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RESPONSE OF THE REPRODUCTIVE SYSTEM MORPHOLOGY TO CHANGES IN THE PITUITARY-OVARIAN AXIS CONTROL IN DIFFERENTLY SELECTED HEN STRAINS DURING EGG-LAYING CYCLE

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Abstract

The biotechnology of producing egg-laying hen hybrids requires a high genetic selection pressure upon the origin inbred strains. The selection may affect the response of the reproductive system to the pituitary-ovarian axis control. In this work, we investigated the changes of ovarian and oviductal histomorphology response to the control exerted by the pituitary-ovarian axis in Lohmann and Leghorn hybrids, versus Sussex hens that has not been subjected to any selection pressure. Determinations were performed on 32-week-old hens, a key-age during the laying cycle. The results revealed differences between the hybrid hens and Sussex hens regarding the blood levels of gonadotropin follicle-stimulating hormone, luteinizing hormones (P<0.01), estrogen (P<0.05), triiodothyronine and tetraiodothyronine, and higher values of egg-laying frequency in hybrids versus Sussex. The number of different types of ovarian follicles (white follicles, small yellow and large yellow follicles), the structure of the follicle perivitelline membrane, and structure of the yolk was also modified in hybrids versus Sussex. Differences in epithelium histology of the infundibulum, magnum, isthmus and shell gland as well as histochemical particularities of these structures were also identified.

Key words: *egg-laying hybrid hen, pituitary control, ovary histology, oviduct histology.*

INTRODUCTION

The histological structure, development, and the physiology of the ovary and oviduct in birds are hormonal controlled, either directly by the pituitary gonadotropic hormones FSH and LH in the case of the ovary, or by estrogens and progesterone in the case of the oviduct. Accordingly, functional changes in the pituitary-ovarian axis generate changes in ovarian and oviduct structure and function (Dojană, 2009). The long-term biotechnology programs of selection and improvement of inbred strains, which are used to obtain performative egg-laying hen hybrids have modified the pattern of control exercised by the pituitary-ovarian axis on the structure and physiology of the ovary and oviduct in the hen. Hanlon et al. (2021) reported that the ovary and oviduct of Lohmann laying hens were significantly heavier than those of unselected Shaver and Smoky Joe hen breeds ($P < 0.05$), suggesting major changes on the control exerted by the hypothalamic-pituitary-ovarian axis on ovarian weight and structure during the biotechnological process of selection.

Researches on the changes induced by gene-tic selection on the control of hypothalamic-pituitary-ovarian axis on the ovary and oviduct in chicken were conducted by Mimura (1937), Joseph et al. (2014), Hanlon et al. (2021) and Bunaciu et al. (2009). Bunaciu and Dojană (1982) incriminate the degree of homozygoty among factors which changed the control of pituitary-ovarian axis upon the ovary and oviduct physiology in inbred strains of chickens and turkeys. On the other hand, according to Sechman (2013), the ovary and oviduct function seems to be under the influence of iodinated thyroid hormones in hen: *in vitro* studies have demonstrated that T3 decreases estrogen secretion from ovary non-hierarchical white follicles and from the theca layer of yellow preovulatory follicles, while, on the other hand, T3 increases progesterone production from the granular layer of these follicles. As the direct effects of progesterone and especially estrogens on the morphology and physiology of the oviduct are well known, one can conclude about the influence of the thyroid (secretion of iodinated hormones, respectively) on this structure.

The present work investigates the extent to which the long-term selection biotechnology processes of the inbred strains used for the production of Lohmann and Leghorn laying hybrids have altered the control pattern of the pituitary-ovarian axis on the structural and functional response of the ovary and oviduct in these hybrids during laying pick. The comparison is done with the Sussex, a chicken breed that has not been subjected to high selection pressure for egg or meat production.

