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Hormonal Correlates of Follicular Development in the Human Ovary*

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Abstract

This review summarizes recent studies pertaining to follicular development in the human ovary. Some of these studies were concerned with characterizing the steroidogenic capacity of the individual cell types of the follicle in relation to whether the follicle was healthy or atretic. Other studies covered in this review concern the oocyte and its hormonal environment both *in vivo* and *in vitro*, the effects of steroids and gonadotrophins on the steroidogenic potentials of follicle cells and also some endocrine and non-endocrine responses of thecal and granulosa cells after being separated and then recombined *in vitro*. The data are integrated in relation to the development of a follicle during the follicular phase of the menstrual cycle.

The studies on the isolated cellular compartments of the follicle show that the biosynthetic and mitotic or developmental activities of granulosa cells and thecal tissues, and the behaviour of oocytes *in vitro*, all correlate with the hormonal microenvironments that they were previously exposed to *in vivo* rather than those in peripheral blood.

The data suggest that the levels of steroid in follicular fluid and ovarian venous blood, collagen synthesis in the thecal tissue and mitotic activity of the granulosa cells are all dependent in part on a functional interaction between the thecal and the granulosa cells.

It is concluded that the fate of a developing antral follicle centres around its ability to generate an oestrogen-enriched intrafollicular environment while simultaneously secreting both androgens and oestrogens into ovarian–venous blood.

Introduction

In the human ovary, follicles begin to form during the fourth month of foetal life (Peters *et al.* 1979). Although some of these newly formed follicles start to grow almost immediately, most remain at rest until some signal(s) (as yet unknown) initiate the resumption of follicular development (see Peters and McNatty 1980 for review). The resumption of ovarian follicular development during foetal, neonatal or adult life begins when the spindle-shaped granulosa cells adjacent to the oocyte transform into cuboidal-shaped granulosa cells and then undergo successive mitotic divisions concomitantly with enlargement of the oocyte. As the granulosa cells proliferate, the cells immediately outside the basement membrane (the thecal cells) become concentrically aligned to the follicle and for the remainder of follicular development, the thecal cells develop synchronously with other components of the follicular apparatus. Throughout follicular development, the thecal tissue is vascularized whereas the granulosa cells and oocyte are separated from the blood supply by the basement membrane of the granulosa cells.

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This review summarizes some recent studies pertaining to follicular development in the human ovary. Some of these studies were concerned with characterizing the steroidogenic capacities of the granulosa and thecal cells in relation to whether the follicle was healthy or atretic. Other studies covered in this review concern: the oocyte and its hormonal environment *in vitro* and *in vivo*; the effects of steroids and gonadotrophins on the steroidogenic potentials of follicle cells; and some endocrine and non-endocrine effects of the thecal and granulosa cells on one another. The data are then integrated in relation to the development of a follicle during the follicular phase of the menstrual cycle.

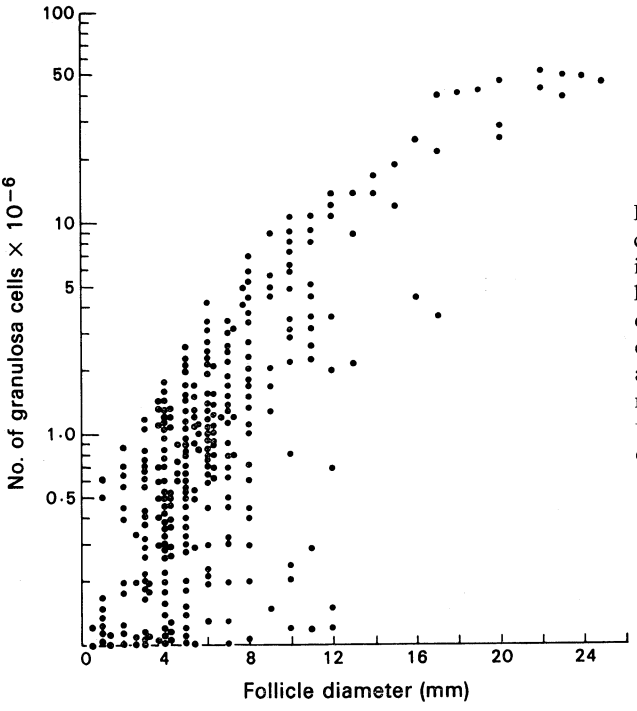


Fig. 1. Number of granulosa cells recovered from 306 individual human follicles of known diameter which were obtained from the ovaries (intact or wedge biopsies) of 73 women at different stages of the menstrual cycle but who were undergoing surgery for a variety of gynaecological disorders.

Growth and Atresia of Ovarian Follicles

Most human follicles ($\simeq 99{\cdot}9\%$) which start to grow degenerate at some stage of their development (Byskov 1978). During preantral development, the incidence of atresia, as assessed by morphological criteria, increases with follicular size. However, the total number of follicles which degenerate before the antrum is formed is probably low (Byskov 1978). In the infant human ovary, only $\simeq 15\%$ of all growing follicles undergo atresia during preantral development (Himelstein-Braw *et al.* 1976); however, quantitative data are unavailable for the adult ovary.

In studies on antral follicles, McNatty *et al.* (1979*d*) have described a series of functional criteria by which the degree of health or atresia of a follicle might be assessed: it was proposed that follicles with more than 50% of their full complement of granulosa cells at any follicular diameter were those which had some potential for further development (healthy follicles). That is, only the follicles with less than 50% of the maximum cell number per follicle size were those undergoing irreversible

atresia (atretic follicles). Healthy follicles were those which contained high concentrations of oestradiol in follicular fluid (>1000 ng/ml) or granulosa cells with the capacity to generate this steroid in response to follicle stimulating hormone (FSH) or both, and a germinal-vesicle stage oocyte which appears healthy at the level of the dissecting microscope (× 100). Atretic follicles were those with low levels of oestradiol (E_2) in follicular fluid and granulosa cells incapable of responding to FSH to generate high levels of intrafollicular E_2. The distribution of granulosa cell populations in human follicles of different sizes is shown in Fig. 1. These data indicate that more than 85% of the antral follicles in the human ovary are smaller than 10 mm in diameter and, in terms of their granulosa cell populations with respect to follicle diameter, most of these small follicles (i.e. ≃92%) could be considered to be atretic (McNatty *et al.* 1979*d*). In contrast, more than 50% of the follicles greater than 10 mm in diameter could probably be considered as healthy structures. Collectively the above data, based on functional criteria, suggest that most human antral follicles undergo atresia during early antral development. These findings are consistent with the morphological data of Block (1951).

