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# HISTOCHEMISTRY OF LIPIDS IN PHEASANT (*Phasianus colchicus* L.) OVARY

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**ABSTRACT.** Pheasant (*Phasianus colchicus L.*)is an important game species. Knowledge on morphological and functional specifics of pheasant reproduction has a significant meaning for its rational breeding. Therefore it is important to clarify the changes in ovaries of these birds according to age and seasonal changes and thus to improve its reproductive abilities in wild and domestic conditions.

Ovary follicle cells in vertebrate animals both with pituitary hormones control the further development of the follicles, namely ovulation and atresion. Since it is accepted that steroid hormones are lipid derivatives the goal of our study is to indicate histochemically the presence and distribution of lipids and the key enzyme δ5-3β HSDH in ovarian follicles. The presence of lipids and δ5-3β HSDH mainly in ovoplasm of granulosa cells and thecal glands allow us to conclude that granulosa cells and thecal glands are a place for steroid production in pheasant ovary.

**KEY WORDS.** pheasant, follicles, lipids, ovary, sex hormones, thecal glands

## INTRODUCTION

Pheasant is a wild or partially domestified species with clearly manifested reproductive cycle. Knowledge on morphological and functional specifics of its reproduction has an important significance for its rational breeding. It is important to clarify the changes in gonads of these birds according to the age and seasonal changes for making more precise the reproductive terms and its potential breeding abilities in wild and domestic conditions.

Recently it is widely accepted that follicle cells in the ovary of vertebrate animals form a complex endocrine system, which produces sex hormones. An opinion has established that ovary steroid substances can modulate the maturation

and functional activity of follicles. The regulatory mechanisms inside the ovary which both with pituitary hormones control the further development of follicles (ovulation and atresion) are in fact locally produced by follicle (granulosa) cells steroid factors most of them with unknown structure and function.

Tests for determining of different lipid substances and their distribution in different ovarian structures in various stages of the ovary development have been carried out for this purpose.

The basic goal of our studies is by using a series of histochemical reactions for establishing neutral lipids, acid lipids and phospholipids and sex hormones to determine their distribution in different parts of the ovary.

## MATERIAL AND METHODS

The investigations have been carried out on populations from the regions of Pleven and Botevgrad. After the opening of the abdominal cavity of the killed birds gonads have been quickly taken and fixed by freezing. The gonads of young and adult birds in spite of their dimensions have been cut in pieces thick 4-5 mm and with diameter 15 mm. Parts of different topographic areas of the organ have been taken to study an ovary condition during the period of its maximum activity.

From the pieces of ovarian tissue cryocuts have been prepared and after that have been treated histochemically by the following methods:

- Oil red O staining for total lipids
- Nile blue staining for neutral and acid lipids
- Baker's test with acid haematein for phospholipids
- δ5-3β HSDH for presence of sex hormones

## RESULTS AND DISCUSSION

The histochemical study has been carried out to clarify the physiological role of the different ovarian components. The results are presented in Table 1.

As a rule in normal follicles neutral lipids and relatively small amount of phospholipids are concentrated. In atretic follicles lipids are converted in an acid form. Their formation coincides with pigment synthesis. This fact gives a reason to speculate that there is significant connection between these two events. In this respect very interesting are various forms of atretic follicles, which would be an object of another study.

Histochemical studies show that follicular cells become active in metabolic respect. During oocyte differentiation and growing the cells of the follicular epithelium develop enzyme systems, which could be considered as indicators for steroid hormones synthesis (Table 1). The presence of enzymes connected with biosynthesis of steroids shows their ability for steroid production that is especially manifested in pre-ovulatory and post-ovulatory follicles (Fig. 2).

The cells of thecal glands show morphological and histochemical characteristics of well developed steroid producing structures (Бояджиева-Михайлова, 1980; Пенков, 1994; Guillette, 1995; Bragdon, 2005). The tests with Oil Red O for total lipids have shown strong positive reaction. The test with Nile

Blue indicates a presence of neutral lipids while the test for δ5-3β HSDH has shown strong positive reaction (Fig. 1).

