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THE OVARIAN FOLLICLE AND FERTILITY

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Summary—The precise roles of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the control of preovulatory follicle growth has been re-examined. Suppression of both pulsatile LH secretion and FSH or specific suppression of FSH results in an inhibition of preovulatory follicle growth beyond 2.5 mm dia. Infusion of sheep FSH alone in physiological amounts in the presence of basal, non-pulsatile LH results in the growth of preovulatory follicles. Co-infusion of large amplitude pulses of LH reduced or abolished this effect of FSH. It is suggested that: (1) FSH controls the number of follicles which develop; (2) selection of the large follicle destined to ovulate is directly related to the decline in the plasma concentration of FSH occurring during the period of follicle selection—thus, only the follicle(s) which can withstand this withdrawal of FSH will continue to develop; and (3) pulses of LH may directly affect the action of FSH on the follicle and play an important, hitherto unrecognized role in the selection of the ovulatory follicle by actively inducing atresia.

INTRODUCTION

It is now beyond dispute that the growth of the preovulatory follicle in the ovary is dependent on both follicle stimulating hormone (FSH) and luteinizing hormone (LH) acting in concert [1]. FSH promotes the growth of the follicle by acting through receptors on the granulosa cells and inducing the aromatase enzyme required to convert androgens to oestrogens, the action of FSH being enhanced initially by androgens [2]. The androgens originate from the theca under the control of LH. While FSH receptors are only present on granulosa cells, in the latter stages of preovulatory development LH receptors, present initially only on the theca, appear in the granulosa cells and are probably coupled to the same second messenger system as FSH receptors. During the preovulatory surge LH acting directly on the granulosa cells initiates luteinization resulting in a reduction, or abolition of aromatase enzyme activity, depending on species, and the enhancement of progesterone synthesis and secretion as the luteinized granulosa cells transform to become the corpus luteum.

During the oestrous and menstrual cycles of most species a variable number of antral follicles are growing and developing yet, in many species, only one or two of these follicles will develop into a viable oestrogenic preovulatory follicle destined to ovulate, thus providing the egg, and forming the corpus luteum. While the actions of FSH and LH on individual follicles is relatively understood, the precise role of these two gonadotrophins in the final selection of the preovulatory follicle is still open to conjecture.

During the follicular phase of the cycle in many species, plasma concentrations of FSH decline, thus withdrawing FSH support to most follicles. Only those follicles which can resist this decline in FSH will be selected. In this paper, the role of LH pulses in the selection of the preovulatory follicle will be examined. These studies have been carried out in the sheep which provide an excellent model for the clinical situation in women since the pattern of follicle growth, steroid secretion, gonadotrophin secretion and follicle selection is very similar to that in women [1, 3].

OESTROUS CYCLE ANOESTRUS

The ewe is a seasonal breeder exhibiting oestrous cycles during the short days of autumn and winter with anovulation and anoestrus, during the long days. Nevertheless, follicle growth and development in the ewe is a continuum throughout the period of oestrous cyclicity and anoestrus [1]. The numbers of preovulatory follicles 5–8 mm dia are related to the ovulation rate of different breeds of sheep [4–7]. The failure to ovulate during anoestrus is related to an

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insufficient frequency of pulsatile LH secretion. Thus, replacement with pulsatile LH [8, 9] or GnRH [10] will result in completion of the final phase of preovulatory follicle development, the generation of sufficient oestradiol to induce a preovulatory LH surge [8] and ovulation. Ovulation does not occur in all ewes treated with GnRH or LH although progesterone pretreatment may increase the numbers ovulating [1]. Suppression of FSH by treatment with bovine follicular fluid will prevent GnRH induced ovulation [12], while addition of FSH to pulsatile LH treatment will enhance the number of ewes ovulating [13]. Thus, during anoestrus LH will only induce adequate preovulatory follicle growth and ovulation in the presence of adequate amounts of FSH.

During the oestrous cycle the number of preovulatory follicles which develop can be increased by the injection of preparations containing FSH although the response is very variable [1]. Treatment with FSH reduces the number of atretic follicles and increases the aromatase activity of the granulosa cells [14]. In contrast, LH does not appear to influence the number of preovulatory follicles [15], suggesting that FSH is the principal gonadotrophin controlling the number of preovulatory follicles which grow and develop.

