



# Gonadal Steroids: Synthesis, Plasmatic Levels and Biological Activities in Sturgeons

# 16

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## Abstract

The information on sex steroids in sturgeons is fragmented and comes from different species. In females, circulating oestradiol-17 $\beta$  increases during vitellogenesis and resulted a good marker of puberty in some species. However, researchers failed to induce oocyte growth and vitellogenesis using treatments with oestradiol-17 $\beta$ . Androgens also increase during this period but remained elevated during ovulation and post-ovulation suggesting a physiological role at peri-ovulatory period. 17,20,21P seems to be a good candidate as mediator of gonadotropin to induce follicle maturation since it is produced *in vitro* by sturgeons ovaries, it has a high potency to induce follicle maturation *in vitro*, and its plasmatic concentration increases after hormonal induction of ovulation; however, other C21 steroids as 17,21-dihydroxy-4-pregnene-3,20-dione (17,21P), 17,20 $\beta$ -dihydroxy-4-pregnen-3-one (17,20 $\beta$ P) and 17,20 $\beta$ -dihydroxy-4-pregnen-3-one (17,20 $\beta$ P) need to be further investigated as possible maturation-inducing steroids. Studies on potency of C21 and C19 steroids to induce resumption of meiosis revealed that C21 steroids are more potent than testosterone and that 11-oxygenated androgens do not induce maturation. Aromatase expression in immature males and plasmatic changes of testosterone and oestradiol-17 $\beta$  suggest their participation in early testicular development. 11-Ketotestosterone increased significantly during spermatogenesis both *in vitro* and *in vivo* as it has been shown in teleosts. The C21 steroid (17,20 $\beta$ P and 17,20,21P) increases in blood plasma after hormonal induction of spermiation suggesting their participation in the control of sperm maturation and release. Biological activity of steroids and *in vitro* steroid production by gonads are almost none studied in sturgeons.

## Keywords

Sturgeons • Sex steroids • Steroidogenesis • Plasma concentrations • Gonads  
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## Introduction

Sex steroid hormones play important roles in the regulation of different processes as sex differentiation, metabolism, immune responses, circadian rhythms, stress response and reproduction of vertebrates. This chapter is focused on gonadal steroids involved in the control of reproduction in sturgeons. A general introduction using the information on teleost fish will be done in order to have a reference to review information reported in sturgeons. The meaning of common abbreviations of steroids used in text is shown in the Table 16.1. The name of enzymes is abbreviated, and the corresponding name of gene is given in the text, and meaning is shown in Table 16.2.

In teleost fish steroid hormones, i.e. oestradiol-17 $\beta$ , testosterone, 11-ketotestosterone (11KT) and 17,20 $\beta$ -dihydroxy-4-pregnen-3-one (17,20 $\beta$ P), are highly produced by ovaries and testis under control of gonadotropins and are key factors regulating gametogenesis (see reviews from Billard et al. 1982; Fostier et al. 1983; Scott and Sumpter 1983; Scott and Canario 1987; Jalabert et al. 1991; Nagahama 1994; Borg 1994; Schulz and Miura 2002; Vizziano et al. 2007; Scott et al. 2010; Schulz et al. 2010).

During early gonad development, Yamamoto (1969) proposed that oestrogens are the natural inducers of female differentiation and androgens the natural inducers of male differentiation. More than 45 years of research suggest that oestrogens play an important endogenous role in the control of female teleost sex differentiation (Baroiller et al. 1999; Piferrer and Guiguen 2008; Guiguen et al. 2010). Nevertheless androgens seem not to be the universal endogenous regulators of male teleost sex

**Table 16.1** Meaning of common abbreviations of steroids used in text following Kime (1995)

Abbreviation	Full name	Other abbreviations or names
P5	3 $\beta$ -Hydroxy-5-pregnen-20-one	Pregnenolone
P	4-Pregnene-3,20-dione	Progesterone
17P	17-Hydroxy-4-pregnene-3,20-dione	17-Hydroxyprogesterone
17P5	3 $\beta$ ,17-Dihydroxy-5-pregnen-20-one	17-Hydroxypregnenolone
17,20 $\beta$ P	17,20 $\beta$ -Dihydroxy-4-pregnen-3-one	DHP
17,20 $\alpha$ P	17,20 $\alpha$ -Dihydroxy-4-pregnen-3-one	
17,21P	17,21-Dihydroxy-4-pregnene-3,20-dione	11-Deoxycortisol
17,20,21P	17,20 $\beta$ ,21-Trihydroxy-4-pregnen-3-one	20 $\beta$ -S
cortisol	11 $\beta$ ,17,21-Trihydroxy-4-pregnene-3,20-dione	Hydrocortisone
A	4-Androstene-3,17-dione	Androstenedione
T	17 $\beta$ -Hydroxy-4-androsten-3-one	Testosterone
11 $\beta$ OHT		11 $\beta$ -Hydroxytestosterone
11KT	17 $\beta$ -Hydroxy-4-androstene-3,11-dione	11-Ketotestosterone
11 $\beta$ OHA	11 $\beta$ -Hydroxy-4-androstene-3,17-dione	11-Beta-hydroxyandrostenedione
Ad	4-Androstene-3,11,17-trione	Adrenosterone, androstenetrione
E1	3-Hydroxy-1,3,5(10)-estratrien-17-one	Oestrone
E2	1,3,5(10)-Estratriene-3,17 $\beta$ -diol	Oestradiol-17 $\beta$
E17 $\alpha$	1,3,5(10)-Estratriene-3,17 $\alpha$ -diol	Oestradiol-17 $\alpha$

**Table 16.2** Meaning of common abbreviations of steroidogenic enzymes used in text and figures following zebrafish nomenclature

Abbreviation	Full name	Gene name
P450 scc	Side-chain cleavage enzyme	<i>cyp11a1</i>
P450-17H	17 alpha-hydroxylase/17,20 lyase	<i>cyp17a1</i>
P450-11H	11 beta-hydroxylase	<i>cyp11c1</i>
P450-arom	aromatase	<i>cyp191a</i>
P450-21H	21-Hydroxylase	<i>cyp21a2</i>
3 $\beta$ -HSDH	3 beta-hydroxysteroid dehydrogenase	<i>hsd3b1</i>
11 $\beta$ -HSD	Hydroxysteroid (11-beta) deshydrogenase 2	<i>hsd11b2</i>
17 $\beta$ HSD-type3	Hydroxysteroid (17-beta) dehydrogenase 3	<i>hsd17b3</i>
17 $\beta$ -HSD-type1	Hydroxysteroid (17-beta) dehydrogenase 1, or	<i>hsd17b1</i>
20 $\alpha$ HSD	Hydroxysteroid (20-alpha) dehydrogenase	
20 $\beta$ -HSD	Hydroxysteroid (20-beta) dehydrogenase2	<i>hsd20b2</i>

differentiation in fish because they are produced only in some species previously instead to sex differentiation (Nakamura et al. 1998; Vizziano et al. 2007; Ijiri et al. 2008; Hattori et al. 2009; Blasco et al. 2013).

