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Research Article

Study on Survival, Serum Immunoglobulin Levels and Some Blood Parameters of Holstein Calves Reared in Individual Calf Hutches

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ABSTRACT

Calf deaths occur most often from birth to two months of age. Sufficient amount of colostrum consumption, type of barn and hygiene are factors that positively affect the survival of calves. Calves that are fed with a sufficient amount of colostrum to obtain healthy and disease-resistant calves should be taken into the hutches prepared for a single calf. The aim of this study was to determine the effect of the birth season on calves living in individual calf hutches, livability, serum immunoglobulin level and some blood parameters. The material of the study consisted of blood samples of 88 female Holstein calves reared in individual fiberglass calf hutches. Birth season had no effect on calves blood serum total protein and glucose levels (P>0.05), while the age and seasonal factors had significant effect on the immunoglobulin levels and blood parameters (P<0.05). Blood serum IgG levels of calves in the first two months of age were significantly higher in autumn and winter than in spring and summer (P<0.001). Serum IgM levels were found to have higher mean scores in autumn than in other seasons (P<0.001). Serum IgA levels of calves in one-day age were found to be similar in spring and summer seasons, but the difference between all seasons at the other day periods was statistically significant (P<0.001). There was no difference in serum total protein levels between autumn and winter months for each measurement period, whereas it was found to be statistically significantly lower than the other seasons at first day of age (P<0.01). Serum albumin levels were found that the summer season was statistically lower than the other seasons (P<0.001). Serum globulin, urea, glucose levels were found to be statistically significant between seasons. As a result; In addition to the barn structure, it can be said that the seasonal effect is important in terms of providing adequate passive immunity between the births of calves to weaning age. It can be said calves in individual hutches reared in summer and spring months have negative effects on passive immunity transfer in Aydin province and surrounding area. In our study, it was found that calf hutches which are suitable for climatic conditions played an important role in preventing heat stress. It was concluded that the colostrum intake of calves will shed light on the studies investigating the interaction of blood parameters and environmental factors, which are indicative of passive transfer mechanism.

Keywords: Calf, Holstein, individual calf hutch, serum immunoglobulin

Bireysel Kulübelerde Barındırılan Holştayn Buzağıların Yaşama Gücü, Serum İmmunglobulin Düzeyleri ve Bazı Kan Parametreleri Üzerine Bir Araştırma

ÖZET

Buzağı ölümlerinin en fazla görüldüğü doğum-iki aylık yaş arası dönemde; yeterli miktarda kolostrum tüketimi, barınak tipi ve hijyeni, buzağıların yaşama gücünü olumlu yönde etkileyen faktörlerdir. Sağlıklı ve hastalıklara karşı dirençli buzağılar elde etmek için yeterli miktarda kolostrumla beslenmiş olan buzağıların, tek buzağı için hazırlanmış kulübelere alınması gerekmektedir. Çalışma, bireysel buzağı kulübelerde barınan buzağıların yaşama gücü, serum immunglobulin düzeyi ve bazı kan parametreleri üzerine doğum mevsiminin ne ölçüde etkilediğinin araştırılması amacıyla yapılmıştır. Araştırmanın materyalini fiberglas bireysel buzağı kulübelerinde barındırılan 88 adet buzağıya ait kan örnekleri oluşturmuştur. Buzağıların serum total protein ve glikoz düzeyleri üzerine mevsimin bir etkisi bulunmamış (P>0,05), immunglobulin düzeyleri ve kan parametreleri üzerine ise buzağı yaşı ve mevsim faktörlerinin önemli düzeyde etkisi olduğu gözlenmiştir (P<0,05). Buzağıların ilk iki aylık yaşta kan serum İgG düzeyleri, sonbahar ve kış mevsimlerinde ilkbahar ve yaz mevsimlerinden istatistiksel olarak önemli derecede yüksek bulunmuştur (P<0,001). Serum İgM düzeylerinin her ölçüm dönemi için sonbahar mevsiminin diğer mevsimlere göre istatistiksel önemde daha yüksek ortalamalara sahip olduğu saptanmıştır (P<0,001). Buzağıların bir günlük yaştaki Serum İgA düzeyi ortalamaları ilkbahar ve yaz mevsimlerinde benzer bulunurken, diğer yaş dönemlerinde tüm mevsimler arası fark istatistiksel olarak önemli bulunmuştur (P<0,001). Serum total protein düzeyleri arasında her ölçüm dönemi için sonbahar ve kış ayları ortalamaları bakımından farklılık bulunmazken bir günlük yaşta yaz ayı ortalamasının diğer mevsimlerden istatistiksel olarak önemli düzeyde düşük olduğu saptanmıştır (P<0,01). Serum albumin düzeylerinin, her bir ölçüm döneminde yaz mevsiminde diğer mevsimlere göre istatistiksel anlamda düşük olduğu saptanmıştır (P<0,001). Serum globulin, üre, glukoz düzeylerinin mevsimler arasında farklılığı istatistiksel olarak önemli bulunmuştur. Sonuç olarak; buzağıların süt kesim yaşına kadar olan dönem mortalitesinin azaltılmasında barınak yapısının yanı sıra mevsimsel etkinin de özellikle yeterli pasif bağışıklık sağlanması bakımından önemli olduğu söylenebilir. Çalışmanın gerçekleştirildiği Aydın ilinde özellikle yaz ve ilkbahar ayları bireysel kulübelerde barındırılan buzağılarda sıcak stresine yol açarak pasif bağışıklık transferini olumsuz yönde etkilediği söylenebilir. Çalışmada bölge iklim koşullarına uygun buzağı barınakları yapılmasının sıcak stresini engellemede önemli rol oynadığı saptanmıştır. Araştırmanın, buzağılarda kolostrum pasif transfer mekanizması göstergesi olan kan parametreleri ile çevresel faktörlerin etkileşimini inceleyen araştırmalara ışık tutacağı sonucuna varılmıştır.

Anahtar Sözcükler: Bireysel buzağı kulübesi, buzağı, Holştayn, serum immunglobulin.

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Introduction

Weaning age of the calves is most important life period in dairy cattle farms from birth until 2 months of age. Calf deaths are seen most common in weaning period. Higher birth weight; colostrum consumption, shelter type and hygiene are the factors that affect the survival of calves positively in this period. In order to obtain healthy and disease-resistant calves, sufficient colostrum feeding should be obtained in individual calf hutches. In modern enterprises, calves are generally breeding in hutches and then transferred to paddocks. It has been reported in some researches about intensive dairy cattle breeding that breeding of calves in individual hutches during weaning period significantly reduce problems of health, growth and maintenance-feeding (Cummins and Brunner, 1991; Gudin, 1991; Quigley et al., 1995; Shukanov, 1992; Virtala et al., 1999; Zhekov et al., 1997). It has been reported that calf hutches made from wood, polyester, water plywood, corrugated cement board, galvanized sheet, cheap and easy to find different materials are most suitable system for calf breeding (Tümer, 1995 Virtala et al., 1999). Macaulay et al. (1995) reported that polythene individual calf hutches deployed inside the barn have the hottest micro-climate; this is followed by wood and polymer hutches outside the barn. Serum immunoglobulin measurement is a practical and important application in newborn calves. Various factors interact with the amount of passive immunoglobulin to detect the presence of the disease. These factors include farm management, environment, hygiene, presence of infection, virulence of infectious organisms and antibody specificity. The risk of disease is high because immunoglobulin passive transfer is not fully realized in neonatal calves. Similarly, newborns which have received adequate passive transfer can easily become ill if housed in unhygienic shelters (Weaver et al., 2000). In a study about some blood parameters of calves housed in hutches using various building materials, the mean total protein, albumin and globulin values of calves in polyethylene hutches were found lower than calves housed in hutches by polymer, wood and metal building materials (McKnight et al., 1999). In some studies on the survival of calves, mortality was observed in summer lower than in winter (Ghose et al., 1994; Mishra and Taneja, 1991; Streit and Ernst, 1992). Some studies on calf shelter types, was researched about environmental, yield and behavioral differences between individual hutches and barn conditions (Hanekamp et al., 1994; Virtala et al., 1999). It has not been observed in any scientific study about this issue in Turkey. It is important to determine the effect of fiberglass individual calf huts, which are becoming more common in intensive dairy cattle breeding, on calf survival, serum immunoglobulin level, and some blood parameters and shelter microclimate.

The aim of this study was to determine the effect of the birth season on calves living in individual calf hutches, livability, serum immunoglobulin G (IgG) which is an important indicator of full immunity against diseases, immunoglobulin A (IgA) and immunoglobulin M (IgM) levels and some blood parameters (total protein, albumin, globulin, urea, glucose, ionized calcium, sodium and potassium levels).

This study; The survival of Holstein calves who spent the weaning period in fiberglass calf hutch, some blood parameters (total protein, albumin, globulin, urea, glucose, ionized calcium, sodium and potassium levels) and serum immunoglobulin G which is an important indicator of full immunity against diseases (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) levels on the effect of birth season were investigated.

Materials and Methods

The research protocol of the study was approved by the Ethic Committee of Adnan Menderes University (Approval Number: 2009/057). The study was conducted in a private dairy cattle farm in Aydin, Turkey. The material of the study consisted of 88 female Holstein calves born between 1 March 2010 and 28 February 2011. The calves were born at the calving pens and were transferred to the fiberglass calf hutches within 24 hours, regardless of gender. Calves were fed with colostrum and ad libitum fresh water with intensive and coarse feed supplements. All of the calf hutches are 110 cm×162×137 cm width and height. The calves were fed twice daily, at 9.00 am and 5.00 pm, with a total of 4 liters of preheated milk per day, 2 liters per meal. The milk was given daily with soft rubber mouthed, plas-

Table 1. Interactions of some blood mean parameters with immunoglobulins (F)

Parameter	In-group (Time)	Between Groups (Seasons)	Factor x Seasons
Immunoglobulin G	310.609***	25.209***	4.334**
Immunoglobulin M	1770.919***	8.545***	204.933***
Immunoglobulin A	756.792***	10.680***	493.934***
Total Protein	4.985*	1.322^{NS}	3.413*
Albumin	332.124***	6.578***	49.450***
Globulin	356.395***	5.658***	43.055***
Urea	588,303***	4,824***	21,219***
Glucose	568,584***	1,136 ^{NS}	132,837***
Calcium	3696,734***	20.297***	63.965***
Potassium	30.068***	2.632**	5.386**
Sodium	77.070***	2.257*	15.037***

*: P<0.05; **: P<0.01; ***: P<0.001; NS: Non-significant

Table 2. Immunoglobulin levels of calves in different seasons in the first two months after birth (g/L, n= spring: 21, summer: 25, autumn: 20, winter: 18; Mean±Standard error)

		Days				
	Seasons	**1th day**	**4th day**	**30th day**	**60th day**	**F**
Immunoglobulin G	Spring	13.1±1.4^{bD}	24.8±2.2^{cC}	43.4±2.9^{bB}	59.0±5.0^{bA}	40.15***
	Summer	13.8±1.3^{bD}	35.2±2.0^{bC}	44.5±2.7^{bB}	62.8±4.6^{bA}	41.28***
	Autumn	26.2±1.5^{aD}	52.4±2.2^{aC}	62.0±3.0^{aB}	93.7±5.1^{aA}	55.43***
	Winter	21.6±1.5^{aD}	47.0±2.3^{aC}	53.6±3.2^{abB}	91.0±5.4^{aA}	51.12***
	F	18.32***	29.97***	8.47***	12.73***	
ImmunoglobulinM	Spring	0.19±0.06^{cC}	0.27±0.09^{cB}	0.90±0.01^{bA}	0.82±0.01^{cA}	552.68***
	Summer	0.27±0.05^{bD}	0.37±0.09^{bC}	0.96±0.02^{bA}	0.79±0.01^{cB}	515.88***
	Autumn	0.50±0.06^{aB}	0.52±0.01^{aB}	1.13±0.27^{aA}	1.08±0.01^{aA}	444.90***
	Winter	0.25±0.06^{bC}	0.30±0.07^{cB}	0.94±0.02^{bA}	0.98±0.01^{bA}	574.39***
	F	487.23***	116.92***	15.13***	65.18***	
Immunoglobulin A	Spring	0.13±0.01^{cD}	2.16±0.6^{dC}	3.02±0.92^{dB}	3.99±0.17^{dA}	69.66***
	Summer	1.57±0.09^{cD}	3.52±0.68^{cC}	4.59±0.85^{cB}	5.98±0.15^{cA}	216.27***
	Autumn	3.16±0.10^{bD}	4.54±0.69^{bC}	6.05±0.95^{bB}	6.65±0.17^{bA}	153.66***
	Winter	3.86±0.11^{aD}	5.04±0.71^{aC}	6.55±0.10^{aB}	7.95±0.18^{aA}	154.63***
	F	133.29***	348.16***	279.05***	84.27***	

*** P<0.001; a, b, c, d: The difference between the means carrying different letters in the same column is statistically significant; A, B, C, D: The difference between the means carrying different letters in the same line is statistically significant (P<0.05)

tic body calf feeding bottles attached to the hangers in front of the hutches. In order to determine blood serum immunoglobulin levels and some blood parameters at various age periods of calves, 5 ml of blood from vena jugularis of each calf in the first 24 hours after birth (1st day), on the 4th, 30th and 60th days of age was collected. Total protein, albumin, globulin, urea and glucose levels were determined by spectrophotometric commercial test kits from serum samples to be obtained from blood Aydin Adnan Menderes University, Faculty of Veterinary Medicine, Department of Biochemistry laboratories in Aydin. IgG, IgA and IgM levels were determined by using commercial Elisa Kits (Cusabio, China) using microtiter plates and an ELISA reader (Anthos 2010, Anthos Labtec Instruments, Salzburg, Austria). Levels of ionized calcium, sodium and potassium, total protein, albumin, globulin, urea and glucose were determined by Microlab 2000 (Merck) using commercially available kits (Biomedical Systems, Barcelona, Spain) from serum samples to be obtained from blood Aydin Adnan Menderes University, Faculty of Veterinary Medicine, Department of Biochemistry laboratories. The analyses were carried out according to the manufacturer's recommendations.

