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Original Research Article

Effect of Different Protocols of Estrus Synchronization on Vaginal Microflora with Antibiotic Susceptibility to Bacteria

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Abstract: To investigate the effect of different protocols for estrus synchronization in ewes on the vaginal tract bacteria and evaluate the sensitivity of bacteria to different antibiotics. Sixteen local breed ewes were allocated to four groups, each containing four. Group A: ewes treated with sponges for 10 days; Group B ewes treated with CIDR for 10 days; Group C ewes treated with sponges for 14 days and finally Group D ewes treated with CIDR for 14 days. Sterile swaps were taken from the vagina's mucous before insertion of sponge or CIDR and after removing it for each group. Vaginal swabs from synchronized ewes were obtained from the vagina's mucous before insertion of sponge or CIDR and after removing it for each group to use in different bacteriological tests. The results showed that the most dominant bacteria were E-Coli 7/7 (100%), and Staphylococcus aureus (71%) before insertion, while in group A, the percentage was 75% and 100% for E-Coli only in group B and group C respectively. According to E-coli, the results showed that it was more sensitive to Doxycycline (3.36±0.3), Enrofloxacin (3.23±0.2), and Ciprofloxacin (3.18±0.3) figure (2). Also, Staphylococcus aureus was sensitive to Doxycycline (3.32±0.4), Enrofloxacin (3.17±0.3), and Ciprofloxacin (3.12±0.3). It was concluded that the E-coli was the most prominent microflora in the vagina in ewes before and after insertion of sponges. Doxycycline, Enrofloxacin, and Ciprofloxacin are the most effective antibiotics against *E-coli* and *Staphylococcus aureus*.

Keywords: Estrus synchronization, ewe, E- coli, Antibiotics susceptibility.

INTRODUCTION

Estrus synchronization is one of the programs that increase the productivity of sheep around the world. Many different methods were used for estrous synchronization such as changes in photoperiod (Fleisch *et al.*, 2015), exogenous hormonal like a vaginal sponge, CIDR, and prostaglandin (Yu *et al.*, 2018; Martinez-Ros *et al.*, 2019; Habeeb and Anne, 2021). There was evidence that using a sponge for 12 or 14 days related to abnormal vaginal discharge which may be purulent or hemorrhagic when removing it (Scudamore, 1988). Vaginitis occurs due to the vaginal sponge causing retention of vaginal secretions that lead to the proliferation of bacteria then cause inflammation and infection (Al-Hamedawi *et al.*, 2003; Sargison *et al.*, 2007; Vasconcelos *et al.*, 2016). On the other hand, many studies recorded that using CIDR cause purulent vaginal discharge but it is lower than when using sponges (Suarez *et al.*, 2006). The most common protocols for using sponges are long protocols (12-14) days or short protocols (6-7) days, the short protocol is related to lower bacterial growth and high fertility rate after laparoscope insemination (Dos Santos-Neto *et al.*, 2015; Martinez-Ros *et al.*, 2018). Many studies, recorded different types of bacteria associated with vaginitis after removing sponges, Martinez-Ros *et al.*, (2018) reported that Salmonella *spp*. and *Staphylococcus aureus* are the most common bacteria withdrawal of sponges. *Staphylococcus aureus* is recorded as the most common bacteria that causes purulent vaginitis in ewes (Donders *et al.*, 2002; Bragança *et al.*, 2017). While Vasconcelos *et al.*, (2016) showed that E-Coli the prevalent bacteria isolated from the vagina after using CIDR or sponges. Altıncekic *et al.*, (2021) revealed that amoxicillin, cefepime, and ceftazidime were

effective antibiotics for Gram-negative bacteria. The study was designed to isolate bacteria after different estrus synchronization protocols by sponges and CIDR with antibiotic susceptibility tests for isolated bacteria.

MATERIALS AND METHODS

The study was conducted to evaluate the changes in bacteria in an ewe's vagina after using different methods of estrus synchronization protocols. Sixteen local breed ewes were allocated to four groups, each containing four. Group A: ewes treated with sponges 40 mg (China) for 10 days; Group B ewes treated with CIDR (USA) for 10 days; Group C ewes treated with sponges 40 mg (China) for 14 days and finally Group D ewes treated with CIDR (USA) for 14 days. Sterile swaps were taken from the vagina's mucous before insertion of sponge or CIDR and after removing it for each group. The vulva of the ewe was cleaned with neutral soap, chlorinated water, and then with water again. From each animal, a sample had been obtained by using sterilized microbiological swabs from the vagina deeply. Disposable medical gloves were used to gather all samples. By using media-transported samples (Afcoswap, Jordan), the entire collected samples were kept at 4 °C and brought to the lab in less than an hour to be cultivated.

In the lab, each collected swabs were placed in 10 mL sterilized tubes containing about 1 mL of Brain Heart Infusion ager. The culture was done on non-selective and selective media in anaerobic conditions by using sixteen streak methods. MacConkey agar, Eosin methylene blue, and SS agar were employed for the selectivity growth of bacteria and also for quantification of these growth bacteria while Mueller-Hinton and horse blood agar as a general medium for bacterial growth.

