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RESEARCH HIGHLIGHT

From bony fishes to mammals: reproductive cycles in vertebrates, hormones and hormone-receptors

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Some reproductive cycles of vertebrates are still little known. Yet, a good knowledge of reproductive cycles and regulation is useful to protect a threatened species, or inversely to control the proliferation of a species which has been recognized as a pest, or still when an animal becomes a model used to study fundamental physiological phenomena with applications to medical research. So, for several years, the studies of our laboratory and associated teams were devoted to the study of reproduction in several vertebrates, related to the external conditions which can be natural (climate, genetics) as well as artificial (pollution). In a first time, variations of both male and female genital tracts were studied in several vertebrates with anatomical and histological methods. To-day, the availability of a large panel of antibodies directed against hormones and their receptors allows the visualization of such molecules in organs according to sexual cycles or submitted to artificial factors such as pollution. Sexual cycles and the importance of hormones and their receptors in regulation are now an important purpose for our own works. Vertebrate models studied to-day are bony fishes living in fresh water, amphibians, reptiles and mammals, living in semi-aquatic, temperate, equatorial, Mediterranean or arid climates. Our purpose is to obtain a large panel of situations allowing the understanding of the complexity and plasticity of reproductive modes, and the effects of external factors of animal species in order to be useful to the preservation of threatened species, regulation of reproduction on animals considered as pests and use of animal models.

Keywords: vertebrate; reproduction; sexual cycle; hormone; hormone receptor; steroid

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Introduction

Some reproductive cycles of vertebrates are still little known or even unknown, and more particularly that of species which are difficult to study consequently to their geographic distribution or mode of life. This lack of knowledge can also be linked to the lack of economical interest. The works done in our laboratory and collaborating teams are devoted for a long time to the study of reproductive cycles in vertebrates. The first purpose of these works was to develop knowledge *per se*. Another reason is now related to the conservation of biodiversity. To protect a threatened species, it is indispensable to know its natural history and more especially its reproductive patterns. It is also useful to know reproductive features of a species which has been contrarily recognized as a pest, in order to control its proliferation. In both these cases, it is necessary to understand the consequences of environmental conditions on reproduction. Another reason, and not the least, is the knowledge of animal biology when a species becomes a model used to study fundamental physiological and/or application to medical aspects.

Several years ago, studies were mainly based on anatomical and histological methods in order to describe the variations of genital tracts in both males and females. Since 1980's, immunohistochemistry techniques permitted to appreciate the presence of hormones in tissues and cells. The first available antibodies were prepared to detect hormones in pituitary cells [1], and lately, antibodies directed against steroid hormones became available. Today, one can also have access to anti-bodies directed against hormone receptors, so it is now possible to visualize the presence of both hormones and their receptors in organisms, and using image analysis, it is also possible to appreciate the variations of such receptors and hormones, related to variations of organs and linked to climatic parameters or pollution. *In situ* hybridization became also useful to visualize and quantify the expression of gene encoding for hormone or hormone-receptors. Knowledge of repartition and action of those receptors is particularly important when animals are submitted to the action of endocrine disrupters which fix on receptors or modify the normal distribution of receptors during embryonic development. For example, it is possible to observe sexual inversion or hermaphrodi-tism in individuals of fishes submitted to the pollution of water [2], in a context on which human populations can also be affected.

The purpose of this paper is to give an overview of our own works on reproductive cycles in vertebrates living in different biotopes and under different climatic factors, emphasizing visualization of hormones and their receptors. For some studies, histological methods have been used to describe anatomical variations of sexual organs and endocrine organs, with immunocytochemical detection of several kinds of hormones. In the most recent works, *in situ* hybridization and immunohisto-chemistry methods have been also used to detect the presence of hormonal receptors.

1. In a bony fish

The first example concerns bony fishes and more particularly *Zingel asper*, whose western limit is Rhône Basin (France). In 2008 population in Rhône and attributed rivers was estimated to 2000 to 4000 individuals living on 400 km and scattered as small populations well localized in some rivers only. These data contrasted with data known at the beginning of 20th century, when *Zingel asper* was abundant [3]. To understand this decreasing, we studied the reproductive pattern of this species using fishes bred in a pond in natural conditions from which were regularly taken fishes during three consecutive years. Oogenesis was continuous with a yearly cycle of reproduction characterized by breeding in spring. The study of ovaries showed the presence of developed follicles, atretic follicles, and empty post-ovulatory follicles which degenerated progressively [3, 4, 5]. 17β estradiol was found in theca of previtellogenic and vitellogenic follicles. The examination of testes in 7 to 39 months-old animals showed four stages of development. A first efficacious spermatogenesis was observed in 22 months-old animals. Finally, the sexual maturity was attempted in 2 years-old animals [6]. In adult, spermatogenesis was continuous from September until May then testes decreased from June. 17β estradiol and testosterone were visualized in interstitial tissue of testes with immunohistochemistry. This work will be useful to understand factors involved in the regression of this species.

