Ovarian inhibin: a hormone with potential to increase ovulation rate in sheep

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ABSTRACT

There is increasing evidence that a non-steroidal compound, inhibin, produced by the ovary is involved in the regulation of follicle stimulating hormone (FSH) production by the pituitary. Our studies have shown that ovarian inhibin is present in follicular fluid, that it is synthesised exclusively by follicular granulosa cells, that androgens regulate its synthesis and that it is predominantly a product of healthy follicles, i.e., follicles with the potential to attain ovulatory maturity. It is suggested that androgens produced by luteinising hormone action on follicular thecal tissue stimulate granulosa cell inhibin production which then suppresses pituitary FSH secretion thereby limiting the number of follicles that can be stimulated to develop to ovulatory maturity. Ovulation rates of Romney ewes actively immunised with a semi-purified preparation of inhibin derived from bovine follicular fluid were significantly higher than those of control ewes (2.06 ± 0.16 *v* 1.31 ± 0.06 ovulations/ewe).

Keywords Inhibin; granulosa cells; theca; androgens; sheep; cow; ovulation rate

Adequate exposure to the pituitary gonadotrophins, luteinising hormone (LH) and follicle stimulating hormone (FSH) is necessary for a follicle to develop and mature sufficiently so that it can ultimately ovulate and release an egg capable of fertilisation. Together LH and FSH act on the follicle to stimulate oestradiol-17ß production, and oestradiol-17ß with FSH promotes the development of the follicle by stimulating processes including antrum development, cell proliferation, receptor and enzyme induction and oocyte maturation. In the absence of FSH, follicles never attain ovulatory maturity but degenerate (i.e., undergo atresia). Conversely, increased exposure to FSH reduces the incidence of follicular atresia thereby allowing more follicles to attain ovulatory maturity. FSH and gonadotrophins with FSH-like activity, e.g., PMSG can be used to increase ovulation rate, albeit unpredictably, in sheep and cattle. Thus, pharmacological manipulation of pituitary FSH secretion provides a potential means of regulating follicular atresia to produce an ovulation rate compatible with a desired prolificacy.

Regulation of Ovarian Inhibin Production

While feedback effects of ovarian steroids is the generally accepted means by which pituitary FSH secretion is regulated, there is increasing evidence that the ovary also produces a non-steroidal compound, named inhibin which regulates FSH secretion (Franchimont *et al.*, 1981; Channing *et al.*, 1982). Inhibin is defined as a non-steroidal compound of gonadal origin which specifically or selectively inhibits pituitary secretion of FSH. Its activity is normally measured by an appropriate bioassay, e.g., suppression of FSH production by cultured rat anterior pituitary cells (Henderson and Franchimont, 1981). Inhibin has not yet been fully characterised. However, data from several laboratories indicate that inhibin is likely to be an acidic protein (isoelectric point $\sim$pH = 5) with a molecular weight $>10\,000$ and it may contain a carbohydrate moiety (Grady *et al.*, 1982). Inhibin activity has been found in the follicular fluid of all primate (monkey and human) and non-primate (cow, sheep, pig, mare) species thus far examined. Studies with bovine ovarian tissues indicate that follicular granulosa cells are the source of ovarian inhibin. Inhibin is secreted *in vitro* by cultured granulosa cells but not by cultured thecal tissue, ovarian stroma nor corpus luteum tissue (Henderson and Franchimont, 1983). In addition, there is a significant positive correlation between inhibin concentrations in bovine follicular fluid and the number of granulosa cells per follicle (Henderson *et al.*, 1984). Inhibin has also been shown to be produced by sheep (Henderson, Franchimont and McNatty, unpublished), pig and monkey (Channing *et al.*, 1982) granulosa cells.

Neither LH, FSH nor prolactin have any effect on the capacity of granulosa cells to produce inhibin *in vitro*, suggesting that granulosa cell inhibin production is not directly regulated by gonadotrophin action on that cell type (Henderson and Franchimont, 1981).

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Androgens do, however, influence granulosa cell inhibin production; androstenedione, testosterone, 5α-dihydrotestosterone and the synthetic androgens mesterolone and methylestrenolone each stimulating inhibin production by granulosa cells in a dose dependent manner (Henderson and Franchimont, 1981; 1983). In contrast to androgens, oestradiol and oestrone have no effect on granulosa cell inhibin production. Theca interna cells, which lie adjacent to granulosa cells in the follicle are the major source of follicular androgens and LH stimulates thecal androgen production (McNatty *et al.*, 1984). Thus, one can envisage an interaction between granulosa cells and theca interna whereby LH acting on the follicle stimulates thecal androgen production and this androgen, in turn, stimulates granulosa cell inhibin production.

The majority of follicles in an ovary never ovulate, but undergo atresia. A study was performed in our laboratory to determine if inhibin was predominantly a product of the relatively few healthy follicles with the potential to attain ovulatory maturity or a product of atretic follicles. It was found that granulosa cells from healthy bovine follicles produced significantly more inhibin than cells from atretic follicles (49 ± 7 *v* 16 ± 4 U inhibin/10^6 cells, $n = 21$). In addition, the mean concentration of inhibin in bovine follicular fluid from non-atretic follicles was also significantly higher than that in fluid of atretic follicles (11 ± 1 *v* 6 ± 1 U/ml follicular fluid, $n = 21$) (Henderson *et al.*, 1984).

