080

26. Mitchell, G., Liu, I.K.M., Perryman, L.E., Stabenfeldt, G.H. and Hughes, J.P. Preferential production and secretion of immunoglobulins by the equine endometrium - a mucosal immune system. J. Reprod. Fertil. (Suppl.) 32:161-168 (1983).

27. Widders, P.R., Stokes, C.R., David, J.S.E. and Bourne, F.J. Quantitation of the immunoglobulins in reproductive tract secretions of the mare. Res. Vet. Sci. 37:324-330 (1984).

28. Widders, P.R., Stokes, C.R., David, J.S.E. and Bourne, F.J. Immunohistological studies of the local immune system in the reproductive tract of the mare. Res. Vet. Sci. 38:88-95 (1985).

29. Asbury, A.C., Halliwell, R.E.W., Foster, G.W., and Longino, S.J. Immunoglobulins in uterine secretions of mares with differing resistance to endometritis. Theriogenology 14:299-308 (1980).

30. Hansen, P.J. and Asbury, A.C. Opsonins of Streptococcus in uterine flushings of mares susceptible and resistant to endometritis: control of secretion and partial characterization. Am. J. Vet. Res. 48:646-650 (1987).

31. Washburn, S., Klesius, P.H., Ganjam, U.K. and Brown, B.C. Effect of estrogen and progesterone on the phagocytic response of ovariectomized mares infected in utero with -hemolytic streptococci. Am. J. Vet. Res. 43:1367-1370 (1982).

32. Evans, M.J., Hamer, J.M., Gason, L.M., Graham, C.S., Asbury, A.C. and Irvine, C.H.G. Clearance of bacteria and nonantigenic markers following intrauterine inoculation into maiden mares: effect of steroid hormone environment. Theriogenology 26:37-50 (1986).

33. Casida, L.E., Graves, W.E., Lauderdalt, J.W., Riesen, J.W., Saiduddin, S., Hauser, E.R. and Tyler, W.J. Studies on the postpartum cow. Wisconsin Res. Bull. no. 270 (1968).

EFFECT OF SUPPRESSION OF PROLACTIN ON REPRODUCTIVE PERFORMANCE DURING THE POSTPARTUM PERIOD AND SEASONAL ANESTRUS IN A DAIRY EWE BREED

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ABSTRACT

The effect of suppression of prolaction with bromocryptine treatment for 15 or 16 d on the interval to estrus and on fertility in Karagounico ewes, a local dairy breed, during seasonal anestrus and the postpartum period was investigated in three experiments. In Experiment 1 (late anestrus), the interval between ram introduction and estrus was shorter in the treated (22 ± 5.6 d) than in the control ewes (mean interval >38 d) during the 43-d testing period. In Experiment 2 (during nursing) and in Experiment 3 (after weaning), the ewes were injected with bromocryptine for 15 or 16 d and subsequently mated at a hormonally induced estrus. In Experiment 2, bromocryptine treatment started during the suckling period and the ewes nursed their lambs until sponge removal. The lambing rate to the induced estrous period was significantly higher in the treated than in the control ewes (70% vs 22%, $P<0.05$). In Experiment 3 bromocryptine treatment started after weaning. Thirty-four percent of the ewes failed to show estrus at the first estrous period. Lambing response for both the induced and the second estrous period, one cycle later, was higher in the treated than in the control ewes (100% vs 66%, $P<0.025$). The results of both experiments suggest that suppression of prolactin during lactation improved fertility.

Key words: sheep, bromocryptine, prolactin, fertility

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INTRODUCTION

Season of lambing and duration of lactation in ewes are the most important factors which influence the interval from parturition to the resumption of cycling (1). Lactation (2,3) and uterine involution and repair (4) extend postpartum anestrus more during the nonbreeding than in the breeding season. In spite of the inhibition due to suckling and lactation, estrus and ovulation can be induced by the use of progestogens and pregnant mare serum gonadotropin (PMSG), but fertility is generally low, particularly during the anestrous season (3,5,6) and was suggested that high prolactin levels may be responsible for the effects of season and lactation on ewe fertility (6).

