تأثير المعاملة بالسائل الجريبي البقري
في فترة انحلال الجسم الأصفر على الخصوبة في النعاج

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الخلاصة

تم تكثيف الشياع لاثنين وثمانين نعجة مضربة السلالة. حقنت هذه الحيوانات تحت الجلد بـ2 أو 5 مل من السائل الجريبي البقري أو بـ2 أو 5 مل من البلازما البقرية (مجموعة السيطرة) عند الساعة 8,00 والساعة 16,00 من اليوم 15 وعند الساعة 6,30 والساعة 16,00 من اليوم 16 من دورة الشبق على التوالي.

وجد أن ليس هناك فروق معنوية في طول دورة الشبق لنعاج التجربة الأولى، بينما كان الفرق معنوياً ($P < 0.05$) في طول الدورة لنعاج التجربة الثانية. في كلا التجربتين لم نحصل على فرق معنوي في معدل الإباضة بين المجموعة المعاملة ومجموعة السيطرة.

أما تأثير المعاملة بالسائل الجريبي البقري على الخصوبة فكان معنوياً

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($P < 0.05$) بالمقارنة مع مجموعة السيطرة حيث عادت 13 من مجموع 41 نعجة للدورة مقارنة بعودة 3 فقط من مجموع 41 نعجة على التوالي إذ اتخذ حدوث الحمل كمقياس على الخصوبة.

Effect of Treatment with Bovine Folliclar Fluid Around the Time of Natural Luteolysis on Fertility in Ewes*

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SUMMARY

Estrus of 82 corss-bred ewes was resynchronized. Subcutaneous injection of 2 or 5 ml bovine follicular fluid (bFF) or 2 or 5 ml bovine plasma (bP) were given at 8:00 h and 16:00 h on day 15 and at 6:30 h and 16:00 h on day 16 of the estrous cycle.

The difference between cycle lengths was not significant in trial (1) while reached significancy ($P < 0.05$) in trial (2).

No significant ($P > 0.05$) differences were observed in ovulation rates (OR) in both trials.

The bFF treated animals (13 returns from 41 ewes) showed significantly ($P < 0.05$) reduced fertility compared to the bP treated control group (3 returns from 41 ewes).

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INTRODUCTION

Follicular fluid from several species is known to contain inhibin, the nonsteroidal regulator of F.S.H, and treatment of animals with steroid-Free preparations of this hormone can result in changes to ovarian function. (Al-Obaidi, 1986).

Bovine follicular fluid (bFF) treatment can produce a delay in the onset of estrus in sheep and cattle (Miller et al., 1979, 1982; Lutjens, 1980; Cummins, 1983; McNeilly, 1984; Wallace and McNeilly, 1984).

Further studies indicated an increase in ovulation rate with bFF treatment given during the luteal phase (Wallace and McNeilly, 1984) or around the time of natural or induced luteolysis (Lutjen, 1980; and Cummins, 1983) in the ewes. Such effects are proposed to result from the suppression of FSH levels producing inhibition of follicular development (delay of estrus) and the subsequent hypersecretion of FSH observed to occur with cessation of bFF treatment (Cummins, 1983).

To farther study such suppression on follicular development and ovarian function this experiment was carried out to determine the effects of two different doses of bFF treatment around the time of natural luteolysis on follicular development by determination of estrous onset, ovulation rate, and fertility in ewes.

MATERIALS AND METHODS

Trial 1:

Injection of 2 ml bFF or 2 ml bP twice daily on days 15 and 16 of the cycle.

Animals from a previous experiment (Al-Obaidi and Kink, unpublished data) were resynchronized by prostaglandin injection (Estrumate, 100*mug*). Ewes not responding and showing estrus prior to the treatment dates were detected and removed from the experiment. The remaining ewes were randomly allocated into two groups (bFF or bP). Subcutaneous injections of 2 ml bFF or bP were given twice daily, at

8:00 h and 16:00 h on day 15 and at 6:30 h and 16:00 h on day 16 of the cycle to ewes in the two groups, respectively.