2. In Amphibia

2.1. Anuran

In Anurans, a present research concerns *Bufo mauritanicus* [7, 8, 9], living in Beni Belaid, a preserved area situated in Northern Algeria. This toad is submitted to a first wet season from September until January, and a second from March until May. An important dry season is observed from June until September. All germ cells were observed throughout the year in testes with a small decreasing in rainy seasons, and an increasing in dry one. Leydig cells containing lipids and testosterone were particularly developed during breeding season. In females, oogenesis was continuous, vitellogenesis was observed on large rainy season; egg-laying occured in April-May, accompanied with the decreasing of follicle number and the disappearance of vitellogenic oocytes. Some atretic follicles were observed throughout the year. 17β estradiol was visualized in the granulosa cells of both previtellogenic and vitellogenic follicle. The presence of receptors of testosterone and 17β estradiol in both males and females were researched. First results have shown their presence in Sertoli cells and spermatogonia in testes, and in oocytes and sometimes granulosa cells in ovaries (Kisserli, unpublished observations).

2.2. Gymnophionans (Caecilians)

Gymnophionans are elongated amphibians with burrowing habits living in tropical areas. Their reproductive patterns were studied on few species. *Typhlonectes compressicauda* lives in South America and our studies concerned a population from French Guiana where it is submitted to a rainy season from January until June, and a dry season from July until December. During rainy season, savanna is flowed, food is abundant, and breeding occurs. At dry season, water level decreases, and animals live in holes burrowed in the mud. At this period, viviparous females give birth to young animals [10, 11, 12].

In males, each testis was segmented with a synchronous gametogenesis in lobes [13, 14]. Reproductive cycle was annual with a period of spermatogenesis during the rainy season. In June, seminiferous tubules were reduced, and in July and August, after a new complete spermatogenesis, testes were full of germ cells in prevision of next reproductive season [10, 15]. Müllerian ducts persisting in adult males were developed during breeding season and secreted substances displayed in cloaca, constituting the sperm; they became empty at the end of this period. From October, they began to develop and reached a maximal size, being filled with secretions at the beginning of a new period of reproduction [16]. Males are equipped with a phallodeum which is a copulation organ corresponding to the posterior part of cloaca which develops at reproduction, bordered with a stratified epithelium. During sexual act, this part of cloaca was reversed and introduced in female's one. So, the proliferating epithelium looking like spines was situated at exterior and it was used to be maintained into the female cloaca. After reproduction, cloaca decreased, and began to develop again on next October [17, 18].

In male pituitary, lactotropic (secreting PRL) cells and gonadotropic (secreting FSH and LH) cells varied according to a cycle perfectly parallel to the variations of reproductive organs. These cells were developed at their maximum at the beginning of reproductive period then they reduced [19]. Corticotropic cells secreting ACTH and somatotropic cells secreting GH increased during the period of reproduction and well decreased at the end of this period. Their size increased again at the beginning of dry season, in July and August, when the animals were in search of food [19]. Quantitative *in situ* hybridization showed an increase in the number of cells with PRL expression at the beginning of dry season. In the middle of this season, this number decreased. In males, PRL mRNAs expression was the same throughout the year [20, 21]. In testes and Müllerian ducts, visualization of PRL-R mRNAs showed variations parallel to the cycle of reproductive activity. The expression of long and short forms of PRL-R mRNAs have been visualized in several organs. In the liver, small intestine and pituitary, mRNAs encoding for the short form were more strongly expressed than mRNAs coding for the long form. It was the contrary in the spleen, stomach and kidneys [22]. Using quantitative *in situ* hybridization we could also show variations of the expression of the long form of PRL-R in male genital tract. In testes, long form of PRL-R mRNAs was expressed in germ cells, Sertoli cells and Leydig cells. In Müllerian ducts, these mRNAs varied consequently. A large increase was observed during the breeding period and a decrease during the period of quiescence [23]. The variations of the long form of PRL-R mRNAs were correlated to the synthesis of PRL in the pituitary.

