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Effect of FSH deprivation at specific times on follicular maturation in the bonnet monkey (*Macaca radiata*)*

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Summary. Cyclic monkeys were deprived of FSH for specific periods on different days of the follicular phase by injecting them with minimal doses of an FSH antiserum characterized for specificity and bioneutralizing ability. The effect of the antiserum on follicular maturation was assessed by determining (a) serum oestrogen concentrations through the midcycle period, (b) serum progesterone concentrations as an index of ovulation and luteal function, (c) laparoscopic examination of the surface of the ovary when necessary, and (d) overall cycle length. While antiserum injection on Day 5 of the cycle caused delay in the oestrogen surge from Days 9 to 11, injection on Day 6 led to the occurrence of two oestrogen surges, on Days 9 and 14. Laparoscopic examination showed that the earlier follicle had disappeared and a new follicle had appeared by Day 14. Antiserum injection on Day 7 of the cycle arrested further growth of the maturing follicle, but a new follicle appeared 9 days later, as indicated by a surge of oestrogen on Day 16. Injection of antiserum beyond Day 7 had no effect on follicular development, ovulation and luteal function. These observations suggest that the mature follicle becomes relatively independent of FSH support about 48 h before ovulation and this event could be a marker for follicular dominance.

Keywords: follicular phase; monkey; ovary; follicular dominance; oestrogen surge; gonadotrophins

Introduction

Follicular maturation in the primate is unique in that, during any given cycle, a single follicle amongst a cohort achieves development into a large mature follicle which subsequently ovulates and forms a corpus luteum. Although it is well known that FSH and LH are required for promoting preovulatory growth of the follicle in most species including the primate (Knobil *et al.*, 1959; Gemzell, 1962; Rabinowitz *et al.*, 1972; Moudgal *et al.*, 1974), information on the precise role these two hormones play in the regulation of follicular growth and differentiation is meagre. The methods used thus far to investigate the problem, such as studying the effect of cauterizing the dominant follicle of cycle and investigating its effect on ovarian function in the ensuing cycle (Hodgen, 1982), studying the effect of partial suppression of FSH with whole pig follicular fluid on cycle events (Channing *et al.*, 1979, 1981; diZerega *et al.*, 1981) and cell culture of granulosa cells from follicles of different sizes (Channing *et al.*, 1982), have all provided only circumstantial evidences for the role of FSH/LH in follicular maturation. The use of FSH antibodies for understanding the FSH requirements for follicular maturation in rodents (Sheela Rani & Moudgal, 1977, 1978, 1979, 1983) and rabbits (Vani, 1986), and for testicular function in monkeys (Murty *et al.*,

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1979; Moudgal, 1981) suggested that a similar approach could be used to study the role of FSH in cyclic follicular maturation of the primate.

Materials and Methods

Animals. Healthy adult female bonnet monkeys (*Macaca radiata*) of proven fertility were used. The details of care, maintenance and feeding were as previously described (Mukku & Moudgal, 1979; Ravindranath & Moudgal, 1987). The animals used in the present series of experiments were 9–12 years of age and weighed approximately 5 kg each.

Antiserum. An antiserum to sheep FSH raised in a donor male bonnet monkey was used. The method of immunization and characterization of the antiserum have been described elsewhere (Murthy & Moudgal, 1987; Ravindranath, 1988). The antiserum, devoid of any contaminating LH antibodies, was tested for its ability to bind and neutralize heterologous primate FSH (human and cynomolgus FSH) before it was used to neutralize circulating concentrations of monkey FSH.

General methodology. The minimal effective dose of FSH antiserum capable of neutralizing circulating concentrations of FSH for approximately 24 h was determined by injecting a group of monkeys with different doses of antiserum on Day 1 of the cycle and analysing serum samples at specific times thereafter for the presence of antibodies capable of binding ^{125}I-labelled sheep and human FSH. The ovine FSH used for radioiodination was purified in the laboratory (purity, 99·9%). Human FSH (hFSH-S11-108B) was obtained from Dr M. R. Sairam (Montreal, Canada). Incubation of the labelled hormone with 100 µl of neat serum in each case in triplicate was done overnight at room temperature (26°C). The antibody-bound label was precipitated with polyethylene glycol (mol. wt 6000) at 10–12% final concentration. The non-specific binding of ^{125}I-labelled hormone, determined using the same volume of a pre-injection serum sample from the same monkey, was deducted from the value for the experimental samples to obtain net binding. The binding ability of the labelled hormone with an excess of the characterized antiserum ranged between 55 and 65% for sheep FSH and 45 and 55% for human FSH. The specific activity of the label ranged from 4·5 to 5×10^4 c.p.m./ng (45–50 µCi/µg) for ovine FSH and 3 to $3{\cdot}5 \times 10^4$ c.p.m./ng (30–35 µCi/µg) for human FSH. Data are expressed assuming maximum binding to represent 100% binding.

