalar

Bu çalışmada, kuzuların yaşamlarının ilk 3 günlük döneminde (özellikle ilk 5-12 saatlik dönemde) şekillenen hipotermi ve hipoglisemi kompleksinin araştırılması amaçlanmıştır.

Çalışma materyalini 20 baş sağlıklı (kontrol grup), 20 baş hafif hipotermili ve 10 baş şiddetli hipotermili kuzu oluşturmuştur. Çalışmaya alınan tüm hayvanların genel klinik muayeneleri (kalp ve solunum sayısı ile vücut sıcaklığı) yapıldıktan sonra *V. jugularis*'ten usulüne uygun olarak kan örnekleri alınarak serumları ayrılmıştır. Kan glikoz düzeyi tam kanda glikometre ile total protein ve albumin tayinleri ise serumda refraktometre ile tayin edilmiştir.

Vücut sıcaklığı, solunum ve kalp frekanslarının ortalama değerlerinin sırasıyla sağlıklı kuzularda 39.36±0.22°C, 55±5.79 ad/dk ve 146.90±9.37 ad/dk; hafif hipotermili kuzularda 37.66±0.48°C, 34.90±5.49 ad/dk ve 72.40±10.15 ad/dk ve şiddetli hipotermili kuzularda 36.49±0.34 °C, 20.00±4.52 ad/dk ve 51.00±4.35 ad/dk olduğu belirlenmiştir. Kan glikoz, albümin ve T.protein ortalama değerlerinin sırasıyla sağlıklı kuzularda 103.20±30.07 mg/dL, 3.22±0.38 g/dL ve 6.18±1.17 g/dL; hafif hipotermili kuzularda 55.25±13.29 mg/dL, 3.02±0.36 g/dL ve 4.91±1.11 g/dL ve şiddetli hipotermili kuzularda 20.20±4.83 mg/dL, 2.53±0.42 g/dL ve 3.90±0.55 g/dL olduğu saptanmıştır.

Klinik (vücut sıcaklığı, solunum ve kalp frekansları) ve biyokimyasal (glikoz, albümin ve total protein) parametrelerin ortalama değerleri açısından tüm gruplar arasındaki farklılıkların önemli olduğu (P<0.05) belirlenmiştir.

Neonatal hipotermi ve hipoglisemili hayvanların tedavilerinde (hafif hipotermili ve şiddetli hipotermili) biberon veya mide sondasıyla kolostrum verilerek veyahut intraperitonal glikoz enjeksiyonuyla glikoz rezervleri tamamlanmaya ve vücut sıcaklığı normal düzeye getirilmeye çalışılmıştır.

Kontrol grubundaki kuzuların hepsinin yaşamasına rağmen, hafif hipotermili kuzuların %25'nin, şiddetli hipotermili kuzuların ise %70'nin öldüğü görülmüştür.

Sonuç olarak; şiddetli hipotermi-hipoglisemi-kompleksi olgularında ölüm oranının yüksek olması nedeniyle hafif vakaların erken tanısı ve zamanında tedavileri önem arz etmektedir.

Anahtar Kelimeler*: Hipotermi, hipoglisemi, yeni doğan, kuzu*

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Introduction

Generally, some factors, such as the exogenous energy supply, environment and climate, type of delivery (parturition), care and feeding conditions, racial and genetic factors, significantly affect the energy metabolism during the first 72 hours of newborn lambs (1-4).

New born lambs lose their body temperature quickly particularly if they are not well fed and also when the environment is cold and humid (1, 4-6). Consequently, hypothermia-hypoglycemia complex (HHC), which is a metabolic disorder characterized by the decrease in body temperature and blood glucose level that leads to significant neonatal lamb losses, emerges (1, 5-7).

HHC, described as the defect of adaptation to the extrauterine environment, is rife in the world and constitutes one of the major problems of newborn lambs (1, 2, 4, 5).

HHC is considered to be a major problem for sheep breeding due to the fact that lambing season generally coincides with the winter period in our region and our country. Furthermore, as a result of the literature studies conducted, no studies on this subject were encountered in our country.

The aim of this study was to detect the clinical and biochemical parameters of HHC cases which are the biggest cause of death in newborn lambs in the first 3-day period and in this way to better define the disease and, additionally, to contribute to the national economy and science by determining the success rate of the treatment.

