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AMAÇ VE KAPSAM

Caucasian Journal of Science Dergisi 2014 yılından bu yana aralıksız olarak yayınlanan uluslararası hakemli bir dergidir. Dergi, haziran ve aralık aylarında olmak üzere yılda iki kez yayınlanmaktadır. Caucasian Journal of Science dergisinin amacı; fen bilimleri, fen eğitimi, mühendislik ve sağlık alanlarında, araştırma makaleleri, kısa bildiriler, olgu sunumları, derleme yazıları ve editöre mektuplar yayınlanmak ve bu sayede akademik çalışmalara destek sağlamaktır. Fen bilimleri, fen eğitimi, mühendislik ve sağlık alanlarında, araştırma makaleleri, kısa bildiriler, olgu sunumları, derleme yazıları ve editöre mektuplar gibi akademik çalışmaların bağımsız ve ön yargısız olarak değerlendirilmesi en önemli ilkemizdir. Bu sebeple, çift kör hakemlik sistemi uygulanmakta ve gerekli durumlarda üçüncü hakeme müracaat edilmektedir. Yazıların değerlendirilmesinde ICMJE standartları gözetilir. Yayınlanan yazıların tam metinlerine erişim ücretsizdir. Dergimizin etik politikası gereği, intihal tolere edilemez. Dergimize yayımlanmak üzere gönderilen tüm makalelerin içeriği, intihal denetimi yazılımı ile kontrol edilmektedir.

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Sığırlarda Böbrek Taşı Oluşumu ve Çeşitleri

Formation and Types of Kidney Stones in Cattle

Aysel GÜVEN[1], Olcay ÖZTÜRKLER[2], Ulviye BUNYATOVA [3]

Makalenin Alanı: Sağlık

Makale Bilgileri

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Böbrek Taşı
Sığır
Biyokimyasal inceleme

Öz

Kars' ta Kasım- Şubat 2021 ayları arasında mezbahanelerde kesimi yapılan 256 erkek ve 200 dişi sığır olmak üzere 456 sığır taş yönünden incelendi. Tüm böbreklerin 37'sinde (erkek:27, dişi:10) taş olgularına rastlandı. Taşlar, kimyasal yöntemlerle fosfat, kalsiyum, ürat, karbonat, kalsiyum okzalat, sistin, ksantin, magnezyum, amonyum ve silikat yönünden analizleri yapıldı. Erkek sığırlarda taş komposisyonları yönünde incelendiğinde %6,64 fosfat, %1,56 karbonat, %10,55 kalsiyum okzalat, %7,03 ürat, %0,78 sistin, %1,56 amonyum, %1,56 magnezyum, %0,39 ksantin bileşikleri saptanırken, dişilerde bu oranlar sırasıyla %1,50 fosfat, %4,00 kalsiyum, %3,00 kalsiyum okzalat, %0,50 ürat, %1,50 amonyum, %0,50 magnezyum bulundu, ancak öte yandan silikat, ksantin, sistin ve karbonat görülmedi.

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Abstract

In this study, 456 cattle, 256 male and 200 female, slaughtered in slaughterhouses in Kars region between November and February 2021 were examined for kidney stones. A total of 37 stones were detected in all examined kidneys (male:27, female:10). The stones obtained were chemically analyzed in terms of phosphate, calcium, urate, carbonate, calcium oxalate, cystine, xanthine, magnesium, ammonium and silicate compositions. There were achieved 6.64% phosphate, 1.56% carbonate, 10.55% calcium oxalate, 7.03% urate, 0.78% cystine, 1.56% ammonium, 1.56% magnesium and 0.39 xanthine compound in male cattle. Female kidney stone examination showed that their structure consist 1.50% phosphate, 4.00% calcium, 3.00% calcium oxalate, 0.50% urate, 1.50% ammonium and 0.50% magnesium. Interestingly that such compounds like silicate, xanthine, cystine and carbonate were not observed in female kidney stone.

1. INTRODUCTION

Agriculture and animal husbandry in the changing the world in parallel with technology and population growth; due to the decreasing food resources, it has become a sector that is increasingly sought and should be given importance. In addition, it has undertaken functions such as providing national development, providing employment to the industry and service

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sector, and correct and balanced nutrition. However, Kars, which has 3.2% of the cattle in Turkey and 39.2% of its land is covered with meadow-pasture, has not been able to provide the necessary existence for various reasons, although there is an important potential for livestock (Aral, 1996). One of these reasons is lack of care and nutrition, insufficient quality of pastureland and kidney and urinary stone formation caused by their contents. Urolithiasis is a disease of intact and castrated male ruminants (sheep, cattle, and goats) and pigs. Uroliths are solid aggregates of minerals that precipitate out of the fluid phase of urine to form one or more pebble-like stones in the urinary tract. Urinary system stone disease (USTH) is a common systemic disease and can cause both acute and chronic kidney damage. Kidney stone formation is defined as crystal nucleation, growth, aggregation and crystal retention within the renal tubular (Kok et al.,1994; Pak,1991; Çakır eta l.,2014). The formed crystal begins to nidus when appropriate conditions are met. Today, the absence of a pharmacological treatment option that can cure or prevent this disease and the ten year recurrence rate reaching 50% maket he picture even more chronic (Forman et ak.,1959; Winneir et al.,2010).

Stone formation is a phenomenon encountered in all ages and animals of all breeds. The formation of the stone begins with a nidus. Cell debris, necrotic tissues, and crystalline substances are substances that play a rol in the formation (Winneir et al.,2010). Their chemical and physical structures, sizes and shapes are different. The main inorganic compounds found in these stones are calcium carbonate, calcium phosphate, silicate, oxalate and urates At the same times, together with the pH factor, it may affect the formation of stones in the mineral structure of the diet and drinking water (Gasthuys et al.,1993; Balley et al.,1963).

The urinary system starts with the kidney and varies between the species and breeds of the animal (Makhdoomi & Gazi, 1913). Anatomically, right kidney in ruminants between 13rd-costa and 2rd-3rd the lumbar vertebrae, the left kidny is pulled into the caudala. Urine flows from the ureter into the urine. The females are also straight, and the flexura sigmioidea is folded with a long narrow urethra channel in males and is ejected by the orificium urethra external (Yüksel and Yaman, 2008; Makhdoomi and Gazi, 2013; Anjara,1967). Kidney stone formation is a complex process that includes the concentration of stone-forming ions, urine pH and flow rate, anaomical abnormalities that may cause urinary stasis, and varieuus metabolic factors (Baştuğ, 2013).

Urolithiasis affects both sexes, but urinary blockade is major problem only in males. Urolithiasis is the retention of urine subsequent to lodgment of calculi anywhere in the urinary conduct from up to urethral orifice. The disease results in heavy economic losses to the livestock industry as it is attributed the fifth most prevalent cause of death in feedlot. Obstructive urolithiasis is a serious, potentially fatal condition, most commonly causing symptoms in castrated male animals, but also occurring in breeding males.

Ruminant urolithiasis is considered primarily as a nutritional disease. The composition of urolithis, also called urinary stones or stones, varies according to tha animal lives. The most common urolihtes are calcium apatite and phosphate based stones (for example, calcium hydrogen phosphate, calcium hydrogen phosphate dihydrate, and magnesium ammonium phosphate or struvite)(Kok and Khan 1994; Breslau et al., 1988). Although the animal is given abundant concentrate feed, giveng very little roughage, not using roughages, and long feding intervals can facilitate the formation of urinery stones.

Urethral obstruction caused by uroliths is most common in show or domestic goats and lambs with a high grain, low roughage diet. Diabets high in grain, phosphorus and magnesium and law in roughage (hay or fresh grass) and calcium will increase the risk of phosphate urolite formation(Güven et al., 2003). Normally, a ruminant removes phosphorus from their body by expelling it into salva and then through feces/dung). High-grain, low-roughage diets reduce saliva formation, so excess phosphorus must be removed from the blood by the kidneys and then excreted in the urine. Diets are too high in phosphorus, urine phosphorus settles and consolidates into stone-like pellets that can be too large to pass. These uroliths icrease the risk of urinary tract infections and can cause life-threatening obstruction of the urethra. Some sheep breeds (such as Texel and Scottish Blackface) may be prone to stone formation as they tend to excrete phosphorus from the urinary tract rather than saliva (Makhodoomi and Gazi, 2013; Güven et al., 2003; Maraşlı et al., 1993). Urolithiasis affects both sexes, but urinary blockade is a majör problem only in males. The intense stone formation in males is due to obstrictions in the flexira sigmoidea (S-fold) area in the breed(Pak, 1991, Çakır et al.,2014, Krzemien et al.,2016;Singh and Rai,2014). The aim of this study is to reval the biochemical and histopathological Picture of kidney Stones, which is a regional problem in czattle, in the Kars region, where animal husbandry is made, and to contribute to studies on the precautions to be taken.

2. MATERIAL VE METOD

The kidneys of 456 cattle (256 males and 200 famales) of various breeds slaughtered in Kars province slaughterhouse were examined for stone formation. Stone samples taken from 37 cattle with stone formation were examined physically and chemically. Recordings of the appearance, weight, color and hardness of the kidneys and stone taken. Systematic stone analysis method was used in the biochemical qualitative examination of stone (Ergun, 1078; Ersoy, 1981). The sones taken according to this method are grround and turned into power. It is defined between 2-30 in the analysis using chemical soutions for each parametrers. The Stones were examined in terms of calcium, phosphate, calcium carbonate, calcium oxalate, silicate, uric acid, systine, xanthine and ammonium.

3. RESULTS

3.1. Physical findings:

In terms of from and density of kidney stones in cattle, 456 cattle kidney stones, 256 males and 200 famales, were examined. The average weight of stone samples taken from 37 cattle with stone formation was 11-2080 mg, while their size was determined as 0,2-1.1 cm. While stone samples, which were generally in irregular shapes, formed hardnesses suitable for their components, their colors were white, white gray, yellowish white, and cream, depending on the density of stone formation and kidney degeneration, and generally showed a rough outer structure (Figure.1).

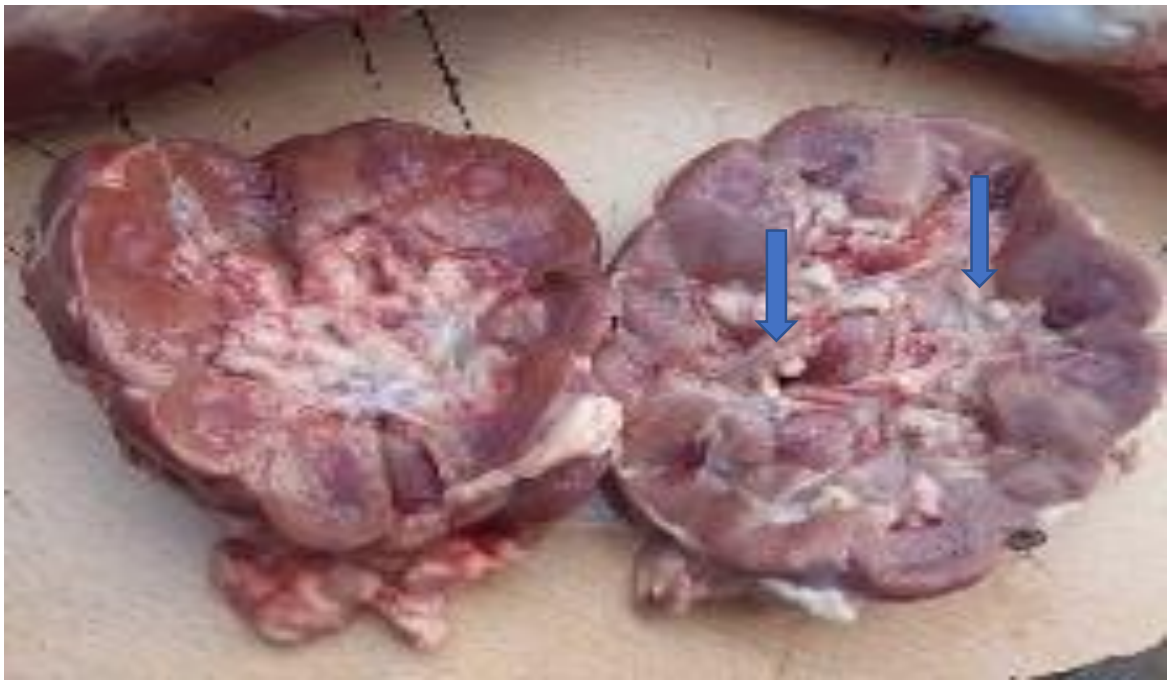

Figure:1 Cattle kidney stone formation examples

Figure:2 Cattle kidney stone formation examples

3.2. Chemical Findings:

The biochemical qualitative examination was used for the systematic stone analysis (Ersoy & Bayşu, 1981; Demir & Aral, 2009). The Stones were examined in terms of calcium, phosphate, calcium carbonate, calcium oxalate, silicate, uric acid, cystine, xanthine, and ammonium.

Table 1. Frequency of the chemical component from kidney stones

Cattle breed/ Parameters	Male	Female
Calcium	27	8
Phosphate	17	11
Calcium Carbonate	34	9
Calciumoxalate	18	7
Silicate	-	-
Urat	2	1
Xanthine	1	-
Cystine	2	-
Ammonium	4	3
Magnesium	4	1
Carbonate	3	0

Table 2. Percentage of kidney stones found (%)

Cattle breed/ Parameters	Male (n:256)	Female(n:200)
Calcium	10,55	4,00
Phosphate	6,64	5,50
Calcium Carbonate Phosphate	13,28	4,50
Calciumoxalate	7,03	3,50
Silicate	0,00	0,00
Urat	0,78	0,50
Xanthine	0,39	0,00
Cystine	0,78	0,00
Ammonium	1,56	1,50
Magnesium	1,56	0,50
Carbonate	1,17	0,00

Table 3. Percentages of chemical combinations in the constituent of kidney stone (%)

Cattle breed/ Parameters	Phosphate+carbonate	Carbonate+Calcium phosphate	Magnezium +amonium phosphate
Male(n:256)	20,00	47,00	25
Female(n:200)	11,00	19,00	15

4. DISCUSSION

Renal diseases in cattle are frequently not recognized due to the subclinical conditions. Uroluthiasis and nephrolithiasis pose major problems in terms of animal husbandry in the Worldwide and in our country. In addition to metabolic effects in the formation of uroliths, endocrinological, consititution, upper urinary tract infections, congenital alkyd malformations and feding patterns can be listed. It is known that chemicals in water and nutrients have important effects on the formation of uroliths. In addition to organic nutrients such as free fatty acids, fat cholesterol, carbohydrates and mucoproteins, organic compounds such as soric acid, sodium urate, potassium urate, cystine, carbonates, xanthine, homogenzitic acid, indigo are stated to be directly or indirectly effective in stone. Stone formation in animals are formed as a result of the combination of salts excreted in the urine with proteins of the precipitation of salts on this matrix after the development of an organic matrix, a dead cell remnant or a blood clot (Krzemien et al2016; Bauza et al., 2018). It has been reported that cases of urolithiasis are formed in the urinary system in the ureters % as it is knowen, ureter and bladder. Stones are shaped as secunder and the most important source of stone formation is the kidneys(Khan et al., 2016).

In this study, cattle were evaluated not only in terms of kidneys but also in terms of bladder and orinary tract. Analyzes of the 48 uroliths revealed that 20% were composed of carbonate+phosphate, 47% were calcium+carbonate+phosphate, 25% were magnezium+ammonium+ phosphate compound. In 1994, kidney stones were found in fattening and pasture cattle at a rate of 10,7% in beef cattle and 8% in pasture cattle in the same region (Maraşlı et al;1995). In the study conducted by Güven et al. (2003) in 807 sheep in Kars, Ardahan and Iğdır, stone cases were found in 45 of them. Compared to our study, it can be said that kidney stones are an increasing problem in animals(Güven et al,2003). In 1994, kidney stones were detected in fattening and pasture cattle at a rate of 10,7% in beef cattle and 8% in pasture cattle. In the study conducted by Güven et al., (2003) in 807 sheep in Kars, Ardahan ve Iğdır, stone cases were found in 45 of them. Compared to our study, it can be said that kidney stones are an increasing problem in animals (Maraşlı et al;1995). The other compound urolith was composed of a center of 90% calcium phosphate carbonate and 10% struvite, surrounded by a shell of 85% magnesium calcium phosphate carbonate and 15% struvite. The formation of stones in the kidneys varies in size and shape. Considering the composition of the components, single formations such as xanthine and cystine are observed,

while the concentration of phosphate in beef cattle and carbonate components in pasture cattle. There are few reports in the worldwide veterinary literature on quantitative analysis of bovine uroliths(Bauza et al,2018). In the studies conduced, it is stated that concentrate feed, environmental conditions, drugs used and vitamin D contribute significantly to stone formation especially in beef cattle. There are many studies supporting that kidney stones may cause degenerative and necrotic changes and tumoral formation in the animal's developmental, circulatory and excretory systems(Maraşlı et al;1995; Sunyeoz,2008).

There are a few reports on quantitative analysis of bovine uroliths in the world's veterinary literature. Urethral obstruction has been extensively reported in ruminant species(Gasthuys et al, 1995). An overall incidence of 5.04 percent in animals has been reported in India(Smith et al., 1989).

Incidence of urolithiasis as high as 13,4%, in Anantnag, 12,6%, Budgam 11,9%, Pulwama 6,9%, and Srinagar 10,5% in male cow calves (Makhdoomi & Gazi, 2013). Outbreaks of urolithiasis in castrated range cattle have been described in Mato Grosso do Sul and Minas Gerais without a determination of causes (Mcintosh et al.,1974). Urolithiasis have also been described in range ruminants in Australia and Turkey (Yüksel and Yaman, 2008; Maraşlı et al;1995; Güven et al, 2003; Mcintosh et al.,1974). In a study conducted to reveal the incidence and pathological findings of kidney lesions of cattle slaughtered in Elazığ slaughterhouses, kidney lesions were found in 64 (4,18 %) of the 1431 cattle (Yüksel &Yaman, 2008). In the light of all these data, as a result of kidney and urinary tract stones, factors such as pain in the animal, inability to urinate or drop by drop, discharge, deterioration of the general condition swelling will adversely affect the milk yield and body mass of the animal, so food that encourages stone formation in fattening or pasture livestock and drug use should be avoided.

Availability of Data and Materials

Datasets analyzed during the current study are available in the author on reasonable request.

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Conflict o Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors state that they have contributed to the study as 50%, 35% and 15%, in order of authorship.

