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Research paper

Oviduct morphology and estrogen receptors ERα and ERβ expression in captive *Chinchilla lanigera* (Hystricomorpha: Chinchillidae)

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ABSTRACT

Chinchilla lanigera is a hystricomorph rodent from South America whose reproductive biology presents particular characteristics that distinguishes it from other Rodentia species, such as low reproductive rate, seasonal breeding pattern, and long estrous cycle. Nevertheless, reproductive features in female chinchillas are still poorly investigated, with a scarce knowledge concerning the estrous cycle and the histology of reproductive organs. In this study, we investigate the morphology, histomorphometry, secretory activity, and immunolocalization of estrogen receptors ERα and ERβ in oviducts of nulliparous chinchillas, euthanized at fall season in Brazil. Follicular phase of estrous cycle of all studied animals was characterized by ovary and uterine morphology inspection, as well as vaginal cytology. Similar to other mammals, the oviduct wall of infundibulum, ampulla and isthmus was composed of mucosa, muscle, and serosa layers. Morphometric data of oviduct layers were used for identifying each oviduct segment. In the follicular phase, the oviduct was characterized by intense secretory activity, mainly in the ampulla, and expression of ERα and ERβ throughout the oviduct epithelium. Both ERα and ERβ were also detected in the connective tissue and smooth muscle cells. Our findings point out to the important role of estrogen in this female organ. Similar wide distribution of both ER proteins has been described for human Fallopian tube. Taken together, our data add to the understanding of the reproductive biology of female chinchillas, and may assist in the intensive breeding of this species and any eventual endeavor for conservation of chinchillas in the wild.

1. Introduction

The mammalian oviduct (or Fallopian tube in human) plays an essential role in reproduction, as it provides a suitable environment for the transport of gametes, fertilization, and early embryo development (Abe, 1996; Lyons et al., 2006; Hunter, 2012; Li et al., 2017). It is a tubular structure composed of three specialized segments, the infundibulum, ampulla, and isthmus, each with distinct morphological, cellular, molecular and functional characteristics (Leese, 1988; Gonella-Diaza et al., 2017). The oviduct mucosa is lined by a single layer of cuboidal or columnar epithelium, which may appear pseudostratified due to crowding nuclei at different height. The epithelium is supported by the lamina propria of connective tissue, followed by the smooth muscle layer and outer serosa (Donnez et al., 1985; Ito et al., 2016; Li et al., 2017).

The oviductal epithelium contains two main cell types: ciliated and non-ciliated secretory cells. The ciliated cells are mainly involved in gamete and embryo transportation, whereas the secretory cells produce the oviductal luminal fluid. For that, the latter cell type presents a typical structure of cells that actively synthesize proteins, amino acids, and various molecules that maintain and modulate dynamic fluid-filled milieu (Ghersevich et al., 2015; Nah et al., 2017).

The cellular composition of the oviductal epithelium, as well as the luminal fluid content, differs along the oviduct and changes temporally in response to alterations in sex-steroid hormone levels during the estrous cycle (Lyons et al., 2006; Winuthayanon et al., 2015). Those hormones, mainly 17β-estradiol and progesterone, play pivotal roles in ciliogenesis, fluid secretion, and cell growth (Reuquén et al., 2015; Gonella-Diaza et al., 2017). In luteal phase, the epithelium exhibits a regressed status with marked deciliation. In contrast, cells reenter in a proliferative status during the follicular phase, with an increased secretory activity and ciliary beating (Chen et al., 2013). At this phase, the estrogens are the main hormones acting in the oviduct. The biological actions of estrogen are mediated by the specific estrogen receptors

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ERα (ESR1) and ERβ (ESR2), which are ligand-dependent transcription factors belonging to the nuclear hormone receptor superfamily. These two ER subtypes are derived from genes located on different chromosomes that appear to have overlapping but different tissue/cell expression and localization (Kuiper et al., 1998). They have been involved in regulating oviductal functions to support fertilization and preimplantation embryo development (Winuthayanon et al., 2015). Despite the importance of estrogen for female reproduction, the tissue and cell distribution of ERα and ERβ in the oviduct of mammals is still a matter of debate.

Several studies have improved the knowledge of the oviduct physiology in different mammalian species (Steinhauer et al., 2004; Ito et al., 2016; Gonella-Diaza et al., 2017; Nah et al., 2017). In this framework, chinchillas (*Chinchilla lanigera*) may become an interesting study model, as they present particular reproductive characteristics that distinguish them from most Rodentia species, such as low reproductive rate and seasonal breeding pattern. The long estrous cycle and gestation period, which lasts around 111 days (ranging from 105 to 118 days), besides the occurrence of haemomonochorial placenta are other reproductive aspects related to this Hystricomorpha rodent species (Weir, 1970; Tam, 1971; Gromadzka-Ostrowska et al., 1985; Mikkelsen et al., 2017). In fact, the reproductive biology of this species has been in focus over the last decade (Busso et al., 2005; Busso et al., 2007; Leal and França, 2009; Celiberti et al., 2013; Gramajo-Bühler et al., 2015; Mastromonaco et al., 2015). However, the female reproductive biology is still poorly investigated, with apparently no reports with respect to puberty, and a scarce knowledge concerning estrous cycle (Busso et al., 2012).

