Detection of the Hormone Receptors Controlling the Female Genital Tract throughout the Sexual Cycle of an Oviparous Caecilian Amphibian, *Boulengerulataitana*

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ABSTRACT

Aims: Hormonal control of the development of the oviducts, as inferred by the distribution of hormone receptors, is described for the first time in the oviparous caecilian, *Boulengerulataitana* (Gymnophiona: Herpelidae).A comparison is made with the aquatic viviparous caecilian, *Typhlonectescompressicauda*.

Study design: The study was performed from histological sections of the left oviduct of three *B. taitana*females collected at different periods of the sexual cyclein the Taita Hills, Kenia, from April 2003 to March 2004. The hormone receptors were localized with indirect immunohistochemical method. Sixty counts were carried out per antibody and per season in the various parts of the oviduct. Analysis of variance (one-way ANOVA) and Fisher test (LSD) permitted to determine the significant differences between the groups ($p \leq 0.05$).

Place and Duration of Study: Sciences and Humanities Confluence research Center, UCLy, cedex 02, 69288 Lyon, France

Methodology: By using an immunohistochemical staining method, the specific receptors of steroid hormones (α- and β estrogen, progesterone) and pituitary hormones (gonadotropin, prolactin) were detected in the tissues of the ostium and the oviduct during the female sexual cycle.

Results: The number of immunostained cells reflects the sensitivity of each part of the genital tract to hormonal control, related to its physiological functions. The large number of progesterone receptors detected in tissues during the preovulation period implies the key role of progesterone in preparing the oviduct for ovulation. The two estrogen receptors are differently detected in the tissues, suggesting a specific physiological function of each isoform. The presence of pituitary hormone receptors in the cells during the reproductive period suggests direct control of these hormones on the development and functions of the genital tract. Similarities are observed in the neuroendocrine control of this oviparous species and the viviparous caecilian *Typhlonectescompressicauda*.

Conclusion: This study indicates the presence of steroid and pituitary hormone receptors in the genital tract of *B. taitana,* with variations closely related to the key events of seasonal reproductive activity, and confirm that oviduct morphology is closely correlated with ovarian function. A comparative study of the detection of these hormone receptors also revealed correspondence between the parts of the oviduct in the oviparous and the viviparous species *T. compressicauda*. Despite differences in reproductive mode, similarity in receptor distribution between the two species suggests conservative physiological control of the reproductive cycle in caecilians.

Keywords: Caecilian; oviduct; sexual steroid receptors; gonadotropin receptors; prolactin receptors.

1. INTRODUCTION

1.1 Receptors of pituitary and steroid hormones

Pituitary and steroid hormones act by binding to receptors expressed on target cells. The FSH and LH receptors (FSHR and LHR) are membrane-bound receptors that belong to the large family of G protein-coupled receptors and constitute the subfamily of glycoprotein hormone receptors [1]. Although the specificity of LH and FSH is somewhat less important in anamniotic vertebrates than in mammals, it has been shown that the two hormones cause different actions in amphibians [2]. The PRL receptor (PRLR) is a membrane-bound protein, that belongs to the cytokine receptor superfamily [3]. The presence of PRLR has been described in a large range of organs of different animals [4]. In the amphibian *Xenopus laevis*, PRLR mRNA was detected in the oviduct [5].

Steroid hormones act on the target tissues by inducing biological response initiated by the hormone binding to an intracellular receptor, which belongs to the superfamily of nuclear receptors. This receptor type acts in the cell nucleus as transcription factor to selectively regulate gene expression [6]. The α- and β estrogen receptors (ERα, ERβ) and progesterone receptor (PR) belong to this superfamily. They regulate protein synthesis, which in some cases is involved in cell proliferation and apoptosis affecting oviduct morphology during the sexual cycle [7]. The localization of steroid hormone receptors has been little studied in amphibians. ERs and PR are detected in the tissues of the oviduct of *Typhlonectescompressicauda*, in the brain of *Pelophylax esculenta* and the level of the hormone-binding molecules quantified during the reproductive cycle [7,8,9].

1.2 Oviparity and viviparity in caecilian amphibians

Gymnophiona are burrowing or aquatic elongate amphibians that mostly inhabit tropical areas. Caecilians constitute an interesting taxon to study modes of parity and evolutionary transitions from oviparity to viviparity, as viviparity has evolved independently at least four times [10-12]. Intermediary forms of reproduction have also been observed [13]. Viviparous species have the advantage of protecting embryos from deleterious environmental influences, but with the disadvantage of potential costs for the mother or the newborns [14-16]. Despite this evolutionary interest, the knowledge of reproductive cycles of caecilians is still imperfect. Some works show correlations between male and female sexual cycles [17], variations of endocrine tissues such as ovarian follicles and corpora lutea in females, or existence of pituitary control of the sexual cycle [18].

In oviparous species, males and females adopt an annual sexual cycle [19, 20]. In viviparous species, the pregnancy is several months long and the female cycle has been found to be biennial [21]. Either mode of reproduction is accompanied by significant changes in the morphology of the genital tract in relation to the elaboration of egg envelopes or the development of embryos [21,22]. Seasonal changes in the oviduct, throughout the ovarian cycle and/or during gravidity, have been studied in several species related to increase in mass, diameter, epithelial height and glandular activity [23,24]. Parental care has also been indicated in some species [25-29].

1.3 Reproductive biology of caecilians

Caecilian reproduction occurs during optimal environmental periods (rainfall, temperature). Environmental factors permit the modulation of the reproductive process, via the control of the hypothalamic-pituitary-gonadal axis [1,30]. The amphibian hypothalamic-pituitary-gonadal axis and the associated neuropeptides and hormones are highly conserved in vertebrates [31]. Activation of the central nervous system by environmental signaling leads to the release of the pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which are heterodimeric glycoproteins formed by a common α subunit and a specific β subunit [32]. These hormones reach the ovaries through the circulation and control oogenesis and gonadal endocrine function there. In all vertebrates, ovarian steroid hormones have a wide range of behavioral and physiological processes [2]. In particular, they control morphology and functions of the oviduct, whose precise synchronization with the ovarian cycle is essential for reproduction [33]. It has been found that cell proliferation, apoptosis and cell differentiation are involved in the tissue remodeling of the oviduct during the sexual cycle of amphibians [7]. Prolactin (PRL) is another hormone also involved in various aspects of reproduction in amphibians [20,34]. In particular, PRL together with sex steroids are necessary for the structural development and formation of oviductal secretions [35, 18]. PRL is a polypeptide hormone, primarily synthetized by the anterior pituitary, but also found in several organs of vertebrates [3].

