The Initiation of Follicle and Oocyte Growth in the Mouse Ovary

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ABSTRACT

The morphological and functional events which surround the initiation of oocyte and follicle growth in the mouse ovary are described. Oocytes of the nongrowing pool were active in RNA synthesis, having relatively low but significant levels of endogenous RNA polymerase activity. Nongrowing oocytes were surrounded by 2–8 follicle cells in the widest cross section. The initiation of oocyte growth was detected by an increase in oocyte nucleolar RNA polymerase activity at 9 follicle cells followed by an increase in oocyte area at 10 follicle cells. Subsequently, increases in nucleolar area, nuclear area and nucleoplasmic RNA synthesis occurred in that sequence.

As the number of follicle cells surrounding nongrowing oocytes increased, there was a transition from flattened (squamous) to cuboidal morphology. At 2–4 cells, 84% of oocytes surveyed were surrounded by a completely squamous follicular epithelium. At 5 follicle cells a transition in follicular morphology occurred: only 8% of oocytes had a completely squamous epithelium whereas all remaining oocytes were surrounded by a follicle envelope containing 1 or more cuboidal cells. Thereafter, the number of squamous follicle cells decreased and the number of cuboidal follicle cells increased. At 9 follicle cells, the point of initiation of oocyte growth, 96% of the follicular epithelium was cuboidal and 68% of oocytes surveyed were surrounded by a totally cuboidal epithelium. Mitotic figures were found only in the follicular envelope of oocytes surrounded by 10 or more follicle cells in the widest cross section.

In the total oocyte population, 81% were found to reside in the nongrowing pool and 52% were surrounded by 4 cells or fewer. Oocytes surrounded by 9 cells, the point at which oocyte growth is initiated, comprised only 2% of the total oocyte population.

INTRODUCTION

In all female mammals the number of oocytes available throughout life is determined during the perinatal period (Zuckerman, 1951). Oocytes become surrounded by a single layer of somatic (follicle) cells believed to originate, in part, from the rete-ovarii (Byskov and Lintern-Moore, 1973; Byskov, 1975). These oocytes constitute a reserve of germ cells which will give rise, after an obligatory period of growth, to all ovulations. However, the number of oocytes is limited and only some commence growth at any one time. The remainder form what is termed the resting pool of small follicles or the primordial follicle population. The initiation of oocyte growth is not restricted to sexual maturity but occurs from the time of appearance of the oocyte population (Peters, 1969; Mauleon, 1969).

The intra- and extraovarian events which cause a particular oocyte or group of oocytes to leave the resting pool and commence growth have not been defined. We examined the morphological and functional characteristics of individual oocytes of the resting pool and investigated the changes which occur in the oocyte and its follicular envelope during the transition from the resting to the growing state.

The earliest event we detected in the initiation of oocyte growth was an increase in the transcription of the nucleolar genes of the oocyte. This was followed by an increase in oocyte size. Oocytes at this stage of development were surrounded by a follicular epithelium composed predominantly of cuboidal cells.

MATERIALS AND METHODS

Thirty-day-old female mice of the Quackenbush strain were used. Mice were maintained in an air

conditioned animal house (21°C; 12 h light:12 h dark) and allowed commercial pelleted food and water *ad libitum*.

The characteristics used to quantitate the morphological and functional status of the oocyte were: the areas of the oocyte nucleolus, oocyte nucleus, oocyte and the follicle excluding the oocyte; the number and morphology of the follicular cells; the transcriptional activity of the oocyte nucleolus and oocyte nucleoplasm. All of these parameters have been shown to be temporally correlated with oocyte growth (Moore et al., 1974).

Ovaries from 12 mice were sectioned at 8 μm on a freezing microtome (-20°C). Cryostat sections were picked up on gelatinized slides, fixed for 5 min in ethanol and acetone (1:1 by volume), air dried and stored in the presence of a desiccant at -15°C. The transcriptional activity of the oocyte was determined using a cytochemical assay for endogenous RNA polymerase activity. The procedure has been described in detail elsewhere (Moore and Ringertz, 1973; Moore, 1978; Moore and Lintern-Moore, 1978). Polymerase I and II activities were distinguished by their nucleolar or nucleoplasmic localizations. RNA incorporating [³H]-uridine monophosphate (UMP) in the oocyte nucleus and nucleolus was quantitated by grain density autoradiography (Moore and Lintern-Moore, 1978).