MATERIALS AND METHODS

Animals and experimental design

To achieve the objectives of this experiment, two groups of 32-week-old hybrid laying hens of 5 cap. each were used: a group of Lohmann hens and a group of Leghorn hens. Comparisons were made with 5 cap. group of 32-week-old Sussex hens. The birds were raised in an industrial system, watered and fed *ad libitum.* The commercial diet recommended by the hybrid supplier was used for feeding. The birds also benefited from a 15-hour light program, from 5:30 to 20:30. Eggs were daily collected for four weeks and the egg-laying frequency was calculated as number of eggs/ cap./week for each group.

Hormone and histology analysis

Blood samples were taken by axillary vein puncture for hormonal determinations. The samples were stored and processed according to the instructions of the kit suppliers for the determination of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, thyroxine (T4) and triiodothyronine (T3). Dedicated kits were used for hormonal determinations, and the analyses were performed based on sandwich enzyme-linked immuno-sorbent assay (ELISA) technology. For this purpose, standard curves were drawn using serial dilutions of reagents and the optical densities were read at 420 nm. After blood was sampled, the birds were slaughtered for the collection of ovaries and oviducts. The ovaries and oviducts were immediately weighed. The weight of the oviduct was determined after removing the ovum or developing egg (as appropriate) possibly located in its lumen. Then tissue samples were taken from these organs and processed histologically and stained with HEA, toluidine blue and periodic acid Schiff (PAS) according to Radu & Diaconescu (1984) and Cornilă & Manolescu (2014).

The systematization of ovarian follicles was done according to Wang et al. (2017) cited by Holkoe et al. (2022), as it follows:

- pre-hierarchical follicles which include:

a) small white follicles, ø < 5 mm

b) small yellow follicles, 5< ø < 10 mm;

- hierarchical follicles, also known as preovulatory follicles, ø > 10 mm.

Measurements were performed by optical micrometry on a sufficient number of follicles (n ≥ 10) from each category, determining the thickness of the follicular granulosa and the average of the large and small diameters of the follicular yolk.

Statistic processing of the data

The obtained data were statistically processed by determining the mean, standard error of the mean and standard deviation using a GLM procedure of SAS statistical package (1999) program. The one-way ANOVA was used to compare the means between hybrid groups and Sussex as control. The statistic difference between hybrid groups and Sussex was considered significant when the probability of the null hypothesis $P < 0.05$.

RESULTS AND DISCUSSIONS

Analysis of the hormonal values of the pituitary-ovarian axis revealed significant differences between Sussex and Lohmann and Leghorn hybrids for LH and estrogen. FHS did not show significant differences between Sussex and hybrids, but higher values were noted in hybrids compared to Sussex (Table 1). Among the iodinated thyroid hormones, only T3 showed significantly higher values in the two hybrids compared to Sussex. Changes in the secretory activity of the pituitary-ovarian and pituitary-thyroid axes were reported by Hanlon et al. (2021) of Lohmann laying hybrids versus unselected Shaver and Smoky Joe hens: higher serum estrogen values, as well as differences in the gene expression of the mRNA of ovarian

FSHr and LHr receptors were reported. Bentley et al. (2003) reported differences in gonado-tropic hormone secretion in song sparrows (*Melospiza melodia*) in different reproductive conditions, versus house sparrows (*Passer domesticus*) relative to chicken-gonadotropin-releasing hormone. The differences in egg laying frequency values can be attributed to a higher ovulation frequency imprinted by higher FSH and LH values to hybrid hens (Liu et al., 2001). Liu et al. (2001) cite papers and authors who showed that administration of exogenous LH in hypophys-ectomized hens (Nakada et al., 1994) or in intact hens (Lang et al., 1984) induced premature and more frequent ovulations, and subsequently eggs, than in controls. Prastiya et al (2022) found that FSH levels were significantly associated with egg laying frequency in ISA Brown hens, which is also verified in our research. On the other hand, according to Stojević et al. (2000) variations in egg production intensity largely follow variations in blood T3 concentration.