1.4 Reproduction modality of *Typhlonectescompressicauda*, a viviparous caecilian species

The reproduction of *Typhlonectescompressicauda*, a South American viviparous species, has been well studied, and therefore provides a good model for comparative purposes. To this end, we summarize the major events during the female sexual cycle of *T. compressicauda*. The oviduct is divided into two parts. After the ostium, the short anterior part of the oviduct is flexuous, with glands involved in the synthesis of egg envelopes. The posterior part is differentiated as uterus, in which embryos develop [36,37]. Significant changes of the genital tract occur during the biennial reproductive cycle of females, which are closely linked to seasonal changes. The first year of the cycle begins during the dry season (October), with vitellogenesis, and continues until the beginning of rainy season (January). Before ovulation, the connective tissue of the anterior part of the oviduct is richly vascularized and supports a pseudo-stratified epithelium where glandular cells and ciliated cells develop. The uterine part increases significantly, the wall being bordered with vascularized crests, and both ciliated cells and glandular cells are observed. At ovulation, the ostium develops, the cilia of the anterior and posterior parts of the oviduct are covered with a layer of mucus. Mating and fertilization take place from the start of the second rainy season (February), and parturition from commencement of warming period (July). During pregnancy, the tubal part gradually involutes, while the uterine wall is subjected to a series of variations closely related to the stages of embryo development [36,37]. After parturition, the ostium and the oviduct become quiescent, bordered with a layer of undifferentiated cells [38]. A new vitellogenesis is observed at the beginning of the second year of the cycle but ovulation does not occur and oviducts are maintained quiescent until the beginning of a new biennial cycle.

The endocrine activity of the ovaries of *T. compressicauda* was studied by histochemical procedures. ∆5 3β hydroxy steroid dehydrogenase, involved in the steroid biosynthesis, were detected in both vitellogenic oocytes and the cells of the corpora lutea [39]. Estriol and 17β-estradiol are mainly observed in the granulosa of follicles containing vitellogenic oocytes [40]. Finally, the presence of progesterone was also detected in cells of the corpora lutea, whose pregnancy-related endocrine activity is similar to that observed in other viviparous Gymnophiona [32,41]. Cytological studies of the pituitary of females at different periods of their sexual cycle reveal volumetric variations in gonadotropic and lactotropic cells [42,43]. These different cells develop from October until April and decrease to reach a minimal size at parturition. In the next October, these cells begin to grow again, but regress in February.

A scheme of hormonal regulation of the female sexual cycle of *T. compressicauda* has been proposed [20]. During vitellogenesis, ovarian follicle cells are filled with estrogens, involved in the development of the genital tract, and with progesterone. When the quantity of the latter was sufficient in the target organs, ovulation is triggered. The genital tract is then fully developed. After ovulation, in follicles becoming corpora lutea, some cells synthesize progesterone. The uterine wall of the posterior part of the oviduct is modified according to the development of the embryo. At the end of pregnancy, the corpora lutea regresses and the genital tract returns to a resting state, indicating that the development and endocrine activity of corpora lutea are closely related to pregnancy. Various changes of gonadotropic and lactotropic cells are correlated with the endocrine activity of the ovary. The pituitary hormone cells developed during vitellogenesis and at the beginning of pregnancy, then decrease to reach a minimal size at parturition. During the second year, they increased again before the theoretical period of ovulation, then abruptly regress, in parallel with degeneration of the follicles and the lack of differentiation of the genital tract [20].

1.5 Reproduction cycle of Boulengerulataitana, an oviparous caecilian species

Boulengerulataitana, a caecilian endemic to the Taita Hills of southeast Kenya, is a burrowing, direct-developing, oviparous species. Its sexual cycle is closely related to annual temperature variation, and is characterized by three principal periods: preparatory phase before ovulation, reproductive period after ovulation and the sexual quiescent period [20,21]. The preovulatory period occurs in the austral spring (September – October), in which the reproductive system is prepared for reproduction, while the postovulatory period occurs during the highest temperatures (November – February), in which the oocytes are released into the coelomic cavity and then transported through the oviduct after internal fertilization. The sexually quiescent period is observed during the prolonged cooler season (March to August) [21,44]. The ostium, that accepts eggs into the *pars recta* during ovulation, is bordered with a single layer of cells with a striated brush-border during the reproductive period, and remains undifferentiated during the sexually inactive period [45]. Following the ostium, the oviduct is differentiated into three segments: the pars recta, the pars convoluta and the pars utera. The mucosa of the pars recta consists of an epithelium, in which some ciliated cells alternate with gland cells placed between the villi. The pars convoluta, that represents the main body of the oviduct, is bordered for half of its circumference with goblet and secreting cells, and glands, and for the other half with ciliated cells during the breeding period. The pars utera is the last portion of the oviduct which connects to the exterior via the cloaca. In this segment, the epithelium is constituted of glands and cells, also ciliated during the breeding period [23]. Each part of the oviduct therefore possesses its own cell morphology and functional properties allowing fertilization and transit of eggs during the reproductive period [18]. Outside this sexually active period, the oviduct is reduced in diameter, the lamina propria is narrower and the epithelium poorly developed, and without ciliated cells [45]. This tissue remodeling of the mucosa, implying proliferative and apoptotic activities, is dependent on the ovarian steroid hormone levels [23,46].

Previous work has not detected ovarian and pituitary hormone receptors in the genital tract. Doing so would provide a better understanding of tissue hormonal regulation of the sexual cycle of oviparous caecilians and how these changes with environmental factors. The aim of this work was to detect when PR, ERs, FSHR, LHR and PRLR in the cells of the different parts of the oviduct occur during the annual cycle of the oviparous caecilian, *B. taitana*, and furthermore to use this to specify some aspects of reproduction regulation. In addition, we discuss similarities and differences with previous observations made on the viviparous species *T. compressicauda* [47], in an attempt to inform on adaptation to viviparity in terms of functional adaptations of the genital tract and modes of hormonal action.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

Specimens of *B. taitana* were collected in the Taita Hills, Kenia, from April 2003 to March 2004 with support from the National Museums of Kenya and the Kenyan Wildlife Service for issuing collecting permits (via the Kenyan Ministry of Education Science and Technology research permit number MOEST13/001/36C 183 to JM: see [44] for details).