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Acil Servis Çalışanlarının Adli Vaka Yönetimine Yönelik Bilgi Düzeyleri: Kars Örneği

Levels Of Knowledge Of Emergency Service Workers On Forensic Case Management: The Case Of Kars

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Makalenin Alanı: Sağlık

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Öz

Araştırma, acil servis çalışanlarının adli vaka yönetimine yönelik bilgi düzeylerinin belirlenmesi amacıyla yapılmıştır. Tanımlayıcı-kesitsel türde olan bu araştırmanın evrenini Kars ilindeki bir üniversitenin Sağlık Araştırma ve Uygulama Hastanesi ve Devlet Hastanesinin acil servislerinde çalışan sağlık personelleri (hekim, hemşire, ebe, paramedik ve tekniker) oluşturmuştur. Araştırma, katılmayı gönüllü olarak kabul eden toplam 74 sağlık personeli ile yürütülmüştür. Çalışmada sağlık personellerine Nisan-Mayıs 2022 tarihlerinde yüz yüze anket uygulanmıştır. Verilerin değerlendirilmesinde sayı yüzdelik hesaplamaları ve ki-kare önemlilik testleri kullanılmıştır. Araştırmaya katılan acil servis çalışanlarının %50'si kadın, %21.6'sı evli ve yaş ortalamaları ise 25.85±3.85'tir. Katılımcıların %52.7'si hemşire, %47.3'ü lisans mezunu, %48.6'sı acil serviste tüm alanlarda ve %54.1'i sabit olarak çalışmaktadır. Çalışanların %19.8'i adli vakalara müdahale konusunda eğitim almış olup %52.7'si adli vakalarla ara sıra karşılaştıklarını belirtmiştir. Acil servislerde adli hekim/hemşire/ebe görevlendirilmesi gerektiğini düşünen ve isteyen (%77) çalışanların %24.6'sı ise uzman kişiler görevlendirilen adli olaylarda daha az sorun yaşandığını ifade etmiştir. Katılımcıların %75.7'si adli hekim/hemşire/ebe olmayı istemediğini, %68.9'u adli hekimlik/hemşirelik/ebelik mesleğinin uzmanlık dalı isteyenlerin, %61.8'i bölümünde uzman olan kişiler tarafından adli vakalara müdahale edilmesini gerektiğini belirtmiştir. Çalışanların %61'i rol ve sorumluluklarını bilmediğini, %73'ü adli hekimlik/hemşirelik/ebelik hakkında bilgi sahibi olmadığını, %73'ü bu konu hakkında okulda ders almadığını, %85.1'i adli hekimlik/hemşirelik/ebelik ile ilgili ders verilmesine ihtiyaç duyulduğunu ve %78.4'ü Türkiye'de adli hekimlik/hemşirelik/ebelik bölümlerine ihtiyaç olduğunu düşünmektedir. İstatistiksel analize göre; acil servis çalışanlarının cinsiyetleri ile meslekleri arasında anlamlı ilişki bulunmuştur ($p<0.05$). Adli vaka ile karşılaşma sıklığı ile Türkiye'de adli hekimlere/hemşirelere/ebelere gereksinim durumu arasında, adli hekimlik/hemşirelik/ebelik dersi alma durumu ile bu konudaki bilgi durumu arasında istatistiksel olarak anlamlı fark bulunmuştur ($p<0.05$). Sonuç olarak; acil serviste çalışan sağlık personellerinin çoğu, adli vakalarla ara sıra karşılaştıklarını, adli hekim/hemşire/ebe olmayı istemediklerini ve adli hekimlik/hemşirelik/ebelik mesleğinin uzmanlık dalı olması gerektiğini düşündüğünü belirtmiştir. Çalışanların geneli adli hekimlik/hemşirelik/ebelik hakkında bilgi sahibi olmadıklarını, okulda ders almadıklarını ve bu dersin ihtiyaç olduğunu ifade etmiştir. Adli hemşirelik/ebelik hakkında çalışanlara alanında uzman olan kişiler tarafından eğitim verilmesi ve bu alanın uzmanlık dalı olması önerilmektedir.

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Abstract

The research was conducted in order to determine the level of knowledge of emergency department employees in forensic case management. It consisted of health personnel (physician, nurse, midwife, paramedic, technician) working in the emergency services of the Health Research and Application Hospital and the State Hospital of a university in Kars. The research was carried out with a total of 74 health personnel who voluntarily agreed to participate. In the study, a face-to-face questionnaire was applied to health personnel between April-May 2022. Number percentage calculations and chi-square significance tests were used in the evaluation of the data. 50% of the emergency service workers participating in the research were women, 21.6% were married, and their average age was 25.85±3.85. 52.7% of the participants are nurses, 47.3% are undergraduate graduates, 48.6% are working in all areas in the emergency department and 54.1% are working permanently. 19.8% of the employees received training on the intervention of forensic cases, 52.7% of them encountered forensic cases occasionally, 77% of them said that forensic nurse/midwife/physician should be assigned and 24.6% of the employees who requested to be assigned stated that there were fewer problems in judicial events by appointing experts. 75.7% of them did not want to be a forensic nurse/midwife/physician, 68.9% of them stated that forensic nursing/midwifery/medicine profession should be a specialty and 61.8% of those who want it to be a specialization in the profession stated that it should be intervened by people who are experts in their department. 61% of the employees do not know their roles and responsibilities,73% of them did not have knowledge about forensic nursing/midwifery/physician, 73% of them did not take lessons on this subject at school,85.1% of them stated that there is a need to give lectures on forensic nursing/midwifery/medicine and 78.4% think that there is a need for a department for forensic nurses/midwives/physicians in Turkey. According to statistical analysis; A statistically significant difference was found between the frequency of encountering a forensic case and the Education Level of Forensic Nursing / Midwifery / Medicine ($p<0.05$). A statistically significant difference was found between the frequency of encountering forensic cases and the education level of the need for forensic physicians/nurses/midwives in Turkey, and between the status of taking forensic medicine/nursing/midwifery courses and the level of knowledge on this subject ($p<0.05$). It was stated that the majority of emergency health workers encountered forensic cases occasionally, they did not want to be a forensic nurse/midwife/physician, and that forensic nursing/midwifery/physician profession should be a specialty. Most of the employees stated that they did not have knowledge about forensic nursing/midwifery/medicine, they did not take courses at school and this course was needed. It is suggested that forensic nursing/midwifery should be trained by experts in the field and this field should be a branch of expertise.

1. GİRİŞ

Adaletin idaresinde öneme sahip olan adli bilim, birçok alanla işbirliği içerisinde olan yasal sorunlara bilimsel yöntemler ile çözüm bulmayı sağlayan kritik bir bilim dalıdır (Özkara & Karaman 2015; Bell vd., 2018). Adli bilimde yer alan adli vakalar, sağlık hizmetleri sisteminde bulunan sağlık çalışanlarının karşılaştığı kaza ya da kasıtlı yaralanmalar, düşmeler, zehirlenmeler, istismar veya kötü muameleye maruz kalan hastalar ile aileler, kadın ya da çocuğa yönelik şiddet, intihar, şüpheli ölüm, tıbbi hatalar veya asılsız suçlamalarla karşılaşılan yasal sorunlar olarak ele alınmaktadır (İlçe ve vd., 2018; Ghofrani ve vd., 2020). Hastalanmaları

veya yaralanmaları sonucunda adalet ve sağlık sistemine ihtiyaç duyan sıklıkla acil servislere ilk adli muayene için başvuranlar adli vakalardır (Çelik 2021). Acil servisler, 24 saat kesintisiz sürede akut tıbbi yardım desteğine ihtiyaç duyan hasta ya da yaralı bireylerin tanılandığı, değerlendirildiği ve tedavisinin uygulandığı sağlık hizmetinin en kısa ve kaliteli şekilde verilmesini sağlayan birimlerdir (Bilir ve vd., 2015; Çelik 2021). Bu birimlerde hastanın tedavisi ve takibi yapılarak, hastanın stabil fonksiyonları sağlandıktan sonra hastada bulunan adli izler avuç içi ya da parmak izi, kan, kıl, tükürük, diş, meni ve vajinal salgılar gibi biyolojik unsurlar ya da patlayıcı ve ateşli silah yaralaması, yanıklar, elektrik çarpması ve kesici delici alet yaralaması gibi kimyasal unsurların bulunup bulunmadığı kontrol edilmelidir (Çelik 2019; Çelik 2021; Silva vd., 2022).Adli vakalarda yer alan hastaların bakımında ön safhalarda bulunan sağlık çalışanlarının yaşamı koruma ve olay yerinde meydana gelen sekelleri azaltma, mağdurda ya da olası saldırganda var olan izlerin korunmasını sağlama, adli izleri tespit etme, toplama ve koruma konusunda sorumlulukları bulunmaktadır (Silva vd., 2022). Bu durumda acil servis çalışanlarının hastaneye başvuran adli vakayı tanımlayabilme, fiziksel olarak değerlendirebilme, mevcut olan kanıtların ne olduğunu tanımlama, toplama, koruma ve kayıt altına alma gibi sorumlulukları hakkında bilgi sahibi olması gerekmektedir (Arslan & Erkan, 2016; Çelik 2019). Bu sorumlulukları yerine getirmede de acil servis çalışanlarının aldığı eğitimlerin önemli olduğu ve bu eğitimleri alan sağlık çalışanının hastanın tedavisi, haklarının korunması ve adalete katkı sağlaması açısından da daha etkin olduğu düşünülmektedir (Arslan & Erkan, 2016). Tüm bu sorumlulukları yerine getiren acil servis çalışanlarının adli vakaları bildiriminin ve ceza sorumluluğunun olduğu adaletin doğru ve hızlı bir şekilde ilerlemesi gerektiği unutulmamalıdır (Odabaş & Taşpınar, 2020). Bu çalışmada acil servis çalışanlarının adli vaka yönetimine yönelik bilgi düzeylerinin değerlendirilmesi amaçlanmıştır.

2. MATERYAL VE METOT

2.1. Araştırmanın Tipi

Bu araştırma tanımlayıcı ve kesitsel türde bir çalışmadır.

2.2. Araştırmanın Evreni ve Örneklemi

Araştırmanın evrenini, Kafkas Üniversitesi Sağlık Araştırma ve Uygulama Hastanesi ve Kars Harakani Devlet Hastanelerinin acil servislerinde çalışan sağlık çalışanı oluşturmaktadır.

Evrenden örneklem seçimine gidilmeden, araştırmaya katılmayı gönüllü olarak kabul eden 74 acil serviste çalışan sağlık personeli çalışmanın örneklemini oluşturmuştur.

2.3. Araştırmanın Veri Toplama Araçları

Bilgi Formu: Araştırmacı tarafından literatür incelemesi (Arslan & Erkan, 2016; İlçe vd., 2018) doğrultusunda hazırlanan veri toplama formunun ilk bölümünde; araştırmaya katılmayı gönüllü olarak kabul eden sağlık personellerinin tanıtıcı özelliklerine, ikinci bölümünde, meslek yaşamına yönelik (adli olgularla karşılaşma sıklığı, konusundaki bilgisi ve çalıştığı birimde adli olgularla ilgili yaşadığı bir problem varsa özelliği…vs.) toplam 30 soru yer almaktadır.

2.4. Araştırmanın Uygulanması

Veri toplama, acil servislerde, sağlık personellerinin araştırmaya katılmak için uygun oldukları zamanlarda, araştırma hakkında açıklama yapılıp, yüz yüze anket yöntemi kullanılarak Nisan-Mayıs 2022 tarihlerinde uygulanmıştır. Anketler bilgilendirilmiş onam formu imzalandıktan sonra katılımcılar tarafından ortalama 10dk.'lık süre içinde doldurulmuştur.

2.5. Verilerin Değerlendirilmesi

Veri analizleri "SPSS for Windows 22" paket programında yapılmıştır. Kadınların sosyodemografik bilgilere ait verileri tanımlayıcı analizlerle belirtilmiştir. Araştırmanın bulguları ortalama ± standart sapma veya ortanca (minimum-maksimum) olarak kategorik değişkenler ise olgu sayısı ve (%) şeklinde belirtilmiştir.Araştırma verilerinin normal dağılıp dağılmadığı kurtosis ve skewness katsayısına göre değerlendirilmiştir. Verilerin değerlendirilmesinde sayı yüzdelik hesaplamaları ve ki-kare önemlilik testleri kullanılmıştır. İstatistiksel anlamlılık düzeyi $p < 0.05$ olarak kabul edilmiştir.

2.6. Araştırmanın Etik Boyutu

Araştırmaya başlamadan önce Kafkas Üniversitesi Sağlık Bilimleri Fakültesi Girişimsel Olmayan Araştırmalar Etik Kurulu'ndan (31.03.2022 tarihli ve 81829502.903/38 sayı) ve çalışmanın yapıldığı Kafkas Üniversitesi Sağlık Araştırma Hastanesi ve Kars Harakani Devlet Hastanesinden yazılı kurum izinleri alınmıştır. Ayrıca katılımcılara çalışmanın amacı hakkında bilgi verilerek gönüllü bilgilendirilmiş onam formu onaylatılmıştır.

3. BULGULAR

Araştırmamıza katılan acil serviste çalışan sağlık profesyonellerinin %50'si kadın, %21.6'sı evli olup yaş ortalamaları (20-40 yaş arası) 25.85±3.25'tir. Sağlık profesyonellerinin %52.7'si hemşire, %13.5'i hekim, %13.5'i paramedik, %14.9'u tekniker ve %5.4'ü ebe'dir. Katılımcıların %47.3'ü lisans mezunu, %48.6'sı acil serviste tüm alanlarda ve %54.1'i sabit bir alanda çalışmakta olup %39.2'sinin acil serviste 2-5 yıl arası çalıştığı, %37.8'inin meslekte çalışma yılının 2-5 yıl arasında olduğu bulunmuştur.

Çalışanların %19.8'i adli vakalara müdahale, %17.1'i sağlık hukuku, %11.7'si adli hekimlik/hemşirelik/ebelik tıbbi teknikerlik, %10.8'i malpraktis, %9.0'u adli rapor, %2.7'si bilirkişilik hakkında eğitim aldığını belirtmiştir. Adli vakalarla % 52.7'si ara sıra karşılaştıklarını, %77'sinin acil servislerde adli hekim/hemşire/ebe'nin görevlendirilmesi gerektiğini, çalışanların %68.9'unun adli hekimlik/hemşirelik/ebelik mesleğinin uzmanlık dalı olması gerektiğini, %75.7'si adli hekim/hemşire/ebe olmayı istemediği bulunmuştur. Acil servilerde adli meslek gruplarının görevlendirilmesini isteyen çalışanların %24.6'sı uzman kişiler görevlendirilen adli olaylarda daha az sorun yaşandığını, %24.6'sı adli olan vakalara yaklaşım ve tedavi alanında bilen kişilerin değerlendirilmesi gerektiğini ve %7.7'si hekim sayısının yetersiz olduğunu belirtmiştir. Sağlık personellerinin %52.7'sinin adli hekimlik/hemşirelik/ebelik ile ilgili düşüncelerinin olmadığı, %17.6'sının uzmanlık alanı olması gerektiğini düşündüğü, %16.2'sinin adli alanlarda ve vakalarda yaklaşım alanında eğitim almış veya bilgili kişiler olması gerektiğini düşündüğü ve %5.4'ünün gereksiz bir alan şeklinde ifade ettiği bulunmuştur. Adli hekimlik/hemşirelik/ebelik meslekte uzmanlık dalı olmasını isteyenlerin %61.8'i adli vakalara bölümünde uzman olan kişiler tarafından müdahale edilmesi gerektiğini, %10.9'u acilde "Adli Alan" adında ayrı bir oda olmasının hem güvenlik için hem de diğer hastaların rahatsız olmaması için yararlı olacağını ve %9.1'i acil serviste hasta sirkülasyonu ve yoğunluğunun fazla olduğunu belirtmiştir. Adli vaka ve kanıtlara yaklaşım konusunda %63.5'inin kısmen yeterli hissettiği, %56.8'inin adli vakalarda kanıt toplama görevinin polise ait olduğunu düşündüğü bulunmuştur. Çalışanların %93.2'si akrabalarında adli tıpla ilgilenen biri olmadığını belirtmiştir.

Adli vaka dendiğinde çalışanların % 22.0'ı ceset, ölüm, cinayet kavramlarının, %16.5'i otopsi, %13.2'si adli rapor, delil ve muayenenin ve en düşük %4.4'ü polis, trafik kazası ve travmanın akla geldiğini belirtmiştir. Çalışanların %32.9'u adli tıbbın amacını bilmediklerini, %30.3'ü şüpheli ölümlerin araştırılması ve aydınlatılması gerektiğini düşündüğünü ifade

etmiştir. %15.8'i adli vakaların tıp ile izlenimi ve adaletin sağlanması şeklinde ifade etmiştir. Çalışanların %61.5'i Adli hekimliğin/hemşireliğin/ebeliğin tanımını bilmediğini, % 10.3'ü suç, ölü ya da mağdurun durumunu inceleme ve %9'u acile gelen adli vakalara bakması gereken branş şeklinde tanımlamışlardır. Çalışanların %61'i adli hekimliğin/hemşireliğin/ebeliğin rol ve sorumluluklarını bilmediğini, %15.9'u adli hekim/hemşire/ebenin yaklaşımlar hakkında süreci yönetebilme ve en düşük %1.2'si adli vakanın olduğu ortam gergin olduğundan idare etme ve sakinleştirme becerisi olmalı şeklinde belirtmiştir.

Çalışanların %73'ü adli hekimlik/hemşirelik/ebelik hakkında bilgi sahibi olmadığını, %73'ü bu konu hakkında okulda ders almadığını, %85.1'i adli hekimlik/hemşirelik/ebelik ile ilgili ders verilmesine ihtiyaç duyulduğunu, %74.3'ü bilgi kaynaklarını bilmediklerini, %16.2'si lisans eğitimde (doktorlar için adli tıp stajında) öğrendiklerini ve %81.1'i adli hekimlik/hemşirelik/ebelik hakkında eğitim verilmesi gerektiğini düşündüğünü belirtmiştir.

Çalışanların %36.5'i adli hekimlik/hemşirelik/ebelik eğitiminin ne zaman verildiğini bilmediğini, %23'ü eğitimin meslekte ve %16.2'sinin üniversitede verilmesi gerektiğini ve %31.1'i Temel mesleki eğitim sırasında dönemlik ders olarak verilmesi gerektiğini belirtmiştir. Çalışanların %55.6'sı adli hekimlerin/hemşirelerin/ebelerin sahip olması gereken özellikleri bilmediğini, %19.8'i adli hekim/hemşire/ebe kimdir sorusuna, adli vakaya yaklaşım, tedavi ve müdahaleyi bilen kişi şeklinde tanımlamıştır. Katılımcıların %33.7'si adli hekimlik/hemşirelik/ebeliğin çalışma alanlarını bilmediklerini, %32.7'si acil ile hastane , %10.9'u adli vaka/adli tıp ve en düşük %1'ı cezaevinde çalıştıklarını ifade etmiştir. Çalışanların %78.4'ü Türkiye'de adli hekimlik/hemşirelik/ebelik bölümlerine ihtiyaç olduğunu düşünmektedir.

Tablo 1. Acil Serviste Çalışan Sağlık Profesyonellerinin Tanıtıcı Özelliklerinin Cinsiyete Göre Dağılımı (n=74)

Sorular		**n**	**Cinsiyet**		**p**	**X^2**
			Kadın	**Erkek**		
Medeni durum	Evli	16	8(%50.0)	8(%50.0)	1,000	0,000
	Bekâr	58	29(%50.0)	29(%50.0)		
Meslek	Hemşire	39	23(%59.0)	16(%41.0)	**0,025**	11,129
	Ebe	4	4(%100)	0(%0)		
	Hekim	10	2(%20.0)	8(%80.0)		
	Paramedik	10	5(%50.0)	5(%50.0)		
	Tekniker (radyoloji, yaşlı bakım, anestezi, ortez-protez)	11	3(%27.3)	8(%72.7)		
	1 ay-6 ay	10	5(%50.0)	5(%50.0)		

Meslekte çalışma süresi	7 ay-1 yıl	12	5(%41.7)	7(%58.3)	0,795	1,676
	2 yıl-5 yıl	28	15(%53.6)	13(%46.4)		
	6 yıl-10yıl	20	9(%45.0)	11(%55.0)		
	11 yıl-20yıl	4	3(%75.0)	1(%25.0)		
Meslekte çalışma süre ortalaması (yıl)	2.51±1.11 (min: 1 ay, max: 20 yıl)					
Acil serviste çalışma süresi	15 gün-6 ay	19	10(%52.6)	9(%47.4)	0,809	1,598
	7 ay-1 yıl	13	8(%61.5)	5(%38.5)		
	2 yıl-5 yıl	29	14(%48.3)	15(%51.7)		
	6 yıl-10yıl	11	4(%36.4)	7(%63.6)		
	11 yıl-20yıl	2	1(%50.0)	1(%50.0)		
Acil serviste çalışma süre ortalaması (yıl)	2.94±1.09 (min: 15 gün, max: 20 yıl)					
Acil serviste çalışma Şekli	Sabit	40	22(%55.0)	18(%45.0)	0,351	0,871
	Rotasyon	34	15(%44.1)	19(%55.9)		

Tablo 1'de acil servis çalışanlarının cinsiyetleri ile meslekleri arasında anlamlı ilişki bulunmuştur (p<0,05).

