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Maturational Effects of Gonadotropins on the Cumulus-Oocyte Complex of the Rat

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ABSTRACT

The temporal relationship between maturational events in the cumulus oophorus and the oocyte of adult rats was examined. The time of preovulatory surge of LH was estimated from analysis of the critical period. Cyclic rats were killed on the afternoon and night of proestrus; hCG-treated rats were injected during the early morning hours of proestrus and were killed up to 13 h later. At necropsy, the ovaries were removed and the oocytes with their cumuli were isolated from the large preovulatory follicles. The intercellular mucification and tissue dissociation characteristic of cumuli around ovulated oocytes began to appear 4.24 ± 0.15 h after endogenous LH secretion or 4.31 ± 0.25 h after hCG. Transformation of the cumulus started at its periphery and spread centripetally. By 5.70 ± 0.21 and 7.17 ± 0.34 h, respectively, the cumuli had completed their differentiation. Maturation of the oocytes began before and ended after maturation of the cumuli. Timing of the maturational events preceding spontaneous cyclic ovulation and hCG-induced ovulation was similar. Small differences were attributed to differences in circulating levels of gonadotropin as a function of time. It was concluded that cumulus maturation is induced by the ovulatory surge of LH.

INTRODUCTION

In the graafian follicle of the rat, the oocyte is surrounded by a compact shell of polyhedral cells, the cumulus oophorus. Following ovulation, the cumulus oophorus is seen to have undergone several changes both in its tissue architecture and in its cellular morphology. The cells are arranged in radial strands near the oocyte and as single cells or small clusters in the periphery of the cumulus mass (Austin, 1961). The cells lose their smooth outline and become "flower-like" in appearance due to the extrusion and resorption of blunt pseudopods or "blebs" Shahar et al., 1971; Dekel et al., 1974).

The postovulatory cumulus mass is readily lysed to a suspension of single cells by hyaluronidase (Leonard and Kurzrok, 1945), suggesting a loss of cell to cell contact and a high concentration of glucosaminoglycans in the intercellular matrix. The loss of cell to cell contact is also evident ultrastructurally (Dekel et al., 1976). Thus, there are at least three changes that have taken place in the postovulatory cumulus oophorus: dissociation of the cells; accumulation of intercellular matrix and the appearance of blebbing. The development of these morphological changes are referred to as cumulus maturation.

The major aim of the present study was to analyze the chronological sequence of cumulus maturation during the hours between the surge of endogenous ovulation inducing hormone (OIH) and ovulation in adult proestrous rats. Ovulation was induced in another group of adult rats by injection of human chorionic gonadotropin (hCG) early on proestrus. By this procedure, ovulation was induced: 1) at a precisely known time and 2) with a hormonal preparation whose hormonal activity is primarily LH-like. (Rowlands and Parkes, 1966). The

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induction of ovulation by hCG avoids the complex secretion of LH and FSH associated with spontaneous cyclic ovulation (Ayalon et al., 1972). The temporal relationship between the morphological changes in the cumulus and the oocyte maturation process was established.

MATERIALS AND METHODS

Young adult female albino rats (100–200 days old) from the breeding colony (originally Wistar strain) of the Department of Zoology, Tel-Aviv University, were used. They were housed in air conditioned quarters with an artificial photoperiod of 14 h. Times were recorded on a 24 h clock, the midpoint of the light period being referred to as 1200 h. Only rats with at least 2 consecutive 4 day cycles, as determined by vaginal smears, were used in this study.

The critical period for preovulatory secretion of the gonadotropin peak was established by injection of 35 mg pentobarbital sodium/kg body weight during the early afternoon of proestrus (Everett and Sawyer, 1950). Sixty-eight rats were killed the following day and their oviducal ampullae examined for ovulated cumulus-oocyte complexes. Follicular oocytes from animals which had not ovulated were also examined.

Eighty-two cyclic rats were killed between 1500 h on the afternoon of proestrus and 0300 h on the morning of estrus. hCG-treated rats were injected i.p. with 15 IU of hCG (Chorigon, Ikapharm) dissolved in 0.1 ml of isotonic aqueous sodium chloride at one of two times, 0500 (47 rats) or 0930 (43 rats) on the morning of proestrus.

Rats were killed by cervical dislocation. The ovaries were quickly removed; the large preovulatory follicles were cut open with microscissors. Their contents were gently expelled into isotonic sodium chloride solution. The ova, surrounded by their cumuli, were collected into a fresh solution.