All injections of the antiserum were done at 10:00 h by the i.p. route. Blood samples were taken using Vacutainer tubes from unanaesthetized monkeys. Serum was separated within 12 h of collection and stored at −20°C until further processing.

Hormone assays. Oestradiol and progesterone in the serum samples were estimated by specific radioimmunoassays standardized in the laboratory (Prahalada *et al.*, 1975). The oestradiol antiserum used in the assay cross-reacted with oestrone (10%) and oestriol (< 1%). The inter- and intra-assay coefficients of variation were 15 and 12·5% respectively. The progesterone antiserum cross-reacted with 17α-hydroxyprogesterone (3%) and 20α-dihydroprogesterone (5%). The inter- and intra-assay coefficient of variations were 9% and 8% respectively. The sensitivity of the assay for progesterone was 0·01 ng and for oestradiol 0·02 ng.

Results

Characteristics of the FSH antiserum used in the current study

The ability of the antiserum to bind ovine and human FSH as well as ovine and human LH before and after affinity purification using an LH sepharose column (Murthy & Moudgal 1987) is shown in Table 1. While LH antibodies were completely removed by this process, the specific FSH antibody concentration remained unaltered. The binding affinity (K_a) and capacity for hFSH determined using Scatchard analysis was $0{\cdot}3 \times 10^{10}$ M^{-1} and 43·8 µg/ml respectively (Vani, 1986).

The ability of the antiserum to bioneutralize human FSH (used here as a representative of primate FSH) was determined using an in-vitro rat granulosa cell system standardized in the laboratory. Human FSH stimulated progesterone production in these cells (50 000 cells/tube/4 h) in a dose-dependent manner (tested over a range of 10–100 ng/tube), 50 ng hFSH producing an optimal response. Preincubating 50 ng hFSH with the antiserum resulted in an inhibition of the hormone response and this was dose-dependent (range 28–77%) and was statistically significant ($P < 0{\cdot}05$; Fig. 1). The bioneutralizing capacity of the antiserum calculated from the above data was 8 µg hFSH/ml and was markedly lower than the overall binding capacity as determined by Scatchard analysis. This is not surprising as only a portion of the total polyclonal antibody pool could be of the bioneutralizing type, namely that capable of preventing hFSH from binding to

Table 1. Relative ability of FSH antiserum to bind ovine and human FSH and LH before and after affinity purification

^{125}I-labelled hormone	Dilution of antiserum	% Specific binding*	
		Before Sepharose–LH treatment	After Sepharose–LH treatment
oFSH	1:10	58	47
	1:10 000	38	28
	1:20 000	25	26
hFSH	1:10	48	41
oLH	1:100	60	12
hLH	1:10	32	0

*The antiserum at the specified dilutions was incubated with labelled hormones at room temperature overnight and the antigen–antibody complex formed was precipitated using polyethylene glycol at a final concentration of 10–12%. The non-specific binding was determined by incubating the labelled hormones with the buffer or normal monkey serum and this was deducted to yield specific binding.

the receptor before evoking a response. The ability of the antiserum to bioneutralize non-human primate FSH was assessed by (a) determining the capacity of the antibody to prevent cynomolgus monkey FSH (kindly provided by Dr G. Bialy, NICHD, Bethesda, MD, USA) binding to FSH receptor in an appropriate radio-receptor assay (100 µl of 1:500 diluted antiserum bound ~30 ng hFSH compared to 50 ng of a relatively cruder monkey FSH) and (b) demonstrating that binding of ^{125}I-labelled hFSH to the antibody can be blocked if serum from a castrated or normal bonnet monkey is used. These studies suggested that, in the absence of purified bonnet monkey FSH, human FSH could be used to assess the binding capacity of the FSH antiserum.

