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# Bidder's organ – structure, development and function

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**ABSTRACT Bidder's organ is an ovary-like structure, which develops from the anterior part of the gonadal ridge in anuran amphibians belonging to the Bufonidae family. Bidder's organs form in both males and females. Because Bidder's organ contains female germ cells (oocytes), the bufonid males are *de facto* hermaphrodites. Due to similarity with the undeveloped ovary, Bidder's organ was, in early literature, described, inaccurately, as a structure present only in males. Due to the fact that Bidder's organ is a unique structure present only in Bufonidae, it is not well studied and its function still remains a mystery. Here we describe the development and structure of Bidder's organs, summarize the knowledge on gene expression and steroidogenic activity in these organs, and present hypotheses regarding Bidder's organ function.**

KEY WORDS: *Bidder's organ, testis, ovary, oocytes, sex hormones*

## Introduction

Bidder's organs are ovarian-like structures present in males and females of majority of bufonid species. Bidder's organs develop, irrespectively of the genetic sex, from the anterior tips of the gonads. The germ cells present in Bidder's organs enter shortened oogenesis and, in the larvae, the diplotene oocytes become arrested in previtellogenesis (Brown *et al*., 2002). Despite the fact that Bidder's organs have been studied for two centuries their function still remains vague. Here we review available information on Bidder's organ structure, development and its proposed functions.

## Short history of Bidder's organ in Bufonidae

Bidder's organ was discovered in 18th century. In 1758 Roesel von Rosenhof presented in his *Historia Naturalis Ranarum Nostratium* the first ever drawing of the pair of lobes at the anterior tips of gonads in *Bufo calamita*. In 1825 Jacobson noted that these peculiar structures are the vestigial ovaries characteristic for bufonids (Takahashi, 1923). In 1846 Bidder described these structures as a part of the testis. In 1876 Spengel used, for the first time, a term 'Bidder's organ', to describe this structure in bufonid gonads. Despite the fact that the presence of Bidder's organ has been described in many bufonid species, and that the removal of testes leads to transformation of Bidder's organs into ovaries, which indicates that Bidder's organs represent developmentally inhibited ovaries, the role of these organs is still unclear (Table 1; Harms, 1923; Ponse, 1924; Tanimura and Iwasawa, 1986; Duellman and Trueb, 1994; Abramyan *et al*., 2010, Piprek *et al*., 2013). Although commonly accepted belief is that Bidder's organs are rudimentary or vestigial structures, some investigators believe that they are fully functional steroidogenic organs important for reproduction control (Davies, 1936; Colombo and Colombo-Belvedere, 1980; Abramyan *et al*., 2010; Scaia *et al*., 2011).

Bidder's organs are present in the majority of studied species from Bufonidae family with the exception of South American *Melanophryniscus setiba* and *Melanophryniscu stelzneri* (Peloso, 2012) and two known species of *Truebella* from Peru (Fig. 1. Type VI) (Graybeal and Cannatella, 1995). This indicates that Bidder's organ is not a synapomorphy of Bufonidae but only of a part of the family (Frost, 2006). It is commonly accepted that the absence of Bidder's organ is a primitive feature for Anura, and its presence in Bufonidae is a derived feature (Duellman and Trueb, 1994).

Stohler (1931) and Dubois (1947) summarised early studies on the presence of Bidder's organs among male and female toads. Bidder's organs are present in all adult males and many adult females as well as in all juvenile males and females of *Bufo*-species. In some species, Bidder's organ is not present in adult females, which indicates its degeneration during the female lifetime. The presence of Bidder's organs in adult both males and females was described

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*Abbreviations used in this paper:* BO, Bidder's organ.

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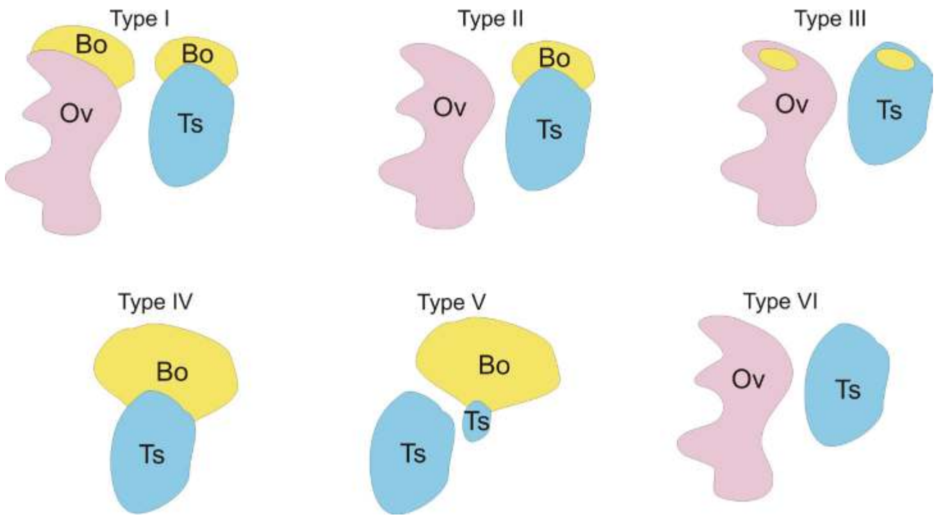