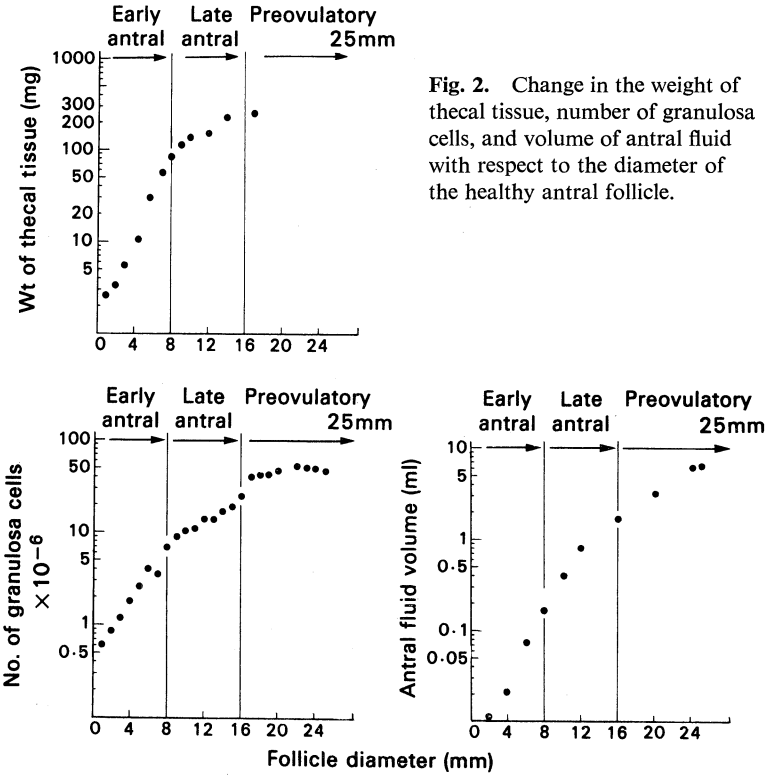
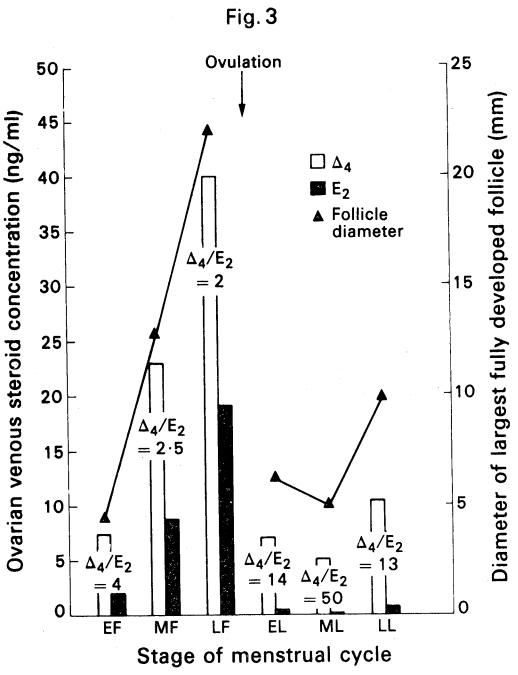


Fig. 2. Change in the weight of thecal tissue, number of granulosa cells, and volume of antral fluid with respect to the diameter of the healthy antral follicle.

Developmental Changes in the Thecal Cell, Granulosa Cell and Antral Fluid Compartments of the Ovarian Follicle during Follicular Development

These changes are summarized in Fig. 2. Substantial thecal mass accumulates during early antral development whereas during the later phases of growth the rate of accumulation declines. In contrast to the theca, the granulosa cells accumulate in

large numbers during both the early and late antral growth phases although the rate of accumulation declines when the follicle reaches the preovulatory phases (see also Delforge *et al.* 1972; Bomsel-Helmreich *et al.* 1979). By the time the follicle has reached its final phase of maturation it has accumulated most of the granulosa cell mass which is ultimately present in a corpus luteum. In contrast to the follicular cells, antral fluid continues to accumulate until the time of ovulation. Indeed, during the preovulatory growth phase, follicular enlargement is due almost entirely to fluid accumulation.



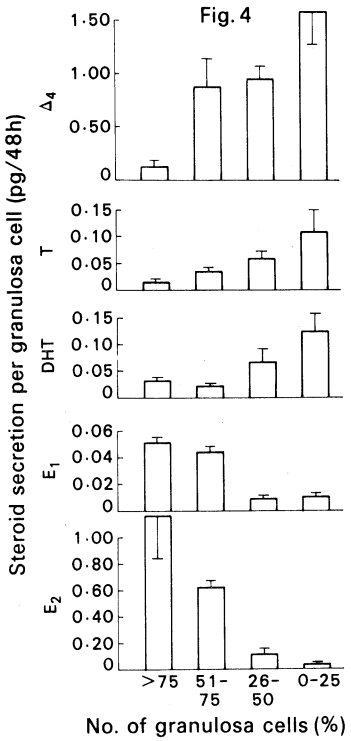


Fig. 3. Relationship between the diameter of the largest healthy follicle and the ovarian secretions of androstenedione (Δ_4) and oestradiol (E_2) during the early (EF), mid (MF) and late (LF) follicular stages and the early (EL), mid (ML) and late (LL) luteal stages of the menstrual cycle. The data for steroid concentrations in ovarian venous blood were determined from three to seven observations at each phase of the cycle.

Fig. 4. *In vitro* production of androstenedione (Δ_4), testosterone (T), dihydrotestosterone (DHT), oestrone (E_1) and oestradiol (E_2) by human granulosa cells containing >75, 51–75, 26–50 and 0–25% of their maximum number of granulosa cells per follicle diameter respectively. Steroid production was that amount produced per granulosa cell during 48 h culture minus the cellular concentration of steroid at the start of culture. The results are expressed as means (histogram) + s.e.m. (vertical bars).

Follicular Development and the Ovarian Secretions of Androstenedione and Oestradiol during the Menstrual Cycle

Normally, healthy antral follicles greater than 10 mm diameter are only observed during the mid- and late-follicular phases of the menstrual cycle (Fig. 3). However, large healthy follicles have sometimes been observed during the late luteal phase

(Fig. 3; McNatty 1978*a*). These late-luteal-phase follicles do not appear to sustain their development into the next follicular phase since large antral follicles during the early follicular phase have been found to be severely deficient in granulosa cells (McNatty *et al.* 1975). During the follicular phase, the ovary containing an enlarging follicle simultaneously increases its output of androstenedione (Δ_4) and E_2 whereas the secretions of these steroids from the contralateral ovary containing small antral follicles remains low (McNatty *et al.* 1976; Serio *et al.* 1976). As the dominant follicle enlarges, the output of Δ_4 continues to exceed that of E_2 although the Δ_4/E_2 ratio decreases progressively (Fig. 3). As a follicle develops into a preovulatory structure, it emerges as a major source of Δ_4 (Abraham 1974) as well as the major source of E_2 (Baird and Fraser 1975). Although antral follicles develop throughout the luteal phase, the amount of Δ_4 secreted by non-luteal tissue does not exceed that generated by ovaries during the early follicular phase. Moreover, non-luteal tissue during the luteal phase secretes only trace amounts of E_2 (Fig. 4).

