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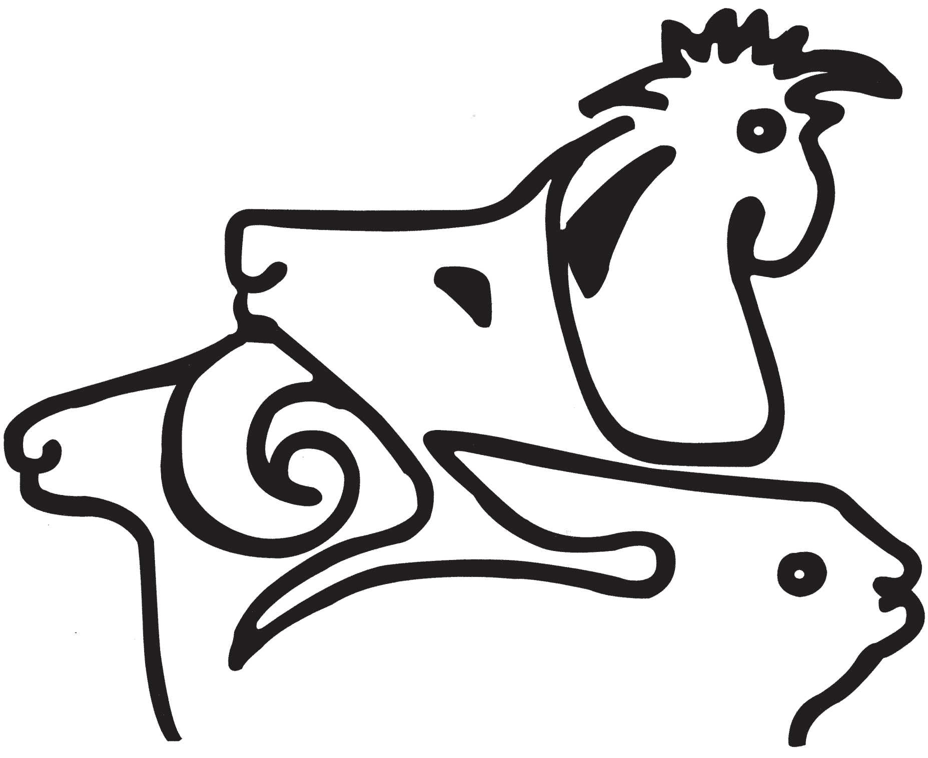

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Importance of Characterization of the Vaginal Microbiota in Ewes and Nannies

Koyun ve Keçilerde Vaginal Mikrobiata Karakterizasyonun Önemi

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Key Words:

Ewes, nannies, intra-vaginal device, vaginal flora, fertility

ABSTRACT

Normal vaginal flora of ewes and nannies creates pheremone effect in ram and buck and increases their sexual attraction. In case of any infection in reproductive tract, the structure of normal vaginal flora gets deteriorated. Deterioration of vaginal flora leads to change in chemical stimulants around vaginal surrounding and loss of attraction of females for males. The change of bacterial flora within vagina might affect the reproduction capacity of animal and significantly decreases to the insemination rate by causing sperm breaks in the reproductive organ even if the detected bacteria are not pathogenic. Since the basis of sustainability in the livestock stockbreeding is the effectiveness of fertility, knowing regular vaginal microbiota and the factors leading a change in this environment has an important role to increase the effectiveness of fertility.

Anahtar Kelimeler:

Koyun, keçi, vagina içi araç, vaginal flora, döl verimi

ÖZ

Koyun ve keçilerde normal vaginal flora koç ve tekelerde feromon etkisi yaratarak cinsel çekiciliği artırır. Üreme kanallarındaki herhangi bir enfeksiyonda antibiyotik kullanımı sonucu normal vaginal floranın yapısı bozulmaktadır. Vaginal floranın bozulması, vaginal çevredeki kimyasal uyarıcıların değişmesine ve dişilerin erkekler için çekiciliğini yitirmesine yol açmaktadır. Vaginadaki bakteriyel floranın değişmesi, saptanan bakteriler patojenik olmasa bile hayvanın üreme yeteneğini etkileyebilmekte, üreme organında sperm kırılmalarına yol açarak döl tutma oranını belirgin ölçüde düşürmektedir. Hayvancılığın sürdürülebilirliğinin temeli döl verimi olduğundan normal vaginal mikrobiatanın ve bu ortamın değişmesine yol açan faktörlerin bilinmesi döl veriminin artırılmasında önemli rol oynamaktadır.