Table 1. FSH, LH, estrogen, T3 and T4 values and egg-laying frequency in 32-wk-old Sussex chicken and in the egg-laying Lohmann and Leghorn hybrids

	FSH (ng/mL)	LH (ng/mL)	Estrogen (pg/mL)	T4 (ng/mL)	T3 (ng/mL)	Egg-laying frequency (eggs/cap./week)
Sussex hens	38±4.2	8.22±1.06	760±34	15.5±11.4	1.35±0.11	5.15±1.77
Lohmann hybrid	40±3.7	11.0**±1.04	890**±111	15.6±6.6	5.55*±2.21	6.43±2.09
Leghorn hybrid	42±6.0	12.0**±3.01	980*±144	16±4.9	5.9±1.02	6.09±0.54
P	0.211	0.005	0.003	0.195	0.002	0.052

Note: each value represents the average of 5-6 determinations
Values are expressed as mean ± standard error of mean
* $P < 0.05$; ** $P < 0.01$ versus Sussex as a control

According to the data presented in Table 2, significantly higher values (P = 0.001) of WF are found in the two lines of egg-laying hybrids compared to Sussex. The two hybrids also present higher values of SYF and LYF compared to Sussex. The number of atretic WF follicles was higher than that of WF (normal) but no data were found in the literature regarding the ratio between the two categories of follicles (Joseph et al., 2002). Joseph et al. (2002) attribute the differences in the number of ovarian follicles to the different sensitivity to photoperiod changes, via the hypothalamic-pituitary-ovary axis. The authors also determined the evolution by age of the number of ovarian follicles in two lines of broiler parent hens. The authors did not analyze the differences between the selected broiler lines. However, at 32 weeks of age, the ovaries showed an average of 14 WF, values that are higher than in the case of Lohmann and Leghorn hybrids, but higher than in Sussex. Other values reported by the authors: 9.8 SYF and 6.2 LYF, to which are added 62 atretic WF and 4 atretic SYF. The number of large yellow follicles was maximum at sexual maturity, numbered at 8.78, decreasing with age. The number of small white follicles (2-5 mm ø) and that of small yellow follicles remained relatively constant with the age of the broiler lines analyzed. Also, according to Du et al. (2020) hens with higher FSH levels tend to have a higher number of (developing) ovarian follicles, which is in agreement with our results.

Table 2. The number of different types of ovary follicles in 32-wk-old hybrid and Sussex hens

Group	n	WF	SYF	LYF	Chistic follicles	Atretic follicles	
						WF	SYF
Sussex	5	9.32±2.30	6.63±0.76	4.44±0.65	2.0±0.19	13.9±4.09	2.12±0.55
Lohmann hybrid	5	16.09±2.90**	8.10±1.98	5.85±1.09*	3.3±0.65	22.09±3.67	4.50±0.45
Leghorn hybrid	5	16.96±4.99**	8.21±2.11	6.32±2.11*	3.0±0.98	22.22±3.08	4.41±0.77

n = number of animals
LYF = large yellow follicles (greater than 10 mm in diameter)
SYF =small yellow follicles (5 to 10 mm in diameter)
WF = white follicles (2 to 5 mm in diameter)
*$P < 0.05$; ** $P < 0.01$ versus Sussex

The histological and histochemical study of the ovary revealed relevant similarities and differences between Lohmann and Sussex hens (Figure 1). Thus, HE-A staining revealed that the follicular granulosa layer is less organized in the small follicles, an apparently thicker granulosa was found in Sussex but with more ordered nuclei in Lohmann. PAS and toluidine staining revealed a more intense PAS+ and toluidine+ material in the cytoplasm of the granulosa cells of the hierarchical follicles in Lohmann. In the perivitelline theca, aromatase cells and interstitial cells with weak PAS+ or toluidine+ of the cytoplasm were identified in both studied taxonomic entities.