Since the number of milking cows in the enterprise was sufficient, blood samples were taken from 22 calves in each season and the data of the calves dying during the study were evaluated as missing data in statistical calculations.

Data are presented with mean and standard error values. The difference between the 1st, 4th, 30th and 60th day measurements, within and between the seasons was determined by the General Linear Model, in terms of blood parameters, IgG, IgA and IgM. For repeated measures, multivariate analysis was performed. A p-value of less than 0.05 was considered to show a statistically significant result.

Results

Five of 88 female calves in the birth season groups have died at different stages of the study from the beginning. One of these calves died in spring and one died in autumn months and three of them died in winter, and the mortality values for spring, summer, autumn and winter were 4.5%, 0%, 4.5% and 13.6%, respectively. The data obtained from these calves as long as they lived were evaluated.

General differences of all parameters within the group (time) and between groups (season) are given in Table 1. There was no effect of season on serum total protein and glucose levels of calves (P>0.05), but it was observed that time and season factors had significant effect on immunoglobulin levels and blood parameters (P<0.05). Calf serum immunoglobulin levels were measured starting with spring in every season starting with birth in the 4th, 30th and 60th days after birth. The highest IgG levels on day 1 and day 60 were 26.2 g/L and 93.7 g/L, respectively. In the same periods, the highest values were found to be 0.50 g/L and 1.08 g/L for IgM, 3.86 g/L and 7.95 g/L for IgA, respectively. It was seen that IgG and IgM levels reach the highest values in autumn and IgA levels reach the highest value in winter months (Table 2). In terms of serum IgG levels, the difference between the seasons was statistically significant for each measurement day (P<0.001). Serum IgG levels determined in autumn and winter were significantly higher than spring and summer values on all four measurement days (P<0.001). Serum IgG levels showed a linear increase between 1-60 days in all four seasons and the difference between the measurement periods was statistically significant (P<0.001). When the serum IgM levels were examined, it was found that the autumn season had statistically higher means than the other seasons for each measurement period (P<0.001). Serum IgM levels increased significantly between 1-30th days in spring, summer and autumn (P<0.001), this level decreased on day 60, but this decrease was only statistically significant in summer compared to the previous period (P<0.001). In winter, there was a statistically significant increase between all measurement periods, and the 30th and 60th day means were significantly higher than the 1st and 4th day measurements (P<0.001). The mean serum IgA levels were found to be similar in the spring and summer means on the first day and the difference between all seasons was statistically significant (P<0.001). It was observed that the means showed a linear increase from spring to winter. When the seasons were examined separately, it was found that serum

Table 3. Total protein, albumin and globulin values of calves in blood in different seasons in the first two months after birth (g/dL, n= Spring: 21, summer: 25, autumn: 20, winter: 18; Mean±Standard error)

		Days				
	Seasons	**1th day**	**4th day**	**30th day**	**60th day**	**F**
Total protein	Spring	5.17±0.14^{acD}	5.50±0.13^{bcC}	5.75±0.13^{bB}	6.14±1.55^{A}	25.40***
	Summer	4.96±0.13^{bcD}	5.12±0.12^{bC}	5.37±0.12^{bB}	5.70±1.42 ^{A}	18.61***
	Autumn	5.59±0.15^{aD}	6.16±0.13^{aC}	6.60±0.13^{aB}	7.05±1.59 ^{A}	71.75***
	Winter	5.60±0.16^{aD}	5.98±0.14^{acC}	6.31±0.14^{aB}	10.34±1.67 ^{A}	34.50***
	F	4.61**	13.24***	18.45***	1.69^{NS}	
Albumin	Spring	2.75±0.07^{aD}	2.97±0.07^{bC}	3.14±0.06^{bB}	3.36±0.05^{bA}	19.46***
	Summer	2.10±0.06^{bD}	2.42±0.06^{dC}	2.68±0.05^{cB}	3.02±0.05^{cA}	54.94***
	Autumn	2.66±0.07^{aD}	3.19±0.07^{abC}	3.52±0.06^{aB}	3.76±0.06^{aA}	62.77***
	Winter	2.77±0.08^{aD}	3.33±0.07^{aC}	3.63±0.06^{aB}	3.86±0.06^{aA}	55.05***
	F	19.94***	34.43***	53.17***	45.20***	
Globulin	Spring	2.47±0.05^{aD}	2.84±0.06^{bcC}	3.01±0.06^{bB}	3.24±0.06^{bA}	36.89***
	Summer	2.10±0.05^{bC}	2.49±0.05^{dB}	2.62±0.05^{cB}	2.89±0.05^{cA}	48.79***
	Autumn	2.54±0.06^{aD}	2.98±0.06^{acC}	3.33±0.06^{aB}	3.58±0.06^{aA}	62.51***
	Winter	2.59±0.06^{aD}	3.15±0.07^{aC}	3.56±0.06^{aB}	3.77±0.06^{aA}	74.94***
	F	15.60***	19.55***	43.16***	40.49***	

**P<0.01; *** P<0.001; NS: Non-significant; a, b, c, d: The difference between the means carrying different letters in the same column is statistically significant; A, B, C, D: The difference between the means carrying different letters in the same line is statistically significant (P<0.05).

IgA levels increased between 1-60 days of age and this increase was statistically different (P<0.001).

The means of total protein, albumin and globulin obtained from blood sera obtained from calves are presented in Table 3. Total protein mean was determined as the first day of measurement in summer (4.96 g/dL) and the highest winter season in day 60 (10.34 g/dL).

The lowest and highest values for albumin were determined as the first day measurement of summer (2.10 g/dL) and 60 days of winter (3.86 g/dL) respectively, while the lowest and highest values for globulin were summer first day measurement (2.10 g/dL) and winter season 60th day measurement (3.77 g/dL). It was found that the mean of summer at 1 day age was significantly lower than the other seasons (P<0.01). Serum total protein 4th day means were not different between winter and autumn and spring, whereas autumn and winter were significantly higher than summer mean and autumn mean (P<0.001). Serum total protein levels in the autumn and winter were significantly higher in the 60th day measurements than in the spring and summer (P<0.001). Serum total protein level increased as the measurement days progressed in each season and a statistically significant difference was found between the measurement days (P<0.001). When the change in serum albumin level is considered, it was found that the summer season was statistically lower than the other seasons at each measurement period (P<0.001). The first day of measurement in summer serum albumin mean is lower than other seasons; the mean taken from since the fourth day were significantly higher in autumn and winter than in spring and summer. Serum albumin level was found to increase linearly with increasing age and this increase was statistically significant in all seasons (P<0.001). Serum globulin levels were also found to have statistically significant differences between the seasons at each measurement day and between days in each season (P<0.001). It was determined that the summer season had a significantly lower value on the first day compared to the other days, and that the spring and summer months had lower values compared to the autumn and winter months in the 4-60 day period. The mean serum glucose lowest level was 54.40 mg/dL on the 60th day of autumn, and the highest level was 118.11 mg/dL on the first day of winter. The difference between seasons of serum urea levels for each measurement day was statistically significant (P<0.001).

In terms of serum urea means on the first, fourth and 30th days, the summer and winter months were found to be significantly higher than the spring and autumn months, whereas the 60th day spring mean was significantly lower than the other seasons. When the serum glucose level was taken into consideration, it was determined that the differences between the measurement days and the seasons were statistically significant (P<0.001). In the 1st, 30th and 60th day measurements, it was found that the spring and winter means were significantly higher than the autumn and summer means, and the calves born during the summer also had significantly higher serum glucose concentrations than the calves born during the autumn season (P<0.001). On the 4th day, the highest serum glucose concentration was observed in the winter. Spring, autumn and summer months' levels followed winter months respectively. It was also found that serum glucose concentration showed a linear decrease with time progressing in all four seasons (Table 4). Calcium, potassium and sodium ratios obtained from blood serum obtained from calves are presented in Table 5. In all seasons, there were statistically significant differences in serum calcium concentrations of calves between all age stages and a linear decrease was observed with increasing age (P<0.001). While calf serum potassium level is maximum 4.55 mEq/L, it occurs in winter in 1 day age period; the lowest level was 3.68 mEq/L with summer 4-day age period. In the first day of summer, serum potassium levels were significantly lower than the other seasons (P<0.05), whereas in the fourth day, summer and autumn months had significantly lower values than spring and winter (P<0.01). The mean of serum potassium levels the fourth day in summer, autumn and winter was significantly lower than the other days (P<0.001, P<0.01). Serum sodium levels were statistically significant between the seasons and between 1-60 days of age blood measurement periods (P<0.001). Sodium levels in calf blood serum taken at 1 and 30 days of age were significantly higher in autumn and winter compared to

Table 4. Blood urea and glucose values of calves in different seasons in the first two months after birth (mg/dL, n= Spring: 21, Summer: 25, Autumn: 20, Winter: 18; Mean±Standard error)

		Days				
	Seasons	**1th day**	**4th day**	**30th day**	**60th day**	**F**
Urea	Spring	13.76±0.45^{bD}	15.38±0.53^{bC}	19.42±0.73 ^{dB}	24.23±0.81^{bA}	55.44***
	Summer	17.00±0.41^{aD}	19.68±0.49^{aC}	24.60±0.67^{acB}	29.80±0.75^{aA}	90.51***
	Autumn	14.20±0.46^{bcD}	16.80±0.55^{bC}	23.35±0.75^{bcB}	28.10±0.84^{aA}	91.27***
	Winter	15.61±0.49^{acD}	20.88±0.57^{aC}	26.61±0.79^{aB}	30.61±0.88^{aA}	81.62***
	F	11.33***	21.56***	16.23***	11.78***	
Glucose	Spring	112.38±1.45^{aA}	101.38±1.42^{bB}	83.90±1.51^{aC}	73.90±1.77^{aD}	65.90***
	Summer	105.08±1.33^{bA}	95.52±1.30^{cB}	76.24±1.39^{bC}	66.92±1.62^{bD}	75.75***
	Autumn	96.90±1.49^{cA}	84.90±1.46^{dB}	65.90±1.55^{cC}	54.40±1.81^{cD}	75.43***
	Winter	118.11±1.57^{aA}	109.61±1.54^{aB}	86.00±1.64^{aC}	74.16±1.91^{aD}	71.04***
	F	36.85***	48.61***	33.59***	25.80***	

*** P<0.001; a, b, c, d: The difference between the means carrying different letters in the same column is statistically significant; A, B, C, D: The difference between the means carrying different letters in the same line is statistically significant (P<0.05)

spring and summer (P<0.001). Fourth day autumn serum sodium levels were significantly higher than the spring and summer seasons (P<0.001), but there was no significant difference between autumn and winter seasons. Blood samples taken at the age of 60 days; again, it was found that serum sodium levels were significantly higher (P<0.001) in the autumn season than in the spring and summer seasons. Serum sodium levels in the 60-day age period were significantly lower than the one and four-day age period (P<0.001); there was no statistically significant difference between the ages of 30-60 days in summer and winter seasons.

Discussion

In our study, 30th and 60th days IgG values were found higher than the values obtained by Broucek et al. (1990). While Serum IgG concentrations of newborn calves were found to be lower than those determined by Streit and Ernst (1992); the values at the age of 30 days were similar and higher than those at the age of 60 days. It can be said that colostral immunoglobulin intake of calves in our study is better and the immune systems of calves are stronger with advancing time. Yuceer, (2008), reported the development of lower passive immunity in calves born in autumn. The lowest colostrum IgG concentration was found in cows giving birth in summer months. Similarly, serum IgG levels of calves born in summer were lower. These data were found to be consistent with the results of our study. Earley and Fallon (1999) reported that the serum IgG levels of calves born in spring were higher than those of calves born in autumn, and that the serum serum IgG levels of one month old calves reached the highest level in autumn compared to 22.6 mg/ml. In our study, it was shown that blood serum IgG levels obtained in autumn and winter months reached significantly higher levels than those obtained in spring and summer months. It can be said that calf blood serum IgG levels may change in different climatic conditions.

Murphy et al. (2005) reported newborn calf blood serum IgG level as 27.1 mg/ml. This result was found to be similar to the values of winter and autumn seasons found in our study, but it was found to be higher than IgG values obtained from calves born in spring and summer months. It can be said that this difference is caused by working with calves of different breeds in both studies. In addition, especially in summer and spring months, it can be said that the climatic conditions of our region have high environmental temperature values which are prone to causing hot stress, and this affects both colostrum quality and passive immunity transfer negatively. In a study conducted by Genç (2015) in Erzurum province, blood serum IgG levels of Brown-Swiss and Holstein calves were 12.6 mg/ml, 9.8 mg/ml, 11.97 mg/ml and 12.81 mg/ml in the spring, summer, autumn and winter months, respectively. In both studies, it was observed that calf serum IgG levels were higher in autumn and winter months.