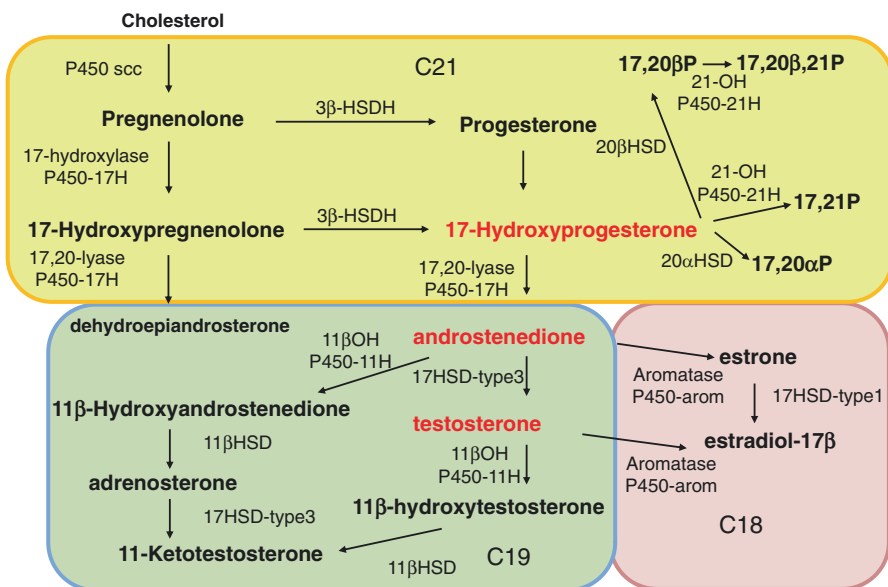
Contrasting with the high number of publications on steroid production and release and its effects in the regulation of gametogenesis reported in teleost fish, the number of studies published in sturgeons is very limited. This review provides an overview about steroid hormone biosynthesis at gonadal level and its release in blood plasma in sturgeons with special emphasis in Siberian sturgeon. It also contributes with original unpublished data on steroid profiles *in vivo* during puberty. There are few data on functional roles of steroids in sturgeons, and they will be introduced in sections of steroid synthesis and its plasmatic level along the biological cycle.

## 16.1 Steroidogenesis and Biological Activity Along the Course of the Biological Cycle

Steroidogenesis occurs in fish primarily in gonads, interrenal gland (Fostier et al. 1983) and brain (Diotel et al. 2011). All classes of steroid hormones are synthesized *de novo* from the common precursor cholesterol (Gower and Fotherby 1975). A key step is the conversion of cholesterol (C27 molecule) into pregnenolone (C21 steroid) by the action of the enzyme p450 side-chain cleavage enzyme (p450 scc) that removes the side chain of cholesterol (Gower and Fotherby 1975) (Fig. 16.1). This step is under the control of steroidogenic acute regulatory protein (star) that transfers cholesterol between the outer and inner mitochondrial membrane. The cytochrome p450 side-chain cleavage is the only enzyme able to convert the cholesterol into the first steroid (pregnenolone) of the steroidogenic route (Gower and Fotherby 1975); thus, its presence defines the steroidogenic capacity of one tissue. Downstream several enzymes modify pregnenolone to reach to the major steroids identified in

fish. The pathway proposed for teleost fish is shown in Fig. 16.1. One of the key enzymes involved in C21 steroid biosynthesis is  $3\beta$ -hydroxysteroid dehydrogenase ( $3\beta$ -HSD, *hsd3b*) that uses different substrates and is able to convert pregnenolone into progesterone and 17-hydroxypregnenolone (17P5) into 17-hydroxyprogesterone (17P) (Fig. 16.1). This enzyme transforms delta 5 steroids (steroids with double bond between carbon 5 and 6 and a hydroxyl group in the carbon 3) in delta 4 steroids (steroids with double bond between carbon 4 and 5 and a ketone group in the carbon 3). 17P is a metabolic crossroad point because it can be converted in other C21 steroids as 17,20 $\beta$ P, 17,20 $\alpha$ -dihydroxy-4-pregnen-3-one (17,20 $\alpha$ P), 17,21-dihydroxy-4-pregnen-3,20-dione (17,21P) and 17,20 $\beta$ ,21-trihydroxy-4-pregnen-3-one (17,20,21P) or in androstenedione, the first step in the androgen (C19 steroid) synthetic route (Fig. 16.1). Depending on tissue and stage of development 17P can be converted in androgens (C19 steroids) or in other C21 steroids (Fig. 16.1).

In tissues in which  $20\beta$ -hydroxysteroid dehydrogenase ( $20\beta$ HSD) is activated, the substrate 17P is converted into 17,20 $\beta$ P, while the activation of  $20\alpha$ -hydroxysteroid dehydrogenase ( $20\alpha$ HSD) is conducted to the synthesis of 17,20 $\alpha$ P. When the enzyme 21-hydroxylase (21-OH or P450-21H) is activated, 17,20 $\beta$ P can also be converted into 17,20,21P (Fig. 16.1).



**Fig. 16.1** Pathways of steroid synthesis in teleost fishes based on Fostier et al. (1983) and Borg (1994). Steroids are grouped following the number of carbons. C21 is highlighted in yellow, C19 or androgens in blue and C18 or oestrogens in pink. In red it is shown two crossroad points of gonad steroid metabolism: 17-hydroxyprogesterone and aromatizable androgens. The meaning of common name of steroid mentioned is given in Table 16.1 and of enzymes in Table 16.2

When 17-hydroxylase/lyase (P450-17H) is active and the substrate 17P is produced, the conversion of 17P (C21 steroid) into androstenedione (C19 steroid) can occur. Androstenedione is the first steroid of delta 4 androgen synthetic route and can be converted in testosterone (C19 steroid) by the enzyme 17-hydroxysteroid dehydrogenase type 3 (17HSD-type3). Testosterone can be converted in 11 $\beta$ -hydroxytestosterone (11 $\beta$ OHT) by the action of the enzyme 11 $\beta$ -hydroxylase (11 $\beta$ OH or P450-11H) and 11 $\beta$ OHT into 11-ketotestosterone (11KT) by the action of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD) (Fig. 16.1, Fostier et al. 1983; Borg 1994).