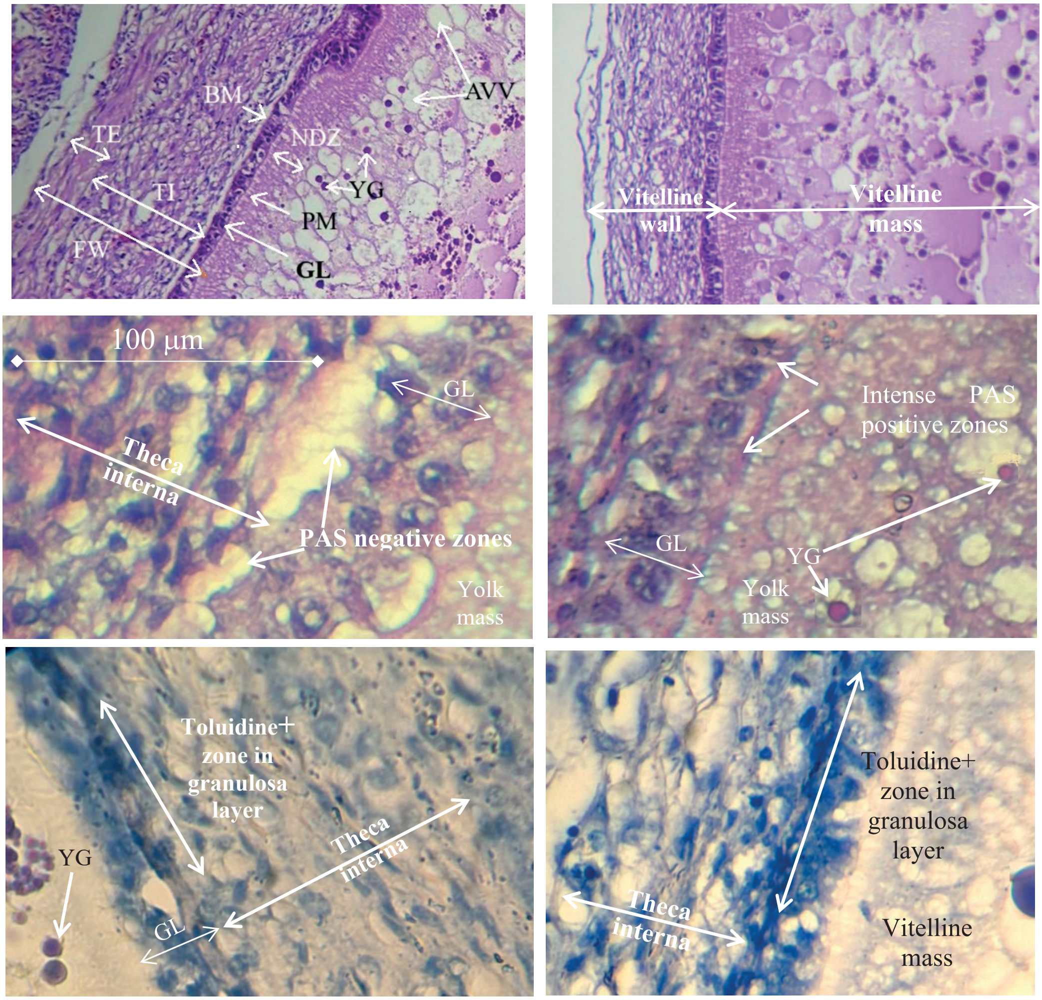

Figure 1. Comparative structural details of ovarian follicles in Sussex (left) and Lohmann hens (right) aged 32 weeks:
Upper left: structural detail of a small yellow follicle. FW - follicular wall; TE - theca externa; TI - theca interna; GL - granuloasa layer; BM - basement membrane; PM - perivitelline membrane; NDZ - undifferentiated zone; YG - yolk granules; AVV- albuminoid vitelline vesicles; ob. 20, HE-A stain;
Upper right: Lohmann follicle of the same size HE-A stained; ob. 20;
Middle left: detail of granulosa, generally negative to PAS-stain; ob. 90;
Middle right: a general PAS-positive reaction is noted, more intense in Lohmann versus Sussex. Two PAS+ yolk granules are present (in red); ob. 90;
Lower left: low concentration of RNA in the cytoplasm of the follicular theca granulosa cells, revealed by toluidine blue stain; ob. 40;
Lower right: high concentration of RNA granules revealed by an intense toluidine stain reaction in granulosa cell cytoplasm; ob. 40