Tablo 2. Acil Çalışanlarının Adli Vaka İle Karşılaşma Sıklığı ile Adli Hekim/Hemşire/Ebe Bilgilerinin Karşılaştırılması (n=74)

Sorular			**Adli Vaka ile Karşılaşma Sıklığı**		**p**	**X^2**
		n	**Her zaman**	**Ara sıra**		
Acil serviste çalışma Şekli	Sabit	40	21(%52.5)	19(%47.5)	0,331	0,945
	Rotasyon	34	14(%41.2)	20(%58.8)		
Adli hekim/hemşire/ebe görevlendirme durumu	Evet	57	24(%42.1)	33(%57.9)	0,101	2,683
	Hayır	17	11(%64.7)	6(%35.3)		
Meslekte uzmanlık dalı olma durumu	Evet	51	23 (%45.1)	28 (%54.9)	0,573	0,318
	Hayır	23	12 (%52.2)	11(%47.8)		
Adli hekim/hemşire/ebe olmayı isteme durumu	Evet	18	6(%33.3)	12(%66.7)	0,173	1,861
	Hayır	56	29(%51.8)	27(%41.2)		
Adli vakada kendini yeterli hissetme durumu	Yeterli	8	5 (%62.5)	3(%37.5)	0,501	1,383
	Kısmen	47	20 (%42.6)	27(%57.4)		
	Yetersiz	19	10(%52.6)	9(%47.4)		
Adli tıpla ilgilenen akrabası olma durumu	Evet	5	3(%60.0)	2(%40.0)	0,662	0,448
	Hayır	69	32(%46.4)	37(%53.6)		
Adli hekimlik/hemşirelik/ ebelik hakkında bilgi durumu	Evet	20	10 (%50.0)	10(%50.0)	0,777	0,080
	Hayır	54	25 (%46.3)	29(%53.7)		
Adli hekimlik/hemşirelik/ ebelik konusunda okulda ders alma durumu	Evet	20	10 (%50.0)	10(%50.0)	0,777	0,080
	Hayır	54	25 %46.3)	29(%53.7)		
	Evet	63	29 (%46.0)	34(%54.0)		

Adli hekimlik/hemşirelik/ ebelik dersi ihtiyaç durumu	Hayır	11	6 (%54.5)	5(%45.5)	0,602	0,272
Adli hekimlik/hemşirelik/ ebelik konusunda eğitim alma durumu	Evet	20	10 (%50.0)	10(%50.0)	0,777	0,080
	Hayır	54	25(%46.3)	29(%53.7)		
Adli hekimlere/hemşirelere/ ebelere Türkiye'de gereksinim durumu	Var	58	22 (%37.9)	36(%62.1)	0,002	9,441
	Yok	16	13(%81.2)	3(%18.8)		

Tablo 2'de adli hekimlere/hemşirelere/ebelere Türkiye'de gereksinim durumu ve adli vaka ile karşılaşma sıklığı arasında istatistiksel olarak anlamlı fark bulunmuştur (p<0,05).

Tablo 3. Acil Çalışanlarının Adli Hekimlik/Hemşirelik /Ebelik Hakkında Bilgi Durumu ile Adli Bilgilerinin Karşılaştırılması (n=74)

Sorular		**N**	**Adli Hekimlik/Hemşirelik/Ebelik Hakkında Bilgi Durumu**		**p**	**X^2**
			Evet	**Hayır**		
Adli vaka ve kanıtlarda yeterli hissetme durumu	Yeterli	8	4(%50.0)	4(%50.0)	0,178	3,448
	Kısmen	47	15(%31.9)	32(%68.1)		
	Yetersiz	19	3(%15.8)	16(%84.2)		
Adli hekimlik/hemşirelik / ebelik ders alma durumu	Evet	20	13 (%65.0)	7 (%35.0)		
	Hayır	54	9 (%16.7)	45 (%83.3)	**0,000**	16,320
Adli hekimlik/hemşirelik/ ebelik dersi İhtiyaç durumu	Evet	63	20(%31.7)	43(%68.3)	0,489	0,301
	Hayır	11	2 (%18.2)	9 (%81.8)		
Adli hekimlik/hemşirelik/ ebelik eğitim verilme durumu	Evet	60	18 (%30.0)	42 (%70.0)	1,000	0,597
	Hayır	14	4 (%28.6)	10 (%71.4)		

Tablo 3'te Adli hekimlik/hemşirelik/ebelik hakkında bilgi durumu ile adli hekimlik/hemşirelik/ebelik dersi alma durumu arasında fark istatiksel olarak anlamlı bulunmuştur (p<0,05).

4. TARTIŞMA

Araştırmamıza katılan sağlık personellerinin yaş ortalaması 25.85±3.25 olup, meslekte çalışma sürelerinin ortalaması 2.51±1.11 yıl ve acil serviste çalışma sürelerinin ortalaması 2.94±1.09 yıl şeklinde saptanmıştır (Tablo 1). Yapılan bir çalışmada sağlık çalışanlarının yaş ortalamasının 30.89 ±7.40 olduğu, meslekte çalışma sürelerinin 9,10±7,40 yıl ve acil serviste çalışma süresinin 5,49±5,22 yıl olduğu bulunmuştur (Çelik, 2019). Başka bir çalışmada acil servisteki sağlık çalışanlarının, yaş ortalamasının 32.4±8.3 olduğu, meslekte çalışma sürelerinin ortalaması 10.1±7.9 yıl ve acil servisteki çalışma süreleri ise ortalama 5.7±4.3 yıl olarak

belirtilmiştir (Şimşek, 2015). Bu araştırmanın sonuçlarına bakıldığında yaş ortalamaları ve acil serviste çalışma sürelerinin literatür ile benzerlik gösterdiği, meslekte çalışma sürelerinin ise yapılan çalışmalarda daha fazla olduğu belirlenmiştir. Araştırmamızda acil serviste çalışan hemşirelerin çoğunluğunun mesleki yaşamlarının meslek hayatlarının başlangıcında olduğunu belirtmektedir.

Araştırmamızın bulgularında acil sağlık çalışanlarının %50'si kadın ve %52.7'si hemşire olup acil servis çalışanlarının kadın cinsiyetleri ile meslekleri arasında anlamlı ilişki bulunmuştur ($p<0,05$) (Tablo 1). Aşçı ve ark. yaptığı çalışmada acil servislerde bulunan sağlık çalışanlarının %58.1'i kadın ve %48.9'u hemşire olduğu belirtilmiştir (Aşçı vd., 2015). Yapılan bir çalışmada %57.2'si hemşire ve %52.4'ü erkek olarak saptanmıştır (Şimşek, 2015). Diğer bir çalışmada ise acil servis çalışanlarının %66.2'si kadın bulunmuştur (Çelik, 2019). Araştırmamızın sonucuna bakıldığında literatür ile hem benzer hem de farklı sonuçlar bulunmuştur. Araştırmamızın yapıldığı hastanede kadın sağlık çalışanının fazla olması bu çalışmanın sonucunu etkilemektedir.

Araştırmamızın sonucunda sağlık çalışanlarının %78.4'ü Türkiye'de adli hekimlere/hemşirelere/ebelere ait bölüme ihtiyaç olduğunu düşünmekte olup adli hekimlere/hemşirelere/ebelere Türkiye'de gereksinim durumu ve adli vaka ile karşılaşma sıklığı arasında istatistiksel olarak anlamlı fark bulunmuştur ($p<0,05$). (Tablo 2). Yapılan çalışmada da %72.0'isi Türkiye'de adli hemşireliğe gereksinim olduğunu belirtmiştir (Şentürk & Büyükaslan, 2013). Diğer bir çalışmada sağlık çalışanlarının %52,9' u adli hemşirenin gerekli olduğunu belirtmişlerdir (Çelik, 2019). Araştırmamızın sonucu benzer olup hastanelerde adli hekimlere/hemşirelere/ebelere ait bölüm olduğunda adli vakalara ait delillerin tespit edilmesi, toplanması ve kanıtların saklanmasında sorun bulunmadan adli olay için kanıt niteliğindeki belge ve delillerin gerekli kurum ve kuruluşlara iletilmesi sağlanacaktır.

Araştırmamızda sağlık çalışanlarının adli hekimlik/hemşirelik/ebelik hakkında eğitim alma durumu incelendiğinde; %73.0'ü bu konu hakkında okulda ders almadığını, %85.1'i adli hekimlik/hemşirelik/ebelik ile ilgili ders verilmesine ihtiyaç duyulduğunu ve %81.1'i adli hekimlik/hemşirelik/ebelik hakkında eğitim verilmesi gerektiğini düşündüğünü belirtmiştir. Adli hemşirelik/ebelik hakkında bilgi durumu ile adli hekimlik/hemşirelik/ebelik ders alma durumu arasında fark istatiksel olarak anlamlı fark bulunmuştur ($p<0,05$). (Tablo 3). Alsaif ve ark tarafından yapılan hemşirelere yönelik çalışmada katılımcıların %77.0'si adli vakaları ele almak için yeterince eğitim almadıkları belirtilmiştir (Alsaif vd., 2014). Soğukbulak ve ark

tarafından yapılan çalışmada hemşirelerin %78.3'ü okul esnasında ve %87.0'si mezuniyet sonrası eğitim almadıklarını saptanmıştır (Soğukbulak vd., 2014). Linnarsson ve ark. tarafından yapılan çalışmada adli hemşirelik için özel eğitim almadıkları saptanmıştır (Linnarsson vd., 2015). Yapılan çalışmada %73.1'i adli hemşirelik dersi almadığını, %92.3'ü adli vakalara yaklaşım konusunda eğitim verilmesinin gerekli olduğu belirtmiştir (Eyüp, 2019). Diğer bir çalışma da %95'i adli hemşirelik eğitimini almadığımı ve %80'i adli hemşirelik hakkında bilgi sahibi olmadığını bildirmiştir (Arslan &Erkan, 2016). Bu durum araştırmamızın sonucunu destekler nitelikte olup hala günümüzde acil servislerde çalışan sağlık prefesyonellerinin adli vaka yönetimi hakkında bilgi yetersizliklerinin olduğunu ve bu konu hakkında çözüm üretilmesi gerektiğini ortaya çıkarmıştır.

Araştırmamızda acil servis çalışanlarının %77'si adli vakalarda adli hekim/hemşire/ebe görevlendirilmesi gerektiğini, çalışanların %68.9'u adli hekimlik/hemşirelik/ebelik mesleğinin uzmanlık dalı olması gerektiğini belirtmiştir. Yapılan bir çalışmada sağlık çalışanlarının %85.7'sinin acil serviste adli hemşireliğin uzmanlık dalı olması gerektiğini düşündüğü bulunmuştur (Bahar 2008). Alsaif ve ark tarafından Suudi Arabistan'da yapılan hemşirelere yönelik çalışmada % 80.0'i adli hemşireliğin alt uzmanlık dalı olması gerektiğini belirtmişlerdir (Alsaif vd., 2014). Araştırmanın sonuçları ile literatürü desteklemektedir. Günümüzde adli vakalara ait sorunlara çözüm sağlama açısından acil servislerde adli hekimlik/hemşirelik/ebelik mesleğinin uzmanlık dalı olması gerektiği düşünülmektedir.

4. SONUÇ VE ÖNERİLER

Acil serviste çalışan sağlık profesyonellerinin adli hekimlik/hemşirelik/ebelik hakkında yeterli bilgi sahibi olmadığı ve eğitim almadığı görülmektedir. Sağlık çalışanlarının adli hekimlik/hemşirelik/ebelik bölümünde çalışmayı istemediği ve acil serviste bu bölümün ayrı uzmanlık dalı olması gerektiğini belirtmişlerdir. Adli bölüme ülkemizde gereksinim duyulduğundan adli hekimlik/hemşirelik/ebelik dersinin üniversitelerde eğitiminin verilmesi düşünülmektedir. Acil serviste sirkülasyon fazla olduğundan sağlık çalışanları adli vakalar ile ilgilenen ayrı bir bölümün bulunması gerektiğini belirtmiştir. Ülkemizde adli vakalar acil servise başvuran olgular olduğundan sağlık profesyonellerinin bu konuda eğitim alması gerekmektedir. Delillerin tespit edilerek toplanmasında, saklanmasında ve gerekli kurum ve kuruluşlara iletilmesinde rolü bulunmaktadır. Bu rolü yerine getirmede sağlık çalışanlarının

adli hekimlik/hemşirelik/ebelik hakkında üniversitede ya da kurumda hizmetiçi programa katılarak simülasyon veya sürekli eğitim alması önerilebilir. Ayrıca acil servislerde adli vakalar ile ilgilenen birimlerin olması acil servis sirkülasyonunu azaltarak sağlık profesyonellerinin hastalara yeterli ve kaliteli bakım vermesini sağlamada da etkili olabileceği düşünülmektedir. Sonuç olarak acil servise başvuran adli hasta tipine göre yapılan girişimlerin ayrıntılı olarak araştırılması gerekmektedir.

Araştırmanın Sınırlılıkları

Bu araştırmadan elde edilen veriler, hekimlerin/hemşirelerin/ebelerin/teknikerlerin bireysel beyanları ile sınırlıdır. Kars Kafkas Üniversitesi Eğitim ve Araştırma Hastanesi'nde ve Kars Harakani Hastanesinin Acil biriminde çalışmanın yapıldığı tarihte orada görev yapan sağlık personelleri ile yürütülmesi araştırmanın sınırlılığıdır ve bu nedenle sadece bu gruba genellenebilir.

Açıklama

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Seksüel Siklus Döngülerinin Proöstrus, Metöstrus-Diöstrus Ve Anöstrus Dönemlerinde Kangal Veya Kangal Melezi Dişi Köpeklerin Uterus Dokusunda Muc1 Ve Muc16 Ekspresyonlarının Araştırılması

Investigation Of Muc1 And Muc16 Expressions In Uterus Tissue Of Kangal Or Kangal Cross-Bred Bitches In Proestrus, Metestrus-Diestrus And Anestrus Periods Of Sexual Cycles

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Öz

Bu çalışmada, seksüel siklus döngülerinin proöstrus, metoöstrus-diöstrus ve anöstrus dönemlerinde Kangal veya Kangal melezi dişi köpeklerin uterus dokusunda MUC1 ve MUC16 ekspresyonları immünohistokimyasal yöntemle incelendi. Çalışmada 3-6 yaş arası sağlıklı ve sahipli Kangal veya Kangal melezi 15 köpeğin uterus dokuları kullanıldı. Köpekler seksüel siklus dönemlerine göre proöstrus (n=5), metöstrus-diöstrus (n=3) ve anöstrus (n=7) olarak 3 gruba ayrıldı. Sonuç olarak, MUC1'in köpek uterusunda hem kornu hem de korpus uteri epitel hücrelerinde, kript epitel hücrelerinde, uterus bezi epitel hücrelerinde, stratum vaskülaris, miyometriyum ve perimetriyum tabakalarından eksprese edildiği tespit edildi. Östrus siklusları karşılaştırıldığında metöstrus-diöstrus döneminde ekspresyonun diğer dönemlere göre daha fazla arttığı saptandı. MUC16'nın ekspresyonu, metöstrus-diöstrus döneminde kornu uteri'de gözlendi. Bu çalışmada MUC1 ve MUC16'nın köpeklerin uterus dokusundan eksprese edildiği ve salınımlarının hormonlardan etkilendiği tespit edildi.

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Abstract

In this study, MUC1 and MUC16 expressions in uterus tissues of Kangal or Kangal cross-bred bitchs in proestrus, metestrus-diestrus and anestrus sexual periods were investigated by immunohistochemical method. Uterus tissues of 15 healthy and owned Kangal or Kangal cross-bred bitches aged 3-6 years were used in the study. The biches were divided into 3 groups according to sexual cycle periods as proestrus (n=5), metestrus-diestrus (n=3) and anestrus (n=7). As a result, MUC1 was found to be expressed in both cornu and corpus uteri epithelial cells, crypt epithelial cells, uterus gland epithelial cells, stratum vascularis, myometrium and perimetrium layers in uterus of bitches. When estrus cycles were compared, it was found that expression increased more in the metestrus-diestrus period compared to other periods. Expression of MUC16 was observed in the cornu uteri during the metestrus-diestrus period. In this study, MUC1 and MUC16 were determined to be expressed in the uterus tissue of bitches and their expressions were found to be affected by hormones.

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1. INTRODUCTION

The estrus cycle is characterised by morphological changes occurring in the ovary, uterus and vagina (Goldman et al., 2007). In mammals, the uterus is a complex and dynamic organ with very important functions as a habitat from implantation of the embryo during pregnancy until birth. During the sexual cycle, many structural differences are observed in the entire uterine, especially in the endometrium, with the effect of sex hormones (Dekel et al., 2010). These structural differences play a crucial role in the regulation and maintenance of implantation and pregnancy (Nikas et al., 2000).

Mucus, which has a slimy and viscous structure, is released by gland epithelial cells and goblet cells. Mucus consists of water, various salts, lipids and glycoproteins. Mucus also contains mucins (MUC) (Bansil et al., 1995). Mucins are glycoprotein aggregates consisting of high amounts of carbohydrates, released from the apical surface of epithelial cells in many systems (Rachagani et al., 2009), especially the apical surface of polarised epithelial cells in the tracheobranchial, gastrointestinal, reproductive system and eyes (Yin and Lloyd, 2001). It was first found on the apical surface of the uterus epithelium in 1980 (Mullins et al., 1980) in a study with pigs. In addition, both in vivo (Braga and Gendler, 1993) and in vitro (Pimental et al., 1996) studies reported MUC1 expression in the mouse uterus tissue.

Although the secretion of MUCs varies according to the organ, their main functions are to protect against bacteria, drugs, toxic substances, digestive enzymes and acids (Hattrup and Gendler, 2008). In addition to these functions, it also has very important functions, especially in the reproductive system, such as protection against pathogens (Argueso et al., 2003), implantation and healthy realisation and maintenance of pregnancy (Meseguer et al., 2001).

MUC1 expression in uterus tissue was first reported in human endometrium (Arklie et al., 1981). In later studies, MUC1 expression was reported in many species such as mouse (McGuckin et al., 1998), rodent (Hewetson and Chilton, 1997), pig (Stenner and Crawford, 1999) and baboon (Ilekis et al., 1997). It was reported that MUC1 played an important role in embryo implantation (Hewetson and Chilton, 1997), was affected by estrogen and progesterone hormones and these hormones increase MUC1 expression (McGuckin et al., 1998). MUC16, similar to MUC1, has protective functions against pathogens by forming a tissue barrier specific to the organ in which it is located and facilitating blastocyst attachment in pregnancy (Argueso et al., 2003).

In this study, it was aimed to investigate the release of MUC1 and MUC16, which were

known to have protective and supportive effects in the uterus tissues of Kangal or Kangal cross-bred bitches in proestrus, metestrus-diestrus and anestrus sexual cycle periods and whose expression was reported in the reproductive system of many species, by immunohistochemical method.

2. MATERIALS AND METHODS

2.1. Ethical Approval

All experiments were approved by Kafkas University Ethics Committee for animal experiments (Approval no: KAU-HADYEK/2016-009).

2.2. Animal Material

In the study, 15 healthy Kangal or Kangal cross-bred and owned bitches aged 3-6 years, which were brought to Kafkas University Faculty of Veterinary Medicine, Department of Obstetrics and Gynaecology for ovariohysterectomy (OH), were used. The bitches were routinely examined for pregnancy before the operation. Detailed clinical examinations were carried out in order to determine that the bitches that were found to be non-pregnant were healthy and OH was performed after fasting for at least 12 hours before the operation.

Vaginal cytology was performed to determine the sexual cycle periods of the bitches before the operation. Cytological samples were taken from the vagina tissue with the help of speculum and two preparations were prepared. After the preparations were stained with giemsa stain used in the laboratory, the cell profile was examined under light microscope and the cycle periods of the dogs were determined. The bitches were divided into 3 groups according to sexual cycle periods as proestrus (n=5), metestrus-diestrus (n=3) and anestrus (n=7).

Before the operation, the bitches were anaesthetised by subcutaneous injection of atropine at a dose of 0.04 mg/kg, followed by intramuscular administration of xylazine at a dose of 12 mg/kg and deep intramuscular administration of ketamine HCl at a dose of 10-20 mg/kg. The uterus tissue along the median line of the operation was removed completely after appropriate ligatures were placed. After this procedure, the peritoneum, muscles and skin were sutured separately and the area was bandaged. Antibiotic treatment was applied for 7 days to prevent complications.

