GONADOTROPHIC ACTIVITIES OF ANTERIOR PITUITARY AND OF BLOOD PLASMA AND OVARIAN RESPONSE TO EXOGENOUS GONADOTROPHIN IN MOULTING HENS

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Summary. The total gonadotrophic activities of the anterior pituitary glands and blood plasma of hens, Gallus domesticus, during the moulting period were estimated using the assay method based on the weight increase of chick testis. The activities in both the pituitary and the blood of hens in the four groups classified according to the progress of moult were significantly higher than those of laying hens. Nevertheless, no detectable level of serum vitellin and a completely regressed ovary were found in moulting hens.

Anterior pituitary glands of moulting and laying hens were assayed for their FSH and LH activities by the use of the HCG augmentation method or the ovarian ascorbic acid depletion test. The FSH activity of the moulting hen was about twofold higher than that of the laying hen, whereas LH activity was about equal during the moulting and the laying periods.

The serum vitellin of moulting hens increased steeply with the injection of oestrogen over 3 days. When chicken anterior pituitary homogenate was injected once daily for 6 days to hens in the moulting period, a satisfactory follicular growth as in the laying hen was induced in all of the treated hens. Injection of PMSG into moulting hens for 6 days resulted in an increase of serum vitellin in all the hens and the formation of yellow follicles in 40% of the treated hens.

These results suggested that the failure of follicular growth in the moulting hen, despite high levels of total gonadotrophic activity in both pituitary and blood, might be due to an unbalanced secretion of endogenous gonadotrophins.

INTRODUCTION

It has been reported that the pituitary gonadotrophin level changes in relation to various reproductive conditions in the hen, Gallus domesticus. Nakajo & Imai (1961) demonstrated that the total gonadotrophin content of the anterior

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pituitary from the non-laying hen was higher than that from the laying hen, the lowest content being found in the broody hen. A similar finding was reported by Riley & Fraps (1942) and Phillips (1942). The blood plasma from the non-laying hen has also been found to contain more gonadotrophic activity than that from the laying hen (Bailey & Phillips, 1952; Imai & Nakajo, 1958). The moultling hen was shown to have an increased content of pituitary gonadotrophin by Saeki, Himeno, Tanabe & Katsuagin (1956), but they determined the hormone content of the pituitary only at a mid-stage in the moultling period.

Recently, Nelson, Norton & Nalbandov (1965) and Imai & Nalbandov (1971) demonstrated changes in the LH and FSH levels of anterior pituitary and blood plasma during an ovulatory cycle of the hen, and Imai & Nalbandov suggested that separate secretion mechanisms existed for FSH and LH activities.

In the present study, changes in total gonadotrophic, FSH and LH activities in the pituitary or the blood of moultling hens and the ovarian response to exogenous gonadotrophin were determined for the purpose of investigating the mechanism underlying the continuance of ovarian inactivity during the moultling period.

MATERIALS AND METHODS

Single comb White Leghorn hens, 10 to 16 months old, were kept in individual cages and were allowed unrestricted access to commercial rations and water. The present work consisted of a series of four experiments.

Experiment 1 was designed to elucidate the changes occurring in the total gonadotrophic activity in the pituitary and blood plasma during the moultling period. The birds were classified into four groups according to progress of moult, and changes in serum vitellin used as an index of ovarian function. The hens of Group M-I were killed shortly before the onset of moult, assessed by failure to produce an egg for 3 to 5 days and a marked decrease in the serum vitellin level on the day of autopsy. The birds in Group M-II had heavy shedding of throat and breast feathers, no egg laying and a low vitellin level. The M-III hens exhibited shedding of four to five primary feathers, no egg laying, and a low vitellin level. The M-IV birds were killed on the day that the serum vitellin level was raised shortly after the termination of the moult while there was still no egg laying. To compare the gonadotrophin level of the moultling with the laying group, the latter hens were killed in the afternoon (14.00 to 15.00 hours) of the day when the first egg of a clutch was laid. At autopsy, all the hens had the soft-shelled second egg of the clutch in the shell gland. Whole blood and anterior pituitaries were collected from hens of each group. To concentrate the gonadotrophic hormones, the plasma pool of each group was extracted with cold acetone, using the procedure described previously (Imai & Nalbandov, 1971). The extracts obtained were stored frozen until assay. Anterior pituitaries were removed immediately after death, weighed and dried in cold absolute acetone. The dried pituitaries from each group were pooled, pulverized and stored in a desiccator until use.