Typhlonectes compressicauda female is viviparous with a biennial reproductive cycle [24, 25, 26]. The first year was characterized by vitellogenesis from October until December-January. Ovulation occurred in February and it was followed with pregnancy during which embryos developed into the uterus which were morphologically and physiologically adapted to embryo feeding [27, 28, 29]. In ovary, functional *corpora lutea* persisted throughout pregnancy. They presented first a central cavity consequently to the expulsion of oocyte. Granulosa cells proliferated in the central cavity. Blood vessels also developed from theca, and invaded each *corpus luteum*. At the end of pregnancy, *corpora lutea* decreased and disappeared being reduced in the connective tissue of ovary. After birth of young animals (July until September), ovaries reduced: no vitellogenic follicle was observed. A new vitellogenesis occurred from October on the beginning of the second year of cycle. In next February, at the theoretical period of ovulation, vitellogenic follicles degenerated and bring on atretic follicles. Atretic follicles coming from degenerative small follicles were always observed whatever their stages of development [30, 25].

Oviducts were submitted to a biennial cycle perfectly parallel to that of ovaries [27, 25, 29]. In October of the first year, oviducts differentiated into an anterior part in which fertilization occurred. The wall was covered with gland cells secreting substances for confection of egg envelope, and ciliated cells. The middle and posterior parts corresponded to uterus in which embryos developed. The uterine wall was first covered with cells secreting substances used for embryo feeding. Then it was transformed according to the developmental stage of fetuses. After parturition, the two parts of oviduct were totally undifferentiated, and a new differentiation began in October of the second year. But, in February, oviducts regressed and let in a quiescent state until the next period of reproduction.

In order to understand the hormonal control of these spectacular variations of female genital tract in *T. compressicauda*, we detected the presence of Δ5 3β hydroxy steroid dehydrogenase, an enzyme implicated in the synthesis of steroid hormones, in granulosa cells of vitellogenic follicles and in *corpora lutea* but never in youngest follicles neither in atretic follicles [30]. Immunocytochemistry permitted to visualize the presence of 17β estradiol in both theca and granulosa cells of vitellogenic follicles and in corpora lutea [31]. Recent preliminary works showed the presence of α and β estrogen receptors (E-R) and progesterone receptors (P-R) localized in cytoplasm or nuclei of both ovaries and oviducts of this species (Raquet, unpublished observations.).

In pituitary of female, gonadotropic and lactotropic cells modified [33]. These cells developed from October until February. In pregnant females, they continued to develop and reached a maximal size in April, when the fetuses just began to be fed with uterine secretions. Then, gonadotropic and lactotropic cells decreased to reach a minimal size at parturition. They increased again from next October but in February, they abruptly decreased to become minimal, and size did not vary until next October when a new cycle starts. Corticotropic and somatotropic cells were developed during the period of breeding without any difference in pregnant and non-pregnant females. Cycles of these cells seemed to be more linked to global activity than reproduction. The number of cells showing PRL mRNAs expression increased at the beginning of dry season. In October, this number decreased inversely correlated to the size of cells. In pregnant females, PRL mRNAs expression remained constant at the beginning of gestation. Then, expression of those mRNAs increased when embryos reached their maximal development. When females remained sexually inactive, PRL mRNAs expression remained the same throughout the year [21].

PRL-R mRNas were observed in the same organs than males. In the female liver, the mRNAs coding for the short form of PRL-R were more strongly expressed than mRNAs coding for the long form [34, 22]. In liver, the values of mRNAs expression were higher mid-way through pregnancy than during the period of quiescence. These results have been correlated with the reserve function of the female liver which increased before the reproduction, then decreased during pregnancy when embryos used the maternal supply of food [35]. At middle and end of pregnancy period, mRNAs coding for the long form of pRL-R were strongly expressed in ovaries with compact *corpora lutea*. During sexual rest, the expression of those mRNAs was lower than this period [34; 22].

The study of hormonal regulation of genital tract in a viviparous species let suppose that mechanisms can also be observed in oviparous species using internal fertilization. So, the oviparous *Boulengerula taitanus* was also studied. This animal living in Kenya (Africa) is submitted to a long rainy season from March until May, and a shorter one in November and December, period at which new-born were found. The female lays several eggs once a year, from November until January. The sexual cycle can be divided into three periods: preparation in September and October, ovulation from November until February and quiescence from March until April [36, 37], correlated with that of males [38]. 17β estradiol has been detected in granulosa and theca of vitellogenic follicles, *corpora lutea* and even sometimes in atretic follicles [37]. Oviducts were also submitted to seasonal variations [39], α and β E-R and PRL-R can be detected in the three parts of oviduct with an increase during seasonal activity [32].