Three females from each of the three periods of the sexual cycle were lethally anaesthetized by submerging into 1% solution of MS222 (Sigma Aldrich, St Louis, MO, USA), fixed in formaldehyde (10%) and stored in 70% ethanol [21]. The left oviduct of each animal was dissected free from the ovary, dehydrated, embedded in paraffin and cut in 7 µm transversal sections. Some sections were stained with hematoxylin-eosin and Romeis' azan techniques for topographic examination.

The hormone receptors were localized in the tissue sections using a classical indirect immunohistochemical method [48]. After deparaffinization, the sections were subjected to antigen retrieval using a water bath for 45 min (Antigen unmasking solution, H-3300, Vector laboratories, Les Ulis, France), incubated in 3% H2O2 for 15 min in order to block endogenous peroxidase activity, and in bovine serum albumin (BSA) for 20 min to block non-specific antibody binding (Kit VECTASTAIN Elite ABC kit, Vector laboratories, Peterborough, UK). The slides were incubated for 1 h at room temperature with primary antibodies directed against the specific steroid hormone receptor (PR: 1:50, AbCys C 10-7068; ERα: 1:50, Santa Cruz sc-7207; ERβ: 1:50, Santa Cruz sc-8974), the specific gonadotropin receptor (FSHR: 1:50, Santa Cruz sc-13935; LHR: 1:50, Santa Cruz sc-25828) or the prolactin receptor (1:100, Santa Cruz sc-20992). The immunoreactions were visualized with a streptavidin-biotin amplification kit using Amino Ethyl Carbazole (AEC) as chromogen (Kit VECTASTAIN Elite ABC kit, Vector laboratories, Peterborough, UK). The slides were counterstained with hematoxylin QS and the preparations were mounted with VectaMount AQ Mounting Medium. Note that the sections were washed with Phosphate Buffered Saline (0.1M, pH 7.4) three times between the different steps of the procedure. For negative controls, deletion of primary or secondary antibodies were performed. Hormone receptor results are expressed as a percentage of labeled cells.

For statistical analysis, three slides from three specimens (equals 9 slides), one from each of the three periods of the sexual cycle (preparatory phase before ovulation, September-October, reproductive period after ovulation, November-February and the sexual quiescent period March-August [21]: total of 9 individuals) were considered, the number of labeled cells per thirty cells observed in the same microscopic field at x100 magnification for each cell type and expressed as a percentage. Connective and epithelial cells can be seen in all sections. The epithelium was constituted by ciliated cells in the ostium, secretory and ciliated cells in pars recta, secretory, goblet and ciliated cells in pars convoluta, secretory and ciliated cells in pars utera. The results were obtained for each antibody and for each season (60 counts were carried out per antibody and per season in the various parts of the oviduct. The Jarque Bera test confirmed the normal distribution of the results. Analysis of variance (one-way ANOVA) and then a Fisher test (LSD) permit to determine if the differences were significant between the groups ($p \leq 0.05$)

3. RESULTS

Steroid and pituitary hormone receptor immunoreactivity was observed in the different tissues of the genital tract throughout the year (Figure 1).

3.1 Detection of sex steroid receptors

The sex steroid receptors were only detected during the reproductive period. No labeling was detected during the sexual quiescent period, except occasionally in the secretory cells. Differences in the distribution of the nuclear steroid receptors were however observed depending on the specific receptor, the cell type, the part of the genital tract and the hormonal status of the female (Figures 2-4).

3.1.1 Progesterone receptors

3.1.1.1 In the ostium

Progesterone receptors (PR) were detected in both connective and ciliated cells. Between the preovulatory and postovulatory periods, the number of anti-PR positive connective cells increased significantly from 0% to 28.3%, while the number of anti-PR positive ciliated cells remained equal (at about 20%), except in sexual quiescent period when there was no signal detection (Figure 2A).

3.1.1.2. In the oviduct

The number of PRs detected varied according to the cell types (Figures 1A, 2B-D): in connective cells, it increased after the ovulation in both the pars recta and the pars convoluta and decreased in pars utera. In secretory cells, it decreased after ovulation in the pars recta, while it increased after ovulation in the pars convoluta and the pars utera. In goblet cells, the percentage of the labeled cells decreased after ovulation. And in ciliated cells, the PRs were detected exclusively after ovulation in both the pars recta and the pars convoluta and during the whole reproductive period in the pars utera.

Fig. 1. Immunohistochemical reactions in the oviduct of *Boulengerulataitana*. The arrows indicate positive labeling. Inserts illustrate negative control. (A) PR immunoreactivity in pars convoluta during the preovulatory period (September). Scale bars: 25 µm. (B): ERα immunoreactivity in pars utera during the preovulatory period (September). Scale bars: 25 µm. (C): ERα immunoreactivity in pars convoluta during the postovulatory period (January). Scale bars: 10 µm. (D): ERβ immunoreactivity in pars recta during the postovulatory period (January). Scale bars: 10 µm. (E): LHR immunoreactivity in ostium during the preovulatory period (September). Scale bars: 10 µm. (F): FSHR immunoreactivity in pars recta during the preovulatory period (September). Scale bars: 50 µm. (G): lack of PRLR immunoreactivity in pars utera during the sexually inactive period (June). Scale bars: 10 µm. (H): PRLR immunoreactivity in pars convoluta during the preovulatory (September). Scale bars: 10 µm