**Fig. 1. The diversity of the external morphology of Bidder's organs and gonads among bufonids. (Type I)** *The Bidder's organs (Bo) are present at the anterior poles of ovaries (Ov) and testes (Ts). The presence of Bidder's organs in both adult males and females was described in* Bufo bufo, Bufo americanus, Bufo terrestris, Bufo quercicus, Bufo ictericus, Pseudophryne bibronii. **(Type II)** *The Bidder's organs presence only in males, and its disappearance in adult females is characteristic for* Bufo viridis, Bufo marinus, Bufo lentiginosus, Bufo canorus, Bufo fowleri, Bufo vertebralis, Bufo rosei, Bufo angusticeps, Bufo gariepensis, *and* Nectophrynoides viviparus. **(Type III)** *The Bidder's organ incorporation into the adult ovaries and testes was described in* Bufo melanostictus, Bufo fowleri, Nectophrynoides occidentalis. **(Type IV)** *In some* B. woodhousii *males the Bidder's organs are well-developed and resemble the ovaries.* **(Type V)** *In some* B. arenarum *males the testes with well-developed Bidder's organs are smaller than the testes lacking Bidder's organs.* **(Type VI)** *In* Melanophryniscus *and* Truebella *the Bidder's organs are absent.*

in *Bufo bufo*, *Bufo* (=*Anaxyrus*) *americanus*, *Bufo* (=*Anaxyrus*) *terrestris*, *Bufo* (=*Anaxyrus*) *quercicus*, *Bufo* (=*Rhinella*, *Chaunus*) *ictericus*, *Pseudophryne bibronii* (Fig. 1. Type I) (Stohler, 1931; Witschi, 1933). However, Rau and Gaterby (1923) indicated the absence of Bidder's organ in *P. bibronii*. This discrepancy between the different investigators' data may result from the analysis of different age individuals. The presence of Bidder's organs only in males was reported for *Bufo* (=*Pseudepidalea*) *viridis*, *Bufo* (=*Rhinella*) *marinus*, *Bufo lentiginosus*, *Bufo* (=*Anaxyrus*) *canorus*, *Bufo* (=*Anaxyrus*) *fowleri*, *Bufo* (=*Poyntonophrynus*) *vertebralis*, *Bufo* (=*Capensibufo*) *rosei*, *Bufo* (=*Vandijkophrynus*) *angusticeps*, *Bufo* (=*Vandijkophrynus*) *gariepensis*, and *Nectophrynoides viviparus* (Fig. 1. Type II) (King, 1908; Stohler, 1931; Witschi, 1933; Vos, 1935; Brown *et al.*, 2002). Bidder's organ has been found also in *Nectophryne*, *Nectophrynoides*, *Pseudophryne*, *Pelophryne*, and *Pseudobufo* (Davies, 1936; Ponse, 1924; Stohler, 1931). In the adult males and females of some species Bidder's organs are not visible macroscopically, which suggests that they became incorporated into the gonad proper. Such a situation was described in adult males of Asian *Bufo* (=*Duttaphrynus*) *melanostictus* in which Bidder's organ is hidden within the testis (Koch, 1934). Similarly, in adult females of *Bufo fowleri*, Bidder's organs do not degenerate but become incorporated into ovarian tissue, however, in juvenile females and all males Bidder's organs and gonads remain as the separate structures. In the European common toad (*B. bufo*), Bidder's organs are present in adults of both sexes, however Dubois (1947), reported singular, 7 cm long, old female toad (*B. bufo*) lacking Bidder's organs, which suggests that Bidder's organ can degenerate over time. In *Bufo arenarum*, atresia of Bidder's organs occurs in females during third winter after metamorphosis and the final resorption of Bidder's organs takes place during the fourth summer (Echeveira, 1990). The males of *B. arenarum* have one or two Bidder's organs, which undergo a cyclic pattern of growth and abatement during a year (Echeveira, 1990). Thus, Bidder's organ cyclicity may contribute to its size variability between individuals of the same species.

Interestingly, in Ethiopian *Nectophrynoides* (=*Altiphrynoides*) *malcolmi* (Bufonidae), in all mature males, Bidder's organ consists of a strip of oocytes curving along the medial axis of the testis from which it is separated by the connective tissue (Wake, 1980). The viviparous *Nectophrynoides* (=*Nimbaphrynoides*) *occidentalis* (Bufonidae) from Ivory Coast and Guinea, do not have separated Bidder's organ, and oocytes are present within the larval (but not adult) testis (Fig. 1. Type III). This suggests that in this species Bidder's organ became incorporated into the testis (Lamote *et al.*, 1973; Zuber-Vogeli and Xavier, 1965). In contrast, closely related Tanzanian ovoviviparous *Nectophrynoides viviparus* (Bufonidae) has a typical well-developed Bidder's organ. This indicates that in this line of African bufonids there is a trend towards modification or elimination of Bidder's organs.

There is a great variability in the degree of Bidder's organ development among individuals, which may depend on significant fluctuations of gene expression in developing Bidder's organs (Abramyan *et al.*, 2010). This variability has also been shown to be associated with environmental pollutants and seasonal activity (Scaia *et al.*, 2008, 2011). There are also examples of individuals with well-developed Bidder's organs and small remnants of testes (*Bufo woodhousii*; Pancak, 1987). In *B. arenarum* some males have one Bidder's organ and two testes, and the testis connected to Bidder's organ is smaller than the other (Fig. 1. Type V). In *B. woodhousii* and *B. bufo* some males from natural population have enlarged Bidder's organs, which may represent the functional ovaries (Fig. 1. Type IV; Witschi, 1933; Pancak, 1987). The naturally occurring hermaphroditic toads frequent among North American hybrids of *Bufo (=Anaxyrus) microscaphus* and *B. woodhousii* have large ovaries (presumably functional Bidder's organs) and small testes, and their gonads produce eggs and sperm (Sullivan *et al.*, 1996).

## The structure of Bidder's organ

In bufonids the female gonad has two regions: Bidder's organ and the proper ovary (connected via the retrobidderian region) (Fig. 2F). In males there is a specific intermediate region between Bidder's organ and testes. In this intermediate region there is a gradual wave of oogenesis, which resembles the pattern of oogenesis in the anterior part of the ovary proper (Zaccanti *et al.*, 1971). Interestingly, the morphology of oocytes in male and female Bidder's organs is the same (Farias *et al.*, 2002, 2004; Tanimura and Iwasawa, 1992).