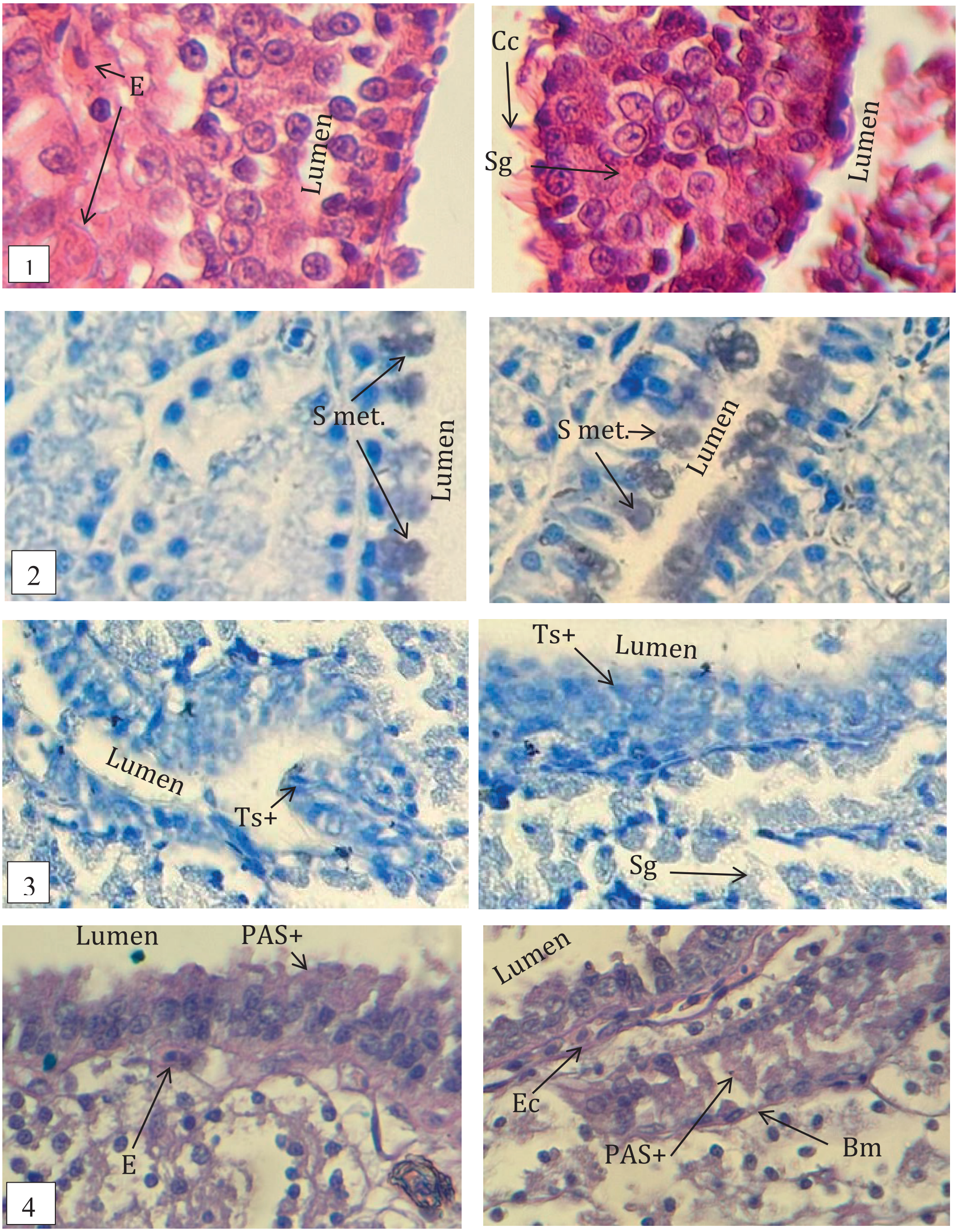

Figure 2. Histology and histochemistry of the oviduct in 32-week-old Sussex (left) and Lohmann (right) hens:
1. HEA stain of infundibulum. E - erythrocytes; Cc - ciliated cells; Sg - groups of pink secretory granules are present in both, hybrid and Sussex hens. Ob. 90;
2. Magnum stained by toluidine blue. Rt+: positive reaction to toluidine; S met - metachromatic (black) stained secretions by toluidine blue. Ob. 40;
3. Isthmus stained by toluidine. Sg: secretion granules; Rt+: positive reaction to toluidine. Ob. 40;
4. Shell gland stained by PAS. PAS+: PAS+ polysaccharide mass; Bm - basal membrane; E - erythrocyte;
Ec- erythrocytes ligned in a capillary vessel, under the granular layer. Ob. 40

The comparative histological and histochemical study of the oviduct allowed highlighting the following aspects (Figure 2):

- the infundibulum presents a pseudostratified epithelium, without breed or hybrid differences. The Schiff and toluidine reactions revealed a high density of secretory cells, without breed/ hybrid differences;
- the magnum presents a simple or pseudostratified epithelium, with pink-colored granulations HE-A stained. The PAS reaction revealed denser PAS+ material in Lohmann versus in Sussex epithelium. On the surface of the epithelial cells and in their upper third, a globular secretory material stained metachromatic in black by toluidine and a more abundant PAS+ polysaccharide secretion is noted in Lohmann versus in Sussex;
- in the isthmus, HE-A staining highlights pink spheroidal agglomerations of secretions in/among the glandular cells. The PAS reaction is generally weakly positive, especially in Sussex. The isthmus epithelium in Sussex and Lohmann shows a very positive cytoplasmic reaction to toluidine but the secretions stained metachromatically in black are absent;
- the epithelium and the wall of the shell gland showed a low reactivity, both in PAS and in toluidine blue staining, without structural or tinctorial affinities differences between Sussex and Lohmann.

