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Effects of aqueous and lipoidal extracts of the wall of preovulatory follicles on the ovary of growing chicks

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Abstract. Effects of continuous intramuscular administration of the lipoidal and aqueous extracts of the wall of the largest preovulatory follicle (3·8–4·0 cm), to chicks from 11 to 29 weeks on alternate days have been studied on the ovarian growth, follicular growth and atresia, and the onset of ovulation. The lipoidal and aqueous extracts appear to have antagonistic effects on the follicular growth and atresia. Chicks injected with lipid extract show increased ovarian weight, enhanced follicular growth and egg laying started at 22nd week which is earlier to 25th week in control birds. The onset of egg laying was delayed in chicks injected with aqueous extract of largest follicle wall by 4 weeks as compared to control birds. Their ovaries showed decreased rate of follicular growth and increased follicular atresia as a result of which abundant amount of lipid-rich interstitial gland tissue of thecal origin accumulates in them.

Keywords. Ovary ; preovulatry follicle ; *Gallus domesticus*.

1. Introduction

A characteristic feature of the ovary of the regularly laying hen is the presence of graded series of yellow follicles in rapid phase of growth. These follicles of the hierarchy differ with regard to the nature of the steroid hormone secreted by them ; growing follicles during their rapid vitellogenic phase secrete estradiol (Kumagai and Homma 1974 ; Shahabi *et al* 1975 ; Shodono *et al* 1975 ; Graber and Nalbandov 1976) and progesterone shortly before ovulation (Furr *et al* 1973 ; Haynes *et al* 1973 ; Etches and Cunningham 1976 ; Huang *et al* 1979). Hammond *et al* (1980) have also demonstrated that the largest follicle, 20–24 hr before ovulation, contains maximum amount of prostaglandins. The possible significance of the higher levels of progesterone and prostaglandins in the preovulatory follicle has been suggested previously mainly in relation to ovulation (Etches and Cunningham 1976 ; Lance and Callard 1979) and oviposition (Hertelendy *et al* 1974) respectively but no report is available indicating the simultaneous effects of the preovulatory follicle secretions on the populations of growing follicles

and other ovarian components. The present study was, therefore, undertaken to investigate the effects of wall of preovulatory follicle on the ovary of growing chicks till the onset of lay.

2. Material and methods

Three-week old chicks were purchased from the local hatchery and were kept in the laboratory under continuous light and provided with feed and water *ad libitum*. The chicks were allowed to grow till they were 11 weeks old. Then they were divided into three groups of 9 chicks each, keeping the average body weight of a chick similar in all the groups. On alternate days intramuscular injections of 0·5 ml doses of lipid and aqueous extract of wall of largest follicle were given to each chick of the first and second group respectively. The chicks of the third group were injected with saline which served as control. Three chicks from each group were sacrificed at 17th, 23rd and 29th weeks to study the ovarian changes. Killing of chicks before 17th week was avoided as our preliminary studies had shown no visible effect on the ovary up to this time. After counting the number of follicles from the ovarian surface they were fixed in Bouin's fluid and calcium-formaldehyde and subjected to routine histological and histochemical techniques for localization of lipids (Pearse 1968).

For preparation of follicular extracts the follicles measuring 3·8–4·0 cm diameter were separated from the ovary of laying hens. The follicular walls which included both the thecal and granulosa layers were obtained after removal of yolk as described by Huang and Nalbandov (1979). The follicular walls from 10 follicles were then homogenized in saline and after homogenization the material was centrifuged for 15 min at 5000 rpm. The supernatant, thus obtained, was diluted to 50 ml and was kept at 5° C during testing procedures. Similarly the lipid extract of the follicular walls from largest follicles was obtained by extracting the material with chloroform and methanol (2 : 1 v/v). The lipid extract was dried and was suspended in saline, with slight heating and stirring.

3. Results

Chicks administered with lipoidal extract and killed at 17th, 23rd and 29th week continue to show higher body, ovarian and oviducal weights as compared to control and aqueous extract injected birds (table 1).

At 17th week, no conspicuous differences were observed in the ovarian surface morphology of the lipid and aqueous extract injected hens. But a study of follicular populations from the serial sections of the ovary revealed that the ovaries of chicks, injected with lipid extract, contained some follicles having dimensions more than 500 μm. The other growing follicles like those of control and water extract injected measure less than 400 μm. The number of follicles at different stages of growth (as given in table 1) is relatively more in lipid extract than those of control and aqueous extract injected birds. Follicular atresia affecting mainly the follicles ranging in size from 200–400 μm was common in the ovaries of all the three groups of hens; no significant differences could be observed among them. The interstitial gland cells existed in irregularly distributed patches (figure 1).