Vitale-Calpe (1969) described Bidder's organ of young males of South American *Bufo arenarum* as a thin strands of connective tissue accompanied by a loose plexus of blood vessels separating different groups of cells. This Bidder's organ has oocyte-containing follicles, enclosed by squamous follicular cells and separated by collagen fibers, capillaries and slender stromal cells resembling fibroblasts (Vitale-Calpe, 1969). The author did not observe the presence of Balbiani body or annulated lamellae (characteristic for ovarian oocytes) in the oocytes of Bidder's organ, however, these structures had been found in Bidder's organ oocytes of *B. bufo* (Gurrieri *et al.*, 1964).

Farias and colleagues (2002, 2004) described the structure of Bidder's organs in South American *Bufo ictericus*. Here Bidder's organ has a typical ovarian structure such as cortex and medulla. The cortex contains oocytes at different stages of oogenesis, which are enclosed by a monolayer of follicular cells. Oocytes have large round nuclei, diffuse chromatin, and prominent nucleoli. The cytoplasm of oocytes shows a well-developed smooth ER, numerous mitochondria, many peroxysomes, poorly developed Golgi apparatus and occasional lysosomes and lipid droplets. There is a discrepancy in the literature concerning the presence of vitellogenic oocytes in Bidder's organs. The majority of studies have shown that the vitellogenic oocytes appear in Bidder's organ only after orchidectomy (testis removal) (Table 1; Ponse, 1927; Pancak-Roessler and Norris, 1991), however, some authors described the presence of vitellogenic oocytes in Bidder's organs of *Bufo marinus* or *Bufo arenarum* during a reproductive season when these organs grow (McCoy *et al.*, 2008; Scaia *et al.*, 2011). Moreover, the lampbrush chromosomes, characteristic of intensively RNA-synthesizing anuran oocytes, were described in the oocytes of Bidder's organs in *B. marinus*, *Bufo terrestris*, *Bufo woodhousii*, which indicates high metabolic activity of bidderian oocytes (Eichler, 1976). In summary, the ultrastructure of bidderian oocytes suggests that Bidder's organs are not vestigial ovaries, but rather organs that are able to produce functional female germ cells (Farias *et al.*, 2002).

Interestingly, the binuclear oocytes had been found in Bidder's organ in *Bufo melanostictus* (Gulhati, 1963). Such binuclear oocytes are also present in *B. bufo* and *B. viridis*, where they constitute about 20% of all diplotene oocytes of Bidder's organs (Fig. 2E; Piprek, unpublished data).

## The development of Bidder's organ

Early studies suggested that Bidder's organ is a residual ovarian structure that develops from progonad i.e. from the most anterior part of the genital ridge (early gonadal anlage) (Ponse, 1951; Pisano and Pizarro, 1958). Newer studies showed that in anuran amphibians the progonad differentiates into the fat body, which suggests that Bidder's organ develops from the most cranial part of the proper gonad (medial part of the genital ridge) instead (Viertel and Richter, 1999). Figure 2 depicts the structure and development of Bidder's organs during the larval life in *B. viridis*. Witschi (1933) stated that Bidder's organ (which does not have medullar cavity characteristic for ovary) develops from anterior gonomeres (metameres of gonad), which do not have typical medullar cavity. The lack of medullar cavity in Bidder's organ of *Bufo japonicus* would support this hypothesis (Tanimura and Iwasawa, 1993; Moriguchi and Iwasawa, 1987; Moriguchi *et al.*, 1991). There is also no distinct medulla in Bidder's organs in *B. viridis* (Fig. 2 A-F). However, there are several instances of the medulla being present in the center of Bidder's organs (Beccari, 1925; Vannini and Busetto, 1945; Talluri and Padoa, 1953). Farias and coworkers (2002) showed a small mass of somatic cells and capillaries immersed in collagen fibers and glycoproteins within Bidder's organ, which they believe represents the gonadal medulla. In fact, this could be a gonadal stroma that is located between the gonadal cortex and medulla and is invaded by capillaries. Falconi and coworkers (2007) showed the presence of small medulla only at the beginning of Bidder's organ development.

Bidder's organs develop at early stage of larval development (during the onset of hindlimb formation) (Beccari, 1925; Ponse, 1925; Ponse and Dovaz, 1943; Falconi *et al.*, 2007). These studies showed that in *B. bufo* and *B. viridis* at the onset of gonadal development at Gosner stage 29 (the hind limb bud development stage) the germ cells are larger and more abundant in the anterior part of genital ridge, i.e. in the region of future Bidder's organ, than

