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The Ultrastructure of the Pineal Body of the Migratory Waterfowl *Anas discors* and its role in Gonadal Maturation.

Julio C. Armas and Susan Tai
Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas,
Universidad de Oriente, Cumaná, Venezuela.

ABSTRACT

The morphology of the pineal body of a migratory duck, *Anas discors* was studied by light and transmission electron microscopy (TEM). This study investigates the functional activity of pinealocytes and their role in the maturation of germ cells during the period between arrival from the north (post-migration) and departure (pre-migration).

During the shortest photoperiod in November and December (post-migration), pinealocytes exhibit ultrastructure which suggests maximal intracellular activity: e.g., a well developed rough endoplasmic reticulum and Golgi apparatus, and abundant secretory granules.

In contrast, the gonad is in an inactive state. As the time for departure (pre-migration), approaches, and the photoperiod gradually lengthens, cells of the gonad differentiate into secondary spermatocytes and spermatids, concomitantly, intracellular synthesis in pinealocytes was reduced.

KEY WORDS

Pineal body, Gonad, Transmission electron microscopy, Light Microscopy.

INTRODUCTION

The pineal body is a neuroendocrine organ. Its activity is stimulated by variations in photoperiod and its signals transmitted through sympathetic nerves [3,17,18].

Several investigators [2,4,5,14], using biochemical and electrophysiological methods, creteria was confirmed by Oksche [9] who showed that photorreceptors are able to transduce photostimulation into chemical reactions.

In the last decade, an antireproductive function for the pineal has been suggested. A considerable body of evidence suggests that the pineal body to modulate photic information and regulates gonadal development [7,8]. For example, Santasri and Maiti [13], measured the pineal activity during the gonadal cycle of the bird, *Dendrocita vagabunda*, and showed it reached its maximum peak at the inactive phase of the gonad. In contrast, minimal pineal activity corresponded to the most active reproductive period of the gonad.

Melatonin is the principal hormone secreted by the pineal body and acts as an antireproductive agent [12,16]. Arendt [1] and Reiter [11] demonstrated that the secretion of melatonine approaches maximum levels in the dark, but returns to minimun levels with daylight illumination. The same results were obtained in birds as well as in mammals.

In addition, monoamine-containing granules were observed with TEM in the pinealocytes of rats and hamsters [10]. Those granules were thought to be secretory products of the pineal body since their numbers varied during day to night cycles.

In the present study, we investigated the pineal body of the migratory Blue winged Teal, *Anas discors*, during their annual visit to the

north-shore of Venezuela (from November to March). In this paper we describe the changes in secretory activity which correlate with gonadal development.

MATERIALS AND METHODS

Blue winged Teals, *Anas discors*, were captured in habitats along the northeast coast of Venezuela. Specimens were collected from November through March. A total of 26 birds were used in the study.

Pineal bodies were dissected and fixed in 2.5% glutaraldehyde in 1.0M phosphate buffer for 3h at 4°C and post - fixed in 1.0% OsO_4 in the same buffer (4°C) for 1h. After rinsing in distilled water and dehydrating in a series of graded alcohols, specimens were embedded in Epon 812 at 60°C. Seventy nm thick sections were obtained with a Ultramicrotome Reichert-Jung UltraCut E and stained with 2% uranyl acetate and lead citrate. Observation and photography were done with a TEM Hitachi H-600.

In addition, the left gonad was collected from each duck and fixed in Bouin´s fixative for 7 days. Specimens were rinsed in the 70% alcohol, deshydrated with 70%, 80%, 90%, 95%, absolute alcohol and xylene, and embedded in paraffin (at 57/58°C). Ten um thick paraffin sections were photographed with a Zeiss photomicroscope III.

Hematoxyline and eosin were used as stains for general histology. The staining technique of Bodian [6] was used to localize nerve cells.

The average diameter of the nucleus of pinealocytes during each month was calculated by measuring 100 cells. Cells had a minimum measurement of 2.60µm for neclear diameters.

All calculated values were subjected to a simple variation analysis to obtain confidence levels at 95% [15]. SNK analysis was used to determine variance among the samples obtained in different months.

