Oviductal and ovarian morphology of a brood parasitic bird, *Molothrus bonariensis* (Passeriformes, Icterinae

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Palabras claves: *Molothrus bonariensis*, Cocha, tamaño postural, patrón de postura, fecundidad, ovario, oviducto.

Las aves parasites de cría, tales como las aves vaqueras (*Molothru*s spp., Ictaridae) ponen sus huevos en nidos de otras especies, abandonándolos para ser incubados y criados por sus hospederos. La ausencia del cuidado parental resulta en una elevada fecundidad para las hembras parásitas. Los parásitos de cría ponen sus huevos en series posturales, separadas por cortos intervalos de no postura, a pesar de esto pueden poner huevos por prolongados periodos de tiempo. En latitudes tropicales, la fecundidad de la hembra, cocha (*M. bonariensis*), se ha estimado alrededor de 120 por ano en una estación reproductiva de 6 meses. Esta fecundidad se asemeja a la encontrada en la gallina. Sin embargo, no hay descripciones morfológicas detalladas del tracto reproductivo de ningún ave parásita de cría. Nosotros estudiamos el tracto reproductivo de la hembra, cocha, en el noreste de Colombia, examinando características morfológicas que pueden estar relacionadas con su elevada fecundidad. No se encontró ninguna diferencia morfológica en el tracto reproductivo ni en la dinámica folicular de las hembras estudiadas respecto a otras aves. Todo lo observado concordó con descripciones previas observadas en aves con diferentes patrones posturales y fecundidades. Lo que sugiere que la elevada fecundidad no requiere un tracto reproductivo especializado. En lugar de esto, las explicaciones de este fenómeno deben ser exploradas en niveles fisiológicos.

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ABSTRACT

TITLE: Oviductal and ovarian morphology of a brood parasitic bird, *molothrus bonariensis* (passeriformes, icterinae)*
Author: Pamela Rueda Cediel**
Key words: *Molothrus bonariensis,* Shiny Cowbird, clutch size, laying pattern, fecundity, ovary, oviduct.

Brood parasitic birds such as cowbirds (*Molothrus* spp., Icteridae) lay their eggs in the nests of other species, abandoning them to be incubated and raised by hosts. Lack of investment in parental care results in high annual fecundities of female parasitic birds. Brood parasites lay eggs in series or clutches, separated by gaps or non-laying intervals of a few days, but may continuously lay for prolonged periods. In tropical latitudes, fecundity of female Shiny Cowbirds (*M. bonariensis*) has been estimated to be 120 eggs per year in a six-month breeding season, a fecundity that is paralleled only by domestic fowl. However, no detailed morphological descriptions of the reproductive tracts of brood parasites are available. We studied the reproductive tract of female Shiny Cowbirds in northeastern Colombia, examining morphological features that might be related to this high fecundity. The reproductive tracts of female Shiny Cowbird, showed no departures from the standard morphology or follicular dynamics known for birds with different postural patterns and fecundities, suggesting that high fecundity in cowbirds does not require a specialized reproductive tract. Instead, explanations must be sought at physiological levels.

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INTRODUCTION

Brood parasitism is a reproductive strategy in which birds lay their eggs in nests of other bird species, thereby not providing any parental care to their offspring (Payne 1977; Rothstein 1990). Because no energy is allocated to parental care, brood parasites such as cowbirds (*Molothrus* spp., Icteridae) and cuckoos (Cuculinae) exhibit higher fecundities than their nonparasitic relatives (Davis 1942a; Payne 1965, 1974, 1977; Scott and Ankney 1983; Kattan 1993). The coevolutionary, ecological, behavioral and reproductive aspects of brood parasitism have been extensively studied (Johnsgard 1997; Rothstein & Robinson 1998). Little attention, however, has been paid to the morphology of the reproductive system in brood parasites, in particular how gonadal morphology may vary in relation to egg-laying rates. Few studies have addressed questions such as: Are higher fecundities of brood parasites related to differences in gonadal morphology or dynamics?

The genus *Molothrus* contains five brood-parasitic species. Bronzed (*M. aeneus*), Brown-headed (*M. ater*) and Shiny (*M. bonariensis*) cowbirds are generalists, parasitizing up to 29, 144 and 200 species respectively (Johnsgard 1997). The Giant Cowbird (*Molothrus oryzivorus*, previously in the genus *Scaphidura*) (Lanyon 1992) specializes on a several species of caciques and oropendolas (Icteridae) and a few other Neotropical species (Johnsgard 1997; Kattan, pers.obs). The Screaming Cowbird (*M. rufoaxillaris*) parasitizes the Bay-winged Cowbird (*Agelaioides badius*, previously in the genus *Molothrus* and the only non-parasitic species among the cowbirds) (Johnsgard 1997).

We studied gonadal morphology in a population of Shiny Cowbirds located in northeastern Colombia. Brown-headed and Shiny cowbirds exhibit higher egg-laying rates than their no-parasitic relatives (Scott and Ankney 1983; Kattan 1993). These cowbirds produce eggs in series or clutches (Payne 1965; Scott and Ankney 1983; Kattan 1993), which are usually ovulated and laid daily, separated by non-laying intervals of a few days (Payne 1965; Scott and Ankney 1983; Jackson and Roby 1992; Kattan 1993). In the North Temperate Zone, Brown-headed Cowbirds lay continuously during the short breeding season, laying up to 40 eggs per season in Canada (Scott and Ankney 1980). In contrast, Shiny Cowbirds lay up to 120 eggs per year during their nine month breeding season in the tropics (Kattan 1993).

No detailed morphological descriptions are available for the reproductive tract of cowbirds, including the ovary, its follicular dynamics and the oviduct. Davis (1942 b, c) described the morphology of the atresic and postovulatory follicles of Shiny Cowbirds, and Payne (1965), briefly described the postovulatory and bursting atresic follicles of Brown-headed Cowbirds. Aditionally, *M. bonariensis* follicular dynamics have only been studied at a macroscopic level (Kattan 1993). It is unknown whether the ability to sustain a high fecundity over an extended breeding season is related to specific morphological traits of the cowbird reproductive tract. In this study, we describe the ovarian and oviducal morphology of adult and juvenile Shiny Cowbirds. Specifically, we examined the follicular dynamics at a microscopic level, and searched for morphological features that might be related to the peculiar reproductive habits of cowbirds.

1. MATERIALS AND METHODS

We captured 55 female *M. bonariensis* on the campus of the Universidad Industrial de Santander, in Bucaramanga, Colombia (07º 07' 47''N, 73º 07' 33''W, 960 m elevation). Mist nets were set up and opened from 0500 to 0600 hours and from 1700 to 1800 hours once or twice each month. In 2004, birds were sampled in July, August, September, November and December. But in 2005, they were sampled in January, February, May and June lastly in 2006 they were sampled only in January. Birds were weighed (± 0.1g) and then euthanized by thoracic compression. Total body size was measured from the tip of the bill to the tip of the longest tail feather. The oviduct and the ovary of each female were removed. Ovaries were weighed (± 0.001 g), and their maximum and minimum diameters measured (± 0.1 mm). All measurements are reported as means ± standard deviations. Vitellogenic and postovulatory follicles were counted to determine clutch size and their maximum and minimum follicular diameters were measured and plotted. Oviducts were also weighed and measured.