However, it has proved difficult to show a clear association between plasma concentrations of FSH and the numbers of preovulatory follicles present in ewes of different breeds with different ovulation rates [1]. There is some evidence that within a breed ewes with twin ovulations had higher plasma concentrations of FSH in the 3–5 days before ovulation during the period of follicle development [14] while ewes in poor body condition had lower plasma FSH, associated with reduced follicle growth [16, 17]. Immunization against androgens [18, 19] or inhibin [20, 21] which results in an increase in ovulation rate is not associated with consistent increases in FSH. However, at least in the case of immunization against androstenedione this appears to be related to an increase in plasma concentrations of inhibin correlated with the increase in the number of large follicles which develop [19]. The failure to detect a significant correlation between an increase in follicle growth and raised plasma FSH physiologically may also relate to the large variation in plasma concentrations of FSH throughout the luteal phase [22] which may obscure any differences [1].

THE RELATIVE ROLES OF FSH AND LH IN FOLLICLE GROWTH

Treatment of ewes with follicular fluid as a source of inhibin to cause the specific suppression of FSH at the start of the follicular phase results in the cessation of follicle development [23–25] with an immediate decline in the secretion of oestradiol, inhibin and androgens and atresia of the follicle [25]. In contrast, inhibition of the pulsatile secretion of LH by either passive immunoneutralization of GnRH [26] or treatment with a potent GnRH antagonist [27] prevents ovulation and ovarian steroid, but not inhibin secretion. However, in contrast to the withdrawal of FSH, which results in the collapse of the follicle, follicles remain viable after the withdrawal of LH pulses, and replacement of LH pulses results in the return of normal steroid secretion [27]. These results support the concept that FSH is the crucial gonadotrophin controlling follicle development and withdrawal of FSH alone during the follicular phase is the major factor causing selection of the preovulatory follicle(s). No role for LH in this process has been suggested. However, our recent studies suggest that LH pulses may play a role in actively suppressing the effect of FSH and thereby accelerating atresia, indirectly playing a role in follicle selection.

Initial studies showed that suppression of the pulsatile secretion of LH and reduction of FSH by 30–50% by either active immunoneutralization of GnRH [28] or chronic treatment with a potent GnRH agonist [29] resulted in a suppression of preovulatory follicle growth with no follicles >2.5 mm dia being present in the ovaries (Fig. 1). A similar effect was seen when ewes were treated throughout the luteal phase with follicular fluid to specifically suppress FSH without affecting the pulsatile secretion of LH (Fig. 1). These results confirmed that the failure of follicle growth was related specifically to the absence of FSH.

In ewes treated with GnRH agonist for 5–6 weeks to suppress FSH to 30–40% of normal, inhibit pulsatile LH secretion and therefore stop preovulatory follicle growth, infusion of pure sheep FSH induced a time-dependent development of preovulatory follicles up to 8 mm dia within 72 h of the start of infusion [30]. This occurred in the absence of pulsatile LH secretion. Infusion of pulsatile LH alone did not cause preovulatory follicle growth [31] but treatment of ewes with antiserum to LH to specifically remove the basal levels of LH