Table 1. Mean body, ovary and oviducal weights and number of follicles in 17, 23 and 29 weeks old chicks.

Age of chicks	17 weeks old			23 weeks old			29 weeks old		
	Pure saline	Lipid extract	Water extract	Pure saline	Lipid extract	Water extract	Pure saline	Lipid extract	Water extract
Weight gain (g)	500	620	500	785	1070	785	860	1080	940
Ovary weight (mg)	38	51	17	883	1578	455	20600	20300	18600
Oviducal weight (g)	0·016	0·026	0·017	11·85	42·88	23·60	42·56	47·6	35·6
Number of follicles (counted from the surface of the ovary) :									
(*a*) Normal follicles	...	...	...	12 (all white)	9 (5 yellow and 4 white)	3 (all large white)	13 (6 yellow) 7 white)	15 (8 yellow 7 large white)	9 (5 yellow 4 large white)
(*b*) Atretic follicles (yellow)	...	...	...	Nil	3	Nil	Nil	3	7
Mean number of follicles (in serial sections):									
(i) 75–160 μm	10	14	6	20	48	2	17	18	15
(ii) 200–270 μm	10	17	4	7	30	9	8	6	4
(iii) 300–400 μm	3	12	3	18	14	4	11	7	8
(iv) 500–1300 μm	...	3	...	6	7	2	3	3	3

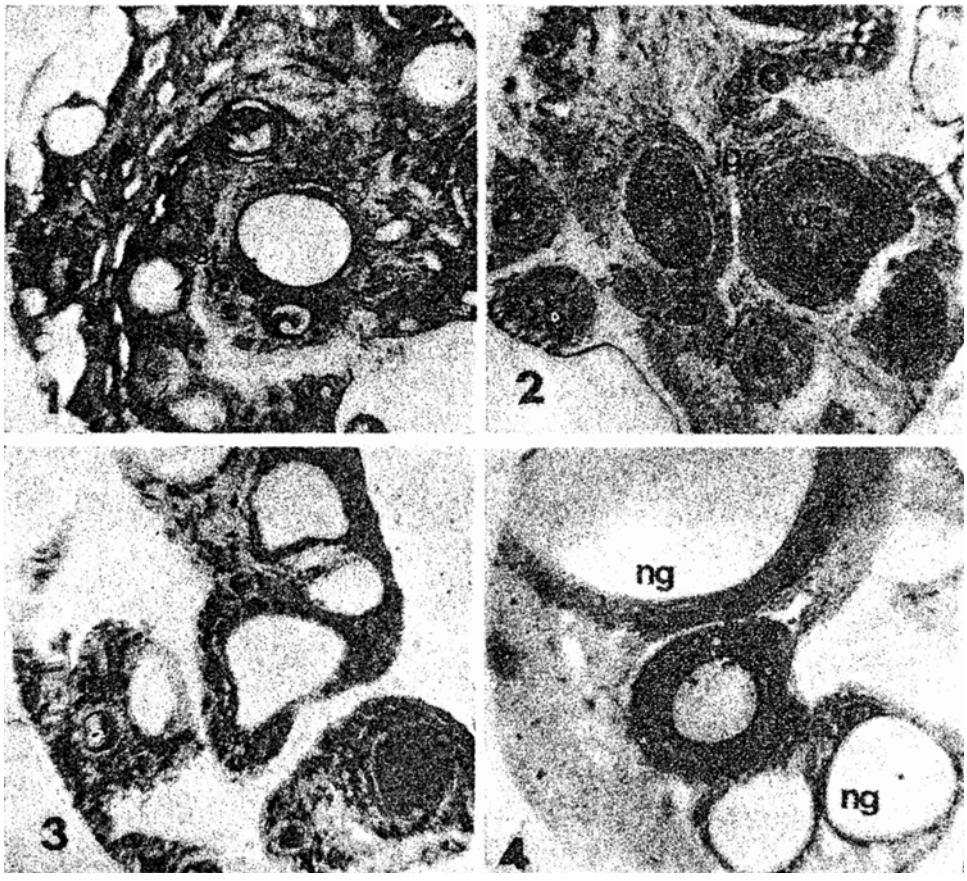
Ovaries of hens at 23rd week possessed follicles of larger size, some of which could be counted easily from the ovarian surface. Marked differences were seen in the ovarian surface morphology of hens which were continuously administered with lipid extract because they started laying at 22nd week. Ovaries of these birds were having normal hierarchical follicles whereas those of control and aqueous extract injected chicks contained only large white follicles, their number was also relatively less in the latter (table 1). Atresia of follicles is increased considerably at 23rd week as compared to the ovaries of 17-week old chicks but it was more in ovaries treated with the water extract and affected the previtellogenic follicles (figures 2, 3). Vitellogenic atretic follicles were also observed in the lipid extract administered hens. The ovarian stroma of the 23-week old chick was found to contain abundant interstitial gland tissue, but their amount was relatively more in aqueous extract injected birds (figures 3, 4). In the latter case, the interstitial gland tissue contained more sudanophilic lipids as compared to those of control and lipoidal extract administered chicks.