Androstenedione and testosterone (C19 steroids) are other metabolic crossroad points, because they can be converted into 11-oxygenated androgens by the action of the enzyme 11 $\beta$ OH or in oestrogens (C18 steroids) by the enzyme aromatase (P450-arom, *cyp19a1*). Aromatase is a key enzyme for oestrogen synthesis and converts androstenedione into oestrone and testosterone into oestradiol-17 $\beta$  (Fig. 16.1, Gower and Fotherby 1975; Fostier et al. 1983).

## 16.1.1 Ovarian Steroidogenesis

### 16.1.1.1 Pre-vitellogenesis and Vitellogenesis

In females of teleost fish, aromatase is active during pre-vitellogenesis, and its activity is highly increased during vitellogenic ovaries converting testosterone in oestradiol-17 $\beta$  which is released to the blood mainly during exogenous phase of vitellogenesis (Fig. 16.1, Fostier et al. 1983). Oestradiol-17 $\beta$  was detected in almost all fish studied at the time of vitellogenesis and decreases during follicle maturation period (Billard et al. 1978; Fostier et al. 1983; Scott and Sumpter 1983; Nagahama 1987, 1994). This oestrogen is the main regulator of vitellogenin production by the liver which constitutes the precursor of exogenous vitellus integrated in oocytes during the growing period of oocytes and ovaries (Lubzens et al. 2010).

Studies on steroid metabolism in female sturgeons are scarce. For pre-vitellogenic and vitellogenic females of Russian sturgeon, major metabolites of pregnenolone were 17-hydroxyprogesterone, androstenedione and testosterone, while those of androstenedione were testosterone and 5 $\alpha$ - and 5 $\beta$ -androstane diols. No evidence was found for the gonadal production of oestrogens studied by TLC/HPLC/reduction/oxidation and crystallization with authentic standard (Bukovskaya et al. 1997). The precursors androstenedione and testosterone are produced by pre-vitellogenic and vitellogenic tissues, but oestrogens were not identified indicating that aromatase were not active. The absence of oestrogen production by vitellogenic follicles of Russian sturgeons is unexpected since plasma concentrations of oestradiol-17 $\beta$  have been measured in vitellogenic females of the species (see Sect. 16.3) opening the question of the primary source of oestrogens released to blood plasma in the species.

Siberian sturgeon ovaries in pre-vitellogenesis express *cyp17a1*—the gene coding for the enzyme P450-17H—that converts C21 into C19 steroids (Table 16.2, Fig. 16.1). At this stage ovaries also express aromatase (*cyp19a1a*, see Table 16.2),

the gene coding for enzyme that converts androgens (C19) to oestrogens (C18) (Vizziano-Cantonnet et al. 2016). These results suggest that ovaries at pre-vitellogenesis are able to produce oestrogens.

In bester (*Huso huso* L. females x *Acipenser ruthenus* L. males), gonads at different stages incubated with precursors as pregnenolone, 17P and testosterone produced oestradiol-17 $\beta$  measured by ELISA (Amiri et al. 1999). The conversion of 17P and testosterone into oestradiol-17 $\beta$  involves the activation of several enzymes of steroid synthesis (P450-17H, 17 $\beta$  HSD-type 3, P450-11H, Fig. 16.1). Oestradiol-17 $\beta$  showed high concentrations in incubation media from primary yolk stage to tertiary yolk stages (vitellogenic ovaries) with respect to oil droplet stage (presence of oil droplets in the periphery of the nucleus) and decreased sharply at migratory nucleus stage, suggesting changes in aromatase activity as reported in teleosts (Kagawa et al. 1983; Young et al. 1983a, b).

Estrogen production has been poorly investigated in sturgeons during pre-vitellogenesis and vitellogenesis, but seems to be produced at least in Siberian sturgeons and in bester. The absence of oestrogen production in Russian sturgeons is unusual when considered the fish literature.

### 16.1.1.2 Follicle Maturation

In teleost fish, during last phases of oogenesis when follicles starts its maturation, 20 $\beta$ -hydroxysteroid dehydrogenase (20 $\beta$ HSD, *hsd20b*) is activated, and the substrate 17P is converted into 17,20 $\beta$ P (C21, steroid) considered as the most potent inducer of follicle maturation in almost all teleost fish studied (Fig. 16.1, Fostier et al. 1973; Fostier et al. 1983; Scott and Canario 1987; Nagahama 1994). 17,20 $\beta$ P is produced by ovaries and released to plasma mainly during the maturation period of several teleost fish under control of gonadotropin (Nagahama 1987; Jalabert et al. 1991). However, in female Sciaenidae 17,20 $\beta$ P can be converted into 17,20 $\beta$ ,21-trihydroxy-4-pregnen-3-one (17,20,21P) by the activity of the enzyme 21-hydroxylase (21-OH or P450-21H) (Fig. 16.1, García-Alonso et al. 2003, 2004). This tri-hydroxylated steroid is largely produced during maturation of the follicles of Atlantic croaker (*Micropogonias undulatus*), and it has been established as the maturation-inducing steroid in Sciaenidae fish (Trant et al. 1986; Thomas et al. 1997; Thomas 2012). In sum, two C21 steroids act as main mediators of follicle maturation in teleost fish: 17,20 $\beta$ P and 17,20,21P.

Few studies have been made in sturgeons during follicle maturation. In bester, 17,20 $\beta$ P has been measured by ELISA in culture media of ovaries at different stages (primary yolk stage, secondary yolk granule stage, tertiary yolk stage, migratory nucleus stage) with an increase at tertiary and migratory nucleus stages, the latter corresponding to the process of follicle maturation or meiosis resumption (Amiri et al. 1999). However, other potential C21 steroids were not measured to conclude about which is the potential mediator of follicle maturation in bester. The major steroids produced *in vitro* by follicles during maturation of Siberian sturgeon incubated with tritiated 17P and analysed by TLC/HPLC/oxidation were androstenedione, 17,20,21P, 17,20 $\alpha$ P and 17,21P (Alberro et al. 2008). 17,20 $\beta$ P was not confirmed by HPLC in the zone of TLC co-migrating with 17,20 $\beta$ P/17,21P, and 17,20,21P resulted the more

prominent C21 steroid produced *in vitro* (Alberro 2009). In white sturgeons among C21 steroids produced by maturing follicles, 17,21P, 17,20 $\beta$ P and 17,20,21P had the major activity as maturation-inducing steroid (MIS) (Webb et al. 2000).

Interestingly, in other sturgeons as sterlet, 17,20,21P increased ten times in culture media of follicles 16 h after gonadotropic stimulated maturation (Semenkova et al. 2006).