*Test drugs: significant from normal control, * $P < 0.05$; ** $P < 0.001$*

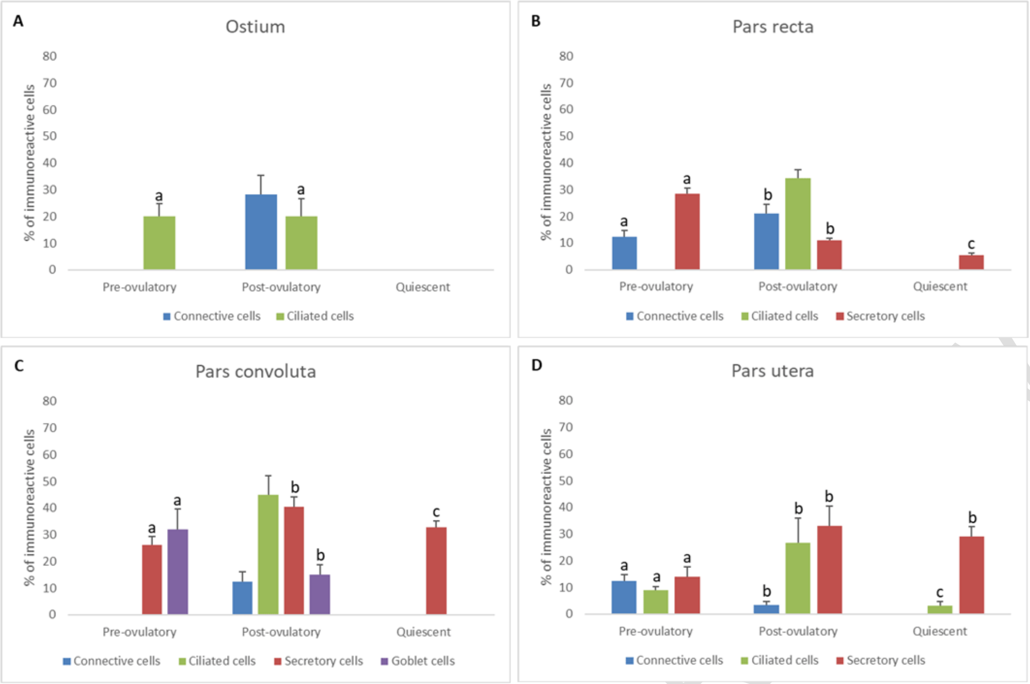

Figure 2. Immunodetection of PR in the different cell types of the genital tract of *Boulengerulataitana* during the sexual cycle. Bars marked with different letters are significantly different from each other (ANOVA, $p \leq 0.05$). (A) Ostium (connective cells: n = 6, Pr > F: 0.006; ciliatedcells: n = 7, Pr > F: 0.532), (B) Pars recta (connective cells: n = 7, Pr > F: 0.028; ciliatedcells: n = 6, Pr > F: 0.001; secretorycells: n = 6 Pr > F: 0), (C) Pars convoluta (connective cells: n = 9, Pr > F: 0.050; ciliatedcells: n = 6, Pr > F: 0.002; secretorycells: n = 8, Pr > F: 0.004; gobletcells : n = 6, Pr > F: 0.017), (D) Pars utera (connective cells: n = 7, Pr > F: 0.001; ciliatedcells: n = 7, Pr > F: 0.001; secretorycells: n = 7, Pr > F: 0.008).

3.1.2. Estrogen receptors

3.1.2.1. In the ostium

The number of both the connective and the ciliated cells with ERα and ERβ detected was significantly greater before ovulation and decreased after ovulation (Figures 3A, 4A).

3.1.2.2. In the oviduct

The different cell types were immunostained (Figures 1B-D, 3B-D, 4B-D). The connective cells of the pars recta were labeled for the two nuclear ER isoforms exclusively during the preovulatory period. In the other parts of the oviduct, the immunohistochemical profile of the cells was different; in pars convoluta, the percentage of labeled cells decreased abruptly after ovulation, while it increased significantly in the pars utera.

In the secretory cells of the pars recta, ERβ alone was expressed before ovulation, while ERα and ERβ together were expressed after ovulation. The increase of detected ERs in these cells during the reproductive period was also observed in the pars convoluta and the pars utera.

The goblet cells contained few detected ERs. Only the cells of females before ovulation were labeled, with a more important labeling for ERα (18.4% versus 2.8% respectively).

The ERs were poorly detected in the ciliated cells of the oviduct. In particular, no estrogen receptor was located in the pars recta, and ERα was the only form detected in the pars convoluta.