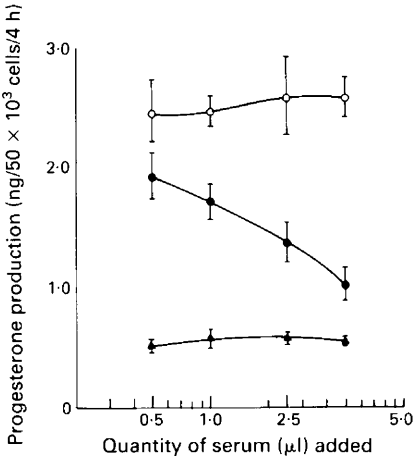

Fig. 1. Bioneutralizing activity of FSH antiserum (No. 603) under in-vitro conditions using rat granulosa cells. ▲——▲, Basal progesterone production; ○——○, progesterone production with 50 ng hFSH/tube in the presence of different doses of normal monkey serum; ●——●, progesterone production with 50 ng hFSH/tube in the presence of different doses of antiserum. Values are mean ± s.d. ($n=6$).

Determination of the in-vivo binding ability of the antiserum injected as a function of dose and time

Cyclic female monkeys were treated i.p. on Day 1 of the cycle with 4, 1 or 0·2 ml of the characterized antiserum and the serum samples collected at specific times thereafter were analysed for the presence of antibodies to ovine and human FSH. It is evident from Fig. 2 that antibodies capable of binding hFSH disappeared at a much faster rate than that for antibodies directed against other epitopes specific to oFSH. This is also a function of the dose of antiserum injected. The observation that antibodies cross-reacting with hFSH disappeared rapidly could be a reflection of its ability to bind endogenous monkey FSH. The times taken to achieve 50% binding with ^{125}I-labelled ovine FSH after injection of different volumes of antiserum (4, 1 and 0·2 ml) were up to 50, 45 and 15 days respectively. For the same volumes of antiserum, the times taken to achieve 50% binding of ^{125}I-labelled human FSH were 22, 16 and 7·5 days respectively.