RESULTS

Microscopic examination of the pineal body of *Anas discors* revealed the presence of three (3) types of cells; pinealocytes, supporting cells and nerve cells. Cells were arranged in follicles which were infiltrated with capillaries (Fig. 1).

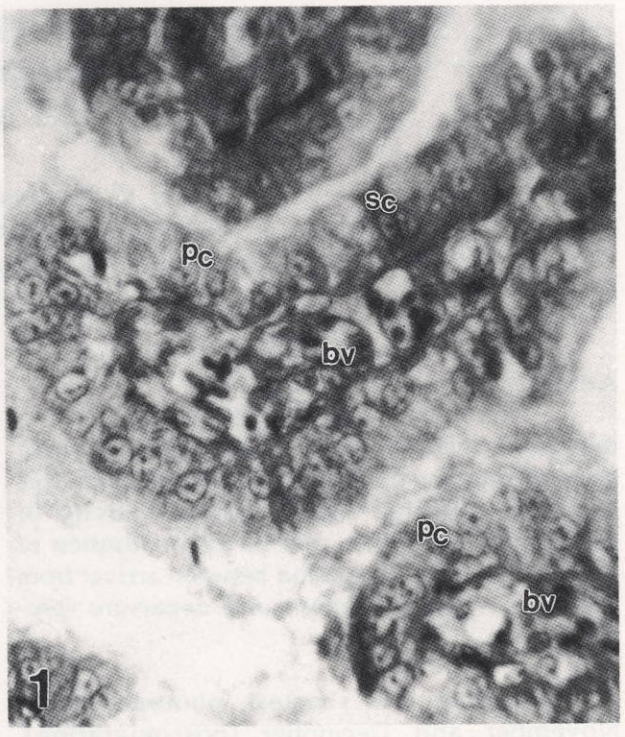

Fig. 1 Low magnification showing pineal cells arranged in follicles with blood vessels in the center. Pc: Pinealocytes. Sc: Supporting cells. bv: Blood vessels. Mag. 720X.

Pinealocytes were located in between the luminal cavity and the capillaries. They were characterized by their cytoplasm; ciliated projections at their apical ends; large amounts of rough endoplasmic reticulum (RER), and numerous mitochondrias. The Golgi apparatus was well developed and the nucleus was irregular in shape (Fig. 2).

The supporting cells were adjacent to the secretory pinealocytes. They were elongated in shape, with rounded nuclei and few organelles. The most significant characteristic of this cell type is the presence of desmosomes at junctions with pinealocytes, next to the lumenal border (Fig. 3).

The third type of cell in the pineal body is the nerve cell. These were situated in the central portion of the tissue and were few in number. These cells were similar in appearance to other nerve cells; possessing a nucleus with an irregular shape, myelinated and non-myelinated processes; and nerve fibers (Fig. 4).