Materials and Methods

In this study, newborn lambs with HHC of Akkaraman species bred in Elazig region and characterized by meat, wool and milk yield were examined. This study was performed in 14 sheep farms in 6 different villages of Elazig city, and it was started in January 2015 and was completed in December 2015. For this study, the ethics committee approval was obtained by the decree (Date 04.04.2013, meeting No. 2013/03 and No. 48) of the Animal Experiments Ethics Board of Firat University.

After the general clinical examination (heart and respiratory rate, body temperature) of the newborns following the birth of all animals included in the study, lambs were divided into three groups according to the body temperature. Twenty healthy (control group), twenty mildly hypothermic, and ten severely hypothermic lambs constituted the study groups. Lambs with the body temperature between 39.0 - 37.0 °C were considered to be mildly hypothermic, and those with the body temperature under 37.0 °C were accepted to be severely hypothermic. The control group was composed of completely healthy lambs with the body temperature above 39.0 °C which were born normal spontaneously, dried by their mother and took colostrum on time and in the sufficient amount.

Attention was paid to excluding lambs with enteritis, septicemia, arthritis, and pneumonia developed due to bacterial or viral infection from the study.

The blood samples of all animals included in the study were collected in accordance with the procedure in blood collection tubes with gel and vacuum features from *V. Jugularis* and sera were separated by centrifugation for 10 minutes at 2500 rpm. The blood glucose level was measured from the total blood samples by a glucometer (Woodley Equipment Company Ltd, G-pet glucose measurement instrument, England) and the determinations of total protein and albumin were performed in the serum by a refractometer (ADVIA 2400 Chemistry System, Japan) using Avia test kits. It was attempted to keep the ambient temperature high by placing electric heaters in certain corners of the sheep pens containing the lambs studied.

For the treatment, it was attempted to complete the glucose reserves and bring the body temperature to the normal level by giving colostrum to the lambs with a weak sucking reflex with a feeding bottle, and to the lambs with insufficient feeding with a stomach tube per os (by fitting a 50 mL syringe to the probe tip) (8) or by means of intraperitoneal glucose injection (injection site was the caudo-lateral side of the umbilical cord, with 45-degree angle and in accordance with the procedure) (9).

Lambs were completely dried for the treatment of mildly hypothermic (37.0-39.0^{o}C) cases. After the birth (in case of the lack of care and unrealized sucking process), colostrum was given by oral gavage or lambs were assisted to suck. Lambs with the rectal temperature returning to normal as a result of these operations were included in the normal herd.

Lambs were dried immediately to treat the cases of severe hypothermia (<37 ^{o}C). Then, glucose solution heated to the body temperature (39 ^{o}C) was administered intraperitoneally. In case the blood glucose level was under 50 mg/dL, 10 % glucose solution was administered in 10 mL/kg doses. If the blood glucose level was between 80-50 mg/dL, 5 % glucose solution was used. Artificial feeding was performed by giving colostrum orally with a stomach tube after the glucose administration. Daily colostrum, which is up to 10 % of the live weight, was given per os several times a day until the sucking reflex was formed. Particular attention was paid to avoiding the leak of colostrum to lungs.

All statistical analyses were evaluated by using SPSS software (MS Windows Release, version 22.0). The significance of the differences between the groups was analyzed with the one-way ANOVA test and the Tukey test was used to determine the difference between the groups.

Results

The general information (date of birth, type of delivery, body weight, life situation, colostrum intake); the arithmetic means of the clinical parameters, the significance of the differences between the groups with the minimum-maximum values; the arithmetic means of the biochemical examination parameters, the significance of the differences between the groups with the minimum-maximum values of all the lambs in this study, healthy (control group), with mild hypothermia and severe hypothermia, are presented in Tables 1, 2 and 3, respectively.

As understood from Table 1, it was determined that all the lambs in the control group were born normal spontaneously, dried by their mothers, and they got enough colostrum on time (5 of them were given colostrum with a feeding bottle), and all survived.

It was observed that four of the lambs with mild hypothermia took colostrum within the first 5 hours of life, others sucked less milk in general and had a weak sucking reflex, and their mothers disowned them, and they were malnourished. It was observed that the lambs with a weak sucking reflex had kyphosis and irregularities in motions, muscle tremors (particularly in the neck and leg muscles), some lambs had mixed hairs, and some had immobile ears. Furthermore, it was highlighted that their ears, extremities, and oral cavities were cold. Some of the lambs were seen lying on the ground with their heads held up. It was observed that 5 lambs with a weak sucking reflex in the group with mild hypothermia died (25%). It was determined that five lambs affected by mild hypothermia were born with dystocia.