The areas of the oocyte, the oocyte nucleus and the nucleolus and its follicle were obtained from the mean of the 2 diameters of each structure measured at right angles to each other in the widest cross section of the follicle containing the oocyte nucleolus. Follicles were classified on the basis of the number of follicle cells in the widest cross section and partitioned into those with between 2–25 follicle cells. Twenty-five follicles were surveyed at each stage of follicular development to quantitate the morphological and functional status of the oocyte. The morphological appearance of the follicle cells (either squamous or cuboidal) was also recorded.

These data provided information on the features which distinguished oocytes and follicles of the resting oocyte pool and those which had commenced growth. The number of nongrowing oocytes, their distribution through the follicular growth compartments and the proportion of the total oocyte population they represented was then determined. Ovaries from 5 30-day-old Quackenbush strain mice were fixed in Bouin's solution and serial paraffin sections were prepared and stained with hematoxylin and eosin using conventional histological procedures. Follicle counts were made according to the methods of Zuckerman (1951) and Mandl and Zuckerman (1951). Nongrowing oocytes were counted in every tenth section and partitioned into those with between 2–9 follicle cells in the widest cross section. Oocytes present in the larger follicular compartments were quantitated in every fifth section using the oocyte nucleolus as a marker. The classification of Pedersen and Peters (1968) was employed with the modification that Type 3a follicles were defined as those with 10–20 follicle cells: that is, follicles in which the oocyte had begun the process of growth towards ovulation. Statistical significance was evaluated by Student's t test (Miller, 1966).

RESULTS

Initiation of Oocyte Growth

Oocytes of the nongrowing pool are surrounded by varying numbers of follicle cells. We therefore started with the simple proposition that the developmental status of the oocyte was related to the number of granulosa cells in the follicular epithelium. The dimensions of the oocyte in follicles of increasing size are shown in Fig. 1. The oocyte, its nucleus and nucleolus, maintained a constant size in follicles composed of between 2–8 cells in the widest cross section. Oocyte area began to increase at 9 cells and was statistically significant at 10 cells. Oocyte nuclear area also showed an increase at 9 follicle cells and was statistically significant at 13 follicle cells. Oocyte nucleolar area increased from a constant 5 μm² at 2–8 follicle cells to 8 μm² at 11 follicle cells. Once growth commenced, oocyte area increased linearly up to 23–25 follicle cells. Oocyte nuclear area did not show a further change in area until 23–25 follicle cells when a further increase was recorded. The area of the oocyte nucleolus also remained relatively constant (8–10 μm²) between 11–25 follicle cells (Fig. 1a, b, c).

Sectioned ovaries that were assayed for RNA polymerase activity showed incorporation of radioactive RNA precursor into the nuclei of oocyte and follicle cells. Oocytes surrounded by 2–8 follicle cells showed relatively constant levels of nucleolar and nucleoplasmic polymerase activities (Fig. 2). However at 9 follicle cells there was a significant, 65%, increase in RNA polymerase activity specifically in the oocyte nucleolus (Fig. 2a). Thereafter, nucleolar polymerase activity increased only slightly between 9–22 follicle cells and then sharply at 23–25 follicle cells. Nucleoplasmic RNA polymerase activity remained constant up to 10 follicle cells and then increased, becoming statistically significant at 16 follicle cells. Further increases in activity were detected in oocytes surrounded by 17–25 follicle cells (Fig. 2b).