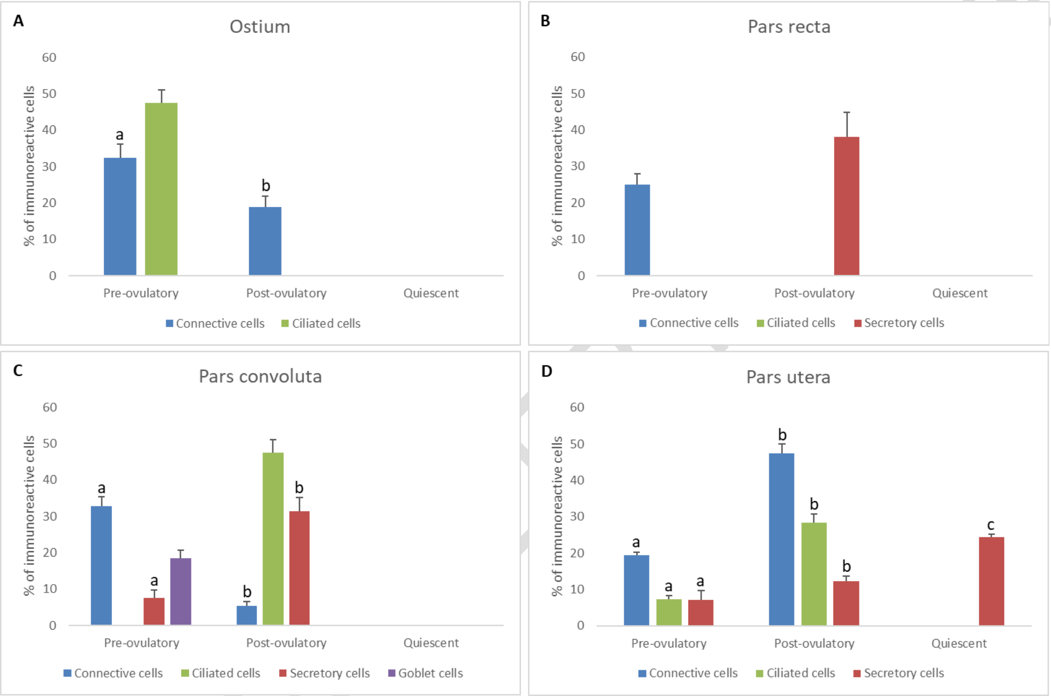

Figure 3. Immunodetection of ERα in the different cell types of the genital tract of *Boulengerulataitana* during the sexual cycle. Bars marked with different letters are significantly different from each other (ANOVA, p ≤ 0.05). (A) Ostium (connective cells: n = 6, Pr > F< 0.002; ciliatedcells: n = 6, Pr > F: 0), (B) Pars recta (connective cells: n = 7, Pr > F: 0; secretorycells: n = 6, Pr > F: 0.003), (C) Pars convoluta (connective cells: n = 8, Pr > F: <0.0001; ciliatedcells: n = 6, Pr > F: 0; secretorycells: n = 8, Pr > F: <0.0001; gobletcells: n = 5, Pr > F: 0.013), (D) Pars utera (connective cells: n = 6, Pr > F: 0.001; ciliatedcells: n = 5, Pr > F: <0.0001; secretorycells: n = 7, Pr > F: 0.002).

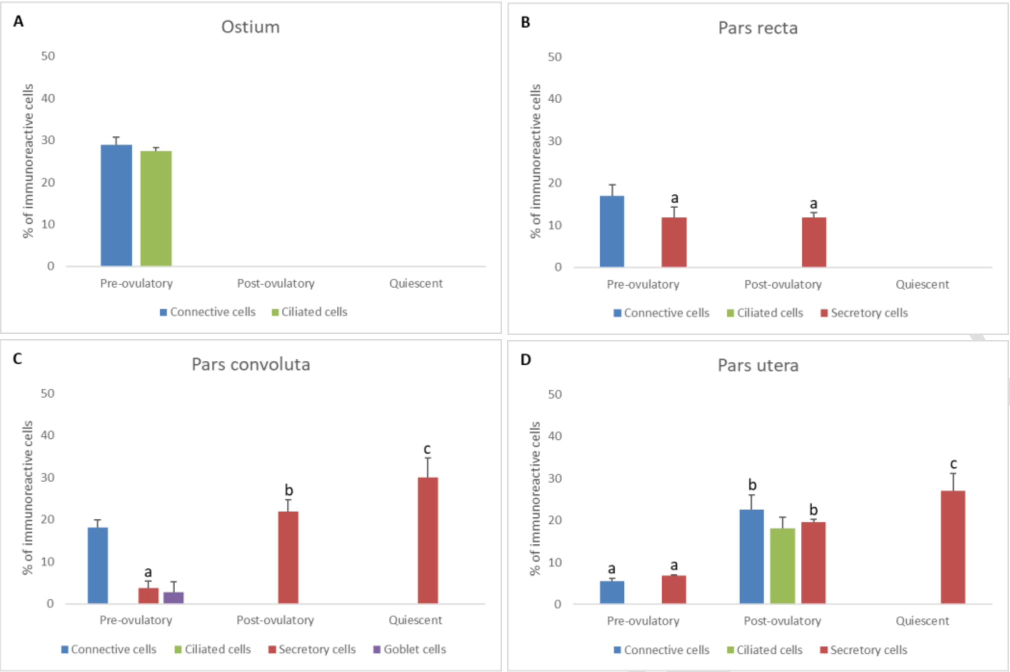

Figure 4. Immunodetection of ERβ in the different cell types of the genital tract of *Boulengerulataitana* during the sexual cycle. Bars marked with different letters are significantly different from each other (ANOVA, p ≤ 0.05). (A) Ostium (connective cells: n = 7, Pr > F: <0.0001; ciliatedcells: n = 6, Pr > F: < 0.0001), (B) Pars recta (connective cells: n = 7, Pr > F: 0.001; secretorycells: n = 6, Pr > F: 0.008), (C) Pars convoluta (connective cells: n = 6, Pr > F: 0.001; secretorycells: n = 8, Pr > F: 0.001; gobletcells: n = 7, Pr > F: 0,246), (D) Pars utera (connective cells: n = 6, Pr > F: <0.0001; ciliatedcells: n = 6, Pr > F: 0.017; secretorycells: n = 6, Pr > F: 0.009).

3.2. Detection of Gonadotropin Receptors (FSHR and LHR) and Prolactin Receptor

3.2.1. In the ostium

While no LHR was detected in the ostium cells, the percentage of FSHR and PRLR labeled cells varied according to the cell type and the period of the sexual cycle (Figures 1E, 5A, 6A, 7A). This percentage increased significantly after ovulation, particularly for ciliated cells, and then decreased abruptly during sexual inactivity.

3.2.2. In the oviduct;

FSH receptors were more intensively detected than LH receptors during the sexual cycle (Figures 5B-D, 6B-D). Abundant cells showed positive signal for FSHR during the reproductive period in the three parts of the oviduct, with variable fluctuations according to the cells between the preovulatory and the postovulatory periods (Figure 1F). An immunostaining was observed in the different cell types of the pars recta during the quiescent period.

Unlike to FSHR, the LH receptors were poorly detected in the cells of the pars recta, except in the ciliated cells during the postovulatory period (48.8%). The LHR detection is more important in the cells of both the pars convoluta and the pars utera during the postovulatory period.

Like the gonadotropin receptors, the PRL receptors were located in different cell types of the oviduct. It has been observed that PRLR was not detected in any tissue during sexual inactivity, except for the secretory cells of the pars recta, and that PRLR was localized in the cells of the pars convoluta during the reproductive period (Figures 1G-H, 7C).