Experiment 2 was conducted to determine the FSH and LH activities of the anterior pituitary during the moultling period and to compare them with those
in the full laying stage. Anterior pituitaries were collected from the moulting hens at the M-II stage and from the laying hens with the second egg of a clutch in the shell gland. They were weighed, quick-frozen and stored until assay.

Experiment 3 was carried out to test the ability of the liver of the moulting hen to produce vitellin substance following the administration of oestrogen. Moulting hens at the M-II stage were injected with diethylstilboestrol (Euves-tin, Takeda Pharm. Co., Osaka) intramuscularly once daily for 3 days—10 mg for the first 2 days and 5 mg on the 3rd day. Control birds at the same period of moulting received soy-bean oil with the same schedule. The birds were killed on the 5th day after the final injection, and anterior pituitaries and blood plasma were collected and ovaries and oviducts were observed.

Experiment 4 was designed to test the responsiveness of the ovary of the moulting hen to exogenous gonadotrophins. Moulting hens at the M-II stage were injected intramuscularly with 100 mg chicken anterior pituitary (CAP) homogenate or 100 i.u. PMSG (Peamex, Tomoda Pharm. Co., Tokyo) once a day for 6 days, while control birds received physiological saline. The birds were killed on the day following the final injection, and ovaries and oviducts were observed and weighed. The CAP used for the injection was collected from broilers of both sexes at a local killing plant, quick-frozen and stored. Immediately before the injection, the CAP pool was homogenized in cold physiological saline in an ice box and centrifuged, the supernatant being used as the injected material.

Serum vitellin was detected by the interfacial precipitin reaction between antivitellin serum and serially diluted test serum, and the titre was expressed as the maximal dilution rate of the test serum in which the precipitin reaction was positive. During the period of the hormone injection in Exps 3 and 4, serum vitellin titres were measured every other day in all experimental hens.

**Bioassays**

In Exp. 1, total gonadotrophic activities of the anterior pituitary and the plasma extract were determined by the use of the chick testis assay described previously (Nakajo & Imai, 1956). In preliminary assays, the linearity of the dose–response line was held within the dose ranges of 1 to 8 mg acetone-dried pituitary powder and 8 to 32 mg plasma extract of the laying hen. Based on these results, 1 mg acetone-dried pituitary powder or 10 mg plasma extract obtained from hens in each of the four groups in the moulting period and from laying hens was homogenized or dissolved in 1 ml distilled water and injected subcutaneously into the dorsal neck region of male chicks 24, 36, 48, 60 and 72 hr after hatching. Control chicks received distilled water only. Chicks were killed 24 hr after the final injection, and the testes were removed, cleaned and weighed. The increased rate of gain in the testicular weight of the treated chicks compared to the controls was calculated, and the estimation of total gonadotrophic activity was assessed in terms of Chick Units (C.U.), constituted by 35% of the rate of increase. The gonadotrophin content/gland, or /25 ml plasma was calculated on the basis of unit potency and the amount of the pituitary powder or plasma extract obtained.

A modification of the hCG augmentation assay by Steelman & Pohley (1953)
was employed for the estimation of pituitary FSH activity. Female Holtzman rats, 21 days old and weighing 40 to 50 g, were used as the assay animals. They received a total of 20 i.u. hCG (Gonatropin, Teikoku-Zoki Pharmac. Co., Tokyo), and NIH-FSH-s3 as FSH standard or hen anterior pituitaries as test substance. A desired amount of each substance to be injected was dissolved or homogenized in 1 ml cold saline and injected subcutaneously on five different occasions over a period of 3 days—once on the afternoon of the 1st day and twice on each of the following 2 days. The rats were killed on the afternoon of the 4th day (24 hr after the final injection) and the ovaries were removed and weighed. In preliminary assays, good parallelism and a satisfactory λ value were found between the standard and the test substance when 5, 10 and 20 mg pooled cock pituitary homogenate and 37-5, 75 and 150 μg NIH-FSH-s3 were used. Parallelism and λ value were also satisfactory when 80, 160 and 320 mg pooled pituitary homogenate from laying hens were used as the test substance against three doses of the standard. In five assays, the average λ value was 0.14 (range 0.10 to 0.18). On the basis of the preliminary assays, two doses (40 and 80 μg) of the standard and one dose (80 mg) of the pituitary homogenate were used in the assay for the pituitary FSH activity of moulting and laying hens. The activity was expressed as μg NIH-FSH-s3/100 mg pituitary (concentration) or /gland (content).

