Evidence to support a follicle-stimulating hormone threshold theory for follicle selection in ewes chronically treated with gonadotrophin-releasing hormone agonist

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Summary. The mean and peak concentrations of follicle-stimulating hormone (FSH) during the luteal phase of a normal cycle were measured in 8 Welsh Mountain ewes. Gonadotrophin secretion and follicle growth were then suppressed by the chronic administration of the GnRH agonist buserelin for 5 weeks. During the 6th week of agonist treatment, each ewe was given a continuous infusion of FSH to produce a peripheral concentration of FSH equal to either the mean or peak of the gonadotrophin measured for that individual in the cycle preceding agonist treatment. Treatment had no effect on the total number of follicles, the number of follicles ≤ 2.5 mm in diameter or the in-vitro production of oestradiol by the small follicles when compared with control animals. None of the animals infused with the mean luteal-phase FSH equivalent developed large follicles > 2.5 mm diameter which could be classified as preovulatory follicles (oestradiol > 1000 pg/follicle/h). All of the animals infused with the peak luteal-phase FSH equivalent developed large follicles, some of which were preovulatory. The results suggest that an individual threshold concentration exists for FSH above which the later stages of preovulatory follicular development are stimulated.

Keywords: FSH; follicle selection; GnRH agonist; sheep

Introduction

In monovulatory species, such as sheep, the exact mechanism by which a single follicle is selected to develop to preovulatory status in preference to other more or less mature follicles remains to be elucidated. On the basis of observations on the induction of ovulation using exogenous gonadotrophins in hypogonadotrophic women, Brown (1978) evolved the concept that there is a threshold concentration of follicle-stimulating hormone (FSH) above which the final stages of follicular development can be induced. Evidence from rhesus monkeys (Zeleznik & Kubik, 1986) and ewes (Picton *et al.*, 1990a) infused with FSH after treatment with gonadotrophin-releasing hormone (GnRH) antagonist or agonist, respectively, provides direct experimental proof in favour of this hypothesis. In agonist-treated ewes, infusion of a high concentration of FSH stimulates the development of an increased number of large preovulatory follicles, whereas infusion of a lower concentration of FSH does not (Picton *et al.*, 1990a). Interpretation of the results from this experiment was complicated by the variation between animals in both the level of suppression of pituitary gonadotrophin secretion produced by the agonist treatment and in the response of individuals to the infused FSH. A marked variation in the peripheral concentration of FSH, within and between animals, has been measured throughout the oestrous cycle in Welsh Mountain ewes

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(Picton, 1989) and other breeds of sheep (L'Hermite *et al.*, 1972; Salamonsen *et al.*, 1973; Findlay & Cumming, 1976; Miller *et al.*, 1981; Bister & Paquay, 1983). In agonist-treated ewes, the response of each individual to exogenous FSH may be determined by the threshold requirement for FSH which has been set for that individual during the normal cycle before the start of agonist treatment.

To address this question, ewes which had been chronically treated with GnRH agonist to suppress their endogenous gonadotrophin secretion were infused with a concentration of FSH which was tailored to reproduce either the mean or peak systemic concentration of FSH measured for that individual during the luteal phase of the normal oestrous cycle preceding agonist treatment.

Materials and Methods

The experimental protocol comprised: (i) measurement of the peripheral gonadotrophin concentration throughout the normal oestrous cycle, (ii) chronic administration of the GnRH agonist D-Ser (Bu)¹GnRH(1-9)-nonapeptide-ethylamide (buserelin: kindly supplied by Dr J. Sandow, Hoechst A. G., Frankfurt, FRG) for 6 weeks and (iii) continuous infusion of ovine FSH (NIADDK-oFSH-16; NIADDK, Bethesda, MD, USA) for 72 h. Throughout the experiments, the animals were housed under natural lighting at the Marshall building, University of Edinburgh, Dryden Field Centre, Roslin, Midlothian.

Experiment 1. The oestrous cycles of 5 Welsh mountain ewes were synchronized by the withdrawal of synthetic progestagen-impregnated, intravaginal sponges (Veramix Sheep Sponge: Upjohn Ltd, Crawley, Sussex, UK) 12 days after their insertion. Oestrus, designated as Day 0, was detected by a raddled, vasectomized Finnish Landrace ram. Jugular blood samples were collected twice a day at 09:00 and 17:00 h using 7-ml heparinized vacutainers (Becton Dickinson UK, Ltd, Oxford, UK) from Day 1 of the ensuing cycle until oestrous activity was detected again some 16–17 days later.