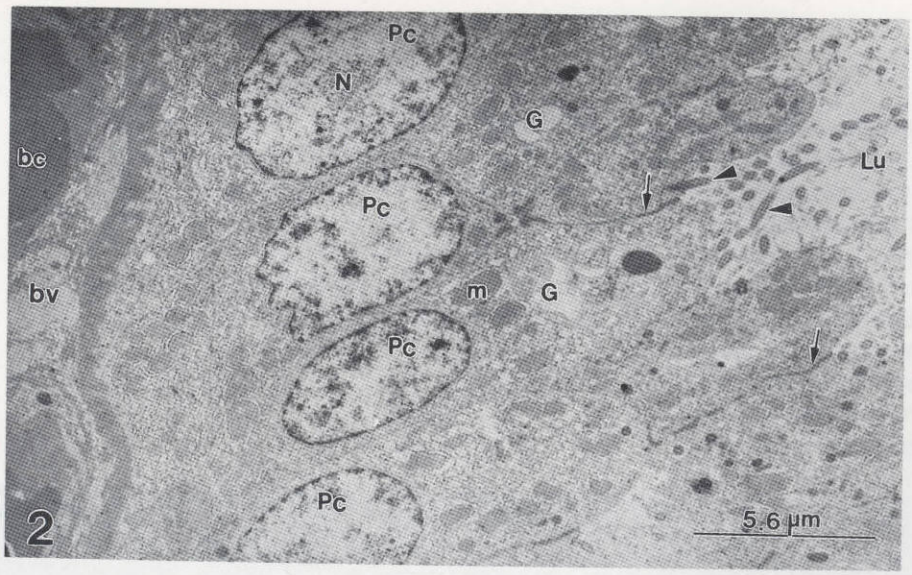

Fig. 2 TEM micrography of a portion of the pineal body revealed that the pinealocytes were situated between the lumenal cavity (Lu) and central blood vessels (bv). They were characterized by the presence of well developed RER, mitochondria (m) and Golgi apparatus (G). Ciliated projections were shown by arrow heads. N: Nucleus. bc: blood cell. Desmosomes shown by arrows.

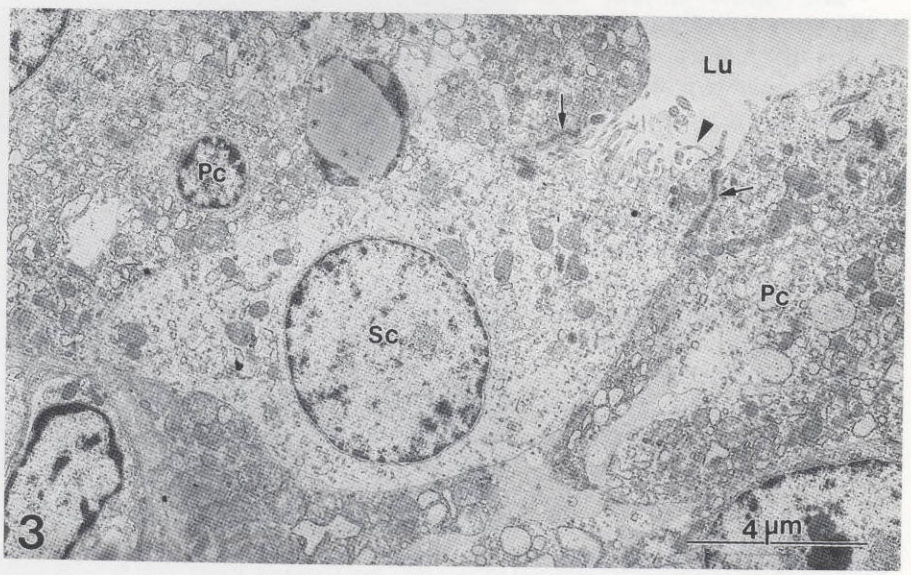

Fig. 3 A supporting cell (Sc) was seen situated between pinealocytes (Pc). These two types of cells were unified by desmosomes (arrows). Supporting cells are less electron-dense, consist of a centrally located, round shaped nucleus, and exhibit less intracellular structure than do pinealocytes. Microvilli (arrow heads) were seen at the luminal side. Lu: Luminal cavity.