The ovarian ascorbic acid depletion test was used to assay pituitary LH activity. Female Holtzman 25- or 26-day-old rats received PMSG (Pemex) and hCG (Gonatropin) pretreatment according to the method of Parlow (1961). The rats prepared for the assay received an injection into the jugular vein of standard NIH-LH-s10 or of pituitary homogenate in 0.5 to 0.8 ml saline under ether anaesthesia. Both ovaries were removed 4 hr ± 10 min later, and were weighed and measured for the ascorbic acid concentration per 100 mg ovarian weight by the method of Mindlin & Butler (1938). When three doses (1, 2 and 4 pituitary equivalents) of pooled pituitary homogenate from laying hens were assayed against 1, 2 and 4 μg or 0.5, 1 and 2 μg NIH-LH-s10, there was no significant deviation from parallelism in any of the assays and the mean λ value was 0.15 (range 0.09 to 0.17). From these preliminary results, a 2×1 assay (0.5 and 2 μg standard and 11 mg pituitary homogenate) was employed for the comparison of pituitary LH activities between moulting and laying hens. The LH concentration/100 mg pituitary and content/gland were expressed in terms of μg NIH-LH-s10.

Statistical analyses were performed, using the methods of Finney (1964) for the parallel line assay, Snedecor (1956) for the t test, and Duncan (1955) and Kramer (1956) for the multiple range test.

RESULTS

Experiment 1

Serum vitellin levels of thirty hens were measured every other day throughout the period from the cessation of lay to its resumption after the conclusion of moult. A marked decrease in the vitellin titre (less than 10) was observed within 5 days of the last lay in almost all the hens, but feather shedding could not yet be
**Table 1**

**TOTAL GONADOTROPHIC ACTIVITIES OF ANTERIOR PITUITARY GLANDS AND BLOOD PLASMA OF MOULTING AND LAYING HENS, ESTIMATED BY THE CHICK TESTIS ASSAY**

<table>
<thead>
<tr>
<th>Group of hen</th>
<th>Result of pituitary assay</th>
<th>GT† activity (C.U.)/mg pituitary powder</th>
<th>GT† content (C.U.)/pituitary</th>
<th>Result of plasma assay</th>
<th>GT† activity (C.U.)/10 mg plasma extract</th>
<th>GT† content (C.U.)/25 ml plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-I</td>
<td>5</td>
<td>8.18 ± 1.07e,d †‡</td>
<td>2.0</td>
<td>6</td>
<td>5.88 ± 0.31b,c †‡</td>
<td>0.6</td>
</tr>
<tr>
<td>M-II</td>
<td>6</td>
<td>7.58 ± 0.33e</td>
<td>1.6</td>
<td>6</td>
<td>6.33 ± 0.42b,c</td>
<td>0.9</td>
</tr>
<tr>
<td>M-III</td>
<td>6</td>
<td>9.15 ± 0.72e,d</td>
<td>2.5</td>
<td>6</td>
<td>6.80 ± 0.27c</td>
<td>1.1</td>
</tr>
<tr>
<td>M-IV</td>
<td>6</td>
<td>9.90 ± 0.74d</td>
<td>3.9</td>
<td>5</td>
<td>5.84 ± 0.20b</td>
<td>0.6</td>
</tr>
<tr>
<td>Laying</td>
<td>5</td>
<td>6.22 ± 0.47b</td>
<td>0.8</td>
<td>6</td>
<td>5.14 ± 0.19b</td>
<td>0.2</td>
</tr>
<tr>
<td>(Control)</td>
<td>8</td>
<td>4.86 ± 0.18a</td>
<td>1.1</td>
<td>8</td>
<td>4.86 ± 0.18a</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S.E.
† Gonadotrophin.
‡ The differences among groups with the same letter were not significant statistically (P<0.05).
detected (Group M-I). The low level of serum vitellin was maintained during the moulting period until the M-IV stage, when the titre rose again to the same level as in the normal layer (80 or more). The first egg was laid by all hens 8 to 12 days after the serum vitellin titre increased. Although there was considerable individual variation in the non-laying period in relation to moult, the average period lasted 41 days.