The cumulus-oocyte complexes were inspected with a stereomicroscope (× 40) for "diffuseness." The structure of the cumulus oophorus was classified as 1) compact; 2) dispersal of peripheral cells and 3) dispersal of all cells. The morphology of individual cumulus cells was also checked for presence of cell membrane activities (extrusion of blebs). The complexes were transferred to a solution of 1 mg ovine testicular hyaluronidase (420 units/mg; Sigma) per ml isotonic sodium chloride at 25°C. The enzymatic effect was rapidly evident and maximal lysis was obtained within 4 min, which was the usual time of incubation. The extent of maximal lysis of the cumulus oophorus was recorded. Sensitivity of cumuli to hyaluronidase treatment was classified as 1) none; 2) removal of cells from the peripheral layers only and 3) detachment of all the cumulus cells, except those of the corona radiata. The last two groups represent the onset and completion of the mucification process, respectively.

After hyaluronidase treatment, the oocytes were mounted for microscopic observation between a glass slide and a cover slip in a drop of medium, ringed with silicone grease containing glass beads, 80–90 μm diameter, which acted as spacers. The ova were examined by differential interference contrast microscopy. In the oocyte, the presence or absence of the germinal vesicle and of the first polar body was checked.

In the analysis of the chronological changes, the time that the animal was killed was recorded as the time of the observation. Completion of the observations of the cumulus-oocyte complexes of one rat was accomplished within 10 min. For purposes of analysis, times of observation were grouped in 1 h intervals, ± 30 min, around each integral hour. Curves of clock time vs frequency of response were analysed by probit analysis (Finney, 1971). From the linearized equation, times of median response and their errors of estimation were calculated.

RESULTS

Timing of Ovulation

The "critical period" for secretion of endogenous ovulatory gonadotropin occurred between 1350 h and 1620 h on proestrus (Fig. 1). The inhibitory effect of pentobarbital was 50% at 1505 h ± 10 min indicating that a full ovulatory quota of gonadotropin had been secreted in 50% of the rats by this time. Ovulation occurred at the median time of 0153 h ± 10 min on the day of estrus, by calculation 10.3 ± 0.23 h after the gonadotropic surge (Table 1).

Injection of 15 IU hCG, either at 0500 or at 0930 h on proestrus, induced ovulation with a median time of 12.45 ± 0.22 h after administration of the hormone (Table 2). The difference

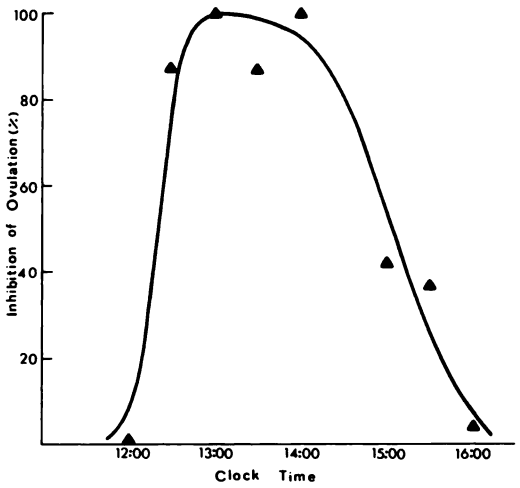

FIG. 1. The critical period for LH secretion as determined by pentobarbital block. Times of pentobarbital injection (8–9 rats per each time point, 35 mg/kg) are shown in the abscissa. The descending limb of the curve, from 1350 to 1620 h is inferred to represent the cumulative frequency of secretion of an ovulatory quota of LH, since it shows the time at which pentobarbital becomes incapable of inhibiting ovulation.

TABLE 1. Timing of ovulatory changes in cumulus-oocyte complexes of spontaneously ovulating rats.

Clock time (h)	NNS[a]		Cumulus mucification onset		Cumulus mucification total		Polar body		Ovulation	
	n[b]	%	n	%	n	%	n	%	n*[c]	%
1500	41	7								
1600	49	4								
1700	88	7								
1800	123	78	18	0						
1900	80	77.5	47	45			25	0		
2000	6	100	18	72	18	0	8	25		
2100			45	87	45	9	10	10		
2200			27	100	27	15	10	50		
2300					18	100	8	50		
2400							8	100	15	7
0100									22	32
0200									27	59
0300									12	92

[a]NNS = no nuclear structure visible in the oocyte.

