Influence of season and sex on the inhibitory effect of ovine follicular fluid on plasma gonadotrophins in gonadectomized sheep

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Summary. The inhibitory effects of follicular fluid on FSH secretion were similar in gonadectomized male and female sheep, and in the anoestrous and breeding seasons. Significant suppression of LH was variable and was observed only at the highest dose of follicular fluid when suppression rarely exceeded 50% of pretreatment values. Basal plasma FSH and LH concentrations were higher in castrated males than in ovariectomized females in both seasons. Plasma FSH concentrations in gonadectomized males and females and LH concentrations in the males were lower in the anoestrous than the breeding season. Therefore, in the absence of the gonads, sex and photoperiod can influence hypothalamic control of basal pituitary gonadotrophin secretion in males and females, whereas the feedback effect of non-steroidal factors in follicular fluid (inhibin) on FSH secretion is not influenced by photoperiod or sex.

Introduction

Ovine follicular fluid and rete testis fluid are both rich sources of inhibin (Hudson *et al.*, 1979; Tsonis *et al.*, 1983) and will suppress FSH secretion in a dose-dependent manner in ovariectomized ewes (Cummins, O'Shea, Bindon, Lee & Findlay, 1983) and castrated rams (Hudson *et al.*, 1979; Blanc, Hochereau-de Reviers, Cahoreau, Courot & Dacheux, 1981). The negative feedback effect of steroids on gonadotrophin secretion in gonadectomized sheep (Pelletier & Ortavant, 1975; Legan & Karsch, 1980) is influenced by photoperiod, but it is not known whether photoperiod influences the degree to which inhibin can suppress the secretion of FSH.

The sex of rats appears to influence the degree to which follicular fluid can suppress FSH secretion, females being more responsive than males at high doses of follicular fluid (de Jong, Welschen, Hermans, Smith & van der Molen, 1978; Hermans, van Leeuwen, Debets & de Jong, 1980). However, there were confounding effects of age (Hermans *et al.*, 1980) and the relative response of FSH was not always based on studies in males and females with the same inhibin preparations given by the same route of administration in the same experiment (Eddie *et al.*, 1980; Lorenzen, Dworkin & Schwartz, 1981; Cummins *et al.*, 1983). In this study, the effects of ovine follicular fluid on peripheral concentrations of plasma FSH and LH were compared in the anoestrous and breeding seasons in gonadectomized male and female sheep matched for age and breed.

Goodman, Bittman, Foster & Karsch (1982) showed that seasonal changes in the pattern of LH secretion can occur, independent of the presence of steroid feedback, in chronically ovariectomized ewes. Similar studies on castrated male sheep have not been reported. We have therefore also compared pretreatment basal plasma gonadotrophin concentrations in the two sexes during both seasons.

Materials and Methods

Sheep and blood sampling. Crossbred Merino ewes and rams (1.5–2 years old) from the same flock were gonadectomized at least 6 weeks before the start of the experiment in the anoestrous season (November). The same animals were tested in the breeding season (April) and again in the following anoestrous season. On the day before blood sampling began (Day 1), the animals were weighed and one jugular vein of each animal was cannulated with Silastic tubing (Dow Corning, MI, U.S.A.) fitted with a three-way tap and kept filled with heparinized saline (0.9 g NaCl/l; 75 U heparin/ml). Blood samples were collected into heparinized syringes, and the plasma was harvested and stored at -15°C . Blood samples were collected hourly for 6 h beginning at 09:00 h on Days 1–4 of the experiment, except in the second anoestrous season, when series of samples were collected on Days 1 and 3 only. Composite plasma samples were formed for each animal on each day and assayed for LH and FSH.

Six ewes remained intact and were run with vasectomized rams to detect oestrus and confirm the times of the breeding and anoestrous seasons. In both anoestrous periods, none of these ewes exhibited behavioural oestrus from 2 weeks before until 2 weeks after the experiment and all ewes had plasma concentrations of progesterone <0.5 nmol/l, indicative of anoestrus. In April, all the entire ewes exhibited at least 2 regular oestrous cycles before and during the experiment and were observed to have plasma progesterone concentrations ranging from 3.4 to 8.4 (mean 6.4 ± 1.7 s.d.) nmol/l in blood samples taken 10 days after oestrus, indicative of ovulation and normal luteal function.