On Days 1–3 of the early luteal phase of the second cycle after synchronization of oestrus, the animals were implanted s.c. in the axillary region of the forelimb with two 0.8 × 0.12 cm rod implants of buserelin as described by Fraser *et al.* (1987). Each implant contained 3.3 mg of the agonist. Jugular blood samples were collected from all of the animals at weekly intervals for 5 weeks. The concentrations of FSH and luteinizing hormone (LH) were measured in duplicate in all of the blood samples collected throughout Exps 1 and 2.

During the 6th week of agonist treatment, both the left and right jugular veins were cannulated and the animals were placed in metabolism crates. All the ewes received a continuous i.v. infusion of oFSH via the right jugular catheter using a Harvard infusion pump (Harvard Apparatus Co., Millis, MA, USA). Further details of the infusion system have been described by Picton *et al.* (1990a). Each animal ($n = 5$) was infused for 3 days with a dose of oFSH so that the peripheral concentration of FSH measured during the infusion period corresponded to the mean concentration of FSH measured for that animal between Days 8 and 16 of the luteal phase of the cycle preceding agonist treatment. The hormonal parameters measured in each animal to determine the quantity of FSH needed to produce the required peripheral concentration during the infusion period are shown in Table 1. The FSH preparation infused had an LH contamination of 0.04 times NIH-LH-S1 standard preparation. The infused FSH concentration was calculated using the formula:

$$\text{Infused FSH} = (\text{luteal phase FSH concentration} - \text{agonist-suppressed FSH concentration}) \times 0.28$$

The luteal phase concentration corresponded to the mean measured for each animal between Days 8 and 16 of the luteal phase of the natural cycle preceding agonist treatment. The agonist-suppressed FSH concentration was measured in the blood sample collected after 4 weeks of agonist treatment. The calculation used the observation that the infusion of 0.28 µg of oFSH-16/h produced an increase of 1 ng/ml in the peripheral plasma FSH concentration in the agonist-suppressed ewe (Picton, 1989).

During the infusion period, jugular blood samples (3.5 ml) were collected at 30-min intervals for 1.5 h immediately before the start of the FSH treatment (time 0, Day 1). Samples were then collected at 30-min intervals for 3 h and at 4, 6 and 8 h after the start of the infusion. On Days 2 and 3, samples were collected at 1-h intervals from 08:00 to 14:00 h and at 16:00 h. All of the blood samples were assayed in duplicate for FSH and LH.

At the end of the infusion period, the animals were killed with an overdose of sodium pentobarbitone and the ovaries were removed. A further 5 ewes which acted as control animals were killed on Day 14 of the luteal phase of a natural cycle. All follicles > 1.0 mm in diameter were dissected from the ovaries and the mean diameter and in-vitro production of oestradiol and testosterone were determined for each follicle using the method detailed by McNeilly & Fraser (1987).

Experiment 2. A second smaller group of 3 ewes was treated according to the protocol for Exp. 1. Each animal was infused with a higher concentration of FSH which corresponded to 110% of the mean value of FSH measured between the mid- and late luteal phases of the cycle preceding agonist treatment.

Hormone assays. The concentrations of LH (McNeilly *et al.*, 1986) and FSH (McNeilly *et al.*, 1989) in plasma samples and the concentrations of oestradiol and testosterone in follicle culture medium (Webb *et al.*, 1985; McNeilly

et al., 1986) were measured in duplicate, using previously described radioimmunoassays. The sensitivities of the assays were 0.2 ng (NIH-LH-S18)/ml for LH, 0.5 ng (NIH-oFSH-S14)/ml for FSH and 5 pg/tube for the steroids. The intra- and interassay coefficients of variation were <10 and <15%, respectively.