In our study it was found that the serum IgM levels were the lowest in the spring months, followed by the summer and winter months, and the highest levels of serum IgM were reached in the autumn months. Earley and Fallon (1999) reported that the highest serum IgM levels of calves at the one month of age were reached in autumn with a rate of 1.37 mg/ml. These results, which are consistent with our study, suggest that calves gain better passive immunity during the cold season. When the total immunoglobulin level is examined, it is seen that higher levels of immunoglobulin are measured in autumn compared to spring months. This result is consistent with the means obtained from our study. Blood serum IgA levels of newborn calves were found to be similar to the values determined by Streit and Ernst (1992) for spring and summer, but higher than those of autumn and winter. It can be said that the climatic conditions of our study are close to the appropriate humidity and temperature values for cattle in autumn and winter months and increase the colostral immunoglobulin level and calf blood serum immunoglobulin level. Earley and Fallon (1999) found that calf serum IgA levels in spring were higher than those of autumn months. According to the results of our study, IgA levels were found to be the lowest in autumn in parallel to IgG and IGM levels, and the highest means for winter months were obtained. When some blood parameters obtained as a result of our study were examined in terms of seasonal changes; Calf blood serum total protein levels were significant in newborn, four day and one month old calves. While serum total protein levels of these age periods decreased to the lowest levels in summer months, they reached the highest levels in autumn and winter months. The total protein values of two-month-old calves were highest in winter and lowest in summer. McKnight et al. (1999) reported serum total protein levels in 42-day-old calves showed differences between seasons. The lowest total protein values in summer were followed by autumn and winter, respectively, and the highest total protein levels were reached in spring. It can be said that the ration quality varies in different seasons, in different regions and this affects the total protein level of colostrum positively or negatively. One and two month

Table 5. Calcium, potassium and sodium levels of calves in blood in different seasons, in the first two months after birth (n= Spring: 21, Summer: 25, Autumn: 20, Winter: 18; Mean±Standard error)

		Days				
	Seasons	**1th day**	**4th day**	**30th day**	**60th day**	**F**
Calcium (mg/dL)	Spring	9.82±0.17^{cA}	9.10±0.15^{cB}	5.21±0.06^{bcC}	4.24±0.03^{bD}	353.58***
	Summer	9.00±0.15^{dA}	8.60±0.13^{bB}	4.85±0.06^{dC}	4.00±0.03^{cD}	352.67***
	Autumn	11.46±0.17^{aA}	10.95±0.15^{aB}	5.64±0.07^{aC}	4.61±0.04^{aD}	532.94***
	Winter	10.51±0.18^{bA}	10.01±0.16^{bB}	5.42±0.07^{acC}	4.60±0.04^{aD}	352.55***
	F	39.80***	48.03***	26.13***	59.73***	
Potassium (mEq/L)	Spring	4.41±0.10^{ac}	4.18±0.09^{a}	4.39±0.07^{D}	4.27±0.07	1.66^{NS}
	Summer	4.13±0.09^{bcA}	3.68±0.08^{bB}	4.41±0.06^{A}	4.33±0.07^{A}	16.73***
	Autumn	4.33±0.11^{acA}	3.79±0.09^{bcB}	4.58±0.07^{A}	4.50±0.07^{A}	16.04***
	Winter	4.55±0.11^{aA}	4.05±0.10^{acB}	4.46±0.08^{A}	4.47±0.08^{A}	6.00**
	F	2.70*	6.55**	1.36 ^{NS}	2.03 ^{NS}	
Sodium (mEq/L)	Spring	136.42±0.69^{bA}	135.00±0.66^{bcB}	133.04±0.66^{bC}	131.42±0.68^{bD}	23.38***
	Summer	135.60±0.63^{bA}	134.52±0.61^{bcB}	133.76±0.61^{bBC}	132.84±0.62^{bcC}	10.82***
	Autumn	141.05±0.71^{aA}	139.05±0.68^{aB}	138.10±0.68^{aB}	136.10±0.69^{aC}	30.68***
	Winter	139.33±0.74^{aA}	136.83±0.72^{acB}	137.22±0.72^{aBC}	135.27±0.73^{acC}	33.30***
	F	13.66***	9.67***	13.85***	9.83***	

*: P<0.05; **: P<0.01; *** P<0.001; NS: Non-significant ; a, b, c, d: The difference between the means carrying different letters in the same column is statistically significant; A, B, C, D: The difference between the means carrying different letters in the same line is statistically significant (P<0.05).

of age Holstein calves serum total protein, glucose and albumin averages were found consistent with Khan et al. (2007[b]). Within the scope of our study, it can be said that the total protein values of calves born during the winter are higher than the age of two months because the calves are given more concentrated feed in order to meet the energy requirement due to environmental temperature change. Khan et al. (2007[a]) found blood serum total protein, glucose and albumin levels of one and two months old Holstein calves, 6.35-6.45 g/dL and 6.40-6.90 g/dL; 93.44-96.62 g/dL and 70.20-84.13 g/dL; 4,11-4,23 g/dL and 4,13-4,45 g/dL respectively. These results are consistent with the total protein, glucose and albumin mean of the one-month-old calves obtained from our study and higher than the average of the two-month-old calves. It can be said that the fact that the winter mean obtained in our study was higher than the mean of serum total albumin and globulin levels were due to the application of a more qualified nutrition program in those months and the positive effect of micro-conditioning conditions on calf's survival and feed efficiency. Swan et al. (2007) determined the maternal colostrum and commercial colostrum substitutes feed serum calves fed the total protein levels of 5.5 g/dL and 4.6 g/dL respectively. In a study designed by Smith and Foster (2007) on Holstein calves, colostrum-fed calf blood serum total protein levels were reported to be 5.4 g/dL, colostrum replacement feed was reported to be 4.4-4.7 g/dL.

Godden et al. (2009) reported maternal colostrum-fed newborn Holstein calves and one and two doses of colostrum substitutes fed with total protein levels of 5.7 g/dL, 4.9 g/dL and 5.5 g/dL respectively. The results of these studies are close to the total protein levels of calf blood serum obtained from our study. Johnson et al. (2007) found serum total protein levels of calves fed with raw and heated colostrum at the age of one day as 5.9 g/dL and 6.34 g/dL, respectively. These levels are higher than the levels obtained as a result of our study. In a study by Pauletti et al. (2003), the effect of IgG levels on total protein level was investigated; Total blood serum protein levels of 1 day old and 1 month of age calves with 20 mg/mL and below IgG levels were found as 6.25 mg/mL, and 7.29 mg/mL; while calves with 30 mg/mL and upper IgG levels were as 8.99 mg/mL and 7.92 mg/mL respectively. These results are higher than the mean serum total protein values obtained on the first day of our study, but are similar to the first and second month values. According to the 42th day data, serum globulin showed a statistically significant difference between the seasons. These results are different from the results of our study. As a result of this research, the highest levels were reached in winter, followed by autumn, spring and summer months. It can be said that this difference is caused by the heat stress that occurs in the summer months and that the studies were conducted in different environments. In the same study, the mean serum urea reached the highest level with 4.4 mmol/L in summer and the lowest level with 2.6 mmol/L in spring. The lowest serum urea levels in the spring months are consistent with the data obtained from our study. Serum glucose and calcium levels were highest in winter and autumn, while the lowest values were achieved in autumn and summer. In terms of both biochemical parameters, the seasonal differences obtained from our study were similar.

As a result; healthy development of newborn calves and reduction of mortality in the period up to weaning; it can be said that the calf house structure and seasonal effect are important, especially in terms of providing adequate passive immunity. Moreover, it can be said that calves receiving adequate and qualified colostrum affect calves' blood serum immunoglobulin levels positively. It can be said that the calves which are housed in individual hutches during the summer and spring months in Aydin province where the study was carried out cause heat stress and affect passive immunity transfer negatively. Our study concluded that the construction of calf houses suitable for the climatic conditions of the region plays an important role in preventing heat stress. It is concluded that our study could be guide to new studies investigating the interaction of blood parameters that are indicative of colostrum passive transfer mechanism in calves and environmental factors.

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Conflict of interest

The authors declare that they have no competing interests.

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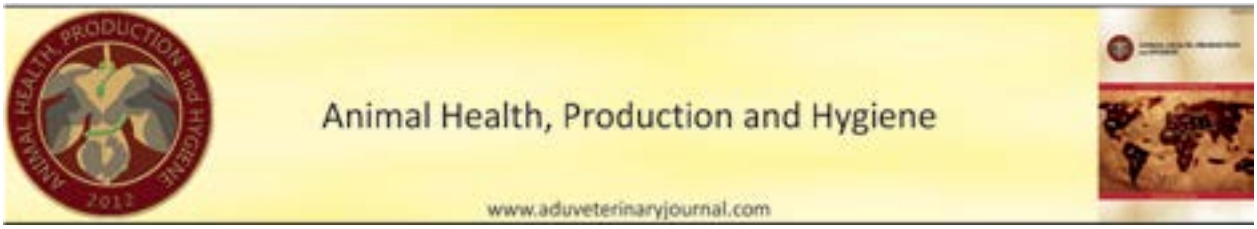

Research Article

Effect of Sildenafil Citrate on Uterine Artery Doppler Indices in Non-pregnant Rabbits

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ABSTRACT

The effect of different doses of sildenafil citrate (SC) administrations on arteria (a.) uterine blood flow was investigated in healthy female rabbits. Twelve healthy non-pregnant New Zealand rabbits were used in the present study. In the first experimental group (n=6), 5 mg SC thawed in 0.9% NaCl solution was administered orally. Arteria uterina was examined with Pulsed Doppler ultrasonography at pre and post (60 minutes) SC administration. Heart rate (HR), pulsatility index (PI) and resistance index (RI) values were recorded from the a. uterina visualized with cervical channels. Similarly, the study was repeated in second experimental group (n=6) by using 10 mg SC for per animal. In the first experiment, the mean HR, PI and RI values were recorded as (223 ± 7.80 & 239 ± 15.00), (1.60 ± 0.08 & 1.63 ± 0.16) and (0.73 ± 0.02 & 0.73 ± 0.03) before and after SC administration, respectively. In the second experiment, the mean HR, PI and RI values were observed as (210 ± 15.00 & 223 ± 14.00), (1.46 ± 0.23 & 1.79 ± 0.08) and (0.68 ± 0.05 & 0.77 ± 0.01) before and after SC administration, respectively.

No significant difference was between pre and post-administration Doppler parameters in both experiments (P>0.05). Consequently, no enhancing effect on the uterine perfusion was observed in oral 5 and 10 mg SC administrations per rabbit.

Keywords: Doppler, uterine artery, sildenafil citrate, rabbit

Gebe Olmayan Tavşanlarda Sildenafil Sitratın Uterin Arter Doppler Bulgularına Etkisi

ÖZET

Sağlıklı dişi tavşanlara farklı dozlarda uygulanan Sildenafil sitratın (SS) arteria (a.) uterinadaki kan akımı üzerine etkisi incelendi. Çalışmada 12 adet sağlıklı, gebe olmayan Yeni Zelanda ırkı dişi tavşan kullanıldı. Birinci deneme grubuna (n=6) 5 mg SS 0,9% NaCl solüsyonu içerisinde çözdürülerek oral yolla uygulandı. Sildenafil sitrat uygulamasından hemen önce ve 60 dk sonrasında a. uterina Pulsed Doppler ultrasonografi ile incelendi. Servikal kanallar ile birlikte görüntülenen a. uterinadan kalp atım hızı (HR), pulsatil indeks (PI) ve rezistans indeks (RI) değerleri kaydedildi. Benzer şekilde, ikinci deneme grubunda da (n=6) hayvan başına 10 mg SC ile çalışma tekrarlandı. Birinci denemede, SC uygulama öncesi ve sonrasında ortalama HR, PI ve RI değerleri sırasıyla (223±7,80 ve 239±15,00), (1,60±0,08 ve 1,63±0,16), (0,73±0,02 ve 0,73±0,03) olarak kaydedildi. İkinci denemede ise 10 mg SC uygulama öncesi ve sonrasında ortalama HR, PI ve RI değerlerinin sırasıyla (210±15,00 ve 223±14,00), (1,46±0,23 ve 1,79±0,08), (0,68±0,05 ve 0,77±0,01) olduğu görüldü. Her iki deneme içinde uygulama öncesi ve sonrası Doppler değerlerinde farklılığa rastlanmadı (P>0,05). Sonuç olarak, tavşan başına 5 ve 10 mg dozda oral SC uygulamasının uterus perfüzyonu üzerinde arttırıcı bir etkisi olmadığı görüldü.

Anahtar kelimeler: Doppler, arteria uterina, sildenafil sitrat, tavşan.

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Introduction

Phosphodiesterase-5 (PDE-5) is responsible for the degradation of the cyclic guanosine monophosphate (cGMP) to guanosine monophosphate. Therefore, inhibiting PDE-5 delays the breakdown of cGMP and induces vasodilatation (Ballard et al., 1998). Sildenafil Citrate, a specific PDE-5 inhibitor, has been shown to potentiate the effects of nitric oxide (Ramesar et al., 2010). Nitric oxide is a key signaling agent involved in smooth muscle cells and activates the cGMP/protein kinase G pathway within smooth muscle relaxation (Hale et al., 2010). Sildenafil citrate acts as a potent vasodilator that enhances and prolongs the action of cGMP by selectively inhibiting PDE-5, which enzymatically converts the intracellular second messenger cGMP into its inactive form (Pellicer et al., 2011). Uterine blood flow has an important role in many reproductive cases, whether it is before and after pregnancy. Thus, its manipulation has been investigating as an additional therapy in lots of cases. In recent years, indications of SC in women have been explored after detection of the arterial dilatation in reproductive tract (Zoma et al, 2004; Hale et al., 2010; Satterfield et al., 2010)nulliparous women. Fifteen women were randomized in a double-blind fashion to receive either placebo or sildenafil (25 or 100 mg**.** In this direction, some investigations have been performing on infertile women (Malinova et al., 2013) especially need improving endometrial thickness (Takasaki et al., 2010). But, the most common usage of SC in pregnancies has been reported in intrauterine fetal growth retardation in women (Villanueva-Garcia et al., 2007; Von Dadelszen et al., 2011; there is no effective therapy for severe early-onset intrauterine growth restriction (IUGRTrapani et al., 2016). Also, similar studies reported in experimental animal models (Sanchez-Aparicio et al., 2008; Satterfield et al., 2010; Pellicer et al., 2011)starting from day 35 of gestation to delivery. Fetuses were delivered by cesarean section. Fetal asphyxia was induced by clamping the umbilical cord at birth for 5 minutes. Results: Sildenafil protected the pups against induced asphyxia at birth in a dose-dependent manner (eg, partial pressure (tension. Accordingly, it was seen that the improving a. uterina blood flow (Ramesar et al., 2010; Batissaco et al., 2013) and abnormal umbilical artery waveforms (Stanley et al., 2012).