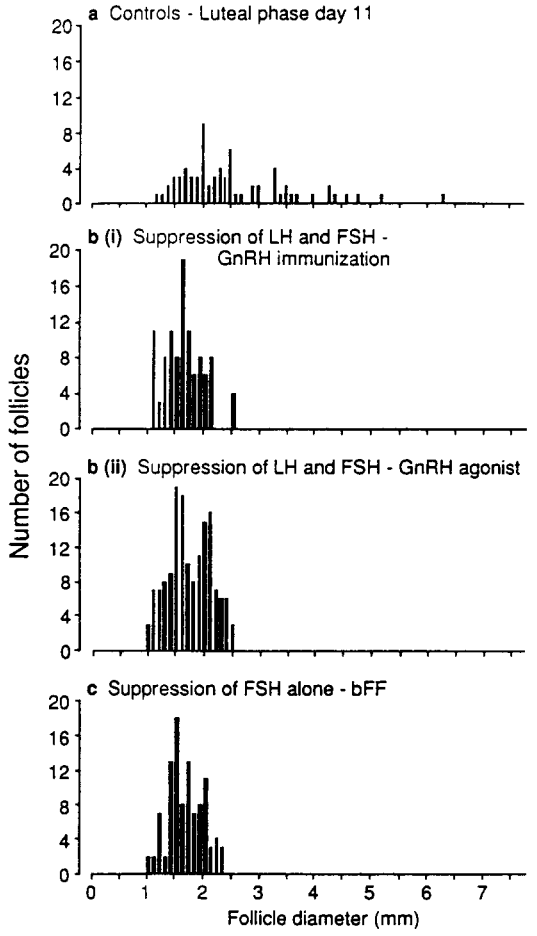

Fig. 1. Distribution of ovarian follicle diameters for follicle populations dissected from: (a) control ewes on day 11 of the luteal phase; (b) ewes actively immunized for 30 months against GnRH (i) and ewes after chronic treatment with a GnRH agonist for 42 days to suppress LH pulsatile and FSH secretion (ii); and (c) ewes on day 11 of the luteal phase after treatment with twice daily injections of bovine follicular fluid to specifically suppress FSH $n = 5$ ewes per group.

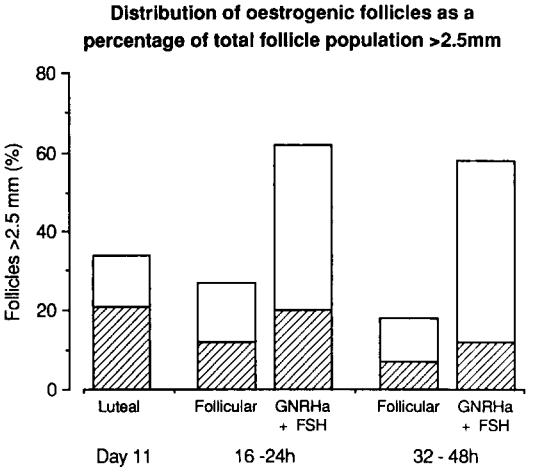

Fig. 2. Percentage of large ovarian follicles >2.5 mm dia which secrete >500 < 1000 pg oestradiol/h *in vitro* (▨) or >1000 ph/h (□) *in vitro*, in day 11 of the luteal phase in control ewes, of the follicular phase in control ewes 16–24 h and 32–48 h after prostaglandin-induced luteal regression and 16–24 h and 32–48 h after the start of FSH infusion in ewes treated with GnRH agonist for 42 days to suppress follicle growth to follicles <2.5 mm dia before FSH infusion.

prevented the induction of preovulatory follicle growth by FSH [32]. Thus, FSH in the absence of pulsatile LH but in the presence of basal LH could induce preovulatory follicle development. These preovulatory follicles were steroidogenically active and a high proportion produced large amounts of oestradiol equivalent to that from preovulatory follicles during the normal oestrous cycle. Indeed, a greater proportion of the large follicles >2.5 mm dia could be classified as oestrogenically active preovulatory follicles than would be expected for the plasma concentration of FSH achieved during the infusion period and in comparison to the follicular phase of the normal oestrous cycle (Fig. 2). During the normal follicular phase the growth of the preovulatory follicle is associated with an increase in the pulsatile secretion of LH [33]. This raised the possibility that LH pulses might reduce the follicle response to FSH. This was indeed the case. Pulsatile injection of small or large amplitude pulses at a frequency equivalent to that seen during the luteal phase, during the period of FSH infusion in GnRH agonist treated ewes reduced or prevented the induction of preovulatory follicle growth [31] (Fig. 3). The effect of LH pulses was dependent on both the amplitude of the LH pulses injected and on the plasma concentration of FSH infused [31]. Thus an increase in the plasma concentration of FSH could overcome the inhibitory effect of an LH pulse of a particular amplitude. The significance of this may relate to the effect of the physiological amplitude LH pulses occurring during the follicular phase of the cycle. Several follicles start to grow but as the negative feedback signals from these follicles, both oestradiol and inhibin, increase this causes a decline in the plasma concentration of FSH. LH pulses of low amplitude which were not inhibitory to the effects of FSH may now become inhibitory as the plasma concentration of FSH falls, and thus act together with the withdrawal of FSH to actively kill off the follicle, i.e. induce atresia.