Statistical analysis. The differences between the mean concentration of FSH measured between Days 8 and 16 of the luteal phase of the normal cycle preceding agonist treatment, the basal concentration of FSH before the start of the infusion, the mean concentration of FSH measured throughout the 72 h of infusion and the basal and mean LH concentration measured during the infusion period were compared for each individual animal using Student's *t* test on paired samples. The effect of treatment on the number of follicles/ewe, the diameter of these follicles and their in-vitro steroid production were assessed by analysis of variance and the differences between treatments were established by Duncan's new multiple range test. The follicle data were transformed by $\sqrt{(x + 0.5)}$ and all the steroid data were transformed by $\log_{10}(x + 1)$ before analysis. These transformations normalized the data as assessed by rankit plotting. Because of the small number of follicles no statistical tests were carried out on the preovulatory follicle data or on the large follicles > 2.5 mm in diameter recovered from animals in Exp. 1. The values presented in Tables 1 and 2 are of untransformed data.

Results

Hormone concentrations

The mean overall changes in the peripheral concentration of FSH and LH throughout the natural cycle and during agonist treatment for Exps 1 and 2 are shown in Fig. 1. The second peak of FSH secretion occurred on Day 1–2 of the early luteal phase in these animals with subsequent but smaller fluctuations in secretion occurring at regular intervals thereafter. Measurement of the highest concentration of FSH during the luteal phase of the cycle varied between animals and occurred between Days 9 and 15 of the cycle preceding agonist treatment. The peak FSH concentration was on average 42% higher ($n = 8$; range 6–97%) than the mean measured between Days 8 and 16 of the cycle. After the onset of agonist treatment, there was a gradual decline in the peripheral secretion of FSH until after 5 weeks the peripheral concentration of FSH was significantly ($P < 0.05$) suppressed below the mean measured between Days 8 and 16 of the luteal phase in 4 of the 5 animals in Exp. 1 and in 2 of the ewes in Exp. 2. Infusion of the different quantities of FSH detailed in Table 1 resulted in a significant ($P < 0.05$) increase in the peripheral FSH concentration over the basal concentration measured before the start of FSH treatment, irrespective of the amount of FSH infused. For 4 of the 5 animals in Exp. 1, there was no significant difference between the mean FSH concentration measured during the infusion period and the mean FSH value measured between Days 8 and 16 of the luteal phase (Fig. 2). In sheep no. 43 the infused FSH concentration was significantly ($P < 0.05$) higher than the mean measured in the luteal phase, but was lower than the peak measured for this animal. The values from sheep no. 43 have therefore been excluded from further analyses. In Exp. 2 there was no significant difference between the mean infused FSH concentration and the peak measured in the luteal phase in the same animal. There was no evidence of fluctuations in endogenous FSH secretion in animals during the infusion period.

The LH profile indicates that during a normal cycle the peripheral concentration of LH initially increased and then declined in the early to midluteal phase of the cycle. The agonist treatment stimulated an increase in the release of LH over the first 20 days of treatment, the concentrations then declined to basal values before the start of the infusion. There was no significant difference between the basal concentration of LH measured immediately before the start of the infusion after 5 weeks of agonist treatment (0.9 ± 0.2 ng/ml; $n = 8$) and the mean LH concentration measured during the FSH infusion period (Exp. 1: 0.9 ± 0.2 ng/ml, $n = 5$; Exp. 2: 0.9 ± 0.06 ng/ml, $n = 3$). There was no evidence of pulsatile LH secretion during the infusion period.

Follicular development and function

There was no significant difference between the total number of follicles or the number and diameter of small follicles ≤ 2.5 mm diameter from the treated animals and the control group (Table 2). There was no significant difference between the production of oestradiol by the small

Fig. 1. Changes in the plasma concentration of follicle-stimulating hormone (FSH, $\square$) and luteinizing hormone (LH, $\blacksquare$) throughout the oestrous cycle and during chronic treatment with gonadotrophin-releasing hormone agonist ($\blacksquare$). Values are means $\pm$ s.e.m. for 8 ewes.

Table 1. Parameters of secretion of follicle-stimulating hormone (FSH) measured during the luteal phase of a normal cycle in ewes and after 4 weeks of chronic administration of gonadotrophin-releasing hormone agonist, and the concentration of FSH required and infused during the FSH treatment period

Exp.	Animal no.	Luteal phase FSH* (ng/ml)	Agonist-suppressed FSH† (ng/ml)	Required FSH (ng/ml)	Infused FSH (μ g/h)
1	34	26.8 $\pm$ 1.4	15.4	11.4	3.2
	37	22.3 $\pm$ 0.8	16.7	5.6	1.5
	43	32.8 $\pm$ 1.4	21.9	10.9	3.0
	50	24.6 $\pm$ 1.2	18.0	6.6	1.8
	51	19.1 $\pm$ 1.7	17.0	2.1	0.6
2	70	23.3 $\pm$ 0.4	12.6	13.0	3.6
	74	19.1 $\pm$ 2.5	14.6	6.4	1.8
	76	28.3 $\pm$ 3.7	8.8	22.3	6.2

*Measured between Days 8 and 16 of the normal cycle. The values are means $\pm$ s.e.m.