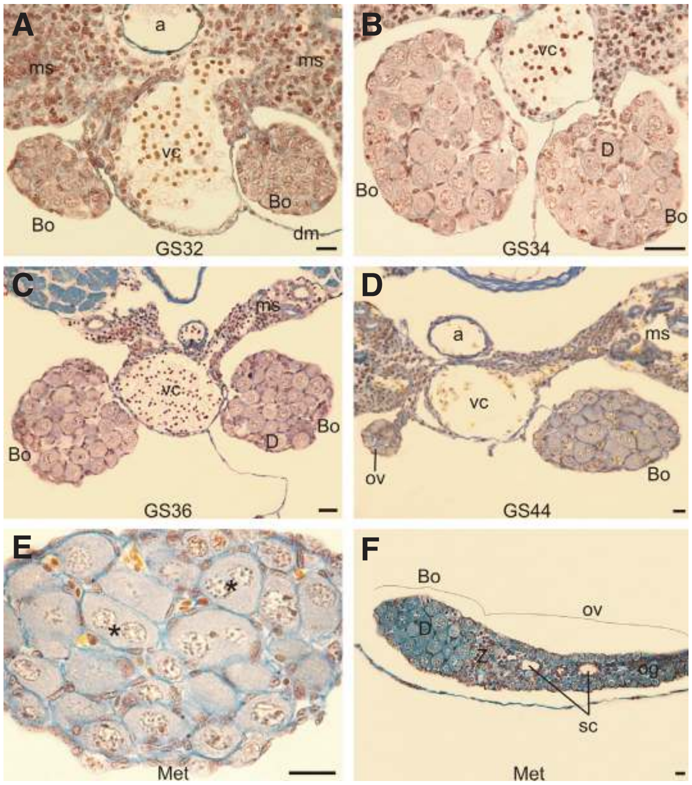

**Fig. 2. The structure of developing Bidder's organ in *Bufo viridis*. (A-E)** *Cross sections through Bidder's organs at subsequent Gosner stages (GS32 to metamorphosis, MET).* **(D)** *Cross section through the ovary (left gonad) and the Bidder's organ (right gonad).* **(E)** *Cross section through the Bidder's organ just after metamorphosis containing diplotene oocytes; asterisks indicate binuclear oocytes.* **(F)** *Longitudinal section through the Bidder's organ (Bo) and the ovary (ov); diplotene oocytes (D) are present in the Bidder's organ, oogonia (og) in the ovary, and the zygotene oocytes (Z) in the transitional retrobidderian region between the Bidder's organ and the ovary. During the development of the Bidder's organ in tadpoles, the germ cells enter diplotene and bidderian follicles form. a - aorta, dm - dorsal mesentery, ms - mesonephros, sc - secondary cavity in the ovaries, vc - vena cava. Debreuill staining, scale bar is equal to 30 $\mu$m.*

in the caudal region (Fig. 2F). The general structure of anterior and caudal region is similar; they both contain the gonadal cortex built of germ and somatic cells and the primary gonadal cavity lined with the basal lamina in the center.

At Gosner stage 34 (the toe differentiation stage) Bidder's organ has three layers: peripheral layer containing somatic cells and germ cells at various developmental stages, intermediate layer containing follicles with diplotene oocytes, and the central layer – medulla (Falconi *et al.*, 2007). Similar to the ovary proper the oogonia are present only in the peripheral layer. At Gosner stage 46 (metamorphosis complete) Bidder's organ contains only two layers: peripheral and intermediate. In contrast to the ovary proper, the medulla of Bidder's organ does not develop further and as a consequence Bidder's organs lack the secondary cavity (Fig. 2 A-F). The oocytes of Bidder's organs are enclosed by follicular cells (granulosa) and the theca interna. The theca externa that develops from medullar cells in the proper ovaries is absent in Bidder's organs (Witschi, 1933). The lack of medulla and the high ratio of germ to somatic cells found in Bidder's organ have been suggested to be the cause of irreversible determination of the anterior end of the bufonid gonad toward the ovarian fate and the formation of Bidder's organ (Witschi, 1933; Lepori, 1980). At Gosner stage 34, Bidder's organ contains synaptic and diplotene oocytes (Fig. 2B). In the ovary proper, the first oocytes appear later (around metamorphosis) than in Bidder's organ. At metamorphosis there are two different size (small and large) diplotene oocytes in Bidder's organs, which reflects the two waves of oogenesis during Bidder's organ development. The first wave begins at Gosner stage 34 and the second wave occurs at the end of metamorphosis (Falconi *et al.*, 2007). The first wave of bidderian oogenesis is so short and fast that some researchers postulated that there is a direct passage of primary oognia into diplotene oocytes without transient synaptic stage (Ponse and Dovaz, 1943; Ponse, 1949). During the first wave of oogenesis the diplotene oocytes grow faster in females than in males, however, there is no size difference in the second wave. Bidder's organ oocytes stop growing at previtellogenesis (stage I of oogenesis according to Dumont; Brown *et al.,* 2002).

The number of germ cells in Bidder's organ increases constantly, in males more slowly than in females (Falconi *et al.*, 2007). In *B. bufo* the differentiation of testes, which begins about Gosner stage 41 (forelimb appearance stage), influences Bidder's organ formation in males: the number of germ cells (oogonia and diplotene oocytes) and the size of diplotene oocytes of first oogonial wave decrease (Falconi *et al.*, 2007). The second wave of oogenesis in Bidder's organ ends, at least in the females, with the degeneration of large previtellogenic oocytes (Zaccanti and Gardenghi, 1968; Zaccanti *et al.*, 1971). Similar to the proper ovary the waves of oogenesis in Bidder's organs occur each year and they proceed from the peripherally located oogonia towards the central part of gonad.

Our studies showed that in *Bufo viridis* and *Bufo bufo* the anterior region of genital ridge grows more rapidly than the rest of the developing gonads due to the increase of germ cell number at the early stage of gonadogenesis (Gonser stage 29). This proliferation of germ cells in the future Bidder's organ leads to the club-like shape appearance of the developing gonads. Because in bufonids the development of Bidder's organ correlates with the appearance of the oocytes in the anterior tip of developing gonad, we hypothesized that the meiotic entry is the major determinant of Bidder's organ development (Piprek *et al.*, 2013). Previously it was shown that retinoic acid (carotenoid compound) induces meiosis in mouse (Bowles *et al.*, 2006; Koubova *et al.*, 2006). We showed previously that inhibition of retinoic acid synthesis by citral as well as blockage of retinoic acid receptors by BMS453 (4-[(1*E*)-2-(5,6-dihydro-5,5-dimethyl-8-phenyl-2-naphthalenyl)ethenyl]-benzoic acid) results in decrease or lack of diplotene oocytes in *in vitro* developing larval Bidder's organs (Table 1; Piprek *et al.*, 2013). This study indicated that the retinoic acid induces meiosis both in Bidder's organ and in developing ovaries. We also showed that there is a higher level of Raldh2 (retinoic acid synthesizing enzyme) in somatic cells of the developing Bidder's organ than in developing ovaries and testes. In contrast, the level of Cyp26b1 (retinoic acid-degrading enzyme) is lower in the developing Bidder's organ at Gosner stage 33 (the time of bidderian oocytes appearance and toe differentiation stage) than in the rest of the gonad (Piprek *et al.*, 2013). This suggests that