Estrus was detected at eight-hourly intervals commencing at 8:00, 16:00 and 24:00 h daily. Entire harnessed rams were used to include a fertility study. This was assessed by detection of any returns to estrus (i.e. non-pregnant ewes) with entire rams harnessed with a different colored raddle by observations at weekly intervals over seven weeks after the initial matings were recorded. Any animals showing uncertain return estrus data (i.e. not clearly raddle marked) we examined by endoscopy to check for pregnancy or non-pregnancy.

Ovulation rate (OR) was assessed for the treatment cycle by endoscopic examination (Oldham and Lindsay, 1980).

Trial 2:

Injection of 5 ml bFF or bP twice daily on days 15 and 16 of the cycle.

The animals used in this trial were 23 ewes removed from trial (1) prior to injection as they were detected in estrus before treatment commenced.

The estrous cycles of these ewes were resynchronized (Estrumate 100μg) and their subsequent estrus recorded, thus giving the exact day 1 of the cycle for each animal. Animals were treated on days 15 and 16 as determined from this observation.

Subcutaneous injections at 5 ml of bFF or bP were given to randomized groups using the same injection protocol as in trial (1). Fertility was determined as in trial (1).

t-tests and analysis of variance with groups of unequal size were carried out (Snedecor and Cochran, 1967).

Estimation of inhibin activity:

Inhibin activity analyzed following the experiments indicated that the bFF used in trial 1 contained 9856 u/ml. Dosage of 2 ml involved

injection of 19712 units inhibin activity twice daily. On the other hand, follicular fluid used in trial 2 had an inhibin activity of 6318 u/ml giving a dosage level at 31590 units per 5 ml injection.

RESULTS

Trial 1:

Mean cycle lengths for bFF and bP treatment groups were compared unsing a t-test for comparison of two samples with groups of unequal size (Table 1). The difference between cycle length was not significant in this experiment ($P > 0.2$).

Mean ovulation rates for the experimental cycle and for the experimental cycle corrected by subtraction of pretreatment ovulation rates were also compared using the same test as mentioned above and the results are presented in Table 1.

No significant differences were observed between experimental cycle ovulation rates ($P > 0.05$) or in experimental cycle ovulation rates adjusted by previous values ($P > 0.4$) with injection of bFF or bP.

Trial 2:

Mean cycle lengths for the two groups were again analyzed using the t-test for groups of unequal size. Comparison of the two treatment groups (Table 1) showed a significant ($P < 0.05$) increase in the length of the cycle in bFF-treated animals.

Comparison of the mean ovulation rates for the experimental cycle and the adjusted mean ovulation rates for the experimental cycle (by subtraction of the original ovulation rate for each animal) was carried out as in trial 1 using t-test for unequal groups. Ovulation rates for both the experimental cycle and for the experimental cycle adjusted by previous ovulation rates were similar for the two treatment groups (Table 1). No significant different occurred ($P > 0.5$).

Table 1. Cycle length and ovulation rate in the cross bred ewes following treatments with two different doses of bFF or bP on day 15 and 16 of the estrous cycle.

Trial No.	Treatment	Number per group	Cycle length (mean ±SEM)	Ovulation rate	
				Experimental cycle	Experimental minus previous cycle
1	2 ml bFF	28	17.98 ± 0.19	1.71 ± 0.51	−0.14 ± 0.17
	2 ml bP	30	17.59 ± 0.21	1.93 ± 0.09	−0.07 ± 0.14
2	5 ml bFF	13	18.86 ± 0.40*	1.71 ± 0.07	−0.43 ± 0.17
	5 ml bP	11	17.28 ± 0.54	1.93 ± 0.09	−0.07 ± 0.14

* Significantly different from control ($P < 0.05$).

Since large numbers of animals are needed to detect small differences in fertility, the data for trial 1 and 2 were combined and analyzed using a Chi squared test.

