EFFECT OF FLUOROGESTERONE ACETATE IMPREGNATED INTRAVAGINAL SPONGES¹ ON VAGINAL BACTERIAL FLORA OF EWES.

J. D. AMIN

Department of Vet. Surgery and Reproduction, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

Received 2 December, 1993; Accepted 3 April, 1995

ABSTRACT

The bacterial flora of the vagina of ten ewes was determined at sponge removal and two days later before insertion of fluorogesterone acetate intravaginal sponge. Using *E. coli* as marker species, there was a significant increase ($P < 0.05$) in bacterial population when samples taken before sponge insertion and at removal were compared, but there was a significant ($P < 0.01$) decrease in bacterial population when samples taken at sponge removal were compared to samples taken two days later. There was no significant difference ($P > 0.05$) when bacterial population before sponge insertion was compared with the bacterial population two days after sponge removal. It is concluded that following use of fluorogesterone acetate intravaginal pessaries (and injection of PMSG at sponge removal), there is increased bacterial growth in the vagina which is largely eliminated two days after sponge removal.

Key words: Fluorogesterone acetate, vagina, sponges, ewe, bacterial flora.

INTRODUCTION

Progesterone containing sponge implants are widely used for oestrus synchronisation in ewes (Arthur, Noakes and Pearson, 1989), but their use has been associated with fetid discharges containing large quantities of bacteria, desquamated cells, cellular debris and leucocytes at removal (Quinlivan, 1970). This could affect fertility of the ewes. Although some workers (Sharaff, Zaki and Saber, 1963) obtained luxuriant bacterial growth in animals treated with progesterone, others (Moore and Robinson, 1967) reported no significant difference in the bacterial population of vaginal mucus or of sponges taken and impregnated with progesterone. Oestrus synchronised ewes do lamb at

optimum times, i.e. when grazing and food are at maximum levels. This is particularly relevant in many parts of Nigeria where pasture is not available on a year-round basis. It is therefore the aim of this study to attempt to determine the effect of fluorogesterone acetate sponge implant on the bacterial flora of the vagina of ewes.

MATERIALS AND METHODS

Ten mature Dorset ewes being bred out of season in Hertfordshire, England were sampled as follows: The ewes were adequately restrained and the vulval lips parted with a gloved hand. A sterile guarded swab (Medical wire and Equipment Ltd. England) was then inserted about 6 cm into the vagina and the swab was pushed out of the protecting tube and rotated several times. The swab was withdrawn back into the protecting tube and removed from the vagina. The swab was then placed in an airtight tube. In the laboratory, the swabs were streaked onto Columbia blood agar and MacConkey agar and where possible direct smears were made, Gram stained and examined. Isolates from the Columbia blood agar and MacConkey agar were identified based on standard microbiological procedures (Barrow and Feltham 1993). The ewes were then treated with intravaginal progesterone impregnated sponges (Cronolone^R (intervet, U.K) which contains 30 mg fluorogesterone acetate) for 11 days. At sponge removal, 500 IU of Pregnant Mare Serum Gonadotrophin (PMSG) was injected into each ewe. Vaginal swabs were taken at sponge removal and then two days later. Using *E. coli* as a marker species, the bacterial population was assessed based on presence of 2 or more colonies on the line of first streak (+), second streak (++) , third streak (+++) and fourth streak (++++) .

RESULTS

E. coli was the commonest isolate. *E. coli* was however not isolated from any of the samples taken from B44. In this study, using *E. coli* as a marker species, the preponderance of growth was significant ($P < 0.05$) when bacterial population was considered before sponge insertion and at sponge removal. There was also a very significant difference ($P < 0.01$) when bacterial population was considered at the time of sponge removal and

the presence of several bacteria which could not be cultured. Detailed result of bacteria isolated before intravaginal sponge insertion, at sponge removal and two days later are given in Table 1

The + sign denotes the presence of two or more colonies on lines of first (+) or fourth (++++) streak.

