Vaginal microflora in ewes after estrus synchronization with intravaginal sponges

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Summary

The aims of this study were to detect the changes in microflora and incidence of vaginitis in ewes after using of intravaginal sponges. Twenty five Awasi ewes were divided randomly into two groups (1st group included 9 animals and 2nd group included 16 animals). Both groups were treated with intravaginal sponges (20 mg) for 12 and 10 days respectively. Swabs were taken from vagina before insertion of sponges and immediately after sponges' removal. The results showed that, only 2 ewes in 1st group exhibited estrus while all ewes in 2nd group exhibited estrus. *Escherichia coli* was the most predominant bacteria before insertion and withdrawal of sponges (66.66 and 68%) respectively. Four from nine ewes in 1st group, whereas only one ewe in 2nd group revealed vaginitis. It was concluded that intravaginal sponges do not change microflora in the vagina plainly but may cause vaginitis.

Keywords: Ewe, Intravaginal sponges, *Escherichia coli,* Estrus synchronization.

Introduction

Sheep are considered as seasonally polyestrous animals which usually affect production of meat and milk; therefore, many researchers were working to follow alternative ways to improve the efficiency of reproduction in sheep (1). Estrus synchronization (ES) in livestock depends on the manipulation of luteal phase, either by artificial prolongation of luteal phase or by creating artificial endogenous luteal phase. In ewes, luteal phase is longer than follicular phase, hence the opportunity for control is greater and more responsive to manipulation (2). Methods of ES and ovulation simplify the use of assisted reproductive technologies (3). Progestin used for mimicking life span of corpus luteum in spite of the stage of ovarian cycle at the time of treatment. (4). Hence, there are many procedures to use progestin for ES such as injection, implantation, in food and intravaginal device like sponge, CIDR and Dico. The most common traditionally method is intravaginal sponge impregnated with progestin in small ruminant (5) which is mainly attributable to their low cost (6) while (7 and 8) found mucopurulent vaginal discharge and signs of vaginitis when sponges were removed, which led to a decrease in pregnancy rate which might be attributed to ascending infection to uterus.

The sponges were considered as a predisposing factor for infections in the vagina (7-11). Other authors demonstrated that intravaginal sponges caused only increment in microflora in the vagina but returned to normal values pre-insertion which did not affect in subsequent fertility or pregnancy rate (12-14). The objective of this study is to investigate the effect of intravaginal sponge on the microflra in the vagina and incidence of vaginitis in Awasi ewes after a program of estrus synchronization.