Follicular Growth

Thus far, the growth status of the oocyte has been related to the number of the accompanying follicle cells. It is clear that follicular area has increased during both the nongrowing and growing phases of the oocyte. This has resulted
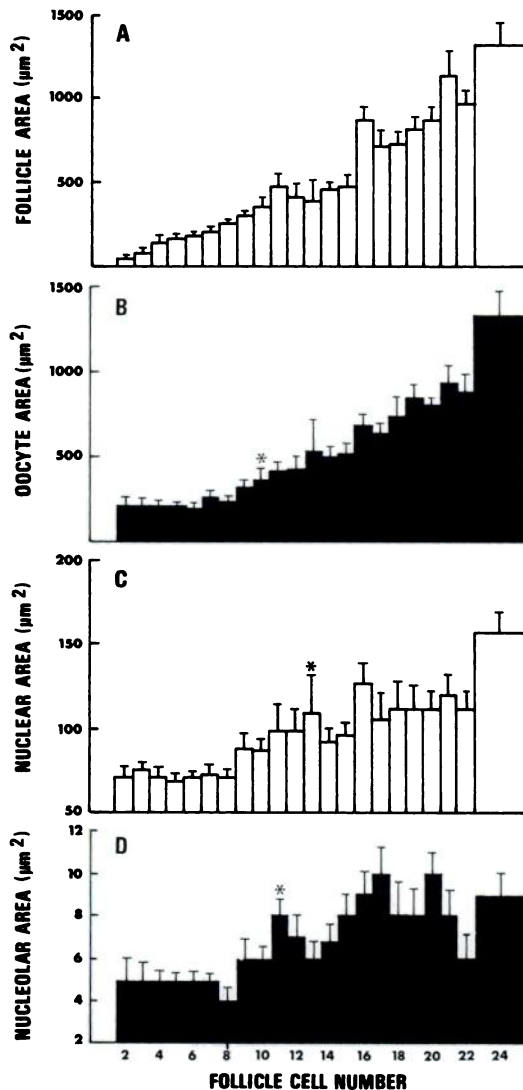
FIG. 1. Areas (μm^2) of (A) the follicular envelope, (B) oocyte, (C) oocyte nucleus and (D) oocyte nucleolus related to the number of follicle cells in the widest cross section of follicles in ovaries of 30-day-old Quackenbush strain mice. Mean ± SEM. *P<0.05.

FIG. 2. Incorporation of [³H]-UMP as measured by the number of silver grains over (A) the oocyte nucleolus and (B) nucleus in mouse oocytes during the initiation of oocyte growth. Mean ± SEM. *P<0.05.

not only from changes in follicle cell number but also from changes in morphology with the transition from a squamous to a cuboidal cell type (Fig. 1d). In the ovary of the 30-day-old mouse, oocytes were associated with at least 2 cells which were, without exception, of a squamous type (Table 1; Fig. 3). Cuboidal cells first became evident in slightly larger follicles containing between 3—4 cells. Sixteen percent of follicles with 4 cells contained at least 1 cuboidal cell. However, in those follicles only
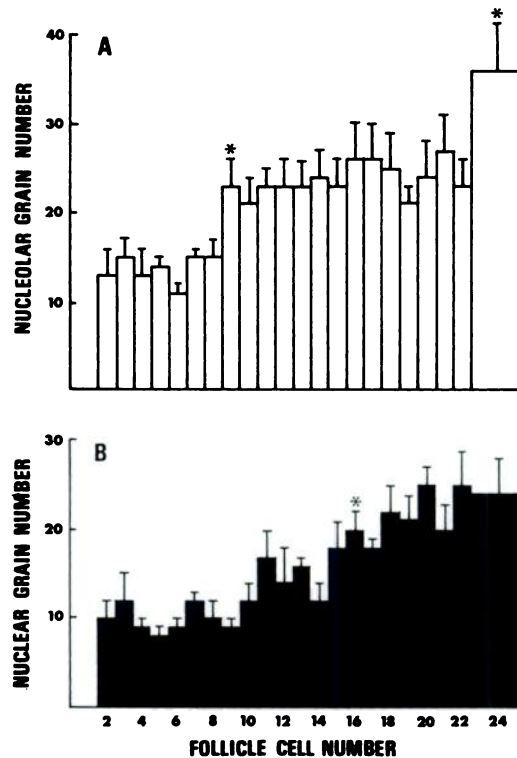
9% of the total number of follicle cells scored were cuboidal. A marked transition in follicular morphology occurred in follicles containing 5 cells. In this category 92% of follicles contained 1 or more cuboidal cells and cuboidal cells formed 33% of the total follicular epithelium. No follicles composed entirely of squamous cells were recorded at 6 cells or more. At 8 follicle cells, 24% of oocytes were surrounded by a totally cuboidal epithelium and this percentage increased to 100% at 12 follicle cells. At the point of initiation of oocyte growth, oocytes surrounded by 9 follicle cells, 68% of oocytes, had a totally cuboidal epithelium. The mean contribution of cuboidal follicle cells to the total follicular epithelium in oocytes surrounded by 9 follicle cells was 96% of all oocytes scored.