TABLE 1

**THE EFFECT OF VARIOUS COMPOUNDS OR SURGICAL MANIPULATION ON THE DEVELOPMENT OF BIDDER'S ORGANS (BO) IN DIFFERENT *BUFO* SPECIES**

| **Compound/manipulation** | **Effect** | **Species** | **References** |
|---|---|---|---|
| Gonadectomy (testis or ovary removal) | Bidder's organ development (full-grown oocytes) | *B. bufo, B. viridis,*<br>*B. woodhousii* | Harms, 1921; Ponse, 1927; Pancak-Roessler and Norris, 1991; Brown *et al.*, 2002 |
| Orchidectomy + gonadotropin injection (pregnant mare serum gonadotropin [PMSG] + human chorionic gonadotropin [hCG]) | Enhancement of Bo development, proliferation of oogonia, meiosis, steroidogenesis | *B. woodhousii* | Pancak-Roessler and Norris, 1991 |
| Gonadotropin injection (pregnant mare serum gonadotropin [PMSG] + human chorionic gonadotropin [hCG]) | Inhibition of Bo oogenesis and steroidogenesis | *B. woodhousii* | Pancak-Roessler and Norris, 1991 |
| Testosterone injection | Atrophy of Bo, inhibition of oogenesis | *B. melanostictus,*<br>*B. vulgaris formosus* | Takahashi, 1956; Deb and Chatterjee, 1963 |
| Spermatin injection | Growth of Bo and oocytes | *B. vulgaris formosus* | Takahashi, 1956 |
| Estradiol, estrone injection | Inhibition of Bo growth, no effect on proliferation of germ cells | *B. vulgaris formosus* | Takahashi, 1956 |
| Inhibition of 5α-reductase by 4-androsten-3-one-17β-carboxylic acid (low level of dihydrotestosterone) | Enhancement of Bo development | *B. bufo* | Zaccanti *et al.*, 1994 |
| LiCl (inhibition of steroidogenesis via 3 βHSD and 17βHSD) | Decrease of Bo size and number of oocytes | *B. melanostictus* | Nandi *et al.*, 1998 |
| Hypophysectomy (pituitary gland removal) | Atrophy of Bo | *B. arenarum* | Houssay, 1949 |
| Hypophysectomy + gonadectomy | Decrease of Bo size and number of oocytes | *B. melanostictus* | Ghosh *et al.*, 1990 |
| Gonadectomy + LH injection | Enhancement of Bo development | *B. melanostictus* | Ghosh *et al.*, 1990 |
| Citral (inhibition of retinoic acid synthesis) and BMS453 (inhibition of retinoic acid receptors) *in vitro* | Decrease or lack of diplotene oocytes in Bo | *B. viridis* | Piprek *et al.*, 2013 |

TABLE 2

**GENE EXPRESSION IN DEVELOPING TESTES, OVARIES AND BIDDER'S ORGANS IN *BUFO MARINUS* (BASED ON ABRAMYAN *et al.*, 2010).**

| Gene | Testes | Male Bidder's organs | Ovaries | Female Bidder's organs |
|---|---|---|---|---|
| *Dmrt1* | +++ | + | +++ | + |
| *Sf1* | +++ | ++ | ++ | ++ |
| *Sox9* | +++ | + | + | +(+++)* |
| *Dax1* | - | +++(-)* | +++ | +++(+)* |
| *p450arom* | - | +++ | ++ | +++ |

* changes in gene expression after metamorphosis

the high level of retinoic acid in the anterior part of genital ridges is responsible for the formation of oocytes and Bidder's organ.

The expression of genes involved in control of gonadogenesis was studied, using qRT-PCR, in developing Bidder's organ of *Bufo marinus* (Abramyan *et al.*, 2010). These studies showed that the gene expression in Bidder's organ is organ specific and more similar to developing ovaries than testes (Table 2). The main genes involved in control of gonad development in amphibians, i.e. *Dmrt1*, *Sox9*, *Sf1*, *Dax1* and *p450arom*, were expressed at different levels in the ovaries, testes and Bidder's organs. The level of *Dmrt1* (doublesex and mab-3 related transcription factor 1 involved in male sex determination) expression was lower in Bidder's organs than in testes and ovaries. The expression of *Sf1* (steroidogenic factor 1, nuclear receptor involved in male sex determination) was higher in testes than in Bidder's organs and ovaries. There was low expression of *Sox9* (SRY-box 9, transcription factor involved in male sex determination) in Bidder's organ and ovary, and in the female Bidder's organ there was a peak of *Sox9* expression after metamorphosis. *Dax1* (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1, a nuclear receptor involved in female sex determination) and *p450arom* (aromatase enzyme) expression was the highest in Bidder's organ, lower in the ovaries and the lowest in the testes. In female, but not in male Bidder's organ, *Dax1* had a second peak of expression after metamorphosis. In male and female Bidder's organs a down-regulation of *Dax1* expression correlated with the increase of *p450arom* and *Sf1* expression. Because *Dax1* inhibits *p450arom* expression, the high level of *Dax1* expression in the male Bidder's organ may be required to prevent estradiol synthesis. This may protect the testes from the feminizing effect of estrogens. Afterwards, when the testes are already differentiated, *Dax1* is down regulated, and *Sf1* and *p450arom* are up regulated in the male Bidder's organs, which enable the synthesis of estradiol. There were large fluctuations in the expression level of genes in developing Bidder's organs among individuals after gonadal differentiation into testes or ovaries, which may be associated with a high variability of Bidder's organs structure. It is interesting that, in spite of structural similarities between ovaries and Bidder's organs, the expression of steroidogenesis genes and sex-determining genes differ between these two organs.