The ovaries of hens in Groups M-I, -II and -III regressed completely, the average weights being 6·1, 4·2 and 2·8 g, respectively, and no growing yellow follicles were present. By contrast, the ovaries from Group M-IV hens were slightly heavier (8·3 g) and one or two small yellow follicles existed.

Total gonadotrophin concentration/mg acetone-dried pituitary powder or /10 mg plasma extract and content/pituitary or /25 ml plasma are shown in Table 1. On the basis of concentration or content, the anterior pituitaries from the four groups within the moulting period contained more gonadotrophin than the laying hen. In the moulting groups, the hormone activity increased gradually as moult progressed, reaching a maximum level at the M-IV stage. In the blood plasma from moulting hens, total gonadotrophic activity was also higher than in the laying hen, the highest level being found at the M-III stage, to be followed by a decrease of activity in the M-IV group.

**Experiment 2**

The anterior pituitaries of Group M-II hens and of the laying hen with the second egg in the shell gland were assayed for their FSH and LH activities. The results are shown in Table 2.

The concentration and content of pituitary FSH in the moulting hen was significantly higher than that of the laying hen, the concentration being approximately a twofold increase over that in the full laying stage. On the other hand, the concentration and content of pituitary LH activity in the moulting hen was approximately equal to that of the laying hen. Thus, the pituitary FSH:LH ratio differed considerably at different stages, and FSH activity was more predominant in the pituitary of the moulting than of the laying hen.

**Table 2**

<table>
<thead>
<tr>
<th>Group of hen</th>
<th>FSH activity</th>
<th>LH activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (µg NIH-FSH-S3 /100 mg pituitary)</td>
<td>Content (µg NIH-FSH-S3 /pituitary)</td>
</tr>
<tr>
<td>Moulting</td>
<td>90·5 (79·1 to 101·9)</td>
<td>6·6 (5·8 to 7·4)</td>
</tr>
<tr>
<td>Laying</td>
<td>46·6 (36·0 to 56·8)</td>
<td>3·9 (3·0 to 4·7)</td>
</tr>
</tbody>
</table>

* 95% confidence limits on the Mean value are shown in parentheses.
Experiment 3

In all five hens which received oestrogen for 3 days, the serum vitellin level increased rapidly within a few days of the first injection and remained at a high titre (320 to 640) until the time of autopsy. The mean ovarian weight of this group, however, was low (3·0±0·3 g) and no yellow follicles were observed in the ovaries. The serum vitellin titre in the five controls injected with oil was maintained unchanged at a level of less than 10, and the mean ovarian weight was 2·8±0·1 g. The total gonadotrophin content of the anterior pituitary of the oestrogen-treated group decreased markedly compared with that of the control, being 1·0 and 2·4 C.U./gland, respectively. Moreover, the potency of plasma gonadotrophin in the oestrogen-injected hen could not be detected, even when 30 mg plasma extract were used as the highest dose in the chick testis assay.