For the entire group a total of 16 returns to estrus (non-pregnant ewes) from 82 animals were recorded (Table 2). The bFF treated group (13 returns from 41 ewes) showed a significant ($P < 0.05$) reduction in fertility compared to the bP treated group (3 returns from 41 ewes).

Table 2. Effects of bFF treatment on fertility (combined trial 1 and 2) as number of returns (non-pregnant).

Treatment	Number per group	Number of returns
bFF	41	13*
bP	41	3

* $\chi^2 = 4.047$; $P < 0.05$

DISCUSSION

Follicular fluid samples used were known to possess inhibin activity by measurement in pituitary cell culture bioassay (J.K. Findlay, personal communication) with varying levels of activity. The results

obtained in trial 1 and 2 were dependent on the dose of bFF and activity of the total dose injected. Injections of 2 ml of bFF with inhibin activity of 9000 u/ml were observed to delay estrus and suppress FSH in Merino ewes (Cummins, 1983). The 2 ml injections given in trial 1 with 9865 u/ml activity had no effect on cycle length. This could be due to body weight differences between the Merinos used by Cummins (1983) (44 Kg) and the cross bred ewes used in these experiments which had an average weight of 60 Kg. An increased dose of bFF in trial 2 resulted in a significant delay to the onset of estrus. This delay to estrus observed to occur with bFF treatment in sheep (Lutjen, 1980; Cummins, 1983; Wallace and McNeilly, 1984) results from deprivation of FSH during the final stages of development of the preovulatory follicle rather than by interference with luteolysis (Miller et al., 1979). Final development of the preovulatory follicle occurs in the three days following luteal regression when basal LH levels increase (Baird, 1983).

Increased estradiol secretion from the preovulatory follicle is responsible for induction of estrous behavior in the ewe (Baird and McNeilly, 1981). FSH is known to be ipmportant in growth of antral preovulatory follicles (Baird, 1983). Therefore, the delay in the onset of estrus may be related to this requirement for sufficient levels of FSH during the time following luteolysis. Lack of FSH will result in low levels of estradiol and consequently estrus will be delayed. Similarly, low levels of FSH at stages of the cycle when follicle growth is important will result in inhibition of development of antral follicles and hence ovulation will also be delayed. Decreased FSH concentration may also promote atresia in antral follicles therefore, inhibiting growth of these follicles not sufficiently developed to withstand such a decrease in FSH.

The expected suppression of FSH by bFF in trial 2 would occur at a time when levels in control animals were at the pretreatment level so normal follicular development was maintained. The hypersecretion (rebound) of FSH during the preovulatory period has been proposed

as the cause of an increase in ovulation rate following treatments with relatively large amounts of bFF (Lutjen, 980; Cummins, 1983; Wallace and McNeilly, 1984). Although in trial 2 ovulation rate was not observed to increase, Lutjen (1980) observed a significant ($P < 0.01$) increase in ovulation rate with four injections of 2 ml of bFF on days 15 and 16 of a normal cycle in Merino ewes. Wallace and McNeilly (1984), by suppressing FSH throughout the luteal phase, observed a rebound response following luteolysis ana d resultant increased ovulation rate.

A further result observed in these experiments was a reduction in fertility in the bFF treated group. The possibility in reduced fertility is that inadequate estrus behavior caused due to decreased secretion of estradiol by the preovulatory follicles compared to control animals may be responsible for poor fertility in these animals. Intensity of estrus was not examined but indications that it is lower in bFF treated ewes were obtained by Cummins (1983). As steroids alone do not appear to control FSH levels completely in the ewe (Goodman et al., 1981) a physiological role for inhibin in addition to steroid action appears probable.

This study has demonstrated that treatment with bFF caused alterations to several aspects of ovarian function through the depression of FSH by inhibin preparations. This indicates a possible important role of inhibin in the raped ovarian control of plasma FSH.

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