Sensitivity Test

The agar well diffusion method was taken on according to (Kavanagh, 1972 and Perez, 1990) for evaluating the antibacterial activity of different antibiotics used against bacteria isolated from the vagina. Standardized bacterial suspensions(1.5×10^8cfu/ml) of *S. aureus* and others for E-coli were mixed separately by using sterilized Mueller-Hinton agar. About twenty-five ml of the sterile agars were spread into sterilized Petri plates and then let them dry for ten minutes at room temperature. The antibiotic discs of Ciprofloxacin, Colistin, Doxycycline, Enrofloxacin, Flumequine, Gentamycin, and Neomycin were added to the plates. The plates were left at 37 C^o for 24 hours, Thereafter, the diameter of the inhibitory zone was measured to the closest millimeter (mm).

RESULTS

The results showed in Table (1) that 7/16 samples were positive for microbiological culture, and the most dominant bacteria were E-Coli 7/7 (100%) Figure (1), Staphylococcus aureus (71%) before insertion sponges, and CIDR. After removing, in group A, the percentage was 75% for E-Coli only. In group B the percentage was 25% for E-Coli only. In group C, the percentage was 100% of E-Coli; finally, there was no bacterial growth in group D. These results indicated a high percentage of bacteria growth was recorded in groups A and C when using sponges.

Table 1: Bacteria isolated from the vagina before and after using sponges and CIDR

Group	Animal No.	Bacteria isolation before insertion	Bacteria isolation after removing
A Sponges for 10 days	1	E-coli Staphylococcus aureus	Nil
	2	E-coli Staphylococcus aureus	E-coli
	3	E-coli Staphylococcus aureus	E-coli
	4	Nil	E-coli
B CIDR for 10 days	5	Nil	E-coli
	6	Nil	Nil
	7	Nil	Nil
	8	Nil	Nil
C Sponges for 14 days	9	E-coli Staphylococcus aureus	E-coli
	10	Nil	E-coli
	11	E-coli Staphylococcus aureus	E-coli
	12	Nil	E-coli
D CIDR for 14 days	13	Nil	Nil
	14	Nil	Nil
	15	E-coli	Nil
	16	E-coli	Nil

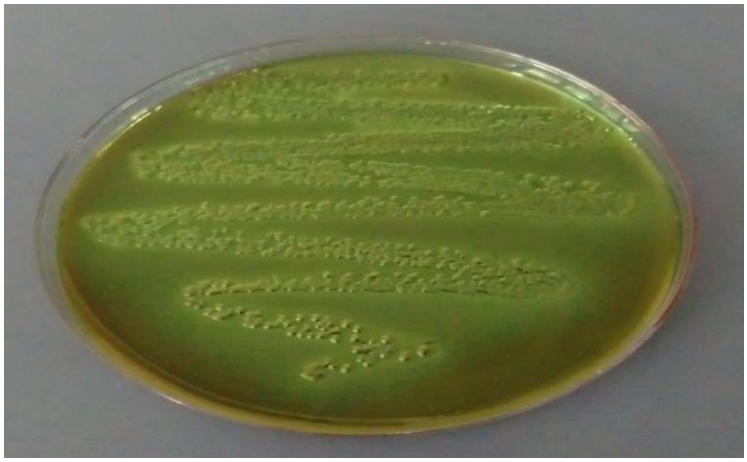

Figure 1: E-coli in Eosin methylene blue agar

The sensitivity test of E-coli and Staphylococcus aureus for antibiotics is illustrated in Table (2), according to E-coli, the results showed that it was more sensitive to Doxycycline (3.36±0.3), Enrofloxacin (3.23±0.2) and Ciprofloxacin (3.18±0.3) figure (2). Also, Staphylococcus aureus was sensitive to Doxycycline (3.32±0.4), Enrofloxacin (3.17±0.3), and Ciprofloxacin (3.12±0.3).

Table 2: Bacteria sensitivity to antibiotics isolated from the vagina in ewes.

Antibiotic	**Zone of inhibition**	
	E-Coli	***Staphylococcus aureus***
Ciprofloxacin	3.18±0.3	3.12±0.3
Colistin	2.77±0.2	2.68±0.2
Doxycycline	3.36±0.3	3.32±0.4
Enrofloxacin	3.23±0.2	3.17±0.3
Flumequine	2.30±0.2	2.25±0.2
Gentamycin	2.39±0.2	2.32±0.3
Neomycin	2.43±0.3	2.37±0.2