Treatments. Follicular fluid from sheep ovaries collected at the abattoirs without regard for time of year was pooled, treated with dextran-coated charcoal according to the method of Tsonis *et al.* (1983), and stored at -15°C in volumes sufficient for each time of treatment. This preparation had an oestradiol-17 β concentration of 0.22 pmol/l by radioimmunoassay (see below) and an inhibin potency of 20.5 kU/ml (19.1–22.0, 95% confidence intervals; index of precision of the bioassay, $\lambda = 0.09$), measured at 3 dilutions in the in-vitro pituitary cell bioassay for inhibin (Scott, Burger & Quigg, 1980) with modifications described by Lee, Quigg, McMaster, Burger & Leversha (1983). Briefly, these modifications involved measuring FSH in the culture medium of pituitary cells prepared from immature females rather than in the pituitary cells of mature male rats. The potencies of a number of follicular fluid samples relative to the inhibin standard from ovine testicular lymph protein (1 U/mg) (Eddie, Baker, Higginson & Hudson, 1979) were similar in the two bioassays (C. G. Tsonis & V. W. K. Lee, unpublished observations).

Treated animals (2–4/group) received about 0.5, 1.0 or 2.0 ml follicular fluid, the final volume being adjusted to give a similar dose of inhibin activity (U/kg live weight) within treatment groups on each occasion. Injections were given s.c. at 08:30 and 17:00 h on each of Days 2 and 3 of the experiment, similar to the treatment regimen described by Cummins *et al.* (1983).

Control animals (4/group) received s.c. injections of 2 ml serum, treated with dextran-coated charcoal as described above, from an hypophysectomized ewe. This preparation had no detectable levels of inhibin (<0.004 kU/ml) or oestradiol-17 β (<0.05 pmol/l).

Hormone assays. The data for hormone assays were computed by the method of Burger, Lee & Rennie (1972).

Plasma concentrations of FSH and LH were determined by radioimmunoassays (Lee *et al.*, 1976; Bremner, Findlay, Lee, de Kretser & Cumming, 1980) using NIAMDD-oFSH-13 (biological potency $15 \times$ NIH-FSH-S1) and NIH-LH-S18 as standards, respectively. All of the samples were assayed within one assay, and were read on that portion of the standard curve where the within-assay coefficient of variation (CV) was $<15\%$ over the range 5–312 ng FSH/ml (minimum 6.7%) and 1.2–61.3 ng LH/ml (minimum 5.1%). The respective sensitivities of the FSH and LH assays were 1.2 and 0.44 ng/ml.

Oestradiol-17 β and progesterone concentrations in follicular fluid, serum or plasma were

measured in solvent extracts by radioimmunoassay using WHO matched reagents (Carson, Trounson & Findlay, 1982). Recovery was >90% and no corrections were made for losses. The sensitivities of the oestradiol-17 β and progesterone assays were 25.0 and 15.0 fmol/tube, respectively and the within-assay CVs were 4.5 and 7.1%, respectively.

Analysis of data. Mean plasma FSH concentrations in males and females on Day 1 did not differ between anoestrous seasons 1 and 2 (t test; $P > 0.05$), therefore the anoestrous data were pooled within sexes. The effects of season and sex on pretreatment (Day 1) plasma gonadotrophin concentrations were analysed by Student's paired and unpaired t tests (Table 1). The gonadotrophin concentrations on Days 2, 3 and 4 were expressed as percentages of the concentrations on Day 1 for each ewe to reduce between-animal variation. These percentages were subjected to (a) analysis of variance for repeated measurements and trend analysis to determine the degree to which the data within treatment groups conformed with a linear trend up or down (Winer, 1971) and (b) analysis of variance and Duncan's multiple range F -test to determine the effects of dose of follicular fluid within days. The effects of sex and season on the response of FSH to ovine follicular fluid in gonadectomized sheep were analysed by comparing, on Day 3, the percentage suppression of FSH at the highest dose of follicular fluid. Day 3 was chosen because it was the period of observation when the suppression of FSH was maximal or approaching the maximum. By Day 4, FSH concentrations in some groups were increasing (Text-fig. 1; Cummins *et al.*, 1983).

Results

Basal gonadotrophin concentrations

The concentrations of FSH and LH on Day 1 were significantly higher in castrated males than ovariectomized females in the anoestrous ($P < 0.005$) and breeding seasons ($P < 0.01$) (Table 1). In the castrated males, the concentrations of FSH ($P < 0.05$) and LH ($P < 0.005$) were significantly lower in the anoestrous seasons than in the breeding season. Similarly, the concentrations of FSH were lower in ovariectomized females in the anoestrous seasons than the breeding season ($P < 0.05$), but the mean concentrations of LH did not differ significantly between seasons (Table 1).