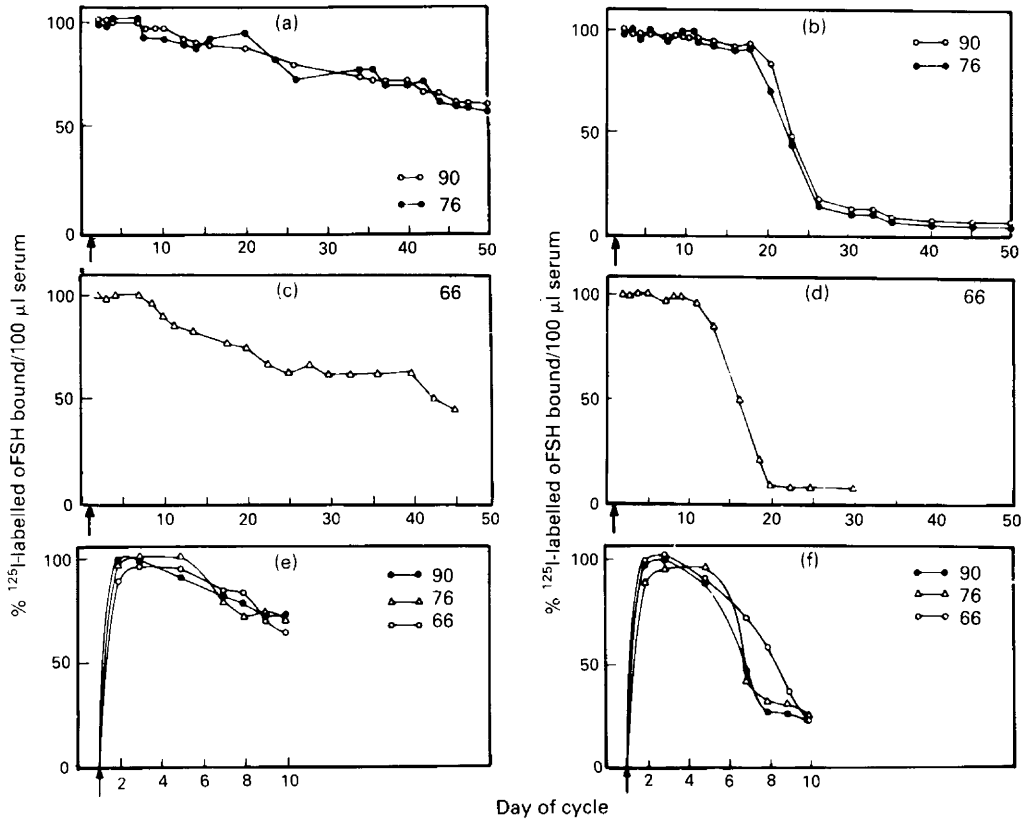

Fig. 2. Percentage binding of labelled hormone to antibodies in the circulation in monkeys injected with different doses of antiserum as a function of time. See text for details of methodology: the nos indicate individual monkeys. (a, c, e) Represent binding to ^{125}I-labelled sheep FSH and (b, d, f) represent binding to ^{125}I-labelled human FSH. (a, b) Monkeys injected (arrow) with 4 ml antiserum on Day 1 of the cycle; (c, d) monkey injected (arrow) with 1 ml antiserum on Day 1 of the cycle; (e, f) Monkeys injected (arrow) with 0·2 ml antiserum on Day 1 of the cycle.

Ability of the antibody to disrupt ovarian function:

In an initial study different doses of antiserum were injected on Day 1 of the cycle. The effect on ovarian function was assessed by monitoring serum oestrogen and progesterone concentrations as

well as noting the time taken for menses to recur. It is evident from Fig. 3 that cycle length was extended because there was arrest of the follicular maturation process.

Follicular maturation appeared to be reinitiated once the antibody titre (that fraction capable of binding ^{125}I-labelled hFSH) fell below 20%. The gradual increase in serum oestrogen values reaching a surge by Day 9 of cycle is an index of normal follicular growth and function (Ravindranath & Moudgal, 1987). Depending on the initial dose of antiserum injected, the appearance of the oestrogen surge was postponed. When the dose of antiserum was 4·0 ml, 20% binding to labelled hFSH was recorded on Day 26 and the oestrogen surge occurred 9 days later in these animals, i.e. around Day 35 of the cycle. Similarly, the monkeys receiving 1·0 ml and 0·2 ml of antiserum exhibited 20% binding to labelled hFSH on Days 19 and 8 respectively. Correspondingly, the oestrogen surge was observed in these groups of monkeys around Day 28 and Day 16 respectively. The follicle that matured after the disappearance of the antibody underwent normal ovulation and luteinization as indicated by normal progesterone patterns (Fig. 3; Table 2).

Since we were interested in arriving at a dose of antiserum which would be effective for no more than 24 h, female monkeys were injected with 100 µl or 25 µl antiserum on Day 7 of the cycle. While 25 µl antiserum lasted for slightly longer than 24 h (computed on 50% binding), the effect of 100 µl lasted for almost 4 days (Fig. 4). Attempts to reduce the antiserum dose further and titrate the circulating values to reach a minimum of 20% of binding by 24 h was not successful. Therefore, in all the subsequent experiments, a dose of 25 µl per monkey was routinely used.

Effect of injecting minimal dose (25 µl) of antiserum during mid follicular phase on ovarian function

Groups of normal cyclic monkeys received a single injection of 25 µl antiserum per monkey on Day 5 or 6 or 7 of cycle. The effect on serum oestrogen and progesterone profiles as well as cycle length was monitored. Whenever necessary recourse was taken to laparoscopic examination of the ovarian surface for evidence of newer follicular growth and ovulation. It is evident from Fig. 5 that the effect was variable depending on the day antiserum was injected. In the normal cyclic monkey oestrogen concentrations start to rise on Day 6–7 and reach a peak on Day 9 or 10 of the cycle (Fig. 5a). Injecting antiserum on Day 5 or 6 had little effect on the high oestrogen levels of Day 9 (Fig. 5b, c): in both groups antiserum injection brought about a transient reduction in serum oestradiol values within 24 h (Day 5 group: from 220 ± 140 on Day 5 to 185 ± 130 pg/ml on Day 6; Day 6 group: from 230 ± 65 on Day 6 to 116 ± 30 pg/ml on Day 7; $P < 0{\cdot}05$). These follicles, however, appeared to return to full activity as seen by normal oestradiol concentrations on Day 9 (Control, 610 ± 300 pg/ml; Day-5 antiserum group, 630 ± 96 pg/ml: Day-6 antiserum group, 730 ± 115 pg/ml). While the serum oestradiol values of the Day 5 group (Fig. 5b) kept rising beyond Day 9 to reach a peak on Day 11, those of the Day-6 group (Fig. 5c) showed an initial fall like the controls but then levels subsequently rose to reach a second higher peak by Day 14. Laparoscopic examinations of the ovarian surface on Days 9 and 14 of a fresh group of 3 monkeys which received antiserum on Day 6 confirmed the suspicion that the dominant follicle that was seen on the surface of one ovary (usually the left ovary) on Day 9 had disappeared on Day 14 and there was no evidence of corpus luteum formation. In the contralateral ovary, however, on Day 14 there was a new dominant follicle which ultimately ovulated and formed a corpus luteum.