All these characteristics prove the suggestion that the cells of thecal glands are with no doubt a place for steroid genesis in the developing bird follicle (Boucek and Savard, 1970; Guraya, 1976, 1977; Marron and Hertelendy, 1983; Erpino, 2005). From the results displayed in Table 1 is seen that with the decreasing of the activity of thecal glands steroid-producing activity of granulosa cells increases. Tests for total lipids and key enzyme δ5-3β HSDH have shown the decreasing of enzyme activity till 20$^{th}$ hour after ovulation. Despite its short life the postovulatory follicle could be included in the production of more that one hormone (Gilbert et al, 1978) but its function is still unclear aspect of the ovarian physiology.

## CONCLUSIONS

1. It is established a great amount of lipids in ovoplasm, cytoplasm of the granulose cells and cells of the thecal glands.
2. Progressive deposition of neutral lipids and phospholipids is established in the ovoplasm.
3. In thecal glands are present a great amount of acid lipids / steroids.
4. Follicular (granulosa) cells indicate presence of steroidogenic potency in growing follicles and steroid production in post-ovulatory ones.

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**Table 1.**

| STRUCTURE | HISTOCHEMICAL REACTION AND RESULTS | | | |
|---|---|---|---|---|
| | OIL RED O STAINING | BAKER'S STAINING | NILE BLUE STAINING | KEY ENZYME δ5-3β HSDH |
| Primary follicles | Significant presence of lipids dominating in the cytoplasm | In granulosa cells and like a net in the ovoplasma | Only neutral lipids like tiny drops spread uniformly | No reaction |
| Growing follicles with diameter 1-2 mm | Lipids are concentrated in the periphery of the oocyte as a tiny droplets; they are not present in the central part; thecal glands are rich in lipids. | Like a net mainly in the oocyte ectoplasm and in granulosa cells | Neutral lipids are in the ectoplasm, acid lipids are spread as a background in granulose cells. In thecal glands there are mainly neutral lipids with light bluish color | Positive reaction in thecal glands |
| Growing follicles with diameter 4-5 mm | Like a background in the ovoplasm; thecal glands are rich in lipids | Like a net on a light background | Weak reaction for neutral lipids – like a background | Intensive reaction in the layer of granulosa cells |
| Growing follicles with diameter 1 cm | Like a background in the ovoplasm and uniformly scattered bigger droplets; thecal glands rich in lipids | Preliminary in the bigger drops and like a net in the ovoplasm | Weak reaction for neutral and acid lipids – light background in thecal glands mainly neutral | Only in granulosa cells |
| Pre-ovulatory follicles | As a background and a lot of in vitelline bodies; they fill in the tiny spaces among them; not very well represented in theca folliculi – only in blood vessels of theca externa | Preliminary in spaces between vitelline bodies | Mainly neutral as a background and among vitelline bodies as granules | Not established |
| Post-ovulatory follicles | Small droplets in intercellular space of granulose layer and in the lumen of ovulated follicle | Phospholipids are present in granulosa cells | Clear effect is not established | Absent in the theca: only small amount in theca interna; mainly in granulosa cells |

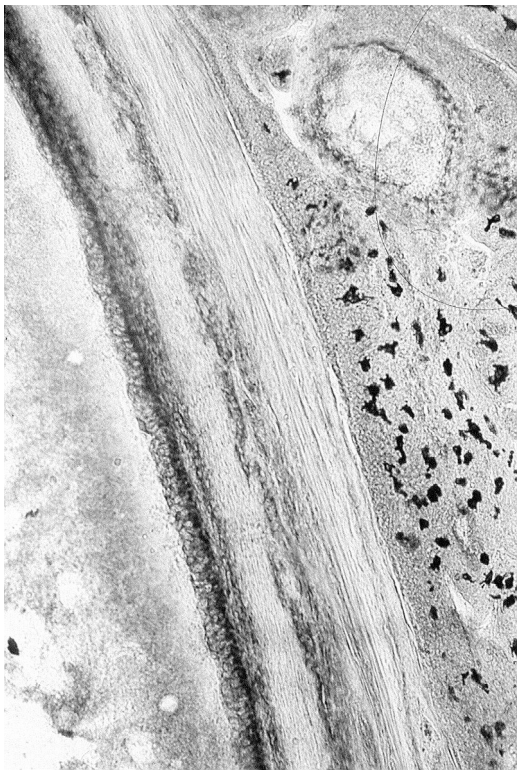

**Figure 1.** Reaction for δ5-3β HSDH in growing follicles; an intensive reaction for the key enzyme is expressed in granulose cells. 180x