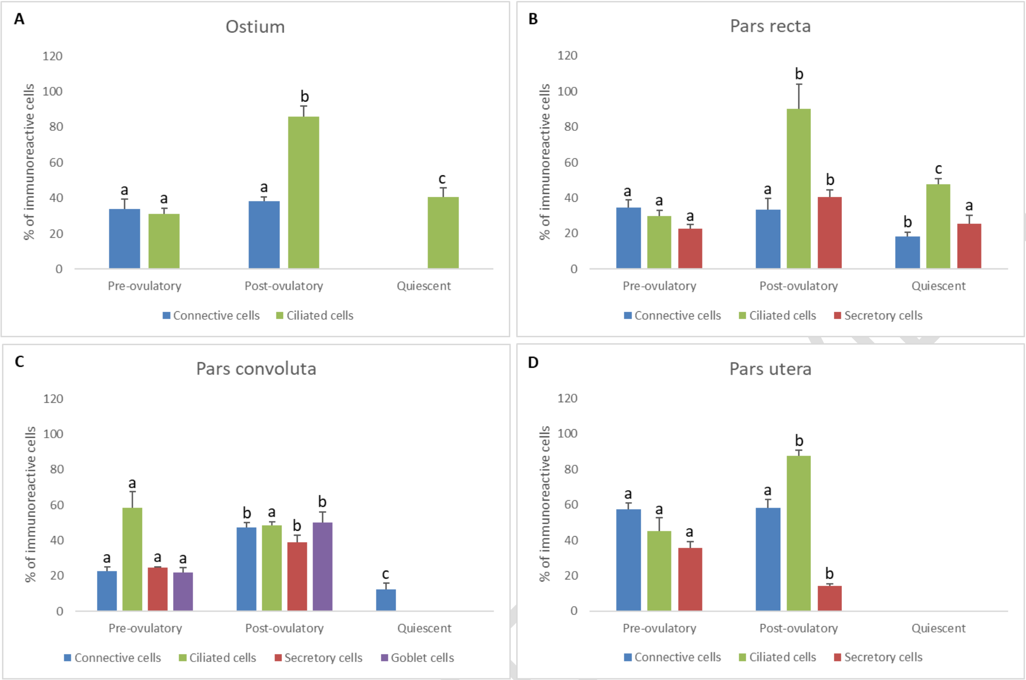

Figure 5. Immunodetection of FSHR in the different cell types of the genital tract of *Boulengerulataitana* during the sexual cycle. Bars marked with different letters are significantly different from each other (ANOVA, $p \leq 0.05$). (A) Ostium (connective cells: n = 6, Pr > F: 0.003; ciliatedcells: n = 6, Pr > F: < 0.0001), (B) Pars recta (connective cells: n = 8, Pr > F: 0.035; ciliatedcells: n = 7, Pr > F: 0.002; secretorycells: n = 9, Pr > F: 0.003), (C) Pars convoluta (connective cells: n = 9, Pr > F: <0.0001; ciliatedcells: n = 6, Pr > F: 0.161; secretorycells: n = 8, Pr > F: 0.001; gobletcells: n = 6, Pr > F: 0.008), (D) Pars utera (connective cells: n = 5, Pr > F: 0.872; ciliatedcells: n = 7, Pr > F: 0.007; secretorycells: n = 6: Pr > F: 0.014).

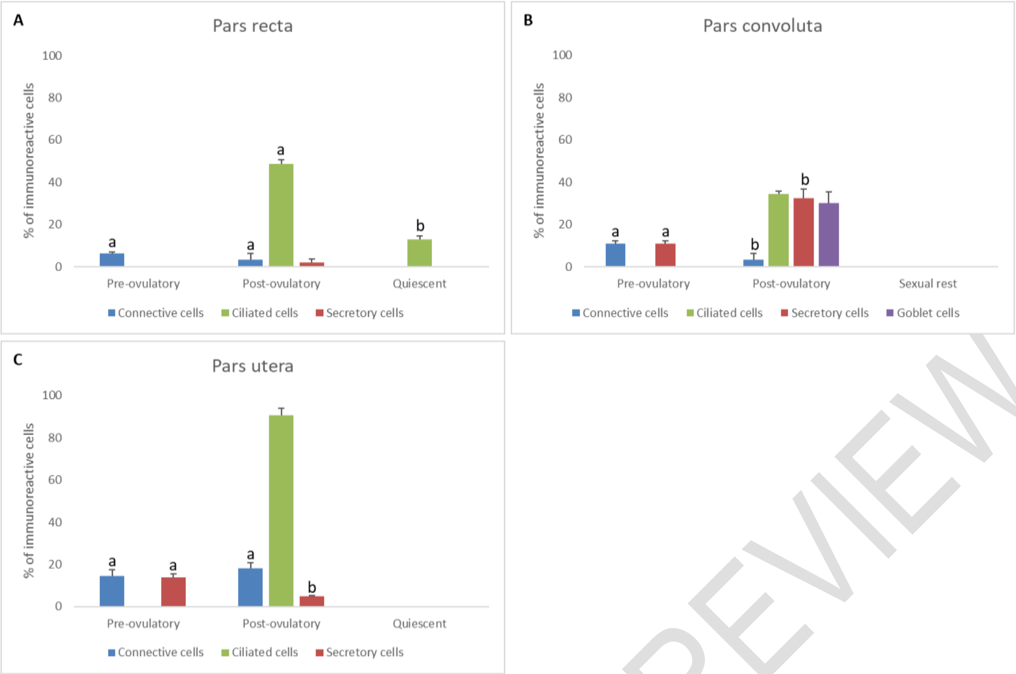

Figure 6. Immunodetection of LHR in in the different cell types of the genital tract of *Boulengerulataitana* during the sexual cycle. No immunoreactive cells was detected in the ostium. Bars marked with different letters are significantly different from each other (ANOVA, p ≤ 0.05). (A) Pars recta (connective cells: n = 7, Pr > F: 0.009; ciliatedcells: n = 7, Pr > F: <0.001; secretorycells: n = 7, Pr > F: 0.053), (B) Pars convoluta (connective cells: n = 7, Pr > F: 0.016; ciliatedcells: n = 6, Pr > F: <0.0001; secretorycells: n = 8, Pr > F: 0.001; gobletcells: n = 6, Pr > F: 0.007), (C) Pars utera (connective cells: n = 5, Pr > F: 0.060; ciliatedcells: n = 6, Pr > F: 0.001; secretorycells: n = 5, Pr > F: 0).