It was detected that the lambs with severe hypothermia did not take colostrum within the first 5 hours of life, most of them did not breastfeed (did not take any food supplement) due to the lack of the sucking reflex, some of them fed inadequately with breast milk. It was observed that frequent muscle contractions occurred in severely hypothermic lambs, they were lying on the floor motionlessly, and they were in a coma, their ears, extremities and mouth cavities were cold. It was also determined that 4 lambs with severe hypothermia were born as twins. It was observed that dystocia occurred in half of the lambs with severe hypothermia (5 of 10), 3 lambs with a weak sucking reflex survived, and 7 lambs with no sucking reflex died (70 %).

Upon examining Table 2, it is understood that the mean values of body temperature, respiration and heart frequencies were 39.36±0.22°C, 55±5.79 pcs/min and 146.9±9.37 pcs/min in healthy lambs, 37.66±0.48°C, 34.90±5.49 pcs/min and 72.40±10.15 pcs/min in mildly hypothermic lambs, and 36.49±0.34 °C, 20.00±4.52 pcs/min and 51.00±4.35 pcs/min in severely hypothermic lambs, respectively.

In Table 3, it is observed that the mean values of blood glucose, albumin and total protein were 103.20±30.07 mg/dL, 3.22±0.38 g/dL and 6.18±1.17 g/dL in healthy lambs, 55.25±13.29 mg/dL, 3.02±0.36 g/dL and 4.91±1.11 g/dL in mildly hypothermic lambs, and 20.20±4.83 mg/dL, 2.53±0.42 g/dL and 3.90±0.55 g/dL in severely hypothermic lambs, respectively.

Table 1. General information about the lambs in the study

Groups	n	Date of Birth	Type of Delivery (parturition)	Gender	Live Weight (g)	Colostrum	Life Status
Healthy	20	27.01.2015 – 20.02.2015	Normal	11 Females 9 Males	3.130 – 5.140	All took	All alive
Mild Hypothermia	20	27.01.2015 – 20.02.2015	5 Dystocia 15 Normal	10 Females 10 Males	2.680 – 4.800	4 of them took 16 of them took insufficiently	5 of them dead 15 of them alive
Severe Hypothermia	10	09.02.2015 – 27.02.2015	5 Dystocia 5 Normal	6 Females 4 Males	2.140 – 3.580	7 of them did not take 3 of them took insufficiently	7 of them dead 3 of them alive

Table 2. The arithmetic means of body temperature, respiration and heart frequencies in the lambs and the significance of the differences between the groups

Parameter	Healthy	Mild Hypothermia	Severe Hypothermia	P
Body Temperature (°C)	39.36 ± 0.22[a]	37.66 ± 0.48[b]	36.49 ± 0.34[c]	0.0001
Respiration Frequency (pcs/min)	55.00 ± 5.79[a]	34.90 ± 5.49[b]	20.00 ± 4.52[c]	0.0001
Heart Rate (pcs/min)	146.90 ± 9.37[a]	72.40 ± 10.15[b]	51.00 ± 4.35[c]	0.0001

These data are presented as mean ± standard deviation.

a,b,c: The difference between the groups with different letters in the same line is statistically significant. Significance was accepted to be $P < 0.05$.

Table 3. The arithmetic means of serum glucose, albumin and total protein values in the lambs and the significance of the differences between the groups

Parameter	Healthy	Mild Hypothermia	Severe Hypothermia	P
Glucose (mg/dL)	103.20 ± 30.07[a]	55.25 ± 13.29[b]	20.20 ± 4.83[c]	0.0001
Albumin (g/dL)	3.22 ± 0.38[a]	3.02 ± 0.36[b]	2.53 ± 0.42[c]	0.0001
Total protein (g/dL)	6.18 ± 1.17[a]	4.91 ± 1.11[b]	3.90 ± 0.55[c]	0.0001

These data are presented as mean ± standard deviation.

a,b,c: The difference between the groups with different letters in the same line is statistically significant. Significance accepted to be $P<0.05$.

Discussion

Neonatal hypothermia is a serious, life-threatening condition characterized by the body temperature in newborns below the normal values (1). There is a balance between heat generation and heat loss in newborn lambs. If heat loss is higher than heat generation, the balance is distorted, and hypothermia occurs (6, 10).

Hypoglycemia is a complex of symptoms manifesting itself in various clinical signs that result in a decrease in the blood glucose level. Hypoglycemia is defined as the level of blood glucose below 80 mg/dL in newborn lambs (5). Hypoglycemia develops as a consequence of the decreased calorie intake and/or increased catabolism. Hypoglycemia is observed in the next hours and days rather than in the first hours of life (1, 2, 11, 12).