From the serum oestrogen profiles it can be observed that the follicles of the Day-7 treatment group were most sensitive to the lack of FSH (Fig. 5d). Within 24 h of injection of antiserum, the oestrogen values dropped from a preinjection value of 360 ± 100 to 100 ± 20 pg/ml ($P < 0{\cdot}05$). The oestrogen values of control animals within the same period increased from 346 ± 160 to 540 ± 150 pg/ml. The Day-7 follicle did not recover from the 24-h FSH deprivation effect and a new follicle developed for ovulation, an oestrogen surge being seen on Day 16 of the cycle. The new follicle apparently ovulated normally as judged by the rise in progesterone concentrations.

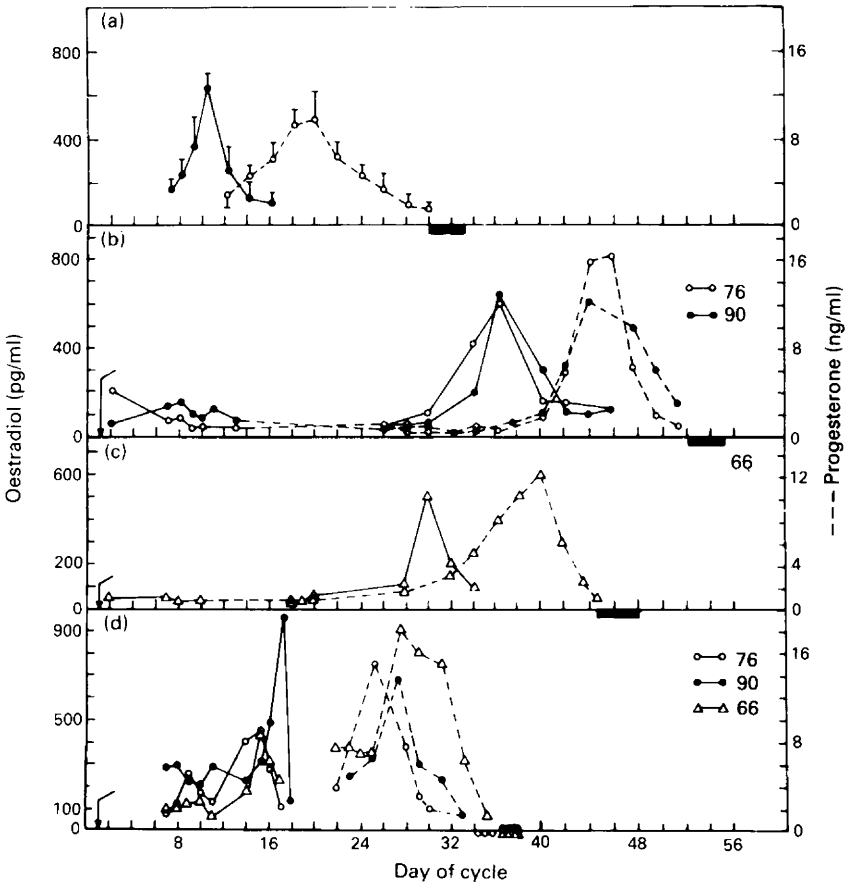

Fig 3. Effect of administering different doses of FSH antiserum (arrows) on Day 1 of the cycle upon follicular maturation and corpus luteum function (serum oestrogen and progesterone profiles). (a) Control monkey pattern (N = 3); (b) monkeys (Nos 76 & 90) injected with 4 ml antiserum; (c) monkey (No. 66) injected with 1 ml antiserum; (d) monkeys (Nos 76, 90 & 66) injected with 0·2 ml antiserum. ■, Duration of menses.

Effect of FSH antiserum injection during the late follicular phase upon ovarian function

The antiserum was ineffective in influencing steroidogenesis when administered on any day between Days 9 and 12 of the cycle (Table 2). However, there was a marginal 25% reduction (statistically not significant) of Day-9 oestrogen concentrations when given on Day 8 of the cycle (from a control value of 540 ± 150 to 406 ± 75 pg/ml). The control animals at the same time showed an increase in oestrogen values from 540 ± 150 to 610 ± 300 pg/ml. Antiserum injection during the late follicular phase had no effect on ovulation or luteal function (Table 2).