3. Amniotes living in arid and semi-arid areas

Several studies concerned reproductive modes of amniotes (reptiles and mammals) living in Saharan desert. Present data are the results of a 20 years-old narrow collaboration with several teams of USTHB (Algiers, Algeria).

3.1 In a reptile

In previous works, reproductive cycles of both males and females have been described in *Uromastyx acanthinura*, an oviparous agamid lizard. The sexual activity of this species was observed in spring and summer, and sexual quiescence in autumn and winter. In males, an active spermatogenesis was observed in spring, with the highest yearly androgen blood level [40, 41]. Male genital tract reduced in summer. A new spermatogenesis began in October but it stopped before spermatozoa formation. In winter, only spermatogonia were observed. In females, vitellogenesis occurred at the end of May. After egg-laying, in spring and autumn, a new activity was observed in ovaries. Follicle growth then slowed down consequently to the lack of food and also the decrease of temperature [42].

Presence of steroid hormones and their receptors has been shown in female [43, 44, 45]. These molecules have been localized in vitellogenic follicles. Progesterone became abundant in vitellogenic follicles, in which progesterone receptors (P-R) were also observed. When follicles began atretic, all hormones and their receptors decreased progressively. Just after reproduction, both hormones and their receptors were shown in previtellogenic follicles. At vitellogenesis, estrogen-receptors (E-Rs) were not expressed in vitellogenic follicles and P-Rs were weakly detected in the nucleus of granulosa cells. After reproduction, E-Rs were observed in previtellogenic follicles like in breeding period. P-Rs were detected in both the cytoplasm and nucleus of granulosa and theca cells. During quiescence, neither E-R nor P-R was observed in previtellogenic follicles [43]. β endorphin was abundant in granulosa cells and oocytes during sexual rest and in females which did not undergo in vitellogenesis. In other hand, β endorphine, a peptide, is also certainly well implicated in the regulation of *U. acanthinura* reproduction [46, 47]. In the same species synthesis of vitellogenin occurs in liver in which only α and β E-Rs were observed with a nuclear or cytosolic location whatever the phase of the cycle [45]. P-Rs were present during luteal phase and sexual quiescence. These receptors disappeared promptly from females treated with 17β estradiol during sexual rest. P-Rs could be implicated on the negative effects exerted by progesterone on vitellogenin synthesis. In pituitary, LH cells were particularly large and numerous during vitellogenesis with abundant LH in cytoplasm. At sexual rest, LH cells were reduced and weakly stained. So, this cycle of activity was well correlated to sexual cycle of ovaries [48]. During vitellogenesis, FSH expression was parallel to synthetic activity with characteristic ultrastructural features. In winter, cells reduced [49].

3.2 In Mammals

Sexual cycle of *Meriones libycus*, a Saharan rodent, is characterized by a short period of activity in summer, and a resting phase between autumn and winter. The reproductive organs of males and more particularly the seminal vesicles vary consequently with obvious tissue modifications [50, 51, 52, 53]. The study of both thyroid-hormone and receptors, suggested the role of thyroid during acquisition of sexual maturity. Presence of thyroid hormone-β1 receptors has been shown in the cytoplasm of Sertoli cells in the first three months after birth, after presence of these receptors decreased, to be finally absent in adult Sertoli cells [54]. Narrow interrelations were observed between testosterone-receptors and TSH-receptors [54]. Experimentation involving animal in natural field, castrated animals, and animals castrated then treated demonstrated the presence of cytosolic androgen-receptors and testosterone hormone in thyroid cells [55]. In this species, testosterone can also modulate suprarenal and kidneys, both organs being implicated in the regulation of the hydromineral balance [56].

During the breeding season all the stages of folliculogenesis, corpora lutea and atretic follicles were observed in the ovaries. During quiescence, non-mature follicles entered atretic process, no corpus luteum was observed [57,58]. In breeding season, 17β-estradiol, progesterone, testosterone and P450 aromatase were well observed in follicles. In resting period, all these molecules became reduced in preantral follicles. Seasonal variations were manifested by significant changes in both ovary morphology and hormonal function [58]. The estrogene and progesterone vaginal receptors in adult females have been observed in the cytoplasm of epithelial and connective cells with variations depending on estral cycle [57].