2.3. Histological Procedure

The uterus tissues were fixed in 10% formalin for 48 hours. After fixation, the samples were processed for routine histological protocols and embedded in paraffin. The tissue sections were taken at thickness of 5 µm from the paraffin blocks prepared and stained with Crossman's triple staining for histological examination (Crossman, 1937).

2.4. Immunohistochemical Procedure

The streptavidin biotin peroxidase complex method was applied in the uterus tissue. Sections of 4-5 µm thickness were collected on adhesive slides. The sections were processed in citrate buffer solution (ph 6.0) for 10 min in a microwave oven at 700 watts. Then, tissues kept on hold in 3% hydrogen peroxide (H2O2) for 15 min. The blocking solution A was dripped to prevent the nonspecific binding by IHC Kit. Sections were incubated with MUC1 primary antibody (ab104978, Abcam, Cambridge, MA 02139-1517 USA, 1/250 dilution), MUC16 primary antibody (ab133419, 1/500 dilution) were applied on the sections in a humid environment at the ambient temperature for 1 h. Seconder antibody and after streptavidin was dripped on the sections for 30 min. The 3,3'-Diaminobenzidine tetrahydrochloride (DAB) used as chromogen for 10 min then Mayer's hemotoxilen was used for the background staining. Rabbit serum without primer antibody served as the negative control. Sections were evaluated using research microscope (Olympus BX51, Tokyo, Japan). Evaluation of immunoreactivity of MUC1, and MUC16 were scored. Immunoreactive cells were categorized as having negative, mild, moderate, and intensive.

3. RESULTS AND DISCUSSION

As a result of vaginal cytology performed to determine the sexual cycle periods of the bitches before the operation, dense erythrocytes and nucleated or non-nucleated superficial cells were observed during the proestrus period (Figure 1a). Increased neutrophil granulocytes, parabasal cells were found in the metestrus-diestrus stage (Figure 1b) and parabasal and intermedier cells were found in the anestrus stage (Figure 1c).

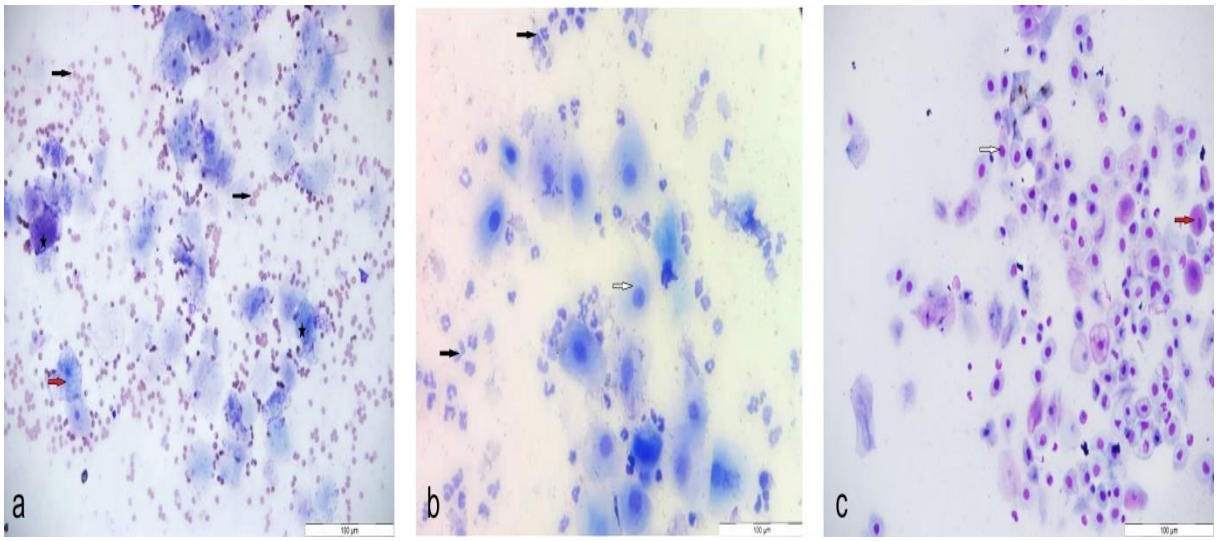

Figure 1. Vaginal cytology in bitchs. a: proestrus: erythrocytes (black arrows), nucleated superficial cell (red arrow) and non-nucleated superficial cells (stars), b: metestrus-diestrus: neutrophil granulocytes (black arrows), parabasal cell (white arrow) c: anestrus: parabasal cell (white arrow) and intermedier cells (red arrow). Giemsa staining.

The estrus cycle in the bitches consists of proestrus, estrus, metestrus-diestrus and anestrus periods. Hormonal changes in the estrus cycle also cause morphological changes in the structure of the uterus (Goldman et al., 2007). The endometrial stroma gains an oedematous appearance in proestrus period when sexual desire begins due to the increase in estrogen hormone. In cross-sections, the lumen looks like an X. In the endometrium, surface epithelial cells proliferate and form superficial recesses into the stroma to create crypts. Increased capillary hyperaemia is associated with extravasation of erythrocytes into the lumen (Van Cruchten et al., 2004). In the study, proliferation of surface epithelial cells, glandular epithelial cells and crypts were detected in the endometrium of both the cornu uteri and corpus uteri in the proestrus period. Thickening of the endometrium due to increased proliferation was observed. In addition, hyperaemic appearance was also noted in the blood vessels. Thickening due to increased muscle cell proliferation was observed in the myometrium. At this stage, the mucosal layers were extremely oedematous and thick (Figure 2a, 2b).

In the metestrus-diestrus period, progesterone hormone expression is at the highest level. Especially in early diestrus, the endometrium and myometrium reach the highest thickness and cellular density (Galabova et al., 2003). At this stage the female accepts the

male. At the beginning of this stage, mucous gland epithelium and surface epithelium acquire high characteristics. The number and secretion of basal glands increases. At the same time, the thickness of the endometrium increases due to increased vascularisation (Wick and Kress, 2002; Vermeirsch, 2001). In the metestrus-diestrus period of this study, the crypts and glands of uterus in the endometrium layer of the cornu and corpus uteri were observed to be extremely enlarged and voluminous. Thickening of the endometrium layer was detected with increasing size. Similarly, the thickness of the layers of the myometrium and perimetrium was also significantly increased. In the stratum vascularis layer, hyperaemia of the blood vessels was observed and their width was extremely increased (Figure 2c, 2d).

If fecundation does not occur, the progesterone level drops rapidly and the anestrus period begins. At this stage, epithelial cells start to take cubic shape. The number of crypt and secretory epithelial cells and the secretions of the uterus glands are reduced (Barrau et al., 1975). The endometrium and myometrium are completely atrophic. All compartments of uterus result in reduced cellular cytoplasm and high nuclear density (Rehm et al., 2007). In the study, it was observed that the growth and enlargement seen in the metestrus-diestrus phase in the anestrus phase gradually weakened in this phase. It was found that the lumen was more enlarged in the anestrus stage and the thickness of the endometrium and myometrium layers decreased due to atrophy. The epithelial layer was extremely thin and the crypts and uterus glands were reduced in size. In addition to the reduction of hyperaemia in the blood vessels, a narrowing of their width was also noted (Figure 2e, 2f).

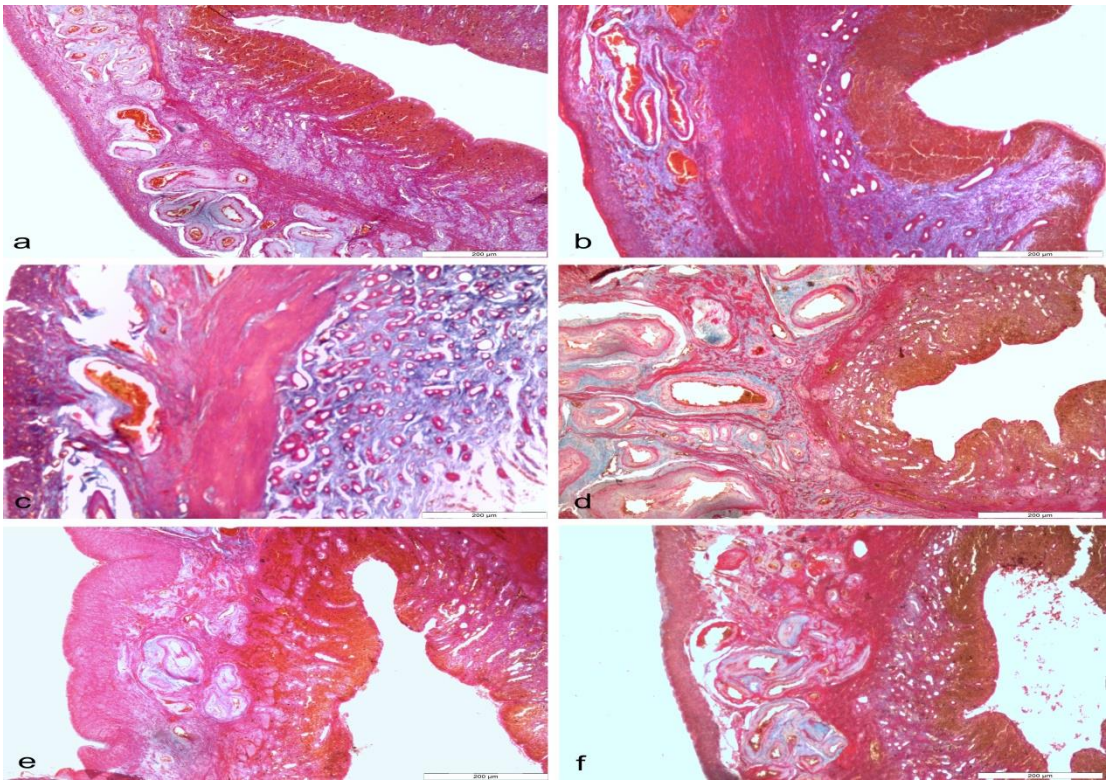

Figure 2. The histological structure of the corpus and cornu uteri in sexuel cycle of bitches. corpus uteri (a) and cornu uteri (b) in proestrus; corpus uteri (c) and cornu uteri (d) in metestrus-diestrus; corpus uteri (e) and cornu uteri (f) in anestrus. Crossman's Triple Staining.

Mucins with glycoprotein content are very important for embryo implantation (Meseguer et al., 2001). In addition, it was reported that mucins also play a protective role by acting as a barrier against enzymatic and microbial attacks (Jentoft N., 1990). It was reported that MUC1 is expressed from the cervix epithelium as well as the uterine epithelium and facilitates the movement of sperm and controls bacterial passage. Deficiency or loss of MUC1 secretion from the surface epithelium of the endometrium has been shown to impair implantation in the uterus of mice (Surveyor et al., 1995) and pigs (Bowen et al., 1996). It was reported that implantation of the embryo into the uterus was not possible when mucins were removed enzymatically from the apical surface of the uterine epithelium (De Souza et al., 1999).

MUC1 expression was reported to be regulated by estrogen, progesterone and glucocorticoids (Dharmaraj et al., 2010). It was reported that the expression of MUC1 is increased in mice during proestrus and estrus periods by hormonal effect (Arklie et al., 1981; Meseguer et al., 2001). In our study, it was found that the release of MUC1, which was

detected in the proestrus period, increased in the metestrus-diestrus period. Moderate MUC1 expression was also detected in the surface epithelial cells, crypt epithelial cells, uterus gland epithelial cells, myometrium and perimetrium layers of the endometrium layer of the corpus uteri during the proestrus period (Figure 3a). In the cornu uteri, moderate MUC1 expression was found in the crypt epithelial cells, uterus gland epithelial cells, myometrium and perimetrium, and intensive MUC1 expression was found in blood vessels in the stratum vascularis (Figure 3d). Intensive MUC1 expression was observed in surface epithelial cells of the corpus uteri, crypt epithelial cells, uterus gland epithelial cells, myometrium, stratum vascularis and perimetrium during the metestrus-dioestrus phase (Figure 3b). It was found that MUC1 expression was intensive in the stroma of the endometrium of the cornu uteri near the lumen, crypt epithelial cells and uterus gland epithelial cells, and moderate expression was noted in the blood vessels of the stratum vasculare (Figure 3e). In the anestrus period, moderate MUC1 expression was observed in the stroma cells and crypt epithelial cells close to the lumen of the endometrium in the corpus uteri, while no reaction was observed in other layers (Figure 3c). In the cornu uteri, a mild reaction was observed in the crypt epithelial cells in the endometrium, while MUC1 expression was not observed in the other layers and blood vessels in the stratum vasculare (Figure 3f).

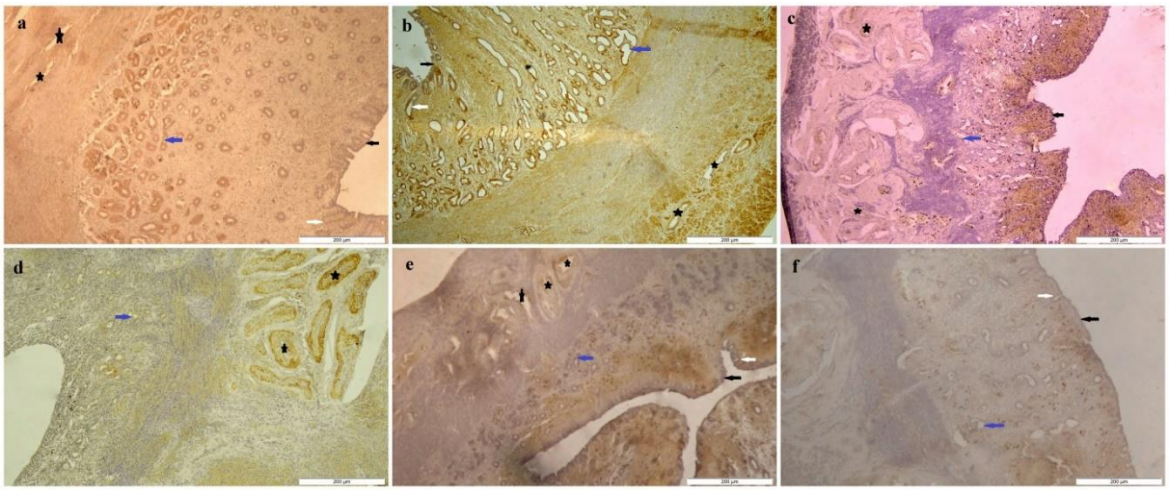

Figure 3. Expression of MUC1 in the bitch uterus during sexual cycle. corpus uteri (a) and cornu uteri (d) in proestrus; corpus uteri (b) and cornu uteri (e) in metestrus-diestrus; corpus uteri (c) and cornu uteri (f) in anestrus. Uterus epithelial cells (black arrow), crypt epithelial cells (white arrow), uterus gland epithelial cell (blue arrow), blood vessel (stars). Immunohistochemical Staining.

MUC16, also known as CA125 antigen, is the largest of the mucins known to date with a molecular weight of 2.5 MDa and a length of 22,152 amino acids (Perez et al., 2008). MUC16 is involved in barrier formation on the surface of epithelial cells (Dharmaraj et al., 2010). In human endometrium, MUC16 was reported to be expressed on the surface of luminal epithelium and glandular epithelium throughout the menstrual cycle (Gipson, 2008). In gynaecological practice, MUC16 serum levels are used as a tumour marker in ovarian cancer (Babic et al., 2017). In addition to the insufficiency of literature data on the relationship between MUC16 expression and reproductive outcome, it was reported that it acted as a chemical barrier to protect the epithelium against harmful environmental conditions and pathogens in the tissue where it was located (Felder et al., 2014; Haridas et al., 2014). Muc-16 expression was detected only in the cornu uteri during the metoestrus-dioestrus period. Similar to other cycle periods, MUC16 expression was absent in the corpus uteri (Figure 4a). MUC16 expression was found to be intensive in the endometrium of the cornu uteri and mild in the blood vessels in the stratum vasculare (Figure 4b).

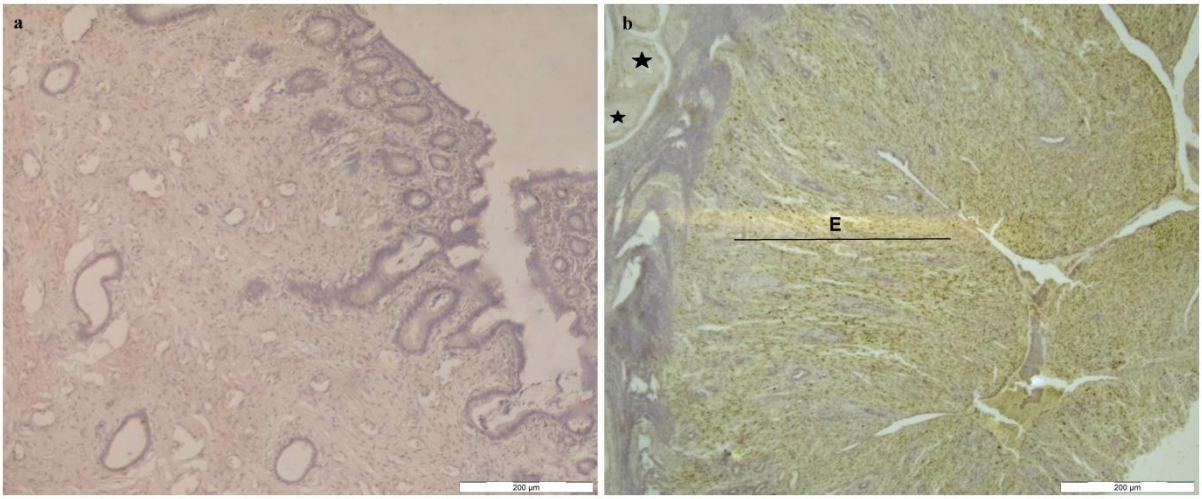

Figure 4. Expression of MUC16 in the bitch uterus in metestrus-diestrus. corpus uteri (a) and cornu uteri (b). Endometrium (E), blood vessels (stars). Immunohistochemical Staining.

The absence or persistence of pregnancy requires a combination of several reasons to be considered. In this study, immunohistochemical expressions of MUC1 and MUC16 were analysed in estrus cycles of bitches and it was observed that MUC1 was released in all sexual cycle periods. MUC1 was found to be expressed in the surface epithelium of both cornu and corpus uteri, crypt and uterus gland epithelium, stratum vasculare, myometrium and

perimetrium layers in bitches uterus tissue. It was noted that its secretion increased during metestrus and decreased during anestrus. MUC16 was found to be expressed in the cornu uteri during the metestrus-diestrus period. In the literature, the importance of mucins in facilitating implantation, protecting against bacterial damage and ensuring healthy continuity of pregnancy is mentioned. In our study, the increase in the release of MUC1 and MUC16, especially in the metestrus period, observed that mucins were affected by hormones. Our results also demonstrated the importance of mucins in implantation and pregnancy.

We believe that our study may provide a different perspective in addition to the existing criteria in the evaluation of complications occurring during pregnancy in different animal species, especially in veterinary medicine.

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Conflict Of Interest

The authors report no conflicts of interest.

Author's Contributions

The authors declare that they have contributed equally to the article.