Data regarding the comparative histochemistry and histology of the ovary and oviduct in birds are relatively few. Dikikh and Pervenetskaya (2022) conducted macro- and microscopic studies on the uterus of Haysex hens (a breed selected for the intensity and persistence of egg laying). The authors describe primary, secondary and tertiary folds of the mucosa, covered with a ciliated epithelium with several rows of cells, of different types, with laterally compressed nuclei located at different levels, and densely arranged tubular glands. Altaey et al. (2023) performed an extensive histological and histochemical study of the oviduct in chickens, turkeys, ducks, and geese. The results showed that the shape, length, width, and weight of the oviduct varied between different bird species. Furthermore, there were signifi-cant differences in anatomical and histological measurements of the oviduct, such as the length of the mucosal folds, the thickness of the oviduct wall tunicae and the distribution of glands. Other authors have conducted studies on the histological features of the oviduct during its ontogenetic development (Blendea et al., 2012) or in different breeds or hybrids (Mimura, 1937; Apperson et al., 2017), but not comparative studies.

CONCLUSIONS

The genetic selection process of hen inbred strains changed the hypothalamo-pituitary and thyroid secretion pattern. LH, estrogen and T3 serum levels in 32-week-old Lochmann and Leghorn hybrids were found significantly increased versus unselected Sussex hens ($P < 0.05$) while FSH and T4 were not significantly modified by genetic selection. Consequently, white and yellow ovary folicle number was found greater in hybrid selected hens versus unselected Sussex. This fact correlates with greater values of laying frequency in hybrid (as egg/cap./week): 6.43 in Lohmann, 6.09 in Leghorn, versus 5.15 in Sussex hens. Changes in histochemical reactions to toluidine and Schiff reagents were found both in the ovarian granulosa tunica and in the the epithelia of the oviductal infundibulum, magnum, isthmus and uterus structures. The thickness, the appearance and density of epithelial cilia, the thickness of the epithelial cell layers of the anatomical elements of the oviduct show differences in relation to the level of the LH, estrogen and iodinated thyroid hormones that control the growth and evolution of these structures.

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