There is limited knowledge regarding the effect of SC on uterine perfusion in rabbits. In this study, the effect of two different doses of SC on the uterine artery Pulsed Doppler ultrasonography findings was investigated in non-pregnant rabbits.

Material and Method

All experiments were performed on 12 healthy, sexually mature; New-Zealand white does weighing 3.6 - 4.1 kg. They were housed individually in wire mesh cages under controlled light (14 h light/10 h dark) and temperature (18 to 24 ˚C) conditions. All does were with free access to water and standard pellets. Animal handling and all procedures were performed in accordance with applicable regulations and guidelines and with the approval of the Animal Research Ethics Committee of Aydin Adnan Menderes University (64583101/14/145). Before the start of the experiments, the does were accustomed to the environment and trained to medical manipulations during three weeks.

In the first experimental group (n=6), 5 mg SC thawed in 5 ml 0.9% saline solution was administered peros way via naso-esophageal catheter. Similarly, the study was repeated second experiment group (n=6) by using 10 mg SC thawed in 5 ml 0.9% saline solution for per animal. In both groups, a. uterina was examined with Pulsed Doppler ultrasonography with an 8-MHz microconvex transducer (MyLab Vet30- Esaote®, Genova, Italy) just before drug administration. Same examinations were repeated 60 minutes after from drug administration. Pelvic transversal scanning was performed for Doppler measurements. Heart rates, PI and RI were recorded from the most cranial part of the artery visualized with cervical channels. After visualizing the a. uterina via color Doppler, Pulsed-wave Doppler examinations were performed. Recordings were obtained for at least regularly three consecutive arterial waveforms. Waveforms were disregarded during doe's movements or cardiac arrhythmias. In order to evaluate the blood flow waveform patterns of the a. uterina, the HR, PI, and RI were measured. Due to the possible thermal and cavity side effects of Doppler sound waves on tissues, Pulsed-wave examinations did not exceed 30 seconds and were recessed for 1 minute. Regarding to stress factors of the study group, all examinations lasted between 9:00-12:00 a.m. and 50-60 minutes totally in silent and dimly lit room, and were performed by the same trained operator.

Average data presented as mean ± SEM. The Mean Doppler findings between pre and post administrations were compared using paired samples t-test and the differences between Doppler findings of first and second experimental groups were evaluated with independent samples t-test. Differences were considered statistically significant at P levels of less than 0.05.

Results

During the study, the does tolerated the ultrasonographic examinations well. The a. uterina was successfully visualized and Doppler trace was recorded in all examined animals. The uterine artery blood flow was characterized low resistance and showed systolic waveform in non-pregnant does. Any pathologic finding (arrhythmia, reverse flow, end-diastolic notch etc.) was not recorded.

Table 1 presents the mean HR, PI and RI values of both experimental groups. In statistical analysis, there was no significant

Table 1: The mean HR, PI and RI values of both experimental groups.

Doppler parameters	Group 1 (n=6)		Group 2 (n=6)	
	Pre 5mg SC administration (Mean±SEM)	Post 5mg SC administration (Mean±SEM)	Pre 10mg SC administration (Mean±SEM)	Post 10mg SC administration (Mean±SEM)
HR (bpm)	223 ± 7.80	239 ± 15.00	210 ± 15.00	223 ± 14.00
PI	1.60 ± 0.08	1.63 ± 0.16	1.46 ± 0.23	1.79 ± 0.08
RI	0.73 ± 0.02	0.73 ± 0.03	0.68 ± 0.05	0.77 ± 0.01

Sildenafil citrate (SC), heart rate (HR), pulsatility index (PI), resistance index (RI) (P>0.05).

difference between pre and post-administration Doppler findings in both experimental group (P>0.05). Also, the results of both experimental groups were found similar (P>0.05). Although, there were a tendency to increase uterine perfusion after SC administrations especially in 10 mg dose, the differences did not reach statistical significance.

Discussion

Uterine blood flow has an important role in many reproductive cases either before or after pregnancy. Thus, the manipulation of blood flow has investigated as an additional therapy in lots of cases. It has been reported that the support the uterine perfusion with SC may improve implantation (Sher and Fisch, 2002; Simon and Laufer, 2012)with normal ovarian reserve and at least two consecutive prior IVF failures attributed to inadequate endometrial development. Intervention(s and fetal growth restriction (Wareing et al., 2005). Also, it can be used in postoperative uterine adhesions (Batukan et al., 2007) as well as Ashermann syndrome (Zinger et al., 2006)Pfizer, Inc., New York, NY.

Although SC is used in females of different species, little reproductive findings obtained from females have been in rabbit model (Lopez-Tello et al., 2017)which had increased systolic peak and time-averaged mean velocities at the MCA. Furthermore, fetuses in the SC group had significantly higher biparietal and thoracic diameters and longer crown–rump lengths than fetuses in Group R. Hence, the SC group had a reduced IUGR rate and a higher kit size at birth compared with Group R. In conclusion, SC may provide potential benefits in pregnancies with placental insufficiency and IUGR, partially counteracting the negative effects of food restriction on placental development and fetal growth. However, the present study also found evidence of a possible blood overflow in the brain that warrants further investigation. López-Tello et al., 2017. In our study, SC was given orally in healthy non-pregnant rabbits and the acute effect on the arteria uterina blood flow was investigated. A Previous study has reported that SC provides 40% bioavailability after oral administration (Villanueva-Garcia et al., 2007). Sildenafil citrate was administered 0.7 mg/kg IV and 1.4 mg/kg PO in previous rabbit studies (Ockaili et al., 2002; Das et al., 2004). These doses were equivalent to clinical dose of 100 mg for 70 kg human patient (Das et al., 2004). The based on these reports, we preferred to use 5 and 10 mg doses per rabbit.

It was shown that SC causes significant increase in uterine blood flow of non-pregnant females in the luteal phase (Hale et al., 2010; Dzieciol et al., 2014, 2015). Taking into account these findings, it would be seen that the different application procedures cause similar results. The results of our study show that no changes of a. uterina blood flow parameters was in response the SC administration in rabbit model in 5 and 10 mg experimental groups. In our study, all the rabbits were in follicular phase of estrus cycle since there was no stimulation (mating or GnRH injection) to induce ovulation subsequently to start luteal phase. Disregarding the doses and routes, unaltered a. uterina blood flow might be related with basal progesterone level of the non-pregnant follicular phase of the rabbits. In further studies, SC use can be done in experimental luteal stage following GnRH injection or artificial cervical stimulations. In this way, it can be examined that whether or not a relation between the level of SC impact and progesterone level. Another result of our study was the well toleration of the SC by study material, and no pathologic finding (reverse flow, end-diastolic notch etc.) seen during Doppler examinations. It was not recorded any general complication after oral administrations. In conclusion, although there was no difference in uterine artery blood flow after SC administration of 5 and 10 mg doses, it is considered that needs further studies reexamining different doses and routes on rabbits in luteal phase.

Conflict of interest

The authors declare that they have no competing interests.

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Research Article

Plasma Lactate Levels Of Single And Multiple Born Chios Lambs

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A B S T R A C T

Hyperlactemia is closely related to the severity of the disease. Besides, the use of lactate as a biomarker has been increased in human medicine and recently in veterinary medicine as well. The aim of this study was to investigate the plasma lactate level and its relationship with survival in single, twin, triplet and quintuplets born Chios lambs. Blood samples were taken from single, twin, triplet and quintuplets lambs before sucking colostrum within 15 minutes after birth.

Differences in the concentaration of plasma lactate, total protein, albumin and triglyceride were determined and there was significant differences between the groups, however no statistically significant difference was found between the groups in glucose, BUN, urea, creatinine, AST, ALT and cholesterol levels.

In this study, high plasma lactate levels were detected in single, twin, triplet and quintuplets born Chios lambs . Plasma lactate levels are an important predictor for the mortality in an emergency veterinary clinic and there is a correlation between survival and plasma lactate concentration. It can be concluded that the increased number of offsprings produced in one litter and lamb deaths were found to be correlated with the plasma lactate concentrations. Monitoring of plasma lactate concentrations can be recommended for the prognosis lamb survival.

Keywords: Lactate, single and multiple born lambs, Chios breed .

Tek ve Çoklu Doğmuş Sakız Kuzularda Plazma Laktat Düzeyleri

Ö Z E T

Hiperlaktemi hastalığın ciddiyetiyle yakından ilişkilidir .İnsan hekimliğinde biyobelirteç olarak kullanılan laktat son yıllarda veteriner hekimlikte de yagın olarak kullanılmaya başlamıştır. Bu çalışmanın amacı yenidoğan tek, ikiz, üçüz ve beşiz yavrularda laktat düzeyini ve bunun sağkalım ile ilişkisini belirlemektir. Gebe sakız ırkı koyunlar takip edilmiş ve doğumdan hemen sonra 15 dakika içinde tek, ikiz, üçüz ve beşiz yavrulardan annelerinden kolostrum emmeden kan örnekleri alınmıştır. Plazma laktat total protein, albumin ve trigliserid düzeylerinde gruplar arası fark istatsistiki olarak anlamlı bulunmuş ancak glukoz, BUN, üre, creatinine, AST, ALT ve kolesterol düzeylerinde istatsitiki olarak anlamlı bulunmamıştır. Bu çalışmada tek, ikiz, üçüz ve beşiz yavrularda yüksek laktat düzeyi belirlenmiştir. Laktat düzeyi acil veteriner klinikte mortalitenin önemli bir göstergesidir ve yüksek laktat konsantrasyonu ile sağkalım arasında negative korelasyon vardır. Bu çalışmada çoklu doğumlarda yavru sayısı arttıkça yavru ölümlerinin de arttığı, laktat ölçümü ile monitorizasyonun korele olduğu ve sağkalımın belirlenmesinde laktatın anlamlı olduğu belirlenmiştir.

Anahtar Kelimeler: Laktat, tek ve çoklu doğum, Sakız ırkı

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Introduction

Multiple born lambs have lower survival rates compared to single born lamb. There are several reasons for this: as such low birth weight, unsatisfactory placental nutrition, intrapartum hypoxia, inadequate thermogenesis and insufficient colostrum intake. Lambs with low birth weight, compared to heavier-born lambs, have not only lower surface areas but also lower energy stores (Dwyer, 2003; Mccord et al., 2017). The causes of lamb deaths in the first three days of life have been investigated pathologically and through clinical observation , and it has been reported that the causes of death in single, twin and triple offspring were mainly due to dystocia, starvation and birth position (Kerslake et al., 2005).

Light metabolic and respiratory acidosis occur in newborn lambs and calves in normal births, for which reduced circulation in the placenta during severe labour pains, prolonged parturition and severe acidosis related diseases in mothers are responsible. . . Along with these causes, hypoxia and hypercapnia can occur in the fetus (Vannuchi et al., 2012). The offspring can either die before birth depending on the degree and duration of acidosis or is born with symptoms of asphyxia. A decrease in fetal growth may occur as a result of maternal malnutrition and fetus's failure to obtain sufficient nutrients from the placenta during pregnancy. Inadequate nutrient supply to the fetus can result in low birth weight of offspring. This leads to insufficient thermoregulation of the offspring and postnatal behaviour such as rising to its feet and sucking (Dwyer, 2003).

However, lactate metabolically plays a protective role in providing ongoing cellular energy during tissue hypoperfusion or hypoxia and in reducing acidosis. Therefore, hyperlactatemia has a close relationship with the disease. This is self-protecting response of the organism (Gillespie et al., 2017). Lactate is usually an essential multifaceted metabolic fuel for cellular bioenergetics. Since lactate is used as a biomarker and as a therapeutic endpoint in the diagnosis of human diseases in emergency medicine and intensive care units, it is also essential to improve its use in the field of veterinary medicine. There are studies about the clinical use of lactate, screening, evaluations, risk determination and prognosis in the human medicine . Plasma lactate concentration is a valuable prognostic marker in trauma, sepsis,septic shock, systemic inflammatory response syndrome (SIRS), cardiac arrest, malaria, head trauma, liver failure, and carbon monoxide poisoning (Vincent et al. 2016). An increase in plasma lactate levels in living organisms is closely associated with life-threatening problems, but it is not seen as a cause. For this reason, while normal level of lactate is associated with a healthy organism, high lactate level is associated with poor prognosis.

Since there is a low survival rate due to many reasons in multiple born lambs, this present study aimed to investigate plasma lactate concentration of single or multiple newborn Chios lambs, which has increased use in veterinary medicine in recent years. Chios breeds of sheep were chosen for the material of this study because of their high fertility and high incidence for the production of larger litters. Thus, it can enable the present study to establish a correlation between plasma lactate concentration and offspring survival and growth rate.

Material and Method

Animals

This study was started with the permission of Adnan Menderes University Ethics Committee of Experimental Animals Local Ethics Committee dated 31/05/2018 and numbered 645831/01/2018/71. Newborn lambs of Chios ewes were provided by the Chios Sheep Production Farm of Aydın Metropolitan Municipality. Blood samples were obtained in the blood sampling tubes from *vena jugularis* in newborn lambs which were born between 01.02.2018 and 01.02.2019. In this time period, 12 singles, 6 twins (6x2 = 12), 4 triplets (4x3 = 12) and 1 quintuplet (5x1 = 5) lambs were born. There was no quadruplet litter during the research period in the farm. As soon as the lambs were born, their blood was drawn into vacuum heparinized tubes to separate out the plasma before they sucked colostrum. After that, they were weighed and rectal body temperatures were measured. The blood plasma was separated into 3 different marked tubes per each sample. One tube was kept at +4 °C to measure lactate within latest 6 hours. The other two tubes were stored at -20 °C in order to perform the analyses of glucose, total protein, albumin, urea, creatinine, cholesterol, triglycerides, AST and ALT. After birth, newborn lambs implemented general care procedures under the same conditions. After the birth and blood collection, vitamin E and selenium were administered to newborn lambs. Besides, newborn lambs were vaccinated twice against enterotoxemia when they were one month and 45 days old.