The increase in the number of follicle cells with increasing follicular area appears to result from an accumulation of cells rather than by division of existing cells. Of the 9,740 cells observed in follicles with 9 or less cells in the

TABLE 1. Mean number of flattened or cuboidal cells surrounding oocytes with 2–15 follicle cells in the widest cross section. Twenty-five oocytes were scored in each category.

No. of follicle cells	No. of flattened cells	No. of cuboidal cells	% cuboidal cells
2	2.00	0	0
3	2.80	0.20	7
4	3.62	0.38	10
5	3.36	1.64	33
6	2.40	3.60	60
7	1.56	5.44	78
8	1.76	6.24	78
9	0.32	8.68	96
10	0.32	9.68	97
11	0.16	10.84	99
12	0	12.00	100
13	0	13.00	100
14	0	14.00	100
15	0	15.00	100

widest cross section, no mitotic figures were recorded.

Follicle Population of the Ovary

The distribution of oocytes in the non-growing oocyte pool in relation to the number of follicle cells in the surrounding follicular envelope is presented in Table 2. Fifty-two percent of the total oocyte population were surrounded by 4 or fewer cells which, as noted earlier, were predominantly squamous in morphology. Of these only half were surrounded by 4 cells. In the majority of follicles
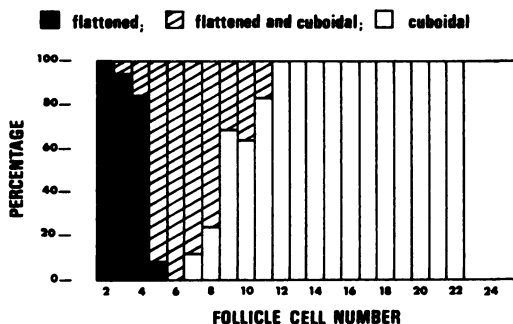

FIG. 3. Relative contribution of cuboidal and/or squamous cells to the follicular envelope during follicular growth. Open bars: % of follicles composed of only cuboidal cells. Hatched bars: % of follicles composed of both cuboidal and squamous cells. Closed bars: % of follicles composed of only squamous cells. Twenty-five follicles were scored in each category.

the transition from 4 to 5 cells was accompanied by the appearance of at least 1 cuboidal cell in the follicular envelope. From 5–8 cells a steady decline occurred in the number of oocytes in these follicular growth compartments. The follicle growth compartment immediately prior to the initiation of oocyte growth, that is, follicles with 8 predominantly cuboidal cells, contained only 3% of the total oocyte population. At the point of initiation of oocyte growth as judged by the increase of nucleolar RNA polymerase activity (i.e., 9 follicle cells of which 96% were cuboidal) only 2% of the total oocyte population were located. Nongrowing oocytes (2–8 cells) represented 81% of the total oocyte population. Of these, 64% were in "primordial follicles" containing 2–4 squamous cells.