[b]n = no. of oocytes.

[c]n* = no. of rats.

in injection time caused no significant difference in timing of ovulation (P=0.7). hCG-induced ovulation occurred significantly later than spontaneous ovulation with respect to the ovulation-inducing gonadotropic impulse ($P=10^{-6}$) (Fig. 2).

Timing of Oocyte Maturation

The breakdown of the germinal vesicle (GVB) occurred in two stages. In the first stage, the vesicular membrane disappeared and in the second, the nucleolus vanished (Figs. 3, 4). The loss of the vesicular membrane could also occur

TABLE 2. Timing of ovulatory changes in cumulus-oocyte complexes following hCG administration (15 IU hCG injected i.p. at 0500 or 0930 h of proestrus).

Hours after hCG	NNS		Cumulus mucification onset		Cumulus mucification total		Polar body		Ovulation	
	n[a]	%	n	%	n	%	n	%	n*[b]	%
1										
2	26	0	26	4						
3	98	56	101	21	49	0				
4	117	67	120	50	19	16	17	0		
5	145	90	146	67	26	0	21	14		
6	133	99	146	82	50	62	48	42		
7			78	100	30	93	28	4		
8					30	100	30	10		
9							40	72.5		
10							43	86		
11									3	0
12									14	29
13									17	76.5
14									8	87.5

[a]n = no. of oocytes.

[b]n* = no. of rats.

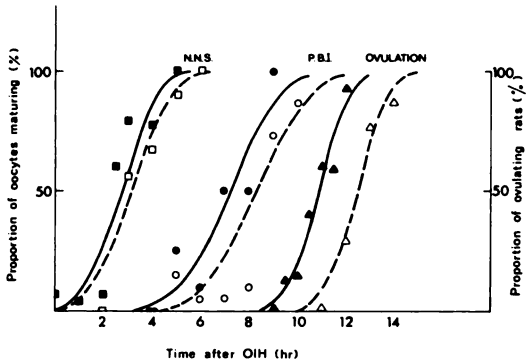

FIG. 2. Timing of maturational changes in follicular oocytes and ovulation. Curves were calculated using the probit transformation. Open symbols represent hCG-treated rats; solid symbols represent spontaneous ovulators.

artifactually during isolation; because of this uncertainty, the timing of the loss of the nucleolus was calculated as marking the onset of oocyte maturation. The disappearance of the nucleolus appeared to be a rapid process. Of the 39 rats killed during the period of its disappearance, 23 (59%) had all of their oocytes either with or without nucleoli. The median time of its disappearance was 1744 h ± 10 min, by calculation 2.67 ± 0.29 h following the gonadotropic surge. After hCG injection, the median time of disappearance of the nucleolus was 3.20 ± 0.24 h which is not significantly different (P=0.3). Oocyte maturation ends with the expulsion of the first polar body (PB). This event is rapid, allowing the ova to be classified as with or without PB. The median time of PB extrusion was 2213 h ± 19 min, or 7.14 ± 0.36 h after the calculated time of gonadotropin release, as compared to 8.33 ± 0.63 h after hCG injection (Fig. 4). The time interval between GVB and PB formation was 4.47 ± 0.46 h in the spontaneously ovulating rats, which is not significantly different from 5.13 ± 0.67 h as found for the hCG-treated rats (P=0.1).

Timing of Cumulus Maturation

Cumulus maturation (i.e., cell detachment), appearance of blebbing cells and intercellular mucification was first seen in the periphery of the cumulus (Figs. 5, 6) and later proceeded centripetally to the cells adjacent to the zona pellucida (Fig. 7). Blebbing cells were found only in dispersed cumuli. The onset and duration of cumulus dispersal and mucification are presented in Fig. 8. The onset of mucification (defined as the time when the hyaluronidase treatment removed only peripheral cells) seemed to occur at the same time following exposure to the endogenous gonadotropin surge (4.31 ± 0.23 h) or to hCG (4.24 ± 0.15 h). By analyzing the time when complete sensitivity to hyaluronidase had developed, it was possible to calculate the period of mucification. The duration of this period was significantly shorter (P=0.02) following exposure to hCG (1.46 ± 0.40 h) than following the gonadotropic surge (2.86 ± 0.41 h).