Gonadotrophins and Intraovarian Control of Follicular Development: Three Concepts

1. Follicular development and ovarian steroid secretion can be stimulated in hypophysectomized women, or in women with gonadotrophin deficiencies, after the administration of exogenous gonadotrophin preparations containing FSH and LH activities (see reviews by Vande Wiele *et al.* 1970; Ross and Lipsett 1978). Antral follicles have not been cited in ovaries of women with hypogonadotrophic hypogonadism. Moreover, in these same ovaries it was observed that preantral follicular development was severely impaired (Tagatz *et al.* 1970; Goldenberg *et al.* 1976). Antral follicular development was also found to be impaired in women who had taken oral contraceptives containing both oestrogen and progestins for at least 2 months (i.e. after gonadotrophin secretion has been suppressed for some time) (Maqueo *et al.* 1972; Starup and Visfeldt 1974). Collectively, therefore, these data support the notion that 'once follicles have started to grow, their continued development is dependent on a certain level of gonadotrophic support' (see also Ross 1974 for review).

2. Within the ovary, the concentrations of steroid are not uniform throughout the gland (see review by Edwards 1974). Indeed, the hormonal milieu within each follicle is different from that in adjacent follicles and plasma. Therefore, the oocyte and granulosa cells within each follicle are exposed to a unique endocrine microenvironment at any one time (McNatty 1978*b*). These findings have led to the suggestion that 'the formation of a fluid-filled cavity within the avascular regions of the follicle provides the means by which intrafollicuar cells of one follicle may be exposed to a different hormonal microenvironment from that in adjacent follicles and peripheral blood'.

3. *In vitro* studies on the isolated cellular compartments of the follicle have shown that the mitotic and biosynthetic activities of granulosa cells, the developmental and biosynthetic activities of thecal tissues and the behaviour of oocytes all correlate with the hormonal microenvironments to which they were previously exposed in antral fluid rather than those in peripheral blood (McNatty and Sawers 1975; McNatty *et al.* 1979*d*; Koob, McNatty and Ryan, unpublished data). These findings support the notion that 'the hormonal composition of a follicle may determine whether it matures or undergoes atresia' (Harman *et al.* 1975; Louvet *et al.* 1975; McNatty and Sawers 1975; Lipsett and Ross 1976).

Functional Correlates of Growth and Atresia

Cause–effect relationships have been established between ovarian hormones and follicular development in experimental animals. In the hypophysectomized and immature female rat, oestrogens have been shown to enhance follicular development whereas androgens, such as testosterone (T) have been shown to antagonize the actions of oestrogen and to stimulate follicular atresia (Ross and Hillier 1979). Although such causal relationships have not been established for the human female, high concentrations of oestrogen in antral fluid have been associated with non-atretic follicles whereas low concentrations of oestrogen have been associated with follicles undergoing degenerative changes (Table 1). For example, if an antral follicle contains more than 75% of its maximal granulosa cell number for a given follicular diameter then it invariably contains an oocyte free of degenerative changes [at the level of the dissecting microscope (× 100), 'healthy-looking'] together with high levels of E_2 and Δ_4 (Table 1) and detectable levels of FSH (data not shown; McNatty *et al.* 1979*d*).

Table 1. Interrelationships in the human follicle between the number of granulosa cells, oocyte viability, and the concentrations of androstenedione (Δ_4), testosterone (T), dihydrotestosterone (DHT) and oestradiol (E_2) in antral fluid

Data (from McNatty 1980) collected from 157 human antral follicles ranging in diameter from 1 to 20 mm

No. granulosa cells (%)[A]	No. of healthy-appearing, GV-stage oocytes/ No. obtained (%)[B]	Mean hormone concn in antral fluid ± s.e.m. (ng/ml) Δ_4	T	DHT	E_2
>95	9/10 (90·0)	1224 ± 431	61 ± 27	168 ± 61	1441 ± 358
76–100	13/18 (72·2)	1453 ± 200	171 ± 62	216 ± 33	972 ± 276
51–75	14/22 (63·6)	1232 ± 182	104 ± 20	220 ± 58	211 ± 53
26–50	24/52 (46·2)	1078 ± 155	68 ± 11	277 ± 44	193 ± 52
0–25	16/55 (29·1)	967 ± 189	125 ± 49	190 ± 25	87 ± 24

[A] Number of granulosa cells is the ratio of the number of granulosa cells recoverable from a follicle of a given size to the maximum number of recoverable cells from a follicle of that size.
[B] GV, germinal vesicle.

Follicles with fewer than the maximum number of granulosa cells for a given follicle size can be considered to be undergoing some degree of atresia: thus the fewer granulosa cells found, the greater the degree of atresia. In such depopulated follicles lower levels of follicular E_2 are found without any significant alteration in the levels of Δ_4, T or dihydrotestosterone (DHT) (see McNatty *et al.* 1979*d*). Moreover, follicles deficient in granulosa cells are less likely to contain a 'healthy looking' germinal-vesicle-stage oocyte.

Meiotic Maturation Potential of Human Oocytes *in vitro*

The interrelationships between the ability of the germinal-vesicle-stage human oocyte to resume meiosis *in vitro*, the diameter of the oocyte and the concentrations of Δ_4 and E_2 in the antral fluid from which the oocyte was recovered are shown in Table 2. Many cumulus-enclosed human oocytes were found to be incapable of resuming meiosis *in vitro* even though they had been removed from all other follicular elements. The cumulus-enclosed oocytes which did not resume meiosis were the smallest of those recovered and they came from follicles with low levels of oestrogen.

Those which resumed meiosis and went on to form polar bodies were amongst the largest of the oocytes recovered and they came from follicles with high levels of oestrogen. Oocytes which lost their germinal vesicles but did not form polar bodies were intermediate in size, and were from oestrogen-deficient follicles. The size of the antral follicle *per se* was found to bear no relationship to the ability of oocytes to

Table 2. Relationship between the ability of the healthy oocyte to resume meiosis *in vitro*, the size of the oocyte and the concentrations of androstenedione (Δ_4) and oestradiol (E_2) in antral fluid

A healthy oocyte is one which appears devoid of degenerative characteristics at the level of the dissecting microscope (×100) (see McNatty *et al.* 1979*d*). Data from McNatty *et al.* (1979*d*). Values sharing a common alphabetical letter are significantly different from one another: [a] $P < 0{\cdot}01$; [b,c] $P < 0{\cdot}001$

Oocyte status after 48 h culture	Oocyte diameter (μm)	Steroid concn in antral fluid (ng/ml) Δ_4	E_2
Healthy	$108{\cdot}2 \pm 0{\cdot}9^{a}$	825 ± 220	160 ± 79^{b}
GV breakdown, no polar body formation[A]	$110{\cdot}6 \pm 2{\cdot}3$	1183 ± 320	87 ± 31^{c}
Polar body	$117{\cdot}2 \pm 2{\cdot}2^{a}$	876 ± 178	$1019 \pm 310^{b,c}$

[A] GV, germinal vesicle.

resume maturation *in vitro*. These findings suggest that intrafollicular oestrogen may itself be important for subsequent maturation of the human oocyte, or that follicular oestrogen synthesis reflects some other aspect of follicular maturation critical to the development of the oocyte.