In sum, di- and tri-hydroxylated progestins (C21 steroids) are produced by sturgeon ovarian follicles during maturation. They were 17,20 $\beta$ P, 17,21P and 17,20,21P for white sturgeon and bester; 17,21P, 17,20 $\alpha$ P and 17,20,21P for Siberian sturgeon; and 17,20,21P and 17,20 $\beta$ P for sterlet. 17,20P was not produced *in vitro* by Siberian sturgeon during the period of follicle maturation. Collectively, the results suggest that the main mediator of follicle maturation could be 17,20,21P in some sturgeon species, including Siberian sturgeon. Other C21 steroid produced could also contribute to regulate the follicle to reach the stage needed to be ovulated and fertilized as it was previously proposed (Webb et al. 2000). The results suggest that some sturgeon species share with Sciaenidae fish 17,20,21P as mediator of follicle maturation.

Another steroid studied during ovarian development of sturgeons was 11KT (Siberian sturgeon, Cuisset et al. 1995; Russian sturgeon, Bukovskaya et al. 1997; white sturgeon Webb et al. 2002). Experiments made *in vitro* showed that the yield of 11KT production was very low in ovarian follicles of Siberian sturgeons at maturation time (Cuisset et al. 1995), while no 11KT was detected using similar stages of ovarian follicle development for the species (Alberro et al. 2008). Moreover, 11KT was not identified in follicles of white sturgeon during maturation (Webb et al. 2002) or in pre-vitellogenic and vitellogenic ovarian follicles of Russian sturgeon (Bukovskaya et al. 1997). The results suggest that 11KT is not a mediator of follicle maturation in sturgeons.

## 16.1.2 Testicular Steroidogenesis

### 16.1.2.1 Spermatogenetic Period

Spermatogenesis comprises three major phases: mitotic proliferation of spermatogonia, meiosis of spermatocytes and spermiogenesis (Schulz and Miura 2002). When spermatozoa are morphologically completed after spermiogenesis, sperm maturation and hydration occurs under regulation of gonadotropins and steroids; see review (Vizziano et al. 2007).

Spermatogonial renewal is stimulated by low doses of oestradiol-17 $\beta$  in eels (Miura et al. 1999) at early phases of testis development when testis were composed of spermatogonias. Physiological role of oestrogens in males has been poorly studied in fish. In Salmonidae, evidence that oestradiol-17 $\beta$  is produced in testes suggest that oestrogens participates in paracrine control of testicular function (see review Vizziano et al. 2007). In sturgeons the oestrogen production by testes has not been reported. However, aromatase—the enzyme that converts androgens to oestrogens—(Fig. 16.1) is expressed in testis of immature (only spermatogonias) male

Siberian sturgeons suggesting that oestrogens are produced very early and could be involved in regulating early testicular development of this species (Vizziano-Cantonnet et al. 2016).

Contrasting with oestrogens, androgens have been well studied in fish. In adult males of teleosts, testis in spermatogenesis converts testosterone in 11 $\beta$ -hydroxytestosterone (11 $\beta$ OHT) by the action of the enzyme 11 $\beta$ -hydroxylase (11 $\beta$ OH or P450-11H, *cyp11b/cyp11c1*) and 11 $\beta$ OHT into 11-ketotestosterone (11KT) by the action of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ HSD, *hsd11b*) (Fostier et al. 1983; Borg 1994). This seems to be the predominant way of synthesis. As consequence spermatogenic period in fish is characterized by the production of high level of 11-oxygenated androgens (11 $\beta$ OHT, 11KT, 11BOHA, Ad) especially 11KT considered as the most potent androgen in fish (Fostier et al. 1983; Borg 1994). Androgens are effective in supporting spermatogenesis (Billard et al. 1982; Fostier et al. 1983; Borg 1994; Nagahama et al. 1994), some early steps as spermatogonial proliferation (Schulz and Miura 2002) and were stimulated by gonadotropins along all testicular cycle (see review Vizziano et al. 2007). It is known that gonadotropin and 11KT induces spermatogonial proliferation in teleosts (Schulz and Miura 2002), and can induce spermiation in some species. But they were clearly less effective than progestins to induce sperm release (Ueda et al. 1985; Schulz et al. 2010).

There are few studies on steroid metabolism of sturgeon testes. Testosterone and 5 $\alpha$ - and 5 $\beta$ -androstane diols, but no 11KT were produced after incubation of pre-spermiating testes of Russian sturgeons with radiolabeled androstenedione (Bukovskaya et al. 1997). Contrasting with that in baster, 11KT has been measured by ELISA in culture media of testis at different developmental stages incubated with inert pregnenolone, 17P and testosterone (Amiri et al. 1999). Levels of 11KT resulted higher during late spermatogenesis and pre-spermiation to decrease at degeneration stage (post-spawning).

The few reports in male sturgeons prevent any general conclusion, but at least in baster, 11KT is high during spermatogenic period as expected.

### 16.1.2.2 Sperm Maturation and Release

In Salmonidae, 17,20 $\beta$ P is produced from immature to spermiating testes and is under gonadotropin regulation both *in vivo* and *in vitro* (Scott and Sumpter 1983; Schulz et al. 1992, 2010; Vizziano et al. 2007; Schulz and Miura 2002; Scott et al. 2010). 17,20 $\beta$ P is considered an essential factor for the initiation of meiosis at least in eels (Miura et al. 2006), but the effect of this C21 steroid on the initiation of meiosis remains to be better studied in other species. A physiological role of 17,20 $\beta$ P has been shown in Salmonidae and Cyprinidae (Ueda et al. 1985), in the amplification of milt volume production (Baynes and Scott 1985; Yueh and Chang 1997) and in acquisition of the potential motility of male gametes (Miura et al. 1992; Miura and Miura 2003). In male Sciaenidae, the main metabolite produced by testes from spermiating fish is 17,20 $\beta$ P (Vizziano-Cantonnet et al. 2015), but its participation as mediator of sperm maturation and release remains to be studied.



Steroids produced by testes of sturgeons are unknown during spermiation, and very scarce data are published on steroid metabolism of testes at different stages of adult cycle. In Russian sturgeons, pregnenolone was added to incubation media of pre-spermiating testes and metabolites analysed by TLC/HPLC/reduction/oxidation and crystallization with authentic standard. The metabolites produced were progesterone, 17P and androgens, but no 17,20 $\beta$ P were reported (Bukovskaya et al. 1997). Contrasting with that, in bester the addition of precursors as pregnenolone and 17P to culture media of testes from males at different testicular stages resulted in detectable levels of 17,20 $\beta$ P measured by ELISA, reaching high levels in late spermatogenesis and pre-spermiation (Amiri et al. 1999). The trends observed in male bester are in agreement with a physiological role of 17,20 $\beta$ P from early spermatogenesis to pre-spermiation as it was observed for Salmonidae. The work made in bester (Amiri et al. 1999) suggest a change in synthesis from 11KT during spermatogenesis to 17,20 $\beta$ P during pre-spermiation, i.e. from C19 to C21, similarly to changes described for teleost fish (Schulz and Miura 2002; Vizziano et al. 2007). However the lack of production of 17,20 $\beta$ P by pre-spermiating testis of Russian sturgeons (Bukovskaya et al. 1997) open the question about the mediators used by this species.