Experiment 4

The findings concerning the serum vitellin, ovary and oviduct of the \textit{pmsg}-treated moulting hens are shown in Table 3. In all ten hens injected with \textit{pmsg}, the serum vitellin level increased within 4 days of the first injection and remained at a high level, resembling that of the laying hen. The vitellin titre at the final determination ranged from 160 to 640. The \textit{pmsg}-treated hens were classified into two groups, i.e. with or without a normal yellow follicle, according to the ovarian response. A growing yellow follicle was present in the ovaries of four of the ten hens treated with \textit{pmsg} (Group A). The number and the size of yellow follicles observed varied with individuals. Two hens had many yellow follicles of varying size at the time of autopsy. By contrast, the ovaries of six of the ten hens (Group B) did not have any normal yellow follicle, but recently formed atretic follicles were found in almost all the ovaries and the weights of such ovaries were slightly heavier than those of the controls. The difference in oviducal weight was statistically significant \((P<0.05)\) between each of the three groups, A, B and the controls.

The findings concerning the ability of CAP homogenate to cause vitellin production and to induce follicular growth in the moulting hens are shown in Table 4. In all the seven hens injected with CAP, serum vitellin was elevated rapidly and maintained at the level found in the laying hen, and follicular growth occurred. Normal yellow follicles of varying size were found in the ovaries, but the number of the follicles indicated some overstimulation of follicular growth by the CAP treatment when compared with the normal graded series of follicles found in the laying hen. Only small atretic follicles were present in the ovaries of two hens. An atretic follicle of large or medium size resembling those found in the ovaries of the \textit{pmsg}-treated hens was not detected in any of the CAP-treated hens. The oviducal weights of the CAP-treated hens were similar to those of the normal laying hen and differed significantly \((P<0.05)\) from those of the B group treated with \textit{pmsg}. No significant difference was observed between the CAP group and the A group treated with \textit{pmsg}.

DISCUSSION

Both the concentration and the content of total gonadotrophin in the anterior
**Table 3**

EFFECT OF PMSG ON THE OVARY AND OVIDUCT OF THE MOULTING HEN

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of hens</th>
<th>Serum vitellin titre at autopsy</th>
<th>Ovarian weight (g) Mean ± S.E.</th>
<th>Total no. of yellow follicles produced Mean ± S.E.</th>
<th>Average no. of yellow follicles (diameter in cm)</th>
<th>Average no. of atretic follicles</th>
<th>Oviducal weight (g) Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMSG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A*</td>
<td>4</td>
<td>160 to 640</td>
<td>26·8 ± 10·2 Mean ± S.E.</td>
<td>4·0 ± 1·2 Mean ± S.E.</td>
<td>1·5</td>
<td>0·8</td>
<td>0·3</td>
</tr>
<tr>
<td>B*</td>
<td>6</td>
<td>160 to 640</td>
<td>4·2 ± 0·5 Mean ± S.E.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>Less than 10</td>
<td>2·8 ± 0·2 Mean ± S.E.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* The hens treated with 100 i.u. PMSG by daily injection for 6 days were classified in two groups (A and B), with or without normal yellow follicles, according to their ovarian responses.
† Diameter was < 1 cm.
‡ Diameter was 1 to 2 cm.
§ Diameter was > 2 cm.

**Table 4**

EFFECT OF CAP ON THE OVARY AND OVIDUCT OF THE MOULTING HEN

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of hens</th>
<th>Serum vitellin titre at autopsy</th>
<th>Ovarian weight (g) Mean ± S.E.</th>
<th>Total no. of yellow follicles produced Mean ± S.E.</th>
<th>Average no. of yellow follicles (diameter in cm)</th>
<th>Average no. of small atretic follicles†</th>
<th>Oviducal weight (g) Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP*</td>
<td>7</td>
<td>160 to 640</td>
<td>43·9 ± 6·0 Mean ± S.E.</td>
<td>10·9 ± 1·7 Mean ± S.E.</td>
<td>2·6</td>
<td>3·0</td>
<td>3·0</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>Less than 10</td>
<td>2·6 ± 0·1 Mean ± S.E.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* CAP was given as a daily injection of 100 mg for 6 days.
† Diameter of small atretic follicle was < 1 cm.
pituitary and blood plasma of hens during the moulting period were significantly higher than those of hens in the full laying stage (Exp. 1). Moreover, total gonadotrophic activity increased gradually as moulting progressed, the highest level being found during the M-IV stage in the pituitary and during the M-III stage in the plasma. Serum vitellin titres throughout the moulting period were uniformly low and undetectable by use of routine quantitative assessment procedure except in hens during the M-IV stage when feather shedding had finished and serum vitellin had just increased. With the exception of the M-IV group, moulting hens had completely regressed ovaries, containing small white follicles of 0.2 to 0.3 cm diameter and sometimes one or two advanced atretic follicles of a small size. The association of failure of follicular growth with high total gonadotrophic activity in the pituitary and the blood during the moulting period might result from any of the following situations: (1) a disturbance in the process of production and/or deposit of the yolk precursor materials, (2) refractoriness of the ovary to gonadotrophins, or (3) an imbalance of the FSH/LH ratio in endogenous gonadotrophins.