DISCUSSION

In the present study, the orderly sequence of changes in the cumulus oophorus of the ovulatory follicle is described. Oocyte and cumulus maturation were simultaneous, during the period between gonadotropic stimulus and ovulation. The sequence in maturation was as follows: 1) The dictyate status of the oocyte nucleus was terminated and meiosis resumed. 2) In the periphery of the cumulus oophorus, the cells separated and began to bleb as mucopolysaccharide filled the intercellular interstices. Within 1.5 to 3 h, the maturational transformation of the cumulus had progressed centripetally to the zona pellucida. 3) The maturational sequence culminated with PB extrusion. As early as 4 h before ovulation, the complex had been transformed into a mucous clot of suspended cumulus cells containing a matured and presumably fertilizable oocyte. It was now indistinguishable from the cumulus complex after ovulation. The timing of oocyte maturation and ovulation reported in this study was similar to that found by Tsafriri and Kraicer (1972); they studied rats of the colony of origin of those used in the present study.

Extensive studies have left little doubt that oocyte maturation is a response to ovulatory gonadotropin secretion (Linder et al., 1974). The close integration of maturational changes in the cumulus oophorus implies that these also are responses to gonadotropins, probably LH. This assumption is based on the following observations: 1) hCG can mimic the endogenous gonadotropic stimulation as shown in the present study; 2) LH added to isolated preovulatory follicles (Hillensjö et al., 1976) as well as to 3) isolated cumuli in culture (Dekel and Kraicer, 1977) will stimulate mucification. There are indications that cAMP is the mediator