Bromocryptine treatment in the breeding season during early postpartum period in a dairy ewe breed resulted in an earlier recovery of ovarian function (7). However, bromocryptine was not effective in restoring the resumption of ovarian activity in Finnsheep-crossbred ewes during the fall (8). Suppression of prolactin did not affect the interval to estrus during the postpartum period (9) and seasonal anestrus (10-13).

In view of the differences in prolactin concentrations and fertility between breeds and seasons and of the alleged association of prolactin with fertility, the effects of suppression of prolactine with bromocryptine on fertility and estrus was investigated during nursing, after weaning, and during late anestrus in a local dairy breed.

MATERIALS AND METHODS

Mature Karagouniko ewes (mean weight 54.9 ± 1.37 kg) from a local dairy breed were used. The breeding season for this breed (>85% of ewes in estrus) is from July to February (14). Ewes involved in these experiments were kept in open-front barns and fed a concentrate-hay diet.

Experiment 1 (Late anestrus)

Ten ewes that lambed in December nursed their lambs for 42 d and were then milked until May. Two to 65 d after termination of milking, they were randomly allocated to two groups. The allocation of ewes to two groups resulted in a balance of days after termination of milking (mean interval from termination of milking to start of experiment 26 and 24 d in Groups 1 and 2, respectively). Group 1 ewes were injected subcutaneously twice daily (830 and 1830 h) with bromocryptine[a] (1.5 mg) for 15 d, starting on May 24 (Table 1). Group 2 ewes were administered a diluent. The drug (2-bromo-alpha-ergocryptine) was dissolved in a minimal volume of 70% (v/v) ethanol and then mixed with water and tartaric acid (same weight as bromocryptine) to a final concentration of 1 mg/ml. A vasectomized ram was introduced for 1 h

[a]Bromocryptine, Sandoz Ltd., Basle, Switzerland.

to the flock every day for 43 d, beginning 4 d before the start of treatment for estrus detection. Jugular blood was obtained daily during treatment. A final sample was taken 7 d after completion of treatment. The plasma was stored at -20°C for prolactin and progesterone measurement.

Experiment 2 (During nursing)

Nineteen ewes that lambed in December and January were randomly allocated to two groups. Ewes with similar lambing dates and litter size were equally allocated in the two groups. On Day 34 ± 2 postpartum, Group 1 (10) and 2 (9) ewes were injected subcutaneously twice daily with bromocryptine (1.4 mg) or diluent, respectively, for 16 days (Table 1).

At the end of treatments, all ewes were given intravaginal cronolone-impregnated (30 mg) sponges (Chronogest)[b] for 12 d and 333 IU PMSG (Folligon)[c] i.m at pessary withdrawal. The following 3 d the treated ewes were hand-mated to rams of proven fertility. The ewes nursed their lambs until pessary withdrawal and were milked thereafter.

Blood samples were taken twice weekly during the bromocryptine treatment and also at 15 and 21 d after pessary withdrawal for plasma progesterone measurements.

Experiment 3 (After weaning)

Thirty-three ewes that lambed between December 15 and January 15 were randomly assigned to one of two groups. Lambing dates and litter size were considered for allocation in the two groups as in Experiment 2. The ewes nursed their lambs for 42 d and were milked thereafter. On Day 56.5 ± 2 postpartum, Group 1 (15) and 2 (18) ewes were injected subcutaneously twice daily with bromocryptine (1.5 mg) or diluent, respectively, for 15 d (Table 1). The day after the last injection all ewes were given intravaginal medroxyprogesterone -impregnated (60 mg) sponges (Veramix)[d] for 13 d and 333 IU PMSG (Folligon) i.m. at pessary withdrawal. The ewes were hand-mated to fertile rams during the 4 d following pessary removal. Following this period the rams remained in the flock for 25 more d. Blood samples were taken twice weekly before the start of the experiment, during the bromocryptine treatment, and at Days 16 and 22 after pessary withdrawal.