In the sand rat *Psammomys obesus* female, the different stages of folliculogenesis were described [50, 60] and seroid activities analysed [61]. Presence of progesterone, androstenediol and estradiol was observed in the different parts of the ovary. Progesterone was obvious in the theca of growing follicles and increased in granulosa; androgenic hormones were weakly labeled in all the follicles; the estradiol labeling weak in preantral follicles, increased in both theca and granulosa. Estradiol was clearly localized in granulosa and totally devoid in theca of antral follicles

In the male, during the breeding season, androgen-receptors (A-Rs) were localized in both nuclei and cytoplasm of principal cells of the caput epididymidis. E-Rs 1 were found mainly in the apical zone of cytoplasm and E-Rs 2 in nuclei. In resting season, label of A-Rs was weak in both cytoplasm and nuclei. E-Rs 1 but not E-Rs 2, showed a strong label in the nuclei. After castration, A-Rs and E-Rs 2 presented a low signal in nuclei but not for estrogen-receptors 1. After castration and testosterone treatment, an androgen-dependence for A-Rs and the restoration of E-R 1 but not E-R 2 were observed. After ligation of the efferent ducts, a considerable reduction of A-Rs was observed in contrast to both E-Rs 1 and 2, which gave a strong signal [62].

Conclusions

Reproductive biology of Vertebrates is always well adapted to external factors, whatever the class they belong, Osteichtya, Amphibian, Reptilia or Mammalia, and whatever the external conditions: aquatic, semi-aquatic, terrestrial, burrowing, temperate, semi-arid or arid conditions. Study of both male and female genital tracts show several resemblances. In males, production of germ cells in testes can be continuous, semi-continuous or discontinuous but they are always perfectly adapted to the fertilization of oocytes at ovulation. In males, hormonal control involves particularly pituitary hormones mainly LH, FSH, or PRL which can be integrated into target-cells with hormone-receptors. Presence of steroid hormones and enzymes of steroidogenesis have been show in Leydig cells. In females, reproductive cycles are also variable according to the external conditions. Certain cycles are continuous with a period devoted to ovulation; sometimes they are discontinuous or still biennial. In all the cases, steroid hormones have been showed in follicular granulosa or theca with variations linked to sexual cycle. Pituitary hormones also vary according to the reproductive cycle. Visualization of several kinds of hormone-receptors or mRNAs encoding for these receptors, show the importance of these molecules dispersed not only in sexual organs but also in all the organs, some of them such as the liver in females being clearly involved in reproductive physiology.

To-day, the possibility to show the presence and variation of hormone-receptors give a new vision of reproductive biology and adaptation to external conditions. For instance, in previous works, we showed modifications of sexual cycle in the black bass, *Micropterus salmoides*, a bony fish in which we studied also several aspects of reproductive cycle resembling *Zingel asper* ones [63, 64, 65, 66]. In altitude, sexual cycle is two years-long and in plain, it is only one year-long [67, 68,69]. Modifications of hormone synthesis under different climatic conditions are probably implicated, but it is not excluded that several kinds of hormone-receptors could be also involved in this modification of cycle. Another example is given by the breeding of the amphibian *Typhlonectes compressicauda* in artificial conditions without any dry season during more than two years. In male, sexual cycle was conserved throughout the duration of breeding, but in female, sexual cycle was deeply modified and totally desynchronized from that of male, so reproduction became impossible. We concluded a dry period was necessary to for a normal sexual cycle in females essentially [70]. These two examples concerning two different taxa and different modes of life show reproduction being well adapted to conditions of life. A deep study of hormone-receptors related to the presence of hormones could give new data and knowledge about adaptation of animals to the external condition. In other hand, we studied a species submitted to a hard pollution and showing deep modifications in both male and female genital tracts [2, 71]. If variations of presence of hormones and hormone-receptor stay identical in all the cases, molecules from water could be ingested by fishes, then transmitted to hormone-receptors and take the place of normal hormone provoking a perturbation of sexual development and sexual cycle.

The works presented here were based on a study of sexual cycle, the first approach to understand reproductive biology, with visualization of hormones and sometimes assay of blood-running hormones. Now, study of hormone-receptors certainly will give new knowledge allowing a more and more deep knowledge of reproduction of well different vertebrates living in very well different conditions.

Conflicting interests

The authors have declared that no competing interests exist.

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