Effect of cervical flora during breeding season on future fertility in Iraqi Awassi ewes

Anas Kh. AL-Saffar

Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Baghdad

Abstract:

Seventeen Iraqi pluriparous healthy Awassi 3-5 years old ewes were included in this study during breeding season selected randomly from flock with relatively low fertility to investigate effect of cervical microflora during breeding season on pregnancy rate, swabs for bacteriological examination collected from cervix pre natural insemination. The study results revealed 100% positive swabs culture with single, double and triple types of isolated microorganisms on culture and different pregnancy rates achieved, 41.17%, 47.05% and 11.76% subsequently. Single, double and triple isolated microorganisms of cervical swabs significantly ($p<0.01$) affect pregnancy rate of ewes, while no significant differences found in between these isolates on pregnancy rate. In conclusion: The study showed influence of microorganisms and combinations in the cervix during breeding season on pregnancy rate of ewes.

Key words: Cervical flora, breeding season, Awassi ewes, fertility.

تأثير العزل الجرثومي لعنق الرحم خلال موسم التناسل على الخصوبة للنعاج العواسية العراقية

انس خضر السفار

فرع الجراحة والتوليد البيطري، كلية الطب البيطري، جامعة بغداد

الخلاصة:

أجريت الدراسة على سبع عشرة نعجة عواسية بحالة صحية جيدة تراوحت أعمارها ما بين 3-5سنوات تم اختيارها عشوائيا من قطيع يعاني من قلة خصوبة وذلك لمعرفة تأثير العزل الجرثومي المتواجد في عنق الرحم خلال موسم التناسل على نسبة الحمل. جمعت مسحات للفحص الجرثومي من عنق الرحم قبل إطلاق الكبش مع النعاج. بينت نتائج الدراسة أن جميع النعاج كانت موجبة للعزل الجرثومي، حيث كانت العزلات مفردة، مزدوجة وثلاثية التواجد الجرثومي على الوسط ألزرعي بمستوى معنوية عالي ($P<0.01$) وكانت نسب الحمل المتحققة في الدراسة (41.17%، 47.05% و 11.76% على التوالي) .أوضحت الدراسة أن جميع أنواع العزلات الجرثومية (المفردة، المزدوجة والثلاثية) أثرت معنويا ($P<0.01$) على نسب الحمل لحيوانات الدراسة مع انعدام الفارق المعنوي بين أنواع العزلات. نستنتج من الدراسة إن هناك تأثير للتواجد الجرثومي في عنق الرحم بأنواع وتآزرات مختلفة على نسب الحمل للنعاج خلال موسم التناسل.

Introduction:

Several factors play an important role in achieving good fertility levels in sheep such as nutrition (1,2) breed and season (3). Research works rise doubts about the effect of vaginal and uterine microflora on future fertility of ewes (4,5). 56.5-88% of genital tracts contains microflora including, Arcanobacterium pyogens, Escherichia coli and Streptococci Spp. are commonly isolated from uteri of ewes (6,7,8) and vagina(9). The microflora have no pathological effect without supporting from other predisposing or stress factors (10,11). In postpartum period, the uterus seems to be able to prevent bacteria from achieving infection unless suppression to uterine defense mechanism occurs (12).

The aim of the current study is to investigate effect of microflora isolated from cervix during breeding season on future fertility of Iraqi Awassi ewes.

Materials and Methods:

Seventeen healthy pluriparous Iraqi Awassi ewes were included in this study during breeding season at the farm of Veterinary medicine college \ Baghdad University. Their ages ranged from 3 to 5 years. The perineal region washed with soap and water then disinfected with povidone-iodine. Swab* for sample collection was introduced through sterile vaginal speculum deep into vagina reaching external uterine os with adequate rolling upon the mucous membrane before naturally inseminated from fertile ram. Swabs were transferred into sterile tubes containing thioglycolate broth as transport media at 4ºC using cool box filled with ice cubes to the laboratory of microbiology department in the college, then cultivated immediately in 37ºC incubator for 24 hours, each sample passed in 1- Tryptic soy broth, 2- Nutrient broth, 3- Thioglycolate broth and incubated at 37ºC for 24 hours.