Table 1. Peripheral concentrations (ng/ml) of LH and FSH in plasma of gonadectomized male and female sheep in the anoestrous and breeding seasons

Hormone	Sex	Anoestrous season 1	Breeding season	Anoestrous season 2
FSH	Male	123 $\pm$ 9 (16)	179 $\pm$ 17 (16)*	95 $\pm$ 8 (16)
	Female	91 $\pm$ 14 (10)†	111 $\pm$ 14 (10)*‡	72 $\pm$ 8 (13)†
LH	Male	14.7 $\pm$ 1.2 (16)	16.9 $\pm$ 1.3 (16)**	—
	Female	10.9 $\pm$ 1.5 (11)†	12.0 $\pm$ 1.0 (11)‡	—

Values are mean $\pm$ s.e.m. for the no. of ewes indicated in parentheses.

* $P < 0.05$, ** $P < 0.005$ versus anoestrous season value, independent t test for FSH; paired t test for LH.

† $P < 0.005$, ‡ $P < 0.01$, versus male value, independent t test.

Treatment with ovine follicular fluid and hypophysectomized ewe serum

Treatment with control serum was not associated with a significant linear trend up or down in plasma LH (Table 2) or FSH (Text-fig. 1) concentrations in any group ($F_{3,12} > 3.49$ for males; $F_{3,8} > 4.07$ for females; $P < 0.05$).

Treatment with follicular fluid had a significant suppression on plasma FSH concentrations in all groups particularly at the highest dose on Day 3 ($P < 0.05$) and Day 4 ($P < 0.05$; except males on Day 4 in the first anoestrous season, $P > 0.05$), but not on Day 2 ($P > 0.05$) (Text-fig. 1). Maximum suppression of FSH was observed on Days 3 or 4, with a tendency for concentrations to

Text-fig. 1. The effects of charcoal-treated serum from an hypophysectomized ewe and various doses of charcoal-treated ovine follicular fluid on plasma FSH concentrations (% of values on Day 1 before treatment) in gonadectomized (a) female and (b) male sheep in the breeding season and anoestrous season 1. Injections (arrows) were given s.c. as 0.5 (△), 1.0 (□) or 2.0 ml (■) follicular fluid or 2.0 ml (○) serum. ^{a-d} Values within days with different superscripts differ significantly, $P < 0.05$.

be increasing by Day 4 in animals treated with lower doses of follicular fluid. Significant suppression of LH was observed only at the highest dose of follicular fluid (Table 2), but the response was variable and the suppression of LH rarely exceeded 50% of pretreatment values, whereas FSH fell to around 30–40% of pretreatment values (Text-fig. 1).

Suppression of FSH (mean $\pm$ s.e.m. % values on Day 1) on Day 3 after the highest dose of follicular fluid was as follows: anoestrus 1; males, 36 ± 8 ; females, 33 ± 5 ; breeding season; males, 39 ± 2 ; females, 34 ± 4 , and did not differ significantly ($P > 0.05$, dependent and independent t test).

Table 2. Mean LH (% of concentrations on Day 1) $\pm$ s.d. on Days 2–4 after treatment of gonadectomized male and female sheep with serum (Control) or the highest dose (2 ml) of ovine follicular fluid (oFF)

Season	Treatment	Day 2	Day 3	Day 4
<i>Males</i>				
Anoestrus I	Serum	113 $\pm$ 32	86 $\pm$ 12 ^a	109 $\pm$ 49
	oFF	115 $\pm$ 41	63 $\pm$ 8 ^a	54 $\pm$ 18
Breeding Season	Serum	94 $\pm$ 11	94 $\pm$ 3 ^b	86 $\pm$ 10
	oFF	91 $\pm$ 5	67 $\pm$ 10 ^b	69 $\pm$ 7
<i>Females</i>				
Anoestrus I	Serum	95 $\pm$ 4	63 $\pm$ 5	82 $\pm$ 18
	oFF	97 $\pm$ 55	44 $\pm$ 17	41 $\pm$ 19
Breeding Season	Serum	83 $\pm$ 10	95 $\pm$ 14	92 $\pm$ 7 ^c
	oFF	91 $\pm$ 19	74 $\pm$ 19	68 $\pm$ 9 ^c

^{a,b,c} Means with same superscript differ significantly, $P < 0.05$. There were no significant differences ($P > 0.05$) in LH between control animals and animals treated with 0.5 and 1.0 ml oFF/injection (Duncan's Multiple Range F-test).