Considering the scarcity of information about the chinchilla oviduct, we aimed to provide site-specific aspects of the histology, morphometry, and ERα and ERβ expression in this female tract segment of captive chinchillas.

2. Materials and methods

2.1. Animals and tissue sampling

Thirty nulliparous female chinchillas (5 to 7 month of age; 458.3 ± 0.08 g) obtained from a commercial farm were used. The animals were kept in individual cages (Fig. 1A) and housed indoors with controlled temperature (21 °C) and natural photoperiod (12:12 h light/dark cycles) from birth, at the coordinates of Nova Lima city (19°59′9″s; 43°50′49″W), state of Minas Gerais, Brazil. The chinchillas received concentrate food (Purina®), alfalfa hay, and water *ad libitum*.

Euthanasia was performed during fall season (April and May), by intramuscular administration of xylazine (0.5 mg/kg) and ketamine hydrochloride (40 mg/kg), as described by Morgan et al. (1981). Vaginal smear samples were collected by the aspiration technique previously described (Bekyürek et al., 2002). Ovaries, uterine horns, and oviducts were removed and dissected (Fig. 1B). This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health recommendations. All the experimental procedures were approved by the Committee on the Ethics and Use of Animal Experiments of the Universidade Federal de Minas Gerais (CETEA/ UFMG process number 221/2006).

2.2. Histology and morphometry

Ovaries, uterine horns, and oviducts were immersed in buffered 4% paraformaldehyde solution, dehydrated in crescent ethanol series (70, 80, 90, and 100%), and embedded in paraffin (Neves et al., 2006). Oviduct sections with 5 µm thickness were obtained using a rotary microtome (RM 2255, Leica Buisystems, Nussloch, Germany), stained with Hematoxylin and Eosin (HE), and qualitatively analyzed under light microscope (Olympus CX40, Tokyo, Japan). Histological sections of ovary were used for identifying the sexual maturity of each animal. To that end, we evaluated the presence of follicles at different developmental stages and corpora lutea (Senger, 2005). Additionally, the endometrial histology and vaginal cytology were used for determining the phase of the estrous cycle.

Other sections of the oviducts were stained with periodic acid-Schiff (PAS), and alcian blue pH 2.5 (Bancroft and Stevens, 1996). The epithelial secretion pattern was scored by its intensity as (1) discrete, when secretory cells were focally labeled either in the apical cytoplasm or glycocalyx, (2) moderate, when approximately 50% of secretory cells were positively labeled either in the cytoplasmic granules or luminal extrusion, and (3) intense, when most secretory cells and lumen presented labeled cytoplasmic granules and luminal extrusion (Sant'Ana et al., 2004).

For morphometric analysis, digital images of the HE stained sections from infundibulum, ampulla, and isthmus were obtained using a digital camera (Olympus DP73, Tokyo, Japan) connected to a light microscope (Olympus BX-53, Tokyo, Japan), and then analyzed with the Image-Pro Plus® 4.5 (Media Cybernetics, Silver Spring, USA) software. Transverse sections were used to measure the thickness (µm) of oviduct wall, mucosa, muscle and serosa layers. The height of mucosal folds and epithelial cells (µm; $n = 40$ cells/section) was also measured.

2.3. Western blotting

Western blot assays were performed on the oviducts to validate the use of the antibodies in chinchillas, and to confirm the immunohistochemistry results. Frozen oviducts and ovary were macerated using dry ice. The pulverized tissue was homogenized in 300 µL of 8 M urea buffer containing 20 mM Tris-HCl pH 7.5, 0.5 mM EDTA pH 8.0, and 10% protease inhibitor cocktail (#P2714, Sigma-Aldrich, Missouri, USA). The samples were sonicated at amplitude of 30% for 30 s (Sonics & Materials, USA) on crushed ice and centrifuged (14.000*g*) for 10 min at 4 °C for total protein extraction.