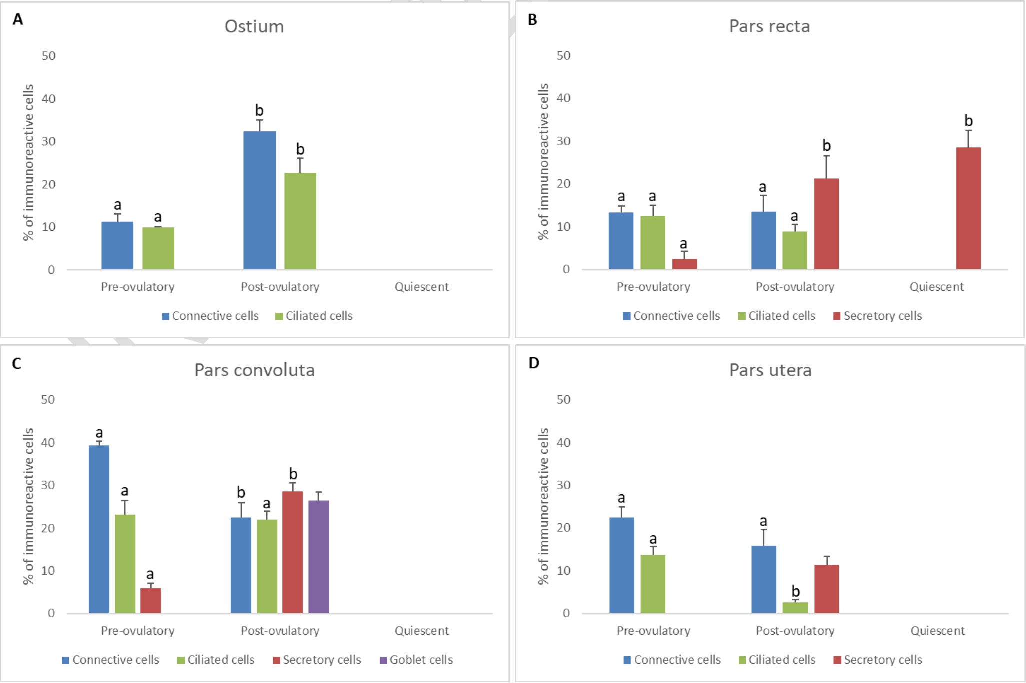

Figure 7. Immunodetection of PRLR in the different cell types of the genital tract of *Boulengerulataitana* during the sexual cycle. Bars marked with different letters are significantly different from each other (ANOVA, $p \leq 0.05$). (A) Ostium (connective cells: n = 6, Pr > F: 0; ciliatedcells: n = 6, Pr > F: 0.040), (B) Pars recta (connectedcells: n = 8, Pr > F: 0.002; ciliatedcells: n = 7, Pr > F: 0.011; secretorycells: n = 7, Pr > F: 0.004), (C) Pars convoluta (connective cells: n = 6, Pr > F: 0.023; ciliatedcells: n = 6, Pr > F: 0.660; secretorycells: n = 8, Pr > F: 0; gobletcells: n = 6, Pr >F: <0.0001), (D) Pars utera (connective cells: n = 7, Pr > F: 0.009; ciliatedcells: n = 5, Pr > F: < 0.0001; secretorycells: n = 6, Pr > F: 0.025).

4. DISCUSSION

This immunohistochemical study indicates for the first time the presence of steroid and pituitary hormone receptors in the genital tract tissues of an oviparous caecilian, *B. taitana*. The variations observed in the detection of receptors are in close relation with key events of seasonal reproductive activity. For comparative purposes, this study of an oviparous species was carried out under the same conditions as studies on *T. compressicauda* in order to compare the modalities of endocrine regulation of reproduction in Gymnophiona. For the immunohistochemical analysis, antibodies directed against human hormone receptors were used after verification of their specificity by checking sequence homologies between amphibian and human receptor proteins reported in the literature (for details see [47]).

PR immunoreactivity, detected in both the ostium and the rest of oviduct, varied throughout the reproductive cycle. In the ostium of *B. taitana*, PR was detected in both connective and ciliated cells during the reproductive period. In the ostium of *T. compressicauda*, only the ciliated cells were immunostained during the reproductive period. In the oviduct of both species, the high sensitivity of cells to PR-antibodies during the reproductive period, before and after ovulation, was consistent with the role of progesterone in preparation and maintenance of the amphibian reproductive tract [49-52]. We observed that the pars recta and the pars utera in *B. taitana* have a similar immunostaining profile to the tubal part and the uterus of *T. compressicauda*. Progesterone is involved in the proliferation of epithelial tissues, in synergy with ovarian activity during the reproductive period [53]. In *B. taitana*, the absence of receptor detection during the sexually inactive period is correlated with the fall of hormone synthesis in ovarian tissue. An exception was found in the secretory cells, where the high percentage of labeled cells might be explained by a possible delay in tissue sensitivity to the hormone. In *T. compressicauda*, the epithelial cells of the different parts of the oviduct were also marked during the resting period. Progesterone is also involved in the regulation of oviductal epithelial secretions in amphibians [32,54,55]. In the glandular cells of the pars convoluta and the pars utera, the significant detection of PRs during the postovulatory period suggested that progesterone facilitated secretion of egg membranes. In the viviparous species *T. compressicauda*, we previously showed an active role for progesterone, via its receptors, in the development of uterine secretions, which participate to the nutrition of embryos [47]. In *Rana temporaria* and *Bufo bufo* (both anurans), secretion of oviductal glands is controlled by progesterone produced in the ovary during ovulation [56] All these data are consistent with an important role for progesterone in controlling the function of the amphibian genital tract.

The estrogen receptors ERα and ERβ have differential distribution profiles, depending on the sexual cycle, the part of the oviduct and the cell type. In the ostium of *B. taitana*, the percentage of immuno-detected ERs was maximal in each cell type during the preovulatory period. The ERα alone was detected in the tissues after ovulation. In the different parts of the oviduct, each isoform had a specific distribution during the reproductive period depending on the cell type. The two ER isoforms were often detected together, and we observed that ERα was systematically detected more than ERβ in all cell types – it was particularly important in the ciliated cells. In the ostium of *T. compressicauda*, ERs were also strongly detected in the tissues before ovulation. After ovulation, ERβ alone was detected in the tissues. In the oviduct of the viviparous species, we had observed that there was an inverse-correlation in the cell detection of the two ERs during the reproductive period. While ERα was the only isoform detected in ciliated cells, ERβ was the nuclear estrogen receptor detected in secretory cells [47]. The particular distribution of the ERs in the different parts of the genital tract of these two species reflects the complexity of endocrine regulation of the oviduct according to the mode of parity. Both ERs appeared to be essential before and after ovulation in the different tissues, but it seemed difficult to find similarity of action of each isoform in each part of the oviduct of each species. In mammals, the primary mediator of female reproduction is ERα. It is the ER subtype required in the genital tract for embryo transport function and uterine reproductive function [57,58].