DISCUSSION

The vagina of ewes like most mucosal surfaces of animals is known to harbour some

TABLE 1 BACTERIAL ISOLATES FROM 10 EWES BEFORE TREATMENT WITH CRONOLONE

Ewe number	Before sponge insertion	At sponge removal	Two days after sponge removal
YYII	<i>E. coli</i> +++ <i>Proteus</i> spp (MacConkey agar)	<i>E. coli</i> ++ Coagulase negative <i>Staphylococcus</i>	<i>E. coli</i> ++
GG2	Coagulase negative <i>Staphylococcus</i> <i>Streptococcus bovis</i>	<i>E. coli</i> ++++	<i>E. coli</i> ++ Coagulase negative <i>Staphylococcus</i>
BB6	<i>E. coli</i> ++	<i>E. coli</i> +++ Coagulase negative <i>Staphylococcus</i>	<i>Bacillus</i> spp <i>Staphylococcus</i>
DD32	<i>E. coli</i> ++ <i>Corynebacterium pyogenes</i>	<i>E. coli</i> +++	<i>E. coli</i> +
D39	<i>E. coli</i> ++ <i>C. pyogenes</i>	<i>E. coli</i> ++++ <i>C. pyogenes</i>	<i>E. coli</i> ++
Y5	<i>E. coli</i> + <i>C. pyogenes</i>	<i>E. coli</i> ++++ <i>C. pyogenes</i>	<i>E. coli</i> ++
B41	<i>E. coli</i> ++ <i>Bacillus</i> spp	<i>E. coli</i> ++ <i>Pasteurella hemolytica</i> <i>Streptococcus</i> spp <i>C. pyogenes</i>	<i>E. coli</i> +
YD20	<i>E. coli</i> + <i>C. pyogenes</i>	<i>E. coli</i> +++ <i>C. pyogenes</i>	Coagulase negative <i>Staphylococcus</i>
B43	<i>E. coli</i> + <i>C. pyogenes</i>	<i>E. coli</i> ++ <i>C. pyogenes</i>	<i>Bacillus</i> spp
B44	<i>Streptococcus</i> spp	<i>Bacillus</i> spp Coagulase negative Staph.	<i>Bacillus</i> spp Coagulase negative <i>Staphylococcus</i>

two days after sponge removal. However, there was no significant difference ($P > 0.05$) when the isolation rate of *E. coli* was considered before sponge insertion and two days after sponge removal (B44 was not considered in the statistics as no *E. coli* were isolated from any of the swabs taken from it).

Other bacteria isolated included *Bacillus* sp, *Corynebacterium* spp, *Pasteurella* spp, *Proteus* spp, *Staphylococcus* spp and *Streptococcus* spp. The direct smears revealed

microorganisms that are considered to be normal flora. Previous studies (Moorthy and Singh 1982; Al-Amin, 1988) have shown that *E. coli* is commonly isolated from the vagina of ewes. Also it has been reported (Quinlivan, 1970) that large quantities of *E. coli* could be isolated from the string hanging from the progesterone sponge implants (especially near the sponge) used in oestrus synchronisation. The results of this study suggest that the presence of progesterone impregnated

intravaginal sponges enhances bacterial multiplication in the vagina. This contrasts the findings of Moore and Robinson (1967). The increased bacterial growth in the vicinity of the sponge was likely to be due to the effects of both the progesterone in the sponge as well as the sponge itself. The focus seems to be particularly favourable for *E. coli* multiplication because of the frequency with which it was isolated. This may be related to the fact that *E. coli* is the commonest bacterial isolate in the vagina of ewes (Al-Amin, 1988). The cervix plays a vital role in the maintenance of adequate sperm numbers in the uterus and fallopian tubes (Hunter, 1980). The presence of large numbers of bacteria within its vicinity is therefore likely to affect fertility. However in a study in cows some authors (Noakes, Wallace and Smith, 1991) suggested that the cervix and other body defenses probably provide an effective barrier even in the presence of heavy vaginal contamination from the environment.