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Koyun Üreme Özellikleri İçin Tanımlanan Aday Genlerden Biri Olan Rora (Rar-Related Orphan Receptor Alpha) Reseptörlerinin Koyun Üremesindeki Rolü

The Role of Rora (Rar-Related Orphan Receptor Alpha) Receptors, One of The Candidate Genes Identified for Sheep Reproductive Traits, In Sheep Reproduction

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Anahtar Kelimeler
RORA (RAR-Related Orphan Reseptör Alfa) 23-bp nükleotit Sekans mutasyonu
İntron 1
Bafra koyunu
MAS (işaretleyici destekli seçim)

Öz

RORA (RAR-Related Orphan Receptor Alpha) steroid ile ilişkili genleri transkribe ederek östrojen sentezini düzenler. Yavru büyüklüğü, östrojen senteziyle ilişkili önemli bir üreme özelliğidir. Bu nedenle RORA geni ile koyun yavru büyüklüğü arasındaki ilişkinin araştırılması kritik önem taşımaktadır. Bu çalışmada, RORA adı verilen bir gende 23 baz çifti nükleotid dizilim mutasyonu ve bu mutasyonun koyun yavrularının verimliliği üzerindeki etkisi araştırılmıştır. Bu çalışmada 35 dişi Bafra koyununun RORA geninin intron 1'inde 23 bp'lik bir nükleotid dizi mutasyonu bulunmuştur. Çalışmaya katılan 35 örneğin her birinden 10 ml süt toplanmıştır. DD genotipinin yavru büyüklüğü, ikinci doğan yavru büyüklüğünde ID genotipinden ve II genotipinden önemli ölçüde daha yüksek saptanmıştır ($p>0.05$). Kombine genotipler ile ortalama yavru büyüklüğü arasındaki ilişki, homozigot (DD) genotipli koyunların heterozigot (ID) genotipli koyunlardan daha büyük kuzulara sahip olduğunu göstermiştir. Birinci ve ikinci yavrulardaki çoğul gebelik oranları, ortalama ölü doğum oranından daha yüksek saptanmıştır ($p> 0.05$). Özetlemek gerekirse, bu çalışma RORA geninin işlevi ve Bafra koyunlarının ıslahı ile ilgili daha ileri araştırmalar için teorik referanslar sağlamıştır. 23-bp indel varyantları, MAS (marker destekli seleksiyon) ıslahında koyunların birinci ve ikinci doğan yavrularının büyüklüğü için moleküler belirteçler olarak kullanılabilir.

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Abstract

RORA (RAR-Related Orphan Receptor Alpha) regulates estrogen synthesis by transcribing steroid related genes. Litter size is an important reproductive trait associated with estrogen synthesis. it is critical to investigate the relationship between the RORA gene and sheep litter size. In this study, a 23 base pair nucleotide sequence mutation in a gene called RORA and the effect of this mutation on the productivity of sheep offspring were investigated. In this study, a 23 bp nucleotide sequence mutation was found in intron 1 of the RORA gene in 35 female Bafra ewes. Ten ml of milk was collected from each of the 35 samples. The litter size of the DD genotype was significantly higher than the ID genotype and the II genotype in the second born litter size ($p> 0.05$). The relationship between combined genotypes and average litter size showed that ewes with homozygous (DD) genotype had larger lambs than ewes with heterozygous (ID) genotype. Multiple pregnancy rates in the first and second litters were higher than the average stillbirth rate ($p> 0.05$). To summarize, this study provided theoretical references for further research on the function of RORA gene and breeding of Bafra sheep. The 23-bp indel variants can be used as molecular markers for the size of first and second born offspring of ewes in MAS (marker assisted selection) breeding.

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1. INTRODUCTION

Sheep breeding is an important aspect of animal husbandry in Turkey due to the country's climatic conditions, topography, and socio-cultural structure. Crossbreeding studies were initiated in 1982 at Bafra Karaköy Agricultural Enterprise to combine the high fertility and milk yield characteristics of Bafra sheep Karayaka and Sakız breeds. As a result, Chios x Karayaka G1 crosses were obtained and bred among themselves to create a new breed called Bafra sheep, which has approximately 75% Chios and 25% Karayaka genotype (Yakan & Ünal, 2010).

Bafra sheep is primarily cultivated in Central and Western Black Sea regions, Central Anatolia, Eastern Anatolia, Aegean, and Mediterranean regions. This breed has high prolificacy, which is inherited from the Sakiz sheep breed. Studies have shown that the litter size of Bafra ewes ranges between 1.78 and 2.20, while the litter size of Akkaraman ewes is between 1.20 and 1.30 (AKÇAPINAR et al., 2008). Overall, Bafra sheep has become an important breed in Turkey due to its unique characteristics and adaptability to different regions.

In order to improve reproductive performance in sheep breeding, various strategies can be applied, such as selecting for traits that are correlated with fertility and using reproductive technologies such as artificial insemination and embryo transfer. However, it is important to note that reproductive traits often have low heritability values, which means that genetic selection may have limited impact in the short term. Nevertheless, by implementing long-term breeding programs and focusing on selecting for other important traits such as milk yield, body weight, and disease resistance, it may be possible to indirectly improve reproductive performance in the herd. Additionally, proper nutrition, management practices, and veterinary care can also play a significant role in improving reproductive efficiency and overall productivity in sheep farming.

The retinoic acid receptor-related orphan receptors (ROR) are a subfamily of nuclear receptors encoded by the RORA-C (or NR1F1-3) genes. These receptors play important roles in various physiological processes such as metabolism, development, and immunity. Nuclear receptors are ligand-dependent transcription factors that translate endocrine and dietary signals into differential gene expression patterns. Although an endogenous ligand for RORs has not been unequivocally confirmed, intermediates and metabolites of cholesterol metabolism have been proposed as potential candidates. RORs have been implicated in a

range of diseases, including autoimmune disorders, metabolic disorders, and cancer, making them potential therapeutic targets (Jetten & Cook, 2020).

The AF2 domain is a highly conserved region among nuclear receptors and is responsible for the ligand-dependent transcriptional activation of target genes. Upon binding of a ligand to the LBD, a conformational change occurs that results in the recruitment of co-activator proteins to the AF2 domain, leading to the initiation of transcription. Co-repressor proteins, on the other hand, are recruited to the AF2 domain in the absence of a ligand, leading to the repression of target gene transcription. The AF1 domain, located in the N-terminus, is less conserved and has been shown to be involved in ligand-independent transcriptional activation, protein-protein interactions, and receptor dimerization. The hinge region, located between the DBD and LBD, plays a role in facilitating the movement of the receptor between the cytoplasm and nucleus and is also involved in receptor dimerization. Overall, the structural features of nuclear receptors allow for precise and regulated transcriptional responses to a variety of endocrine and dietary signals. (Y. Zhang et al., 2015). Recent studies have shown that RORs play a critical role in regulating lipid and glucose metabolism, making them potential targets for the treatment of metabolic disorders such as obesity and type 2 diabetes. RORs have also been implicated in the regulation of the immune system, with RORγt playing a key role in the differentiation of Th17 cells, which are involved in autoimmune and inflammatory diseases such as multiple sclerosis and rheumatoid arthritis. In addition, RORs have been linked to cancer, with RORα and RORγt both showing both oncogenic and tumor-suppressive roles depending on the cancer type. The identification of ROR ligands with therapeutic potential has led to the development of several ROR-targeting drugs, some of which are currently in clinical trials (Marciano et al., 2014).

RORA has also been linked to neurodevelopmental disorders such as autism spectrum disorder (ASD) and intellectual disability (ID). Several studies have reported altered RORA expression in individuals with ASD and ID, and genetic variants in RORA have been associated with an increased risk of developing ASD. RORA has been shown to regulate the expression of genes involved in synapse formation and function, neuronal migration, and differentiation, which are all critical processes in neurodevelopment. Furthermore, RORA has been implicated in the regulation of the immune system, and dysregulation of RORA expression has been linked to autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (Sarachana & Hu, 2013). In addition to its potential role in animal fertility and

infertility, RORA has also been implicated in a variety of other physiological processes. For example, it has been shown to play a role in lipid and glucose metabolism, and its dysregulation has been linked to the development of obesity and type 2 diabetes. RORA has also been found to be involved in the regulation of inflammatory responses and autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis. Moreover, recent studies have suggested that RORA may have a role in regulating the sleep-wake cycle, with dysregulation of RORA expression leading to sleep disorders. Thus, RORA is a promising target for the development of therapeutics for a wide range of diseases and disorders (Findlay et al., 2010).

Marker-assisted selection (MAS) is a breeding method that uses genetic markers, such as single nucleotide polymorphisms (SNPs), to identify and select animals with desirable traits. This method can help to accelerate the breeding process and increase the accuracy of selection, particularly for traits that are difficult to measure directly or have low heritability.
In sheep breeding, MAS has been used to improve a variety of traits, including reproductive performance, wool quality, and meat quality. For example, several studies have identified SNPs associated with litter size and other reproductive traits in sheep, which can be used to select animals with higher fertility.

In addition to MAS, other molecular breeding technologies, such as genomic selection and gene editing, have also shown promise in improving the hereditary character of sheep. These methods can help to identify and manipulate specific genes that affect desirable traits, allowing for more targeted and efficient breeding.

Overall, the development of molecular marker technology has provided new opportunities for improving the genetic potential of sheep and other livestock species, leading to more efficient and sustainable animal production systems (Platten et al., 2019). SNP (Single Nucleotide Polymorphism), CNV (copy number variation), and indel (insertion/deletion) are the most common MAS methods. Indel is gradually becoming a common method in MAS that is widely used for breeding because it is easily and quickly identified (Hui et al., 2020). To sum up, RORA is a nuclear receptor that plays a crucial role in various physiological processes, including circadian rhythm, neuronal cell development, and immune cell differentiation. It is also linked to several pathologies, including autoimmune, inflammatory, and metabolic diseases. RORA has been identified as a potential candidate gene that may affect animal reproductive traits, and its expression has been found to regulate the synthesis of androgens and estrogens. In addition, various MAS methods, such as SNP, CNV, and indel, have been

used to identify molecular markers associated with sheep reproductive traits, providing a foundation for future research into the functions of RORA (Wang et al., 2020).

2. MATERIALS AND METHODS

10 ml of milk was collected from each of the 35 samples participating in the study. DNA was obtained from all samples using a commercial DNA extraction kit (invitrogen) according to the manufacturer's protocol.

The Rora gene 23bp indel variant were genotyped in all the subjects by the polymerase chain reaction analysis. The total reaction mixture (20 ul) contained 2 ul of genomic DNA (10 ng/ul), 1 ul of each primer (10 uM), 1 ul of d NTP mix (10 uM), 1.5ul $MgCl^2$, and 0.2 ul Taq (1 units of Taq DNA polymerase) and 13.3 ul ddH2O. A set of primers, P1-rs604927153 Intron 1 F1: GGATGGGGCTTGGTGGATTA and R1: CAGGTGGTGAGCCATCTTGG was used to amplify the Rora gene inton 1 variant 23 bp indel site.

The amplified products were carried out by electrophoresis using a 2.5% agarose gels stained with ethidium bromid. The insersion alleles yielded 195 bp. The deletion allele gave a band of 172 bp. All of the samples were taken and reworked. No difference in results was observed and no bias in genotyping.

2.1. Statistical Analysis

SPSS v22 for Windows (IBM Corp., Armonk, NY, USA). Categorical variables are presented as n (percentage frequency) and continuous variables as mean±standard deviation. Chi-square test was used for comparisons of categorical data. P values less than 0.05 were considered statistically significant.

3. RESULTS

The study included 35 female Bafra sheep. Descriptive data of these sheep are given in Table1.

Table 1. Descriptive data

Parameter		Female Bafra sheep n:35
Mean age X±SD		3.02±0.92
Body height (cm) X±SD		71.66±3.52
Hip height (cm) X±SD		72.17±3.84
Hip width (cm) X±SD		20.49±1.56
Abdominal circumference (cm) X±SD		95.69±12.52
Chest circumference (cm) X±SD		89.74±7.33
History of mastitis	**available n (%)**	3 (08.57)
	none n (%)	32 (91.43)
RORA_23bp_indel genotype frequencyn (%)	**DD**	21 (60.00)
	DI	13 (37.14)
	II	1 (02.86)
RORA_23bp_indel allele frequency n (%)	**D**	55 (78.57)
	I	15 (21.43)
2021 (1st parity) X±SD	**Average multiple pregnancy**	2.14±1.26
	Average stillbirth	1.74±0.56
	Average lamb weight (kg)	3.71±0.76
2022 (2nd parity) X±SD	**Average multiple pregnancy**	2.14±1.24
	Average stillbirth	1.80±0.53
	Average lamb weight (kg)	4.06±0.69

The relationship between genotype frequencies and the number of pregnancies in the first and second litterings was evaluated in Table 2.

Table 2. Relationship between genotype frequencies and number of pregnancies in thefirst and second litter

Parity	Number of pregnancies	Genotype n			X^2	DF	P
		DD	DI	II			
1. parity	**Singular**	7	4	0	0.496	2	0.780
	Plural	14	9	1			
2. parity	**Singular**	7	4	0	0.496	2	0.780
	Plural	14	9	1			

There was no significant relationship between genotype frequencies and number of pregnancies in both the first and second litters.

Stillbirth averages and lamb average weights in the first and second calving were evaluated according to genotype frequencies in Table 3.

Table 3. Comparison of stillbirth averages and lamb average weights according togenotype frequencies in the first and second calving

Parity		Genotype			P
		DD	DI	II	
1. parity	stillbirth averages	1.78	2.00	2.00	0.083
	lamb average weights	3.64	3.90	3.80	0.514
2. parity	stillbirth averages	1.83	1.85	2.00	0.848
	lamb average weights	4.23	3.89	3.65	0.442

There was no significant difference between the mean stillbirths and mean lamb weights according to genotype frequencies in the first and second calving.

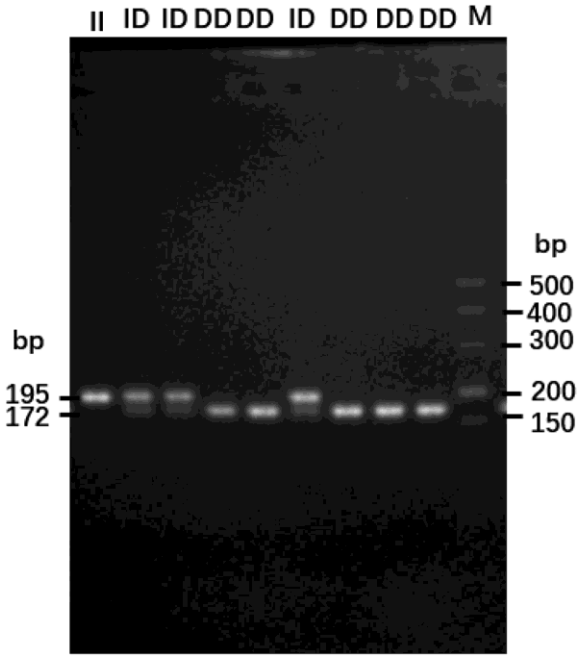

Figure 1. Genotyping of primer pair P1 23-bp indel deter- mined by PCR amplification (3.5 % agrose gel). *Note*: II, inser- tion/insertion. ID: insertion/deletion; DD: deletion/deletion.

4. DISCUSSION

Bafra sheep is a crossbreed of Chios (75%) and Karayaka (25%) sheep breeds. Body-color is white and covered with fleece. Black spots are seen around the mouth, eyes, and ears. They are generally raised in the middle of the Black Sea Region and are well suited to the harsh climate, poor pasture, and severe climatic conditions. Lambs of Bafra breeds are grown for

meat production .Average live weight is 62 kg, height at withers 68 cm, body length 71 cm (Yakan & Ünal, 2010).

Sheep play a vital role in the livestock industry, and improving their growth and reproductive traits is essential for efficient breeding. Increasing litter size is particularly important for the sheep industry. Previous research has suggested that the RORA gene, which regulates sex hormones and is associated with autism, may also influence animal fertility. However, few studies have explored the relationship between sheep genetic mutations and litter size. Therefore, the aim of this study was to identify the RORA gene as a potential molecular marker for further research, which could lead to the use of MAS in sheep breeding. The study identified five transcript variants of the RORA gene in sheep, and it was found that different variants were related to the development of sheep tuberculosis and the protective immune response to infection. The RORA gene was found to be expressed in various tissues, including the testis, kidney, fat, cerebellum, liver, as well as normal breast, prostate, and ovarian epithelial cells (Jetten & Ueda, 2001). The RORA gene is known to regulate the expression of genes associated with autism spectrum disorder (ASD), including A2BP1, CYP19A1, HSD17B10, ITPR1, NLGN, and NTRK2. Among these, CYP19A1 and HSD17B10 are responsible for the conversion of androgens to estradiol, crucial for the growth of follicles and development of oocytes. Thus, RORA plays a critical role in regulating steroid metabolism in sheep, which affects the levels of androgens and estrogens. Downregulation of CYP19A1 and HSD17B10 due to reduced RORA expression can result in decreased levels of androgens and estrogens in sheep. (Lardone et al., 2017).

There are no studies on Bafra sheep and genotyping in the literature. For this reason, this study is important in terms of being the first study in the literature.

A research study was conducted to investigate the potential association between the Inhibin βB Gene Exon 2 region and FSHB gene Exon 3 region with offspring production in Akkaraman and Bafra Sheep Breeds. The results of the study revealed that no significant correlation was found between the identified polymorphisms in INHBB and FSHB genes and offspring production in either Bafra or Akkaraman sheep (Unlusoy & Ertugrul, 2016).

In a study conducted by Zhang et al., a polymorphism was identified in exon 3 of the FSHB gene in goats, resulting in a change from glutamine (Gln) to arginine (Arg). The genotypes of this polymorphism were denoted as AA, AB, and BB in four goat breeds, and it was found to have a significant effect on litter size. Additionally, Chu et al. discovered a SNP at the intron

of the INHBB gene in prolific Hu sheep, and the genotypes of this SNP were classified as AA, AB, and BB. Their results indicated that the BB genotype had a significant impact on litter size in comparison to the AA genotype (C. Y. Zhang et al., 2011).

A study analyzing genetic polymorphism in Turkish domestic sheep breeds using microsatellite analysis found that Turkish Merino had the highest level of heterozygosity. The shortest genetic distance was observed between Sakız and Morkaraman breeds, indicating a close genetic relationship between these two breeds. In contrast, the farthest genetic distance was found between Turkish Merino and Ivesi breeds, suggesting a greater genetic distance between these two breeds (Yildiran & Cakir, 2012).

Yang et al. conducted a study on 532 female Australian White Sheep and identified a 23-bp nucleotide sequence mutation in intron 1 of the RORA gene. The locus had a polymorphic information content (PIC) value of 0.219. The ID genotype was found to have a significantly better second born litter size compared to the II and DD genotypes ($p<0.05$), and the ID genotype was dominant in relation to the third born litter size ($p<0.05$). The study also revealed that sheep with heterozygous (ID) genotypes had larger litters on average compared to homozygous (DD and II) genotypes (Yang et al., 2022).

Our study found a higher frequency percentage of DD genotype compared to both ID and II genotypes in RORA_23bp_indel. This finding is consistent with previous studies in the literature. Additionally, our study also found a low frequency of II genotype, which is consistent with other studies. When evaluating the RORA_23bp_indel allele frequency, we observed a higher frequency of the D allele compared to the I allele.

Our study did not find any statistically significant association between genotype frequencies and the number of pregnancies in the first and second litters. However, there was a higher number of multiple pregnancies compared to singleton pregnancies. Interestingly, although not statistically significant, the DD, ID, and II genotypes were more frequent in multiple pregnancies compared to singleton pregnancies. These findings suggest a potential association between RORA_23bp_indel genotype and multiple pregnancies in sheep, which warrants further investigation.

The non-significant results in our study regarding genotype frequencies may be attributed to the limited sample size. Thus, we propose that our study can serve as a preliminary groundwork for future research with larger sample sizes.

5. CONCLUSION

The non-significant results in genotype frequencies in our study could be attributed to the limited sample size. Therefore, we propose that our study can serve as a preliminary investigation and recommend conducting future studies with larger sample sizes to confirm our findings**.**

Author contributions: SY and RA designed the experiment, performed the experiments and drafted the manuscript. SMU and RA contributed to the experimental studies and drafted the manuscript. All authors reviewed and approved the final manuscript.

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Conflict Of Interest

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Author's Contributions

The authors declare that they have contributed equally to the article.