## Sex hormones and surgical experiments

Harms (1921) first demonstrated that in *B. bufo* (former *Bufo vulgaris*) Bidderian oogenesis in males could be enhanced by orchidectomy (testis removal). He stated that although the orchidectomized male toads produce the full-grown oocytes within their Bidder's organ, they develop only rudimentary oviducts and therefore cannot lay eggs (Harms, 1921; Pancak-Roessler and Norris, 1991). However, it is interesting whether Bidderian oocytes are fertilizable. It can be tested via oocyte transplantation of marked bidderian oocytes into an appropriate female host. Figure 3 depicts the effects of various experiments on Bidder's organs development and the hormonal interactions between the organs.

Ponse (1927) showed that gonadectomy in females and males of *Bufo viridis* leads to the development of Bidder's organs into functional ovaries with vitellogenic oocytes (Table 1). Other authors also described the development of Bidder's organs into functional ovaries after orchidectomy (Witschi, 1933, Pancak, 1987, Pancak-Roessler and Norris, 1991; Brown *et al.*, 2002). Brown and coworkers (2002) described in the detail the resumption of Bidder's organ development after orchidectomy. Bidder's organs of orchidectomized individuals become highly vascularized, Bidderian oocytes grew (from 100 to 580 μm), accumulated yolk (onset of vitellogenesis), advanced to Dumont stages II-III, and within 1 month post-orchidectomy the whole Bidder's organ increased considerably in size. During the growth of bidderian oocytes after testes removal, the lamina-associated polypeptide 2 (LAP2 β) located under the nuclear envelope is replaced by LAP2ω (Brown *et al.*, 2002). Similar lamina isoform replacement takes place during oocyte growth in the ovary proper; small oocytes and somatic cells of the ovary proper contain LAP2β isoform, which during oocyte growth is replaced by LAP2ω isoform. This isoform replacement is probably associated with the increase in oocyte transcriptional activity.

Pancak-Roessler and Norris (1991) showed that in *Bufo woodhousii* the administration of gonadotropins (pregnant mare serum gonadotropin [PMSG] + human chorionic gonadotropin [hCG]) after orchidectomy enhances growth of Bidder's organ and stimulates proliferation of oogonia and development of oocytes towards later stages of oogenesis and vitellogenesis (Table 1). In addition, the orchidectomy enhances steroidogenic activity (3 beta-hydroxysteroid dehydrogenase [3 beta-HSD] and 17 beta-HSD) in Bidder's organ. Importantly, in the presence of testes (in sham-operated toads) Bidderian oogenesis remains inhibited despite the high level of gonadotropins in blood plasma. Thus, the high level of gonadotropins does not overcome the inhibitory effects of testes. The administration of testosterone in *Bufo melanostictus* caused atrophy of Bidder's organs, which indicates that testes inhibit Bidder's organ development (oogenesis) via androgens (Deb and Chatterjee, 1963). This, in turn, indicates that the testes (being the major source of the androgens) are responsible for the inhibition of oogenesis and the maintenance of Bidder's organ (Fig. 3).

Takahashi (1956) showed that the administration of testosterone in *Bufo vulgaris formosus* led to diminution of Bidder's organ size, inhibition of bidderian oocyte growth and inhibition of oogonia proliferation, but never to Bidder's organ disappearance or reversal of its sex (Table 1). Spermatin (a substance present in semen, allied to alkali albumin and mucin) administration led to the growth of Bidder's organ and increase of oocyte size and number. Administration of estradiol and estrone inhibited the growth of Bidder's organ, however, did not inhibit the proliferation of oogonia. All these results show that both androgens and estrogens produced by testes or ovaries inhibit the development of Bidder's organ.

Also more active form of androgens – dihydrotestosterone – has inhibitory effect on Bidder's organ. Zaccanti and colleagues (1994) showed that inhibition of 5α-reductase, that converts testosterone into dihydrotestosterone (DHT), via 4-androsten-3-one-17β-carboxylic acid, caused resumption of Bidder's organ development in *B. bufo* (Table 1). The decrease of DHT synthesis resulted in the increase of Bidder's organ volume and oocyte growth.

Petrini and Zaccanti (1998) showed that administration of testosterone to the tadpoles' water or inhibition of aromatase lead to the differentiation of the gonads into testis in the genetic females (sex reversal). Interestingly, they showed the strong tendency of Bidderian germ cells to develop toward oogenic fate regardless of sex genotype and steroid treatment. Neither the exogenous androgens nor the inhibition of estradiol synthesis (inhibition of aromatase) are able to alter the differentiation of germ cells present in Bidder's organ into oogenic cells.

The influence of steroid hormones on Bidder's organ was shown in *B. melanostictus* via subcutaneous injection of LiCl that inhibits steroidogenetic enzymes – 3βHSD and 17βHSD, important for androgen and estrogen production (Fig. 3) (Nandi *et al.,* 1998), and in the *in vitro* study of Bidder's organs cultured in the medium containing LiCl (Nandi *et al*., 1999). Both studies showed that the inhibition of androgen and estrogen production results in decrease of size and number of bidderian follicles (Table 1). This proves that balanced level of sex hormones are important for development of Bidder's organ and its inhibition in appropriate stage of gonad development.

Bidder organ development and maintenance depends not only on gonadal hormones but also on the hypothalamus-pituitary-gonadal pathway of endocrine system, which is depicted in Figure 3. The removal of pituitary gland (hypophysectomy) in *B. arenarum* leads to the atrophy of Bidder's organs (Fig. 3; Table 1; Houssay, 1949). Bidder's organ does not enlarge in the specimens with removed both gonads and pituitary gland. Bidder's organ resumes its growth and the vitellogenesis in its oocytes only when the pituitary gland is intact or if the toads are injected with solution of the *pars distalis* of pituitary gland. Ghosh and colleagues (1990) showed that in *B. melanostictus* the removal of gonads (gonadectomy) led to the increase of Bidder's organ weight, number of bidderian follicles and *corpora lutea*. However, the removal of both gonads and pituitary gland led to decrease in Bidder's organ weight, number of bidderian follicles and *corpora lutea* (Table 1). Administration of luteinizing hormone (LH, pituitary hormone) led to the resumption of Bidder's organ development. Thus, pituitary hormones are necessary for development of Bidder's organs into functioning ovaries but the gonads suppress the inhibitory influence of pituitary hormones towards Bidder's organs (Fig. 3).