†Measured after 4 weeks of agonist treatment.

follicles from the treated ewes and similar sized follicles from Day 14 control animals, but the in-vitro production of testosterone was significantly ($P < 0.05$) lower in the follicles from animals in Exps 1 and 2.

Large follicles of >2.5 mm diameter were recovered from the control ewes and from all of the animals infused with the higher concentration of FSH (Exp. 2), but there were significantly ($P < 0.01$) more large follicles in the control group. In contrast, only 1 of the 4 animals (no. 34 infused with the lower, mean luteal phase concentrations of FSH (Exp. 1)) possessed a large follicle of 2.7 mm diameter, with an in-vitro oestradiol and testosterone production of 39 and 255 pg/h, respectively. The in-vitro secretion of oestradiol and testosterone was higher in the follicles from the animals in Exp. 2 which were infused with the higher FSH concentration than in the follicles from control animals. These differences were not, however, significant.

Potential ovulatory follicles

Large follicles >2.5 mm in diameter with an in-vitro oestradiol secretion exceeding 1000 pg/follicle per h were classified as potential ovulatory follicles (Webb & England, 1982). The data

Fig. 2. Mean (■) and peak (▒) plasma follicle-stimulating hormone (FSH) values measured between Days 8 and 16 of the luteal phase of a normal cycle in ewes compared with the concentration measured after 5 weeks of chronic treatment with gonadotrophin-releasing hormone agonist (□) and after infusion of exogenous FSH (▨) to correspond to (a) the mean luteal-phase FSH concentration (Exp. 1) or (b) 110% of the mean luteal-phase FSH concentration (Exp. 2). * $P < 0.05$ and ** $P < 0.01$, compared with the mean luteal-phase FSH concentration. Standard error bars have been plotted for all but the peak FSH concentrations, but in some cases are too small to be seen.

Table 2. Number and diameter of steroid production by follicles in control ewes on Day 14 of the luteal phase and in ewes treated with an agonist of gonadotrophin-releasing hormone after the infusion of FSH to produce a peripheral FSH concentration equivalent to the mean luteal-phase concentration of FSH (Exp. 1) or 110% of the mean luteal-phase FSH concentration (Exp. 2)

	Day 14 control ($n=5$)	Exp. 1 ($n=4$)	Exp. 2 ($n=3$)	Significance
Total no. of follicles/ewe	28.8 ± 4.3	23.5 ± 2.3	23.7 ± 1.4	NS
Range	20–45	20–30	21–26	
<i>Follicles ≤ 2.5 mm diameter</i>				
No. of follicles/ewe	18.4 ± 3.2	23.2 ± 2.5	20.0 ± 1.1	NS
Range	13–30	19–30	18–22	
Diameter (mm)	2.0 ± 0.1	1.8 ± 0.09	1.7 ± 0.5	NS
Oestradiol (pg/follicle per h)	155 ± 41	65 ± 20	80 ± 55	NS
Testosterone (pg/follicle per h)	502 ± 113 ^a	232 ± 33 ^b	264 ± 12 ^b	$P < 0.05$
<i>Follicles > 2.5 mm diameter</i>				
No. of follicles/ewe	10.4 ± 1.7 ^a	0.3 ± 0.3 ^{b†}	3.7 ± 1.2 ^c	$P < 0.01$
Range	7–15	0–1	2–6	
Diameter (mm)	3.8 ± 0.2	—	3.6 ± 1.2	NS
Oestradiol (pg/follicle per h)	231 ± 45	—	939 ± 444	NS
Testosterone (pg/follicle per h)	1049 ± 316	—	3448 ± 1538	NS
<i>Preovulatory follicles*</i>				
Total no.	2	0	6	
Diameter (mm)	4.1 ± 1.2	—	4.5 ± 0.5	
Oestradiol (pg/follicle per h)	1172.4 ± 170.0	—	1839.0 ± 26.0	

Values are means ± s.e.m.