Neonatal hypothermia-hypoglycemia complex is sometimes understood as the life-threatening heat loss and the lack of energy in newborn lambs (13).

Body temperature (39.0-39.7 ºC) and blood glucose level (80 mg/dL) decrease below the normal values in HHC. The lambs with the body temperature between 39.0-37.0 ºC are considered to be mildly hypothermic, and the lambs with the body temperature below 37.0 ºC are considered to be severely hypothermic (1, 2, 5, 9). These body temperature values were taken as the basis for the formation of groups in this study.

As stated in the literature (14), the body temperature of the lambs was determined by a thermometer, and the blood glucose levels were also easily determined by an inexpensive portable glucose meter. Glucometers, which can be easily used especially by cowmen, will provide great convenience for measuring the blood glucose levels in the field.

In the references (1, 3, 13, 15), muscle tremors, kyphosis and irregularities in movements, bedridden cases and even deaths in some cases reported to be observed in HHC lambs are similar to the findings in the study.

It is considered that significant decreases in the clinical parameters (body temperature, respiration and heart frequencies) in mildly, and especially severely hypothermic lambs are due to the coma.

In lambs, the blood glucose concentration in neonatal hypoglycemia is less than 80 mg/dL (5). It has been reported by the researchers (2, 5, 11) that the blood glucose levels in severely hypothermic lambs fall from 80 mg/dL to 50 mg/dL or lower and become complex with hypoglycemia.

In the study, it was observed that the blood glucose values detected in mildly and severely hypothermic lambs were consistent with these notifications (2, 5, 11), and it was also determined that there were significant differences between the blood glucose values in the control, mildly and severely hypothermic lambs. However, it is noteworthy that the glucose levels were very low (average 20.20 ± 4.83 mg/dL) in severe cases. This is probably due to the fact that the lamb births in the study were in the winter months when the air was very cold and that the lambs did not take colostrum for a long period, in other words, they were hungry because environmental temperature and feeding are important factors in the regulation of the blood glucose values (1, 4-6). Therefore, high-energy colostrum, which regulates blood glucose, needs to be taken timely and in the adequate amount exogenously (4).

Hypothermia and hypoglycemia complex is an important disease that results in not taking colostrum during the first 5 hours of newborn lambs. If the ambient temperature of newborn lambs is not warm enough, this complex emerges and causes large-scale losses in this period. Especially when the body temperature falls below 37 °C, the disease in lambs becomes heavy and may result in irreversible conditions (1, 5). Death events in severely hypothermic lambs among the studied animals support this opinion. However, we believe that dystocia (1, 4) and twin (1, 16) births are effective in lamb deaths due to their effects on the HHC formation.

Albumin is the most important one among negative acute phase proteins. Although albumin levels in the study did not differ significantly among all groups, it was detected there were decreases in the mild and severe groups. Although these decreases suggest an acute phase response, when assessed together with decreases in the total protein values, the reason for these reductions can be explained by inadequate sucking or the insufficient intake of colostrum by lambs, in other words, starvation (17).

Passive transfer failure may be detected by determining the serum protein level. The serum protein level above 6 g/dL indicates sufficient passive immunity

(18). Since the main source of passive immunity is colostrum, the total protein values in mildly and severely hypothermic lambs in the study were below 6 g/dL, which indicated that they did not take colostrum or took it inadequately.

In the literature (1, 5, 8), it is stated that hypoglycemia can be ameliorated by intraperitoneal glucose injections and hypothermia can be regulated by creating hot environments. In the study, 10% glucose was injected intraperitoneally in the direction of these statements, and the lambs were moved to a warm environment.

The fact that lambs older than five hours are in a coma may be due to the inadequacy of the herd observations because they have remained hungry for 1-2 days, have used all their glycogen and glucose reserves and brown fat tissue (8). Lambs are born with the limited energy reserves (13), such as glycogen and brown fat tissue, and especially twin-born lambs have a low body weight as well as less energy after birth (6, 19).

It has been stated by Deinhofer (13), Huffmann et al. (20) and Owens et al. (21) that there is a significant correlation between lamb mortality and birth weights. This is supported by the fact that the mortality rates among the lambs with a low birth weight in this study are 25% in mildly hypothermic lambs and 70% in severely hypothermic lambs.

As a result, the early diagnosis and timely treatment of mild cases are important because of the high mortality rate in the cases of severe hypothermia-hypoglycemia-complex.

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