Gram stain for each sample was performed before cultivation on Blood, Nutrient, MacConkey, Tryptic soy and Sabouraud's dextrose agars for 24 - 48 hours. Colonies were purified on facultative medias (13). Identification of bacteria was based on colony characteristics, gram stain, morphology, hemolysis, sugar utilization and catalase, coagulase, indole production, methyl red, Voges-Proskauer, citrate production tests. Sabouraud's dextrose broth and agar supplemented with Cyclohexamide 0.5 gm/L, Chloramphenichol 250 mg/L used for fungi cultivation (13,14).

Pregnancy rate was adopted as a criterion of fertility, diagnosis of pregnancy done using abdominal palpation (15).

Statistical analysis: Chi-square (χ^2) test according to Contingency tables method within the statistical analysis system –SAS (2001) program was used for significant comparing between different percentages in this study (16).

(*): Henry Schein® made in China

Results:

Results of the study showed that all of the seventeen ewes had significant ($p<0.01$) positive swabs culture of cervix during breeding season.

Table 1 summarizes significant ($p<0.01$) isolation of seven microorganism types from cervical swabs of ewes during breeding season in different percentages, Escherichia coli 41.4%, Listeria spp. 20.7%, Arcanobacterium pyogens 17.24%, Klebsiella spp. 6.9%, Pseudomonas spp. 6.9%, while the lowest percentage was for Strepto-coccus 3.44 and Candida 3.44%.

Table 1: Percentage of microorganisms' prevalence in cervix during breeding season.

Species of microorganism	Frequency of isolated microorganism	Prevalence (%)
Escherichia coli	12	41.40
Listeria spp.	6	20.70
Arcanobacterium pyogens	5	17.24
Klebsiella spp.	2	6.90
Pseudomonas spp.	2	6.90
Streptococcus spp.	1	3.44
Candida	1	3.44
Chi-square (χ^2) value	-----	7.336 ***

** ($P<0.01$).

The significant ($p<0.01$) prevalence of microorganisms on cultured cervical swabs of the study ewes which is single 41.17%, double 47.05% or triple 11.76% shown in table 2.

Table 2: Number of cervical isolates on culture during breeding season.

No. of cervical isolates on culture	No. of cases	Percentage of isolates (%)
Single	7	41.17
Double	8	47.05
Triple	2	11.76
Chi-square (χ^2) value	-----	6.904 **

** ($P<0.01$).

Statistical analysis of results shown no significant differences found according to number of cervical isolates in the cultured swabs on pregnancy rates achieved in this study (table 3).

Table 3:Effect of isolates number on Pregnancy rate of ewes in the study.

No. of cervical isolates on culture	No. of cases	Pregnancy rate (%)
Single	7	57.14
Double	8	50.00
Triple	2	50.00
Chi-square (χ^2) value	-----	1.337 Ns

Ns: Non-significant.

Different pregnancy rates achieved in this study according microorganisms species prevalence the cervix of ewes (table 4). When E. coli prevalence cervix in this study

pregnancy rate was 66.66%, A. pyogens 100%, Pseudomonas spp. 100% and Klebsiella spp. 0.0% (P<0.01). While when two species types of microorganisms are in culture, pregnancy rate was 75.0% with E. coli + Listeria spp., 33.33% with E. coli + A. pyogens and 0.0% with A. pyogens + Candida (P<0.01). In ewes with three species types of microorganisms presented in culture E. coli + Listeria spp. + Streptococcus spp. pregnancy rate was 100%, in E. coli + Listeria spp. + Pseudomonas spp. pregnancy rate was 0.0% (P<0.01), while no significant differences found when comparing between single, double and triple types of isolated microorganisms on pregnancy rate.

Table 4:Effect of various isolated microorganisms on pregnancy rate.