During the normal oestrous cycle the preovulatory follicle(s) destined to ovulate emerge from a pool of 2–3 mm dia follicles around the time of luteolysi some 36–60 h before the start

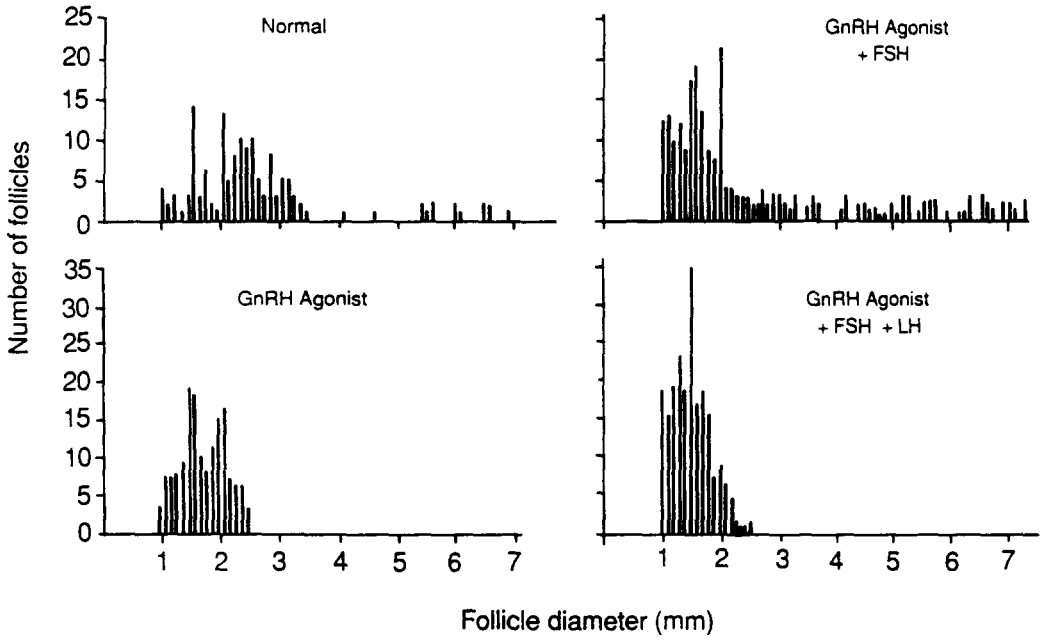

Fig. 3. The effect of large amplitude pulses of LH on the growth of follicle >2.5 mm dia induced by the infusion for 72 h of physiological amounts of sheep FSH in ewes treated with a GnRH agonist to suppress follicle growth. Ovine LH was given as an i.v. bolus injection every 4 h throughout the period of FSH infusion. Results are compared to the distribution of ovarian follicles in control ewes on day 8 of the luteal phase and to ewes treated with GnRH agonist but without FSH or pulsatile LH infusion.

of the preovulatory LH surge [1]. Infusion of FSH during this period to prevent the drop in FSH results in an increase in the number of preovulatory follicles which develop. This increase in FSH would effectively counteract the detrimental effects of the pulsatile secretion of LH.

A similar situation arises in rats where induction of superovulation with FSH is significantly reduced as the amount of LH at the same time is increased [34, 35]. In human clinical studies the quality of the oocytes in *in vitro* fertilization patients was impaired if LH was too high [36], while the probability of conception or pregnancies continuing to term was reduced in patients with a higher than normal concentration of LH in the follicular phase of the cycle [37].

These results suggest a hitherto unrecognized role for pulsatile LH in directly affecting the ability of FSH to induce preovulatory follicle growth and perhaps to affect the process of oocyte maturation with the subsequent risk to fertility.

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