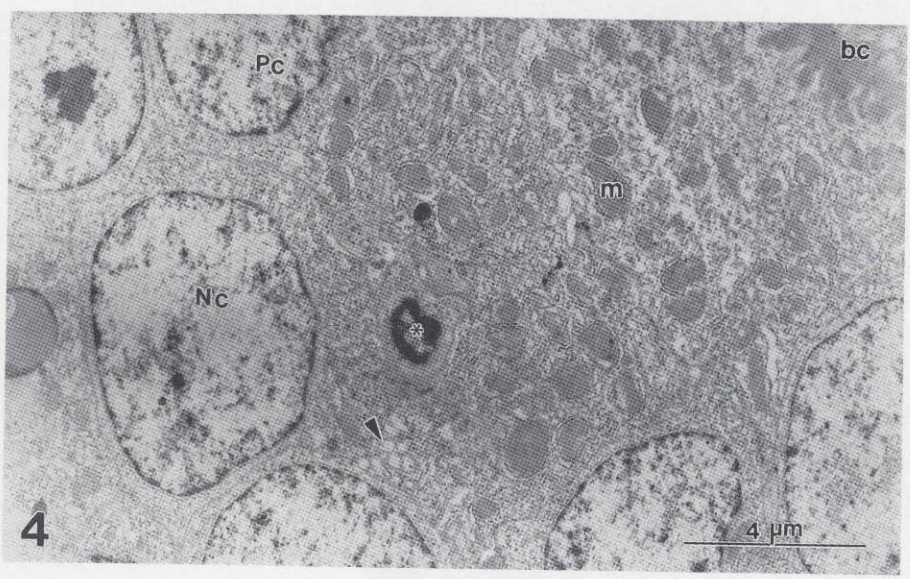

Fig. 4 TEM micrography revealing the presence of a nerve cell (Nc) which consist of myelinated (*) and non-myelinated fiber (arrow heads). Pc: pinealocytes. bv: blood vessel.

Post-migration, pinealocytes revealed a highly active appearance, caracterized by presence of numerous mitochondria, extended Golgi apparatus, and abundant granules ready to release from the Golgi cisternae (Fig. 5). This cellular structure was observed to modulate its appearance as the photoperiod lengthened from january to March (pre-migration). In these pinealocytes, the Golgi apparatus exhibited a tendency to be less active and the size and number of the Golgi cisternae were significantly reduced (Fig. 6).

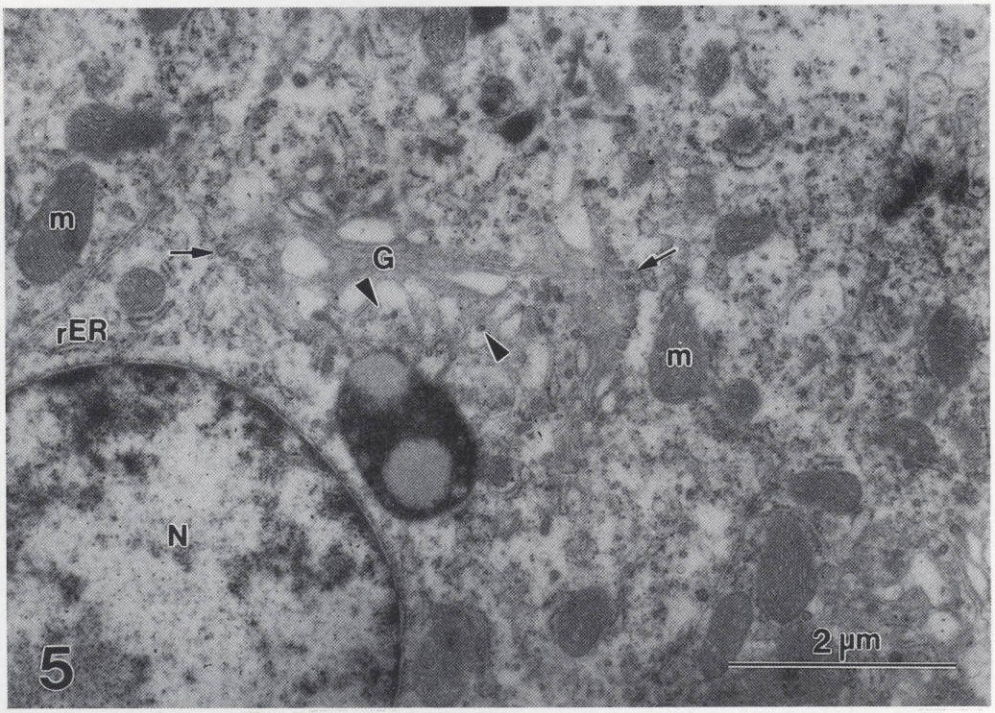

Fig. 5 High magnification showing a portion of a pinealocytes from the bird captured in November. The Golgi apparatus (G) was highly active. Golgi cisternae were extended. Abundant granules were in synthesis (arrow heads), or ready to release (arrows) from the Golgi cisternae. RER: rough endoplasmic reticulum. N: nucleus. m: mitochondria.