We classified females as juveniles, reproductive adults or non-reproductive adults, according to their total body length and the ovarian and oviductal morphology at macroscopic and microscopic levels. Gonads and oviducts were fixed with Bouin´s solution for 24 h. The oviducts were separated into the: infundibulum, magnum, isthmus, uterus and vagina (Aitken 1971; Lofts and Murton 1973; Hodges 1974). Tissues were washed with running water, dehydrated in graded ethanol, cleared in xylene, and embedded in paraplast. Tissues of ovaries and oviducts were sectioned on a rotary microtome at 7 µm. Sections of the ovaries

and oviducts were stained with Mayer´s hematoxilin and eosin, and with a double coloration of PAS and Alcian blue (2.5 pH). Qualitative comparisons were done among different tissues at different reproductive stages. In the case of the oviduct, descriptions were mainly based on characteristics of the mucosa, including the presence, development, and activity of the glands (indicated by presence of any secretory substance), epithelial height means were compared. The stage of development of each follicle type in the ovary was observed, including the width of the theca, height of the follicular epithelium, type of epithelium, ooplasm organization, presence of the Balbiani´s complex, position of the nucleus and its meiotic phase. In addition, atresia at different reproductive stages was observed and recorded.

To obtain microscopic counts of the different follicles for establishing clutch size, eleven complete ovaries at different reproductive stages were serially sectioned every 10 μm and stained with Mayer´s hematoxilin and eosin. Measurements of the largest and smallest diameters of preovulatory vitellogenic follicles, vitellogenic atresic follicles, and recent postovulatory follicles were taken with an ocular micrometer and plotted to show their hierarchical order and clutch size.

2. RESULTS

2.1 GROSS MORPHOLOGY

The ovary was located over the left kidney and was suspended by the mesovarian ligament in the peritoneal cavity. Macroscopically, active ovaries resembled a cluster of grapes and showed a follicular hierarchy of 3-4 vitellogenic follicles and 2-3 postovulatory follicles. Overall coloration was creamy yellow with a dark reddish hue due to high vascularization. Ovaries of reproductive females had a mean diameter of 12.5 $\pm$ 2.3 mm (n = 23) whereas regressed ovaries in non-reproductive females were 5.2 $\pm$ 2.5 mm (n = 17) and in juveniles were 2.6 $\pm$ 2.6 mm (n = 10). The regressed ovaries of adult non-reproductive birds had a pyramidal shape and a granulose compact texture due to the presence of several small previtellogenic follicles. The greatest diameter observed was 2.3 $\pm$ 1.6 mm, (n = 4) and they lacked vitellogenic follicles. As in the regressed ovaries, the juvenile ovaries were pyramidal, small and lightly granulated. The texture was hardly visible to the naked eye because the follicles were small, with the diameter not exceeding 0.9 $\pm$ 0.5 mm, (n = 5).

The oviduct was located in the left side of the abdominal cavity and it was attached by ventral and dorsal ligaments to the peritoneal cavity. The oviduct also varied in size according to reproductive condition, with reproductive females having the longest oviducts (218.4 $\pm$ 56.8 mm, (n = 13). Oviducts were convoluted and contained numerous wide folds. They were highly vascularized, particularly in the posterior region, which was highly expanded. The oviducts were generally pink in color. Oviduct length was reduced in non-reproductive females (126.5 $\pm$ 102.6

mm). They were sometimes surrounded by a wide band of highly vascularized connective tissue (Anexo. 1). Other primordial follicles were located in small nests around previtellogenic follicles deeper in the ovarian stroma (Anexo. 1).

The primordial follicles consisted of a primary oocyte with a conspicuous round nucleus, surrounded by separate granulose cells (Anexo. 1). There was no defined theca around them. The ooplasm was fibrous and sometimes minute, light eosinophilic granules were visible, but in general it did not strongly stain with the colorants used. The nucleus was centrally located; its nucleoplasm was granulated and contained a fibrous chromatin in the prophase of the first meiotic division. In most of these nuclei, there were compact chromosomes very close to each other, and several nucleoli were apparent. Some other nuclei had reached the diplotene phase and exhibited evident chiasmata. The Balbiani complex was located in the ooplasm close to the nucleus (Anexo. 1). This was a crescent or oval shaped, granulated, fibrous and highly eosinophilic structure.

There was no apparent synchrony in the development of the primordial follicles; in some of them the granulosa cells were flat and appeared to surround the oocyte, whereas in others this epithelial layer was complete and the cells were cuboidal. The Balbiani complex varied in different size on the different kinds of primordial follicles. Biovular primordial follicles were also seen sharing a single and incomplete granulosa.

2.2.1.2 *Previtellogenic follicles.* The diameter of previtellogenic follicles ranged from 156 to 905 µm. They were spherical or oval in shape. Two stages of

previtellogenesis were easily discernible. The main difference between them was the presence of abundant vacuoles in the ooplasm at the advanced stage. The follicles at early stages had a thin theca (13 ± 5 µm, n = 31) that stained lightly with PAS, and contained few fibers of connective tissue (Anexo. 2). The first step of theca formation (fibroblasts and fibers of the stromal tissue surrounding the granulosa) was observed in some early follicles. The vascularization was conspicuous on the external side of the theca, but it was moderate in general. There was a single, flat to cuboidal follicular epithelium (8 ± 4 µm, n = 31). The follicular epithelium completely surrounded the oocyte, even though it was not very compact. The nucleus of the granulose cells was heterochromatic and central. The zona pellucida was thin. The ooplasm was highly eosinophilic, granulated and compact. The Balbiani complex had begun to disperse through the ooplasm. The oocyte nucleus was eccentric: its nucleoplasm stained lightly with PAS and showed more conspicuous granules than in the previous stage, and it also had many nucleoli with some lampbrush chromosomes still visible.

At advanced stages of follicular development in previtellogenesis, the mean follicular diameter, the granulosa and the theca width were higher than in early previtellogenic follicles (551 ± 201 µm, n = 21; 16 ± 6 µm , n =21; 25 ± 13 µm, n = 21, respectively). The granulosa was cuboidal but it had begun to change into a pseudostratified epithelium, as some of their nuclei were centrally located whereas others were apical or basal (Anexo. 2). The zona radiata was more evident than in the early stage (1 ± 1 µm thick, n = 5). The ooplasm became vacuolated, with two small vacuole rings initially appearing at the periphery, and some unorganized

vacuoles at the center. At posterior stages, the vacuoles started to increase in size and number. The Balbiani complex was not visible. The nucleus changed from having notorious and evident chromosomes to having them not readily noticeable. It still had some nucleoli, however, the absence of chromosomes might be indicative of the final stages of prophase I.

2.2.1.3 *Vitellogenic follicles.* As in previtellogenesis, vitellogenesis was divided into earlier and advanced stages. The diameter range of the vitellogenic follicles was 905 μm – 8 mm. Early vitellogenesis was characterized by a wide and highly vascularized theca, its thickness was 63 $\pm$ 18 μm, (n = 23) (Anexo. 2). The inner theca was formed by dense and uniform connective tissue with several fibroblasts, being less vascularized and dense than the outer theca; the latter was irregular and contained fibroblasts with round nuclei. The zona radiata was conspicuous, 2 $\pm$ 1 μm in width (n = 14), and was wider near the oocyte nucleus (≈ 7 μm).