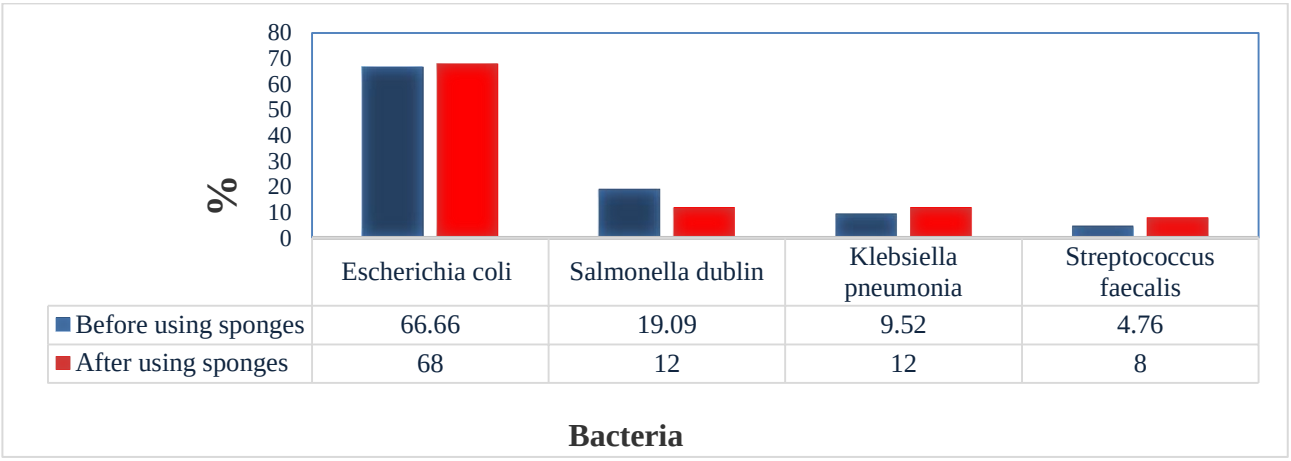
Materials and Methods

The study was conducted in Al-Yusufiyah township-Baghdad province. Twenty-five Awasi ewes were allocated into two groups (1st group included 9 animals and 2nd group included 16 animals) and underwent vaginal examination before insertion of sponges. Chronogest CR (Intervet, B.V. Holland) are intravaginal sponges impregnated with 20 mg of a synthetic progesterone-like hormone, cronolone (flugestone acetate) were used. Both groups were treated with intravaginal sponges for 12 and 10 days respectively. Sterile swabs without rubbing of vaginal wall were used for mucous collection before insertion and

immediately after removal of sponges. Samples were transported by medium (Difco™, Becton, Dickinson and Company, USA) until cultured. The specimens were plated on the culture media depending upon the bacteria suspected; the appropriate culture media were selected. Nutrient agar, blood agar and more selective media (MacConkey agar, SS agar (for Salmonella), Thermo Fisher Scientific, USA) were the culture media that were used for isolation and purification of obtaining bacteria while Gram stain was the useable stain in this research. The incubation of inoculated media was aerobically for 24 hours at 37 ºC. The storage of isolates was in brain heart infusion broth with (15%) glycerol at (-20˚C) for further use. After Gram stained slides of bacteria isolates were prepared; the diagnosis of obtaining bacteria was first examined microscopically to study their cellular morphology, size, consistency and color. Then biochemical tests including catalase, oxidase, (indol, methyl red, vogas-proskauer and citrate utilization) IMVIC tests and (triple sugar iron) TSI were performed (15). Finally Vitek 2 system was performed to identify species level of bacterial isolates.

Results and Discussion

Vaginitis with purulent mucous discharge was seen in 1st group in 4/9 ewes (44.4%), while in 2nd group 1/16 ewe and was (6.25%). Only 2 ewes in 1st group showed estrus and all ewes in 2nd group showed estrus. It was found that the most predominant bacteria before insertion of sponges were Gram (-) bacteria with percentage 95.24% (*Escherichia coli, Salmonella dublin, Klebsiella pneumonia*) and Gram (+) in a ratio 4.76 % (*Streptococcus faecalis*); otherwise the percentage of Gram (-) and Gram (+) bacteria was 92% and 8% respectively after withdrawal of sponges. *E-Coli* is the most presumptive bacteria found before and after using sponges in a percentage of 66.66 and 68% respectively. (Fig. 1) showed the distribution of other bacteria isolated before and after using sponges.



Figure, 1: Distribution of bacteria before insertion and after removal of sponges.

It was reported that 20% of ewes displayed vaginitis with mucopurulent discharge; this observation is relative with (16) who found that 100 % of treated animals with intravaginal device showed signs of vaginitis, also (8) referred to the same observation after using intravaginal sponges for (6, 9 and 12) days. Other researchers (7 and 10) reported that 100 % of ewes showed vaginitis with increment in the mucous secretion that was chiefly hemorrhagic and purulent. While (17) recorded that vaginal sponge created a local inflammation with increasing in bacterial load. In other studies, (13) noted that intravaginal sponge lead only to increase in colony-forming units (CFUs) in goat and then, there was a rapid reestablishment of normal flora after removal of intravaginal sponge. Also (12 and 14) concluded that intravaginal sponges caused rise in bacterial count but it returned to normal value before insertion. The study revealed that the most predominant bacteria were Gram (-) 95.24 % (*Escherichia coli, Salmonella dublin, Klebsiella pneumonia*) in

