قسم : الميكروبيولوجيا
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الفحص البكتريولوجى للمهبل والفتحة الخارجية لعنق الرحم فى الأغنام خلال فترة ما قبل الولادة وبعدها فى حالات نقص الخصوبة

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تم فحص نسبة وجود البكتريا والفطريات فى المهبل والفتحة الخارجية لعنق الرحم فى الأغنام (٧٥ غنما) خلال الفترة ما قبل الولادة وبعدها وفى بعض حالات نقص الخصوبة . تبين وجود البكتريا العنقودية والسبحية والكولى والسود وموناس بنسبة كبيرة خلال فترة النفاس عنها فى فترة ما قبل الولادة

لوحظ أن الكانديدا كانت من الأسباب الرئيسية لنقص الخصوبة فى الأغنام وكذلك من الفطريات الريزوبس والميوكر والأسبيريا كانت بنسبة عالية فى خلال فترة النفاس .

لوحظ أن الكانديدا كروزى وتروبيكاليس فى حالات نقص الخصوبة قادرة على أنتاج أنزيم الفوسفاتاز بينما العترات المماثلة فى الحالات الطبيعية غير قادرة على ذلك .

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MICROFLORA OF THE VAGINA AND EXTERNAL OS OF EWES DURING LATE PREGNANCY, PUERPERIUM AND INFERTILE CASES

(With 5 Tables)

By

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SUMMARY

Bacteria and Fungi present in the vagina and external os of the cervix were studied in a total of 75 ewes during late pregnancy, puerperium and cases suffering from infertility. The percentagte of Staph. aureus, Strep. Pyogenes, E.coli and Ps. aeruginosa were higher during puerperium than during late pregnancy.

Candida spp. were the main cause of infertility, while the revealed Rizopus, Mucor and Absida were found at high percentage during puerperium. Candida Krusei and Candida tropicalis recovered from infertile cases were able to produce phosphatase enzyme while similar strains isolated from the normal animals were not.

INTRODUCTION

Several studies on the microflora of the genital tract and its effect on sheep reproduction were investigated by GUNTER, et al. (1955); ZAKI and SABER (1962); QUINLIVAN (1970).

MARINOV (1967); GRYSEWSKI and PIER, (1968); SHALASH and ELGINDI (1968); DENNIS and NAIRN (1970); THURSTON, et al. (1978) and YOUSEFF, et al. (1984) proved that different bacteria were incriminated in lowering of fertility rate or even sterility in sheep.

In Egypt, it is evident from the available literature that mycotic and bacterial flora in ovine female genital tract have received a little attention. The yeast and fungi together, with pathological changes in the female genitalia plays a great role infertility. The scopy of the present work is to study the effect of bacterial and mycotic microflora during late pregnancy, puerperium and reproductive disorders in sheep in upper Egypt.

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MATERIAL and METHODS

A total of 75 ewes (40 pregnant and 35 infertile) were used in this study. Out of 40 pregnant ewes, only 35 swabs were collected during late pregnancy, while after birth swabs were taken from all parturent ewes. Seventeen out of 35 infertile ewes showed vaginal discharge. Clinical examination of the used ewés were recorded in the Farm of Fac. of Agric., Assiut Univ. and private farm. The collected swabs were taken aseptically from the external os and vaginal mucosa for bacteriological and mycological examination. Care was taken in order that the cervical swab would not touch the mucosa of the vagina. Briefly, the samples which were obtained were inoculated on blood agar, selenite broth, alkaline peptone water and sabouraud's dextrose broth containing chloramphenical (50 mg/litter). The selenite enrichment broth and alkaline peptone water were incubated over night, then it was subeultured on salmonella - shigella agar (S.S.A.) for isolating Salmonella and Shigella. The inoculated sabouraud's dextrose broth was incubated for 7 days, then subcultured on sabouraud's agar. Duplicat cultures were made, one being incubated at 25°C and the other at 37°C for 4 days periode. In case of no fungal growth at the end of this period, it was considered as negative (AL-DOORY, 1980). Any resulting colonies on sabouraud's dextrose agar were identified according to their colonial morphology, microscopical and biochemical activities (AL-DOORY, 1980). Phosphatase activity of candida spp. was also determined according to FARID, et al. (1980). Due to varied causes of reproductive disorders, the inoculated blood agar was incubated aerobically, than subcultured on Mac Conkey's agar, Hoyl's medium, Mannitol salt agar and cetrimide agar, while blood agar incubated under 5% Co was subcultured on brucella agar. For anaerobs, the material was plated on agar which was incubated anaerobically. Suspecious-looking colonies from the above media were screened morphologically as well as biochemically and were confirmed serologically according to the methods described by BUCHNAN and GIBBONS (1974) and BAILEY and SCOTT (1978).