Values followed by different superscripts are significantly different.

*Follicles secreting > 1000 pg oestradiol/h; values are means ± s.e.m. for the number of follicles shown.

†Only one animal developed 1 follicle > 2.5 mm diameter (see text for details).

indicate that all 5 control animals developed follicles of > 2.5 mm diameter, but only 2 of these animals developed ovulatory follicles. All of the control ewes developed at least 1 follicle with an in-vitro oestradiol production within the range of 500–1000 pg/h. In the agonist-treated animals in Exp. 1, an increase in the peripheral FSH concentration to the mean value measured during the

luteal phase of the cycle stimulated the development of only one follicle >2.5 mm in diameter (animal no. 34) and no preovulatory follicles. In contrast, sheep no. 43, which was infused with higher than its mean luteal phase FSH concentration, developed 9 large follicles >2.5 mm in diameter and 1 ovulatory follicle of diameter 6.5 mm with an in-vitro oestradiol production of 1464 pg/h. Furthermore, in Exp. 2, when all animals were infused with the higher concentration of FSH which corresponded to the peak value measured during the luteal phase, all 3 animals developed large follicles >2.5 mm in diameter and in 2 animals (nos 70 and 76) at least 1 of these large follicles was classified as an ovulatory follicle (Table 2).

Discussion

These results confirm our previous observations that FSH in the presence of only basal concentrations of LH stimulates the later stages of follicle growth and development (Picton *et al.*, 1990a, b). In this model system the FSH requirement for folliculogenesis can be regarded as the summation of the administered dose of exogenous FSH and the suppressed endogenous FSH concentration after 5 weeks of agonist treatment. We have previously shown that the basal gonadotrophin concentration present after 5–6 weeks of agonist treatment is insufficient to stimulate follicle growth beyond 2.5 mm (Picton *et al.*, 1990a, b). The patterns of gonadotrophin secretion measured during both the normal cycle and the agonist treatment period for each animal were similar to the profiles of these hormones reported in detail for normal Welsh Mountain ewes and ewes treated with agonist implants (Picton, 1989).

These data support the hypothesis proposed by Brown (1978) and agree with the evidence reported for monkeys (Zeleznik & Kubik, 1986) and humans (Polson *et al.*, 1987; Glasier *et al.*, 1989; Remorgida *et al.*, 1989) that a threshold exists above which FSH induces preovulatory follicular development. In this study, when the FSH concentration was maintained below the threshold requirement, there was no stimulation of the later stages of follicular development. This was the case in 4 of the animals in Exp. 1. In the remaining animal (no. 43) the infused FSH concentration was some 16% higher than the average measured during the luteal phase; this was above the threshold requirement for this individual and was sufficient to stimulate the development of 9 large follicles, one of which could be classified as a potential ovulatory follicle. In contrast, in Exp. 2 the higher infused FSH concentration was above the threshold and stimulated preovulatory follicular development in 2 of the 3 animals. Although preliminary, these data suggest that within an individual ewe the difference between a subthreshold and a stimulatory dose of FSH can be as little as 16% in one animal or $>50\%$ in another. The FSH threshold therefore differs greatly between individuals. These results could explain why it is so difficult to control accurately the increases in follicular development and ovulation rate induced by the superovulatory treatments currently available commercially. The results suggest that a dose of FSH which will induce a superovulatory response in one animal may be below the threshold requirement for the stimulation of follicle development in another, but it is not clear whether the putative FSH threshold varies from cycle to cycle for each ewe. Recent evidence from women with polycystic ovarian disease has shown that the FSH threshold within individuals varies between successive uniovulatory cycles (Polson *et al.*, 1987; Remorgida *et al.*, 1989). The results of the present study in sheep imply that the growth response of follicles to FSH, or perhaps more importantly the FSH threshold requirement of the follicles, reflects the endogenous pattern of FSH secretion and is set by the ovary during the mid- to late luteal phase of the cycle.