Studies also showed that Bidder's organs are a source of steroid hormones and can have important role in regulation of seasonal reproduction. The enzymes responsible for synthesis of androgens and estrogens (3βHSD, 17βHSD) from cholesterol are present in the ooplasm of bidderian oocytes and in the follicular cells in *B. arenarum*, *B. melanostictus* and *B. woodhousii* (Ghosh, 1982; Pancak-Roessler and Norris, 1991; Scaia *et al*., 2011). Bidder's organ is capable of converting pregnenolone or progesterone into several steroid hormones such as 17-hydroxyprogesterone, androstendione, testosterone, 17β-estradiol and estrone (Colombo and Colombo-Belvedere, 1980). In *B. arenarum* the enzyme converting cholesterol into pregnenolone was detected in follicular cells only in few follicles, however, aromatase was present in all bidderian follicles (Scaia *et al*., 2011). In bufonids *B. marinus*, *B. bufo*, *B. arenarum* testes do not produce estradiol, however, the estradiol synthesis was detected in their Bidder's organs (Kime and Hews, 1978; Colombo and Colombo Belvedere, 1980; Canosa *et al*., 1998). Orchidectomy in *B. woodhousii* led to decrease in androgens but did not influence the level of estradiol (Pancak-Roessler and Norris, 1991). Thus, Bidder's organ is a source of estradiol in females and males. There is a correlation between the weight of Bidder's organ and level of plasma estradiol (Scaia *et al*., 2013). Estradiol is necessary for the regulation of cyclic reproduction activity and proliferation of spermatogonia in post-reproductive season. The removal of Bidder's organs disturbed spermatogenesis in bufonid

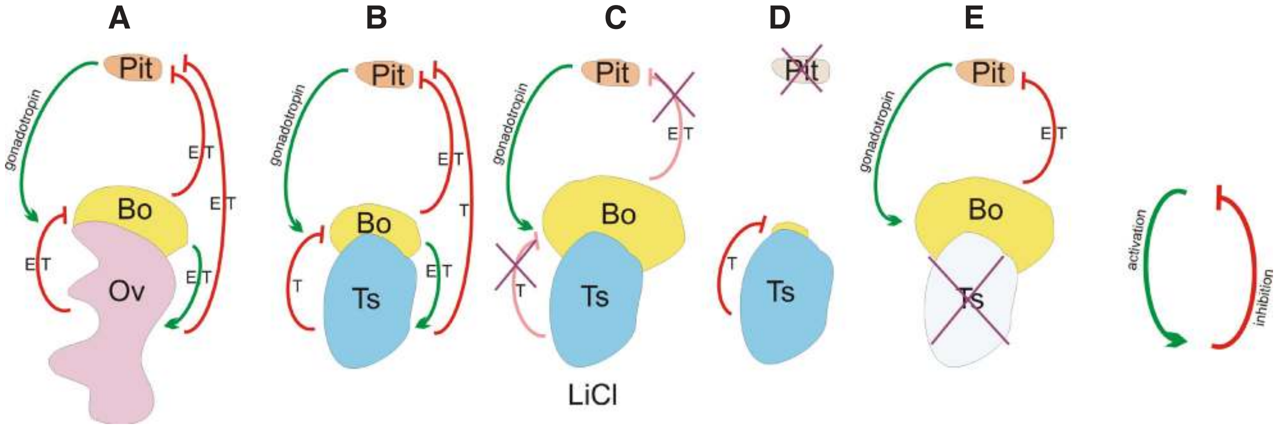

**Fig. 3. The hormonal interactions between Bidder's organs, gonads and pituitary gland. (A)** *In the females the Bidder's organs (Bo) produce estradiol (E) and testosterone (T) that stimulate (green arrow) ovaries (Ov) and together with E and T synthesized in the ovaries inhibit (red arrow) the pituitary gland (Pit). E and T produced in the ovaries inhibit the stimulating effect of gonadotropins on the Bidder's organs, which blocks their development and ovarian fate differentiation.* **(B)** *In the males, the mechanism of Bidder's organ regulation is similar to the females, however, the inhibition of the pituitary gland and thus the Bidder's organ development is due to the great load of testosterone produced by testes (Ts).* **(C)** *The block of steroidogenesis by LiCl administration leads to derepression of stimulating effect of gonadotropins on the Bidder's organs and thus to derepression of their development.* **(D)** *The removal of pituitary gland (hypophysectomy, cross) eliminates the stimulating effect of gonadotropins and the gonadal hormones and leads to the atrophy of the Bidder's organs.* **(E)** *The removal of the gonads (gonadectomy) eliminates the inhibitory effects of gonadal hormones on the Bidder's organs and the stimulating effect of gonadotropins, which results in the development of the Bidder's organs.*

males, however, administration of estradiol to such males normalized the spermatogenesis. Thus, estradiol derived from Bidder's organ is necessary for proper spermatogenesis. In *Rana esculenta* (Ranidae) high plasma and testicular level of estradiol was found in males (Fasano *et al.*, 1989). The synthesis of the estradiol in the testes may explain abnormalities in the male gonad structure in this species and the presence of oocytes (*testisova*) in their testes. It seems that in bufonids the production of estrogens had been shifted from testes to Bidder's organs, and this may prevent the exposure of testes and spermatogonia to high levels of female hormones. This may be one of the putative roles of Bidder's organ.