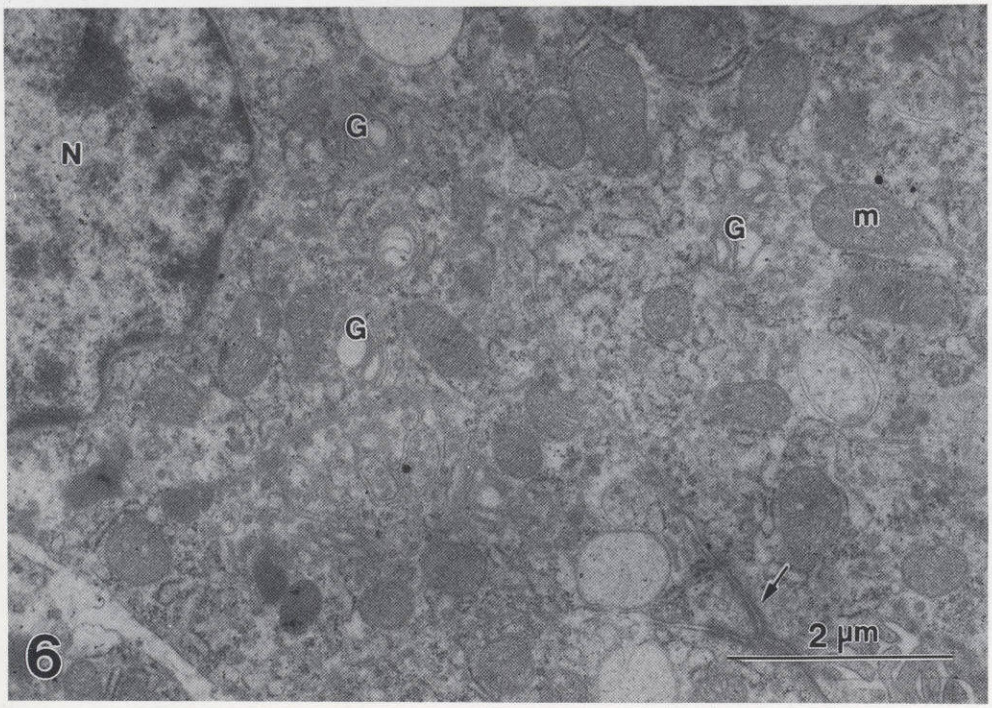

Fig. 6 A pinealocytes obtained from the bird captured in March. The size of the Golgi apparatus (G) was significantly reduced compare to that seen in November. N: nucleus. m: mitochondria. desmosomes: arrows.

Average nuclear diameters of pinealocytes in February and March was also noted to be smaller than those obtained in November and December. Fig. 7 shows that larger nuclear diameters were obtained in the post-migratory period, reaching largest diameters.