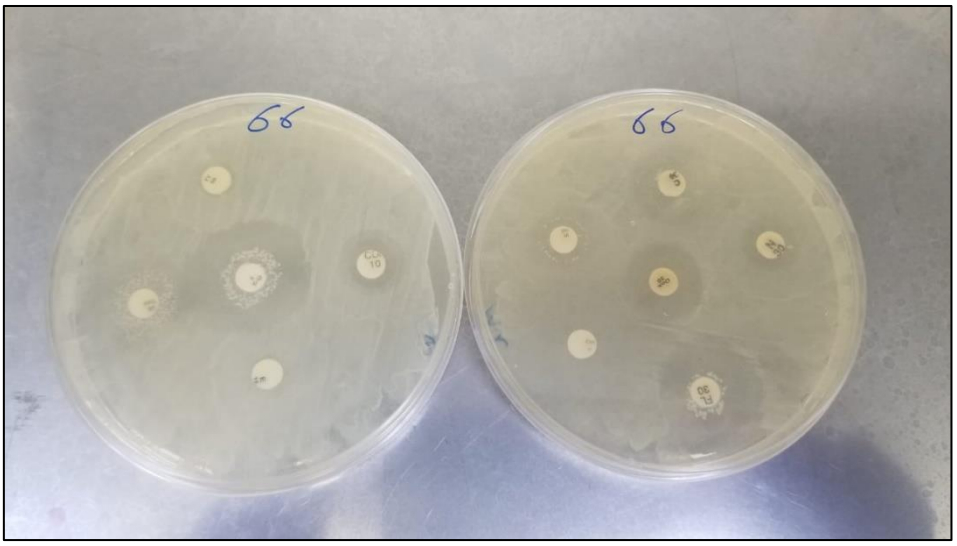

Figure 2: The susceptibility of bacteria to different antibiotics

DISCUSSION

Using an Intravaginal device may result in aberrant discharges upon device withdrawal and also retention of the vaginal secretions, which may alter the usual bacterial flora in the vagina, 2015; Manes *et al.*, 2010) with a reduced rate of fertilization (Scudamore, 1988 and Martinez *et al.*, 2018). These alterations in the vaginal bacterial flora may facilitate the development of pathogenic bacteria that are opportunistic and may need to be treated with antibiotics (Gatti, *et al.*, 2011; Ojeda *et al.*, 2019). Furthermore, the rise and spreading of antibiotic resistance highlight the necessity of "antibiotic-free" methods for veterinary disease control. The results indicated a high percentage, and the most prevalent bacteria were

recorded in groups A and C when using sponges. In the same pattern, the researcher Majeed *et al.,* (2012) discovered that, before sponge insertion, the most common kind of bacteria belonged to the genus Staphylococcus spp., accounting for 57.9%. In contrast, the percentage of Gram-negative bacteria was 42.1%. On the other hand, Manes *et al.,* (2010) found that the majority of the bacterial flora present upon insertion of the device was mostly Gram-positive, in contrast, the percentage of Gram-negative bacteria was zero. Regarding antibiotic sensitivity tests, the findings presented in this study as well as those of other research by (Oliveira *et al.,* 2013) who showed that Staphylococcus spp. were among the typical vaginal germs that had found in the small-ruminants, which indicated the need to stop the development of opportunistic bacteria like E. coli. Antimicrobials are frequently used concurrently to lessen the adverse effects that the sponges may generate (Suarez *et al.,* 2006; Martins *et al.,* 2009). Different antibiotics were used to assess the sensitivity of the discovered strains of vaginal bacterial flora that were collected in this investigation, either as a single isolate or mixed (including more than one type of bacterial colony) to determine Staphylococcus aureus and E-coli sensitivity to various antibiotics, by using the disc diffusion technique. Doxycycline was effective against most bacteria isolates after sponge insertion followed by Ciprofloxacin and Enrofloxacin. This result is in line with earlier studies published in 2008 by Carson *et al.,* (2008), Gabriel Martins *et al.,* (2009), and Bruno Penna *et al.,* (2013). Conversely, Suarez *et al.,* (2006) found that the best drugs to stop pathogenic bacteria from growing after insertion of intravaginal sponges that were used on ewes were cefazolin and gentamycin. Still, concurrent antibiotic administration is frequently advised to lessen the negative effects produced by sponges; however, this approach may result in antibiotic resistance. (Mohammed *et al.,* 2017). The percentages of different antibiotic susceptibilities for each bacteria isolated from the vaginas of the ewes are shown in Table 2. Every strain that was isolated for this investigation is susceptible to every antibiotic that has been tested, but the following antibiotics Doxycycline, Enrofloxacin, and Ciprofloxacin treatments were most effective against isolated strains in decreasing their growth provoked by sponge treatments. blocking the production of nucleic acids. Members of this class of antibiotics can passively diffuse into cells through porins, which are protein canals filled with water that are found on the outer membrane of bacteria. Commonly, these medications prevent the replication of bacterial DNA by interfering with the DNA gyrase (topoisomerase II) activity during the growth and reproduction of bacteria (Hangas *et al.,* 2018). It was concluded that the E-coli was the most prominent microflora in the vagina in ewes before and after insertion of sponges. Doxycycline, Enrofloxacin, and Ciprofloxacin are the most effective antibiotics against *E-coli* and *Staphylococcus aureus*.

CONCLUSION

It was concluded that the E-coli in the vagina of ewes was the most prominent microflora before and after insertion of sponges. Doxycycline, Enrofloxacin, and Ciprofloxacin are the most active antibiotics against E-coli and Staphylococcus aureus.

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