Species of isolated microorganisms on culture	No. of animals	No. of pregnant and %	No. of non pregnant and %	Chi-square (χ^2) value
Single type of microorganism				
E. coli	**3**	**2 (66.67%)**	**1 (33.33%)**	**8.341 ****
A. pyogens.	**1**	**1 (100%)**	**0 (0.0%)**	**15.00 ****
Pseudomonas spp.	**1**	**1 (100%)**	**0 (0.0%)**	**15.00 ****
Klebsiella spp.	**2**	**0 (0.0%)**	**2 (100%)**	**15.00 ****
	7	**4 (57 %)**	**3 (43 %)**	**---**
Double microorganism types				
E. coli+ Listeria spp.	**4**	**3 (75.0%)**	**1 (25.0%)**	**10.50 ****
E. coli+ A. pyogens.	**3**	**1 (33.33%)**	**2 (66.67%)**	**8.341 ****
A. pyogens. + Candida	**1**	**0 (0.0%)**	**1 (100%)**	**15.00 ****
	8	**4 (50 %)**	**4 (50 %)**	**---**
Triple microorganism types				
E. coli+ Listeria spp.+ streptococcus spp.	**1**	**1 (100%)**	**0 (0.0%)**	**15.00 ****
E. coli+ Listeria spp. +pseudomonas spp.	**1**	**0 (0.0%)**	**1 (100%)**	**15.00 ****
	2	**1 (50 %)**	**1 (50 %)**	**---**
Chi-square (χ^2) value compare between 3 types	**--**	**1.348 ns**	**1.277 ns**	
Total	**17**	**9 (52.94%)**	**8 (47.06%)**	**1.683 Ns**

** (P<0.01), Ns: Non significant.

Discussion:

The results of this study revealed a prevalence of some non-specific microorganisms and combinations in the cervix associated with significant ($P<0.01$) reducing reproductive efficiency of ewes (17,18,19), the prevalence of E. coli 41.40%, A. pyogens 17.24%, Pseudomonas spp., Klebsiella spp. 6.9%, and the lowest percentage was Streptococcus spp. 3.44, these percentage are higher than results recorded by Clyde A. (17) and lower than recorded by Gabriel M. et. al. (20), these differences may be explained according to the influence of bad hygienic condition and management during previous parturition (4,17,21). The results of cultured swabs collected from cervix showed single, double or triple species types of microorganisms. Acceptable pregnancy rate were achieved in ewes with single non specific microorganism species isolated on culture (E. coli, A. pyogens, Pseudomonas spp.) whereas ewes with non specific single microorganism species (Klebsiella spp.) recorded low pregnancy rate. The presence of non specific microorganism species (E. coli, A. pyogens, Pseudomonas spp.) in genital tract did not give rise to endometritis without synergism to other ascending contaminated microorganisms (22). The identification of Klebsiella spp. (a specific cause of low fertility in mare) (23) in some ewes could become from other animal species accommodated in same field. Ewes with double microorganisms species isolated on culture had low pregnancy rate in different synergistic levels, minor effect of E. coli + Listeria on pregnancy rate, whereas E. coli + A. pyogens and E. coli + Candida induce low pregnancy rate, this results indicated that synergism of the second microorganism had severe effect on reproductive tract and resulted in pathological changes which lowered pregnancy rate (5,19), ewes with triple isolates of microorganisms species on culture had different levels of pregnancy rate depending on the type of synergistic microorganism (21). In general, ewes with low pregnancy rate in the third synergism; Candida may cause endometritis and early embryonic loss (24). Later the prevalence of triple type of microorganisms species showed no synergistic effect of E. coli+ Listeria spp.+ streptococcus spp., while E. coli+ Listeria spp. + pseudomonas spp. synergism affect's pregnancy rate, the presence of microorganisms in the uterine cavity increase secretion of PGF2a which have a detrimental effect on pregnancy (25). Results of the study revealed total low pregnancy rate 47.05%, due to presence of microflora with synergistic effect interfering pregnancy rate due to genital tract environmental changes inducing the presence of phagocytes engulfing spermatozoa (26), impaired embryo development and low pregnancy rates (27).

From this study, microflora of cervix may play an important role in uterine

infection as microflora could stay dormant in the genital tract tissues, passed to next breeding season or could be localized after coitus due contamination from livestock, microflora under the elevated progesterone levels during pregnancy play a role in genital tract infection. Further cytopathological studies required to investigate microfloral influence on genital tract tissue.

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