Protein content was determined by Bradford reagent using bovine serum albumin as a standard. Total protein extract (80 µg) was mixed in loading buffer containing 1% sodium dodecyl sulfate, 30 mM Tris-HCl pH 6.8, 2-mercaptoethanol, 20% (v/v) glycerol and bromophenol blue. After 5 min of boiling, the samples were subjected to continuous electrophoresis using 10% SDS-PAGE. A prestained protein molecular weight standard (PageRuler, Thermo Scientific, Waltham, USA) was used as a reference, and the separated proteins were transferred to a nitrocellulose membrane (Immobilon NC, Merck Millipore, Massachusetts, USA). The membranes were blocked with 10% normal goat serum for 1 h at room temperature, and then incubated overnight in a cold chamber with mouse monoclonal anti-ERα (#NCL-L-ER-6F11, Novocastra Laboratories, Newcastle, UK), diluted 1:200, or mouse monoclonal anti-ERβ antibody (#NCL-ER-beta, Leica Biosystems, Newcastle, UK), diluted 1:150. After washing in PBS-0.05% Tween (PBST), the membrane was incubated with a biotinylated goat anti-mouse secondary antibody (Dako, Glostrup, DK), diluted 1:1000, followed by incubation with avidin–biotin complex (Vectastain Standard ABC kit, Vector Laboratories, Burlingame, USA). Finally, the reaction was visualized by the addition of 0.1% 3,3′diaminobenzidine in PBS containing 0.05% chloronaphthol, 16.6% metanol and 0.04% H_2O_2. Protein extract from rat efferent ductules and ventral prostate was also loaded in assays as positive control from ERα and ERβ, respectively. The β-actin signal was used as internal control.

2.4. Immunohistochemistry

Oviducts fixed in buffered 4% paraformaldehyde and embedded in paraffin were sectioned (5 µM) and processed for immunohistochemistry to detect ERα and ERβ. Sections were blocked for endogenous peroxidase activity with 0,6% H_2O_2 in methanol and subjected to microwave antigen retrieval followed by blocking of endogenous biotin and avidin using a commercial kit (Avidin/Biotin