The granulose layer had a columnar and pseudostratified epithelium (14 $\pm$ 7 μm, n = 22). The cortical ooplasm was granulated. It stained intensely with eosin. It had small, dispersed granules and vacuoles. Moving inward the number and size of vacuoles increased to the point where the whole ooplasm was saturated with them. Yolk platelets in the peripheral vacuoles were small, homogeneous round lipid droplets. Towards the center of the follicle they were smaller and scarce. A regionalization of yolk platelets becomes evident. Closer to the nucleus, the yolk platelets were smaller. The nucleus was eccentric, close to the granulose, and its

nucleoplasm was granulated and uniform. The medullar ooplasm was vacuolated, eosinophilic, fibrillar and finely granulated.

The theca was still thick (88 ± 43 μm, n = 13) in advanced vitellogenesis the inner layer was more compact than the external one, and vascularization in the former increased (Anexo. 2). The granulosa changed into a single, cuboidal and flat epithelium (4 ± 2 μm, n= 13) and the zona radiata was barely visible. The cortical ooplasm was reduced and there was a narrow line of small yolk platelets (initially homogeneous and not composed of small spheres). In an advanced stage, the ooplasm was full of yolk platelets which kept their size regionalization. The outer yolk granules and those surrounding the nuclei were smaller and more spherical than those in the medulla. The medullar yolk platelets were hexagonal and composed of small, round yolk spheres. The nucleus was eccentric; it was completely polarized and adjacent to the granulosa.

2.2.1.4 *Post ovulatory follicles.* Post ovulatory follicles (POFs) of different sizes were observed. The largest and more evident POFs were the recent ones found in birds that contained an oviductal egg as a result of a recent ovulation (Anexo. 3). At this stage, the follicle looked like a shrunk empty bag; its theca was thick (288 ± 117 μm, n = 12). The highly vascularized outer theca was very dense, irregular and eosinophilic. The inner theca was disorganized and highly basophilic. It weakly stained with PAS. There was a layer of fibroblasts with small and oval shaped nuclei and a few small blood vessels between the two thecal layers (Anexo. 3). The granulosa was wider as it lost its epithelial shape because some cells were

detached from it; a few cells were visible underneath the inner theca. It was highly basophilic and it stained less intensely than the inner theca for PAS.

Less recent POFs had essentially the same morphology of the latter, but they were smaller and their lumen had become occluded by granulosa cells, other cells that came off from the theca and some blood cells (Anexo. 3). Old POFs were smaller than those already described and they had an occluded lumen filled with thecal cells and granulosa cells. These two kinds of cells had pycnotic nuclei and their cytoplasm did not stain at all with the colorants used (Anexo. 3). This type of follicle may be easily confused with old atresic follicles. Finally, POFs disappeared within the ovarian stroma.

2.2.1.5 *Follicular atresia.* Early atresia was characterized by the vacuolization of granulose cells (Anexo. 4). These cells swelled and became edematous but were still organized as a normal epithelium. Their nuclei were pycnotic. The theca still appeared to be in good condition, although the follicle had shrunk. The ooplasm was not homogeneous; it had patches that stained more intensely than other regions. Subsequently, the granulose cells invaded the ooplasm. Atresia was common in primordial and previtellogenic follicles.

In late atresia the follicular shape was lost and it was very difficult to differentiate from advanced POFs. The ooplasm was occluded by granulose cells that were vacuolated and had pycnotic nuclei; thecal cells and stromal fibers were surrounded the granulose layer and were not as vascularized as in the center of the follicle where abundant blood vessels with erythrocytes and several lymphocytes were observed. A cellular arrangement forming layers was observed. These layers

had different amounts of dense connective tissue, so some of them appeared compact while others did not (Anexo. 4).

Atresia was also observed in vitellogenic follicles (Anexo. 4). It was of the bursting type, which included a rupture site where the yolk was discharged into the ovarian stroma. This type of atresia was characterized by a swelling of the thecas. Both thecal layers were vascularized, but abundant blood vessels were also seen under the granulosa, which was projecting into the ooplasm (Anexo. 4). In the ooplasm the yolk granules were not hexagonal in shape and most were broken. In advanced atresia, the follicular shape was completely lost, the outer theca was highly eosinophilic, thick, and highly vascularized, and the fibroblast nuclei appeared in good condition. However, some areas of connective tissue in this theca were similar to those in the early POF, showing only early signs of atresia. The inner theca was basophilic, thinner and with less vascularization than the outer theca. The granulose layer occupied the ooplasm where there were abundant blood cells, fibroblasts and broken yolk platelets. At the point of rupture where the theca was broken as well, there were theca strands, blood vessels, and blood cells emerging through the opening (Anexo. 4).

In general, atresia was more frequently seen in previtellogenic follicles than in other follicular stage, and in reproductive birds than in other reproductive phases. Atresia of vitellogenic follicles was relatively uncommon.

2.2.2.2 *Non reproductive ovary*. The ovary was compact and its stroma was denser and more highly vascularized than in reproductive ovaries (Anexo. 5). The ovary had previtellogenic follicles at different stages of development, but there were

several follicles with small and sparse ooplasmic vacuoles. Follicles at advanced stages of previtellogenesis or postovulatory follicles were absent. Each follicular stage of previtellogenesis had the same morphology as in a reproductive ovary.

2.2.3 *Juvenile ovary*. Ovaries of juveniles had morphological features similar to non-reproductive, inactive ovaries (Anexo. 5). The stroma was the most tightly organized of the three ovarian stages; follicles were very close to each other. The stroma contained many blood vessels in dense and irregular connective tissue. In the youngest birds, the earliest ovary consisted of just a double layer of primordial follicles, with a few early previtellogenic follicles underlying the ovarian epithelium (Anexo. 5).

2.2.4 *Reproductive oviduct.* The oviduct was regionalized proximodistally in five sections (infundibulum, magnum, isthmus, uterus and vagina) recognizable by the epithelium type and mucosa features (presence and type of glands) and folding sotle.

The infundubulum. The infundibulum contained several small, thin folds, a reduced mucosa without glands and a narrow lumen. Its scarce and thin connective and muscular tissues stained positively with PAS. At the infundibular ostium, the mucosal folds were the smallest compared to other regions of the female's reproductive tract (Anexo. 6). The luminal epithelium was simple, columnar and ciliated; however, it also had some non-ciliated cells with apical nuclei containing several nucleoli. The connective layer of the mucosa was thin and poorly vascularized by small capillaries. Few longitudinal muscular fibers enveloped the mucosa, and a very thin serosa surrounded the oviduct externally. The secretion of

the non-ciliated cells of the luminal epithelium was finely granular, eosinophilic, and PAS positive. At the posterior infundibulum, the general morphology was the same but there were some differences (Anexo. 6). The mucosal folds were higher and had primary, secondary and tertiary folds. The blood vessels were larger than in the ostium and the wall was wider, with a thicker layer of muscle bands. The epithelial secretion was less finely granular than in the anterior region.

2.2.4.2 *The magnum*. At the infundibulum-mangum joint, several glands were visible in the connective tissue of the mucosa and stained PAS positive. In the magnum, the folds were higher and simple (Anexo. 6). The luminal epithelium of the magnum was columnar with ciliated and non-ciliated cells; the latter were alcian blue positive and had an apical round nucleus with several nucleoli. The mucosa was filled with branched tubulo-acinar glands; the ducts of the glands had a columnar epithelium and their lumen was filled with a fine, homogeneous light PAS secretion. The glandular acinus was formed by approximately six pyramidal cells, with several cytoplasmic fine granules that strongly stained with PAS and with eosin. The nuclei of the acinar cells were flat and basal with many nucleoli. Abundant small capillaries were observed among the glands. The muscular layers were wider than in the infundibulum. The inner layer had circular bundles of muscle cells while in the outer layer they were longitudinal; surrounding the muscular layers, a thin layer of loose connective tissue with small capillaries and a flat epithelium were observed. When there was an egg in the magnum, the acinar glands looked hypertrophied.