Table 3. Ability of human oocytes to resume meiosis in culture medium alone or in culture medium supplemented with 50% follicular fluid

Follicular fluid for each oocyte culture was obtained from the same follicle as the oocyte. Oocytes were cultured in 20 μl of media which consisted either of 10 μl antral fluid plus 10 μl of medium containing Ham's F-10 supplemented with L-glutamine, sodium pyruvate (0·055 mg/ml), penicillin (100 i.u./ml), streptomycin (50 μg/ml) and 20% newborn calf serum (referred to as AF + medium) or 20 μl medium alone. Data from McNatty *et al.* 1980*c*

Stage of oocyte maturation at the time of recovery	No. of oocytes	Oocyte treatment	Percentage of oocytes at each stage of maturation at the end of 40–50 h culture *in vitro*[A] GV	GVBD	PB	Necrotic	Unknown stage
Germinal vesicle	18	AF+medium	31·5	15·8	36·8	15·8	—
Germinal vesicle	96	Medium alone	27·0	22·0	39·0	11·0	1·0

[A] GV, germinal vesicle; GVBD, germinal vesicle breakdown but without polar body formation; PB, polar body.

A non-steroidal substance in human follicular fluid has been found to prevent oocytes from resuming meiosis *in vitro* (Channing *et al.* 1978). However, when human oocytes were cultured in their own follicular fluid (50% v/v) supplemented with culture medium (50% v/v), their meiotic maturation potential still correlated

with the hormonal microenvironment that they had previously been exposed to *in vivo* (data not shown) and was not influenced by the presence of follicular fluid in the culture dish (Table 3). These data suggest that the existence of 'inhibitory substances' in follicular fluid in sufficient concentrations to be of physiological importance must remain equivocal at the present time.

Follicular Steroids: Possible Cellular Origins

It seems that the fate of a developing follicle centres around its ability to generate an oestrogen-enriched intrafollicular environment while simultaneously secreting both androgens and oestrogens into ovarian venous blood. A greater understanding of follicular development may therefore be derived from examining the possible cellular sources of follicular androgen and oestrogen and the role of the gonadotrophins in regulating their secretions.

Steroidogenesis by Granulosa Cells in vitro

Androgens and oestrogens

The first 48 h of production of androgens and oestrogens by human granulosa cells in a tissue culture medium supplemented with 20% (v/v) foetal calf serum is shown in Fig. 4. Irrespective of whether they were recovered from small (<8 mm diameter) or large (⩾8 mm diameter) follicles, human granulosa cells were found to have the biosynthetic capacity to produce some Δ_4, T, DHT, oestrone (E_1) and E_2. These findings are consistent with the earlier results of Ryan and Smith (1965) and Channing (1969). The patterns of secretion of these hormones were found to be related to the health of the follicle from which the cells were harvested. When granulosa cells were harvested from follicles with more than 75% of their maximum cell population, they preferentially secreted oestrogens and smaller amounts of androgens. However, when the cells were derived from follicles with proportionately smaller populations of granulosa cells per follicle size, they were found to be less capable of secreting oestrogens but not androgens *in vitro*. When granulosa cells from healthy follicles (with ⩾50% of their maximum granulosa cell population) between 2·5 and 10 mm in diameter were cultured in the presence of excess Δ_4, their ability to produce oestrogen was enhanced when they were exposed to additional amounts of FSH (McNatty *et al.* 1979*c*). However, when granulosa cells from healthy follicles >10 mm in diameter were exposed to excess Δ_4 *in vitro*, their ability to produce oestrogen was not enhanced by the addition of FSH to the culture medium. Thus in healthy growing follicles, it seems that granulosa cells probably function as oestrogen-secreting cells and that the level of aromatase (oestrogen synthetase) activity in these cells is acutely sensitive to FSH stimulation during the early antral phases of development. In contrast to the granulosa cells recovered from healthy follicles, those from atretic follicles (i.e. with <50% of their maximum granulosa cell population) were generally incapable of metabolizing Δ_4 to oestrogen. Moreover, even after exposure to additional FSH their capacity to metabolize Δ_4 to oestrogen remained low. Thus as a follicle degenerates its granulosa cells appear to lose their capacity to secrete oestrogen and eventually, the cells lose their capacity to respond to FSH. The effect of LH on androgen and oestrogen synthesis by granulosa cells is unknown.

Progesterone

As a follicle matures into a preovulatory structure, its granulosa cells transform from an oestrogen-secreting tissue into a progesterone (P)-secreting one (McNatty and Sawers 1975; McNatty *et al.* 1979*b*). The capacity of the granulosa cells to make this transformation appears to depend on their ability to respond to LH (Table 4) which in turn depends on the appropriate 'priming' of the cells to FSH and oestrogen within the follicle (McNatty and Sawers 1975; Table 4). High levels of P in the antral fluid of large follicles before ovulation are indicative of granulosa cells undergoing luteinization (Fig. 5; Baird *et al.* 1975).

Table 4. Relationship between the endocrine microenvironment of the human antral follicle and the average daily secretion of progesterone by granulosa cells exposed LH or LH-free media *in vitro*

Data from McNatty (1979*a*)

Hormones in antral fluid *in vivo*[A]	Stage of menstrual cycle[B]	Mean daily secretion rate (range) of progesterone by granulosa cells *in vitro* (pg/cell)	
		LH[C] (30 m u./ml)	LH-free media[D] (<0·02 m u./ml)
$-FSH, -E_2$	F	0·57 (0·01–0·88)	0·13 (0·04–0·21)
n		12	3
$+FSH, -E_2$	F	0·55 (0·08–1·10)	—
n		10	
$+FSH, +E_2$	MF	0·64 (0·14–1·10)	0·12 (0·09–0·18)
n		8	5
$+FSH, +E_2$	LF	5·0 (2·8–8·0)	0·85 (0·30–1·16)
n		16	5

[A] E_2, oestradiol-17β; $-FSH$, <2·8 m u./ml; $-E_2$, <250 ng/ml; $+FSH$, >2·8 m u./ml; $+E_2$, >250 ng/ml; *n*, number of follicles.

[B] F, any stage of follicular phase; MF, mid follicular phase; LF, late follicular phase.