At 29th week, the ovaries of control and lipid extract injected birds showed the normal features of those of laying hens, since the control birds also started laying at the 25th week. But the aqueous extract injected birds started laying at the end of the 29th week. The ovaries of these birds contained more number of medium-sized vitellogenic atretic follicles as judged from the shrinkage and prominence of the stigmal site. The ovarian stroma at this stage appeared relatively loose in all the three treatment groups. The interstitial gland cells were abundant and continued to show more lipids in aqueous extract injected hens.

4. Discussion

The present observations have shown that the lipid and aqueous extracts of walls of larger yellow follicles have antagonistic effects on the ovarian functions in the growing chicks. The lipoidal extract initiates the follicular growth from the pool and enhances the rate of growth of follicles at all stages leading to their early maturity. However, the aqueous extract appears to have the reverse effect. The enhanced rate of growth of follicles, thus, indicates the presence of some lipid-like growth-promoting substance elaborated by the larger follicles. *In vivo* and *in vitro* studies have shown that the largest follicle secretes progesterone (Furr *et al* 1973 ; Shodono *et al* 1975 ; Shahabi *et al* 1975 ; Huang *et al* 1979) and prostaglandins (Hammond *et al* 1980) shortly before ovulation. Thus the presence of these two substances in the lipid homogenate of the follicular walls is expected but the possibility of the existence of any other lipoidal substance cannot be excluded. Prostaglandins do not appear to influence ovarian steroidogenesis in hen (Hertelendy and Hammond 1980), but their role in initiating and promoting follicular growth is not known. The involvement of progesterone in promoting follicular growth cannot be overlooked since it is known to play a key role in endocrine control of the hypothalamo-pituitary-ovarian axis.

The effect of growth-promoting substance expected to be present in the lipoidal extract becomes more marked after 17th week. Thus it appears that pituitary-ovarian axis after 17th week probably becomes more responsive to the growth-promoting factor contained in lipid extract of the walls of the largest follicle.



Figures 1–4. 1. Section of 17-week old chick administered with aqueous extract showing small growing follicle(s) and patches of interstitial gland cells in stroma. Sudan black B× 50. 2. Section of ovary of 23-week old chick administered with aqueous extract showing degenerating follicles (*dg*) and abundant lipid-rich interstitial gland cells (*igc*). Sudan black B × 50. 3. Section of the ovary of 23-week old chick injected with aqueous extract showing abundant interstitial gland cells (*igc*) in the stroma. Sudan black B× 50. 4. Section of ovary of 23-week old chick injected with lipoidal extract showing normal growing follicles (*ng*) and stroma with lesser interstitial gland cells (*igc*). Sudan black B× 50.

In contrast to the lipid extract, the aqueous extract of the follicular wall inhibits the follicular growth and simultaneously enhances follicular atresia. A water-soluble factor was extracted from the largest preovulatory and postovulatory follicles which could induce premature oviposition (Tanaka and Nakada 1975) but no mention is made until now regarding its effect on the ovary itself. Preliminary studies on the estimation of soluble proteins have indicated that the amount of proteins abruptly increases in the follicles of 3·8–4·0 cm diameter (unpublished observations). Possibly, there may be same protein in aqueous extract which exerts inhibitory influence on the follicular growth and stimulation of follicular atresia but this suggestion needs to be extended and confirmed.

Our observations on the ovary of growing chicks after treatment with lipoidal and aqueous extracts have clearly shown that the larger yellow follicles in the laying hen elaborate two different kinds of substances, one stimulates and the second possibly inhibits the follicular growth. But the exact mechanisms of action of these two factors in maintaining the normal and regular pattern of laying remains to be determined.

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