Using this system of oestrus synchronisation, ewes are inseminated 57 hours after removal of pessaries (Laing, Brinley-Morgan and Wagner, 1988; Arthur et al., 1989). This study suggests that normalcy is restored about 2 days after sponge removal following increased bacterial multiplication due to the effect of the sponge. However, some authors (Quinlivan and Robinson, 1969) reported reduced fertility using Artificial Insemination following use of progesterone sponges for oestrus synchronisation. This may not be unconnected with the bacterial load in the vagina. The restoration of normal bacterial population is probably due to large amounts of oestrogen secreted just before ovulation as a result of the effect of the PMSG administered.

It has been shown (Ritar, Salamon, Ball and O' May, 1989) that administration of PMSG stimulated ovulatory activity in female goats treated with intravaginal devices. Therefore, varying the time of administration could affect the rate of bacterial clearance from the vagina. The genital tract is known to be more resistant to infection at estrus (Arthur et al.; 1989). The

bacteria isolated from the vaginal swabs in this study are compatible with findings from normal ewes (Al-Amin, 1988), suggesting that only the quantity of the bacteria changed rather than the quality following treatment with intravaginal sponges. In this study only aerobic bacteria were cultured. It may be worthwhile to determine the effect of other intravaginal sponges treatment regimes on the vaginal flora and fertility taking into consideration strict anaerobes.

ACKNOWLEDGEMENTS

The assistance received from Dr. A. J. Wilsmore, RVC London is gratefully acknowledged.

REFERENCES

- AL-AMIN, J. D. (1988). Bacteriology of genital tract of ewes: aetiology of non-specific infections. *Bull. Anim. Hlth Prod. Afr.* 36:225-227
- ARTHUR, G. H., NOAKES, D. E. and PEARSON, H. (1989). *Veterinary Reproduction and Obstetrics*, 6th Ed. ELBS London.
- BARROW, G. I. AND FELTHAM, R. K. A. (1993). *Cowan and Steel's Manual for the identification of Medical bacteria*. Third Edition, Cambridge University Press
- HUNTER, R. H. F. (1980). *Physiology and Technology of Reproduction in female Domestic Animals*. Academic Press, London.
- LAING, J. A., BRINLEY-MORGAN, W. J. AND WAGNER, W. C. (1988). *Fertility and Infertility in Domestic Animals*. Second Edition, Bailliere Tindall Co. London. p. 119.
- MOORE, M. W and ROBINSON, T. J. (1967). *Control of the ovarian cycle in the sheep*. Sydney University Press.
- MOORTHY, A. R. S. and SINGH, S. P. (1982). Studies on the bacterial flora of the female genital tract of sheep. *Bull. Anim. Hlth. Prod. Afr.* 30: 15-18.
- NOAKES, D. E.; WALLAGE, L. and SMITH, G. R. (1991). Bacterial flora of the uterus of cows after calving on two hygienically contrasting farms. *Vet. Rec.*, 128:440-442.
- QUINLIVAN, T. D. (1970). The bacteriology of the cervical mucus of ewes at oestrus following treatment with fluorogesterone impregnated intravaginal sponges. *Austr. Vet. J.* 46: 11-16
- QUINLIVAN, T. D. and ROBINSON, T. J. (1969). Numbers of spermatozoa in the genital tract after artificial insemination of progesterone treated ewes. *J. Reprod. Fert.* 19:73-86.
- RITAR, A. J., SALAMON, S.; BALL, P. D. and O' MAY, P. J. (1989). Ovulation and fertility in goats after intravaginal device-PMSG treatment. *Small Rumin. Res.* 2:323-331.
- SHARAFF, A. A., ZAKI, K. and SABER, M. S. (1963). The effect of oestrogen and progesterone on the growth of normal bacterial flora of the external os uteri in Egyptian cattle. *J. Arab Vet. Med. Ass.* 23: 214-226.