Biochemical Analyses

The blood samples were taken to Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Internal Medicine Laboratory. Plasma lactate measurements were made colourimetric with the Eurolyser Solo device (Austria) according to the producer instruction. The final point measurement absorption was 546 nm wavelength. The measurement range of the method is 0.1-15 mmol/L. Plasma glucose, BUN, urea, creatinine, total protein, albumin, triglyceride, cholesterol, AST and ALT level measurements were performed in autoanalyser (Rayto Chemray120, China) using commercial test kits (Archem Diagnostic, Turkey).

Statistical Analysis

The 'SPSS for Windows Version 22 (SPSS Inc, USA) package program was used for all statistical analysis. The level of statistical significance was accepted as $p<0.05$. Whether the variables showed normal distribution or not was examined by the Shapiro-Wilk test. Average and standard deviation values were specified for descriptive statistics. The difference between groups was determined by one-way variance analysis (One-way ANOVA)which is suitable for normal distribution. Tukey test was performed to determine the group which caused this difference. Non-parametric tests (Kruskal Wallis) were used to show the differences between the groups which were not normally distributed.

Results

The individual data (ear numbers, date of birth, sexes, birth weights, rectal temperatures, lactate levels and postnatal follow-up) of the lambs used in the study are presented in Table 1. Mean plasma lactate, glucose, BUN, urea, creatinine, total protein, albumin, triglyceride, cholesterol, AST and ALT levels of single, twin, triplet and quintuplet groups are presented in Table 2.

According to the present results, 3 of 12 single-born offspring were died when they were 15 days and three months old. There was no mortality in the twin group. Two lambs died in the triplet group within the first month following their birth. There was no, quadruplet deliveries of ewes in the study, and only one quintuplet delivery occurred at the farm. Quintuplet lambs had lower live weight and higher lactate level than all other lambs, but only one lamb died in this group in the third month after the birth. Of the 41 lambs included in the study, six lambs died between 15 days and three months old of age. . Among them, lamb number 841 was treated for general condition disorder and weakness, and lamb number 824 was treated for respiratory disease, but both of them died. The other four

Table 1 The individual data of the newborn lambs.

Litter size	Animal number	Sex	Birth Weight	Date of Birth	Lactate mmol/L	Rectal Temp.	Status	Date of Death
Single	920	M	5	05.02.2018	8,6	37,7	Alive	
Single	897	F	3,9	08.02.2018	9,4	38,7	Alive	
Single	865	M	6,1	11.02.2018	7,34	37,8	Alive	
Single	864	F	4,5	12.02.2018	14,52	38,2	Alive	
Single	854	M	4,4	18.02.2018	3,44	37,9	Alive	
Single	853	M	4	20.02.2018	4,54	36,5	Alive	
Single	851	M	4,8	20.02.2018	8,74	37,8	Alive	
Single	850	M	4,3	20.02.2018	5,1	38,4	Alive	
Single	846	M	3,3	22.02.2018	>15	37,2	Dead	14.03.2018
Single	841	F	3,8	22.02.2018	3,98	39,1	Dead	16.05.2018
Single	590	F	3,5	31.12.2018	>15	37,6	Dead	09.01.2019
Single	774	F	4,3	07.01.2019	6,94	37,2	Alive	
Twin	914	F	2,7	06.02.2018	4,56	37,8	Alive	
Twin	915	F	3,8	06.02.2018	10,01	38,3	Alive	
Twin	909	F	2,8	06.02.2018	6,44	38,5	Alive	
Twin	910	F	3,6	06.02.2018	4,76	38,2	Alive	
Twin	906	F	3,1	07.02.2018	>15	38,2	Alive	
Twin	905	F	3,6	07.02.2018	4,7	39,1	Alive	
Twin	895	M	4	08.02.2018	8,59	38,6	Alive	
Twin	896	F	3,8	08.02.2018	4,41	38,5	Alive	
Twin	881	M	4,8	09.02.2018	5,86	38,4	Alive	
Twin	882	M	4,5	09.02.2018	6,38	38,5	Alive	
Twin	622	M	5	12.12.2018	2,69	37,4	Alive	
Twin	621	M	4,5	12.12.2018	7,35	37,8	Alive	
Triplet	900	M	4,3	07.02.2018	3,45	37,6	Alive	
Triplet	901	M	3,6	07.02.2018	10,74	38,1	Alive	
Triplet	902	M	2,8	07.02.2018	4,49	37,3	Alive	
Triplet	822	F	2,8	23.02.2018	3,85	37,5	Alive	
Triplet	823	M	3,6	23.02.2018	>15	37,8	Alive	
Triplet	824	M	2,2	23.02.2018	2,58	37,1	Dead	05.03.2018
Triplet	728	F	2,1	07.07.2018	>15	37,5	Dead	18.08.2018
Triplet	727	F	2,6	07.07.2018	10,25	37,6	Alive	
Triplet	726	M	3,6	07.07.2018	6,51	37,4	Alive	
Triplet	582	M	2,6	05.01.2019	4,82	37,6	Alive	
Triplet	583	F	3,7	05.01.2019	5,21	37,4	Alive	
Triplet	584	F	4,6	05.01.2019	7,74	37,2	Alive	
Quintp.	921	F	2	05.02.2018	>15	37,5	Alive	
Quintp.	922	F	1,5	05.02.2018	>15	36,7	Dead	31.05.2018
Quintp.	923	M	2,7	05.02.2018	>15	37,1	Alive	
Quintp.	924	F	1,7	05.02.2018	>15	37,7	Alive	
Quintp.	925	F	2	05.02.2018	12,2	37,2	Alive	

lambs (ear-numbered 846, 590, 922, 728) were recorded as sudden death without receiving any treatment.

According to the results, differences in metabolic parameters between single and multiple born lambs were evaluated. Statistical differences in plasma lactate, total protein, albumin and triglyceride levels were determined between the groups, while glucose, BUN, urea, creatinine, AST, ALT and cholesterol levels were not different statistically between the groups.

Average plasma lactate levels were 6.05 ± 4.1 mmol / L, 6.70 ± 3.6 mmol / L, 12.47 ± 4.3 mmol / L, 14,44 ± 1.25 mmol / L in single, twin, triplet and quintuplet born lambs respectively. As the number of offspring in one litter increased, the average lactate levels of the offspring also increased, and the highest level was identified in quintuplets. The mean lactate levels of triplet and quintuplets groups were higher (p <0,001) compared to single and twin offspring groups.

Total protein level was 4.69 ± 0.29 g / dL, 4.08 ± 0.19 g / dL, 4.21 ± 0.25 g / dL and 4.07 ± 0,16 g / dL in single, twin, triplet and quintuplet born lambs respectively. The lowest level was in the quintuple offspring group. Similarly, serum albumin level was 3.19 ± 0.12 g / dL, 3.04 ± 0.11 g /dL, 3.07 ± 0.12 g / dL and 2.95 ± 0.09 g / dL in single, twin, triplet andquintuplet born lambs and the mean albumin level of the quintuplet offspring group was lower than the other groups. The difference between total protein and albumin levels were found to be significant (p <0.001). Triglyceride levels were 57.91 ± 20.89 mg / dL, 51.53 ± 16.36 mg / dL, 53.50 ± 23.93 mg / dL and 72.50 ± 7.63mg / dL in single, twin, triplet and quintuplet born lambs respectively, and the level in the quintuplet offspring was higher than all other groups. No statistically significant difference was found between the groups in terms of glucose, BUN, urea, creatinine, cholesterol, AST and ALT concentrations.

Discussion

The neonatal period requires extra physiological, morphological and behavioural changes to ensure successful adaptation to the extrauterine environment. In some cases this adaptation is slowly shaped and there exist differences between species (Piccione et al., 2007; Piccione et al., 2008). Newborns are under metabolically unstable conditions. Therefore, newborns, especially in the first week of life, are vulnerable to diseasesand this condition can result in a high mortality rate (Piccione et al., 2010). One of the most important problems in cattle breeding is neonatal deaths which occur at an approximate rate of 15%, which is also one of the reasons for economic losses in small ruminant husbandary (Dwyer, 2008). Efforts to prevent neonatal deaths should be the main aim of veterinary neonatology studies. For these reasons, clinical follow-up of newborn lambs may provide an early diagnosis in determining adaptation failure. With all these clinical follow-ups, it may be possible to establish corrective procedures in cases such as inability to correct the acid-base balance and failure to provide thermal or metabolic hemostasis, which causes mortality.

Chios sheep are fed and cultivated in Turkey because of their high fertility and delivery of multiple lambs in a litter. However, multiple lambs lead to low survival rate due to various reasons such as low birth weight, hypersensitivity to placental insufficiency, intrapartum hypoxia, inadequate thermogenic mechanism and insufficient colostrum intake. While normal lactate levels at lambing are associated with better survival rate, however high lactate levels are associated with poor prognosis. The most effective cause of this may be lactic acidosis developing due to hyperlactatemia). Metabolic acidosis is evident in small ruminants within 10-15 minutes after birth. Increased lactic acid is one of the causes of metabolic acidosis. Acidosis, hypercapnia and hypoxia in newborns stimulate carotid chemoreceptors showing an increase in O_2 sensitivity and affect the respiratory centre, which causes an increase in tidal volume and respiratory rate. This may be responsible for the significant increase in pO_2 in the days after birth so that it may provide an improvement in respiratory function due to increased gas diffusion capacity through vascularization of the alveolar-capillary membrane and lung capillary (Piccione et al, 2006). Furthermore, the cause of acidosis after birth is explained by decreased blood circulation in the placenta, prolonged delivery process and severe acidosis of ewes. Therefore, hypoxia and hypercapnia occur in the fetus (Sahal et al., 1994). Prolonged deliveries, changes in offspring position and conditions that require support for delivery are defined as difficult births. It may cause acute hypoxia and elevated plasma lactate due to cord compression (Mellor and Stanford, 2004). Utero-placental blood flow may be decreased because number of placentomes are lower in lambing withtwo or more offspring than single one (Kenyon et al., 2007; Vonnahme et al., 2008). Heat production of lambs with placental insufficiency is also limited, and a negative correlation was found between heat production and plasma lactate levels in the first 6-8 hours of life (Kerslake et al., 2010). Since the capacity of the placenta to carry oxygen and nutrients depends on the surface area of the placenta, high lactate concentration can be formed in multiple deliveries because it affects the number of placentomes and uteroplacental blood flow (Kerslake et al., 2010).

Glucose is the main energy substrate for fetal and placental metabolism in all mammalian species (Brolio et al., 2010). Anaerobic glycolysis is a process that uses glucose to produce lactate in an oxygen-free environment (Barroso et al., 2006). Lactate plays a key role as a marker of fetal and neonatal distress in both human and veterinary obstetrics (Armstrong et al., 2006), and it is one of the main components of metabolic acidosis (Borruto et al., 2008). It has been reported in many studies that hyperlactatemia occur physiologically in newborns (Peternelli-Silva , 2012 and 2013; Regazzi, 2015). The increase in lactate levels in newborns can be observed physiologically. It takes 24 hours for lactate to return to normal levels. Hyperlactatemia after birth is associated with placental production

Table 2 Average concentration of plasma biochemical parameters in lamb litters (mean, and standard deviation).

Groups	Lactate mmol/L $X \pm S_x$	Glucose mg/dL $X \pm S_x$	BUN mg/dL $X \pm S_x$	Urea mg/dL $X \pm S_x$	Creatinin mg/dL $X \pm S_x$	Total protein g/dL $X \pm S_x$	Albumin g/dL $X \pm S_x$	Tryglyceride mg/dL $X \pm S_x$	Cholesterol mg/dL $X \pm S_x$	AST IU $X \pm S_x$	ALT IU $X \pm S_x$
Single n=12	6,05±4,1^{b}	53,50±20,25	14,60±2,70	31,25±5,80	1,35±0,38	4,69±0,29^{a}	3,19±0,12^{a}	57,91±20,89^{ab}	34,16±6,73	23,41±4,62	3,33±1,65
Twin n=12	6,70±3,6^{b}	34,76±11,51	13,76±2,80	29,16±5,99	1,53±0,58	4,08±0,19^{b}	3,04±0,11^{b}	51,53±16,36^{b}	29,00±5,30	19,38±4,07	2,69±0,63
Triplet n=18	12,47±4,3^{a}	45,72±30,19	14,24±3,77	30,85±8,44	1,53±0,48	4,21±0,25^{b}	3,07±0,12^{ab}	53,50±23,93^{ab}	34,33±11,04	23,16±3,91	3,83±2,85
Quintuplets n=5	14,44±1,25^{a}	41,33±13,45	15,88±0,73	34,00±1,58	1,86±0,27	4,07±0,16^{b}	2,95±0,09^{b}	72,50±7,63^{a}	33,80±1,64	20,20±3,27	2,60±0,54
p	***	NS	NS	NS	NS	***	***	**	NS	NS	NS

*: p<0.05 ** : p<0.01 ***:p<0.001 NS :Not significant

of lactate. Hyperlactemia in neonatal animals and its gradual reduction in 24 hours was demonstrated for sheep by Peternelli-Silva et al. (2013) and Castagnetti et al (2010), and for horses by Cruz (2014). According to a research by Peternelli-Silva et al. (2018) in which lactate levels in ewes at lambing, in placenta and newborn lambs were examined, the lactate level was 3.09 mmol/L in ewes at lambing, 5.78 mmol/L in placenta, and in newborn offspring the maximum level was 5.98 mmol/L at lambing and the minimum level was 3.80 mmol/L at 24 hours after lambing. Şahal et al (1994) reported the lactate concentration in newborn lambs as 4.84 mmol/L. A study conducted by Peternelli-Silva et al. (2013) on 18 sheep (9 Suffolk and 9 hybrid sheep) and 20 healthy newborn lambs, average lactate level in newborn lambs was 3 mmol/L. Kerslake et al. (2010) reported postnatal plasma lactate concentration in single, twin and triplet lambs as 7.9±1.15, 7.7±0.54 and 7.6±0.57 mmol / L, respectively. The same researcher pointed out that glucose concentrations were lower in twin and triplet groups.