Prolactin concentrations in peripheral plasma were determined by a double antibody radioimmunoassay (15). Ovine prolactin for preparation of standards and iodination (OPRL-I-1,16) and ovine prolactin antiserum (anti-OPRL-1) were provided by the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases (NIADDK), National Hormone and Pituitary Program. The second

[b] Chronogest, Intervet, Angers, France.

[c] Folligon, Intervet, Angers, France.

[d] Veramix, Upjohn Co., Kalamazoo, MI 49001.

Table 1. Schedule for treatments of ewes during late anestrus (Experiment 1), during nursing (Experiment 2), and after weaning (Experiment 3)

Item	Experiment 1	Experiment 2	Experiment 3
Check of estrus, Days -4 to 39	May 20	—	—
Weaning of lambs (Experiment 3)	—	—	January 30 to February 24
Bromocryptine or diluent injection, Days 0 to 16	May 24 to June 8	February 1 to February 17	February 24 to March 11
Insert sponges, Day 20 (Experiment 2; Day 16 (Experiment 3)	—	February 21	March 11
Remove sponges/PMSG injection, Day 32 (Experiment 2), Day 29 (Experiment 3)	—	March 5	March 24
Weaning of lambs (Experiment 2)	—	March 5	—
First induced estrous period, Days 33 to 35 (Experiment 2), Days 31 to 33 (Experiment 3)	—	March 6 to March 8	March 26 to March 28
Second estrous period, Days 46 to 54 (Experiment 3)	—	—	April 12 to April 18

antibody was antirabbit-γ-globulin cultured in donkey[e]. The sensitivity of the assay was 3 ng/ml. Concentrations of progesterone were measured by radioimmunoassay (17). The sensitivity of the assay was 0.06 ng/ml. The within and between assay coefficients of variation were 12.5 and 15.7%, respectively. The concentrations of progesterone in plasma were corrected for recovery (70 ± 3%).

Comparisons between treatments were made using Student's t-test and the Chi-square analyses.

RESULTS

Experiment 1 (Late anestrus)

Plasma prolactin concentrations were high at the start of

[e] Anti-rabbit-γ-globulin, Wellcome Reagents Ltd. U.K.

blood sampling. During the bromocryptine treatment mean prolactin concentrations were 103.0 ± 4.9 and 12.7 ± 1.5 ng/ml in control and treated ewes, respectively (Figure 1).

The mean interval between ram introduction and estrus exhibition was shorter in the treated (22 ± 5.6 days, $P<0.05$) than in control ewes (mean interval > 38 d); three out of five ewes did not show estrus during the testing period and the remaining two had intervals of 24 and 37 d.

Plasma progesterone concentrations were lower than 0.5 ng/ml in all ewes except one (3.5 ng/ml) at Day 0 of treatment (Table 2). The ewes which exhibited estrus within the testing period had progesterone concentrations ≥ 1 ng/ml, similar to cyclic patterns. All ewes in the control group had similar patterns of progesterone levels during the sampling period. Progesterone levels of these ewes were low at Days 0 and 6 and increased the following days, suggesting the occurrence of ovulations.

Table 2. Concentrations of progesterone in plasma samples of anestrous ewes during and after bromocryptine treatment

Days after start of treatment	Progesterone (ng/ml)[a]	
	Control	Bromocryptine-treated
0	0.26 ± 0.08	0.98 ± 0.63
3	2.03 ± 0.85	2.86 ± 0.81
6	0.25 ± 0.08	2.51 ± 0.54
10	4.48 ± 0.57	1.07 ± 0.28
14	0.77 ± 0.40	1.00 ± 0.19
21	0.75 ± 0.07	2.06 ± 0.38

[a] Each value is the mean ± SEM (n = 5).

Experiment 2 (During nursing)

All ewes exhibited estrus within the 3 d following PMSG. The lambing rate in the bromocryptine group was significantly ($P<0.05$) greater (Table 3). Progesterone concentrations during bromocryptine treatment were <0.5 ng/ml in most of the samples of all ewes. Concentrations >1 ng/ml found at Day 13 of treatment in both groups were of short duration. Progesterone concentrations at Days 12 to 14 after mating were similar in pregnant and nonpregnant ewes (Table 4).