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Huzurevinde Kalan Yaşlıların Sağlık Algısı ile Yaşam Doyumu Arasındaki İlişki

The Relationship Between Health Perception and Life Satisfaction of Elderly People Living in Nursing Homes

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Öz

Bu araştırma, huzurevinde yaşayan yaşlı bireylerin sağlık algısı ile yaşam doyumu arasındaki ilişkinin incelenmesi amacıyla yapılmıştır. İlişki arayan tanımlayıcı araştırma ilkelerine uygun olarak yapılan bu çalışma, Mart -Temmuz 2019 tarihleri arasında Samsun ilinde bulunan beş tane huzurevinde yaşayan yaşlı bireyler ile yapılmıştır. Araştırma kapsamında örneklem seçimine gidilmemiş olup 65 yaş ve üzeri 129 yaşlı bireye ulaşılmıştır. Araştırmanın verileri, "Kişisel Bilgi Formu'', ''Sağlık Algısı Ölçeği'' ve ''Yaşam Doyum Ölçeği" kullanılarak toplanmıştır. Araştırmadan elde edilen veriler SPSS 20.0 programında değerlendirilmiştir. Araştırma verileri değerlendirilirken; sayı, yüzdelik, T testi, One Way ANOVA, korelasyon ve çoklu regresyon analizi kullanılmıştır. Katılımcıların %27.9'u kadındır. Katılımcıların %10.1'i evli olup %81.4'ü çocuk sahibidir. Katılımcıların % 79.8'inin huzur evinde kalma kararlarının kendilerine ait olduğu ve % 66.7'sinin ziyaretçisinin olduğu belirlenmiştir. Katılımcıların %72.9'unun kronik bir sağlık sorununun olduğu, %31.8'inin sağlıklarını kötü olarak değerlendirdiği belirlenmiştir. Katılımcıların %13.2'si kendini çok yaşlı olarak değerlendirirken, %17.1'i yaşlılığı kötü bir durum olarak değerlendirmektedir. Katılımcıların sağlık algısı ölçek toplam puan ortalamaları 50.21±6.40 olup, (min-max=31-65), yaşam doyum ölçek toplam puan ortalamaları 14.4±4.81, (min-max=5-25) dir. Katılımcıların herhangi bir kronik sağlık sorunu olma durumları ve yaşamak istedikleri yerler ile sağlık algısı ölçeği arasında istatistiksel olarak anlamlı bir ilişki bulunmuştur ($p<0.05$). Katılımcıların yaşamak istedikleri yerler, sağlığını değerlendirme, yaşını ve yaşlılığı algılama durumları ile yaşam doyum ölçeği arasında istatistiksel olarak anlamlı bir ilişki bulunmuştur ($p<0.05$). Huzurevinde yaşayan yaşlı bireylerin sağlık algıları ile yaşam doyumlarının orta düzeyde olduğu tespit edilmiştir. Huzurevinde yaşlıların sağlık algıları ve yaşam doyumlarını artırıcı sosyal faaliyetler planlanmalı ve bu yöndeki çalışmalar arttırılmalıdır.

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Abstract

This research was conducted to examine the relationship between health perception and life satisfaction of elderly people living in nursing homes. This study, which was

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conducted in accordance with the descriptive research principles seeking relationship, was conducted with elderly individuals living in five nursing homes in Samsun between March and July 2019. Sample selection was not made within the scope of the research, and 129 elderly individuals aged 65 and over were reached. The data of the study were collected using the "Personal Information Form", "Health Perception Scale" and "Satisfaction with Life Scale". The data obtained from the research were evaluated in the SPSS 20.0 program. While evaluating the research data; number, percentile, T test, One Way ANOVA, correlation and multiple regression analysis were used. 27.9% of the participants are women. 10.1% of the participants are married and 81.4% have children. It was determined that 79.8% of the participants made their own decision to stay in a nursing home and 66.7% had visitors. It was determined that 72.9% of the participants had a chronic health problem and 31.8% evaluated their health as bad. While 13.2% of the participants consider themselves to be very old, 17.1% consider old age as a bad condition. Participants' health perception scale total score averages were 50.21±6.40, (min-max=31-65), and life satisfaction scale total score averages were 14.4±4.81, (min-max=5-25). A statistically significant relationship was found between the participants' state of having any chronic health problems and the places they want to live, and the health perception scale ($p<0.05$). A statistically significant relationship was found between the places where the participants wanted to live, their health assessment, their age and old age perception, and the life satisfaction scale ($p<0.05$). It has been determined that the health perceptions and life satisfaction of the elderly individuals living in the nursing home are at a moderate level. Social activities that increase the health perceptions and life satisfaction of the elderly in nursing homes should be planned and studies in this direction should be increased.
for the size of first and second born offspring of ewes in MAS (marker assisted selection) breeding.

1. INTRODUCTION

The increase in living standards in the world and in Turkey, developments in medicine, decrease in infectious diseases, improvement of nutritional conditions and improvement of hygiene conditions have been effective in prolonging the average life expectancy and accordingly the increase in the elderly population (Elkin, 2016; Erol et al., 2016). According to the United Nations' 2019 World Population Aging report, the population aged 65 and over has reached 703 million 711 thousand 487 people in the world, and according to this report, 9.3% of the world's population is composed of the elderly population. According to the data of the Turkish Statistical Institute (TUIK) for 2020, the population of 65 and over, which is considered as the elderly population, was approximately 6 million 193 thousand people in 2014, and increased by 21.9% in the last five years to approximately 7 million 551 thousand people in 2019. While the proportion of the elderly population in the total population was 8.0% in 2014, it increased to 9.1% in 2019 (TUIK, 2020).

The increase in the elderly population brings with it some health problems (Uçku & Şimşek, 2012). With the prolongation of life expectancy, diseases that occur in old age have

also brought the quality of the years lived to the agenda. With aging, individuals experience physical inadequacies, chronic diseases, pain and social isolation, a perception of poor health occurs, and as a result, the life satisfaction of the elderly may be adversely affected (Boylu, 2013; İlhan et al., 2016; Kulakçı et al., 2012; Uçku & Şimşek, 2012).

Individuals' personal feelings, thoughts, prejudices and expectations for their own health are defined as health perception. Health perception is how the individual evaluates his/her biological, psychological and social dimensions and health status in general and is a strong indicator in health assessment. Health perception is directly related to the process of improving the health of the individual and is one of the important criteria used in determining the health status in recent years (Ağaçdiken Alkan et al., 2017; Altay et al., 2016; Çimen & Temel, 2017; Doğanay & Uçku, 2012). An individual's health status and health perception affect life satisfaction (Arpacı et al., 2015; Kankaya & Karadakovan, 2017). Life satisfaction is defined as the general evaluation of the quality of life according to the criteria chosen by the individual and is expressed as the goals that the individual desires and achieves (Dağlı & Baysal, 2016; Erci et al., 2017). Life satisfaction is not the satisfaction related to any situation, but the morale, happiness satisfaction, etc. in the whole life in general. It refers to the state of being well in different aspects such as Since life satisfaction is related to whole life satisfaction, it is thought that life satisfaction can be affected by various factors (Canlı et al., 2020). Life satisfaction in the elderly; Many factors affect personality traits, physical and psychological well-being, health status, economic well-being, relations with the social environment, methods of coping with problems, reaching goals and objectives, and meaning attributed to life (Boylu & Günay, 2018; Dağlı & Baysal, 2016; Elkin, 2016; Kahraman et al., 2011; Kankaya & Karadakovan, 2017; Softa et al., 2015). Feeling worthless, dysfunctional and powerless, distressing events, conflicts, frustrations, and having poor health perception are important determinants that can lead to a decrease in life satisfaction of individuals (Ata & Ekinci, 2020; Elkin, 2016; Erol et al., 2016). Studies show that depression affects life satisfaction negatively, while strong self-esteem and positive health perception affect life satisfaction positively (Arpacı et al., 2015; Durak et al., 2010; Elkin, 2016).

In the old age, there are changes in social roles with retirement, increase in physical losses, especially chronic diseases, economic losses and various psychosocial problems (Kankaya & Karadakovan, 2017; Özdemirkan et al., 2020). Despite all the efforts made to

ensure that the elderly people live in a happy and healthy way in nursing homes, living in a nursing home is perceived by the elderly as being removed from their family and being unwanted, negatively affecting their expectations and outlook on life (Genç et al., 2015). It is not an easily acceptable situation for the elderly person, who has a respectable place and authority in the family, to be placed in a nursing home, which is a care institution outside the family. It is a difficult phenomenon for the elderly to lose their usual status in the society and to have to give up the life they are accustomed to at home. As older individuals start to continue their lives under the roof of an institution, the possibilities of acquiring, recognizing and controlling an environment that will accept and respect them disappear; The elderly person may experience feelings of isolation, being denied, feeling useless and worthless (Altay & Aydın, 2009). Due to all these factors, elderly individuals, who constitute a risk group in terms of mental health, encounter more intense problems in nursing homes (Altıparmak, 2009; Birinci & Quadir, 2017).

Since it is thought that the life satisfaction of the elderly living in a nursing home is related to the perception of health, it is of great importance to know the life satisfaction of the elderly, their perception of health and the factors affecting both, in displaying the behaviors of protecting and improving the health of the elderly individuals living in the nursing home and reintegrating them into the society. Although there are studies in the literature investigating the relationship between loneliness, depression and death anxiety variables and life satisfaction in elderly individuals, no study examining the relationship between health perception and life satisfaction of the elderly has been found (Elkin, 2016; Erol et al., 2016; Kahraman et al., 2011). In old age, which is a developmental crisis period; It is very important to evaluate the psychosocial determinants of health, such as health perception, in order to effectively cope with the problems of old age in the nursing home, to protect and maintain their health, to increase their quality of life and life satisfaction. In line with these inferences; This study, which was planned in accordance with descriptive research principles, was conducted to determine the relationship between health perception and life satisfaction of elderly individuals living in nursing homes.

Research Questions

- Is there a relationship between Health Perceptions and Life Satisfaction of the elderly living in a nursing home?

- Is there a relationship between the scores obtained from the Health Perception scale and the Satisfaction with Life scale and the demographic characteristics of the elderly?

2. MATERIAL AND METHOD

2.1. Type, Place and Time of Research

This study, which was carried out in accordance with the descriptive research principles seeking relationship, was conducted between March and July 2019 with elderly individuals living in five nursing homes affiliated to the Ministry of Family and Social Policies in the province of Samsun.

2.2. Population and Sample of the Research

The population of the research consists of 282 elderly individuals living in five nursing homes in Samsun. In the study, it was aimed to reach the entire universe without selecting the sample. All individuals aged 65 and over, able to communicate, without hearing problems and dementia, who wanted to participate in the study voluntarily and who could fully answer the data collection form were included in the study, and the study was completed with 129 elderly individuals.

2.3. Data Collection Tools

Data collection tools; It consists of three parts, namely, “Description form”, “Health Perception Scale” and “Satisfaction with Life Scale” and a total of 35 questions.

Introduction Form: Introductory form developed by the researcher by scanning the literature (Arslan et al., 2016; Çevik Akyıl et al., 2018); It includes questions that will determine the socio-demographic characteristics of the participants (age, gender, department, class, family type, family income status, working status of parents, educational status of parents, etc.) and consists of a total of 15 questions.

Health Perception Scale: It was developed by Diamond et al. in 2007. The Turkish validity and reliability of the HPS was made by Kadıoğlu & Yıldız (2012), and it is a five-point Likert-type scale consisting of 15 items and four sub-factors. 1st, 5th, 9th, 10th, 11th and 14th items consisted of positive statements, 2nd, 3rd, 4th, 6th, 7th, 8th, 12th, 13th and 15th items consisted of negative statements. Positive statements were scored as “strongly agree= 5”,

“agree= 4”, “undecided= 3”, “disagree= 2”, “strongly disagree=1”. For negative statements, reverse scoring was done. A minimum of 15 and a maximum of 75 points can be obtained from the scale. Cronbach Alpha Values according to the sub-factor groups of the scale: Control center 0.90; Self-awareness 0.91; Precision 0.91; The importance of health is 0.82. The increase in the scoring indicates that the perception of health increases positively (Kadıoğlu & Yıldız, 2012).

Life Satisfaction Scale: The Turkish validity and reliability of the scale developed by Diener, Emmons, Larsen, and Griffin (1985) was verified by Dağlı and Baysal (2016). The scale is a self-evaluation scale consisting of 5 questions. The alpha reliability value of the scale was determined as 0.88. In the evaluation of the scale, 5-point Likert-type evaluation, scoring ranging from “I strongly disagree (1), I slightly agree (2), I agree moderately (3), I mostly agree (4) and I totally agree (5)” is used. The minimum score that can be obtained from the scale is 5 and the maximum score is 25. An increase in the scoring indicates an increase in life satisfaction (Dağlı & Baysal, 2016).

Evaluation of Data: IBM SPSS 20.0 (Statistical Package for Social Sciences) statistical package program was used to evaluate the data obtained from the research. Quantitative data are presented as median (min-max), frequency (percent). Participants' health perception scores, life satisfaction scores and t-test, Pearson correlation and Anova analysis were performed between the variables. It was decided whether the relationship between the groups was statistically significant or not according to the p values and the statistical significance level was accepted as $p<0.05$.

Ethical Aspect of Research: Before starting the research, ethics committee approval (B.30.2.ODM.0.20.08/153) and necessary written permissions from the relevant institution were obtained to evaluate the ethical suitability of the research. In addition, the study was completed in accordance with the principle of voluntariness by obtaining the verbal consent of the elderly.

Limitations of the Research: The fact that the research was conducted in nursing homes in Samsun constitutes the limitations of the research.

3. RESULTS

This study was carried out with a total of 129 elderly individuals who live in five nursing homes in Samsun and agreed to participate in the study between March and July 2019. The data obtained as a result of the study are given in this section.

Table 1. Distribution of the participants according to their socio-demographic characteristics (n=129)

Features	n	%
Age		
65-74	61	47.3
75-84	51	39.5
85 and over	17	13.2
Gender		
Woman	36	27.9
Man	93	72.1
Marital status		
Single	116	89.9
Married	13	10.1
Educational status		
Illiterate	26	20.1
Literate	58	45.0
Secondary education	41	31.7
High school	2	1.6
Higher Education and above	2	1.6
Status of having children		
There is	105	81.4
No	24	18.6
Retirement status		
Retired	84	65.1
Not retired	45	34.9
Regular income		
There is	96	74.4
No	33	25.6
Total	129	100.0

47.3% of the participants included in the study are in the 65-74 age group, 13.2% are in the 85 and over age group. 27.9% of the participants are women, 10.1% are married and 81.4% have children. 45.0% of the participants are literate and 1.6% are higher education graduates. 65.1% of the participants are retired and 74.4% have a regular income (Table 1).

Table 2. Distribution of the findings regarding some characteristics of the participants regarding being in a nursing home, health and age perceptions (n=129**)**

Features	n	%
Decision to stay in a nursing home		
Own	103	79.8

It does not belong to you	26	20.2
Visitor arrival status		
Yes	86	66.7
No	43	33.3
where he wants to live		
Home alone	10	7.8
At home with their children	58	45.0
Rest home	61	47.2
Having a chronic health problem		
Yes	94	72.9
No	35	27.1
Health assessment status		
Good	39	30.2
Middle	49	38.0
Bad	41	31.8
Age perception status		
I don't consider myself old	11	8.5
Middle aged	54	41.9
Old	47	36.4
Too old	17	13.2
Perception of old age		
Bad situation	22	17.1
Uselessness	25	19.4
Discomfort	28	21.7
Part of life	54	41.8

It was determined that 79.8% of the participants included in the study made their own decision to stay in a nursing home and 66.7% had visitors. It was determined that 47.2% of the participants wanted to live in a nursing home, and 45.0% wanted to live at home with their children. 72.9% of the participants state that they have a chronic health problem, 31.8% consider their health as bad and 13.2% consider themselves as very old. 17.1% of the participants consider old age as a bad condition, 19.4% as useless and 41.8% as a part of life (Table 2).

Table 3. The participants' health perception and life satisfaction scale score averages

Scales and sub-dimensions	Mean ± standard deviation
Health perception scale	50.21±6.40
life satisfaction scale	14.4±4.81

The health perception scale mean score of the participants included in the study was 50.21±6.40, and the mean life satisfaction scale score was 14.4±4.81 (Table 3). There was no statistically significant relationship between the health perception scale and the life satisfaction scale (r=0.179; p=0.139).

Table 4. Comparison of the participants' health perception and life satisfaction scale mean scores with some variables (n=129)

Variables	n	Health Perception Scale X±SD	Life Satisfaction Scale X±SD
Gender			
Woman	36	50.1±6.5	13.6±4.6
Man	93	50.2±6.3	14.7±4.9
		p>0.05	p>0.05
Marital status			
Single	116	50.4±6.4	14.3±4.8
Married	13	48.5±5.8	13.5±4.7
		p>0.05	p>0.05
Retirement status			
Retired	84	50.9±6.6	14.3±4.8
Not retired	45	48.8±5.7	13.6±4.7
		p>0.05	p>0.05
Regular earning			
There is	96	50.7±6.3	14.6±4.8
No	33	48.7±6.5	13.8±4.9
		p>0.05	p>0.05
Having a chronic health problem			
Yes	94	**50.3±6.7**	13.8±4.7
No	35	**49.7±5.5**	15.8±4.9
		p<0.05	p>0.05
Place to live			
Home alone	10	**45.0±5.8**	**15.3±4.1**
At home with their children	58	**49.6±6.1**	**13.1±4.7**
Rest home	61	**51.6±6.2**	**15.5±4.8**
		p<0.05	**p<0.05**
Educational status			
Illiterate	26	49.7±5.8	15.7±4.7
Literate	58	51.5±5.8	14.0±4.6
Secondary education	41	49.6±7.6	14.3±5.7
High school	2	45.6±5.0	14.1±3.5
Higher Education and above	2	48.0±5.6	16.5±0.7
		p>0.05	p>0.05
Health assessment			
Good	39	50.3±5.5	**16.8±4.9**
Middle	49	50.3±7.1	**14.7±4.4**
Bad	41	50.0±6.3	**11.7±3.9**
		p>0.05	**p<0.05**
Detecting your age			
I don't consider myself old	11	52.7±6.0	**18.0±5.1**
Middle aged	54	50.0±6.3	**15.2±4.4**
Old	47	50.4±7.0	**13.8±4.2**
Too old	17	48.5±4.4	**11.0±5.5**
		p>0.05	**p<0.05**
Sensing old age			
Bad situation	22	49.8±5.4	**12.5±4.5**
Uselessness	25	50.5±8.1	**12.7±4.0**
Discomfort	28	49.0±6.0	**13.6±4.2**
Part of life	54	50.8±6.1	**16.3±4.9**
		p>0.05	**p<0.05**

In Table 4, the comparison of the participants' health perception and life satisfaction scale mean scores with some variables is given. It was determined that there was a statistically significant difference between the chronic health problems of the participants and the place they wanted to live and the health perception scale ($p<0.05$). It was determined that there was a statistically significant difference between the place where the participants wanted to live, evaluating their health, perceiving their age and old age, and the life satisfaction scale ($p<0.05$). It was determined that there was no statistically significant difference between the participants' gender, marital status, retirement status, regular income status, education level, health assessment, perception of age, perception of old age and health perception scale ($p>0.05$). Likewise, it was determined that there was no statistically significant difference between the participants' gender, marital status, retirement status, regular income, chronic health problems, education status and life satisfaction scale ($p>0.05$) (Table 4).

4. DISCUSSION

The findings of this study, which examines the relationship between health perception and life satisfaction of elderly people living in nursing homes, will be discussed in this section. It was determined that the average life satisfaction scale score of the elderly individuals included in the study was 14.4±4.81 (min-max=5-25) and their life satisfaction was moderate. In a study conducted with the elderly living in nursing homes, the life satisfaction of the elderly was found to be moderate (X= 9.70) (Birinci et al., 2017). In another study conducted with the elderly, the average score of the life satisfaction scale of the elderly was found to be 21.89±5.87 (Kankaya & Karadakovan, 2017). In the study of Softa et al. (2015), the average score of the life satisfaction scale of the elderly living in a nursing home was 7.90 points, and the average of the life satisfaction scale score of the elderly people in the study conducted by Tel et al. (2020) in the elderly living at home was 15.81 points. In a study conducted with elderly people living in nursing homes, the life satisfaction score was found to be 20.6±5.9 (Altay & Avci, 2009). In a study conducted with the elderly, the life satisfaction score was found to be 23.6±8.2 in the elderly living at home and 12.3±5.7 in the elderly living in a nursing home (Arslan et al., 2016). In another study investigating the effect of social environment on life satisfaction of elderly individuals, it was stated that elderly individuals with a large social environment had higher life satisfaction (Tomini et al, 2016). Looking at the studies, life satisfaction scores differ and are generally at medium and low levels. In many studies

investigating the relationship between life satisfaction and different demographic variables, it has been determined that there is a negative correlation between increasing age and life satisfaction (Bakış & Çınar, 2007; Erci et al., 2017). The life satisfaction of individuals may decrease as they become consuming and needy people with old age, from a position that meets their own needs and is productive for the society before old age (Başterzi & Yalçın, 2005). The advancing age and the occurrence of chronic diseases may have affected the decrease in life satisfaction.