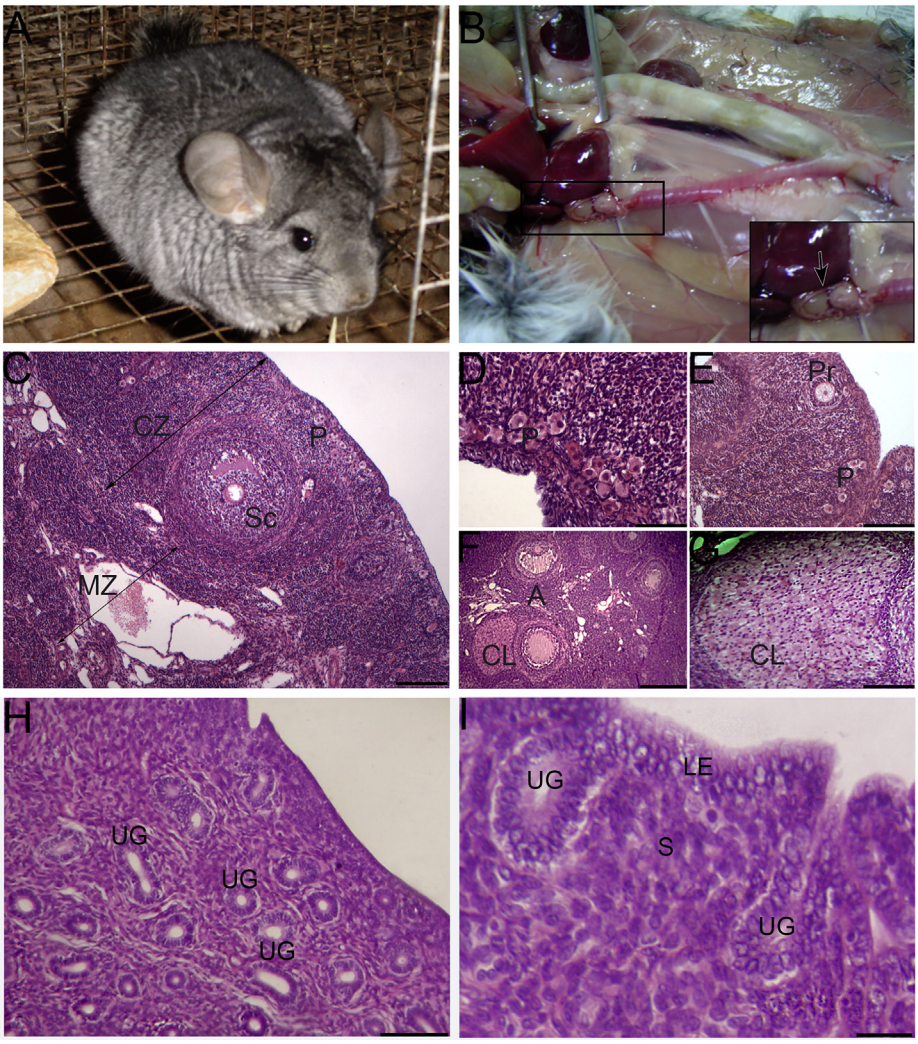

Fig. 1. Morphology of the ovary, oviduct and endometrium of captive *Chinchilla lanigera*. (A) External aspect of the chinchilla. (B) Anatomy of the female reproductive system. Higher magnification of the depicted area (insert) shows the ovary and oviduct (arrow). (C and D) Histology of the ovary shows ovarian follicles in different stages of development. (H) Endometrium showing straight and narrow uterine glands. (I) Detail of the endometrium. A = antral follicles; CL = corpus luteum; CZ: cortical zone; LE = luminal epithelium; MZ = medullar zone; P = primordial follicles; Pr = primary follicle; Sc = secondary follicle; S = stroma; UG = uterine glands. Hematoxilin-eosin staining (HE). Scale Bars (C) 100 μm, (D, G and I) 20 μm, (E and F) 40 μm, (H) 50 μm.

blocking kit, Vector Laboratories, Burlingame, USA). Then, non-specific binding was blocked with 10% normal goat serum prior to the incubation for 40 h at 4 °C with the monoclonal anti-ERα (#NCL-L-ER-6F11, Novocastra Laboratories, Newcastle, UK) or anti-ERβ (#NCL-ER-beta, Leica Biosystems, Newcastle, UK) primary antibodies, diluted 1:25 in phosphate buffer solution (PBS, pH 7.4). For negative controls, the sections received PBS in place of the primary antibodies. After washing in PBS, the sections were exposed for 1 h at room temperature to a biotinylated goat anti-mouse secondary antibody (Dako, Carpinteria, CA, USA), diluted 1:100 in PBS. After this step, the sections were incubated with the avidin–biotin complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, USA, for ERα) or with Streptavidin (Streptavidin/Biotin Blocking Kit, Vector Laboratories, Burlingame, USA, for ERβ) for 30 min. The immunoreaction was visualized using diaminobenzidine containing 0.01% H_2O_2 in 0.05 M Tris–HCl buffer, pH 7.6. Sections were slightly counterstained with Mayer's hematoxylin and mounted.

2.5. Statistical analysis

Analysis of variance (ANOVA) was used to compare histomorphometric parameters of the oviduct considering regions as factors (infundibulum, ampulla, and isthmus) and histomorphometric parameters as response variables. Then, we performed a posteriori Student-Newman-Keuls test to account for the differences between each pair of factors (Zar, 2010). All of our response variables followed the ANOVA premises, which are normal distributions and homogeneous variances (Zar, 2010). The statistical analysis was performed using statistical software (GraphPad Software, Inc., San Diego, CA, USA). Differences were considered significant when $P < 0.05$. Results were expressed as mean ± standard deviation (*SD*).

3. Results

3.1. Characterization of ovary and endometrium histology, and vaginal cytology

The ovary presented evident outer cortical zone and inner medullar zone (Fig. 1C). The former contained primordial follicles and other follicles in advanced developmental state (Fig. 1C–F). The follicles were surrounded by the stroma, which was composed of connective tissue with abundant collagen fibers and fibroblasts (Fig. 1C–F). Corpus luteum in apparent functional state exhibiting luteal cells and blood vessels (Fig. 1F–G), besides corpora lutea in regressing stages were also observed in the cortical zone (data not show). The medullar zone comprised nerves and blood vessels immersed in the connective tissue (Fig. 1C).

The endometrium in all studied animals showed a simple columnar epithelium and an underlying stroma of connective tissue (Fig. 1H–I). The epithelium was found lining the endometrial lumen (luminal epithelium) and forming the uterine glands, which were short, straight and narrow, with no secretion in the lumen (Fig. 1H–I). Finally, vaginal smears presented high number of superficial cells, small and large intermediate cells, as well as parabasal cells and neutrophils in trace

Table 1
Biometry of the oviduct of captive *Chinchilla lanigera* during the follicular phase of estrous cycle (mean ± *SD*).

Parameters	Left oviduct	Right oviduct
Length (cm)	4.37 ± 0.25	4.42 ± 0.77
Weight (mg)	27.0 ± 9.0	21.0 ± 6.0

amounts (data not shown).

3.2. Morphology of the oviduct regions

The gross anatomy showed the chinchilla oviduct as a single, long, and convolute tube, surrounded by the mesosalpinx. Each oviduct presented the distal part in the vicinity of the ovary, and the proximal portion connected to the uterus (Fig. 1B). The tube diameter was approximately 1 mm in the left and right oviducts. The total length and weight were also similar when left and right oviducts were compared (Table 1).

Three regions in the oviduct of chinchillas were histologically identified, the infundibulum (Fig. 2A–F), ampulla (Fig. 2G–L), and isthmus (Fig. 2M − R). The oviduct wall comprised the mucosa, composed of epithelium and lamina propria, followed by the muscle layer, composed of smooth muscle, and the serosa layer, composed of a thin layer of connective tissue and the mesothelium (Fig. 2A, G and M).