Serum vitellin is known to be the precursor of ovo-vitellin which constitutes the main part of yolk substances. Serum vitellin levels, therefore, reflect the production of yolk precursor materials in the liver and of their utilization by the ovary. Although undetectable levels of serum vitellin in the moulting hen indicated a lack of yolk precursor production in the liver, the increase in the level of serum vitellin which occurred following treatment with oestrogen (Exp. 3) showed that the process of production and utilization of the yolk precursor was still functional during this period if the liver was stimulated by oestrogen. However, no follicular growth occurred in the ovary of the oestrogen-injected moulting hen despite a marked increase in yolk precursor material. This may have been due to suppression of endogenous gonadotrophin resulting from injection of oestrogen. The total gonadotrophin content of the pituitary of the oestrogen-injected hen decreased to 1/2.4 that of control pituitary at the same period of moulting. Moreover, the gonadotrophic activity of the plasma from the oestrogen-injected hen could not be detected at the dose level which was threefold higher than that used in the assays for plasma gonadotrophin of the moulting hen.

The results following injection of CAP and pMSG (Exp. 4) show that the ovary of the moulting hen can respond to gonadotrophins with respect to follicular growth and the secretion of oestrogen by these follicles. It is assumed that the failure of follicular growth which occurred in about half of the pMSG-treated hens is attributable to the use of a preparation of mammalian origin. It has been reported that chicken pituitary preparations were more effective than mammalian preparations in inducing and maintaining follicular growth in hens with quiescent ovaries (Das & Nalbandov, 1955; Morris & Nalbandov, 1961). Imai (unpublished) suggested that the superiority of the chicken pituitary material was due to the difference between avian and mammalian gonadotrophins but not to that between crude and partially purified preparations. In the present experiments, many recently formed atretic follicles of various sizes from large to small were found in the ovaries of the pMSG-treated hens, but very few atretic follicles were observed in any of the hens treated with CAP. These
findings suggest that PMSG may be effective in initiating follicular growth in the inactive ovary but not in stimulating further follicular development.

Nakajo & Imai (1956), comparing assays based on increases of ovarian and uterine weights in immature mouse or rat with the chick testis assay, reported that the latter was most sensitive for chicken gonadotrophin and adequate for use as quantitative assay when anterior pituitaries from cockerels or hens were used as the test substance. It is commonly accepted, however, that the increase in chick testis weight is not specific for either FSH or LH (Breneman, Zeller & Beekman, 1959). The activity estimated by the use of this assay must, therefore, be considered as representing the total gonadotropic activity in the test substance. The data from Exp. 2 showed that the FSH activity of the anterior pituitary of the moulting hen, as estimated by the HCG augmentation reaction in the immature rat, was approximately double that of the laying hen. On the other hand, pituitary LH activity, which was measured by depletion potency of OAA concentration in the pseudopregnant rat, was almost equal in the moulting and laying conditions. From these results, it was suggested that the increase of FSH activity was responsible for the higher potency of total gonadotrophin during the moulting period, and that the ratio of FSH : LH in the endogenous gonadotrophins of the moulting hen differed from that of the laying hen. If the FSH : LH ratio in the laying period is particularly favourable for the occurrence of follicular growth, the ratio in the moulting period may be out of balance and unfavourable for follicular growth in the ovary.

Further experiments are required to investigate what gonadotrophin constitution is effective in inducing follicular growth in the chicken ovary.

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Pituitary gonadotrophins and ovary in moulting hen