In this study, no statistically significant relationship was found between the health perception scale and the life satisfaction scale. It was determined that health perception did not affect life satisfaction. In a study conducted with individuals over the age of 60, a low level of positive correlation was found between life satisfaction and health perception (Ayna & Gümüş, 2021). The results obtained differ with the literature.

When the elderly people included in the study evaluated their health status in general, it was determined that the majority of them evaluated their health status as "moderate" and "poor". In a study conducted with the elderly living in nursing homes, 35.5% of the elderly stated their moderate health status and 9.2% stated their physical health status as bad (Birinci et al., 2017). In another study conducted with the elderly, 44.4% of the elderly evaluated their health perception as good and 15.1% as bad (Doğanay & Uçku, 2012). In a study conducted with individuals over the age of 65, 41.2% of the elderly rated health perception as moderate and 28.5% as bad (Günay et al., 2005). In another study, it was determined that 51.0% of elderly individuals manage their health, 37.7% perceive it as bad (Yalınkılıç et al., 2020). In a study conducted with 4065 people over the age of 65 in the USA, 34.2% of individuals stated a perception of moderate-bad-very bad health (Jylha et al., 2006). In a study conducted in China in individuals aged 50-70 years, the rate of perception of poor health was found to be 68.0% (Haseli-Mashhadi, 2009). In a study conducted with elderly individuals who applied to a family health center, 54.5% of the elderly evaluated their health as moderate and 12.6% as bad (Ekin, 2016). In a study conducted with elderly individuals living in nursing homes, 47.7% of the elderly evaluated their health as moderate and 6.2% as poor (Altıparmak, 2009). When we look at the studies conducted in general, it is seen that the perception of health is moderate or bad. Due to the increase in chronic health problems and physiological changes with age, individuals may evaluate their health status as worse (Altay et al., 2016; Altıparmak,

2009; Bayık Temel et al., 2009; Bayık, Temel & Çimen, 2017). Therefore, in this study, it can be thought that the high average age of the elderly group and the presence of chronic disease have an effect on the assessment of health as moderate/poor.

While 13.2% of the elderly included in the study considered themselves very old, 36.4% old, 41.9% middle-aged, 8.5% stated that they did not consider themselves old. The life satisfaction of the elderly, who consider themselves to be very old, is significantly lower than the others. The increase in the loss of sensory skills and abilities of individuals with increasing age, the difficulty in taking care of themselves, the decrease in decision-making ability and the increase in addiction situations such as loss of physical abilities and the increase in the need for social support may have caused a significantly lower life satisfaction in individuals who perceive their age as too old.

In the study, it was determined that those with chronic diseases had higher health perceptions than those without chronic diseases. Conditions such as the regular follow-up of the elderly living in the nursing home by the health personnel in the nursing home and the regular use of their medications may have had a positive effect on their perception of health.

In the study, it was determined that those who want to live in a nursing home have higher health perceptions and life satisfaction than those who want to live alone at home. In a study, it was determined that the issue that elderly individuals are most affected by emotionally is loneliness (Ayna & Gümüş, 2021). Kapikaran (2016) found a significant negative and weak relationship between loneliness and life satisfaction in his study with 110 elderly individuals living in cities, towns and villages, Erol et al. (2016) in their study with 210 elderly individuals, the elderly living in villages were less lonely and more found that they experienced more life satisfaction. In the study conducted with elderly individuals who applied to the family health center, the average life satisfaction score of the elderly living with their families was found to be higher than the elderly living alone, but there was no statistical significance between the living environment and life satisfaction. In Özer's (2004) study, it was stated that the mean score of the life satisfaction scale was 9.07 in individuals living in nursing homes and 11.98 in those living in a family environment. Looking at the literature, it is stated that loneliness is a factor that negatively affects life satisfaction (Erol et al., 2016; Gümüş et al., 2018; Kapıkıran, 2016; Mellor et al., 2008; Swami et al., 2007; Tel et al., 2020). In this respect, it can be thought that the nursing home environment, where they can socialize and make

friends instead of living alone at home, removes the feeling of loneliness and is effective in increasing their life satisfaction.

In the study, it was determined that the participants who evaluated their health as good had higher life satisfaction than those who evaluated their health as moderate or bad. In a study conducted with elderly people living in nursing homes, it was determined that as their physical health increased and their chronic diseases decreased, their life satisfaction increased (Birinci et al., 2017). In a study conducted by Erol et al. (2016), the life satisfaction of the elderly without chronic disease was found to be higher than those with chronic disease. In a study conducted with elderly individuals, it was found that physical health status was a significant predictor of life satisfaction scores of the elderly (Boylu & Günay, 2018). Individuals feel closer to death due to many changes and chronic diseases that occur in old age, and decrease in physical strength (Üstüner Top et al., 2010). It is stated that the elderly who experience intense death anxiety are not satisfied with life and spend this period unhappy and alone (Engin et al., 2016). In a study conducted with elderly people living at home, it was determined that as death anxiety increases, life satisfaction decreases (Tel et al., 2020). The individual, who is in good health until old age, meets his own needs and is in a productive position for the society, can become a consuming individual with an increased need for help and a decrease in life satisfaction as a result of his health condition worsening in old age.

In the study, it was determined that those who perceive old age as a part of life have higher life satisfaction than those who perceive old age as discomfort, uselessness and a bad situation. In another study, it was stated that the elderly had negative thoughts about old age and their health, and this thought was caused by many factors such as education level, difficulty in performing activities of daily living, inability to do any activity and chronic diseases (Zanesco et al, 2018). In another study, it was reported that those who do not actively participate in social life, those who actively participate in social error, those who care for their grandchildren, those who do not care for their grandchildren, those who are retired, define themselves as older than their peers (Liu et al, 2019). The fact that individuals become dependent on others as a result of the decrease in productivity and the emergence of health problems with aging may have been effective in the elderly's evaluation of old age as useless and a bad situation.

5. CONCLUSION AND RECOMMENDATIONS

In this study, which was carried out with the aim of determining the relationship between health perception and life satisfaction of elderly people living in nursing homes; It has been determined that there is no relationship between health perception and life satisfaction, there is a statistically significant difference between the status of having a chronic health problem, the place they want to live and the health perception scale, the place they want to live, evaluating their health, perceiving their age and old age, and the life satisfaction scale.

In line with the results obtained; To make evaluations and plans to protect and maintain the physical and mental health of the elderly in nursing homes, to increase the social activities and social relations of the elderly in the nursing home in order to reduce the feeling of loneliness and loneliness, to direct the elderly to activities that will support their life satisfaction, to improve the health perceptions and life satisfaction of the elderly. It has been suggested to plan and implement the elderly individuals by considering their individual characteristics such as age, education level, chronic disease status, as well as environmental factors such as living space, and to conduct qualitative studies on loneliness, life satisfaction and health perception in the elderly.

Conflict of Interest

There is no conflict of interest between the authors.

Author's Contributions

The authors declare that they have contributed equally to the article.

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İnkübasyon Süresinin Kefir Kültürü ile Hazırlanan Kefir İçeceklerinin Raf Ömrüne Etkisi

The Effect of Incubation Period on the Shelf Life of Kefir Beverage Prepared with Kefir Culture

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Makale Bilgileri

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Anahtar Kelimeler
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Lactococcus türleri,
Maya

Öz

Bu çalışmanın amacı farklı inkübasyon sürelerinin kefirin raf ömrüne olan etkisini incelemekti. Bu amaçla kefir örnekleri ilk önce 8, 12, 18, 24 ve 36 saat inkübasyona tabi tutularak 5 farklı kefir grubu oluşturuldu. Daha sonra 4±1°C'de 21 gün boyunca depolandı. Muhafaza süresi boyunca kefir gruplarının mikrobiyolojik (*Lactobacillus* spp., *Lactococcus* spp. ve maya), kimyasal (pH, titre edilebilir asitlik) ve duyusal analizleri yapıldı. Uzun süre inkübasyon uygulanan kefir gruplarında Laktobasil (7.80 log10 kob/mL), laktokok (7.30 log10 kob/mL) ve maya (6.14 log10 kob/mL) sayılarının daha yüksek olduğu, muhafaza sürecinde de inkübasyon süresine bağlı olarak değişimlerin olduğu görülmüştür. İnkubasyon süresi ve muhafaza süresi uzadıkça titre edilebilir asitliğin arttığı belirlenmiştir. Sonuçlar inkübasyon süresinin *Lactobacillus* spp. ve *Lactococcus* spp. sayıları, pH, asitlik üzerinde etkili olduğunu ($P<0.05$) göstermiştir.

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Abstract

The aim of this study was to examine the effect of different incubation times on the shelf life of kefir. For this purpose, kefir samples were first incubated for 8, 12, 18, 24 and 36 hours, creating 5 different kefir groups. It was then stored at 4±1°C for 21 days.. Microbiological (*Lactobacillus* spp., *Lactococcus* spp., and yeast), chemical (pH, titratable acidity), and sensory analyses of the kefir groups were performed during the storage period. The *Lactobacillus* (7.80 log10 cfu/mL), *Lactococcus* (7.30 log10 cfu/mL) and yeast (6.14 log10 cfu/mL) counts were higher in the kefir groups that were incubated for a long time, and changes were observed during the storage process depending on the incubation time. It was determined that the titratable acidity became higher as the length of the incubation and storage time increased. The results showed that the incubation time had an effect on *Lactobacillus* spp. and *Lactococcus* spp. counts, pH and acidity ($P<0.05$).

1. INTRODUCTION

The word probiotic is a Latin term that means "for life". Probiotics are foods of live microbial origin that benefit humans by maintaining the intestinal flora (FAO/WHO 2001). Probiotics ensure a stable environment for beneficial bacteria in the gastrointestinal system

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and support their function by protecting the intestinal microflora. Thus, the intestinal flora plays an active role against infections and prevents the proliferation of harmful microorganisms (Güven et al., 2021; Karahan & Güvener, 1999).

Probiotics are found naturally in fermented dairy products. One of these is kefir, which is formed as a result of ethyl alcohol and lactic acid fermentation. Kefir is a slightly acidic, refreshing fermented milk product obtained by adding kefir grains to the milk of various animals, including that of sheep, goats, camels, mares, and especially cows (Kakisu et al. 2011 Kurman et al., 1992). Kefir can be prepared from whole, semi-skimmed, or skimmed milk, as well as from vegetable sources such as rice, nuts, coconut and soy milk (Dahiya & Nigam, 2023; Gocer & Koptagel, 2023; Otles & Cagindi 2003; Rosa et al., 2017).

It has been stated that kefir was first made by the Turks in Southwest Asia (Yüksekdağ & Beyatlı, 2003). Kefir contains all the nutrients of milk as well as essential fatty and amino acids that are extremely important for the body. In addition, kefir is rich in B vitamins, vitamin K, and folic acid. Kefir is a good source of calcium and also contains potassium, iron, copper, phosphorus, magnesium, cobalt, zinc, and manganese. Kefir, which is of great importance in a healthy diet, can be consumed without problems by individuals with lactose intolerant, because it contains less lactose than milk (Saloff-Coste, 1996). During kefir formation, the metabolites produced by lactic bacteria (López-Cuellar et al., 2016, Zhang et al., 2014), strengthen the immune system by suppressing the growth of pathogenic microorganisms (Amirbozorgi et al., 2016; Erdoğmuş & Bostancı, 2020). Erdoğmuş and Bostancı (2020) reported in their study that the substances obtained from lactic acid bacteria isolated in kefir samples exhibited high antimicrobial effects on pathogenic microorganisms. In addition to its especially positive effects on the digestive system, kefir has been reported to be highly effective in controlling obesity, regulation of blood sugar, reduction in levels of serum cholesterol, regulation of blood pressure, and prevention of allergy and tumor formation (Bengoa et al., 2018; Kadıoğlu, 2017). These features are provided by kefir grains, 3 to 35mm in size and having the appearance of hard, yellowish pieces of cauliflower (Arslan, 2015). Kefir grains are composed of gelatinous colonies formed by a combination of several bacteria and yeast species. Many kinds of bacteria and yeast with symbiotic metabolic activity are effective in the formation of kefir. They ferment milk to form substances such as lactic acid, ethyl alcohol, CO_2, acetone, and acetaldehyde, diacetyl, all of which provide the organoleptic

properties of kefir (Anonymous, 2016). Furthermore, the taste and composition of kefir may vary depending on the type and characteristics of the milk used, the kefir production technique, the fermentation temperature of the milk, the time and temperature of incubation and storage conditions. The diverse microflora of the kefir culture, living or dead, are effective in the formation of the characteristic properties of kefir (Kadıoğlu, 2017). Many microorganisms form the microflora of kefir, including bacteria like *L. acidophilus, L. brevis, L. casei, Leuconostoc mesenteroides* subsp*. dextranicum, Streptococcus lactis* ssp*. cremoris, S. citrovorum, L. fermantum, L. caucasicus, L. Helveticus, Acetobacter rasens, Acetobacter aceti, S. durans, and S. diacetylactis,* with many microorganisms such as *Saccharomyces cerevisiae, Kluyveromyces marxianus* subsp. *marxianus*, and *Torulaspora delbrueckii* (Güzel-Seydim et al., 2011; Witthuhn, 2005). The types of microorganisms in the grains and their ratio to each other may vary according to the origin of the grains. In all kefir production, lactic fermentation, alcohol fermentation, kefir-specific yeast flavor formation, and slow proteolysis fermentation occur during fermentation (Konar & Şahan, 1989). In addition, the largest changes in kefir formation occur during the fermentation phase (Gawel & Gromadka, 1978). The chemical and biochemical events that began at this stage continue in the kefir storage phase (Kılıç et al., 2001). In kefir production, the fermentation time, storage time, and changes in production temperature can affect the number and type of microorganisms in the kefir and its sensory, physical, and chemical properties (Yaygın, 1995).

From this point of view, with this study, kefir groups were formed and incubated at different times to determine changes in the microflora and chemical and sensory properties of kefir samples kept for 21 days in cold storage (4±1°C) depending on the incubation times.

2. MATERIAL AND METHODS

2.1. Material

The cow milk used in kefir production was obtained from the Kafkas University Veterinary Faculty Farm, and the Lyophilised kefir culture was obtained from the Wisby company.

2.2. Preparation of Samples

Kefir production by culture; First of all, a few of the lyophilized cultures Kefir culture was prepared by making a passage. Milk with a fat-free dry matter of 9.4% and a fat content of 3.2% was pasteurized at 90°C for 5 min, cooled to 25°C, put into sterile glass bottles and inoculated with kefir culture at a rate of 4.5%. Afterwards inoculation with the culture, the milk was divided into five groups: 8 h, 12 h, 18 h, 24 h, and 36 h. Each group was incubated separately at 25±1^{o}C for the specified times. After the incubation period was completed, kefir groups were separated from the culture under sterile conditions and stored at 4°C.

2.3. Analytical Methods

To monitor the development of the kefir microflora, a 1 mL sample was taken under aseptic conditions and mixed with 9 mL of 0.1% peptone water in a sterile tube using a vortexer. Ten-fold dilutions were then prepared, taking into account the estimated number of bacteria.

For the *Lactobacillus* spp., MRS agar (Oxoid CM 361) was used. Using the spread plate method, 0.1 mL of inoculation was carried out on the medium with the pH adjusted to 5.7. After 36 h incubation at 35°C anaerobically (AnaeroGen-Oxoid), the petri dishes were evaluated. Typical colonies of 1 to3 mm in diameter were counted on the petri dishes after microscopic verification (IDF, 1983). For counting the *Lactococcus* spp., M17 Agar (Oxoid CM785) adjusted to pH 6.9 was used. After inoculating the spread plates, the samples were incubated at 35°C for 36 h aerobically. Typical colonies of 1 to 2 mm in diameter were counted after confirmation by microscopic examination (Dave & Sha, 1996). Potato dextrose agar (Oxoid CM 139) adjusted to pH 5.6 was used for counting. After inoculation and incubation at 22°C for five days, the counted colonies were evaluated. After counting the typical colonies growing in all the morphologically evaluated growth media, the amount of cfu/mL was calculated (Dave & Sha, 1996; Elmer & James, 2001). All analyses were repeated twice.

The pH analysis of the samples was carried out using a pH meter (Hanna HI 8521), and acidity in terms of lactic acid (LA)% was determined by the titration method (Meyer et al., 2007).

Sensory analysis was applied to kefir samples whose incubation period was completed. Five experienced panelists evaluated the kefir samples sensorially based on appearance, consistency, odor and taste. After the panelists had first interpreted the appearance and then

the consistency, the kefir was mixed and analyzed in terms of smell and flavor. Water was provided to refresh the mouth between samples. Each panelist evaluated the kefir according to the specified qualities using the 5-point hedonic test scale (1-worst, 5-very good) indicated on the form (Clark et al. 2008; Metin, 2006). All of the analyses were done twice.

2.4. Statistical Analysis

SPSS 18 package program was used to interpret the data obtained from the analyzes performed in duplicate. The Tukey test was used to evaluate the difference between groups. The results were presented as; mean (±) and standard error (x±Sx). (Pripp 2013).

3. RESULTS

During storage, the kefir samples showed statistically significant ($P<0.05$) changes depending on the last incubation time. In the kefir groups incubated for 8, 12, 18, 24, and 36 hours, the initial lactic acid bacteria count was determined as 5.17, 5.17, 7.64, 7.10, and 7.80 log cfu/g, respectively. On day 21 of cold storage, the lactic acid bacteria count of the kefir samples (incubated for 8, 12, 18, 24, and 36 hours) was determined to be 5.15, 5.15, 7.55, 4.95 and 7.76 log cfu/g, respectively. While Lactococcus bacteria counts in the groups were 4.80-7.30 log10 cfu/ml at the beginning, they changed between 4.46-7.76 log10 cfu/ml at the end of the 21st day. At the end of the 21-day storage period, the lowest yeast count was 3.42 log10 cfu/ml (8 h.) and the highest yeast count was 5.15 log10 cfu/ml (36 h.) ($P<0.05$). The difference between the kefir groups was statistically significant. The changes in the kefir groups (0, 1, 3, 7, 10, 14, 18, and 21 days) are given in Table 1.

At the end of the incubation, the highest pH value (5.10) was in the kefir group incubated for 8 h, and the lowest (4.40) was in the kefir group incubated for 36 h. This did not change during the cold storage period. At the end of the 21-day storage period, the acidity of the groups varied between 0.42-0.69%. The changes in the kefir groups (0, 1, 3, 7, 10, 14, 18, and 21 days) are given in Table 2. After the sensory analyses, the group with the highest score from the panelists was the kefir group incubated for 18 hours. The results of the sensory analysis are given in Table 3. and Figure 1.