In Siberian sturgeon, *in vivo* studies after hormonal stimulation of spermiation show a high increase in plasma concentrations of 17,20 $\beta$ P together with an increase in the volume of sperm released (Vizziano et al. 2006) suggesting that 17,20 $\beta$ P is a main mediator of sperm maturation and release as it was reported in teleost fish; see review (Vizziano et al. 2007). In Stellate sturgeon males, plasmatic 17,20,21P showed an increase after LHRH analogue stimulation of spermiation. However, other C21 steroids as progesterone and 17,20 $\beta$ P were not stimulated. Testosterone and cortisol were also stimulated during induced spermiation (Bayunova et al. 2006). Other studies in stellate sturgeon showed that only testosterone was increased significantly after hormonal stimulation of spermiation, while progestins 17,20,21P and 17,21P increased significantly only at post-spawning period (Bayunova et al. 2008). Finally, in another report on stellated sturgeon, progesterones were clearly increased in blood plasma after injection of sturgeon pituitary extracts, but 17,20,21P was not measured (Semenkova et al. 2002). For Persian sturgeon testosterone, 11KT and progesterone resulted more elevated in spermiating males stimulated by LHRH analogue when compared to non-spermiating males (Aramli et al. 2014). Similar results were obtained for testosterone and 11KT in Russian sturgeon males (Artyukhin et al. 2006).

The *in vivo* studies after hormonal stimulation of spermiation show that C21 steroids as 17,20 $\beta$ P and 17,20,21P can be increased, while in other cases androgens were stimulated.

Results on C21 steroid production in sturgeons females and males at the end of gametogenetic cycle show that two major steroids seem to be MIS in females (17,20 $\beta$ P and 17,20,21P), while 17,20 $\beta$ P could be the progestin involved in the regulation of sperm maturation and release of Siberian sturgeons; however, other steroids as 17,20,21P remain to be studied for the species.

A physiological role of androgens during spermiation cannot be discarded.

## 16.2 Circulating Steroids During Puberty of Females

Puberty is a critical endocrine period that comprises the transition between immature juvenile to a mature adult state of the reproductive system; see review of Taranger et al. (2010). The fish became capable to reproduce for the first time, and this implies that hypothalamus-pituitary-gonad axis became functional (Taranger et al. 2010). During the entry in puberty of females—considered as the transition to first batch of oocytes accumulating cortical alveoli—oestradiol-17 $\beta$  increases in some salmon and yellowtail together with circulating fish (Taranger et al. 2010).

In farmed fish it is interesting to control puberty to advance or delay it. In natural conditions Siberian sturgeon reach puberty at different ages depending on the river or lake in which it lives, ranging from 9 to 34 years for females and 8 to 29 years for males (see Chap. 1). In captivity, this species reaches puberty at 5 years old for males and 7 years old for females in France (Pelissero and Le Menn 1991). In Uruguayan culture conditions, males are spawning at 3 years old, while females reach the puberty at 5 years old (Ryncowski, personal communication). In Russian sturgeon sexual maturity is reached at 8–13 years for males and 10–16 years for females under natural conditions (Vlassenko et al. 1989). Puberty occurs in farms of Israel at 6 years old, and oestradiol-17 $\beta$  was a very good indicator of ovarian development for the species (Hurvitz et al. 2008).

Studies made during puberty in Siberian sturgeon maintained in captivity in France (females and males at 4, 5 and 10 years old) showed that plasmatic concentration of oestradiol-17 $\beta$  was not different between males and females (Pelissero and Le Menn 1988) as expected considering the trends observed in teleosts. Moreover, oestradiol-17 $\beta$  showed high concentrations in pre-vitellogenic fish and do not show a sharp increase during vitellogenesis (see Table 16.5). In addition the maximum levels of oestradiol-17 $\beta$  in plasma were reached at pre-ovulating time and not in vitellogenic females as expected. Oestrone increased between pre-vitellogenic and vitellogenic females, but remained high during pre-ovulation period. Oestrogens do not follow the trends observed in other fish, and the authors measured oestrogens in food to better understand their results. They suggested that oestrogens measured in males and females could be the result of an alteration of the natural production altered by the exogenous supply coming from food.

Studies made by our laboratory in cooperation with sturgeon farmers of Uruguay showed that Russian sturgeon reaches puberty at 5 years old as indicated by the presence of females at stage 3 (follicle diameter ~2 mm) and stage 4 (~2.5 mm). Fish data and maturity stages are given in Table 16.3. Serum anti-oestradiol-17 $\beta$  was very specific as it is shown in Table 16.4. Plasmatic concentrations of oestradiol-17 $\beta$  increased significantly ( $p < 0.05$ ) between immature females (stage 1) and females at stage 2 (small white oocytes) and stage 3 (follicle diameter of 2 mm) (Fig. 16.2). At stage 4 (follicle diameter of 2.5 mm) oestradiol-17 $\beta$  decreased significantly ( $p < 0.05$ ). Oestradiol-17 $\beta$  resulted in a good indicator of female puberty for Russian sturgeon. We used oestradiol-17 $\beta$  for the diagnosis of a delayed cohort of 5-year-old fish that were not well developed and for which oestradiol-17 $\beta$

**Table 16.3** Plasma concentrations of E2 in female *A. gueldenstaedtii* at immature and first maturity stages in fish farmed in Uruguay