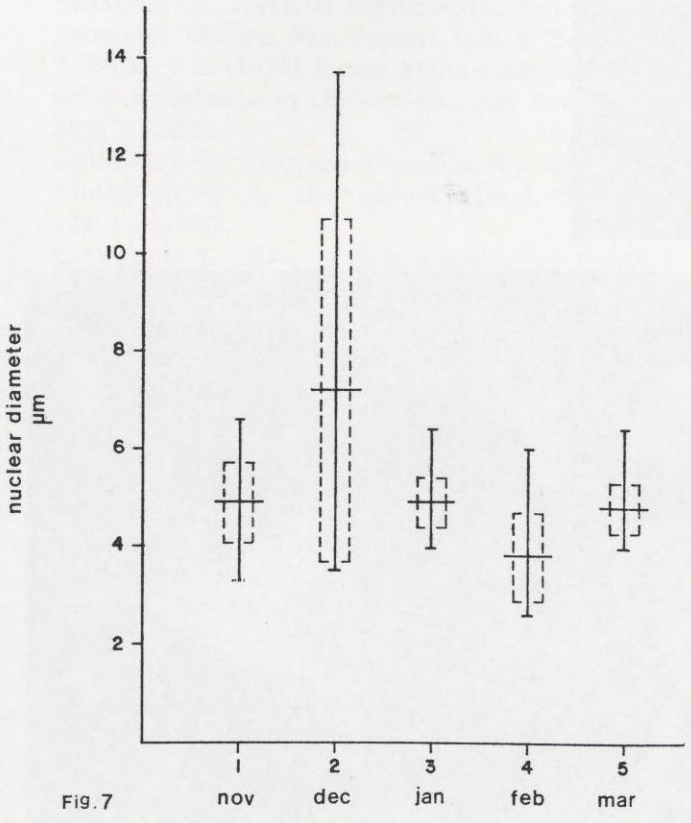

Fig. 7

Fig. 7 According to the results obtained in those progressive months and analyzed by SNK test, values for nuclear diameter can be divided into 2 groups: the first 3 months, (November, December and January) forms one group for which the nuclear diameter is significantly larger than the second group (February and March).

The majority of cells in gonads of post-migratory birds were spermato-gonia. These cells lined the internal surface of seminiferous tubules and were observed in mitosis (Fig. 8). In January, few non-differentiated germ cells (spermatogonia), were present. However, numerous cells in the central portion of the seminiferous tubules were in mitosis and can be characterized as primary spermatocytes (Fig. 9). In March, just before the departure to the north, there were both primary and secondary spermatocytes as well as spermatids in the gonads (Fig. 10).

DISCUSSION

The result obtained in the present study, using light and electron microscopy, have demonstrated the relation between the activity of pinealocytes and the maturation of the gonadal germ cells from the post-migratory through the pre-migratory state.

Cell structure suggesting high levels of synthetic activity of pinealocytes was observed upon the arrival of the birds in November and December. This activity started to decrease during January through March when the photoperiod was gradually lengthened.

Reiter [10], has pointed out that the daily photoperiod is an important factor which influences the endocrine and antireproductive function of the pineal body in many species of mammals. It has been suggested that a short photoperiod can stimulate the secretion of pineal anti-gonadotrophin, whereas longer photoperiod inhibit the antigonadotropic action.

Our present results from the ultrastructure of the pinealocytes and the histological features of avian gonads support suggestions made by Reiter [10]. In addition, we have demonstrated that, upon arrival from their journey in November, birds contain only spermatogonia in the gonads. Germ cells begin to differentiate in January and progressively reach the spermatid stage before departure in March. This suggested that a lengthened photoperiod stimulates pineal hormone production which can only effect the maturation of gonads before migration. Furthermore, we conclude that the remainder of the maturation procedure will be completed arrival in the north.

In conclusion, we hope to study the final stage of maturation of migratory avian gonad and its regulation by factors such as photoperiod, pineal secretion and other hormones (e.q. prolactin and gonadotrophin) from the pitiutary.

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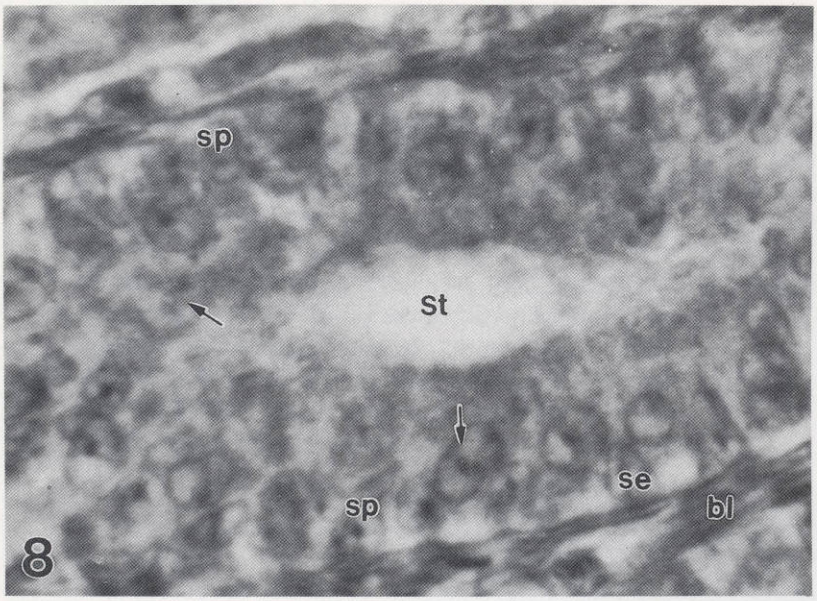

Fig. 8 LM micrography showing a portion of gonadal tissue from a bird captured in November. Spermatogonia (Sp) can be observed. Mitosis shown by arrows. St: seminiferous tubules. Se: Sertoli cells. bl: basal lamella. Mag. 1530X.