INTRODUCTION

All kinds of effectiveness in livestock depend on the continuity of the sperm effectiveness. Growing of herds and increase in their yields is only possible by a healthy fertility. Increase in sperm effectiveness of ewe and goat can be provided at first by performing the inseminations on time and regularly at herd level. Besides, since sheep and goat are among the species that come in heat seasonally, they show mating desire only in certain times of the year (Kaymakçı and Sönmez, 1996). At this point, creating the highest estrus reply during anoestrus period and control of estrus cycles through hormonal practices in order to obtain high pregnancy rate is the most effective instrument of management in increase of reproduction productivity (Kusina et al., 2000). Progestogen impregnated sponges (FGA-fluorogestone acetate), (MAP-medroxyprogesterone acetate) and hard medical silicons containing progesterone called CIDR (controlled internal drug release) are commonly used in order to stimulate ovarium activity in ewe and goats (Dellal and Cedden, 2002; Emsen and Koşum, 2009; Rowe et al., 2009; Souza et al., 2011; Zohara et al., 2014).

However, the vaginal devices used may create an environment that paves a way for infection by causing change in profile and number of bacteria in uterus and vagina (Padula and Macmillan, 2006). This environment causes inflammatory reaction by triggering the mucus accumulation and the bacterial load increase in vagina. Consequently, increase in vaginal flora and bloody and stinky discharge takes place during the removal of vaginal devices. As a result of these problems, opportunistic secondary pathogens in reproductive organs of animals cause vaginitis, and fertility capacity of the animal is blocked (Root Kustritz, 2006). The used intravaginal devices cause mechanic irritation in vagina as well as reduce lymphocyte increase and $PGF_{2\alpha}$ production due to progesterone hormone in their structure, and this weakens the capacity of animals to block or cope with infections (Manes et al., 2010).

Abnormal vaginal secretion was associated with high rate of unfertilized egg existence in ewes to which artificial insemination was applied, and resulted by this low rate of pregnancy and embryo development (Scudamore, 1988). Increase in vaginal infection during pregnancy period may result with pre-term birth (Swartz et al., 2014). On the other hand, it is known that normal vaginal flora increases the sexual attraction in ewes. Structure of normal vaginal flora gets deformed due to antibiotic use in any reproductive tract infection, female animals lose their attraction for male animals as a result of changes in chemical stimulants around vaginal environment which leads to losses in sperm effectiveness (Ungerfeld and Silva, 2005).

In this context, the knowledge about normal vaginal bacteria flora is very important for the right diagnosis and treatment of pathologic abnormalities of the reproductive system. Under stressful conditions, the bacteria lead to genital infections which generally cause sperm holding deficiency in ruminants. Understanding the types of bacteria which are colonized in vagina and uterus will allow to determine antimicrobial sensitivities of these bacteria, and consequently, management of genital infection will be revealed in sheep and goat (Mshelia et al., 2014).

Normal Vaginal Flora and Vaginal pH in Ewes and Nannies

The microorganism societies, which are present in healthy animals' bodies and live there without causing any harm and sometimes even have benefits and live together with the organism are called "normal microbial flora" of the body. In order to detect disease factors caused by microorganisms, receive clinical sample in this context, carry and keep them if required and plant them into normal environments; normal body flora should be known. Normal flora members generally provide ecologic balance and prevent settlement of pathogen microorganisms, and increase body resistance against disease formation. However when the immune system is deformed, the bacteria here may cause opportunistic infections (Levinson and Javetz, 1996). In other words; although it is generally harmless for settled microbiota, some of these organisms become potential pathogens in the presence of trauma or infection and may cause disease (Martins et al., 2009).