Experiment 3 (After weaning)

Twenty-one (65.6%) ewes were observed in estrus within 4 d after pessary withdrawal (Table 5). Two successive peaks of lambing activity were observed which differed by the duration

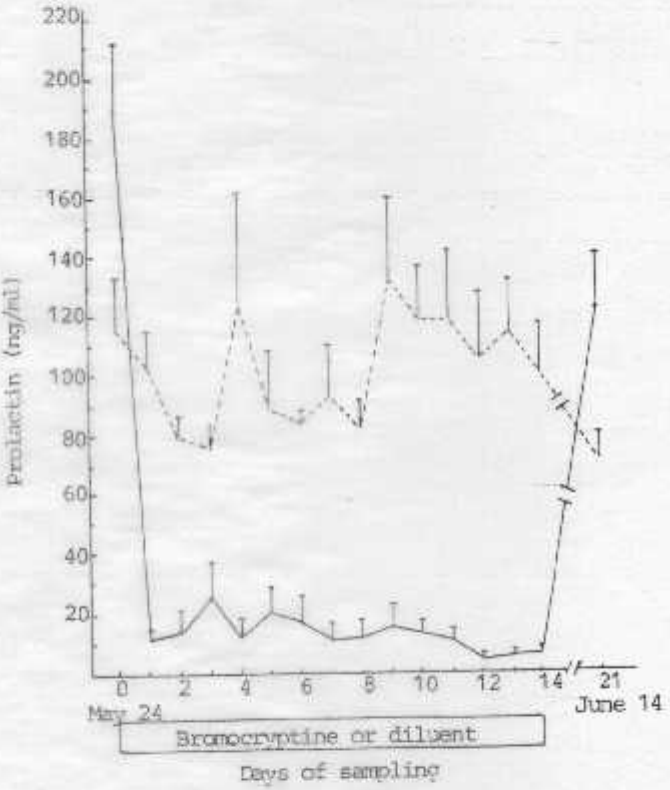

Figure 1: Plasma prolactin concentrations (mean ± SEM) in control (.- - - -.) and bromocryptine treated (. _____ .) ewes during late anestrus (Experiment 1).

Table 3. Estrus and lambing responses of control and bromocryptine treated ewes during nursing

Group	No. of ewes	Ewes responding within 3 d to PMSG: First induced estrus No.	(%)	Lambing rate No.	(%)	No. born
Control	9	9	(100)	2	(22.2)[a]	1.0
Bromocryptine	10	10	(100)	7	(70.0)[a]	1.29 ± 0.18[b]

[a] $P<0.05$.

[b] No. born is the mean ± SEM.

Table 4. Plasma progesterone concentrations in postpartum ewes at various times after mating (Experiments 2 and 3)

Days after mating	Progesterone (ng/ml)[a] Pregnant No.	ng/ml	Nonpregnant No.	ng/ml
Experiment 2[c]				
12 to 14	10	1.8 ± 0.24	9	1.5 ± 0.25
18 to 20	10	2.2 ± 0.30	9	<0.25
Experiment 3[d]				
12 to 14	15	2.9 ± 0.40[b]	17	1.9 ± 0.15[b]
19 to 21	15	2.26 ± 0.22	17	<0.3

[a] Each value is the mean ± SEM. Prediction of pregnancy was based on levels ≥ 1 ng/ml at Days 18 to 21 after mating.

[b] The values are significantly different from each other ($P<0.05$).

[c] Blood samples taken at Days 15 and 21 after pessary withdrawal.

[d] Blood samples taken at Days 16 and 23 after pessary withdrawal.

of one estrous cycle. Lambing response for both estrous periods after PMSG injection was greater (P<0.025) in the bromocryptine than in the control group (100 vs 66.7%). Progesterone concentrations before and during bromocryptine treatment were low (<0.2 ng/ml) in most of the samples of all ewes. Values of 1 ng/ml observed in 42% of the animals in both groups were of 3 to 5 d duration. Progesterone concentrations at Days 12 to 14 after mating were significantly higher (P<0.05) in pregnant than in nonpregnant ewes (Table 4).