DISCUSSION

Nongrowing oocytes of the mouse ovary are surrounded by 2–8 granulosa cells. Oocyte growth is initiated with a marked increase in RNA polymerase activity in the oocyte nucleolus. This is interpreted as an increased synthesis of ribosomal RNA. At progressively later stages of follicle growth there are increases in oocyte area, nucleolar area, nuclear area and nucleoplasmic RNA polymerase activity. Changes in RNA synthesis are characteristic of cells which have been stimulated to grow. In particular, a marked rise in the production of ribosomal RNA by target cells follows hormone administration (Tata, 1968). In the case of

TABLE 2. Composition of the follicle population of the mouse ovary. Mean ± SEM. Data expressed as numbers of follicles in each growth compartment and as percentages of the total follicle population.

Follicle type	No. of follicle cells	No. of follicles	% of total
2	2	264 ± 16	5.0 ± 0.3
2	3	824 ± 41	16.0 ± 0.6
2	4	1560 ± 81	31.0 ± 2.4
2	5	748 ± 85	15.2 ± 1.3
2	6	402 ± 70	7.7 ± 1.0
2	7	192 ± 39	3.6 ± 0.7
2	8	152 ± 28	3.0 ± 0.6
2	9	110 ± 10	2.0 ± 0.0
3a	10– 20	362 ± 40	7.0 ± 0.8
3b	21– 60	264 ± 32	5.2 ± 0.6
4	61–100	102 ± 12	1.8 ± 0.2
5a	101–200	66 ± 7	1.2 ± 0.2
5b	201–400	23 ± 3	0.6 ± 0.2
6	401–600	25 ± 5	0.6 ± 0.2
7	>600	7 ± 1	0.1 ± 0.0
Total	. . .	5101 ± 188	100

quiescent cells it has been suggested that a resumption of development may be dependent on the availability of ribosomal RNA (Van Blerkom and Brockway, 1975). Our observations are consistent with this view because the oocytes initiated to grow have been quiescent since their formation some 30 days earlier.

Although a clear delineation exists between nongrowing and growing oocytes, no such distinction could be drawn between nongrowing and growing follicles. Follicular area increased in both growing and nongrowing oocytes. How then can the often used term "initiation of follicle growth" be defined? First, it is significant that the mitotic index of follicle cells was zero for follicles containing 9 or fewer cells in the widest cross section. If follicle cells do not arise by division of existing cells, they must accumulate from a separate cell population within the ovarian stroma. Certainly in the infant period follicle cells arise from the rete ovarii (Byskov and Lintern-Moore, 1973). The present data suggest that follicle cells are progressively accumulated around the nongrowing oocyte. Second, the major proportion of nongrowing oocytes (64%) is surrounded by 4 or fewer follicle cells which are predominantly squamous in morphology. The remaining nongrowing oocytes were surrounded by 5–8 follicle cells and the proportion of oocytes in these compartments decreased with an increasing number of follicle cells. Oocytes devoid of

follicle cells do not persist in the ovary beyond the infant period (Peters, 1969). We suggest that follicles with 4 or fewer cells represent the "primordial" follicle population. The initiation of follicle growth appears to be heralded by a transition of the follicle from one consisting predominantly of squamous cells to one containing cuboidal cells. This transition begins at 5 follicle cells (Table 2). However, the nongrowing oocyte laid down in the ovary at birth remains in a structurally and functionally quiescent state until 8 cuboidal follicle cells accumulate around it. A second transition stage is then evident at 9 follicle cells when oocyte growth is initiated. At this point 96% of the follicle cells are cuboidal. In follicles containing 10 cells occasional mitotic figures were seen.

The physiological mechanism which directs particular oocytes to start to grow and develop is not known. Both in vivo (Lintern-Moore, 1978) and in vitro studies (Baker and Neal, 1973; Challoner, 1975) indicate that the initiation of the first wave of follicular growth during infancy requires gonadotropins. However, no such requirement has been demonstrated for subsequent waves of follicular growth after this developmental period (Peters et al., 1974; Lintern-Moore and Pantelouris, 1976). Whether the stimulus is intraovarian or extraovarian, the present data show an apparent requirement by the oocyte for a predominantly cuboidal follicular epithelium before oocyte growth is stimulated. This may indicate a role

for the follicle cells in the initiation of oocyte growth. The decreasing proportion of oocytes with 5—9 follicle cells suggests that passage through these stages becomes increasingly rapid with increasing follicle cell number. Only 2% of the total oocyte population were in the compartment at which oocyte growth is initiated.