Pena et al. (2013) and Oliveira et al. (2013) reported that *Staphylococcus spp.* was clinically the most common type of bacteria in vaginal microfloras of healthy sheep and goats. Swartz et al. (2014) identified that the most common types in ewes' vaginas were *Aggregatibacter spp.* and *Streptobacillus spp*. Sawyer (1977), and Manes et al. (2010) reported that, *Bacillus spp., Corynebacterium spp., Escherichia spp., Staphylococcus spp.*ve *Streptococcus spp.* types were also often isolated in ewes. Swartz et al. (2014), stated that *Lactobacillus spp.* kinds were rarely seen in vaginal microbiota as the vaginal pH value in ewes is close to neutral unlike in human vaginal flora. Because *Lactobacillus spp.*

types are intensely present in vagina means that a large number of lactate is produced, so pH value becomes low (<4.5) at that point. Manes et al.(2010) reported that vaginal pH varied between 7.0 - 7.6 before they applied sponge in ewes, but 53 hours after sponge application, that it decreased to 6.8. In goats, ruts of which are synchronized by CIDR; Widayati et al. (2010) detected vaginal pH as 8.55 in estrous and 36 hours after CIDRs are removed as 9.33. They stated that vaginal pH value varied between 6-7 range throughout dioestrus. Manes et al. (2013) also identified vaginal pH in goats as the highest (7.0) on estrous day, as 7.7 before vaginal devices were installed, as 7.9 on day 6 and 7.4 on day 11. Mahmoud (2013) stated vaginal pH as 6.74 for Ossimi sheep to which sponge was applied and as 6.80 to which sponge was not applied. Dogra et al. (2016) reported vaginal pH as 6.8 - 7.0 in goats to which progesterone implant was applied. Doğaneli et al. (1978) reported that vagina was more resistant during estrous period since pH was higher in that period than in luteal period, and that microorganisms were unable to settle easily in estrous period as there was much leukocyte increase.

Factors Leading to Change in Vaginal Microbiota

The vagina ecosystem consists of various components living in a sensitive balance. This ecosystem continuously contains metabolic products such as acids, carbohydrates and proteins as well as many types of bacteria that develop with distortion of dead bacteria cells and secrete cellular debris i.e. nucleic acids, fatty acids and sugars. This microflora consists of other organisms as well as of Gram positive and Gram negative aerobic facultative and obligate anaerobe bacteria. Among the countless current bacteria, there are pathogenic and non-pathogenic bacteria. When ecosystem is destroyed or becomes unbalanced, pathogenic bacteria become dominant and a possible threat for human health (Faro, 2006). It means that the changing vaginal flora consists of pathogenic microorganisms and permanent flora members which become dominant from time to time by various effects. Vasconcelos et al. (2016) also stated that microorganisms responsible from vaginitis were not different from the microorganisms (*Staphylococcus spp.)* that were commonly present in normal vaginal flora before internal vagina devices were installed. Vaginal bacterial flora is a sensitive system and varies under the effect of many endogenous and exogenous factors such as estrogen concentration, vaginal pH, temperature, diseases, medication, immunological conditions and microbial interactions (Bjurström and Linde-Forsberg, 1992). Differences that takes place in pH value during estrous or pregnancy may cause change in vaginal environment and consequently cause change in microbial flora. Thus, reproduction performance weakens and important economic losses takes place (Al-Hilli and Ajeel, 2015).

Opportunistic bacteria among microflora members play role in development of vaginitis and infections in genital organs when they find the suitable environment. Vaginitis in sheep and goat is a genital system disease which is mostly caused by coliform bacteria (Ababneh and Degefa, 2006; Root Kustritz, 2006; Martins et al., 2009). *Staphylococcus aureus* is the most common microorganism which is isolated from ewes with vaginitis. Severe erythema may occur associated with yellowish discharge and abundant vaginal leukocyte (Donders et al., 2002). *Escherichia coli* is known as an opportunistic second agent for bacterial vaginitis (Sargison et al., 2007). Sargison et al. (2007) reported that *Escherichia coli* causes abortus in Suffolk sheep on days 133 and 142 of pregnancy.