Species/country	Puberty in captivity	Maturity stage	Oocyte diameter (mm)	E2 (ng mL <sup>-1</sup> )
<i>A. gueldenstaedtii</i> /Uruguay	5 years old	Stage 1—undistinguishable oocytes (n = 10)	nd	0.074 ± 0.09
		Stage 2—white tissue, undistinguishable oocytes (n = 13)	nd	1.53 ± 3.25 (p < 0.05)
		Stage 3—grey oocytes (n = 8)	2.05 ± 0.23 (n = 100)	4.37 ± 2.03 (p < 0.05)
		Stage 4—grey oocytes (n = 11)	2.47 ± 0.11 (n = 159)	0.64 ± 0.86 (p < 0.05)

nd = not determined

**Table 16.4** Cross-reactivity of serum anti-oestradiol-17β used for radioimmunoassay

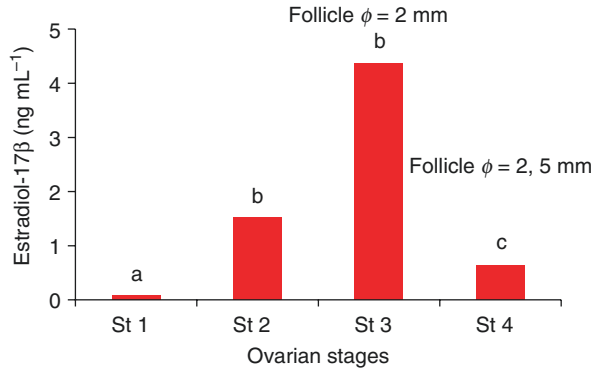
Steroids	Anti E2
17,20,21-P	nd
17P	nd
P4	nd
17,21P	nd
cortisol	nd
T	<0.01
E2	100%
A	nd
11BOHA	nd
17,20BP	<0.01
E3	<0.01
E17α	<0.01
11KT	<0.01

nd: assay not done

concentrations were undetectable. We were able to answer to farmers that this cohort was unable to produce caviar in next months.

In order to control vitellogenesis, experiments with oestradiol-17β implants during immature period of females have been made in *Huso huso*, but growing of oocytes was not observed (Akhavan et al. 2015), while oestradiol-17β causes suppression of growth in stellate sturgeon (Khara et al. 2013). A more ample knowledge on the axis of hypothalamus-pituitary-gonads during puberty remains to be further studied in sturgeons.

**Fig. 16.2** Concentrations of plasmatic oestradiol-17 $\beta$  (ng mL<sup>-1</sup>) during ovarian development at puberty of Russian sturgeons of 5 years old in farmed conditions in Uruguay. Statistical differences come from an ANOVA



## 16.3 Circulating Steroids During Adult Phase

### 16.3.1 Females

During gonadal cycle, oestrogens (C18 steroids) are produced by vitellogenic ovaries, and a shift towards progestins (C21 steroids) occurs when follicles enter to the process of maturation (resumption of meiosis). The shift from the gonadal production of oestradiol-17 $\beta$  to 17,20 $\beta$ P has been first observed in teleosts (Kagawa et al. 1983; Young et al. 1983a, b; Yaron 1995). Steroids are released to the blood, and measurements of plasmatic steroids reflect in some extent the gonadal steroid production of ovaries. 17,20 $\beta$ P is produced in some teleosts during ovarian maturation induced by gonadotropins and is released and measured in plasma during maturation and ovulation (Fostier et al. 1981; Young et al. 1982, 1983b; Ueda et al. 1984). In this section the steroid changes in blood plasma are reviewed for female sturgeons.

#### 16.3.1.1 Pre-vitellogenesis and Vitellogenesis

In Siberian sturgeon (Hamlin et al. 2011) and in bester (Amiri et al. 1996a), oestradiol-17 $\beta$  showed a significant increase of 6–15 times from pre-vitellogenic to vitellogenic fish reaching 4–7.5 ng mL<sup>-1</sup> with a significant decline at post-vitellogenic and maturation (migratory nucleus) stages (Table 16.5). Similar results have been observed for Russian sturgeons during puberty (see Sect. 16.3). These are the expected profiles of oestradiol-17 $\beta$  taking account the information coming from teleost fish and are coherent with a functional role of oestradiol-17 $\beta$  as stimulator of exogenous vitellogenesis. However, the primary source of oestradiol-17 $\beta$  needs to be further investigated at least for Russian sturgeons, for which ovaries at vitellogenesis are not able to produce oestradiol-17 $\beta$  when precursors are given.

Testosterone concentrations in blood plasma of sturgeons can be one or two orders higher than oestrogens for a given species (Table 16.5). A significant increase

in testosterone concentrations occurs from pre-vitellogenic to vitellogenic sturgeons (bester, Amiri et al. 1996a, Siberian sturgeon, Hamlin et al. 2011, Table 16.5) as it was observed for oestradiol-17 $\beta$ . Oestrogens decreases significantly at the end of oogenesis during migratory nucleus stage in sturgeons (Hamlin et al. 2011, Table 16.5) as it is the case in teleosts. Contrasting with that Siberian sturgeons, testosterone can increase significantly at follicle maturation stage (Pelissero and Le Menn 1988; Hamlin et al. 2011) and remain at high concentrations during post-ovulated period in Stellate sturgeon (Bayunova et al. 2011) or during post-vitellogenesis in bester (Amiri et al. 1996a, Table 16.5). The high testosterone concentration around the time of ovulation is discussed below. C21 steroids as progesterone, 17,20 $\beta$ P and 17,20,21P remain low (0.05–0.75 ng mL<sup>-1</sup>) and stable from pre-vitellogenesis to post-vitellogenesis (Table 16.5) suggesting that this C21 steroids are not involved in the regulation of this process.

### 16.3.1.2 Follicle Maturation

As previously mentioned progestins measured in blood plasma (P and 17,20 $\beta$ P) are very low (order of pg mL<sup>-1</sup>) and do not change between immature and post-vitellogenic fish (Table 16.5). Progesterone and 17,20 $\beta$ P remain very low ( $\leq 0.3$  ng mL<sup>-1</sup>) except for European sturgeon (*Acipenser sturio*) that shows  $\sim 1$  ng mL<sup>-1</sup> during maturing stages (Table 16.5). The progestins measured in sturgeons without hormonal stimulation were in general low, but 17,20,21P is highly increased in blood plasma after hormonal stimulation of follicle maturation (Sect. 16.2.1.2). In fact in sterlet females 17,20,21P, but not 17,20 $\beta$  P was clearly stimulated after hormonal treatments to induce follicle maturation (Bayunova 2016). In addition, in Stellate sturgeon a surge of 17,20,21P has been observed after hormonal stimulation (GnRH) of follicle maturation (Semenkova et al. 2002; Bayunova et al. 2006; Semenkova et al. 2006; Bayunova 2016). In accordance with these data 17,20 $\beta$  P became detectable but very low (0,09 ng mL<sup>-1</sup>) after hormonal stimulation (GnRH) of follicle maturation (100% of GVBD) of Siberian sturgeon females (Vizziano et al. 2006). These *in vivo* data together with *in vitro* studies (see 16.2.1.2) suggest that 17,20,21P is the main mediator of follicle maturation in several sturgeon species including Siberian sturgeon

Siberian sturgeon females show high plasmatic levels of 11KT during the whole period of ovarian development and reached its maximum concentrations at early and mid vitellogenesis. Moreover, a huge and significantly high amounts of testosterone (120 ng mL<sup>-1</sup>) were described during follicle maturation of this species (Table 16.5, Williot et al, Chap. 17 of this book, Hamlin et al. 2011). A possible explanation is that testosterone acts in sturgeons as follicle maturation mediator of LH. However, studies on potency of C21 and C19 steroids to induce resumption of meiosis (follicle maturation or GVBD) in sturgeons revealed that C21 steroids (17,21P, P, 17P, 17,20P, 17,20,21P and cortisol) are more potent than testosterone and that 11-oxygenated androgens do not induce maturation (Webb et al. 2002). This result discards androgens as follicle maturation inducers.