2.2.4.3 *The isthmus*. At the transition site between the magnum and the isthmus/uterus there was a very short ring zone (less than a millimeter). This region was very narrow and highly folded inward. The wall was composed of loose connective tissue and two flat layers of muscle, the inner circular and the outer longitudinal (Anexo. 6). The mucosa was reduced and had thin folds and no glands. The luminal epithelium was columnar and ciliated, with round nuclei at different levels. It was possible to observe magnal and isthmus glands simultaneously in the same fold in some cases, suggesting an intrusion between both tubes. When this region was evident it was not preceded by a transition region, rather there was an abrupt change. The luminal epithelium of this zone was higher when compared to magnal or uterine luminal epithelia.

2.2.4.4 *The uterus*. The isthmus/uterus was a region with a distinctive mucosa; their folds were leaf shaped, simple and irregular. The luminal epithelium was columnar, pseudostratified and with some ciliated cells whose nuclei were apical and rounded, and these cells did not stain with PAS nor alcian blue. The mainly non-ciliated cells had basal and ovoid shaped nuclei with many nucleoli and were strongly PAS positive. The connective tissue was not as compact as in the magnum; however it was highly vascularized with small capillaries and their glands were not as close together as in the magnum (Anexo. 6). Glands were branched tubulo-acinar, their secretion was fine and the cytoplasm of acinar cells was clear. The glandular lumen was generally filled with a transparent substance; however, hypertrophy of glands cells, which contained eosinophic and secretory granules facilitate the presence of lumen occluded glands. Each acinus was shaped as in the

magnum, but in the uterus they were smaller and their cells were lighter than in the magnum. The nuclei of the acinar cells were euchromatic and the cytoplasm was poorly eosinophilic and almost translucent. A conspicuous irrigation was seen in the uterine folds, since in the middle of the fold there was a strain of connective tissue with several blood vessels. The muscular layers of the uterus were similar but wider than those in the magnum.

2.2.4.5 *The sperm storage tubules.* The sperm storage tubules (SST) were located at the transition site between the uterus and the vagina (Anexo. 6). These tubules were mucosal invaginations; their epithelial cells were columnar with a basal euchromatic nucleus. At the start of the invagination there were visible cilia but no cilia were evident when the tubule was completely formed. The bottom of the SST epithelial cells was filled with an eosinophilic secretion. Some SSTs contained sperm in bundles or dispersed in their lumens. The sperm cells stained slightly PAS positive and were highly basophilic. These storage glands were seen in both actively reproductive females and those that were in the regression period (Anexo. 6).

2.2.4.6 *The vagina.* The most posterior segment of the oviduct was the vagina, functioning as a sphincter because of its strong and thick circular muscle layers (Anexo. 6). The mucosa of this region was thin, and its primary and secondary folds were small. The luminal epithelium was columnar, pseudostratified and ciliated, and Alcian blue positive. The connective tissue of the mucosa was thin with few small blood vessels, and there were few invaginations of the same size as

a SST. The muscle layer was composed of a wide layer of circular muscle, plus a layer of longitudinal bundles in a matrix of loose connective tissue.

2.2.5 *Non reproductive oviduct.* The morphology was similar to that of the active oviduct. The differences were related to the development of the duct. When a bird was reproductively inactive, the most anterior regions regressed first. Birds that had recently stopped reproductive activity had an active uterus, with sperm in their SSTs, and a distended vagina. Epithelial heights in the different regions also varied between reproductive and regressing females. In general cells were taller in reproductive females, with the exception of the isthmus where there was no apparent change (Anexo.7).

2.2.5.1 *The infundibulum*. The infundibulum was barely visible with the naked eye. Histologically, it was in generally, as evidenced by the smaller mucosa folds (Anexo. 8). The mucosa was completely irregular; it had primary folds with many small invaginations, had loose connective tissue and was poorly vascularized. The luminal epithelium was cuboidal with some ciliated cells. The wall was composed of thin circular layers of smooth muscle and irregular connective tissue.

2.2.5.2 *The magnum*. The magnum had a reduced mucosa. The connective tissue was extensive but the glands were atrophied (Anexo. 8). In some cases, glands were located just under the columnar epithelium, and in other cases the whole fold was filled with inconspicuous acinar glands, which had small cells with heterochromatic nuclei, no nucleoli and empty cytoplasm. The gland lumens were occluded, and the connective tissue was extended between the small acines. The

luminal epithelium was columnar and mostly ciliated. The wall of this region was thin, with few muscular fibers. The magnum did not stain with PAS or Alcian Blue.

2.2.5.3 *The isthmus*. The luminal epithelium of the isthmal ring, the isthmus and the magnum did not have secretory granules, and only the glicocalix stained positively with Alcian blue. The short isthmal ring, as in the reproductive female, was folded inward. The isthmal ring had primary and secondary folds, which were small, had a reduced mucosa with loose connective tissue and small capillaries, but no glands (Anexo. 8). The luminal epithelium was columnar, even though in some regions its nuclei gave the appearance of a pseudostratified epithelium, which was scantly ciliated.

2.2.5.5 *The uterus*. The uterine folds were simple with short grooves (Anexo. 8). The uterine mucosa was composed of irregular and dense connective tissue with few capillaries or few glands, all of them empty and located immediately underneath the luminal epithelium. The luminal epithelium was simple and cuboidal; it was also scantly cilliated and lightly stained with PAS. The mucosa was enveloped by a layer of longitudinal smooth muscle, along with large arterioles and small capillaries.

At the utero-vaginal junction there were some SSTs (Anexo. 8). These tubules seemed to be active. They were formed of columnar cells, and their apical cytoplasm stained intensely with eosin. Mitotic figures and sperm were observed in the SST cells of one bird.

2.2.5.5 *The vagina*. The vagina was similar to that of a reproductive bird. Its mucosa was reduced and had primary folds with short grooves (Anexo. 8). The

mucosa was composed of a thin layer of loose connective tissue with small capillaries and no glands. The luminal epithelium was pseudostratified, low, and scantily ciliated. It stained poorly with PAS. The wall was still highly engrossed. It had few capillaries and two layers of smooth muscular tissue, with the circular layer being thicker.

2.2.6 *Juvenile oviduct.* In juvenile birds, oviductal morphology was similar to the former stage but there was no sign of activity, as neither glands nor ciliature were seen (Anexo. 9). A few glands were evident in some cases but at a very early developmental stage, epithelial cells were low, and they did not stain for PAS or Alcain blue. Therefore, the different regions of the oviduct were barely differentiated.

2.3 FOLLICULAR DYNAMICS

2.3.1 *Microscopic and macroscopic clutch size.* Macroscopic mean clutch size was 3.7 $\pm$ 1.4 (n = 15) and microscopic mean clutch size was 3.7 $\pm$ 1.1 (n = 7) (Anexo. 10). Macroscopically, the number of POFs was 1.9 $\pm$ 0.8 (n = 18), and microscopically it was 2 $\pm$ 0.8, (n = 7). Laying rate, calculated using the method of Scott and Ankney (1980) was 0.6 eggs per day. Microscopic and macroscopic analyses showed that these birds lay clutches of 3-5 eggs. Atresia in vitellogenic follicles, which were preovulatory follicles (according to their diameter), seems to indicate lapses of few days or gaps between clutches. These gaps were also observed at macroscopic level.