Discussion

The follicular maturation process of the primate can be categorized into three phases, an early phase (Days 1–4) involving recruitment and initiation of growth of a cohort of follicles, a mid-follicular phase (Days 5–7) involving selection of the follicle which achieves dominance and initiation of active steroidogenesis, followed by a late follicular phase (Days 8–11) when a spurt in the growth of the dominant follicle occurs to take it to an ovulable state. The completion in follicular

Table 2. Effect of neutralizing FSH on different days upon ovarian function

Group	Day of cycle when antiserum was injected*	No. of monkeys	Effect on oestrogen conc. on Day 9 of cycle†	Day on which oestrogen surge occurred	Length of luteal phase (days)‡
1	5	4	None	11	19·0 ± 0·8
2	6	3	None	9 and 14	15·0 ± 1·0¶
3	7	9	Reduced by 80%	16	12·1 ± 1·1§
4	8	4	Reduced by 25%	9	19·5 ± 1·3
5	9	4	None	9	17·0 ± 0·8
6	10	4	None	9	19·3 ± 0·9
7	12	4	None	9	19·5 ± 1·1

*Except for those in Groups 6 and 7, which received 50 µl per monkey, all animals received 25 µl antiserum i.p. on the specified day.
†Normal oestrogen concentration on Day 9 = 400 ± 140 pg/ml.
‡Days (mean ± s.d.) from oestrogen peak to next menses.
§Length of luteal phase was significantly shortened compared to controls (18·0 ± 2·2 days); $P < 0·05$.
¶Calculated from Day 14 oestrogen surge.

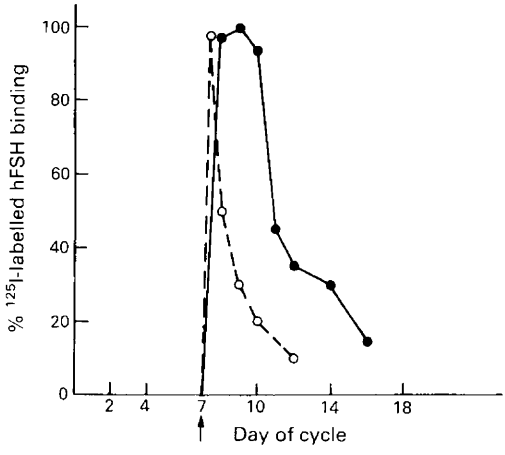

Fig. 4. Binding of ^{125}I-labelled human FSH to antibodies in the circulation in monkeys injected (arrow) with 25 µl (○) or 100 µl (●) FSH antiserum.

growth can perhaps be attested by the occurrence of oestrogen surge and in control bonnet monkeys this occurs on Day 9 or 10 of the cycle.

In the current study, an attempt has been made to deprive adult cyclic monkeys of FSH support during the three different phases of follicular growth. The antiserum used specifically neutralizes primate FSH and, as shown by in-vitro binding (current study) and in-vivo neutralization studies, has no effect on primate LH activity (Ravindranath, 1988). The overall effect the antiserum produces is directly linked to the dose as well as the time of administration, and the capacity of the antiserum injected at any given time was sufficiently large to neutralize the existing FSH concentrations of that day. In the bonnet monkey the FSH concentrations between Days 5 and 7 of cycle ranged between 2 and 7 ng hFSH equivalent/ml and the surge levels at Days 10–11 ranged between 12 and 18 ng/ml (Ramasharma *et al.*, 1978). After neutralization of FSH from Day 1 of the cycle, the initiation of the follicular maturation process was arrested. The postponement in the initiation was linked to the dose of antiserum injected, i.e. the larger the dose the greater the postponement. The 20% binding level chosen in the current study as the termination of the FSH antibody effect is