**Table 16.5** Plasma steroid concentrations (ng mL<sup>-1</sup>) measured in female sturgeons

Species	Ovarian stage/month	E2	E3	T	11KT	17,20P	17,20,21P	P	Source
<i>A. baerii</i>	Pre-vitellogenic	11.6 ± 1.2	5.1 ± 3.2	8.2 ± 0.3	-	-	-	-	Pelissero and Le Menn (1988)
	Vitellogenic	16.6 ± 1.34	10.8 ± 7.18	5.8 ± 0.54	-	-	-	-	
	Pre-ovulation	19.3 ± 2.68	10.3 ± 1.53	109 ± 25.5	-	-	-	-	-
	Migratory nucleus	0.15-0.41	-	16-133	-	nd	-	-	Vizziano et al. (2006)
<i>A. baerii</i>	Follicle $\phi = 3.3-4.7$ mm	-	-	-	-	-	-	-	Hamlin et al. (2011)
	Pre-vitellogenic (St2)	~0.5	-	~10	~5	-	-	0.15	
	Follicle $\phi = 0.6$ mm	-	-	-	-	-	-	-	
	Early-vitellogenic (St3)	~3.2 ( $p < 0.05$ )	-	~40 ( $p < 0.05$ )	~20 ( $p < 0.05$ )	-	-	0.1	
	Follicle $\phi = 0.6-1.8$ mm	-	-	-	-	-	-	-	
	Mid-vitellogenic (St4)	~7.5 ( $p < 0.05$ )	-	~65	~20	-	-	0.12	
	Follicle $\phi = 1.8-2.2$ mm	-	-	-	-	-	-	-	
	Migratory nucleus (St5)	~6 ( $p < 0.05$ )	-	~120 ( $p < 0.05$ )	~10 ( $p < 0.05$ )	-	-	0.13	
	Follicle $\phi = 1.8-2.2$ mm	-	-	-	-	-	-	-	

<i>A. baerii</i>	Migratory nucleus Follicle $\phi = 3.3$ – 4.7 mm	~0.7–2.7	–	~40–160	~58–90	~1.8–3.8	–	Williot et al see Chap. 17
<i>A. sturio</i>	Mature female	~33	–	12–15	<2	<2	–	Davail- Cuisset et al. (2008)
<i>A. sturio</i>	Maturing (IP = 0.1–0.3)	4.5	–	9.4	1.7	0.05	–	Davail- Cuisset et al. (2011)
	90% GVBD/ ovulation	7.9	–	9.6	2.2	1	–	
<i>Huso huso</i> L. Females x <i>Acipenser</i> <i>ruthenus</i> L. males	Immature Vitellogenic Post- vitellogenic	<0.6 2–4 ( $p < 0.01$ ) 1–2 ( $p < 0.01$ )	– – –	5–15 20–80 ( $p < 0.05$ ) 20–80	– – –	0.05–0.3 0.05–0.3 0.05–0.3	– – –	Amiri et al. (1996a)
<i>A. gueldenstaedtii</i>	Pre- vitellogenic oocytes	0.2–0.48	–	–	–	–	–	Hurvitz et al. (2008)
<i>A. gueldenstaedtii</i>	Stage III Stage IV Stage V	0.68 ± 0.93 – –0.11 ± 0.22	– – –	46 ± 67 105 ± 30 19 ± 7	13.7 ± 3.4 – 28.8 ± 6.7	– – –	– – –	Barannikova et al. (2006)

(continued)

Table 16.5 (continued)

Species	Ovarian stage/month	E2	E3	T	11KT	17,20P	17,20,21P	P	Source
<i>A. stellatus</i>	Spring	~3	-	~55	~70	~0.025	-	-	Ceaça et al. (2002)
	Early May	<2.5	-	~35	~18	~0.25	-	-	
	Late May	~6	-	~30	~39	~0.2	-	-	
	Autumn	~2.5	-	~48	~78	~1	-	-	
<i>A. stellatus</i> <sup>a</sup>	Stage IV (April–May)	0.9 ± 0.09	-	172 ± 13	26 ± 1.3	-	-	0.9 ± 0.3	Barannikova et al. (2002)
	Stage III–IV (May–June)	0.4	-	98 ± 7	35	-	-	0.6 ± 0.1	
	Stage IV (June)	0.38 ± 0.2	-	99 ± 9	28 ± 5.3	-	-	0.9 ± 0.1	
	Stage IV (July)	-	-	29 ± 8	-	-	-	0.3 ± 0.1	
<i>A. stellatus</i>	Post-ovulated	-	-	111 ± 43	9.5 ± 2	-	-	0.4 ± 0.12	Bayunova et al. (2011)
<i>A. stellatus</i>	Pre-spawning	~1.4	-	~180	~25	~0.1	~0.25	~0.8	Bayunova et al. (2006)
<i>A. stellatus</i>	Captured in wild and delivered to the farm	1.8 ± 1	-	94 ± 14	25 ± 4	-	<0.5	0.15 ± 0.1	Semenkova et al. (2002)
	14 days holding	1.6 ± 0.8	-	167 ± 21	40 ± 4	-	<0.5	0.33 ± 0.05	
<i>A. ruthenus</i>	Pre-spawning	-	-	~18	~10	~0.5	~0.75	-	Semenkova et al. (2006)

<sup>a</sup>Stages III–IV, incomplete gametogenesis; Stage IV, complete gametogenesis; Stage V, mature gonads (Barannikova et al. 2002)



The high concentrations of testosterone in female before and after ovulation (Vizziano et al. 2006, Hamlin et al. 2011, see Chap. 17, Bayunova et al. 2011) and in LH-stimulated culture media of maturing follicles (Semenkova et al. 2006) suggest an important function during peri-ovulatory stage (Table 16.5). The presence of androgens in coelomic fluid leads instead to propose that androgens are functional to maintain the viability of ovules in the abdominal cavity (Bayunova et al. 2003).

A shift from oestrogens (C18 steroids) to progestins (C21 steroids) is observed at circulating levels of sturgeons as it is the case in some teleost fish.