RESULTS

Table (1) revealed the incidence of cases with positive and negative findings for bacteria and fungi at various conditions of ewes (late pregnant ewes, at puerparium and infertile ewes).

Table (2) showed the different bacterial species isolated from ewes at various conditions. Staphylococci and E.coli were the most predominant during puerperium and during pregnancy, while Strept. pyogenes was the most predominant in infertile ewes.

Table (3) revealed the different mycotic species isolated from ewes at various conditions. The mould was the most predominant during late pregnancy, while candida spp. were the most predominant in infertile ewes.

From table (4) and table (5), it was observed that all strains of Candida tropiclis and Candida krusei which were isolated from pathological materials of infertile ewes, were strongly positive phosphatase producers after 24 hours incubation, while some strains of Candida pseudotropicalis produced only weak reactions after 48 hours. All the isolates of Candida albicans were unable to produce phosphatase after the different incubation times. On the other hand, all strains isolated from normal conditions were unable to produce phosphatase. The enzymatic activity of the studied Candida tropicalis and Candida krusei strains grown in media of different pH, was found to be unrelated to changes in pH of the culture medium.

DISCUSSION

Examination of the ewes at late pregnancy revealed a variaty of microorganisms, these were Micrococcus tuteus (11.4%), E.coli (11.4%), Staph. epidermidis (8.6%) and Strept. faecalis (8.6%). Most of the organisms isolated from the pregnant ewes may be harmless saprophytes, only causing troubles under certain unfavourable conditions. However, the isolation of E.coli with low percentage; either before or after parturition showed that the foetal membrane and amniotic fluid may have probably bactericidal effect or immune bodies which may act as a normal barrier against bacteria (ROBERTS, 1971 and ARTHUR, et al. 1982).

Staph. aureus, B-Haemolytic streptococci, E.coli and Ps. aeruginosa as well as predominant mould infection were higher during post-partum period than during late pregnancy stage. Similar findings were reported by GUNTER, et al. (1955) in cattle. This results indicate that infection may be due to the unhygenic environments or contamination during parturition. In addition, ZAKI, et al. (1963) recorded during puerperium in cattle the isolation of different bacteria which were as follows: Micrococi, Streptococci, Anthracoids, Corynibacteria, E.coli, Gaffkya, Diplococci, Proteus and Sarcina.

Concerning the infertile animals, our findings seems to agree with the results obtained by SYKORA, (1932) in cattle who found that only 75% of the healthy genital organs were bacteriologically free, while GUNTER, et al. (1955) recorded that 38% of samples from the reproductive tract of normal dairy animals were negative. However, in Egypt, ZAKI and SABER (1962) have isolated micrococci, Gaffkya and unidentified sarcina species from the non-pregnant ewes while YOUSEFF, et al. (1984) reported the isolation of main bacteriological causes of endometritis in ewes which were in their order of frequency, as follows; Staph aureus, C.pyogenes, Strept. pyogenes, C.ovis and E.coli.

It is noteworthy to mention that the pyogenic organisms, are frequently considered as a causal agents of many diseases in domestic animals (ARTHUR, 1964 and WATSON, 1970). Moreover, ZAKI and SABER, (1962) and QUINLIVAN (1970) reported that there were greater number of E.coli during estrus cycle in ewes.

E.coli may be met with in cases of outbreaks of abortion in flocks of ewes as reported by HOWARTH (1932) and MARINOW (1967). They found that E.coli had the greatest effect on the embryonic mortality.

As regards proteus species and Ps. aeruginosa and yeast isolated in incidence 13.33, 3.33% and 84.22% respectively. These findings are in accordance with the results obtained by QUINLIVAN (1970) who isolated Ps. aeroginosa from the cervical mucous of ewes.

From table (4) and (5) it is observed that the results are in agreement with the findings of SMITH, et al. (1974) and FARID, et al. (1980).

Finally, it could be concluded that candida tropicalis and candida Krusei strains recovered from pathological materials were able to produce the phosphatase enzyme while similar strains isolated from normal conditions were not. In addition, the phosphatase production test may be used as differential test between C. albicans and the other yeast species.

REFERENCES

Al-Doory, Y. (1980): Laboratory medical mycology. Lea and Febider, Philadelphia, U.S.A.

Arthur, G.H. (1964): Wright's Veterinary Obstetrics, 3rd Ed. Bailliere, Tindall and Cox. Chapter 28.