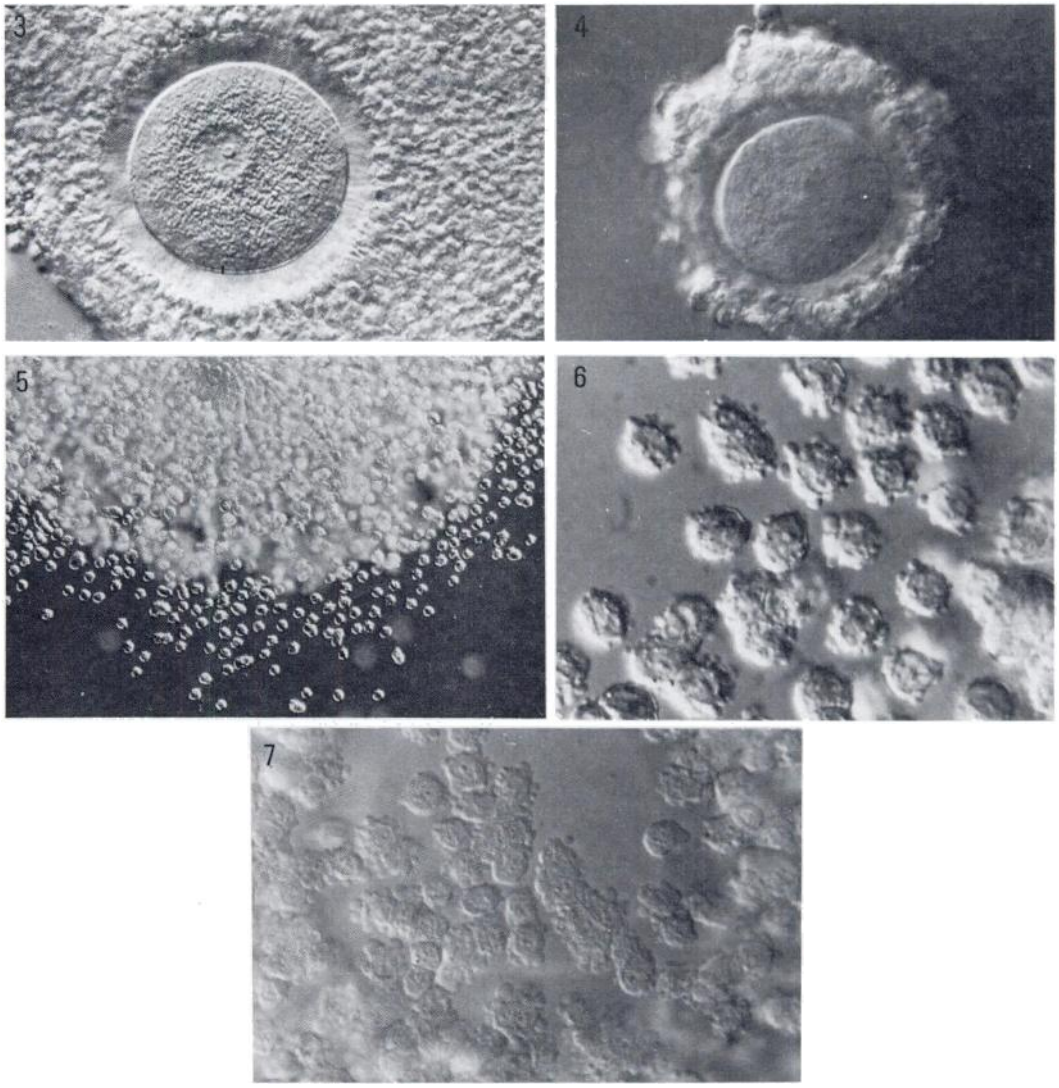

FIG. 3. A dictyate oocyte surrounded by cumulus cells freshly isolated from the follicle of a rat before noon on the day of proestrus. Differential interference contrast. × 408.

FIG. 4. A NNS oocyte (no visible nuclear structure), freshly isolated from the follicle of a rat during the afternoon of proestrus. Differential interference contrast. × 342.

FIG. 5. Dispersal of cumulus cells on the periphery of a follicular cumulus oophorus during the late evening of proestrus. Differential interference contrast. × 196.

FIG. 6. Cells on the periphery of the cumulus mass shown in Fig. 5, showing the typical rosette shape of blebbing cells. Differential interference contrast. × 782.

FIG. 7. Appearance of blebbing in the cells adjacent to the zona pellucida. The position of the oocyte can be seen, although it is not in focus. This cumulus mass was isolated from the follicle of a rat 5.5 h before the time of expected ovulation. Differential interference contrast. × 782.

of this LH effect on the cumulus (Dekel and Kraicer, 1977; Hillensjö, 1977).

With regard to oocyte maturation, the mechanism of OIH action is less clear. It has long been known that dictyate oocytes, released from the follicle, will mature spontaneously in culture (Pincus and Enzmann, 1935). Recent studies strongly suggest that LH acts by removing an inhibitor of oocyte maturation which is secreted by the granulosa cells (Foote and Thibault, 1969) or cumulus oophorus (Gwatkin and Andersen, 1976) and, at least in

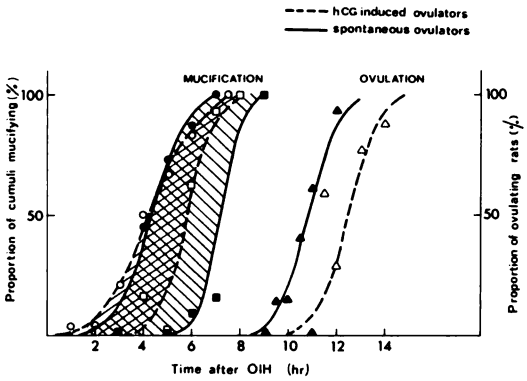

FIG. 8. Timing of cumulus mucification and ovulation. For further details, see legend to Fig. 2.

the sow, accumulates in follicular fluid (Tsafriri and Channing, 1975; Tsafriri et al., 1976). There is both morphological (Odor, 1960; Dekel et al., 1976) and functional (Epstein et al., 1976) evidence to suggest intercellular communication between the cumulus cells and the oocyte. One function of the compact, preovulatory cumulus oophorus may, therefore, be to maintain the oocyte in meiotic arrest. The ovulatory LH surge would then be envisioned to act on the cumulus cells to stop the transfer of oocyte maturation inhibitor (Gilula et al., 1978).

Two differences in the timing of the response to hCG were noted. Cumulus maturation was accelerated and ovulation was delayed, in comparison with spontaneous ovulators. The explanation of these differences may be related to the rate at which the gonadotropic stimulus is applied. The endogenous gonadotropic stimulus appears gradually whereas hCG acts immediately upon injection.

There is some evidence to suggest that the mediation of responses to gonadotropins within different follicular compartments is not identical (Channing, 1970; Cho et al., 1972; Pharris and Shaw, 1974; Dekel and Kraicer, 1977). This implies several sets of receptors and of reactive systems in the follicle. Injection of hCG, in a physiologically adequate dose, presumably triggers a response in all of these systems at the same time. Endogenous ovulatory gonadotropin secretion triggers these systems in sequence, as the rising levels reach and then exceed individual thresholds. In other words, all ovulatory events should begin simultaneously following hCG treatment, but not in spontaneous, cyclic ovulators.

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