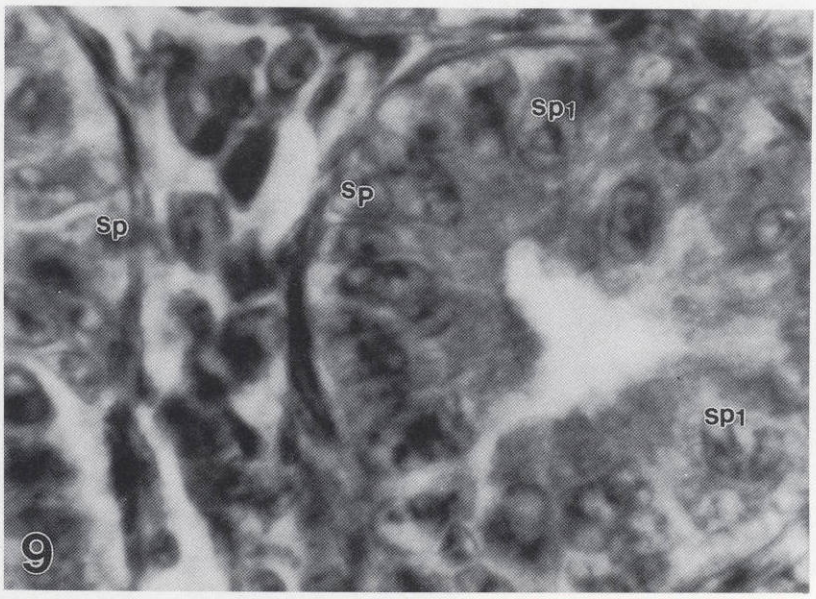

Fig. 9 LM micrography showing a portion of gonadal tissue of a bird captured in January. Most gonadal cells were primary spermatocytes (Sp1). Few spermatogonia (Sp). Mag. 1530X.

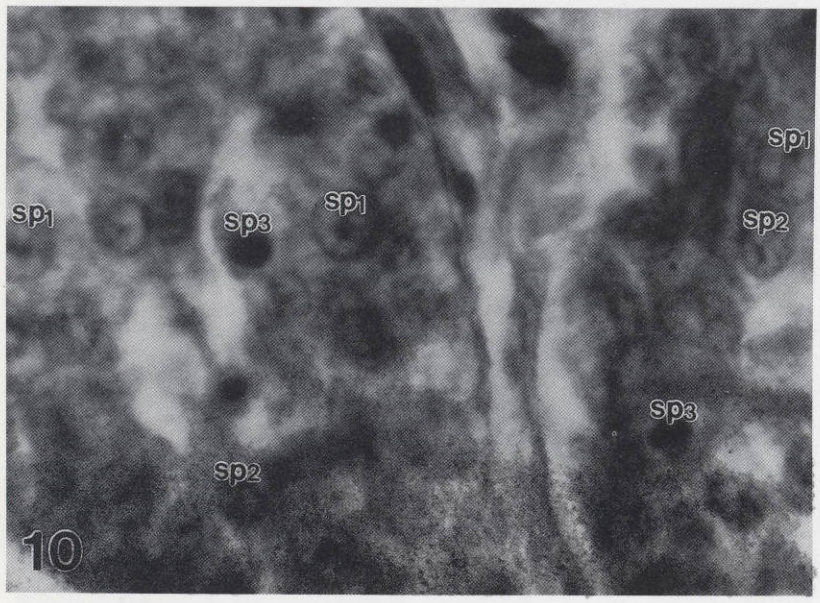

Fig. 10 LM micrography showing a portion of gonadal tissue of a bird captured in March. Primary spermatocytes (Sp1), secondary spermatocytes (Sp2) and spermatids (Sp3) are present. Mag. 1530X.

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