During these experiments it was assumed that the endogenous FSH concentration measured after 5 weeks of agonist treatment represented biologically active FSH. This assumption is supported by the observation made in rams that detectable concentrations of LH measured after chronic agonist treatment were shown to be biologically active in the mouse Leydig cell bioassay (Lincoln *et al.*, 1986). It is possible that agonist treatment decreased the ratio of biological to

immunological activity of the endogenous FSH as has been reported for women after treatment with a GnRH antagonist (Mortola *et al.*, 1989) and/or may have altered the rate of metabolic clearance of the infused hormone (Picton, 1989). If this is true, the proportional difference in the biological activities of the subthreshold and threshold concentrations of FSH required to stimulate the later stages of follicle growth may be greater than the difference measured by radioimmunoassay in these experiments.

The induction of a critical granulosa cell aromatase activity and LH responsiveness is believed to serve as a type of 'on-off' switch which is thrown in the responding follicle when the FSH threshold concentration is achieved (Brown, 1978; Hillier, 1981; Hillier *et al.*, 1988). The selected follicle will be the first to aromatize thecal androgen at a rate sufficient to increase its oestrogen biosynthesis. This increased oestrogen production throws the switch and sensitizes the granulosa cells of the selected follicle to FSH, so protecting it from the deleterious effects of the decline in FSH during the follicular phase of the cycle (Baird *et al.*, 1981; Wallace *et al.*, 1988; Picton, 1989). The factors which set the level of the hypothetical threshold remain obscure, but may include one or more variables such as: (i) the local blood supply which regulates the differential delivery of FSH to each follicle; (ii) number of follicle cells or gonadotrophin receptors per cell as this would determine the gonadotrophin uptake and the amplification of the response of each follicle; and (iii) the intrafollicular concentrations of substances which modulate the action of the gonadotrophins such as oestrogen, androgens (Hillier *et al.*, 1988) or perhaps intrafollicular growth factors and peptides (reviews: Cahill, 1984; Hsueh *et al.*, 1984; Ying, 1988; Tonetta & diZerega, 1989), which may establish a variable sensitivity of the follicles to mixed amounts of the gonadotrophins and thus contribute to the establishment of the threshold.

The observation in these experiments that multiple follicles mature upon prolonged stimulation by concentrations of FSH which are within the physiological range, but which are above the threshold, argues against the hypothesis that the maturing follicle inhibits the growth and development of other follicles directly at the ovarian level by decreasing their responsiveness to the gonadotrophins. However, the infusion of a constant concentration of FSH throughout the treatment period may have by-passed any intraovarian regulation of follicle growth; or the absence of a dominant follicle and/or the presence of a highly synchronous population of small antral follicles at the start of the FSH infusion period may have rendered any intraovarian control mechanism redundant in this model situation. Also, if an intraovarian mechanism of follicle selection was operative in this model environment, we would have expected a single follicle to mature to preovulatory status regardless of the duration of exposure of the follicle population to the infused FSH. In this situation the selected follicle would establish its dominance by the secretion of the putative intraovarian factor(s), so preventing the continuing infusion of FSH from stimulating the development of other less advanced follicles. The results of Picton *et al.* (1990a) show that this is not the case. Once the FSH concentration increases to the stimulatory range the subsequent duration of the FSH treatment influences the number of follicles which develop (Zeleznik & Kubik, 1986; Picton *et al.*, 1990a). This suggests that the physiological determinant of the number of follicles destined to ovulate in the ewe is the systemic concentration of FSH in the 48–72 h before the onset of spontaneous luteal regression. This suggestion is supported by the evidence of Driancourt & Cahill (1984) and Tsonis *et al.* (1984) who have demonstrated that selection of the ovulatory follicle occurs around the time of luteolysis. McNatty *et al.* (1985) have shown that the plasma concentration of FSH before, but not after, luteolysis was significantly higher in ewes which subsequently had double ovulations than in animals which had single ovulations. Once selected, the ovulatory follicle(s) actively inhibit the development of other less mature follicles, through the negative feedback influences of increased oestradiol and inhibin secretion, by suppressing the FSH concentration below the threshold during the early follicular phase of the cycle. This hypothesis is further supported by the data of Driancourt & Fry (1989), who demonstrated in ewes that blocking the follicular-phase fall in FSH secretion, by the administration of exogenous FSH, significantly increased ovulation rate.

These experiments highlight the variability of the follicular growth response of individual animals to exogenous preparations of FSH and suggest that this variability is directly related to the profile of gonadotrophin secretion which occurs during the normal oestrous cycle. The results from this study clearly support the hypothesis of Brown (1978) and suggest that in ewes the FSH threshold for follicle selection is set by the ovary in the mid- to late luteal phase of the cycle. The intrafollicular requirement for FSH operates within a narrow range and the difference between a substimulatory and a threshold dose of FSH may be as little as 34%, depending on the individual.