The close relationship between the stimulation of gene transcription in the oocyte and follicle cell number suggests that the follicle cells themselves may play a significant role in the stimulation of the oocyte genome. This is further supported by the finding that oocyte growth is initiated only in follicles in which a transition from a squamous to a largely cuboidal epithelium has occurred. We propose that a stimulation of nucleolar RNA synthesis as the first event in the initiation of oocyte growth is in response to an inductive stimulus from the surrounding follicular cells.

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REFERENCES

Baker, T. G. and Neal, P. (1973). Initiation and control of meiosis and follicular growth in ovaries of the mouse. Ann. Biol. Amin. Bioch. Biophys. 13, 137—144.

Byskov, A. G. (1975). The role of the rete ovarii in meiosis and follicle formation in the cat, mink and ferret. J. Reprod. Fertil. 45, 201—209.

Byskov, A. G. and Lintern-Moore, S. (1973). Follicle formation in the immature mouse ovary: the role of the rete ovarii. J. Anat. 116, 207—227.

Challoner, S. (1975). Studies of oogenesis and follicular development in the golden hamster. 2. Initiation and control of meiosis in vitro. J. Anat. 119, 149—156.

Lintern-Moore, S. (1978). Initiation of follicular growth in the infant mouse ovary by exogenous gonadotropin. Biol. Reprod. 17, 635—639.

Lintern-Moore, S. and Pantelouris, E. M. (1976). Ovarian development in athymic nude mice. V. The effect of PMSG and oestrogen on the number and growth of follicles in the early juvenile ovary. Mech. Ageing Dev. 5, 259—265.

Mandl, A. and Zuckerman, S. (1951). The relationship of age to the number of oocytes. J. Endocrinol. 7, 190—193.

Mauleon, P. (1969). Oogenesis and folliculogenesis. In: Reproduction in Domestic Animals. 2nd ed. (H. H. Cole and P. T. Cupps, eds.). Academic Press, New York and London. p. 187.

Miller, R. G. (1966). Simultaneous Statistical Inference. McGraw-Hill, New York.

Moore, G.P.M. (1978). RNA synthesis in fixed cells by endogenous RNA polymerases. Exp. Cell Res. 111, 317—326.

Moore, G.P.M. and Lintern-Moore, S. (1974). A correlation between growth and RNA synthesis in the mouse oocyte. J. Reprod. Fert. 39, 163—166.

Moore, G.P.M. and Lintern-Moore, S. (1978). Transcription of the mouse oocyte genome. Biol. Reprod. 18:865—870.

Moore, G.P.M., Lintern-Moore, S., Peters, H. and Faber, M. (1974). RNA synthesis in the mouse oocyte. J. Cell. Biol. 60, 416—422.

Moore, G.P.M. and Ringertz, N. R. (1973). Localization of DNA dependent RNA polymerase activities in fixed human fibroblasts by autoradiography. Exp. Cell Res. 76, 223—228.

Pedersen, T. and Peters, H. (1968). Proposal for a classification of oocytes and follicles in the mouse ovary. J. Reprod. Fert. 17, 555—557.

Peters, H. (1969). The development of the mouse ovary from birth to maturity. Acta Endocrinol. 62, 98—116.

Peters, H., Byskov, A. G., Lintern-Moore, S., Faber, M. and Andersen, M. (1973). The effect of gonadotrophin on follicle growth initiation in the neonatal mouse ovary. J. Reprod. Fertil. 35, 139—141.

Tata, J. R. (1968). Hormonal regulation of growth and protein synthesis. Nature (London) 219, 331—337.

Van Blerkom, J. and Brockway, G. O. (1975). Qualitative patterns of protein synthesis in the preimplantation mouse embryo. Dev. Biol. 46, 446—451.

Zuckerman, S. (1951). The numbers of oocytes in the mature ovary. Rec. Progr. Horm. Res. 6, 63—109.