The infundibulum was characterized by the presence of numerous long finger-like projections of the mucosa going into the lumen (Fig. 2A and B). Mucosa folds were observed along the entire oviduct, but exhibiting varying number and height depending on the segment analyzed (Fig. 2B, H, N; Table 2). The infundibulum wall was the thinnest compared to other oviduct segments ($P < 0.05$; Table 2). Although there was no significant difference among oviduct segments regarding the thickness of the mucosa ($P > 0.05$; Table 2), higher mucosa folds and epithelium were found in the infundibulum compared to ampulla and isthmus ($P < 0.05$; Table 2). In the ampulla (Fig. 2G–H), the mucosa fold and epithelium heights as well as the muscle layer thickness were comparable to those of the isthmus ($P > 0.05$; Table 2). Finally, the isthmus presented the thickest serosa layer and overall oviduct wall (Fig. 2M) compared to other segments ($P < 0.05$; Table 2). The remarkable characteristic of this region was the presence of few and shorter mucosa folds (Fig. 2M − N) when compared to the infundibulum (Fig. 2A and B).

Regardless the oviduct segment, the mucosa was lined by a columnar epithelium composed of ciliated cells (Fig. 2H) and non-ciliated secretory cells (Fig. 2N). The epithelium was supported by the lamina propria (Fig. 2B, H and N), mainly visible in the mucosa folds from infundibulum (Fig. 2B) and ampulla (Fig. 2H). The muscle layer was organized into an inner, relatively thick circular layer of smooth muscle cells, and an outer, thinner longitudinal layer of cells. The boundary between these layers was easily visible in all segments (Fig. 2H and 2N), except infundibulum (Fig. 2B).

3.3. Characterization of the epithelium secretion

The intensity of PAS-positive secretion was discrete in most infundibulum sections (Table 3), which was mainly detected in the apical portion of the epithelium (Fig. 2C). Other sections from this oviductal segment (37.5%) were scored as moderate and intense (Table 3). Inversely, most ampulla sections were scored as intense secretion, followed by discrete and moderate (Table 3). Moderate intensity of PAS-positive secretion was observed in ampulla and isthmus (Table 3; Fig. 2I and O). However, the latter segment presented most of its sections scored as discrete, and only 10% scored as intense secretion (Table 3).

Moreover, the intensity of alcian blue-positive secretion was discrete in most infundibulum sections (Table 3; Fig. 2D), whereas the secretion in ampulla was mostly scored as moderate followed by discrete (Table 3; Fig. 2 J). Similarly, isthmus segment presented few sections showing intense secretion activity (Table 3; Fig. 2P), being mostly scored as moderate followed by discrete secretion (Table 3).

3.4. Expression of ERα and ERβ along the oviduct in chinchillas

ERα and ERβ proteins were detected in all segments of the chinchilla oviduct. Intense immunopositivity for both ERα and ERβ were observed in the nuclei of the epithelial secretory and ciliated cells, as well as the smooth muscle cells in infundibulum (Fig. 2E–F), ampulla (Fig. 2K–L), and isthmus (Fig. 2Q–R). Many nuclei of the connective tissue cells from the lamina propria were positive for ERα and ERβ, but some nuclei were negative. There was no remarkable difference in the ERα and ERβ expression when infundibulum, ampulla, and isthmus were compared.

The specificity of the antibodies used was confirmed by detection of main double bands of around 68 and 70 KDa for ERα and 54 kDa and 48 kDa for ERβ (Fig. 3), both within the expected molecular mass previously described for these receptors (Kuiper et al., 1996, 1998; Leav et al., 2001; Saunders et al., 2001; Zhou et al., 2002; Rago et al., 2007; Oliveira et al., 2012; Dominguez and Micevych, 2010; Dominguez et al., 2013; Morais-Santos et al., 2015).

4. Discussion

The results provide pioneer information concerning the oviduct of captive chinchillas, focusing on morphology, histomorphometry, secretory activity, as well as expression of estrogen receptors ERα and ERβ, during the follicular phase of the estrous cycle. In fact, the female reproductive physiology, female tract morphology, and sexual behavior of chinchillas have been poorly explored (Busso et al., 2012). The duration of estrous cycle, for instance, is still controversial in the literature, ranging from 15 days to 90 days, depending on the technical approach used (Weir, 1970; Tam, 1971; Bekyürek et al., 2002; Busso et al., 2012). Therefore, our data add to the understanding of the reproductive biology of this species, which is best known as fur-bearing animals (Hoefer, 1994; Jiménez, 1996), besides being important as pet, as well as animal model for biomedical studies over human pregnancy and auditory researches (Prior, 1986; Shimoyama et al., 2016; Mikkelsen et al., 2017).