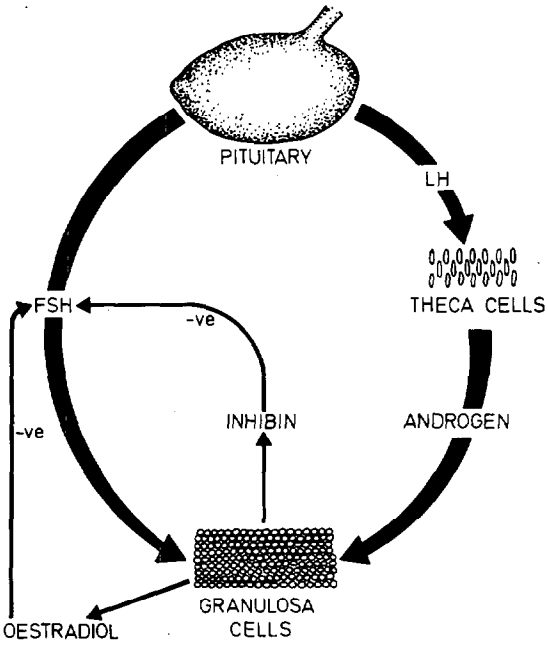

FIG. 1 Relationship between pituitary gonadotrophin secretion and ovarian inhibin production.

Inhibin and the Regulation of Pituitary FSH Production.

Taking these findings together and using the sheep as a model, one can propose that during the follicular phase the following scheme of events might occur, as outlined schematically in Fig. 1. Following the initiation of luteal regression, plasma progesterone concentrations start to fall. This releases the pituitary from the negative feedback effects of progesterone and there is a rise in the peak frequency and amplitude of LH, causing an increase in the plasma concentration of LH. This together with elevated plasma FSH concentrations promotes the growth and maturation of the follicle(s) which will ultimately ovulate, which respond with an increased production of oestradiol. Follicular inhibin production would also be stimulated by androgens, produced by LH action on the theca interna, stimulating granulosa cell inhibin production. While plasma LH and oestradiol concentrations continue to rise throughout the follicular phase, plasma FSH concentrations fall during the mid-follicular phase (Baird *et al.*, 1981) due to the suppressive actions of oestradiol and inhibin on the pituitary. The falling plasma concentrations of FSH prevent any additional follicles being stimulated to mature and thereby limit the number of follicles that can attain ovulatory maturity. Inhibin concentrations in both peripheral and ovarian vein blood are too low to be measured by current bioassays for inhibin. Thus, one cannot determine if there is indeed an inverse relationship between blood concentrations of FSH and inhibin during the follicular phase. However, if inhibin is physiologically important in the regulation of FSH secretion in sheep, then active immunisation against inhibin might neutralise any circulating inhibin and thereby reduce the supression of FSH occurring during the follicular phase, so allowing additional follicles to attain ovulatory maturity. To test this hypothesis, inhibin was partially purified from bovine follicular fluid by chromatographic methodology (Franchimont *et al.*, 1983). Adult parous Romney ewes on day 10 of an oestrous cycle were actively immunised with this preparation emulsified in Freund's complete adjuvant (1 ml emulsion containing 1 mg protein was injected subcutaneously into the gracilar or axillary region). Control ewes were immunised with Freund's complete adjuvant alone. Each ewe was immunised twice at 30-day intervals. The number of ovulations occuring in each of 4 successive oestrous cycles immediately following the first immunisation was determined by laparoscope examination of the ovaries of each ewe, 9 to 12 days after each display of oestrous behaviour, and counting the number of corpora lutea present. The number of sheep on which the effects of active immunisation against inhibin could be studied was restricted to 4 because of the limited amount of inhibin preparation available. Nevertheless, despite this limitation, a significant effect

TABLE 1 Ovulation rates in 4 successive oestrous cycles of 4 control and 4 inhibin immunised Romney ewes.

	Sheep No.	No. of ovulations oestrous cycle 1	2	3	4	Mean No. of ovulations/ ewe	Mean ovulation rate ± s.e.m.
Control	934	1	1	2	1	1.25	
	962	1	1	1	2	1.25	1.31 ± 0.06
	738	2	1	1	1	1.25	
	662	1	2	1	2	1.50	
Inhibin immunised	782	2	2	2	2	2.00	
	701	3	2	3	2	2.50	2.06 ± 0.16
	716	1	2	2	2	1.75	
	651	2	2	2	2	2.00	

on ovulation rate was observed (Table 1), the inhibin immunised ewes having a higher mean ovulation rate compared to the controls (2.06 ± 0.16 *v* 1.31 ± 0.06 ovulations/ewe, mean ±s.e.m. for $n = 4$, $P < 0.05$; Wilcoxon rank sum test). This finding not only provides further support for the notion that inhibin is physiologically important in the regulation of ovarian function in the ewe but suggests that immunisation with inhibin to increase ovulation rate might have some potential as a means of increasing fecundity.

It is of interest to note that immunisation with androgens increases ovulation rate and fecundity (cf. fecundity drugs Multi-Lamb and Fecundin). Androgens stimulate follicular inhibin production (Henderson and Franchimont, 1981; 1983) and immunisation against inhibin increases ovulation rate. Thus it is conceivable that the effects of the fecundity drugs are mediated by causing changes in follicular inhibin production. Immunisation against androgen may reduce circulating androgen concentrations thereby reducing follicular inhibin production which in turn reduces the suppression of pituitary FSH secretion and so allows more follicles to be stimulated to develop to ovulatory maturity.

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