[C] Culture media contained 1·7 m u./ml LH, 1·8 m u./ml FSH and 5 ng/ml prolactin.

[D] Culture media contained <0·02 m u./ml LH, 2·6 m u./ml FSH and 6 ng/ml prolactin.

Contribution of intrafollicular cells to steroids in antral fluid

Granulosa cells are a major source of steroid in antral fluid. In the appropriate tissue culture medium, granulosa cells can generate levels of P, T, E_1 and E_2 that are similar to those in antral fluid from which the cells had been recovered (Table 5; McNatty *et al.* 1979*a*). Indeed, of the major steroids in antral fluid only Δ_4 is present in significantly higher levels than the granulosa cells are capable of producing *in vitro*. The oocyte–cumulus cell complex is also capable of secreting steroids such as T, E_1 and E_2, and of modifying an antral-fluid endocrine environment *in vitro* (McNatty *et al.* 1980*c*). Therefore, it is likely that most (if not all) of the cell types within the avascular tissues of the follicle influence the steroid composition of antral fluid.

Steroidogenesis by Thecal Tissue *in vitro*

Production of androgens and oestrogens by minced human thecal tissue *in vitro* in the first 48 h is shown in Fig. 6. In contrast to the secretory bchaviour of granulosa

cells, the major secretory product of the thecal minces was always Δ_4 (see also Tsang *et al.* 1979; Batta *et al.* 1980) irrespective of whether the tissue was recovered from healthy or atretic follicles (Fig. 6). In healthy but not severely degenerating follicles, E_2 was also a major secretory product. It seems unlikely that the thecal output of oestrogen was due to granulosa-cell contamination, nevertheless conclusive proof for

Fig. 5

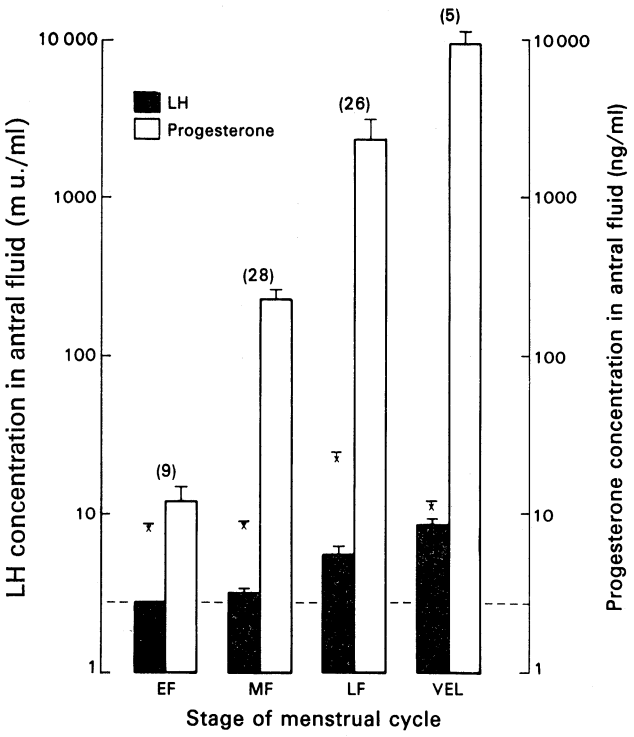


Fig. 6

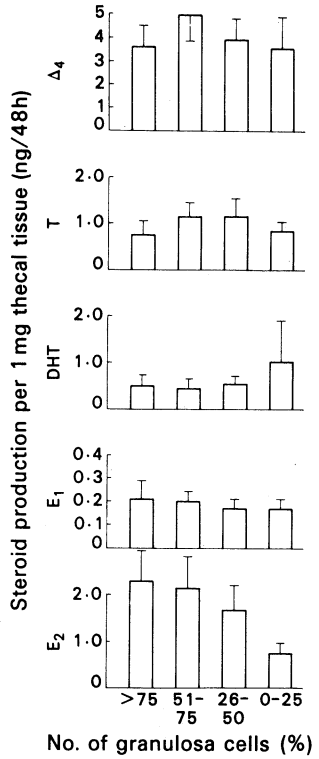


Fig. 5. Relationship between the concentrations of LH and progesterone in large antral follicles ($\geqslant$8 mm diameter) and in recently ovulated follicles during the early (EF), mid (MF) and late (LF) follicular phases and the very early luteal (VEL) phase of the menstrual cycle. The mean concentrations of LH in plasma are also shown (×). All results are expressed as means + s.e.m. The number of observations are in parentheses. The dotted line was the limit of detection of LH (2·8 m u./ml). The limit of detection for progesterone was 10 ng/ml. Data from McNatty 1979*a*.

Fig. 6. *In vitro* production of androstenedione (Δ_4), testosterone (T), dihydrotestosterone (DHT), oestrone (E_1) and oestradiol (E_2) by human thecal tissue from follicles containing >75, 51–75, 26–50 and 0·25% of their maximum number of granulosa cells per follicle diameter respectively. Steroid production was that amount produced per granulosa cell during 48 h culture minus the cellular concentrations at the start of culture. The results are expressed as means (histograms) + s.e.m. (vertical bars). The number in parenthesis represent the numbers of observations.

thecal aromatase activity must await the isolation of a 'pure line' of thecal cells (McNatty *et al.* 1979*b*). On a per unit mass basis, thecal tissue secreted more steroid than the surrounding stromal tissues under the *in vitro* conditions employed (McNatty *et al.* 1979*b*). The thecal output of androgen and oestrogen exceeded that of the stroma by about 50- to 100-fold. Irrespective of follicle size, the capacity of a given amount of thecal tissue to secrete steroid *in vitro* remained relatively constant. Thus,

during follicular development any increased output of steroid by thecal tissue may partly be related to the amount of tissue present.

Table 5. Relationship between steroid production by the total population of granulosa cells from each healthy follicle *in vitro* and the levels of steroid in the antral fluid from which cells were harvested

Healthy follicles were those containing >50% of their maximum number of granulosa cells for the size that the follicle has reached. a, $P<0{\cdot}01$; b, $P<0{\cdot}001$; c, $P<0{\cdot}002$. Data from McNatty *et al.* 1979*a*

Healthy follicles[A]	Follicle diam. (min)	Mean steroid concentrations in the culture medium and antral fluid ±1 s.e.m. (ng/ml)[B] P	Δ_4	T	DHT	E_1	E_2	*n*
In vivo	<8	36± 14	231± 37[a]	26±10	29± 10[b]	29± 11	355± 76	8
In vitro	<8	29± 8	687±158[a]	19± 4	179± 40[b]	27± 5	498± 41	8
In vivo	≥8	334±119	368±114[c]	41±11	174±113	271±106	808±250	4
In vitro	≥8	269±102	909±267[c]	41±11	231± 73	231± 73	1171±403	4

[A] *In vitro* concentrations are the amounts of steroid produced by the total population of granulosa cells from each follicle into 1 ml of culture medium consisting of 20% foetal calf serum + 80% medium 199 + Hank's salts + HEPES buffer; *in vivo* concentrations are mean concentrations of the steroids in the antral fluid from which the cells were harvested.