Females were reproductive during the months of January, February, May, June and July. March and April were not sampled, and only one female was captured in August and in September. Reproductively inactive females were

collected at the end of the rainy season (November through January). This suggests that Shiny Cowbirds breed during the rainy season. Molting and breeding were not simultaneous. Molting females were in their resting period (dry season) or juveniles that were changing their plumage; however, one reproductive female was found molting in June 2005.

3. DISCUSSION

We found no morphologically unique traits that may be related to the high fecundity of *M. bonariensis*. Macroscopically, the reproductive of this species did not exhibit any departures from previously described avian morphology. Ovarian morphology resembled that of domestic fowl, *Gallus gallus domesticus,* and other bird species studied by Hodges (1974) and Guraya (1989). Moreover, ovarian morphology was similar to that of other members of Archosauria, such as caiman, *Caiman crocodiles,* and alligator, *Alligator mississipiensis,* (Uribe and Guillette 2000; Calderón *et al*. 2004).

Generally, germ cells are absent in mature birds (Torkaz 1978; Guraya 1989; Callebaut *et al.* 1997). Instead, nests of primordial follicles containing primary oocytes were found. With respect to the arrangement of germ cells, *M. bonariensis* was similar to the house crow, *Corvus splendens*, but the arrangement differed from other birds such as common quail, *Coturnix coturnix,* common myna, *Acridotheres trisitis,* and *G. gallus.* In the former, germ cells formed compact nests surrounded by highly vascularized tissue bands, whereas in the latter they formed loose groups. This compact arrangement apparently increases physical support and nutrient acquisition for this group of cells (Guraya 1989).

The general morphology of primordial follicles was similar to previous descriptions by Hodges (1974) and Guraya (1989). Other follicular types (previtellogenic and vitellogenic follicles) were also morphologically similar to those of *G. gallus* (Hodges 1974) and other birds (Guraya 1989), and also to *A. mississipiensis* (Uribe and Guillette 2000) and *C. crocodiles* (Calderón *et al*. 2004).

The arrangement of the yolk granules according to their size and shape was similar to that described for birds and crocodilians. Polyovular follicles were found in the ovary of *M. bonariensis*. This type of follicle is also found in *G. gallus* (Guraya 1989) and in *A. mississipiensis* (Guillette et al. 1994; Guillette and Bermúdez 2001). In the latter species, Guillette et al. (1984) suggested that the presence of polyovular follicles at high frequency is a gonadal response to estrogenic xenobiotic contaminants. Polyovular follicles occurred at a low frequency in *M. bonariensis*. Atresic and postovulatory follicles were also morphologically similar to those already described for other birds (Hodges 1974; Erpino 1969; Guraya 1989), and specifically for cowbirds (Davis 1942b, c; Payne 1966), and *C. crocodiles* (Calderón *et al.* 2004). This suggests that at an ovarian level the folliculogenesis process is similar to that in previously studied bird species.

Oviductal morphology was as conservative as ovarian morphology. Living archosaurians diverge from other reptiles in oviductal anatomy; the eggshell membrane is produced in a differentiated region of the oviduct (isthmus in birds and anterior uterus in crocodilians) and the calcite eggshell is produced in another (uterus in birds and posterior uterus in crocodilians). This separation of oviduct function in egg formation is a synapomorphy for archosaurian reptiles (Palmer and Guillette 1992). Crocodilians, however, exhibit simultaneous ovulation, whereas birds show sequential ovulation, shelling and laying each egg individually. Our observations agreed with previous descriptions made for the different regions of the oviduct: the infundibulum (Aitken 1971; Hodges 1974; Evencio-Neto *et al*. 1997; Bakst 1998), the magnum (Aitken 1971; Hodges 1974; Palmer and Guillete 1992;

Evencio-Neto *et al.* 1997; Romero and Ramírez-Pinilla 2002; Oashi et al. 2003; Sultana *et al.* 2003), the non-glandular zone anterior to the isthmus (Gupta and Maiti 1987; Romero and Ramírez-Pinilla 2002; Sultana *et al.* 2003), the uterus (Breen and De Bruyn 1969; Hofer 1971; Aitken 1971; Hodges 1974; Palmer and Guillette 1992; Romero and Ramírez-Pinilla 2002), the vagina (Aitken 1971; Hodges 1974; Evencio-Neto *et al.* 1997; Bakst 1998; Holm and Riderstrale 2002; Romero and Ramírez-Pinilla 2002) and the sperm storage tubules (Aitken 1971; Hodges 1974; Bakst 1998; Chiba and Nakamura 2001; Holm and Riderstrale 2002).

The general histology of ovaries in birds at different reproductive stages was similar; only the lack of vitellogenic follicles and a more compact stroma differentiated non-reproductive animals. A top-down regression was observed in the oviduct of Shiny Cowbirds, as observed in the Zebra Finch, *Taenopigya gutata,* (Williams and Ames 2004). In early regression stages, the proximal segments of the oviduct had a remarkably fast regression when compared with the posterior segment. It was also noticeable that glands, ciliature, fold sizes and epithelium height were reduced with duct inactivity. In addition, every region changed but the isthmus remained almost constant, showing only a small reduction. All these changes were also common for the Pied Myna, *Sturnus contra contra,* studied by Gupta and Maiti (1987), except for the non-glandular zone previous to the isthmus.

Clutch size of *M. bonariensis* observed in this study was in the range previously reported in *M. ater* by Friedman (1929) (4-5 eggs), Payne (1965;1976) (1-6; 3-4.5 eggs), Scott and Ankney (1983) (4.0-4.5 eggs), and finally it also coincided with the previous reported clutch size range of 1-6 eggs in *M. bonariensis*

(Kattan 1993). Contrary to Jackson and Roby's (1992) suggestion about the absence of real clutches in *M. ater*, due to the ability of individual females to lay an egg each day for extremely extended periods of time, we found a laying pattern of clutches or series separated by non laying intervals, as previously reported for cowbirds (Scott and Ankney 1980; Kattan 1993). The nonlaying intervals may be part of a physiological strategy to sustain a high seasonal fecundity, or may be a response to external factors. For example, follicles may become atresic because females are nutritionally challenged, or because of a lack of host nests to parasitize. Limitation of host nest availability in a population of Brown-headed Cowbirds in Canada resulted in low female fecundity over the breeding season (Woolfenden et al. 2003).

The only way to rigorously establish the annual fecundity of a particular female cowbird would be to follow her daily laying history throughout the year (Wooldfenden et al. 2003). In the absence of such data, annual fecundity of female cowbirds can be inferred from the reproductive seasonality of the population. *M. bonariensis* did not reproduce continuously. Previous data showed that a cowbird population in the tropical Andes breeds throughout the year, interrupting egg laying only during the three month dry season (Kattan 1993). Thus, the breeding season was nine months long. During the breeding season, females only stopped breeding to molt; therefore, the laying season was estimated at six months, and annual fecundity was 120 eggs (Kattan 1993). This trend was also observed in our population. Additionally, most reproductive females were found during the rainy season supporting the findings of Kattan's (1993) study. In tropical locations, hosts

are available year-round for this generalist parasite, so lack of hosts is not likely to be a limiting factor, although lack of nests ready to parasitize may be a temporarily limiting factor for females.