The steroidogenesis in Bidder's organ is independent of the gonadal steroidogenesis; the destruction of Leydig cells in the testes via administration of ethane 1,2-dimethane sulphonate, has no effect on steroidogenesis or oogenesis in Bidder's organ (Scaia *et al.*, 2011). Presumably, in addition to the steroid hormones secreted by gonads that inhibit differentiation of Bidder's organ into functional ovaries, other factors may influence development of Bidder's organs. Although the glucocorticoids do not influence Bidder's organ development, the environmental pollutants have a significant effect on its presence and weight (Scaia *et al.*, 2008). McCoy with colleagues (2008) showed that in *B. marinus* Bidder's organ abnormalities increase in a dose-dependent fashion with the increase of the pollutant level. This suggests that the variability of the structure of Bidder's organs observed in nature may result from the presence of environmental pollutants.

## Seasonal changes in Bidder's organ

Already early studies described the seasonal changes in Bidder's organs (Alexander, 1932; Roessler *et al.*, 1990; Pancak-Roessler and Norris, 1991). Recently, the correlation between steroidogenesis and seasonal changes of Bidder's organ size was described in details for South American *Bufo arenarum* (Scaia *et al.*, 2011). Bidder's organ size (weight) and the number of vitellogenic and atretic oocytes are the highest in the reproductive season (from September to December) and the lowest in the pre-reproductive season (from May to August) and post-reproductive season (from January to April) in *B. arenarum*. The testes are also larger during the reproductive season, however, the T/B ratio (testis/Bidder's organ ratio) is lower in this season due to the significant growth of Bidder's organ. Echeveria (1990) described the growth of Bidder's organ during reproductive season and its degeneration during non-reproductive season in *B. arenarum*. In contrast, in *B. woodhousii* Bidder's organ diminishes during the reproductive season (Calisi, 2005). There is correlation between the weight of Bidder's organs and testes in *B. woodhousii* (Calisi, 2005), but not in *B. arenarum* (Scaia *et al.*, 2011). The difference between these two bufonid species may result from the fact that in *B. woodhousii* the reproduction takes place when the level of androgens is high and in *B. arenarum* when the level of androgens is low. This indicates that Bidder's organ size is influenced not by the reproductive or non-reproductive season but by the testosterone level.

In *B. arenarum*, there is increase of estradiol concentration during the reproductive season and its decrease in pre-reproductive season (Scaia *et al.*, 2013). In contrast, the level of testosterone is low during the reproduction and high in pre-reproductive season. The same changes in sex hormones level occur in amphibians from temperate zone: the low level of testosterone during summer (reproduction season) correlates with proliferation of spermatogonia and formation of spermatocytes. The high level of testosterone during winter (non-reproductive season) correlates with spermiogenesis and inhibition of Bidder's organ. Estradiol (high level in summer) is required for spermatogonia proliferation. Thus, in *B. arenarum* in the pre-reproductive season, when the expression of aromatase is low, the level of estradiol is low and the level of testosterone is high, the weight of Bidder's organ is low, however, in the reproductive and post-reproductive season when the aromatase expression is high, estradiol load is high, and the level of testosterone is low, the weight of Bidder's organ is high.

Similar to all amphibians where the sex hormones control the reproductive activity, in male bufonids the production of estradiol had been shifted from the testes to Bidder's organ. The frequent total disappearance of Bidder's organs in bufonid females may be associated with the fact that ovaries produce sufficient amount of estradiol.

In spite of extensive literature on Bidder's organs development and structure their function still remains a mystery. Some researchers suggested that Bidder's organ is a by-product of the evolutionary loss of hyperfecundity among bufonids (Roessler *et al.*, 1990). These authors proposed that the bufonids are in the transitory state of evolutionary transformation from extensive, hyperfecund ovaries to smaller, less fecund ones. In this scenario, some members of *Bufo* retain hyperfecundity, while other such as derived bufonid species show a spectrum of ovarian reduction. In the latter cases Bidder's organs, by performing steroidogenic functions, may compensate for the reduced ovaries.

## Conclusions

Bidder's organs are present in males and females of the majority of bufonid species. They usually disappear in females during the lifetime.

*Melanophryniscus* and *Truebella* are basal bufonid species with (probably) primary absence of Bidder's organs. The *Nectophrynoides* show gradual reduction (to total disappearance) of Bidder's organs.

Bidder's organs develop, in both males and females, during the early gonadogenesis from the anterior parts of the gonads.

The gene expression profile in Bidder's organ is organ specific, different than in ovaries or testes. The expression of genes involved in sex determination and gonadogenesis in Bidder's organ is more similar to the expression in ovaries than in testes.

The analysis of retinoic acid synthesis and degradation suggests that the higher production of retinoic acid in the anterior ends of genital ridges in bufonids may be responsible for the formation of Bidder's organs.

Bidderian oocytes are produced in waves and become arrested in the previtellogenic stage.

Bidder's organs contain small and temporary medulla and lack of secondary ovarian cavity.

Bidder's organs grow and resume oogenesis after the removal of testes. Bidder's organs grow when the testosterone level is low, which occurs depending on the species during a reproductive or non-reproductive season.

The pituitary hormones are necessary for the development of Bidder's organs.

Bidder's organs are individually and seasonally variable.

The role of Bidder's organ still remains unknown. Proposed role are the steroidogenesis and a by-product of the loss of hyperfecundity in Bufonidae.

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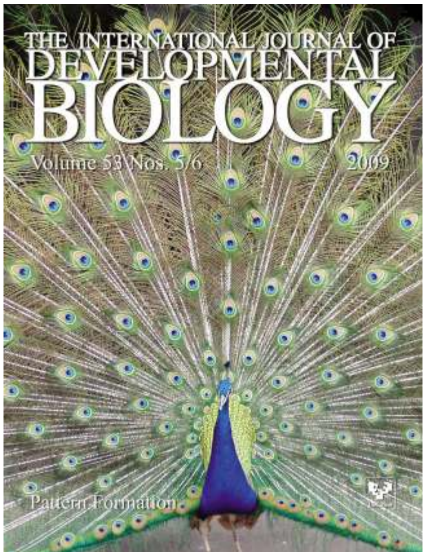

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