DISCUSSION

The interval from ram introduction to estrus was shorter in the bromocryptine compared with the control group. The high progesterone levels found in one ewe of the bromocryptine group at Day 0 of treatment suggests that cycles started in this ewe before ram introduction. The progesterone profiles of the ewes during the period before ram introduction are not known. A proportion of ewes of this breed showed estrus or had silent ovulations during May and silent ovulations in June (14). The number of ewes in this work do not permit a conclusion on the effect of bromocryptine treatment on the interval to estrus.

Results (Experiments 2 and 3) indicated that the reduced fertility of ewes mated during lactation was improved by bromocryptine treatment. These results are consistent with studies of reduced fertility of lactating ewes, particularly during the anestrous season (3,5,18). Therefore, elevated prolactin levels may be responsible for the effects of season and lactation on ewe fertility (6). The results from suckled ewes (Experiment 2) are consistent with the suggestion that higher prolactin levels specifically induced by suckling are responsible for the suppression of ovarian activity during lactation (19). It has been suggested that high prolactin levels may interfere at early stages of follicular development with the resultant failure of normal luteal function (20). The exhibition of estrus at two estrous cycles (Experiment 3) is in contrast with a previous study using long-term bromocryptine treatment of Greyface ewes that returned to anestrus after the induced estrus (13). Breed differences in the depth and length of anestrus may have contributed to these results.

Plasma progesterone concentration at Days 12 to 14 after mating were significantly higher in pregnant than in nonpregnant ewes (after weaning). This difference was not observed in nursing ewes. The lower progesterone levels at the days preceeding luteolysis at Day 15 in pregnant nursing ewes are in agreement with results of others (5) and suggest a possible association with greater embryonic mortality in these ewes. In the ewes that failed to lamb to the induced estrus during nursing, the progesterone levels at Days 12 to 14, were similar to those of nonbred ewes, and they decreased to low levels at Days 18 to 20, indicating fertilization failure or embryonic death before Days 10 to 14 (21,22).

REFERENCES

1. Hunter, G.L. Increasing the frequency of pregnancy in sheep. I. Some factors affecting rebreeding during the post-partum period. Anim. Breed. Abstr. 36:347-378 (1968).

2. Whiteman, J.V., Zollinger, W.A., Thrift, F.A. and Gould, M.B Post-partum mating performance of ewes involved in a twice-yearly lambing program. J. Anim. Sci. 35:836-842 (1972).

3. Rhind, S.M., Robinson, J.J., Chesworth, J.M. and Phillippo, M. Effects of season, lactation and plane of nutrition on the reproductive performance and associated plasma LH and progesterone profiles in hormonally treated ewes. J.Reprod. Fertil. 58:127-137 (1980).

4. Van-Niekerk, C.H. Limitations to female reproductive efficiency. In: Tomes, G.J., Robinson, D.E. and Lightfoot, R.J. (eds.). Proceedings International Sheep Breeding Congress. Western Australian Institute Technology Press. pp. 299-309 (1976).

5. Cognie, V., Hernandez-Barreto, N., and Saumande, J. Low fertility in nursing ewes during the non-breeding season. Ann. Biol. Anim. Bioch. Biophys. 15:329-343 (1975).

6. Rhind, S.M., Robinson, J.J., Chesworth, J.M. and Crofts, R.M.J. Effects of season, lactation and plane of nutrition on prolactin concentrations in ovine plasma and the role of prolactin in the control of ewe fertility. J. Reprod. Fertil. 58:145-152 (1980).

7. Kann G., Martinet, J. and Schirar, A. Modifications of gonadotrophin secretion during natural and artificial hyperprolactinaemia in the ewe. In: Crossigniani, P. and Robyn C (eds.). Prolactin and Human Reproduction. Acad. Press pp. 47-59 (1977).