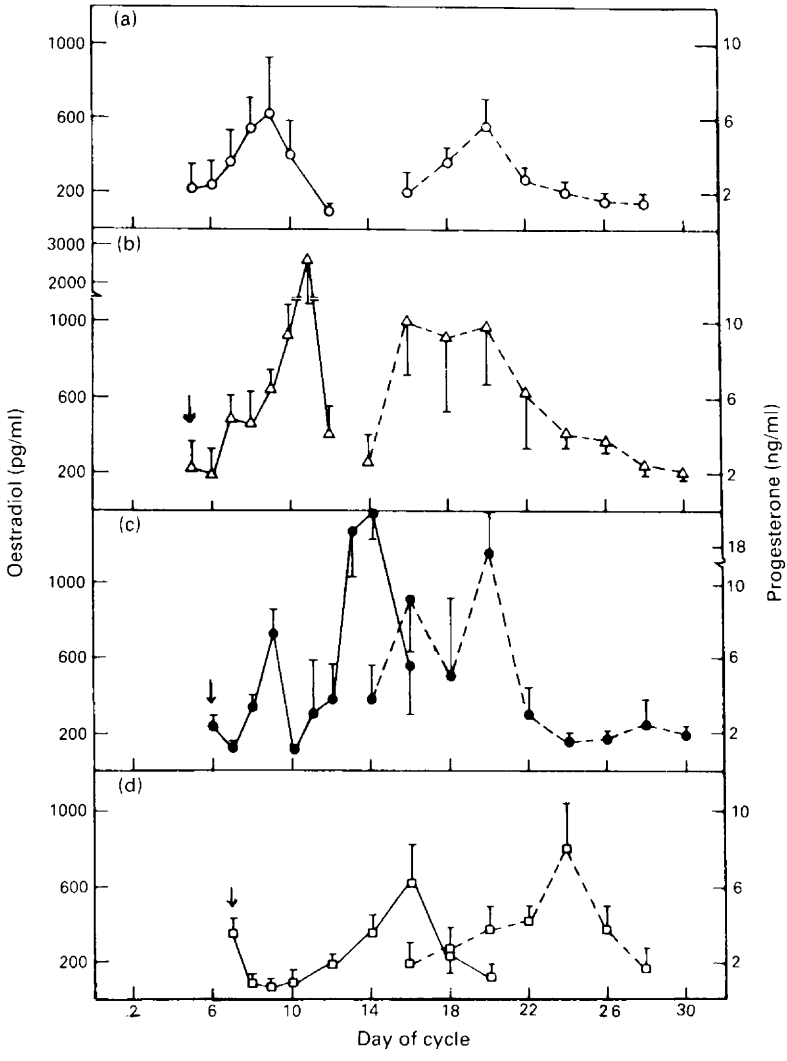

Fig. 5. Effect of injecting (arrow) 25 µl FSH antiserum on Day 5, 6 or 7 of cycle on serum steroid profiles during the cycle. (a) Serum oestrogen and progesterone profiles in control monkeys (N = 3); (b) Day 5 antiserum treatment group (N = 4); (c) Day 6 antiserum treatment group (N = 3); (d) Day 7 antiserum treatment group (N = 9).

arbitrary and was considered to coincide in most cases with the starting point of follicular activity, which was inferred by retrospectively deducting 9 days from the day of the oestrogen surge (observed) to obtain the start of the follicular activity. This would suggest that, as long as FSH levels are kept below a threshold, oestrogen production and follicular growth are kept in abeyance and the 20% binding level referred to above perhaps indicates the turning point when follicular activity is reinitiated. It would have been prudent to determine circulating FSH concentrations at this time but the available methodology does not permit us to estimate free FSH in the presence of antibody. A direct consequence of the reduced FSH and hence oestrogen concentrations is the lengthening of the follicular phase leading to extension of cycle length. Zeleznik (1981), using Silastic implants of oestrogen to affect feed back inhibition of FSH secretion, has also observed an extension of cycle length. In our study, the effect of 0·2 ml FSH antiserum given as a single injection

on Day 1 lasted up to 8 days (based on the time taken for hFSH antibody level to fall to the 20% binding limit). The follicular activity in this group began by Day 7–8 as indicated by the increase of oestrogen values, the surge of oestrogen itself occurring 8–9 days later (Fig. 3). Crude pig follicular fluid, a source of inhibin, has also been used by Stouffer & Hodgen (1980) to reduce FSH values by 40% during Days 1–3 of cycle in rhesus monkeys and this led to a reduction in oestrogen secretion during the follicular phase followed by a luteal defect.