[B] P, progesterone; Δ_4, androstenedione; T, testosterone; DHT, dihydrotestosterone; E_1, oestrone; E_2, oestradiol; *n*, number of observations.

Table 6. Amounts of steroid produced by thecal tissues from healthy and atretic follicles *in vitro*

Data from McNatty *et al.* 1980*b*. Median values and 95% confidence limits are given. All numbers sharing a common alphabetical letter are significantly different from one another: a,b,c,d,e,f, $P<0{\cdot}05$. Abbreviations *P*, Δ_4, T, E_2 as in Table 5

Follicle treatment	No. of follicles	Amount of steroid produced (ng/15 h) P	Δ_4	T	E_2
		Thecal tissue from healthy follicles			
Control	5	1·3[a]	24·9	1·7	1·9[c]
		(0·8, 1·8)	(8·0, 120·1)	(0·2, 4·6)	(0·7, 3·7)
+LH	5	2·4[ab]	77·4	3·9	7·6[cd]
(10 ng/ml)		(1·3, 8·9)	(33·8, 220·3)	(1·4, 6·9)	(2·2, 6·2)
+LH	5	1·9[b]	23·0	1·7	1·5[d]
(50 ng/ml)		(1·2, 2·2)	(11·5, 143·9)	(0·5, 2·9)	(0·5, 6·1)
		Thecal tissue from atretic follicles			
Control	14	2·0	11·6[e]	1·4	1·4
		(1·3, 2·8)	(4·3, 29·0)	(0·8, 2·1)	(1·0, 2·2)
+LH	14	2·6	40·8[ef]	2·2	2·1
(10 ng/ml)		(1·3, 8·4)	(23·0, 79·7)	(1·4, 3·2)	(1·2, 3·2)
+LH	14	4·6	9·6[f]	1·9	2·2
(50 ng/ml)		(1·1, 10·9)	(2·4, 34·0)	(1·1, 3·5)	(0·9, 3·6)

Responsiveness of Thecal Tissue to Pituitary Hormones

Human thecal tissue is responsive to LH but not FSH (Tsang *et al.* 1979). However, the responsiveness of thecal tissue to LH *in vitro* is variable and depends partly on the mass of tissue (i.e. size of the follicle), the health of the follicle, and the concentration of LH (Tsang *et al.* 1979; McNatty *et al.* 1980*b*). *In vivo*, it probably also

depends on the nature of the LH stimulus in blood (i.e. a pulsatile stimulus *v.* constant level) (Table 6 and McNatty *et al.* 1980*b*). Small incremental increases in LH (from 1 to 10 ng/ml) during a 15-h incubation period were found to increase markedly the thecal output of Δ_4 from healthy or atretic follicles or both. Theca from healthy follicles were also stimulated to increase their output of P and E_2 whereas theca from atretic follicles produced more variable amounts of P and were unable to generate E_2. When theca from healthy or atretic follicles were exposed to high concentrations of LH (50 ng/ml) *in vitro*, the net output of most steroids remained comparable to those produced by the unstimulated controls.

Table 7. *In vitro* metabolism of androstenedione by granulosa cells recovered from healthy follicles of different sizes

Data from McNatty *et al.* 1979*c*

Follicle diameter (mm)	TP (%)[A]	DHT formed (%)[B]	Oestrogen formed / (%)[C]	No. of follicles tested
23	49	22	61	1
17	54	17	78	1
10	22	58	22	1
7–8	16	44	25	2
4·5–6	9	44	16	3
2·5–4	15	78	10	2
0·5–2	2	69	5	1

[A] TP(%) is the sum (in percentages) of testosterone (T), dihydrotestosterone (DHT) and oestrogen [oestrone (E_1) + oestradiol (E_2)] formed after incubation of granulosa cells with 1 μg of androstenedione + 0·1 μg FSH for 48 h.

[B] DHT(%) is the percentage of DHT in metabolite TP.

[C] Percentage of $E_1 + E_2$ in the metabolite TP. The amount of T (data not shown) in the metabolite TP is 100% − [DHT(%) + oestrogen (%)].

Metabolism of Androstenedione by Granulosa Cells and the Possible Role of Androstenedione Metabolites in Atresia

Since human thecal tissue is a major source of Δ_4, it has been suggested that the granulosa cells could utilize this hormone to form more biologically potent androgens or oestrogens (see Henderson 1979 for review). The ability of human granulosa cells from different-sized healthy follicles to metabolize Δ_4 to either DHT or oestrogen (i.e. E_1 and E_2) *in vitro* is shown in Table 7. The ability of a given population of granulosa cells to metabolize Δ_4 was found to increase as a function of follicle diameter. Granulosa cells from small follicles (<8 mm diameter) preferentially metabolized Δ_4 to DHT irrespective of the amount of FSH present. However, granulosa cells from healthy large follicles (>12 mm diameter) preferentially metabolized Δ_4 to oestrogen.

The preferential conversion of Δ_4 to DHT in small follicles may partly explain why most small antral follicles in the human ovary undergo atresia (see Fig. 7). As demonstrated earlier, substantial thecal-cell mass is formed before the follicle reaches a diameter of 10 mm. During this early phase of growth the theca is sensitive to changes in the concentrations of LH and is capable of secreting large amounts of Δ_4. Consequently, excessive or repeated LH stimulation of the theca, in small follicles,

may result in the granulosa cells preferentially metabolizing thecal Δ_4 to DHT. DHT is known to inhibit aromatase activity in granulosa cells (Hillier *et al.* 1980), and to significantly reduce the rate of granulosa cell accumulation *in vitro* (McNatty 1980). Because DHT and E_2 are detectable in antral fluid of most follicles, the potential exists for the mutually antagonistic actions of androgens and oestrogen to occur simultaneously during antral follicle development. Thus for small antral follicles in which the aromatase enzyme activity is dependent upon FSH stimulation, growth may be dependent on a limited output of thecal androgens or the appropriate balance of LH and FSH in plasma or both (Brown 1978; Ross and Lipsett 1978).