In the present study, lactate values in newborn lambs were obtained between 6.05 and 14.44 mmol / L . The highest lactate concentrations were determined in multiple born offspring groups (triplets, quintuplets). In some studies, lactate levels in newborn lambs were lower than our findings (Şahal et al. 1994; Peternelli-Silva et al. 2013; Partidge, 2017), and in others, they were higher (Kerslake, 2010; Aridos et al. 2017). The reason for the differences may be the effect of breed or the method of analysis. However, in all of these studies, lactate values in newborn lambs are higher than sheep's reference values, and this means hyperlactatemia develops mostly in newborns. It was concluded in the present study that the sudden death pattern in 4 lambs after birth whose birth lactate concentrations were very high, was associated with the inability to compensate the lactic acidosis. This condition has been interpreted as the cause of high lactate concentrations in multiple offspring with low birth weight, especially in quintuplets.

In the present study, blood glucose levels were examined and no statistical difference was found between offspring groups, but there was a tendency to be lower in single offspring than the multiple born offspring. Similarly, Hannock et al (2012) determined the birth weight and blood sugar levels in twin offspring to be lower than single offspring. This study determined that birth weights of multiple born lambs were lower than single born offspring, and the lowest birth weights were detected in the quintuplets group. This is due to the mother's inability to feed the fetus because there is more than one offspring in the uterus. As a matter of fact, in multi-fetus pregnancies, decrease of blood glucose and increase of plasma free fatty acids and beta-hydroxybutyrate levels are indicative of this inadequacy (Hancnock et al. 2012). The same researchers reported that the blood glucose levels of twin-born offspring were lower than those of single offspring.

Vannucchi et al. (2012) reported in a clinical and hemogasometric study that blood glucose levels of new born Santa Ines lambs were 30 mg/dL at and after the birth. Peternelli-Silva et al. (2018) found out that the average blood glucose of lambs was 46.86 mg / dL in 60 minutes after lambing. The same researchers also reported that glucose levels increased to normal levels in 24 hours. In the present study, blood glucose concentrations were similar with this studies, and the highest value was 53.50 ± 20.25 mg / dL in single born offspring, 34.76 ± 11.51 mg / dL in twins, 45.72 ± 30.19 mg / dL in triplets and 41.33 ± 13.45 mg / dL in quintuplets. Although there was no statistically significant difference between the groups, low glucose levels in multiple born lambs were interpreted as the mother's inability to feed the fetus sufficiently. Moreover, it can suggested that there is a correlation between high lactate concentration and low glucose.

In a study on newborn lambs, total protein levels of healthy lambs were reported to be between 5.9 g / dL and 6.3 g / dL (Nozarian et al., 2010). Lepherd et al. (2009) reported concentrations of total protein and albumin 5.7 g / dL and 3.4 g / dL in lambs respectively. Bornez et al. (2009) determined total protein levels in newborn lambs between 5.7 and 6.4 g / dL. Total protein and albumin levels obtained in the present study are consistent with the studies conducted on lambs previously. However, total protein and albumin levels were found to be higher in single-born offspring than multi-born offspring, and the lowest level was found in the quintuple offspring group. The difference between groups was statistically significant. In the literature, no research was encountered comparing the total protein and albumin levels of multiple offspring and single offspring. The results of the present study can be interpreted that low total protein and albumin levels of the offspring, especially of quintuplets, could be a consequence of low birth weights and insufficient placental nutrition.

In this study, no statistically significant difference was found between single and multiple new born offspring in terms of plasma BUN and urea levels. The average BUN levels in new born lambs were found to be nonsignificant between groups and the min and max concentrations were 13.76 ± 2.80 and 15.88 ± 0.73 mg / dL. Vanucchi et al (2012) found out 14.60 ± 3.54 mg / dL BUN level in hour zero and 16.20 ± 3.23 mg / dL in the 60th minute in their research on newborn lambs (bu cümle anlaşılmıyor !!!!!), and they reported that this BUN level was within the reference ranges which is 10-26 mg / dL and also confirmed by Lester et al. (2009). This study determined that triglyceride concentration in newborn quintuplets lambs was statistically higher than that of the other groups. Atakişi et al (2013) interpreted that the increasing concentration of triglycerides after birth was due to the usage and conversion of energy sources for the increased energy requirements. Indeed, low glucose levels are an indicator of this fact. In this study, it is believed that the high triglyceride concentration in quintuplets was due to the efforts of lambs with low birth weight to produce more energy in order to provide thermoregulation and metabolic hemostasis. In the present study, no difference was found between groups in terms of cholesterol concentrations. Since cholesterol is not transmitted to the offspring through the placenta, the offspring receive large molecules (such as immunoglobulin, fat-soluble vitamins, and cholesterol) for growing up through the colostrum and mother's milk. Therefore, blood cholesterol levels of mother and postnatal lambs are related to lipid levels in the feed taken. The lower level of post-natal cholesterol level than that of a healthy adult was interpreted as normal. There was no significant difference between the groups in terms of concentrations of AST and ALT enzymes, which are indicative for liver functions, and it was interpreted that these lambs had a similar state of liver functions immediately after the birth.

Lactic acidosis occurs when plasma lactate level exceeds 4-5 mmol / L (optimum range: 0.5-1.5 mmol / L). However, previous studies showed that birth and especially multiple lambs births may cause lactic acidosis (Vannucchi et al., 2012; Silva et al., 2013). Six out of the 41 studied newborn lambs died, and the lactate levels of 4 dead lambs (% 66.6) were determined to be 15 mmol / L at lambing. Based on the present results, low birth weight and high lactate concentration can be an important indicator for offspring survival.

When the number of offspring in a litter increases, the lactate levels is also increasing, and this creates a risk for lactic acido-

sis. This situation applies to all single, twin, triplet and quintuplet births of Chios sheep. Therefore, early intervention and treatment of lactic acidosis in newborn lambs are essential. Based on the data of the present study, clinical and laboratory monitoring of the offspring of this breed which is characterised by high fertility and multiple offspring will be important to increase the survival rate.

The possibility to run lactate analysis in the field and on farms is increasing because of the development of fast test kits. The development of practical individual patient-side devices areeasy-to-use and provide cheap analysis. In light of the study, lactate analysis is considered an usefull test in postpartum monitoring. The routine application of lactate analysis as an important marker in controlling and preventing newborn lamb deaths due to lactic asidosis, which poses a major problem in herd and farm management. It is thought that the result of the present study will economically be beneficial for the farms if they can be implemented into practice and used effectively in farm management.

Conflict of interest: The authors declare that they have no conflict of interest.

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Research Article

Evaluation of Clinical and Haematological Findings of Mono- and Co-Infection with Hepatozoon canis in Dogs

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ABSTRACT

Hepatozoon canis (*H.canis*) which is transmitted by *Rhipicephalus sanguineus*, is the most common cause of canine hepatozoonosis in Europe, Asia, Africa and Latin America. The objective of this study was to evaluate risk factors and clinical signs in 32 *H. canis* infected dogs (16 dogs with *H. canis* mono-infection, 15 dogs with *H. canis and Ehrlichia canis* co-infection and one dog with *H. canis and Babesia canis co-infection)* and compare the haematological findings between the groups. For this purpose, signalment, anti-parasitic drug application, living environment, the presence of tick and general condition of the dogs were noticed, clinical and haematological variables were determined. Nine out of 16 (56.25%) mono infected dogs and ten out of 16 (62.5%) co-infected dogs were living in the house. Nine out of 16 (56.25%) mono-infected and 12 out of 16 (75%) co-infected dogs were unfrequently treated with anti-parasitic drugs. Consequently, 4 out of 16 (25%) mono- and 9 out of 16 (56.25%) co-infected dogs had tick infestation on clinical examination. In both groups, the most common clinical signs included Inappatence (93,75%), lymphadenopathy (59,38%), tachypnoea (53,13%) and fever (50%). Thrombocytopenia (84,38%) and anaemia (56,25%) were the most frequent haematological abnormalities. There were no significant differences in the haematological variables between the groups. In conclusion, H. canis mono and co-infection should be considered in the diagnosis of the dogs presenting inappetence, lymphadenopathy, tachypnea, fever, thrombocytopenia and anaemia with previous tick exposure.

Keywords: H. canis, mono- and co-infection, clinical and haematological findings

Hepatozoon canis ile Mono ve Ko-Enfeksiyonlu Köpeklerde Klinik ve Hematolojik Bulgularının Değerlendirilmesi

ÖZET

Rhipicephalus sanguineus tarafından bulaştırılan *Hepatozoon canis (H. canis),* Avrupa, Asya, Afrika ve Latin Amerika'daki köpeklerde hepatozoonosis'in en yaygın nedenidir. Bu çalışmada, *H. canis* ile enfekte 32 köpekte (*H. Canis* ile mono enfekte 16 köpek, *H. canis* ve *Ehrlichia canis* ile ko-enfekte 15 köpek ve *H. Canis* ve *Babesia canis* ile ko-enfekte bir köpek) risk faktörleri ve klinik bulguların değerlendirilmesi ve gruplar arasında hematolojik bulguların karşılaştırılması amaçlanmaktadır. Bu amaçla, köpeklerin eşkâlleri, anti-paraziter ilaç uygulamaları, yaşadıkları ortam, kene varlığı ve genel durumları not edilmiş, klinik ve hematolojik değişkenler belirlenmiştir. Mono-enfekte 16 köpekten 9'u (%56,25) ve ko-enfekte 16 köpekten 10'u (% 62,5) ev ortamında yaşıyordu. Mono-enfekte 16 köpekten dokuzu (%56.25) ve ko-enfekte 16 köpekten 12'sinin (%75) anti-paraziter ilaç uygulamaları düzensizdi ve buna bağlı olarak, 16 mono enfekte köpeğin 4'ünde (% 25) ve 16 ko-enfekte köpeğin 9'unda (% 56.25) klinik muayene sırasunda kene görüldü. Her iki grupta da en sık görülen klinik bulgular iştahsızlık (% 93,75), lenfadenopati (% 59,38), taşipne (% 53,13) ve ateş (% 50) olarak tespit edildi. Trombositopeni (% 84,38) ve anemi (% 56,25) en yaygın görülen hematolojik anormalliklerdi ve gruplar arasında hematolojik değişkenler açısından anlamlı bir fark bulunmadı. Sonuç olarak, daha önce keneye maruz kalmış, iştahsızlık, lenfadenopati, taşipne, ateş, trombositopeni ve anemi görülen köpeklerin tanısında *H. canis* mono ve koenfeksiyonlarının da dikkate alınması gerektiği kanısına varıldı..

Anahtar Kelimeler: H. canis, mono- ve ko-enfeksiyon, klinik ve hematolojik bulgular

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Introduction

Hepatozoon species are apicomplexan parasites from the family of Hepatozoidae and are phylogenetically closely related to the piroplasms and haemosporinids (Ivanov & Tsachev, 2008; Baneth, 2011; Aydin et al., 2015) Two *Hepatozoon* species have been identified in dogs: *Hepatozoon Canis* (*H. canis*) and *Hepatozoon americanum* (*H. americanum*) (Gavazza et al., 2003; Little et al., 2009; Baneth, 2011; Aydin et al., 2015; Senthil et al., 2015). While *H. canis* is mainly transmitted by *Rhipicephalus sanguineus*, *H. americanum* is transmitted by *Amblyoma maculatum* (Baneth, 2011; Paşa et al., 2011; Aktas et al., 2015). *H. canis* is the most common species associated with canine hepatozoonosis in Europe, Asia, Africa and Latin America. (Baneth & Vincent-Johnson, 2005; Karagenc et al., 2006; Littleet al., 2009; Baneth, 2011; Aktas 2014; Kaewkong et al., 2014; Aydin et al., 2015). Tüzdil (1933) was firstly described canine hepatozoonosis in Turkey, and then the small number of epidemiological surveys in the different region of Turkey was published (Voyvoda et al., 2004; Karagenc et al., 2006; Pasa et al., 2009; Aktas et al., 2013; Aktas et al., 2015; Aydin et al., 2015). In these studies, the prevalence of canine hepatozoonosis caused by *H. canis* ranged from 3.61 % to 36.8 % by the different methods.