Change in bacterial flora in the vagina may affect reproduction capacity of the animal even the identified bacteria are not pathological, and significantly reduces the rate of sperm holding by causing sperm breaks in the reproductive organ (Hawk et al., 1981). In animals with vaginitis, although number of vaginal bacteria returns to basal values until estrous day, normal vagina flora composition changes. For example, the presence of opportunistic *Enterobacteriaceae* family is associated with vaginitis even 72 hours after the removal of vaginal devices. Likewise, the *Escherichia coli* which is observed most common after the removal of vaginal devices, reduces sperm mobility by causing sperms to stick and cluster and leads to morphological changes (Martins et al., 2010). Bacteria causing vaginitis may also cause a toxic effect on spermatozoa (Fraczek and Kurpisz, 2007). This effect prevents normal sperm activity by causing sperms not to penetrate into oocyte as a result of sperm membrane components and DNA fractionation (Aitken and Baker, 2006). These negative effects on sperm parameters are actually a result of a few mechanisms. Slight changes in pH and osmolality values lead to loss of sperm mobility. Actually, pH value increases after the use of internal vagina device, and spermatozoa vitality is adversely affected from excessive alkalinity in cervical mucus.

As the presence of intravaginal devices also stimulate bacterial growth, it leads to leukocyte and inflammatory accumulation at the inflamed area. The presence of these polyphormonuclear leukocytes is associated with subacute inflammatory response. The presence of biologically active substances during inflammation i.e. reactive oxygen tubes released by leukocytes lead to structural, metabolic and functional changes in sperm cells. All those reasons explain the significant decline in sperm quality (Manes et al., 2016). However, cervical mucus filters the sperms which have weak morphology and motility, and allows only a small percentage of the sperm to en womb. In short, spermatozoa are selected by natural mucus according to their progression ability. This progress is associated with fertilization capacity of spermatozoa. Spermatozoa penetration into cervical mucus is a reliable indication of sperm function (Suárez and Pacey, 2006).