We conclude that the morphology of the reproductive tract (ovary and oviduct) of *M. bonariensis* did not exhibit any particular features related to a high fecundity. These organs conserved the typical morphology of birds with lower fecundities and varied laying patterns, following conservative bird/archosaurian morphology. Our results suggest that the high fecundity of cowbirds is not based on the morphology of their reproductive tract, but instead has a physiological basis associated with their reproductive behavior.

4. ACKNOWLEDGMENTS

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ANEXOS.

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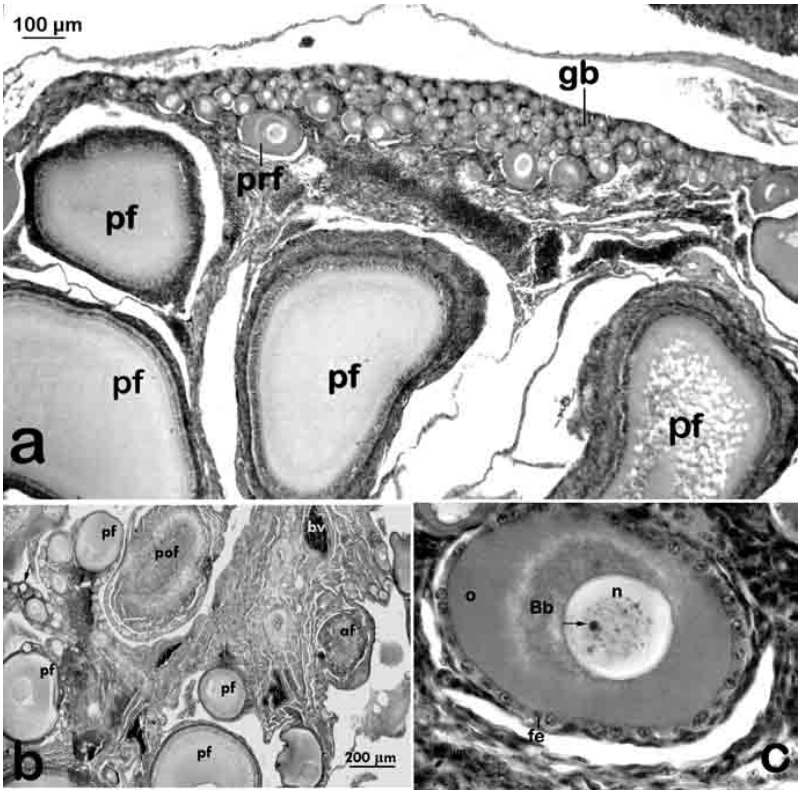

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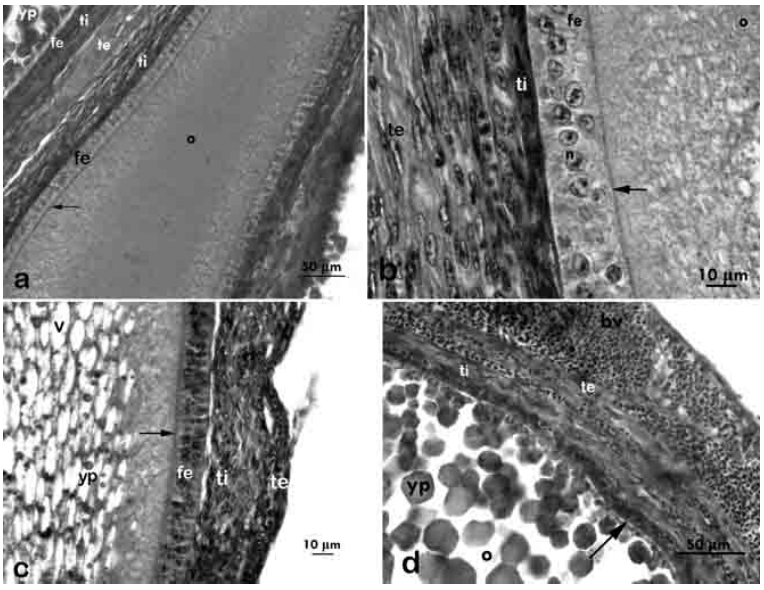
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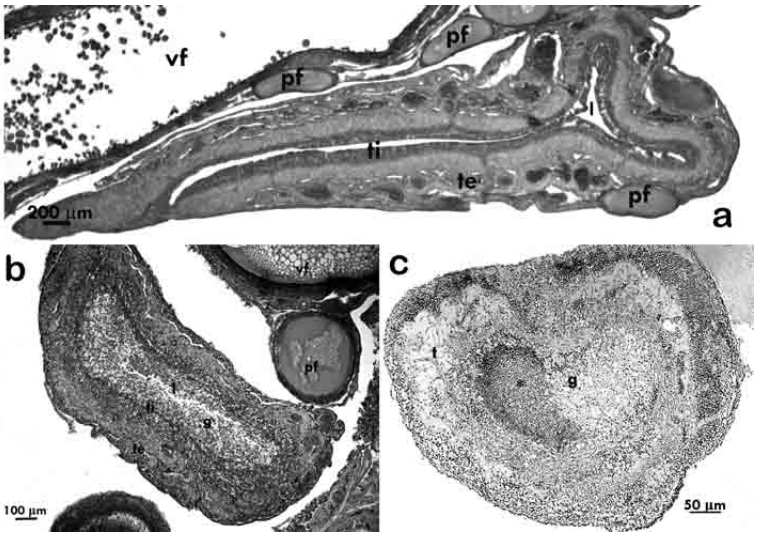

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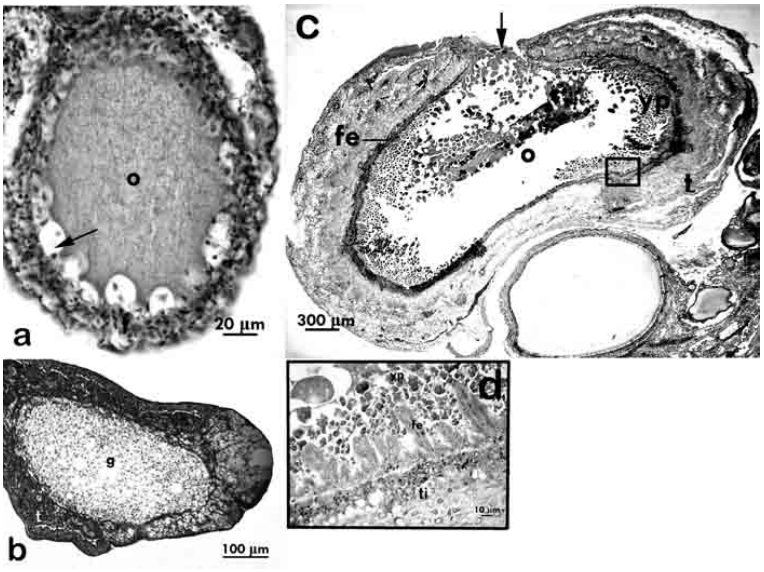