Awad, H.H.; Zaki, K.; El-Sawaf, S. and Afiefy, M.M. (1978): Bacterial flora of puerperial disturbances in buffaloes and cows and its sensitivity to different antibiotics. J. of Egypt. Vet. Asoc., 38, 2, 9-14.

Bailey, W.R. and Scott, E.G. (1978): Diagnostic Microbiology 4th ed., A text book for the isolation and indentification of pathogenic microorganisms. The V.V. Mosby Company, Saint Louis.

Buchanan, R.E. and Gibbons, N.E. (1974): Bergey's Manual of determinative bacteriology. William and Wilkins Company, Baltimore.

Crysewski, S.J. and Pier, A.C. (1968): Mycotic abortion in ewes produced by Aspergillus fumigatus. Pathologic changes Am. J. Vet. Res. 29, 1135-1139.

Dennis, S.M. and Nairn, M.E. (1970): Prenatal lambmortality in a Marino flock in Western Australia. Aust. Vet. J. 46, 432-438.

Dardner, D.E. (1967): Abortion associated with mycotic infection in sheep. N.S. Vet. J. 15, 85-86.

Farid, A.; Atia, M. and Hassouna, N. (1980): Production of phosphatase enzyne by candida species. Mykosen, 23, Heft. 11, 640-645.

Gunter, J.J.; Collins, W.J.; Sorensen, A.M.; Scaies, J.W. and Alford, A. (1955): A survey of the bacteria in the reproductive tract of dairy animals and their relationship to fertility. Am. J. Vet. Res. 16, 282-286.

Howarth, J.A. (1932): E.coli like organism. Causing abortion in sheep. Cornell Vet., 22, 253-260.

Marinov, P. (1967): Effect of certain microorganisms on fertility and embryonic mortality in sheep. Ii- Their effect on viability and dehydrase activity of sperm. Vet. Med. Nauki, Sofia 4, 73-78.

Quinlivan, T.D. (1970): The bacteriology of cervical mucous of ewes at certens following intravaginal sponges. Aust. Vet. J., 46, 11-16.

Roberts, S.T. (1971): Veterinary Obstetrics and genital direases. CBS publisher & Distribulars.

Shalash, M.R. and Elgindi, M. (1968): Preliminary studies on some reproductive characters of Ausimi ewes. Veterinaria Saiy. 17, 145-149.

Smith, I.D. and Frost, A.I. (1968): The pathogenicity to pregnant ewes. Aust. Vet. J. 44, 17-19.

Smith, R.; Dianna, B. and Doyton, L. (1974): Phosphatase activity among candida species and other yeasts isolated from clinical materials. Applied Microbiology, 26, 364-369.

Sykora, F. (1932): The presence of bacteria in health and diseased genital organs of cattle Klin, Spisy, Skaly Brno, 9, 1-33.

Thurston, J.R.; Cysewski, S.F.; Pier, A.C. and Richard, J.L. (1978): Precipitions in serum from sheep infected with Asp. Fumigatus. Am. J. Vet. Res., 33, 5, 929-936.

Watson, W.A. (1970): Other infections diseases of the reproductive tract. Fertility and Infertility in the Domestic Animals. 2nd Ed. J.A. Laing (Ed.), Bailliere Tindell and Cassell, London.

Youseff, H.I.; Hegazy, A.; Ragab, E.S.A.; Seida, A.A. and Ismail, M. (1984): Bacteriological and histopathological studies on endometritis of ewes J. Egypt. Vet. Med. Assoc., 44, 2, 75-84.

Zaki, K. and Saber, M.S. (1962): The bacterial flora of the external os and prepuce of Marino sheep. Vet. Med. J. Giza, 8, 175-179.

Zaki, O.; Zaki, K. and Mousa, A. (1963): The bacterial flora of normal puerperium in Friesian cows in Egypt. Vet. Med. J., Fac. of Vet., Cairo Univ., IX, No. 10.

Table (1)
Incidence of positive cases for culture at various conditions

Cases	Site of study	total No.of samples	Bacteriological cases				Mycotic cases			
			Positive No.	cases %	Negative No.	cases %	positive No.	cases %	Negative No.	cases %
Late pregnancy	vagina	35	14	40.00	21	60.00	13	37.14	22	62.86
	cervix		9	25.71	26	74.29	7	20.00	28	80.00
During peuroperium	vagina	40	23	57.50	17	42.5	19	47.50	21	52.50
	cervix		20	50.00	20	50.00	10	25.00	30	57.00
Infertile ewes	vagina	35	27	77.14	8	22.86	15	42.86	20	57.64
	cervix		18	51.43	17	48.57	12	34.29	23	65.71