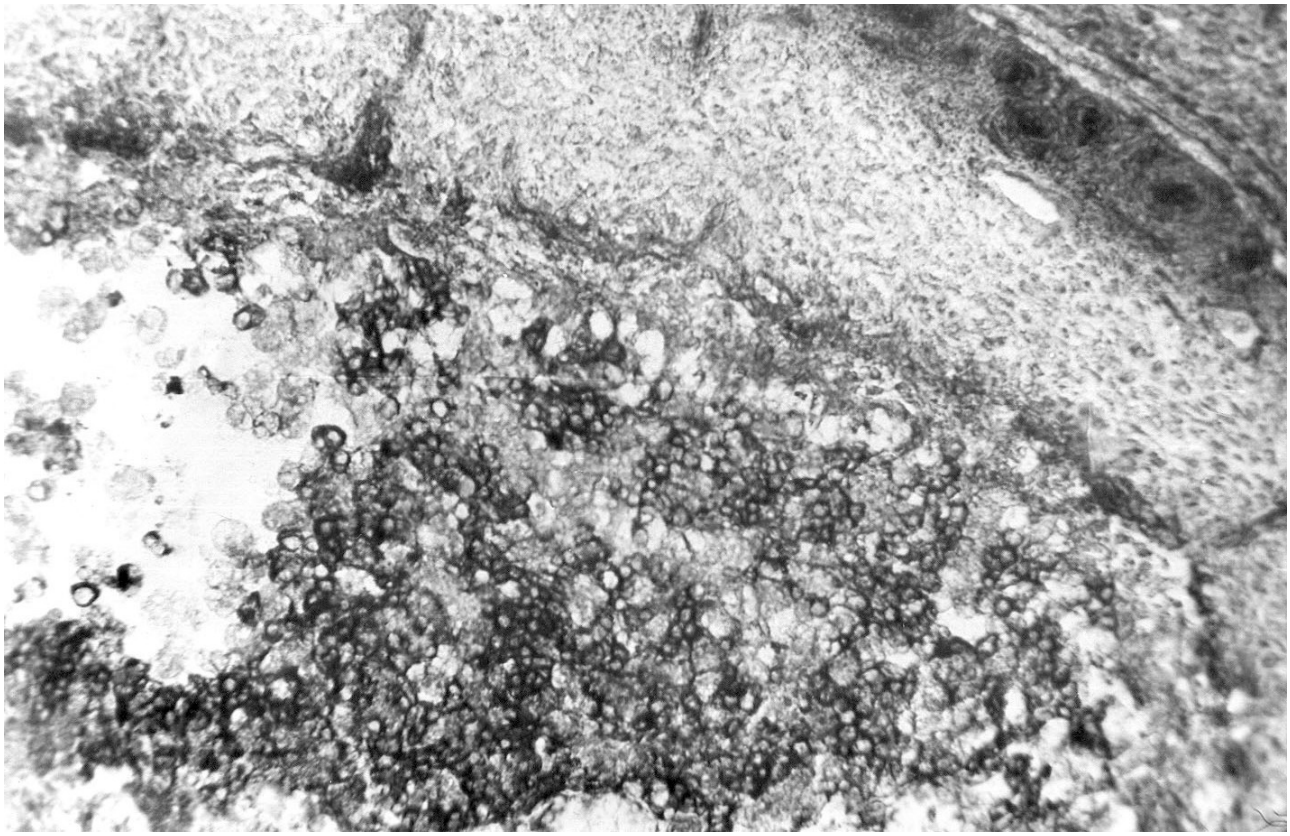

**Figure 2.** Intensive reaction for δ5-3β HSDH in the granulose cells and especially in the thecal cells of early post-ovulatory follicle. 180x