Intravaginal devices are reported as factors which pave the way for vaginitis caused by opportunistic microorganisms. In various conducted studies, independently of progesterone presence and type of implant, along with typical clinical findings in ewes i.e. mucopurulent discharge and increased local sensitivity 6 days after vaginal devices are installed, it was stated that vaginitis was identified which caused an important increase in microbial load. Consequently, although these results make think that intravaginal devices might be a factor that creates a tendency to vaginitis in healthy ewes, it also shows the effect of physical activity of vaginal devices (Suárez et al., 2006; Sargison et al., 2007; Yeşilmen et al., 2008). As a result of sponge application, vaginal flora composition changes in ruminants and particularly there are significant increases in number of Gram negative *Enterobacteriaceace* (Suárez et al., 2006; Manes et al., 2010; Gatti et al., 2011). It is known that liposaccharides of gram negative bacteria lead to failing in sperm hold, abnormal embryonic development, pregnancy losses and infertility (Gorga et al., 2001; Deb et al., 2004a,b). But in goats, regardless of progesterone impregnated sponges' staying times in vagina, Gram-pozitive *Streptococcus,* coagulase-negative *Staphylococcus* and coagulase-positive *Staphylococcus* are the most common isolated bacteria (Penna et al., 2013). Vasconcelos et al. (2016) reported that vaginitis agents were the coliforms which replace normal vaginal microbiota 48 hours after implants were removed. And probably while clinical vaginitis is present, bacteria from coliform group originating from stools become dominant. Also in a study they made on ewes, they identified coliforms as vaginitis agent replacing *S. Aureus* which is mostly known as opportunistic bacteria in *Staphylococcus* population. Those findings show that after the use of intravaginal implants, vaginal microbiotics have increased in quantification as well as undergone an important change qualitatively. Penna et al. (2013) reported that on the day sponges were removed, vaginitis indications were observed in all goats 70.3% of which as bloody blurry and 29.7% as stinky discharge. Donders et al. (2002) pointed out that vaginal infections were characterized by a blurry discharge as well as by a plenty of leukocytes. Such changes in vaginal flora arise from physical actions of the used devices to hold and suck vaginal secretions during the time they stay in vagina, (Al-Hamedawi et al. 2003), and two days after the devices are removed, bacterial population in vagina return to numbers before the devices were installed (Amin, 1996). This decline in CFU/ ml arises from elimination of the irritant and also from the increase in local immunity response caused by estrogenic phase during proestrus and estrus (Martins et al., 2010). Manes et al. (2013) stated that there was no significant difference between the application dates in terms of numbers of vaginal bacteria, however; that the number decreased to the levels before devices were installed on the day estrus were observed. Yeşilmen et al. (2008) reported that progesterone had no significant effect on the increase in number of bacteria in vaginal flora and that the number of bacteria also increased in the ewes to which empty sponge was attached. On the other hand, number of vaginal bacteria decreased to values before sponge was applied as progesterone applied ewes showed estrus when sponges were removed, and no decrease occurred in the number of vaginal bacteria in ewes to which empty sponge was attached as no estrus developed after sponges were removed. However, Vasconcelos et al. (2016) identified in a study they carried out on ewes that although vaginitis was observed in all MAP and CIDR applied ewes including the control group after vaginal devices were removed, that CFU analysis of the control group was lower than the ewes to which MAP and CIDR were applied. This result shows that unlike the findings of Yeşilmen et al. (2008), hormones -synthetic or regardless of their natural origin - also increase bacterial load as well as physical movement of vaginal devices. Sheldon et al. (2006) stated that by blocking the defense mechanism of uterus, progesterone hormone reduces its resistance power against microorganisms and hence much more live microorganism and more inflammatory activity are seen in animals' uterus in luteal period than in estrus period. Manes et al. (2013) detected that dominant bacteria flora was gram positive and the most common seen type was *Bacilllus spp.* before vaginal devices were installed. On days 6 and 11 after vaginal devices were installed, they identified that bacteria flora was gram negative and the most common isolated type of bacteria was *Arcanobacterium pyogenes*. According to results of the study made by Oliveira et al. (2013), *Staphylococcus spp.* types which are member of normal vaginal microflora in ewes are replaced by bacteria from *Enterobacteriaceae* family i.e. *Klebsiella pneumoniae* and *Escherichia coli* 24 and 48 hours after sponges are removed. This situation is considered to arise from local inflammatory effect of vaginal devices that promotes the growth of opportunistic bacteria or as the device containing progestogen is a predisposing factor that carries external microorganisms into reproductive system. After one week the sponges are removed, staphylococci return as being the dominant microorganisms.