8. Forè, J.J. Postpartum reproductive performance of Finn sheep-crossbred ewes. J.Anim.Sci. 49:1043-1050 (1979).

9. Fitzgerald, B.P. and Cunningham, F.J. Effect of removal of lambs or treatment with bromocryptine on plasma concentrations of prolactin and FSH during the post-partum period in ewes lambing at different times during the breeding season. J.Reprod.Fertil. 61:141-148 (1981).

10. McNeilly, A.S. and Land, R.B. Effect of suppression of plasma prolactin on ovulation, plasma gonadotrophins and corpus luteum function in LH-RH-treated anoestrous ewes. J.Reprod.Fertil. 56:601-609 (1979)

11. Land, R.B., Carr, W.R., McNeilly, A.S. and Preece, R.D. Plasma FSH, LH, the positive feedback of oestrogen, ovulation and luteal function in the ewe given bromocryptine to suppress prolactin during seasonal anoestrus. J.Reprod.Fertil. 59:73-78 (1980).

12. Schanbacher, B. Relationship of daylength and prolactin to resumption of reproductive activity in anestrous ewes. J.Anim.Sci. 50:293-297 (1980).

13. Rodway, R.G., Robinson, J.J. and Phillippo, M. Ovulation rate in induced oestrous cycles of anoestrus ewes given bromocryptine. J.Reprod.Fertil. 68:265-267 (1983).

14. Bosniakou, A.G. Comparative study of annual oestrual and ovarian activity of Greek breeds of sheep, Chios, Karagouniki and Serres. Doctorate Thesis, Aristotle University of Thessaloniki (1983).

15. Walton, J.S., Evins, J.D., Fitzgerald, B.P. and Cunningham, F.J. Abrupt decrease in daylength and short-term changes in the plasma concentrations of FSH, LH and prolactin in anoestrous ewes. J.Reprod.Fertil. 59:163-171 (1980).

16. Goldfine, L.D., Amir, S.M., Peterson, A.W. and Ingbar, S.H. Preparation of biologically active ^{125}I-TSH. Endocrinology 95:1228-1233 (1974).

17. Furr, B.J.A. Radioimmunoassay of progesterone in peripheral plasma of the domestic fowl in various physiological states and in follicular venous plasma. Acta Endocr. 72:89-100 (1973).

18. Dawe, S.T. and Fletcher, I.C. The affect of post lambing interval on fertilization in lactating ewes treated with progestagen impregnated sponges and gonadotrophins. Proc.Aust.Anim.Prod. 11:137-140 (1976).

19. Kann, G. and Martinet, J. Prolactin levels and duration of post-partum anestrus in lactating ewes.Nature (Lond) 257:63-64 (1975).

20. McNeilly, A.S., Glasier, A., Jonassen, J. and Howie, P.W. Evidence for direct inhibition of ovarian function by prolactin. J.Reprod.Fertil. 65:559-569 (1982).

21. Dooley, M. and Wodzicka-Tomaszewska, M. Progesterone levels in ewes following early embryonic death. J.Reprod.Fertil. 28:150-151 (1972).

22. Moor, R.M. and Rowson, L.E.A. Influence of the embryo and uterus on luteal function in the sheep. Nature (Lond) 201:522-523 (1964).

EVALUATION OF THE UTERINE ENVIRONMENT IN EXPERIMENTAL AND SPONTANEOUS BOVINE METRITIS

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ABSTRACT

This stucy describes a model for experimental induction of metritis in cattle. Cows were given two intrauterine inoculations of a mixture of Corynebacterium pyogenes and Bacteroides melaninogenicus at 12 and 36 h following calving. The endometrium was subjected to minor irritation just prior to inoculation with bacteria. All cows showed signs of severe metritis within 48 h following the last intrauterine inoculation.

The study also evaluated intrauterine oxygen reduction potential (E_h) and pH following calving in control cows, cows with retained fetal membranes, and cows with induced metritis. The results revealed that cows with retained fetal membranes or induced metritis had lower E_h values ($P < 0.05$) than control cows on Days 2, 3, or 4 postpartum. This suggests that antibiotics that are effective under anaerobic conditions should be considered for intrauterine therapy during this period.

Key words: metritis, uterine environment, retained fetal membranes

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