Discussion

This study shows that the response of FSH to ovine follicular fluid in adult gonadectomized animals was not influenced by the sex of the sheep or the season (photoperiod). It confirms the effects of treatment with preparations rich in inhibin activity on plasma FSH concentrations and, at the highest doses, on LH concentrations in gonadectomized sheep (Hudson *et al.*, 1979; Blanc *et al.*, 1981; Cummins *et al.*, 1983).

The study shows that the changes in FSH responses with time were similar in gonadectomized male and female sheep when follicular fluid was given by s.c. injection, and confirm an earlier observation for ewes by Cummins *et al.* (1983). This time course of suppression is very likely to be influenced by the route of administration of follicular fluid. Tsonis (1984) has shown that maximum suppression of FSH occurs within 12 h when ovine follicular fluid is given as a single i.v. injection to ovariectomized ewes.

Two studies of rats indicated that the relative responses of FSH to bovine and porcine follicular fluid, respectively, were greater in gonadectomized females than males, particularly at high doses (Hermans *et al.*, 1980; Lorenzen *et al.*, 1981). However, the response depended on the age of the rats and the time after surgery (up to 72 h). When dose-dependent decreases in plasma FSH concentrations were observed 8 h after injection of bovine follicular fluid into male and female rats gonadectomized 48 h earlier, the relative responses appeared to be similar, except at the highest dose when FSH suppression appeared greater in females (de Jong *et al.*, 1978). In this study no statistical difference was observed in the ability of ovine follicular fluid to inhibit FSH in gonadectomized male and female sheep.

The absence of a change with season in the relative FSH response to follicular fluid in both sexes suggests that inhibin is not involved in the seasonal regulation of gonadal function. Therefore, the lower concentrations of FSH in intact ewes (Findlay & Cumming, 1976) and rams (Lincoln & Short, 1980) in the anoestrous compared with the breeding season are likely to be due to enhanced responsiveness to oestradiol-17 β (Legan & Karsch, 1980) and testosterone (Pelletier & Ortavant, 1975), respectively, and to steroid-independent decreases in the hypothalamic stimulation of pituitary function (Goodman *et al.*, 1982; this study). It is possible that the relative response of plasma FSH to follicular fluid is enhanced in the presence of steroids, particularly in the anoestrous season. There is evidence of synergistic actions of inhibin and oestradiol-17 β or testosterone in the control of FSH secretion in gonadectomized rodents (Bronson & Channing, 1978; Hopkinson, Fritze, Chari, Sturm & Hirschhauser, 1977; Peek & Watkins, 1980) and mares (Miller, Wesson & Ginther, 1981), but no such studies in sheep have been reported.

The higher mean FSH concentrations in gonadectomized sheep in the breeding than in the anoestrous season are consistent with previous reports for intact sheep (Findlay & Cumming, 1976; Lincoln & Short, 1980). The pulse amplitude and frequency of LH has been observed to change inversely between seasons in chronically ovariectomized ewes, resulting in similar mean LH concentrations throughout the year (Goodman *et al.*, 1982). We confirmed that mean LH does not change significantly with season in ovariectomized ewes in this study, but in castrated males LH concentrations were higher in the breeding than anoestrous seasons. These effects attributed to season are unlikely to be a function of time after gonadectomy of both sexes, because gonadotrophin concentrations in plasma plateau by 20 days after surgery (Blanc *et al.*, 1981; Wright, Stelmasiak & Anderson, 1983).

The higher mean LH and FSH concentrations in gonadectomized male than female sheep, independent of season, has not been reported previously. Hermans *et al.* (1980) showed data indicating a sex difference in FSH but not LH in 35-day-old rats 8 h after gonadectomy. The reasons for the higher concentrations of gonadotrophins in chronically castrated male sheep are not known. It may reflect greater GnRH pulse frequency and amplitude, greater sensitivity of the pituitary to GnRH stimulation or lower clearance of FSH in the males. The pituitary content of FSH and LH in intact rams is only 10–20% of that in cyclic ewes (Robertson, Ellis, Foulds, Findlay & Bindon, 1984).

In conclusion, this study has shown that the feedback effect of non-steroidal factors in follicular fluid, presumably inhibin, on FSH secretion in gonadectomized sheep is not influenced by photoperiod or sex. In contrast, basal pituitary secretion of gonadotrophins in chronically gonadectomized sheep does change with sex and photoperiod, indicating an influence on hypothalamic control of pituitary function which is independent of the gonads.

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