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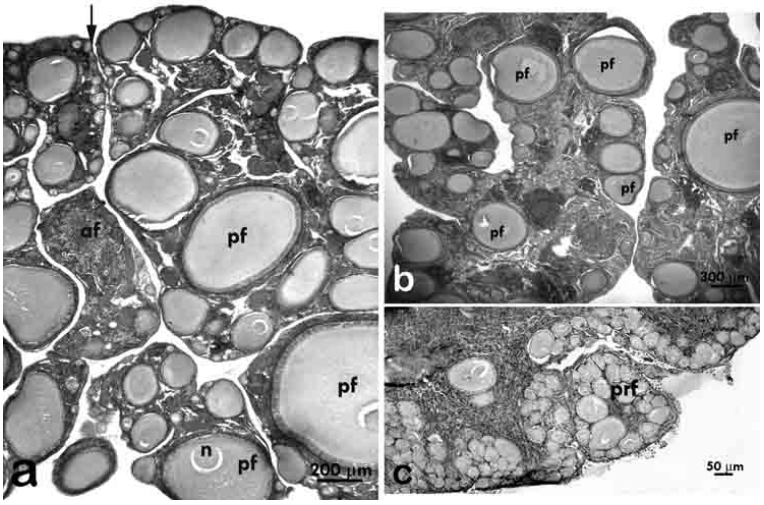

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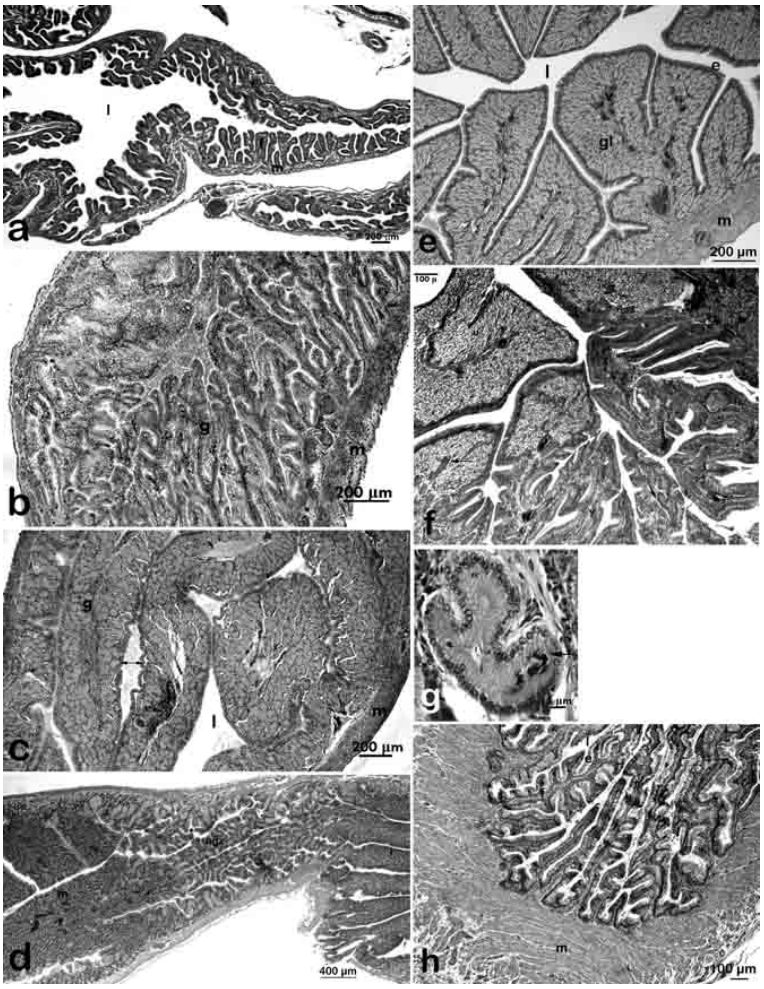

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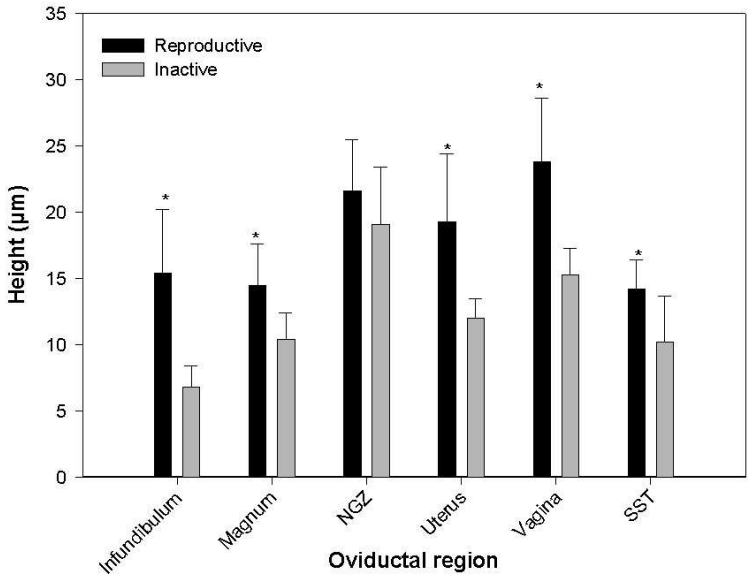
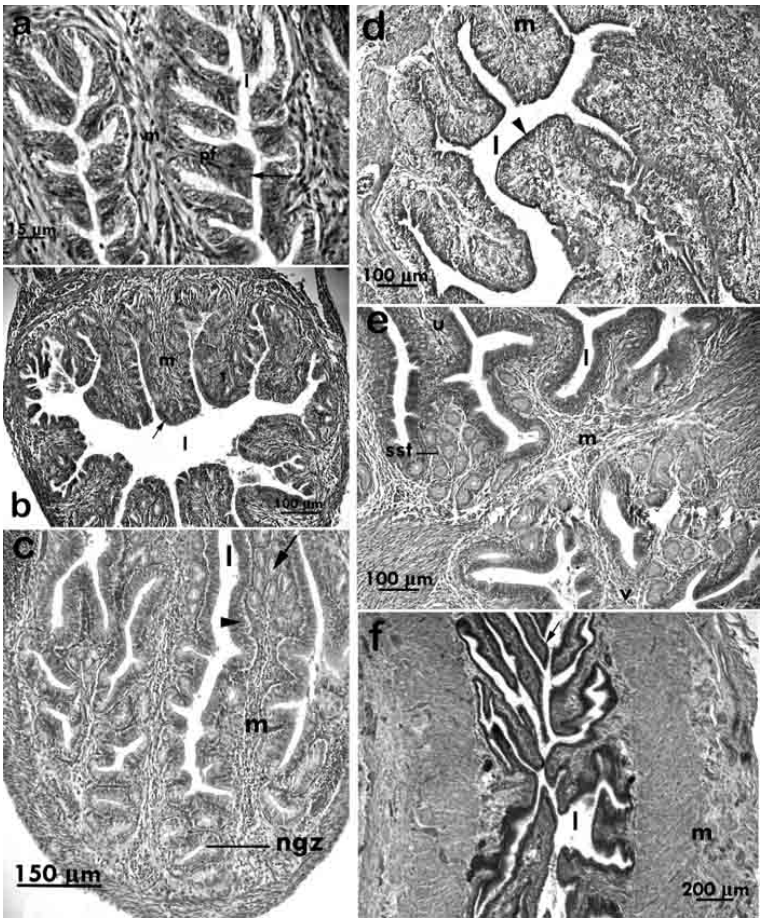

Reproductive
Inactive
Height (µm)
35
30
25
20
15
10
5
0
*
Infundibulum
Magnum
NGZ
Uterus
Vagina
SST
Oviductal region

ANEXO 8.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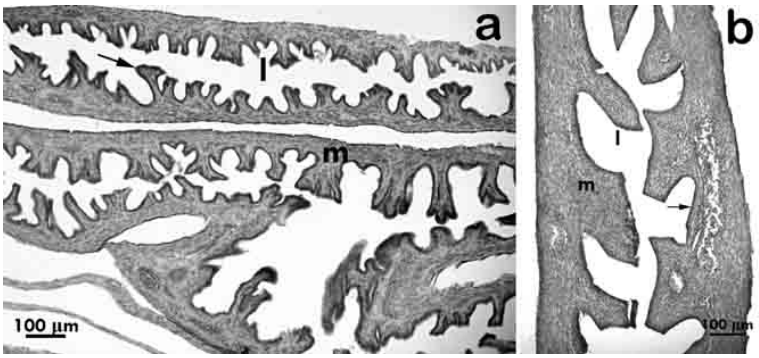
ANEXO 9.