Herein, the vaginal cytology and endometrial histology outcomes indicated that both organs were under the influence of estrogen, which is the main hormone regulating the follicular phase of estrous cycle (Senger, 2005). The morphological and functional changes of uterus and vagina are sex steroid hormones dependent, so that they are important targets for assessing cyclic changes throughout the estrous cycle (Steinhauer et al., 2004; Arai et al., 2013; Arnon et al., 2014). In chinchillas, although changes in the vaginal mucosa along the estrous cycle have been previously described (Bekyürek et al., 2002; Celiberti et al., 2013), information concerning endometrial features is still unknown.

The ovarian histology confirmed the sexual maturity of the chinchillas examined, and presented morphological features compatible with the follicular phase (Weir, 1970; Senger, 2005). Herein, the ovarian architecture found for nulliparous animals was similar to that observed for adult chinchillas, as well as for other Hystricomorpha rodents in the follicular phase of the cycle (Cooper and Schiller, 1975; Sánchez-Toranzo et al., 2014). It has been determined that, during the follicular phase, the adult chinchilla ovary usually presents follicles in different developmental stages, including antral follicles, whereas senile females show few developing follicles (Sánchez-Toranzo et al., 2014). Moreover, it is possible to observe corpora lutea in different stages of development including regressing stages, as functional luteolysis occurs before the morphological luteolysis (McCracken et al., 1999).