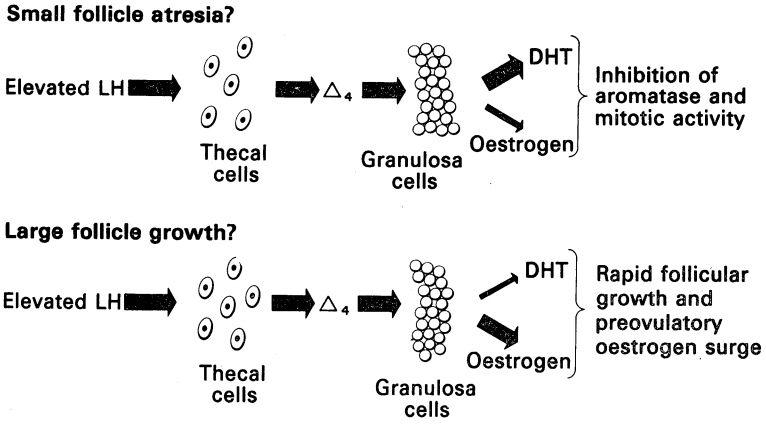


Fig. 7. Possible mechanisms by which LH promotes atresia of small follicles and growth of large follicles in the human ovary. Aromatase activity = oestrogen synthetase activity.

Plasma Prolactin and the Microenvironment of Antral Follicles

Prolactin has been shown to have both inhibitory and luteotrophic effects on progesterone synthesis in human and porcine granulosa cells (McNatty *et al.* 1974; Veldhuis and Hammond 1980). In the porcine ovary these divergent effects can be regulated by oestrogen (Veldhuis and Hammond 1980). In granulosa cells from the immature, hypophysectomized, oestrogen-treated rat, high concentrations of prolactin have been shown to suppress basal and gonadotrophin-induced increases in oestrogen production *in vitro* (Wang *et al.* 1980). All the *in vitro* evidence from the above three species suggests that prolactin may, in some circumstances, have a direct inhibitory effect on the development of ovarian follicles. Irrespective of whether prolactin exerts an 'antigonadal effect' at the level of the ovary or via the hypothalamic–pituitary axis (Glass *et al.* 1975; Bohnet *et al.* 1976; Jacobs *et al.* 1976) hyperprolactinaemia can be associated with a marked reduction in intraovarian activity and the extent of this reduction may not always be apparent from the levels of FSH and E_2 in plasma or from the numbers of antral follicles (McNatty 1979*b*). This latter conclusion was reached after the levels of prolactin, FSH and E_2 were measured in plasma and follicular fluid of women undergoing hysterectomy. Also in this study the number of granulosa cells were determined in the individual excised antral follicles. A summary of these data for ovaries in the follicular phase is shown in Table 8. When the plasma levels of prolactin exceeded 100 ng/ml, 95% of the follicles (19 out of 20) were found

to contain undetectable levels of FSH, levels of E_2 below 500 ng/ml, and none of the follicles contained more than 50% of their maximal granulosa cell number per follicle size. In contrast, when the prolactin levels were below 100 ng/ml, between 20 and 62% of the follicles contained measurable amounts of FSH, high levels of E_2 and $\geqslant$50% of their maximum granulosa cell number.

Table 8. Relationship between prolactin levels in plasma, number of human antral follicles with detectable FSH levels and distribution of follicles with certain granulosa cell populations during the follicular phase of the menstrual cycle

Plasma prolactin levels at ovariectomy (ng/ml)[A]	No. of follicles with FSH >1·3 m u./ml in antral fluid	No. of follicles with E_2 $\geqslant$500 ng/ml in antral fluid[B]	No. of follicles (%) with granulosa cell populations[C] >75%	51–75%	26–50%	<26%	No. of follicles[D]
>100	1	1	0	0	35·0	65·0	20
50–100	12	6	20·0	15·0	35·0	30·0	20
<50	18	9	27·6	24·1	31·0	17·2	29

[A] The lowest and highest prolactin levels recorded were 11 and 260 ng/ml respectively.
[B] Oestradiol (E_2) levels $\geqslant$500 ng/ml indicate active contribution from the granulosa cells (McNatty and Baird 1978).
[C] A follicle with a granulosa cell population of 100% is one with its full complement of cells for whatever diameter it has reached. A follicle with a granulosa cell population of 0% is devoid of granulosa cells (modified from McNatty 1979*b*).
[D] Follicles studied ranged in diameter between 4 and 20 mm.

Steroid and Non-steroidal Interactions between Granulosa Cells and Thecal Tissues

Steroid Interactions

The amounts of steroid entering the bloodstream from human follicles are probably not simply the summation of those secreted from each follicular compartment in isolation. As has already been indicated, it is likely that the cells from each follicular compartment (theca and granulosa) utilize steroid substrates from one another and thereby enhance or lower the output of one steroid over another (see Bjersing 1978 and Henderson 1979 for reviews). Recent *in vitro* studies have demonstrated that the output of P, Δ_4 and E_2 from the recombined granulosa and thecal tissues from the same follicles can sometimes be significantly greater than that generated by the tissues in isolation (Batta *et al.* 1980; McNatty *et al.* 1980*c*). In short-term (4 h) incubations, recombined granulosa and thecal tissues were capable of producing greater than additive amounts of E_2 but only when the tissues were recovered from large follicles ($\geqslant$8 mm diameter) and exposed to elevated concentrations of LH and FSH (50 ng/ml of each hormone; $P<0{\cdot}05$). In long-term incubations (48 h), granulosa cells plus thecal tissue from small follicles produced significantly greater than additive amounts of Δ_4 in the presence of low concentrations of LH and FSH ($\simeq$2 ng/ml of each hormone; $P<0{\cdot}05$) however, in the presence of high concentrations of LH and FSH they produced significantly greater than additive amounts of E_2 ($P<0{\cdot}05$). In contrast, granulosa cells plus thecal tissue from large follicles produced significantly more P in the presence of low concentrations of LH and FSH ($P<0{\cdot}05$) but significantly more P, Δ_4 and E_2 in the presence of high concentrations of LH and FSH compared with the respective amounts produced by granulosa cells and thecal

tissue in isolation ($P<0{\cdot}05$). These findings are consistent with the hypothesis that there is increasing collaboration between granulosa cells and thecal tissue in the production of E_2 as the follicle enlarges (McNatty *et al.* 1979*c*; Hillier *et al.* 1980). Moreover, they suggest that peak secretions of E_2 from large follicles without concomitant luteinization may be critically dependent on the nature of the gonadotrophin stimulus and the length of time the tissues are exposed to elevated levels of gonadotrophin. In summary they also suggest that peak secretions of all the major ovarian steroids (i.e. P, Δ_4 and E_2) are the results of synergistic interactions between different ovarian cell types, namely the thecal cells, the granulosa cells and the stroma (McNatty *et al.* 1980*a*).