Hepatozoonozis in dogs may be asymptomatic or display a severe, life-threatening illness, with fever, lethargy, weight loss, lymphadenomegaly, and anaemia in varying combinations, depending on the level of parasitaemia (Baneth et al., 1995, Baneth and Weigler 1997, Harrus et al., 1997; Moreira et al., 2003). The severe clinical signs are characteristic for high parasitaemia reaching 100% and often, is associated with marked leukocytosis (up to150,000 /µl) whereas the low parasitaemia with gamonts in less than 5% of neutrophils is generally related to asymptomatic or mild illness (Baneth et al., 1995, 1997). Furthermore, co-infections with other vector-borne pathogens (VBPs) or intrinsic factors specific to the host (age, breed, physical condition, immune status, or stress) may contribute to a more severe expression of the disease by impairing the host immune responses (Baneth et al., 1998, 2001). In this regard, co-infections of *H. canis* with *E. canis* (Baneth and Weigler, 1997), *B. canis* (Cardoso et al., 2010) and *L. İnfantum* (Rioux et al., 1964) are described. In endemic areas, canine vector-borne disease-causing pathogens may infect the same dog with three (*H. canis, Babesia spp., E. canis*) [Karagenc et al., 2006; Sasanelli et al., 2009] or even four agents (*H. canis, Babesia spp., E. canis, L. infantum*) [Otranto et al., 2010]. In this content, *H.canis* and *E. canis* are two of the most common and widely distributed canine haemopatogens. Anaemia and leucocytosis with neutrophilia are the most common blood abnormalities in dogs that have been naturally or experimentally infected with *H. canis* (M'ghirbi et al., 2009).

Veterinarians easily misdiagnose *hepatozoon canis* infection as general symptoms are similar to those seen in other tick-borne diseases such as ehrlichiosis and babesiosis (McCully et al., 1975; Murata et al., 1991, Spolidorio et al., 2011). Additionally, scarce information is available regarding the implication of co-infections with other vector-borne pathogens. The purposes of the study were therefore to evaluate risk factors and clinico-haematological findings in IFAT-confirmed naturally H. *canis*-infected dogs around the Aegean Region, Turkey, and to assess the potential impact of co-infections with other vector-borne pathogens.

Table 1. Breeds distribution of *H.canis* mono- and co-infected dogs

Total		Mono-infected Dogs		Co-infected Dogs	
Breed	**n**	**Breed**	**n**	**Breed**	**n**
Crossbreed	8	Crossbreed	4	Crossbreed	4
Terrier	5	German Shepherd	2	Terrier	4
Golden Retriever	3	Boxer	2	Golden Retriever	2
German Shepherd	3	Terrier	1	German Shepherd	1
Boxer	2	Golden Retriever	1	Cocker Spaniel	1
Cocker Spaniel	2	Cocker Spaniel	1	Doberman	1
Pekinese	1	Bull Terrier	1	Kangal	1
Bull Terrier	1	Basset Hound	1	Pekinese	1
Basset Hound	1	Rottweiler	1	Siberian husky	1
Rottweiler	1	Dogo Argentino	1		
Dogo Argentino	1	King Charles	1		
King Charles	1				
Kangal	1				
Siberian husky	1				
Doberman	1				

Materials And Methods

The investigation was carried out on 32 *H. canis* infected dogs of both sexes, different breeds and ages. All of the animals were from the Aegean region of Turkey (Aydın, İzmir, Denizli,

Table 2. Risk factors of *H.canis* mono- and co-infected dogs

Risk factors	Total	Mono-infected	Co-infected
Age			
< 1	1/32 (3.13 %)	0/16 (0%)	1/16 (6.25%)
≥ 1	31/32 (96.88 %)	16/16 (100%)	15/16 (93.75%)
Sex			
Male	17/32 (53.13 %)	9/16 (56.25%)	8/16 (50%)
Female	15/32 (46.88 %)	7/16 (43.75%)	8/16 (50%)
Residing conditions			
House	19/32 (59.38 %)	9/16 (56.25%)	10/16 (62.50%)
Garden	13/32 (40.63 %)	7/16 (43.75%)	6/16 (37.5%)
Anti-parasitic Application			
Frequently	11/32 (34.38%)	7/16 (43.75%)	4/16 (25%)
Infrequently	21/32 (65.63%)	9/16 (56.25%)	12/16 (75%)
Presence of tick			
Existent	13/32 (43.75%)	4/16 (25%)	9/16 (62.5%)
Nonexistent	18/32 (56.25%)	12/16 (75%)	7/16 (43.75%)

Muğla, Manisa) and all dogs were owned. The dogs were admitted to the Department of İnternal Medicine, Faculty of Veterinary Medicine, the University of Adnan Menderes for vaccination, clinical examination and therapy applications. As being informed by the owner's age, breed, sex, anti-parasitic drug application, residing conditions, presence of tick and general condition of the dogs were recorded. All animals were examined for general clinical investigation and all data were recorded.

Blood samples for haematological, serological and molecular analysis were obtained by cephalic venipuncture into tubes with and without anticoagulant. Anti-coagulated bloods were analysed shortly after collection for haematological analysis. Haematological analysis (erythrocyte count, haemoglobin concentration, haematocrit, leucocyte and platelet count) were performed using the Abacus Junior Vet haematology cell counter (Diatron MI Ltd, Hungary).

Serology of *H. canis* was performed by use of the indirect fluorescent antibody test (IFAT) (Shkap et al., 1994; Baneth et al., 1998). Briefly sera were diluted at 1:32 as the cut off titre for IgG seropositivity determined previously (Shkap et al., 1994, Baneth et al., 1998). Rabbit-antidog IgG fluorescein conjugate (Sigma–Aldrich Biotechnology LP) was used at 1.100 dilution.

IFAT was used to detect *E. canis* IgG antibodies. The latter technique was applied according to the manufacturer's recommendations (VMRD®, Inc.). Sera were diluted at 1:100 in saline solution and the used conjugate was a rabbit IgG anti-dog IgG, diluted in 0.01% concentrated Evans Blue (Sigma E0133) PBS according to the manufacturer's recommendations (Karagenç et al., 2005)

The other laboratory procedures included the diagnosis of *Babesia canis* with PCR (Kırlı, 2006), serological screening for *Leishmania infantum* (*L. infantum*) by immunofluorescent antibody test (Abranches et al., 1991).

Statistical analysis was performed using SPSS version 19.0 for Windows (SPSS, Armonk, NY: IBM Corp). Independent-samples T-test was used to compare haematology parameters between mono- and co-infected dogs. Chi-squared tests (x^2) were conducted to examine whether the dogs' breed, sex, age, residing conditions, ant parasitic application and presence of tick were associated with mono- and co-infected dogs. $P < 0.05$ was considered significant. Results were given as mean ± standard deviation.

Results

Out of 32 dogs, 16 dogs were mono-infected and 16 dogs were co-infected (co-infection of 15 dogs with *H. canis* and *E. canis* and one dog with *H. canis* and *B. canis*).

The most commonly represented breed was crossbreed (n=8), followed by Terrier (n=5) and Golden Retriever (n =3) (Table 1). Dogs were evaluated against risk factors such as age, sex, residing conditions, tick presence, and anti-parasitic applications (Table 2).

Seventeen dogs (53.13%) were male and 15 dogs (46.87%) were female. Only one dog was smaller than 1-year-old and it was also co-infected with *B. canis*. Nine (56.25%) mono infected dogs and 10 (62.5%) co-infected dogs were living in the house. Nine out of 16 (56.25%) mono-infected and 12 out of

Table 3. Clinical signs *of H.canis* mono- and co-infected dogs

Clinical Signs	Total	Mono-infected	Co-infected
Inappetence	30/32 (93.75%)	15/16 (93.75%)	15/16 (93.75%)
Lymphadenopathy	19/32 (59.38%)	9/16 (56.25%)	10/16 (62.5%)
Tachypnoea	17/32 (53.13%)	10/16 (62.50%)	7/16 (43.75%)
Fever	16/32 (50%)	9/16 (56.25%)	7/16 (43.75%)
Tachyarythmia	10/32 (31.25%)	10/16 (62.50%)	7/16 (43.75%)
Pale mucose membranes	9/32 (28.13%)	7/16 (43.7%)	2/16 12.50(%)
Arthritis	6/32 (18.75%)	2/16 (12.5%)	4/16 (25%)
Eye lesions	5/32 (15.63%)	3/16 (18.75%)	2/16 (12.50%)
Skin lesions	4/32 (12.5%)	3/16 (18.75%)	1/16 (6.25%)
Haemorrhage	3/32 (9.38%)	2/16 (12.5%)	1/16 (6.25%)
Neurological signs	1/32 (3.13%)	1/16 (6.25%)	0/16 (0%)
Haematuria	1/32 (3.13%)	0/16 (0%)	1/16 (6.25%)

16 (75%) co-infected dogs were unfrequently treated with anti-parasitic drugs. Consequently, 4 (25%) mono- and 9 (62.50%) co-infected dogs had tick infestation on clinical examination (Table 2).

The data about clinical signs are summarised in Table 3. In both groups, the most common clinical signs included inappetence (93.75%), lymphadenopathy (59.38%), tachypnea (53.13%), fever (50%), tachyarrhythmia (31.25%), pale mucose membranes (28.13%). Other findings were observed arthritis, eye lesions, skin lesions, haemorrhage, neurological signs and haematuria.

Haematological variables and Haematological abnormalities of *H. canis* mono- and co-infected dogs are shown in Table 4 and Table 5, respectively. Thrombocytopenia (84.38%) and anaemia (56.25%) were the most frequent haematological abnormalities in both of the groups and there were no significant differences in the haematological variables between the groups ($p>0.05$). Other haematological findings were leucocytosis (25%), leukopenia (25%) and pancytopenia (21.88%).

Discussion

Hepatozoon infection in the dog, caused by *H. canis* is widely spread in Europe, Asia, Africa and Latin America (Ivanov & Tsachev, 2008; Baneth 2011) and presence of this disease has been reported in various regions of Turkey (Voyvoda et al. 2004; Karagenç et al. 2006; Paşa et al. 2009; Aktaş et al., 2013; Aktaş et al., 2015; Aydin et al., 2015).

Habitat, environmental conditions and epidemiological factors, such as the presence of the vector are essential factors in the development of *H. canis* infection (Craig, 1990; Gavazza et al. 2003; Baneth & Vincent- Johnson, 2005; Paşa et al., 2011). *Rhipicephalus sanguineus* is widely distributed in the world, but it is mainly in tropical and subtropical regions and also well adapted to the indoor environment where owned dogs are kept (Uspensku & Ioffe-Uspensky, 2002; Dantas-Torres, 2010; M Ansari-Mood et al., 2015). In this study 21 dogs (% 65.625) were infrequently treated with ectoparasitic drugs, and as a result of this situation, 43.75 % of dogs infected ticks. Co-infected dogs (56.50%) were exposed to ticks more than mono-infected dogs (25%). Co-infection with other hematozoa can be attributed to the presence of the common tick vector, *Rhipicephalus sanguineus,* which is also a transmitter of *E. canis* and *B. canis* (Gondim et al., 1998; O'Dwyer et al., 2001; Mundim et al., 2008). Several studies indicate that a small percentage of ticks are responsible for harbouring multiple pathogens and successfully transmitting all pathogens to host (Kaur et al., 2011, Chhabra et al., 2013).

Although male dogs were slightly more affected than females by canine hepatozoonosis, gender differences were not significant in the present study. This situation is consistent with previous reports of no correlation of gender with the presence of infection (Gomes et al., 2010, Aktaş et al., 2015). Furthermore, no significant difference was found between mono- and co-infected dogs concerning gender in our study.

Clinical findings of dogs with *H. canis* vary from mild to severe signs, depending on the parasitemia and the dog immune status. Dogs with a low parasitemia may be normal or show only mild clinical signs, whereas more severe clinical signs including fever, lethargy and emaciation are noted with high parasitemia. Most frequently observed clinical signs in both groups were inappetence, lymphadenopathy, tachypnea, fever, tachyarrhythmia, pale mucose membranes (Table 3). These findings showed similarity to other researchers (Paşa et al., 2009; Chhabra et al., 2013). Some investigators reported that *H. canis* to be non-pathogenic and attributed clinical signs of infected dogs to other causes such as ehrlichiosis, leishmaniasis or babesiosis, (McCully et al., 1975; Banrth et al., 2003; Gavazza et al., 2003; Mylonakis et al., 2004; Voyvoda et al., 2004; Paşa et al., 2011). In contrast these report, clinical findings in 16 mono infected dogs in this study were associated with primary H. canis infection. None of the signs was attributable to concurrent

Table 4. Haematological variables of *H. canis* mono- and co-infected dogs([a] Raskin & Wardrop, 2010)

Parameters	Mono	Dual	Referances Value
WBC (×10³ cells/µL)	4.73-24.40 13,00±6.49	4.48-28.67 12,04±7.07	6-17[a]
RBC (×10⁶ cells/µL)	1.40-10.90 5,69±2,37	4.25-7.89 5,56±1,02	5.5-8.5[a]
Hct (%)	10.60-59.30 36,72±14,24	27.70-45.76 35,82±6,31	37-55[a]
PLT (x10³/µL)	0-322 121.63±98.67	0-366 108.88±107.94	200-500[a]

Table 5. Haematological abnormalities of *H. canis* mono- and co-infected dogs

Haematological results	Total	Mono-infected	Co-infected
Thrombocytopenia	27/32 (84.38%)	14/16 (87.5%)	13/16 (81.25%)
Anaemia	18/32 (56.25%)	8/16 (50%)	10/16 (62.5%)
Leucocytosis	8/32 (25%)	5/16 (31.25%)	3/16 (18.75%)
Leukopenia	8/32 (25%)	4/16 (25%)	4/16 (25%)
Pancytopenia	7/32 (21.88%)	3/16 (18.8%)	3/16 (18.75%)

disease states, and we were observed similar clinical signs in both groups.