The current study shows that follicles between Days 5 and 7 become increasingly sensitive to FSH deprivation, the Day-7 follicle showing maximal sensitivity to the lack of FSH. The follicles of Days 5 and 6 of cycle show an immediate reduction in oestrogen output after antiserum injection (based on serum oestrogen levels 24 h later) and regain their ability to produce oestrogen in normal quantities by Day 9 of the cycle. From the binding data after a 25 μl antiserum injection one would assume that 24 h later there is still considerable amount of FSH inhibitory activity (50% binding). However, this appears not to be correct if the efficacy of the antibody is assessed by noting the period it takes for oestrogen production to restart. Serum FSH concentrations between Days 5 and 7 do not significantly alter and the capacity of the antibody is more than adequate to neutralize FSH production for 1 day. The current studies therefore suggest that it is better to use return of steroidogenic response as an end point of antibody efficacy rather than % binding to ^{125}I-labelled hFSH.

From the serum oestradiol patterns of monkeys treated with antiserum on Day 5, it can be inferred that either ovulation is postponed by 2 days (as day of oestrogen surge is shifted from Day 9 to 11) or that the dominant follicle ovulates in response to normal oestrogen levels on Day 9 and the increase in oestrogen values seen up to Day 11 is due to secondary follicular activity. The second possibility, however, seems highly unlikely. Laparoscopic examination of the ovarian surface on Days 9 and 14 as well as serum progesterone concentrations of monkeys receiving antiserum on Day 6 indicated that the first dominant follicle in the Day-6 group did not ovulate despite producing normal levels of oestrogen on Day 9. The oestrogen levels would normally have signalled the midcycle surge of FSH and LH (not measured). This suggests that the follicle of Day 6 is sensitive to lack of FSH in more than one way. It is possible that the reduction in oestrogen output between Days 6 and 7 brought about by antiserum injection on Day 6 or the absense of FSH *per se* must have initiated other metabolic changes in the Day 6 follicle leading to follicular atresia. It appears as though this process, once started on Day 6, cannot be reversed by a resumption in the follicular ability to synthesize oestrogen. The nature of these changes remains to be elucidated. It is also not clear whether the reduction in oestrogen concentrations between Days 6 and 7 is adequate to act as a positive feedback effect on FSH release leading to initiation of fresh follicular maturation and ovulation beyond Day 14. The reason for obtaining significantly higher levels of oestrogen in the Day 5 (on Day 11, $P < 0{\cdot}05$) and Day 6 (on Day 14, $P < 0{\cdot}01$) groups over the control Day-9 levels is currently not clear. These high concentrations of oestrogen, however, do not lead to a situation similar to the polycystic ovary syndrome as the oestrogen values return to normal within 24–48 h of the surge. The oestrogen/progesterone secretory pattern in the Day-6 group was suggestive of a new follicle growing towards maturation. Similar observations were made by Zeleznik *et al.* (1985), i.e. administration of oestradiol antibodies from Days 5 to 10 of the cycle in cynomolgus monkeys leads to recruitment of newer follicles to mature. The recovery in the ability of the Day-6 follicle to produce oestradiol could indicate that the follicle has, by this time, developed enough LH receptors to respond to LH in the absence of FSH (Hillier *et al.*, 1981, 1983), but this is belied by the fact that the Day-7 follicle totally succumbs to the lack of FSH for 24 h and does not recover preovulatory status or oestrogen synthesizing ability by Day 9. Once again, the observation that the follicle from Day 8 onwards becomes less sensitive to the lack of FSH suggests that between Days 6 and 8 some differential changes must be occurring in the follicle making it transform from an FSH-dependent to a mostly independent state. It could at this time point become totally LH dependent. Zeleznik & Kubik (1986) have shown that, after initiation of follicular growth by elevated FSH concentrations, these follicles can continue to mature in the presence of an FSH

concentration which by itself is unable to support the growth of less mature follicles. The LH concentrations of these monkeys, however, were unaltered, suggesting that LH may have an important role once follicular dominance is achieved. If follicular dominance can then be redefined as that state when the follicle by virtue of developmental changes becomes mostly independent of FSH support, it can be observed that this is acquired by Day 8 of the cycle. In support of this, recent studies by Harlow *et al.* (1988) demonstrate that granulosa cell steroidogenesis is highly sensitive to hFSH during preovulatory follicular development in marmosets. However, in granulosa cells isolated from large preovulatory follicles, androgen suppressed hFSH-stimulated aromatase activity suggesting a development-dependent change to FSH responsiveness.

In conclusion, the current study shows that initiation of the cyclic follicular maturation process is dependent on the presence of FSH and the requirement for FSH is confined to the first 7 days of the total 9–10 days the follicle takes to reach the ovulable state. In particular, a critical need for FSH appears to be felt when follicular growth has reached the stage seen on Days 6–7 of the cycle and this could be the signal for asserting follicular dominance.

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