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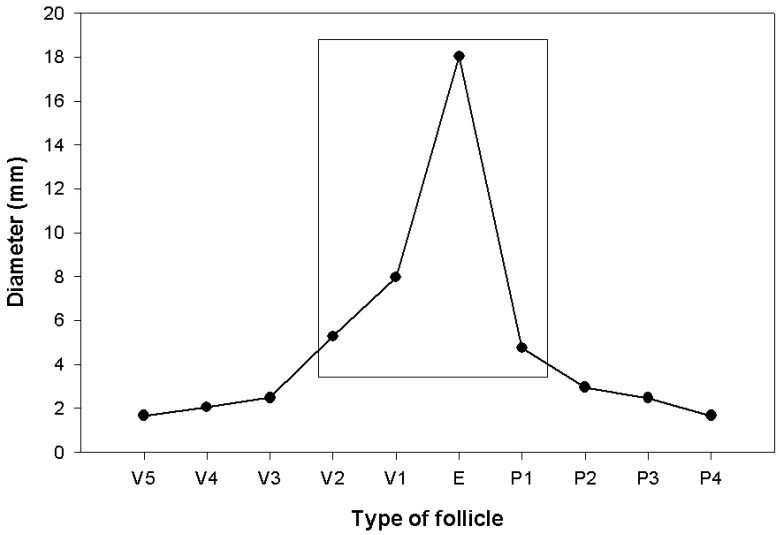
Diameter (mm)
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P1
P2
P3
P4
Type of follicle

ANEXOS LEGENDS

Anexo. 1–Light micrograph of a transverse section through an ovary of a reproductive female, showing its general morphology. H and E stain. **–A**. Ovarian cortex. gb, germinal bed; prf, primordial follicles; pf, previtellogenic follicles. **–B**. Ovarian stroma morphology. bv, blood vessels; arrow, primordial follicles; pf, previtellogenic follicles; af, atretic follicles; pof, post ovulatory follicles. **–C**. Morphology of primordial follicle. fe, follicular epithelium; o, ooplasm; n, nucleus; arrow, nucleoli; Bb, Balbiani complex.

Anexo. 2– Light micrograph of a transverse section through previtellogenic follicles and vitellogenic follicles. H and E stain. **–A.** Early previtellogenic follicle. ti, inner theca; fe, cuboidal follicular epithelium; arrow, zona radita; o, ooplasm; yp, yolk platelets. **–B.** Advanced previtellogenic follicle. ti, inner theca; te, outer theca; fe, pseudostratified follicular epithelium; n, nuclei; arrow, zona radiata. **–C.** Early vitellogenic follicle. v, vacuoles; yp, yolk platelets; fe, follicular epithelium is pseudostratified; arrow, zona radiate; ti, inner theca; te, external theca. **–D.** Advanced vitellogenic follicle. bv, blood vessels; arrow, flat follicular epithelium; o, ooplasm; yp, yolk platelets.

Anexo. 3– Light micrograph of postovulatory follicles showing their morphologies at different stages. H and E stain. **–A.** Early POF. l, lumen; ti, theca interna, te, theca externa; pf, previtellogenic follicle; vf, vitellogenic follicles. **–B.** POF at an intermediate stage. l, lumen; g, granulosa cells; ti, inner theca; te, outer theca; pf, previtellogenic follicle; vf, vitellogenic follicle. **–C.** Old POF. g, granulosa cells; t, thecae.

Anexo. 4– Micrograph of atresic follicles. H and E stain. **–A.** Early atresia. Arrow, follicular epithelium; t, theca; o, ooplasm. **–B.** Adavanced stage of atresia. g, granulosa cells; t, thecae. **–C.** Atretic vitellogenic follicle. arrow, rupture site; yp, yolk platelets; fe, follicular epithelium; t, thecae is swollen; o, ooplasm. **–D.** Detail of the follicular wall. fe, follicular epithelium; t, theca; yp, yolk platelets.

Anexo. 5– Micrograph of non reproductive ovaries at different stages, showing their general morphology. H and E stain. **–A.** Adult female. Arrow, primordial follicles; pf, previtellogenic follicles; af, atretic follicles. **–B.** Juvenile female. pf, previtellogenic follicles of different sizes. **–C.** Youngest female. prf, primordial follicles.

Anexo. 6– Mircrograph of a reproductive oviduct, showing separately the morphology of all its regions. H and E stain. **–A.** Anterior infundibulum. l, lumen; f, primary and secondary folds; m, muscularis. **–B.** Posterior infundibulum. g, tubular glands; asterisk, secretion; m, musuclaris. **–C.** Magnum. l, luminar space; g, acines

of the glands; arrowheads, columnar epithelium; m, muscularis. **–D.** Transition zone between the magnum (m) and isthmus. m, magnum; i, isthmus; ngz, no glandular zone. **–E.** Uterus. l, lumen; e, pseudostratified epithelium; g, glandular tissue of the mucosa; m, muscularis. **–F.** Transition zone between the uterus and the vagina. u, uterus. v, vagina; arrows, sperm storage tubules. **–G.** Detail of a sperm storage tubule. l, lumen; cc, columnar cells; arrow, spermatozoa. **–H.** Vagina. m, muscularis; f, primary folds; e, columnar epithelium; l, lumen.

Anexo. 7– Epithelial height per oviducal region in active and inactive females. NGZ, no glandular zone of the isthmus; SST, sperm storage tubules. Asterisk, significant difference, ($P < 0.05$) Mann-Whitney U test.

Anexo. 8– Mircrograph of an adult inactive female, showing separately the morphology of each oviducal region. H and E stain. **–A.** Infundibulum. m, mucosa; pf, primary folds; arrow, cuboidal epithelium. **–B.** Magnum. m, mucosa; arrowhead, glands; arrow, columnar epithelium; l, lumen. **–C.** Isthmus. ngz, no glandular zone; m, mucosa; arrow, glands; arrowhead, columnar epithelium; l, lumen. **–D.** Uterus. arrowhead, cuboidal epithelium; l, luminal space; m, mucosa with few glands. **–E.** Utero vaginal junction. U, uterus; v, vagina; sst, sperm storage tubules; m, mucosa; l, luminal space. **–F.** Vagina. m, muscularis; l, lumen; arrow, luminal epithelium.

Anexo. 9– Juvenile oviduct, showing its two main regions. H and E stain. **–A.** Anterior region. m, mucosa; arrow, epithelium; l, lumen. **–B.** Posterior region. m, mucosa; arrow, epithelium; l, lumen.

Anexo. 10– Follicular diameter plot to show the macroscopic clutch size. v, vitellogenic follicle; e, egg; p, post ovulary follicle; rectangle, clutch size, which is of 3 eggs.