Table 9. Numbers of granulosa cells after 6 days of culture after the cells had been cultured alone (G) or in combination with thecal tissue (G+T) with or without exposure to elevated concentrations of LH plus FSH

Co-culture of granulosa cell with other minced human tissues such as foetal heart, kidney or skin did not result in a statistically significant change in granulosa cell number compared with that when granulosa cells were cultured alone. Data from McNatty *et al.* (1980*a*)

Treatment	Follicle diameter (mm)	Cell ratio[A]	No. of observations
G + T	<8	$3{\cdot}43 \pm 0{\cdot}45^{a}$	43
G	<8	$1{\cdot}39 \pm 0{\cdot}22^{a}$	43
G + T	⩾8	$3{\cdot}40 \pm 0{\cdot}53^{b}$	12
G	⩾8	$1{\cdot}17 \pm 0{\cdot}25^{b}$	12
G + T + LH/FSH	<8	$2{\cdot}83 \pm 0{\cdot}09^{c}$	4
G + LH/FSH	<8	$1{\cdot}79 \pm 0{\cdot}07^{c}$	4
G + T + LH/FSH	⩾8	6·4	2
G + LH/FSH	⩾8	1·5	2

[A] Ratio of the number of granulosa cells after 6 days of culture to the number of 'live' cells at the start of culture. [a], $P<0{\cdot}001$; [b], [c], $P<0{\cdot}01$ (two-way analysis of variance).

Granulosa Cell Accumulation and Thecal Collagen Synthesis

In addition to the follicle cell interactions *in vitro* influencing steroidogenesis, granulosa and thecal cells interact to influence the rate of accumulation of granulosa cells (Table 9) and also the amount of collagen formed in the thecal tissues (Koob, McNatty and Ryan, unpublished data). Apparently, thecal tissue contains some substance(s) which can act on the membrana granulosa to enhance cell proliferation (McNatty *et al.* 1980*a*).

Thecal tissue is very rich in collagen: its concentrations may range from 30 to 230 μg/mg tissue (Koob, McNatty and Ryan, unpublished data). In contrast the membrana granulosa are entirely free of collagen. Theca from healthy follicles was found to increase its collagen content by $\simeq 32\%$ during 6 days in tissue culture, but if the same tissue was co-cultured with granulosa cells from the same follicles, the collagen content in the theca was increased by $\simeq 93\%$ ($P<0{\cdot}05$). In contrast, theca from atretic follicles either alone or in co-culture with granulosa cells was not found to increase its collagen content after 6 days of culture (Koob, McNatty and Ryan, unpublished data).

These findings, although preliminary, suggest that proliferating granulosa cells may influence certain structural changes in the thecal compartment. Similarly, as the theca accumulates its secretions may enhance granulosa cell accumulation.

Concluding Remarks

On Ovarian Follicular Steroidogenesis

It is suggested that steroidogenesis during follicular maturation can be summarized as follows:

(1) Both the granulosa and thecal cells have the capacity to synthesize and secrete progestins, androgens and oestrogens. Therefore, differences in the patterns of steroidogenesis between the two cell types are quantitative rather than qualitative.

(2) Throughout most of antral follicle development, thecal tissue is responsive to LH but not FSH: small increases in LH concentrations stimulate the production of Δ_4 and, to a lesser extent of E_2, from healthy follicles but only Δ_4 from atretic follicles; large increases in LH concentrations (>50 ng/ml) do not enhance Δ_4 and E_2 production from either healthy or atretic follicles above the levels generated by unstimulated tissues.

(3) Thecal tissue is probably the major source of the Δ_4 in antral fluid. Indirectly, therefore, the theca is likely to exert a major influence on the intrafollicular levels of T, DHT, E_1 and E_2.

(4) Collectively, the granulosa cells, the oocyte–cumulus cell complex and, to a lesser extent, thecal tissue determine the intrafollicular levels of P, T, DHT, E_1 and E_2. In healthy small antral follicles ($\leqslant 10$ mm diam) granulosa cells are responsive to FSH and have a capacity to generate high levels of oestrogen. In degenerating follicles granulosa cells eventually lose their capacity to respond to FSH and although they become incapable of metabolizing Δ_4 to E_2, they retain a capacity to metabolize Δ_4 to DHT. Prolactin appears to have both stimulatory and inhibitory effects on steroidogenesis by granulosa cells but its role in the development of human follicles is unknown.

(5) 'FSH–oestrogen primed' granulosa cells eventually develop a responsiveness to LH thereby enabling them to transform into luteal cells.

(6) Peak secretions of P, Δ_4 and E_2 from human follicles are probably the outcome of synergistic interactions between the granulosa and thecal compartments.

On Integration of Intraovarian and Extraovarian Events Associated with Follicular Maturation

It is suggested that our current understanding of follicular development in the human can be summarized as follows:

(1) Follicular growth is a continuous event. Follicles leave the non-growing pool of small follicles continuously throughout childhood and adult life. The initiation of follicular growth is a process which is not interrupted during infancy, pregnancy or any other period of anovulation (Peters *et al.* 1978).

(2) The follicle which goes on to ovulate starts to grow before the follicular phase in which it will ovulate. At the onset of the follicular phase, the

presumptive preovulatory follicle will already have reached a diameter of about 4 mm (McNatty *et al.* 1979*d*).

(3) It is probably reasonable to assume that the selection of a follicle to ovulate is made during or before the early follicular phase of the menstrual cycle. Presumably, a follicle is selected because it happens, by chance, to be the most responsive of all healthy follicles to the changing patterns of gonadotrophin secretion at the time when the corpus luteum is regressing.

(4) It appears that the successful maturation of small antral follicles during the early follicular phase is dependent on a certain 'threshold level' of FSH (Brown 1978) with respect to the circulating levels of LH in plasma (Ross *et al.* 1970).

(5) At the beginning of the mid-follicular phase, the best developed follicle will have reached a diameter ranging between 8 and 16 mm. At this time the thecal compartment of the follicle is well developed and the granulosa cells have developed sufficient aromatase activity to enable them to sustain an oestrogen-enriched follicular microenvironment despite an increased output of thecal androgen.

(6) During the mid- to late-follicular phase, the preovulatory follicle will have developed a certain degree of autonomy: its granulosa cells no longer require stimulation by FSH in order to metabolize androgen to oestrogen; the capacity of the follicle to produce oestrogen is limited only by the amount of aromatizable substrate; and subject to being adequately stimulated by LH (Yen *et al.* 1974) this follicle is established as the major ovarian source of Δ_4 and E_2 (Fig. 3). Thus such a follicle has the ability to suppress FSH but not LH secretion (Baird *et al.* 1975) thereby reducing the possibility of any other emerging follicles being stimulated by FSH to generate or sustain an oestrogen-enriched follicular microenvironment.

(7) As the late follicular phase approaches, the rapidly proliferating granulosa cells of the presumptive preovulatory follicle probably contribute markedly to the follicular output of oestrogen which in turn results in the discharge of preovulatory LH.

(8) Finally, the exposure of follicle cells to preovulatory levels of LH results in a reduced output of androgen and oestrogen secretion by the theca, luteinization of the granulosa cells and the transformation of the follicle from an oestrogen-secreting tissue into a progesterone-secreting one with the concomitant release of a large healthy oocyte.

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