### 16.3.2 Males

Among steroids measured in plasma of males (oestradiol-17 $\beta$ , T, 11KT, 17,20 $\beta$ P, 17,20,21P, 17,21P, P), the major concentrations were observed for androgens as T (13–257 ng mL<sup>-1</sup>) and 11KT (1–82 ng mL<sup>-1</sup>), while low and stable concentrations were measured for estradiol-17 $\beta$  (<0.7 ng mL<sup>-1</sup>) in most of the cases studied (Table 16.6). Contrasting with that, oestrogens measured in males were very high in Siberian sturgeons farmed in France, and these unusual high levels could come from the oestrogens given in food (Pelissero and Le Menn 1988). In the case of Siberian sturgeon cultured in Florida (USA) amounts of testosterone and oestradiol-17 $\beta$  increased significantly between pre-meiotic (only spermatogonias) and meiotic (spermatogenesis) fish (Hamlin et al. 2011, Table 16.5), while in bester 11KT increased significantly at pre-spermiation testicular stage (Amiri et al. 1996b). During first reproductive cycle of Siberian sturgeon, high concentrations of 11KT were detected in blood plasma of males during spermatogenesis (Cuisset et al. 1994), while plasmatic testosterone was high both during spermatogenesis and spermiation (Pelissero and Le Menn 1988). The high level of androgens observed in sturgeons during spermatogenesis plaids in favour of a role in the control of male gametogenesis as it was stated for teleost fish (Schulz and Miura 2002; Vizziano et al. 2007; Schulz et al. 2010). Plasma levels of steroids observed in sturgeons are coherent with a participation of C18 and C19 steroids in the control of fish spermatogenesis as it was proposed for teleosts (see Sect. 16.2.2.1).

C21 steroids were poorly studied in sturgeon blood plasma and are generally low. 17,20 $\beta$ P were not detectable or low (0.5 ng mL<sup>-1</sup>) during pre-spermiation (Vizziano et al. 2006; Bayunova et al. 2006, 2008) and changes with seasons in Stellate sturgeons reaching 1 ng mL<sup>-1</sup> in Autumn (Ceapa et al. 2002, Table 16.6). After hormonal induction of spermiation, 17,20 $\beta$ P increases in blood plasma of Siberian sturgeons with the volume of sperm released (Vizziano et al. 2006), and 17,20,21P increased in Stellate sturgeons (Bayunova et al. 2006) suggesting a physiological role in the control of sperm maturation and release.

**Table 16.6** Plasma steroids concentrations (ng mL<sup>-1</sup>) measured in male sturgeons

Species	Maturity stage/ month	E2	T	11KT	17,20P	17,20,21P	17,21P	P	Source
<i>A. baerii</i>	Spermatogenesis	14 ± 0.95	104 ± 47	-	-	-	-	-	Pelissero and Le Menn (1988)
	Spermiation	27 ± 4.7	178 ± 6.5	-	-	-	-	-	
<i>A. baerii</i>	Pre-spermiating	0.05–0.23	70–116	-	nd	-	-	-	Vizziano et al. (2006)
<i>A. baerii</i>	Pre-meiotic	~0.1	~18	~18	-	-	-	~0.14	Hamlin et al. (2011)
	Meiotic	~0.2 ( <i>p</i> < 0.05)	~80 ( <i>p</i> < 0.05)	~80	-	-	-	~0.15	
<i>A. sturio</i>	Mature male	<1	~40	~6.6	-	-	-	-	Davail- Cuisset et al. (2008)
<i>A. gueldenstaedtii</i>	Stage A—primary spermatogonias	-	-	<1	-	-	-	-	Hurvitz et al. (2008)
	Stage B—small number of germ cells	-	-	~4	-	-	-	-	
	Stage C—whole spermatogenesis	-	-	~8	-	-	-	-	
<i>A. gueldenstaedtii</i>	May	0.018 ± 0.01	75.5 ± 27	15.6 ± 2.8	-	-	-	-	Barannikova et al. (2006)
	May	-	185 ± 23	-	-	-	-	-	
	May	-	49 ± 13	26.9 ± 4.6	-	-	-	-	

<i>A. stellatus</i>	Spring	~<0.5	~55	~70	~0.025	-	-	Ceapa et al. (2002)
	Early May	<0.5	~35	~18	~0.25	-	-	
	Late May	~0.7	~30	~39	~0.2	-	-	
	Autumn	~0.7	~48	~78	~1	-	-	
<i>A. stellatus</i> <sup>a</sup>	Stage IV (April–May)	0.042 ± 0.009	257 ± 11	-	-	-	0.37 ± 0.04	Barannikova et al. (2002)
	Stages III–IV (May–June)	0.015	117 ± 10	32.9	-	-	0.6 ± 0.06	
	Stage IV (June)	0.034 ± 0.004	157 ± 18	82 ± 11	-	-	1 ± 0.14	
	Stage IV (July)	-	22 ± 16	-	-	-	0.24 ± 0.08	
<i>A. stellatus</i>	Pre-spermiating	-	46 ± 12	54 ± 17	0.3 ± 0.09	0.9 ± 0.19	1.1 ± 0.24	Bayunova et al. (2008)
<i>A. gueldenstaedtii</i>	In spermatogenesis	-	~50	15–20	-	-	-	Artyukhin et al. (2006)
<i>A. stellatus</i>	Pre-spermiating	-	~20	~15	~0.5	~3	<0.5	Bayunova et al. (2006)
<i>Huso huso</i>	Spermatogonial proliferation	-	13	12	-	-	-	Amiri et al. (1996b)
L. Females x <i>Acipenser ruthenus</i> L. males	Early spermatogenesis	-	28 (p < 0.01)	55 (p < 0.01)	-	-	-	
	Pre-spermiation	-	28	72 (p < 0.01)	-	-	-	
	Post-spawning	-	28	10	-	-	-	
		-	<10	<10	-	-	-	

nd = not detected; -; not determined

<sup>a</sup>Stage III, incomplete gametogenesis; Stage IV, fully completed spermatogenesis (Barannikova et al. 2006)

## Conclusions

Some general patterns emerge for sturgeons. In females androgens and oestrogens are the main steroids produced by ovaries and released to blood plasma during gonadal growth, while di- and tri-hydroxylated progestins (C21 steroids) have a maturational activity. Androgens are the precursors of oestrogen steroid production, but the higher levels detected at peri-ovulatory period suggest a physiological role at the last phases of biological cycle of females. In males androgens are the main steroids during the spermatogenesis, while C21 could have a maturational activity. Very poor efforts have been made to understand the biological activity of steroids in sturgeons. The role of other gonadal steroids in sturgeon reproduction remains to be studied. Many gaps need to be filled into the current knowledge of gonad regulation by steroids.

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