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References

- Baird, D.T., Swanston, I.A. & McNeilly, A.S. (1981) Relationship between LH, FSH, and prolactin concentration and the secretion of androgens and estrogens by the preovulatory follicle in the ewe. *Biol. Reprod.* **24**, 1013–1025.
- Bister, J.-L. & Paquay, R. (1983) Fluctuations in the plasma levels of the follicle-stimulating hormone during estrous cycle, anestrus, gestation and lactation in the ewe: evidence for an endogenous rhythm of FSH release. *Theriogenology* **19**, 565–583.
- Brown, J.B. (1978) Pituitary control of ovarian function-concepts derived from gonadotrophin therapy. *Aust. N.Z.J. Obstet. Gynaecol.* **18**, 47–54.
- Cahill, L.P. (1984) Folliculogenesis and ovulation rate in sheep. In *Reproduction in Sheep*, pp. 92–98. Eds D. R. Lindsay & D. T. Pearce. Cambridge University Press, Sydney.
- Driancourt, M.A. & Cahill, L.P. (1984) Preovulatory follicular events in sheep. *J. Reprod. Fert.* **71**, 205–211.
- Driancourt, M.A. & Fry, R.C. (1989) Mechanisms involved in the control of the differentiation of the ovulatory follicle in sheep. *J. Reprod. Fert., Abstr. Ser.* **3**, 54.
- Findlay, J.K. & Cumming, I.A. (1976) FSH in the ewe: effects of season, live weight and plane of nutrition on plasma FSH and ovulation rate. *Biol. Reprod.* **15**, 335–342.
- Fraser, H.M., Sandow, J. & Seidel, H. (1987) An implant of a gonadotrophin releasing hormone agonist (buserelin) which suppresses ovarian function in the macaque for 3–5 months. *Acta. endocr., Copnh.* **115**, 521–527.
- Glasier, A.F., Baird, D.T. & Hillier, S.G. (1989) FSH and the control of follicular growth. *J. Steroid Biochem.* **32**, 167–170.
- Hillier, S.G. (1981) Regulation of follicular oestrogen biosynthesis: a survey of current concepts. *J. Endocr.* **89**, 3–18.
- Hillier, S.G., Harlow, C.R., Shaw, H.J., Wickings, E.J., Dixon, A.F. & Hodges, J.K. (1988) Cellular aspects of pre-ovulatory folliculogenesis in primate ovaries. *Hum. Reprod.* **3**, 507–511.
- Hsueh, A.J.W., Adashi, E.Y., Jones, P.B.C. & Welsh, T.H. (1984) Hormonal regulation of the differentiation of cultured ovarian granulosa cells. *Endocr. Rev.* **5**, 76–127.
- L'Hermite, M., Niswender, G.D., Reichert, L.E., Jr & Midgley, A.R. (1972) Serum follicle stimulating hormone levels in sheep as measured by radioimmunoassay. *Biol. Reprod.* **6**, 325–332.
- Lincoln, G.A., Fraser, H.M. & Abbot, M.P. (1986) Blockage of pulsatile LH, FSH and testosterone secretion in rams by constant infusion of an LHRH agonist. *J. Reprod. Fert.* **77**, 587–597.
- McNatty, K.P., Hudson, N., Gibb, M., Ball, K., Henderson, K.M., Heath D.A., Lun, S. & Kieboom, L.E. (1985) FSH influences follicle viability, oestradiol biosynthesis and ovulation rate in Romney ewes. *J. Reprod. Fert.* **75**, 121–131.
- McNeilly, A.S. & Fraser, H.M. (1987) Effect of GnRH agonist-induced suppression of LH and FSH on follicle growth and corpus luteum function in the ewe. *J. Endocr.* **115**, 273–282.
- McNeilly, A.S., Jonassen, J.A. & Fraser, H.M. (1986) Suppression of follicular development after chronic LHRH immunoneutralization in the ewe. *J. Reprod. Fert.* **76**, 481–490.
- McNeilly, A.S., Swanston, I.A., Crow, W., Tsonis, C.G. & Baird, D.T. (1989) Changes in plasma concentrations of inhibin throughout the normal sheep oestrous cycle and after infusion of FSH. *J. Endocr.* **120**, 295–305.
- Miller, K.F., Nordheim, E.V. & Ginther, O.J. (1981) Periodic fluctuations in FSH concentrations during the ovine estrous cycle. *Theriogenology* **16**, 669–679.
- Mortola, J.F., Sathanandan, M., Pavlou, S., Dahl, K.D., Hsueh, A.J., Rivier, J., Vale, W. & Yen, S.S.C. (1989) Suppression of bioactive and immunoreactive follicle-stimulating hormone and luteinizing hormone levels by a potent gonadotropin releasing hormone antagonist; pharmacodynamic studies. *Fert. Steril.* **51**, 957–963.
- Picton, H.M. (1989) *The gonadotrophic control of ovarian follicle growth in the ewe*. Ph.D. thesis, University of Edinburgh.
- Picton, H.M., Tsonis, C.G. & McNeilly, A.S. (1990a) FSH causes a time-dependent stimulation of preovulatory follicle growth in the absence of pulsatile LH secretion in ewes chronically treated with gonadotrophin-releasing hormone agonist. *J. Endocr.* **126**, 297–307.