The most common haematological abnormalities was thrombocytopenia in both groups (Table 5). In co-infected dogs, thrombocytopenia can be caused by *E. canis* infection which thrombocytopenia is most common hematologic findings in each stage of the disease (Tuna & Ulutaş, 2009; Carlos, et al., 2011; Maazi et al., 2014). Thrombocytopenia that is observed in *E. canis* infection may be due to destruction and consumption of platelets, increased hepatic or splenic platelet sequestration, decreased platelet production following bone marrow hypoplasia and production of antiplatelet antibodies (Woody & Hoskins, 1991; Gaunt et al., 2010; Maazi et al., 2014) Mechanisms of thrombocytopenia in *H. canis* mono-infected dogs are not well understood, but it may be the result of general causes of thrombocytopenia. There was no significant difference found between mono- and co-infected dogs concerning thrombocyte count in current study. Some authors report that common haematological abnormality in *H. canis* infected dogs with or without concurrent infection; have been anaemia (Baneth &Weigler, 1997; Gondim et al., 1998; Kontos and Koutinas, 1991; O'Dwyer et al., 2006, Mundim et al., 2008; Marchetti et al., 2009, Baneth, 2011, O'Dwyer, 2011). Similar to these reports, anaemia also was common hematologic findings in our study. The leucocyte count is usually normal or increased in *H. canis*-infected dogs (Gaunt et al., 1983; Baneth, 2006, Miyama et al., 2011). The alterations in leukocyte count may be due to the parasite's invasion and multiplication of the parasite in animal's organ, leading to an inflammatory response exacerbated by secondary bacterial infections. Gaunt et al. (1983) reported that anaemia and neutrophilia are probably secondary to necrosis and inflammation of the spleen, lymph nodes, liver and lungs.

These results may provide important information about risk factors clinical and haematological abnormalities in *H.canis* mono- and co-infected Dogs. *H. canis* infection should be considered in tick existence and/or a history of tick infestation. In dogs with thrombocytopenia and anaemia, *H. canis* infection should also be taken into consideration.

Conflict of interest

The authors declare that they have no competing interests.

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Case Report

Clinical Features and Surgical Outcomes of Suture Granulomas Following Ovariohysterectomy in Two Dogs

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ABSTRACT

Granuloma formation following ovariohysterectomy (OVH) can be caused by ligatures of nonabsorbable suture material and poor aseptic technique. To describe the clinical characterization of granulomas following OVH utilizing silk suture, two spayed bitches were reported. The variable clinical findings, ultrasonographic diagnosis and effectiveness of surgical therapy for treatment are discussed. Foreign body granulomas are important clinically cause of misdiagnosis tumours and stimulating new tumours. Practitioners should always consider elective OVH with ligation by nonabsorbable suture, however, often require surgical correction.

Keywords: Ovariohysterectomy, granuloma, bitch.

İki Köpekte Ovaryohisterektomi Sonrası Oluşan Dikiş Granulomalarının Klinik Özellikleri Ve Cerrahi Sağıtım Sonuçları

ÖZET

Ovaryohisterektomi (OVH) sonrası şekillenen granulomalar emilmeyen dikiş materyali içeren ligatürler ve yetersiz aseptik koşullar nedeniyle oluşmaktadır. Bu sunumda, iki köpekte OVH sırasında ligasyon için ipek iplik kullanımı sonrası gelişen yabancı cisim granulomalarının klinik, ultrasonografik ve postoperatif bulguları sunuldu. Farklı klinik bulguları, ultrasonografik tanısı ve cerrahi yaklaşımın tedavideki etkinliği verildi. Yabancı cisim granulomaları hatalı tümör tanısı ve olası yeni tümörlerin tetiklenmesi açısından klinik öneme sahiptir. Klinisyenler emilmeyen dikişlerle ligasyon uygulanan OVH operasyonlarında bazı olguların ilerleyen süreçte yaşamsal önem taşıyan sorunlara yol açabileceğini ve yeni bir cerrahi yaklaşımın gerekebileceğini göz önünde tutulmalıdır.

Anahtar kelimeler: Ovaryohisterektomi, granuloma, köpek.

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Introduction

In canine practice, the most frequently method of pet population control is ovariectomy and ovariohysterectomy operations (Stockner, 1991; Goethem et al, 2006). Several short-term and long-term postoperative complications associated with reproductive organs were reported as stump pyometra, ovarian remnant syndrome, adhesions of genital tract to other intra-abdominal organs and granulomas (Spackman et al, 1984; Wallace et al, 1991; Stone, 2003; Goethem et al, 2006). Uterine and ovarian stump granulomas are usually classified as major and short-term complications. The most common cause of granulomas was a foreign body reaction to multifilament nonabsorbable suture material, surgical sponge, poor aseptic technique, or excessive residual devitalized tissue at a previous neutering procedure in female dogs (Spackman, 1984; Werner et al, 1992; Mai et al, 2001, Goethem et al, 2006; Boza et al 2010). If a retained surgical sponge or another foreign body is encapsulated by an aseptic granuloma, there may be no clinical signs other than a mass. This type of lesion is unlikely to be ever detected in veterinary patients (Merlo and Lamb, 2000). The dogs in the present study represent examples of the infected foreign-body granuloma develop after surgery, which is associated with variable clinical signs and surgical outcome.

Case Description

Case 1

A 4-year-old Terrier bitch underwent OVH for elective spaying before a year was referred to our clinic for a history of lethargy, poor appetite, and intermittent- slight haemorrhagic vaginal discharge for two months. In physical examination, her temperature and pulsation values were normally. Haematological analysis showed that her haematological parameters were normal except white blood cell (WBC) count was 22×10^9 g/dl. Transabdominal ultrasonography revealed that a mass surrounded by significant capsule and hypoechoic cavity (Figure 1A) caudal to right kidney. Besides, the remnant of right ovary was detected close to mass (Figure 1B).

Case 2

A 10-year-old mongrel spayed bitch underwent OVH for elective spaying before four months was referred to our clinic for a history of intermittent purulent vaginal discharge for three months. Her temperature, pulsation values and haematological analysis results were normally. In transabdominal ultrasonography, an irregular shape mass including cystic structure surrounded by hyperechogenic tissue was detected caudal to right kidney (Figure 2A). Besides, another spherical hyperechoic mass adjacent to bladder was detected (Figure 2B).

After the clinical and ultrasonographic examinations, it was decided to perform a ventral midline laparotomy as treatment in both cases. Dissociative anaesthesia was applied with 1.1 mg/kg xylazine hydrochloride (Alfazine®, Alfasan) and 10 mg/kg ketamine hydrochloride (Alfamine®, Alfasan) intramuscularly after premedication with 0.04 mg/kg atropine sulphate (Atropan®, Vetas) subcutaneously. The patients were placed in dorsal recumbency, and the ventral abdomen was aseptically prepared in standard fashion.

In Case 1, a mass close to right kidney and with the right ovary remnant was found and removed surgically. The encapsulated mass was adjacent to the right ovary remnant. On cut section of the mass, there were serous-purulent fluid and knots with 1/0 black silk suture observed in cavity (Figure 3A). During laparotomy, no more pathological structures related to OVH complication (stump granuloma and pyometra) were detected. Postoperatively, antibacterial therapy was applied with 400 000 IU penicillin (Iecilline®, IE Ulagay), daily for five days and an Elizabethan collar was used until removing skin sutures. After removing skin sutures, her general condition, appetite and, WBC was normal. Her owners reported that no vaginal discharge during six months.

During operation of Case 2, abdominal inspection revealed two masses. First mass was solid, irregular shape, covered with the omentum and close to right kidney. After resection of mass, cut section was performed. Three separate knots by 2/0 black silk suture observed inside (Figure 3B). The second granuloma was complicated and adjacent to bladder severely. A spheroidal mass tightly adhere to bladder. Moreover, a thick omental tissue was closely connected to this combination (Figure 4A). The embedded silk suture residues were observed in the adhesion area (Figure 4B). Cause of this omental tissue was not separated adequately, and neither ureters was visible in this complicated mass, it was decided that only its drainage but not resection of this structure. A thick infectious haemorrhagic fluid was collected after an 18-gauge needle inserted into the granuloma. Following the irrigation of peritoneal cavity using saline solution, abdominal wall was closed. For antibacterial therapy, Amoxicillin, (Amoxyphen®, EgeVet) 15mg/kg was administered intramuscularly daily for 7 days. In the first week postoperatively, vulvar oedema and vaginal discharge were decreased gradually and lasted.

Discussion

Foreign body granulomas following OVH are occurring ligation by using non-absorbable material (Werner et al, 1992; Kanazono et al 2009, Boza et al, 2010) or retained surgical sponge (Mai et al, 2001; Miller et al, 2006; Frank and Stanley, 2009; Putwain and Archer 2009; Rayner et al, 2010) in dogs. These lesions have clinical importance cause of several reversible or irreversible disorders. Kanazono et al (2009) reported that ovarian pedicle granulomas related to a reaction to the suture material can cause urethral obstruction and hydronephrosis. Frank and Stanley (2009), reported that an enterocutaneous fistula related to before surgery was detected. Moreover, it was well known the development and progression of foreign-body-induced tumours (Rayner et al, 2010) is likely accelerated by the foreign-body-associated inflammation itself and reactive oxygen species produced by the inflammatory cells (Okada, 2007; Kim et al, 2009). In this report, either the any masses in their histories or new masses progressed during six months after surgery was not detected.

The other problem of patients with granuloma is well-known that a suture or stitch granuloma can have a complex appearance and mimic a soft-tissue tumour at imaging (Carroll et al, 1996; Kise et al, 1999; Gan and Wastie, 2007). In some cases, they create imaging findings that may be confused with a malignant lesion, which can lead to unnecessary surgical treatment (Kim et al, 2009). It is important for radiologists and clinicians to be alert to this condition and to consider a history of previous surgery when evaluating images of a patient presenting with an abdominal or pelvic mass (Deschamps and Roux 2009; Gan and Wastie 2007). There are few reports of ultrasonographic appearance of foreign body granuloma in dogs (Boza et al 2010, Mai et al 2001; Merlo and Lamb 2000). In human medicine, suture granulomas are often seen as irregular masses with central necrosis (Carroll et al 1996; Kise et al 1999). But the findings in the present cases that had ultrasonography had some difference from these reports. In our cases, the lesions were likely an abscess with fluid-filled cavities surrounded by hyper echoic area but not a retained sponge including high amplitude echoes cause of calcification, gas pockets or interfaces due to the fibres of the sponge as reported earlier (Wan et al, 1992).

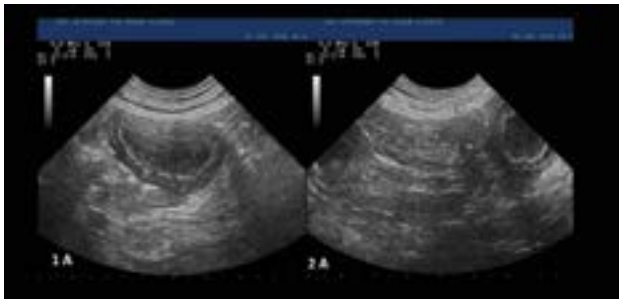

Figure 1: 1A Ultrasonographic image of abdominal mass in Case 1, 1B: Ultrasonographic image of remnant right ovarian tissue in Case 1

Figure 2: 2A Ultrasonographic image of abdominal mass in Case 2, 2B: Ultrasonographic image of pelvic mass adjacent to bladder in Case 2

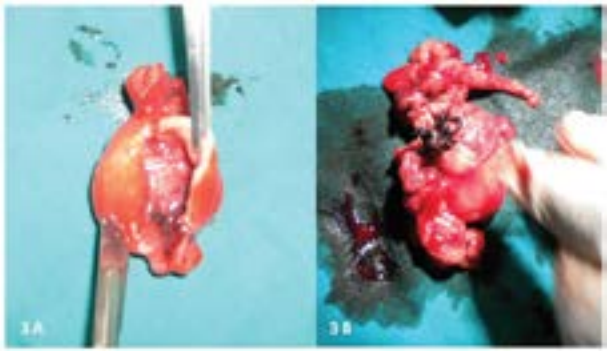

Figure 3: 3A Macroscopic appearance after cut of the abdominal mass and remnant ovarian tissue in Case 1, 3B: Macroscopic appearance the abdominal mass and remnant ovarian tissue after cut off in Case 2

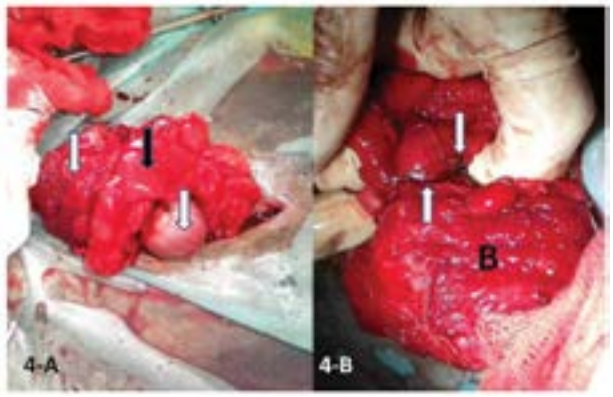

Figure 4: 4A Macroscopic appearance in operation in Case 2. Bladder (white arrow), omental tissue (black arrow) and pelvic mass (white arrow), 4B: Macroscopic appearance of the embedded silk suture (arrows), and bladder (B) in Case 2.

In this report, stitch granulomas were observed on the right ovarian pedicle in both of cases. Cause a more cranial and deeper anatomic location of the right ovary, this ligation procedure can be more difficulty. Therefore, OVH which is performed by paralumbal area on the left side can be related to this complication. Regarding to patient's size, it should be prefer to perform on the median or right paramedian area for OVH than paralumbal area to decrease of this complication.

When comparing to these granulomas in their areas, it can be specified that the mass localized in cervical ligation has more risky and cause irreversible outcome. In both of cases, although the abdominal granulomas in ovarian pedicle were removed successfully, pelvic granuloma in case 2, which was adhered to bladder, was not excised with surgical application. In addition, it was detected that the thick silk materials (2/0) were used in previously operation especially in case 2. Regarding to adhesion of other pelvic organs (bladder, intestines, colon and ureters), clinicians should be aware of this complication and care to cervical ligation by using appropriate suture materials and technique.

Conflict of interest

The authors declare that they have no competing interests.

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