The presence of gonadotropin receptors has also been investigated in the different tissues of the genital tract. In the ostium of *B. taitana*, FSHR was the only gonadotropin receptor detected in cells during the sexual cycle. In the oviduct, the percentage of anti-FSHR and anti-LHR labelled cells was particularly high in the different tissues during the reproductive period, and low or absent during the sexually inactive period. These results indicate that both FSH and LH are directly involved in the regulation of the oviduct morphology, whereas FSH alone is involved in the control of ostium morphology. The synergic action of the two gonadotropic hormones on the development of the histological structures of the oviduct has also been shown in *T. compressicauda* [47]. In the ostium of this species, the two gonadotropic hormone receptors were detected together in the ciliated cells. In the oviduct, we have observed that FSH and LH receptors were detected together in the tissues during the preparatory and breeding periods. The percentage of ciliated cells reacting with anti-FSHR was significant after ovulation in the uterus (*T. compressicauda*) and the pars utera (*B. taitana*), whereas the percentage of these same cells reacting with anti-LHR was significant after ovulation only in the pars utera (*B. taitana*). In the two species, the cells of the genital tract were therefore direct targets of pituitary hormones. The variations observed in the detection of FHSR and LHR were dependent on the hormonal status of the females. After ovulation, the difference in sensitivity of tissues, especially to FSHR-antibodies, showed similarity in the functioning of the pars recta (*B. taitana*) and tubal part (*T. compressicauda*), the pars utera (*B. taitana*) and the uterus (*T. compressicauda*). In *T. compressicauda*, Exbrayat and Morel [59] have shown that the activity of the gonadotropic cells of the pituitary was correlated with that of the genital tract. In the oviparous species *Ichthyophisbeddomei*, Bhatta [60] indicated that the size of gonadotropic cells reached its maximum before ovulation and decreased after breeding. These data are consistent with those obtained in different anuran species, which show important hormonal seasonal variations related to seasonal reproductive activity [31]. Taken together, these results suggest a role of tissue revival through FSH during the pre-ovulation period.

Prolactin receptors were detected in the cells of the genital tract of *B. taitana*. The percentage of PRLR-positive cells was high during the reproductive period, whereas they were largely absent during the sexually inactive period, except in the secretory cells of the pars recta. Before ovulation, PRLR was detected in connective and ciliated cells; little or no labeling was detected in secretory cells. PRLR was particularly prominent in the tissues of the pars convoluta during the reproductive period. Comparatively for *T. compressicauda*, we had previously observed a significant signal in secretory cells of the tubal part and the uterus before ovulation. After ovulation, it was observed that connective and epithelial cells with detected PRLR were most numerous in the posterior part, transformed into the uterus. The structural development of the oviduct of both caecilians studied appears to depend on prolactin. The immunohistochemical profile of the pars convoluta in *B. taitana* appears closer to that of the uterus in *T. compressicauda*. In *B. taitana*, Raquet et al. [45] have previously shown the importance of glandular cells of the pars convoluta in the oviparous process, the secretory cells being involved in the synthesis of the glycoprotein sub-layer of eggs and the goblet cells in the production of mucus allowing the propulsion of the egg towards the posterior zone of the oviduct. The importance of prolactin in the reproduction of other amphibians has also been shown [61]. In the Japanese newt *Cynopspyrrhogaster*, it has been indicated that without PRL, estradiol could not stimulate the synthesis of jelly substances [62]. In *T. compressicauda*, volumetric variations and number of lactotropic cells vary considerably throughout the cycle. An increase of PRL-encoding mRNAs was observed from the beginning to the end of pregnancy, whereas the ratio of PRL mRNAs remain constant and low during the other periods of the cycle [43,63]. These data suggest that PRL is a crucial endocrine factor that controls the development of the genital tract during the reproductive period of both viviparous and oviparous caecilian species.

This global comparison between the two species shows that the immunohistochemical profile of the pars recta of *B. taitana* corresponds well with the tubal part of *T. compressicauda*, and that the pars utera has greater similarity with the uterus. However, the pars convoluta has similarities with the both parts of the oviduct of *T. compressicauda*.

5. CONCLUSION

This immunohistochemical study indicates the presence of steroid and pituitary hormone receptors in tissues of the genital tract of an oviparous caecilian. The variations observed in the detection of receptors are in close relation with the key events of seasonal reproductive activity, and confirm that oviduct morphology is closely correlated with ovarian function. Prior to ovulation, progesterone release seems necessary to permit oviductal development and the acquisition of its secretory capacity. Estrogen activity is also probably necessary to facilitate oocyte maturation under progesterone influence. After ovulation, the two steroid hormones are involved in propulsion of the oocytes, which facilitates the deposit of the secretions generating the formation of jelly coats.

The detection of pituitary hormone receptors in different parts of the oviduct suggests that pituitary hormones directly control various aspects of oviductal development and function during the ovarian cycle. In addition to triggering ovulation through a surge of LH and acceleration of vitellogenin uptake into oocytes by FSH, the gonadotropins directly regulate oviductal functioning. Similarly, the detection of prolactin receptors suggests that prolactin plays an important role in controlling morphological changes in the oviduct. However, further studies are needed to clarify the relationship between hormone receptor amounts and hormone levels.

The comparative study of the detection of ovarian and pituitary hormone receptors in the cells of the different parts of the oviduct during the sexual cycle revealed correspondence between parts of the oviduct in the oviparous and the viviparous species. Despite differences in reproductive mode, similarity in receptor distribution between the two species suggests conservative physiological control of the reproductive cycle in caecilians.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Ethical review and approval were waived for this study due to the use of museum specimens belonging to a collection of preserved animals collected before 2005 under Kenyan Ministry of Education Science and Technology research permit number MOEST 13/001/36C 183."

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