Antimicrobial Susceptibility

Vasconcelos et al. (2016) stated that while vaginal devices are applied on site, it would be useful in order to minimize the contamination to take care of hygiene to prevent entrance of fecal gram negative bacteria into vagina and to reduce the holding time of devices in vagina. Some commercial laboratories recommend a wide antibiotic spectrum to be included to the sponge to prevent vaginitis. According to results of susceptibility tests they conducted on goats, Oliveira et al. (2013) stated that most bacteria resist to Penicillin and Tetracycline type antibiotics which are ones of the most common used by breeders, and that it was not suitable to include such antibiotics to intravaginal devices. They stated that *E. coli* isolates were the most resistant bacteria to antibiotics which are followed by *K. Pneumoniae* and *Staphylococcus spp.* types. Penna et al. (2013) detected that 62.5% of coliforms were resistant to at least one tested drug and only Tetracycline and Gentamicin were effective to all isolates. They recommended a drug named Ciprofloxacin for treatment of vaginitis caused by Gram positive or Gram negative bacteria. Suárez et al. (2006) reported Cephalothin and Gentamicin type antibiotics, and Manes et al. (2013) reported Amoxicillin, Ampicillin and Streptomycin as the most effective antibiotics in preventing bacterial growth. As Martin et al. (2009) included in the study they made only the healthy animals which do not have antimicrobial treatment history, they expected the members of normal vaginal microflora to be rather sensitive to antibiotics that were tested. However, they unexpectedly identified that 66% of isolates resisted to at least one of the antibiotics that were tested. Among the isolates; they reported Penicillin resistance as 40%, Ampicillin resistance as 26.6%, Tetracycline as resistance 20%, Amoxicillin resistance as 20% and Gentamicine resistance as 13.3%. Along with all isolates obtained from the vagina of healthy ewes, they stated that the most active antimicrobial agent against *Staphylococccus spp.* was Ciprofloxacin. They also detected that resistance of Gram positive bacteria to antibiotics (77.7%) were much higher compared to negative bacteria (50%). Mulu et al. (2015) identified that the most frequent isolates that are isolated from ewes vagina were *E.coli, Pseudomonas spp.* and *S.aureus* They identified that these bacteria were highly sensitive to Norflokxacin (75.6%), Ciprofloxacin (79.6%) and Gentamicine (77.6%), while put up high resistance for Amoxicillin (82.2%), Tetracycline (63.3%) and Cotrimoksazole (62.2%). In the studies of Al-Qasi and Ahmed (2016), *S. aureus* was found totally resistant to Amoxicillin (100%) and Penicilline (100%), low resistant to Rifampicin (33%), and completely sensitive to Vancomycin (0%). *E.coli* is highly resistant to Amoxicillin (98%) and Penicilline (95%) while sensitive to Vancomycin (10%) and Ciprofloxaine (20%). *P. vulgaris* was found highly resistant to Amoxicillin (97%), Penicillin (95%) and Tetracycline (91%), but completely sensitive to Ciprofloxacin (0%) and Cefotaxine (0)%. *P. aeruginosa* and *Klebsiella spp.* are highly resistant to Amoxicillin (96%) and Penicilline (100%), but low sensitive to Ciprofloxacine (2.0-8.6%). *Streptococcus spp.* was sensitive to Ciprofloxacin (6%) but put up high resistance to Streptomycin (100%) and Tetracycline (93%). Al-Qasi and Ahmed (2016) reported that broad spectrum antibiotic use in treatment and using the same antibiotics for a long time have made resistance in bacteria. Aziz et al. (2017) identified *E. coli (*41.94%), *Klebsiella spp. (*29.03%*), Enterobacter spp. (*16.13%), *Pseudomonas aeruginosa* (6.45%) and *Proteus spp.*

(6.45%) in the samples they took from the uterus of ewes. They detected that all isolates were resistant to Oxacilline (%100). Along with that, they reported Ampicillin resistance as 93.64% and Tetracycline resistance as 41.92%. Besides, all isolates showed 100% sensitivity to Cefamandole and their sensitivity to Ceftriaxone and Gentamicin were identified as 90.32%. Based on these results, Aziz et al. (2017) reported that Cefamandole, Ceftriaxone and Gentamicin activitated very well in preventing bacterial activities in ewes. Moreover, they stated that wrong or excessive use of broad spectrum antibiotics, particularly Cephalosporins, caused formation of resistant bacteria and that this constituted a very important problem for animal health and accordingly for human health. Mohammed et al. (2017) detected that *Staphylococcus spp*. and *E. coli* were the most common observed isolates. They separated the sheep into 3 groups and applied progesterone containing vaginal sponge (G1) to the first group, Ciprofloxacin containing vaginal sponge (G2) to the second group and Ciprofloxacin+Clotrimazole containing vaginal sponge (G3) to the third group. As a result of this application, they determined the pregnancy rates as 66.7% in G1, as 100% in G2, and as 82.4% in G3. They identified that particularly the *Staphylococcus spp*. isolates showed high sensitivity to Ciprofloxacine. As a result of the study, they reported that simultaneous use of antibiotic with vaginal sponge could provide benefit in reducing undesired effects caused by sponges and in increasing reproductive performance. However, they suggest the antibiotic sensitivity test towards bacteria detected in the vagina to be certainly made before the application.

CONCLUSION

Vaginal flora consists of settled the pathogen and the non-pathogen microbiological populations. Getting knowledge about settled microbiological populations is important in determining pathophysiology of diseases. When vaginal system is destroyed or become unbalanced for any reason, opportunistic pathogen microorganims become dominant. Thus cause infections and become a threat for animal health. If this situation is taken into account, keeping the animals under healthy conditions without any stress and to prevent weakening of immune system will considerably allow to stay away from opportunistic bacterial infection.

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