Table (2)
Bacterial species isolated from ewes at various conditions

Species	Pregnant				During peurperium				Infertile ewes			
	vagina		cervix		vagina		cervix		vagina		cervix	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Micrococcus lateus	4	21.05	2	16.67	6	9.23	1	4.00	4	8.00	1	3.33
Micrococcus rubrum	-	-	-	-	-	-	-	-	1	2.00	-	-
Staph. aureus	1	5.26	1	8.33	11	16.92	5	20.00	7	14.00	5	16.67
Staph. epidermidis	3	15.79	1	8.33	-	-	-	-	1	2.00	-	-
B.Haemdlytics strep-tococci (Strept. pyogenes)	1	5.26	1	8.33	7	10.77	2	8.00	9	18.00	7	23.34
-Haemolytic strept-ococci (Strept. facealis)	3	15.79	2	16.67	5	7.69	3	12.00	3	6.00	2	6.68
Bacillus spp.	1	5.26	2	16.67	5	7.69	3	12.00	3	6.00	3	10.00
E.coli	4	21.05	3	25.00	11	16.92	7	28.00	6	12.00	3	10.00
Proteus mirabilis	-	-	-	-	1	1.54	-	-	5	10.00	3	10.00
Pr. morganii	-	-	-	-	4	6.15	1	4.00	2	4.00	-	-
Pr.vulgaris	-	-	-	-	-	-	-	-	-	-	1	3.33
Pr.rettgeri	-	-	-	-	1	1.54	1	4.00	-	-	-	-
Ps.aeruginosa	-	-	-	-	1	1.54	-	-	5	10.00	1	3.33
Ps.flourscence	-	-	-	-	3	4.61	1	4.00	-	-	-	-
Klebsiella	-	-	-	-	2	3.07	1	4.00	3	6.00	1	3.33
Aerobicales aerogores	-	-	-	-	-	-	-	-	1	2.00	1	3.33
Serratia merescence	-	-	-	-	-	-	-	-	-	-	1	3.33
C.pyogenes	-	-	-	-	-	-	-	-	-	-	1	3.33
Mixed culture	2	10.54	-	-	8	12.31	-	-	-	-	-	-
Total	**19**		**12**		**65**		**25**		**50**		**30**	

Table (3)
Mycotic species isolated from ewes at various conditions

Species	Pregnant				During Peurperium				Infertile ewes			
	Vagina		Cervix		Vagina		Cervix		Vagina		Cervix	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Candida albicans	2	12.50	1	14.28	3	15.00	-	-	16	64.00	13	68.44
C.Krusei	2	12.50	-	-	-	-	-	-	1	4.00	-	-
C.tropicalis	-	-	1	14.28	-	-	-	-	2	8.00	1	5.26
C. pseudo-tropicalis	-	-	-	-	-	-	-	-	2	8.00	2	10.52
Tor.glabrata	2	12.50	1	14.28	-	-	-	-	1	4.00	1	5.26
Asp. niger	3	18.75	1	14.28	-	-	-	-	2	8.00	1	5.26
Asp. fumigatus	2	12.50	1	14.28	-	-	-	-	1	4.00	1	5.26
Asp. terreus	3	18.75	1	14.28	-	-	-	-	-	-	-	-
Rizopous	-	-	-	-	7	35.00	3	30.00	-	-	-	-
Mucor	2	12.50	1	14.28	5	25.00	4	40.00	-	-	-	-
Absida	-	-	-	-	5	25.00	3	30.00	-	-	-	-
Total	**16**		**7**		**20**		**10**		**25**		**19**	

Table (4)
Phosphatase Profduction by candida species recovered from various condition

Condition	Organism tested	Number of strains tested	Number of positive strains 24 hours	48 hours	72 hours
Infertile ewes	C. albicans	29	-	-	-
	C. krusei	1	1	-	-
	C. tropicalis	3	3	-	-
	C. psudotiopicalis	4	-	1	1
Normal Condition	C. albicans	6	-	-	-
	C. krusei	2	-	-	-
	C. tropicalis	1	-	-	-

Table (5)
Enzymatic activity as related to change in the medium pH.

Candida species	strain number	24 hours pH 5	pH 4.7	pH 4.2	48 hours pH 5	pH 4.7	pH 4.2
C. tropicalis	1	+3	+3	+3	+3	+3	+3
	2	+4	+4	+4	+4	+4	+4
	3	+4	+3	+3	+3	+3	+3
C. krusei	1	+4	+4	+4	+3	+3	+3

+4 deep pink colour
+3 pink colour
+2 Jaint pink colour
+1 very paint pink colour