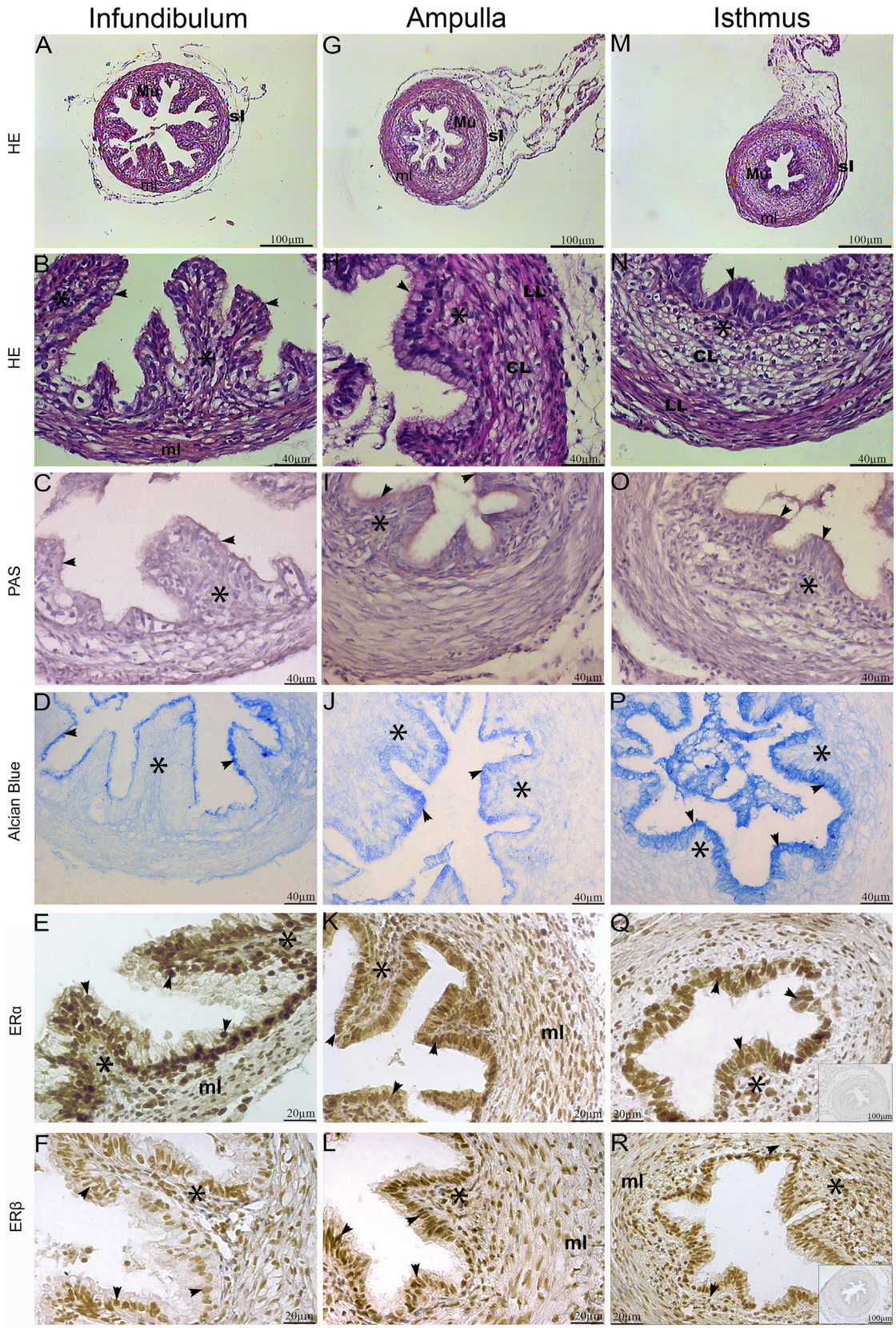

Fig. 2. Histology, secretory activity and estrogen receptor ERα and ERβ expression in the oviduct of captive *Chinchilla lanigera*. (A, G and M) General morphology of the oviduct infundibulum, ampulla and isthmus. (B, H and N) Detail of the oviduct segments' wall. (C, I and O) PAS staining of the oviduct segments. (D, J and P) Alcian blue staining of the oviduct segments. (E, K and Q) Expression of ERα in the oviduct segments. Insert in Q = negative control. (F, L and R) Expression of ERβ in the oviduct segments. Insert in R = negative control. Arrowhead = epithelium; asterisk = lamina propria; CL = circular muscle layer; LL = longitudinal muscle layer; ml = muscle layer; Mu = mucosa; sl = serosa layer; HE = Hematoxilin-eosin staining; PAS = periodic acid-Schiff staining.

Table 2
Histomorphometry of the oviduct of captive *Chinchilla lanigera* during the follicular phase of estrous cycle.

Parameters	Oviduct segments		
	Infundibulum	Ampulla	Isthmus
Oviduct wall thickness (μm)	0.09 ± 0.01[a]	0.18 ± 0.01[b]	0.23 ± 0.01[c]
Mucosal folds height (μm)	0.196 ± 0.021[a]	0.096 ± 0.053[b]	0.13 ± 0.061[b]
Mucosa layer thickness (μm)	0.051 ± 0.015[a]	0.093 ± 0.019[a]	0.082 ± 0.006[a]
Epithelium height (μm)	0.032 ± 0.02[a]	0.028 ± 0.01[b]	0.029 ± 0.02[b]
Muscle layer thickness (μm)	0.05 ± 0.01[a]	0.13 ± 0.02[b]	0.16 ± 0.01[b]
Serosa layer thickness (μm)	0.009 ± 0.005[a]	0.013 ± 0.001[a]	0.021 ± 0.001[b]

Mean ± *SD*. [a,b,c]Different superscript letters in the same row indicate significant differences between these measurements ($P < 0.05$) by ANOVA with post hoc Student-Newman-Keuls.

Table 3
Epithelial secretion intensity in the oviduct of captive *Chinchilla lanigera* during the follicular phase of estrous cycle.

Parameters	Oviduct segments		
	Infundibulum	Ampulla	Isthmus
PAS (Periodic acid-Schiff)			
Discrete	15 (62.5%)	10 (35.71%)	18 (62.07%)
Moderate	4 (16.67%)	6 (21.43%)	8 (27.59%)
Intense	5 (20.83%)	12 (42.86%)	3 (10.34%)
Number of sections analyzed	24 (100%)	28 (100%)	29 (100%)
Alcian Blue pH *2.5*			
Discrete	13 (68.42%)	14 (28.57%)	10 (31.25%)
Moderate	5 (26.31%)	23 (46.93%)	19 (59.37%)
Intense	1 (5.26%)	12 (24.49%)	3 (9.37%)
Number of sections analyzed	19 (100%)	49 (100%)	32 (100%)

Secretion intensity: Discrete – secretory cells focally labeled either in the apical cytoplasm or glycocalyx; Moderate – approximately 50% of secretory cells positively labeled either in the cytoplasmic granules or luminal extrusion; Intense – most secretory cells and lumen positively labeled in the cytoplasmic granules and luminal extrusion (according to Sant'Ana et al., 2004).

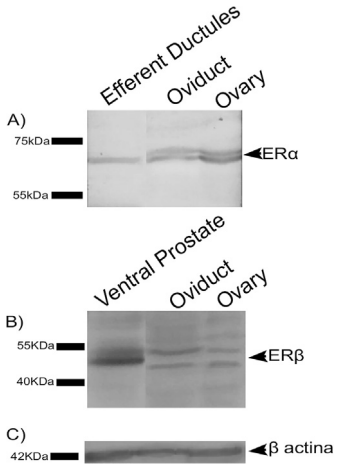

Fig. 3. Western blotting assays confirming the specificity of the antibodies used in chinchilla tissues. (A) Estrogen receptor ERα. (B) Estrogen receptor ERβ. The respective molecular weights are shown on the left. Rat efferent ductules and ventral prostate were used as positive control. β-actina (42KDa) was used as internal control.