Table 1. Average values for the microbiological parameters of the samples during the storage period (log10 cfu/mL±Std deviation)

	Groups	0. day (X±Sx)		1. day (X±Sx)		3. day (X±Sx)		7. day (X±Sx)		10. day (X±Sx)		14. day (X±Sx)		18. day (X±Sx)		21. day (X±Sx)		P
	Culture	5.10	0.02^{BCa}	5.10	0.02^{BCa}	5.11	0.04^{Ca}	5.12	0.03^{Ca}	5.12	0.02^{Ca}	5.10	0.02^{BCa}	4.98	0.02^{Cb}	4.95	0.03^{Cb}	*
	8. h.	5.17	0.04^{b}	5.16	0.04^{b}	5.17	0.03^{b}	5.16	0.02^{b}	5.16	0.03^{b}	5.16	0.03^{b}	5.15	0.03^{b}	5.15	0.02^{b}	-
Anaerobic	12. h.	5.17	0.02^{b}	5.17	0.02^{b}	5.16	0.04^{b}	5.16	0.04^{b}	6.17	0.02^{b}	5.17	0.02^{b}	5.16	0.04^{b}	5.15	0.02^{b}	-
Lactobacillus spp.	18. h.	7.64	0.02^{Ba}	7.62	0.03^{Ba}	7.62	0.03^{Ba}	7.60	0.02^{Ba}	7.58	0.04^{Aa}	7.58	0.03^{Aa}	7.58	0.02^{Aa}	7.55	0.02^{Aa}	-
	24. h.	7.10	0.02^{BCa}	5.10	0.02^{BCb}	5.11	0.04^{Cb}	5.12	0.03^{Cb}	5.12	0.02^{Cb}	5.10	0.02^{BCb}	4.98	0.02^{Cc}	4.95	0.03^{Cc}	*
	36. h.	7.80	0.04^{a}	7.82	0.03^{a}	7.81	0.02^{a}	7.85	0.02^{a}	7.83	0.03^{a}	7.81	0.02^{a}	7.80	0.02^{a}	7.76	0.02^{a}	-
	p		*		*		*		*		*		*		*		*	
	Culture	4.60	0.04^{Dd}	4.60	0.03^{Dd}	4.60	0.02^{Dd}	4.58	0.02^{Dd}	4.55	0.03^{Dd}	4.53	0.02^{Dd}	4.49	0.04^{Cd}	4.46	0.02^{Cd}	-
	8. h.	5.12	0.03^{b}	5.12	0.02^{b}	5.11	0.04^{b}	5.12	0.02^{b}	5.12	0.02^{b}	5.12	0.03^{b}	5.11	0.02^{b}	5.11	0.04^{b}	-
	12. h.	4.80	0.03^{Dc}	4.80	0.02^{Dc}	4.85	0.03^{Dc}	4.84	0.02^{Cc}	4.86	0.02^{Cc}	4.82	0.04^{c}	4.80	0.02^{Bc}	4.76	0.03^{Bc}	-
Lactococcus spp.	18. h.	5.11	0.02^{Cb}	5.11	0.03^{Cb}	5.11	0.02^{Cb}	5.11	0.04^{Cb}	5.11	0.02^{Cb}	5.11	0.03^{Cb}	5.10	0.02^{Bb}	4.98	0.02^{Bc}	-
	24. h.	5.18	0.03^{Cb}	5.18	0.02^{Cb}	5.18	0.03^{Cb}	5.18	0.02^{Cb}	5.17	0.04^{Cb}	5.17	0.02^{Cb}	5.17	0.03^{Bb}	5.16	0.02^{Bb}	-
	36. h.	7.30	0.02^{Ba}	7.33	0.03^{Ba}	7.35	0.02^{Ba}	7.34	0.03^{Ba}	7.38	0.02^{Ba}	7.32	0.03^{Ba}	7.29	0.03^{Aa}	7.26	0.04^{Aa}	-
	p		*		*		*		*		*		*		*		*	
	Culture	6.50	0.03^{Aa}	6.48	0.02^{Aa}	6.48	0.02^{Aa}	6.45	0.02^{Aa}	6.40	0.02^{Aa}	6.38	0.04^{Aa}	6.37	0.02^{Aa}	6.35	0.03^{Aa}	-
	8. h.	3.50	0.02^{Cd}	3.50	0.03^{Cd}	3.51	0.02^{Cd}	3.53	0.02^{Cd}	3.51	0.03^{Cd}	3.49	0.02^{Cd}	3.47	0.03^{Bd}	3.42	0.02^{Cd}	-
	12. h.	3.50	0.02^{Cd}	3.51	0.02^{Cd}	3.53	0.04^{Cd}	3.55	0.02^{Cd}	3.57	0.02^{Cd}	3.60	0.02^{Cd}	3.62	0.02^{Cd}	3.65	0.03^{Cd}	-
Yeast	18. h.	4.90	0.04^{Bc}	4.90	0.02^{Bc}	4.92	0.02^{Bc}	4.95	0.03^{Bc}	4.93	0.03^{Bc}	4.95	0.03^{Bc}	4.93	0.03^{Bc}	4.94	0.03^{Ac}	-
	24. h.	4.80	0.02^{Bc}	4.80	0.02^{Bc}	4.82	0.02^{Bc}	4.84	0.03^{Bc}	4.86	0.02^{Bc}	4.87	0.02^{Bc}	4.85	0.02^{Bc}	4.89	0.02^{Bc}	-
	36. h.	6.14	0.02^{Ab}	6.14	0.04^{Ab}	6.15	0.02^{Ab}	6.14	0.02^{Ab}	6.15	0.03^{Ab}	6.16	0.04^{Ab}	5.15	0.02^{Ac}	5.15	0.02^{Ac}	*
			*		*		*		*		*		*		*		*	

Capital letters (A, B, C,...) indicate statistical difference between groups in the same column, while miniscule letters (a, b, c,..) indicate the statistical difference between groups on the same line. *: The statistical difference is important (P<0.05).

Table 2: Average values for the chemical parameters of samples during the storage period (log10 cfu/mL±Std deviation)

	Groups	0. day (X±Sx)		1. day (X±Sx)		3.day (X±Sx)		7.day (X±Sx)		10.day (X±Sx)		14.day (X±Sx)		18.day (X±Sx)		21.day (X±Sx)		P
pH	8. h.	6.20	0.03^{Aa}	6.10	0.02^{Aa}	6.00	0.02^{Ab}	6.00	0.03^{Ab}	5.80	0.02^{Ac}	5.60	0.02^{Ad}	5.20	0.02^{Ae}	5.10	0.02^{Ae}	*
	12. h.	6.10	0.02^{Aa}	6.00	0.02^{Aa}	6.00	0.02^{Aa}	5.90	0.02^{Ab}	5.70	0.02^{Ac}	5.30	0.02^{Bd}	5.30	0.02^{Ad}	5.00	0.02^{Ae}	*
	18. h.	6.05	0.02^{Aa}	5.80	0.04^{Bb}	5.30	0.03^{Bc}	5.20	0.02^{Bc}	5.10	0.03^{Bc}	4.90	0.03^{Bd}	4.80	0.03^{Bd}	4.70	0.02^{Be}	*
	24. h.	6.00	0.02^{Aa}	5.60	0.02^{Bb}	5.30	0.02^{Bc}	5.20	0.02^{Bc}	5.00	0.02^{Bd}	4.90	0.02^{Bd}	4.80	0.02^{Bd}	4.60	0.03^{Be}	*
	36. h.	5.80	0.04^{Ba}	5.30	0.03^{Cb}	5.10	0.02^{Cb}	5.00	0.02^{Cc}	4.80	0.03^{Cd}	4.70	0.02^{Cd}	4.60	0.02^{Ce}	4.40	0.02^{Cf}	*
	p		*		*		*		*		*		*		*		*	
	8. h.	0.21	0.01^{Cg}	0.23	0.00^{Df}	0.30	0.00^{Ce}	0.31	0.00^{Bd}	0.33	0.00^{Cd}	0.38	0.01^{Cc}	0.40	0.00^{Bb}	0.42	0.01^{Ba}	*
	12. h.	0.31	0.01^{Be}	0.35	0.00^{Cc}	0.32	0.00^{Bd}	0.35	0.00^{Bc}	0.39	0.01^{Cb}	0.40	0.01^{Bb}	0.42	0.00^{Ba}	0.45	0.01^{Ba}	*
	18. h.	0.37	0.00^{Bd}	0.42	0.01^{Cd}	0.50	0.01^{Ac}	0.55	0.01^{Ac}	0.58	0.00^{Bb}	0.61	0.00^{Ab}	0.65	0.00^{Aa}	0.66	0.00^{Aa}	*
Acidity	24. h.	0.43	0.01^{Ad}	0.49	0.00^{Bc}	0.52	0.00^{Ac}	0.57	0.00^{Ab}	0.59	0.01^{Bb}	0.62	0.00^{Ab}	0.66	0.01^{Aa}	0.68	0.00^{Aa}	*
	36. h.	0.50	0.00^{Ad}	0.53	0.01^{Ac}	0.55	0.00^{Ac}	0.59	0.01^{Ab}	0.60	0.01^{Ab}	0.63	0.00^{Ab}	0.67	0.01^{Aa}	0.69	0.00^{Aa}	*
	p		*		*		*		*		*		*		*		*	

Capital letters (A, B, C,…) indicate statistical difference between groups in the same column, while miniscule letters (a, b, c,..) indicate the statistical difference between groups on the same line. *: The statistical difference is important (P<0.05).

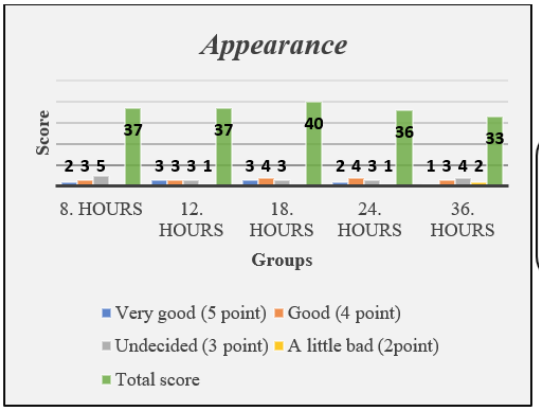

Figure 1. Appearance analysis results of kefir samples

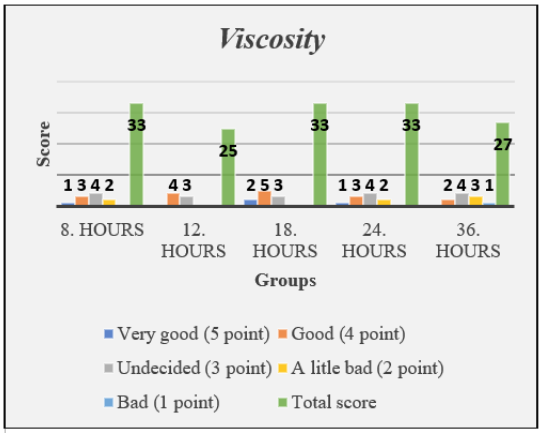

Figure 2. Viscosity analysis results of kefir samples

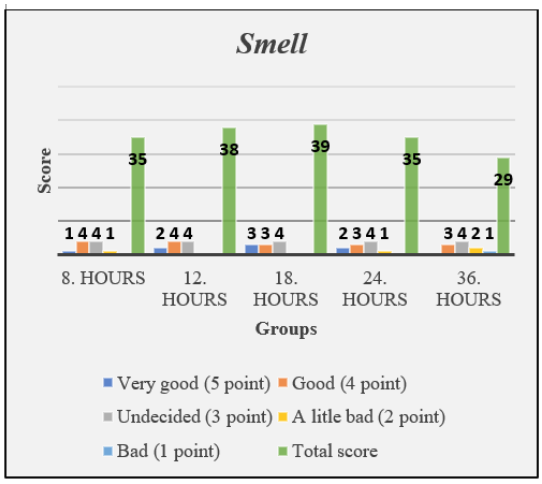

Figure 3. Smell analysis results of kefir samples

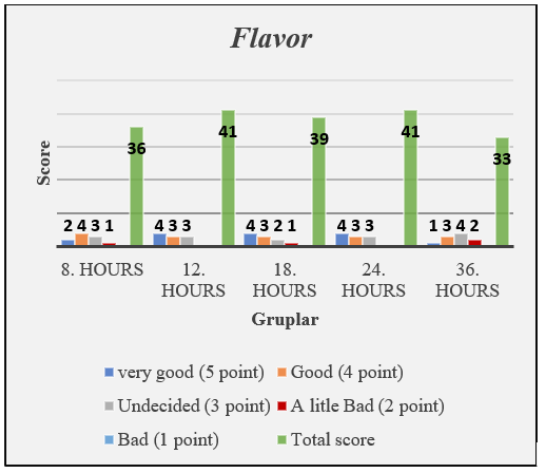

Figure 4. Flavor analysis results of kefir samples

4. DISCUSSION

The yeast and bacteria in the microflora of kefir culture are directly effective in the formation of the microflora of the kefir without any heat treatment. Accordingly, the bacterial load in kefir may vary depending on the microflora characteristics of the kefir culture used. The location of the yeasts in the kefir grain and their ability to ferment lactose are properties that can directly affect the microflora. These active flora have been reported to actively maintain their vitality in the final product (Farnworth, 2005). In this study, the kefir culture retained its vitality during storage and, during the storage process, depending on the incubation period, changes occurred in the kefir groups prepared using the kefir culture.

The bacterial load and microflora of the kefir samples varied depending on the incubation time. It was observed that the number of lactic acid bacteria and yeasts forming the kefir microflora increased significantly as the incubation period increased.

The initial lactobacillus counts were found to be quite high in the kefir groups with an incubation period of 8 and 36 h. At the end of the storage period, the highest lactobacillus count was also in parallel with these groups. This may have been due to the fact that increased acidity is well-tolerated in lactobacilli.

Güzel-Seydim et al. (2000) stated that lactic acid bacteria (LAB) increased during the fermentation period because of the increase in the amount of lactic acid. Similarly, in this study, it was observed that with the increase of acidity, the LAB increased throughout the storage period. During the storage period, the results showed statistically significant changes in proportion to the final number of fermentation hours. During the trial period, the lactobacilli counts in the kefir groups were between 4.95 and 7.85 log10 cfu/mL, which were similar to those reported by Kesmen and Kaçmaz (2011) but lower than the values of Garrote et al. (2001).

Güzel-Seydim et al. (2005) stated in their study that at 0, 5, 10, 15, and 22 h of kefir fermentation, the LAB numbers gradually increased compared to the initial numbers. In this study, it was observed that the LAB numbers increased in parallel with the fermentation period. Throughout the cold storage duration, the bacterial load in the kefir groups changed in proportion to the bacterial load at the end of the incubation period, and the lactobacilli count decreased, similar to the findings of Iriyogen et al. [35]. This decrease in kefir culture was statistically significant in kefir groups incubated for 24 hours. ($P<0.05$).

Fontan et al. (2006). stated that lactococci were dominant in the microbial flora in the first 48 h of fermentation, but that the number of lactobacilli surpassed them after 48 h. In this study, lactobacillus counts were more dominant in the first hours of fermentation (5.1 log cfu/mL), whereas after 36 h, lactococci were more dominant (7.3 log cfu/mL), with numbers increasing in parallel with the increase in fermentation time and approaching the lactobacillus counts. The study of Kök-Taş et al. (2012) reported the lowest number of *Lactococcus* spp. as 6.3 log cfu/mL, and the highest as 9.1 log cfu/mL, whereas the lactococcus counts in the kefir groups in this study during the trial period exhibited lower values of between 4.46 and 7.38 log cfu/mL. This difference may be due to the microflora variations in the kefir cultures used or differences in the production technique.

Irigoyen et al. (2005). and Öner et al. (2010). stated in their studies that there was a reduction in the number of lactococcus bacteria during the cold storage period. Similarly, in this study, compared to the initial numbers, there was a decrease in the number of lactococci in the kefir groups on the 21st day of cold storage. Statistically significant decreases were seen in the culture during storage. The 12- and 36-h kefir incubation groups were found to have 4.76, and 7.26 log cfu/mL at the end of the 21st day, respectively.

The Turkish Food Codex Fermented Milks Communiqué states that kefir must contain yeast at a level of at least 10^4 cfu/mL (Anonymous, 2009). The results obtained from this study were determined to be in accordance with the Turkish Food Codex Communiqué on Fermented Milk. Although the amount of yeast in the samples decreased compared to the kefir culture at the end of the first 8 h of incubation, it increased in parallel with the increase in the incubation period, as in the study of Güzel-Seydim (2005)., and approached the number in the kefir culture at the end of the last 36 h of the incubation period. As in the studies of Güzel-Seydim et al. (2005) and Öner et al. (2010), during the storage period, an increase in the kefir cultures was seen in the kefir groups incubated for 12, 18, and 24 h. However, a statistically significant decrease in kefir cultures was observed in the kefir groups incubated for 36 h ($P<0.05$). During the trial period, a change varying between 3.420 and 6.500 log cfu/mL was found which was lower than in the study of Karagözlü (1990).

As the incubation time of kefir samples increased, the pH value decreased. In the study of Graciela et al. (2001), the pH value of kefir samples was measured between 3.5 and 4. Another study found pH values of kefir groups to be 4.08-4.10 at the 24th h of the storage period (Sezer, 2003), whereas Ergin et al. (2017) reported kefir pH values of between 4.54 and

4.59. In this study, the pH values of the kefir groups were determined to be 4.40-5.10 at the end of the 24th h. In a study in which pH analysis was performed on kefir samples incubated for 24 h, pH changes were between 6.60 and 3.79 during the storage period (Tan & Ertekin, 2017). In this study, pH values of the kefir groups incubated for 24 h were between 6.00 and 4.60. It was thought that the pH differences determined in the kefir samples might have occurred because of the chemical properties of the milk used in the study or the microflora of the kefir culture.

As in the studies of Sezer (2003) and Karagözlü (1990) the pH values of the kefir groups decreased and acidity increased during the storage period. This may be associated with the decrease in pH as a result of the bacteria of the lactobacillus group increasing the production of lactic acid in the environment. Ergin et al. (2017) stated that the acidity value of their samples varied between 0.73% and 0.87% during storage, whereas the acidity of the kefir samples in this study during storage was between 0.21% and 0.69%. In a study, different types of kefir were produced and the acidity value of plain kefir was determined as 0.300% after 12 hours of incubation (Harmankaya, et al. 2019). Similarly in this study, upon chemical analysis of the kefir groups, acidity values were the similary (0.31% LA) at the end of the 12^{th} hour incubations. Among all groups, at the end of the storage period, the kefir group incubated for the longest time (36 h) showed the highest acidity (0.69% LA). At the end of the study, it was concluded that change in the duration of the incubation affected the acidity and the acidity increased as the storage time was extended ($P<0.05$).

In a number of studies it is stated that changes in the chemical and microbiological properties of kefir directly affect its sensory properties (Tekinşen & Atasever, 1994; Toklu, 1999). In this study, it was observed that chemical and microbiological changes that occurred depending on the incubation period also affected the sensory properties.

Sensually, good kefir should have a fluid consistency, a homogeneous and light appearance, and a light yeasty taste. The aroma should be experienced when consumed, and it should have a refreshing quality (İnal, 1990). In the sensory analysis of the kefir evaluated considering these properties, the kefir group incubated for 18 h received the highest score in appearance (40). The highest score for viscosity was found for the kefir incubated for 8 h, 18 h, 24 h and the lowest for that incubated for 36 h. The panelists stated that the consistency of the kefir increased as the duration of incubation increased and it became unacceptable by the end of 36 h. In the smell analysis, the kefir group that was least liked was the one that had

been incubated for 36 h. This was thought to have been the result of the increase in acidity. In the flavor analysis, the 12th (41) and 24th (41) h incubation groups had the highest scores. This may have been because of the amounts of flavor-affecting substances such as CO_2, lactic acid, ethanol, acetone, and acetaldehyde formed during the fermentation period (Vedamutlu, 1997).

5. CONCLUSION

In conclusion, it was observed that the incubation period was effective on the acidity and pH values of the kefir, especially for the long storage period. The acidity of the kefir groups increased with the long incubation period and the kefir was not acceptable in terms of sensory aspects. Although the lactobacillus counts exhibited a decrease in the kefir groups incubated for 12, 18, and 24 h, they increased with rising titratable acidity at the end of the 36th h. At the end of the study, the highest lactobacillus counts were found in the kefir groups incubated for 8 and 36 h. This can be explained by the ability of lactobacilli to tolerate high acidity well. Similarly, at the end of the study, lactococcal counts were found to be the highest in kefir incubated for 36 h. By resisting the developing acidity as the kefir incubation period increased, the yeast reached the highest counts in the kefir groups that were incubated for the longest time. At the end of the study, it was concluded that the incubation period had affected the chemical, microbiological, and sensory properties of the kefir.

Conflict Of Interest

The article authors declare that there is no conflict of interest between them.

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