the day of insertion; this result is in contrast to other authors (12-14 and 16) who found the percentages of bacteria were (100, 77, 63.6 and 90%) respectively. And (18-20) informed that Gram (-) bacteria was the most predominant bacteria in female reproductive tract in ewes in Iraq. The percentage of Gram (-) was 92% after removing sponges; this result agreed with (14) who found that 100% of bacteria was Gram (-) after removing of sponges in goats. Moreover, (7) reported that 89.9% of bacteria were (*E-coli* 72.7% + *Klebsiella pneumonia* 18.2%). And (8) recognized that *coliform* constitute the most commons bacteria in goat with vaginitis. Also, (12, 13 and 16) observed that the percentage of Gram (-) bacteria was (90.9, 82.7 and 79%) respectively.

The incidence of vaginitis and change in vaginal microflora may be attributed to sponge itself; also contamination of string of sponge through environment and fecal materials. Furthermore, infection might occur during insertion of sponges in septic procedure or due to the effect of flugestone acetate. In addition, previous infection that did not recognize by routine examination of vagina before insertion of sponge may lead to increase in opportunistic bacteria specially *E-coli*. While (17 and 20) suggested that, the change might be due to physical retention or absorption of vaginal secretion by sponges. (10 and 11) determined that sponge itself may be responsible for rise in microflora in spite of being impregnated with progesterone derivatives or not. Others (3 and 21) speculated that sponge itself was responsible for lower pregnancy rate. According to the effect of progesterone, it might be due to its immune suppressor effect in which (22) confirmed that progesterone down regulate uterine immune thus lead to proliferation of bacteria. Whilst (12 - 14) approved the effect of progesterone in provoked rise in bacteria specially *E-coli*. It was concluded that Gram (-) bacteria especially *E-Coli* is the most prominent bacteria in the vagina before insertion and after removal of sponges. The incidence of vaginitis might be attributed to sponges itself (it composition, contamination through it string) and flugestone acetate which cause immune suppression. So, there is a controversy in the incidence of vaginitis between authors attributed to different types of sponges and their composition; moreover different breeds and their resistance to infection.

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مايكروفلورا المهبل في النعاج بعد توحيد الشبق باستعمال الاسفنجات المهبلية

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الخلاصة

إن هدف الدراسة تحديد التغيرات في المايكروفلورا ونسبة حدوث التهاب المهبل في النعاج بعد استخدام الأسفنجات المهبلية. استخدمت ٢٥ نعجة وقسمت الى مجموعتين (المجموعة الأولى ضمت ٩ حيوانات والمجموعة الثاية ضمت ١٦ حيواناً). عولجت المجموعة الأولى والمجموعة الثانية بالاسفنجات المهبلية (٢٠ ملغم) لمدة ١٠ و ١٢ أيام على التوالي. أخذت مسحات من المهبل قبل إدخال الاسفنجات وبعد رفعها مباشرة. بينت النتائج أن نعجتين اثنتيين فقط في المجموعة الأولى أظهرت الشبق في حين جميع النعاج في المجموعة الثانية اظهرت الشبق. شكلت الإشريكيا القولونية غالبية الجراثيم قبل وبعد إزالة الاسفنجات (٦٦,٦٦ و٦٨%) على التوالي، كما أن اربع من تسع نعاج في المجموعة الأولى، في حين نعجة واحدة من المجوعة الثانية أظهرت التهاب المهبل. نستنتج من هذه الدراسة أن الاسفنجات المهبلية لاتحدث تغييراً في المايكروفلورا في المهبل ولكن قد تسبب التهاب المهبل.

الكلمات المفتاحية: نعجة، أسفنجات مهبلية، الإشريكيا القولونية، توحيد الشبق.