In the follicular phase, estrogen is essential for smooth muscle contractility and ciliary beating, besides modulation of the secretory activity of the oviduct epithelium, providing a supportive environment for fertilization and embryo development (Reuquén et al., 2015; Winuthayanon et al., 2015; Kowalik et al., 2016; Li et al., 2017). The key to the biological effects of estrogen is the activation of the ERα and ERβ (Kuiper et al., 1998). Within the female reproductive system, ERα appears to be the predominant subtype, whereas ERβ predominates in the ovary and mammary gland (Ulbrich et al., 2003). In relation to this, ERα mRNA and protein have been detected in the epithelium, connective tissue and smooth muscle layers of oviducts from diverse mammals' species (Sar and Welsch, 1999; Jefferson et al., 2000; Pelletier et al., 2000; Mowa and Iwanaga, 2000; Wang et al., 2000; Okada et al., 2003; Ulziibat et al., 2006; Valle et al., 2007; Orihuela et al., 2009; Kenngott et al., 2011; Horne et al., 2009; Li et al., 2017). Expression of ERβ has been controversial, as some authors have found the receptor in few epithelial cells of rat oviduct (Wang et al., 2000; Okada et al., 2003), restricted to the cilia of ciliated cells in mice oviduct (Li et al., 2017), sparse mRNA in the rat muscle layer (Mowa and Iwanaga, 2000), or absence at all in rats and mice oviducts (Sar and Welsch, 1999; Jefferson et al., 2000; Pelletier et al., 2000). Conversely, others have found ERβ mRNA and/or protein in the epithelial cells, connective cells and muscle cells of human Fallopian tube (Taylor and Al-Azzawi, 2000; Ulziibat et al., 2006; Horne et al., 2009). In the current study, both ERs were detected in ciliated and secretory epithelial cells, smooth muscle cells, as well as some connective cells from all oviduct segments. The widespread of both ERs in all compartments of the oviduct provides further evidence for a functional importance of the estrogenic responsive system in the follicular phase of the chinchilla estrous cycle. Furthermore, our findings are in line with those previously described for human Fallopian tube (Ulziibat et al., 2006; Horne et al., 2009), corroborating that chinchilla may be a suitable animal model for human obstetric research (Mikkelsen et al., 2017).

Concerning the oviducts, the histological features presently described are similar to those reported for other mammals such as bitch, sow, rat, and cow (Steinhauer et al., 2004; Chen et al., 2013; Reuquén et al., 2015; Gonella-Diaza et al., 2017). In chinchillas, the morphometric data of the oviduct wall and layers were used for identifying each oviduct region during the follicular phase.

Similarly to uterus and vagina, the oviduct morphology changes markedly during the estrous cycle, and steroid hormones strictly modulate the epithelium and mucosa fold heights (Gonella-Diaza et al., 2017). While the oviduct epithelium exhibits substantial reduction in cellular height in luteal phase, there is increased epithelial height and secretory activity during the follicular phase (Chen et al., 2013). The increase in epithelial height may increase the area of intercellular space available for ion transport, whereas the increased folding of the mucosal surface may result in greater secretory capacity (Leese et al., 2001; Gonella-Diaza et al., 2017). In this context, the secretion pattern of neutral and acid glycoproteins and carbohydrates, as well as mucins presently found are in accordance with the estrous stage predominantly influenced by estradiol. Indeed, there is evidence that the amount of oviductal glycoproteins is highest during the periovulatory period in response to estrogen stimulation (Leese et al., 2001; Kowalik et al., 2016; Albors et al., 2017). Furthermore, the secretory activity was mainly detected in the ampulla segment compared to isthmus and infundibulum of the chinchillas. These data corroborate others showing the ampulla producing more fluid than isthmus, thus reflecting the greater surface of the ampulla cells (Leese et al., 2001; Gonella-Diaza et al., 2017). There is paucity of information concerning the morphological changes in the infundibulum, with special regard to its secretion capacity during follicular phase. At this phase, the oviductal fluid has important role in reproductive events, such as mediation of sperm-oviduct epithelial cell interaction, modulation of sperm functions in the oviduct, and promotion of sperm-zona pellucida interaction culminating with fertilization. These events mostly occur in ampulla and

isthmus microenvironments (Leese et al., 2001; Ghersevich et al., 2015), thus corroborating the present findings concerning chinchilla oviduct.

It is known that chinchilla females do not routinely achieve either maximum fertility or fecundity at breeding facilities (Busso et al., 2012). The low egg-recovered rates from chinchilla oviducts reported by Weir (1973) might be related to this fact. Hence, oviduct features described herein concerning epithelium secretion and expression of ERs may be associated with fertilization failure and early embryonic loss. However, as we did not present data about fertilization and embryonic development, this statement should be taken with care. Nevertheless, our findings might be considered a baseline to future studies testing the possible mechanisms involved in the low fertility of this species. Therefore, collectively, our findings are the first pieces of information about the oviduct physiology in captive chinchillas that may assist to establish reproductive strategies in the commercial breeding, as well as in any eventual endeavor for conservation of chinchillas in the wild.

5. Compliance with ethical standards

Declaration of interest None of the authors have any conflict of interest to declare.

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