- Picton, H.M., Tsonis, C.G. & McNeilly, A.S.** (1990b) The antagonistic effect of exogenous LH pulses on FSH-stimulated preovulatory follicle growth in ewes chronically treated with a gonadotrophin-releasing hormone agonist. *J. Endocr.* **127**, 273–283.
- Polson, D.W., Mason, H.D., Saldanha, M.B.Y. & Frank, S.S.** (1987) Ovulation of a single dominant follicle during treatment with low-dose pulsatile follicle stimulating hormone in women with polycystic ovary syndrome. *Clin. Endocr.* **26**, 205–212.
- Remorgida, V., Venturini, P.L., Anserini, P., Lanera, P. & De Cecco, L.** (1989) Administration of pure follicle stimulating hormone during gonadotropin-releasing hormone agonist therapy in patients with clomiphene-resistant polycystic ovarian disease: hormonal evaluations and clinical perspectives. *Am. J. Obstet. Gynaecol.* **160**, 108–113.
- Salamonsen, L.A., Jonas, H.A., Burger, H.G., Buckmaster, J.M., Chamley, W.A., Cumming, I.A., Findlay, J.K. & Goding, J.R.** (1973) A heterologous radioimmunoassay for follicle-stimulating hormone: application to measurement of FSH in the ovine estrous cycle and in several other species including man. *Endocrinology* **93**, 610–618.
- Tonetta, S.A. & diZerega, G.S.** (1989) Intraovarian regulation of follicular maturation. *Endocr. Rev.* **10**, 205–229.
- Tsonis, C.G., Cahill, L.P., Carson, R.S. & Findlay, J.K.** (1984) Identification at the onset of luteolysis of follicles capable of ovulation in the ewe. *J. Reprod. Fert.* **70**, 609–614.
- Wallace, J.M., Martin, G.B. & McNeilly, A.S.** (1988) Changes in the secretion of LH pulses, FSH and prolactin during the preovulatory phase of oestrous cycle of the ewe and the influence of treatment with bovine follicular fluid during the luteal phase. *J. Endocr.* **116**, 123–135.
- Webb, R. & England, B.G.** (1982) Relationship between LH receptor concentrations in thecal and granulosa cells and in-vivo and in-vitro steroid secretion by ovine follicles during the preovulatory period. *J. Reprod. Fert.* **66**, 169–180.
- Webb, R., Baxter, G., McBridge, D., Nordblom, C.D. & Shaw, M.P.K.** (1985) The measurement of testosterone and oestradiol-17 β using iodinated tracers and incorporating an affinity chromatography extraction procedure. *J. Steroid Biochem.* **23**, 1043–1051.
- Ying, S.-Y.** (1988) Inhibins, activins, and follistatins: gonadal proteins modulating the secretion of follicle-stimulating hormone. *Endocr. Rev.* **9**, 267–293.
- Zelevnik, A.J. & Kubik, C.J.** (1986) Ovarian responses in macaques to pulsatile infusion of follicle stimulating hormone (FSH) and luteinizing hormone: increased sensitivity of the maturing follicle to FSH. *Endocrinology* **119**, 2025–2032.

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