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Steroidogenesis in Pre- and Postspawned Ovaries of Feral African Catfish, *Clarias gariepinus*

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ABSTRACT

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Steroidogenesis in pre- and postspawned ovaries of *Clarias gariepinus*, collected in the Hula Nature Reserve in Israel, was studied following in vitro incubation with [^{3}H]-pregnenolone or [^{3}H]-androstenedione as precursors.

In both experimental groups the ovaries synthesized progesterone, 17α-hydroxyprogesterone, 17α,20β-dihydroxy-4-pregnen-3-one, androstenedione, testosterone, estrone, estradiol-17β, and several 5β-reduced C_{21}- and C_{19}-steroids. Testosterone and most of the 5β-reduced C_{21}- and C_{19}-steroids were also identified in a conjugated form as steroid glucuronides. In postspawned ovaries the production of these steroid glucuronides, especially testosterone-glucuronide and 5β-androstane-3α,17β-diol-glucuronide, and the steroid 5β-pregnane-3α,17α,20β-triol had increased significantly in comparison with prespawned ovaries. In these prespawned ovaries, on the other hand, androgen production, mainly testosterone, prevailed. It is suggested that the water-soluble compounds formed in relatively high amounts in postspawned ovaries are excreted into the external environment, where they may function as sex pheromones during the spawning period.

INTRODUCTION

In nature the African catfish, *Clarias gariepinus*, shows an annual reproductive cycle with a spawning period in early summer, immediately following gonadal recrudescence (Van Den Hurk et al., 1986; Van Oordt et al., 1987). Rainfall followed by a rise in water level, resulting in inundation of grassland bordering the shallow lakes in which the African catfish live, appear to trigger spawning

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behaviour in several catfish species, i.e. *C. gariepinus* (Van Der Waal, 1974; Bruton, 1979), *C. batrachus* (Van Der Waal, 1974), and *Heteropneustes fossilis* (Sundararaj and Vasal, 1976; Lamba et al., 1983). In the absence of these environmental changes, for instance under laboratory and husbandry conditions, catfish do not spawn spontaneously, and the ovaries remain in a post-vitellogenic stage. Maturation of oocytes and ovulation can, however, be induced by artificially raising the plasma gonadotropin (GTH) level, either by injecting exogenous GTH (Eding et al., 1982), or by inducing pituitary GTH release by means of pimozide plus LHRH-analogue injections (De Leeuw et al., 1985).

In several teleosts, oocyte maturation and ovulation are accompanied by changes in ovarian steroidogenesis and steroid blood plasma levels (Scott and Baynes, 1982; Zohar et al., 1982; Theofan and Goetz, 1983; Young et al., 1983). This was also observed in a preliminary study on ovarian steroidogenesis of laboratory-reared African catfish following gonadotropin administration by Lambert and Van Den Hurk (1982). These findings imply that steroidogenesis in the ovaries of catfish before, during and after artificially induced ovulation under laboratory conditions may be comparable with that of feral catfish. To test this hypothesis a detailed qualitative and quantitative investigation was set up into the bioconversion of tritiated precursors by using tissue samples of pre- and postspawned ovaries of African catfish collected in nature during the spawning period, and of laboratory-reared African catfish, treated with pimozide and LHRHa. The latter experiment in particular, because of a good time schedule, may give a better insight into the changes in steroidogenesis immediately preceding, accompanying and following oocyte maturation and ovulation.

The effects of pimozide and LHRHa on steroidogenesis in ovaries of laboratory-reared African catfish will be published elsewhere. The situation in feral African catfish during the spawning period is presented here. Steroidogenesis in the ovaries was investigated by in vitro incubations with tritiated pregnenolone, to get a general impression of the steroidogenic capacity, and with tritiated androstenedione, to determine the capacity for androgen synthesis.

MATERIALS AND METHODS

Materials

[7-^{3}H]-Pregnenolone (spec. act. 9.4 Ci/mmol) and [7-^{3}H]-androstenedione (spec. act. 9.2 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, and purity was checked by thin-layer chromatography. Reference steroids were obtained from Steraloids and Makor. Hepes was obtained from Boehringer, and Leibovitz-15 medium from Serva. All chemicals and solvents (Baker) were of analytical grade.

Animals

African catfish (*C. gariepinus*, Burchell) were collected in the Hula Nature Reserve, 30 km north of Lake Kinneret in Israel, during the breeding period in May 1984. Five animals were caught in inundated grassland (temperature 30°C), while showing spawning activity (Bruton, 1979; Lambert et al., 1986). Three other animals, not showing nuptial behaviour, were caught near the spawning grounds.

The animals were transported to the nearby laboratory, anesthetized with phenoxyethanol and sacrificed. The ovaries were removed, weighed to determine the gonadosomatic index (GSI) and prepared for histology and for in vitro incubations with tritiated steroid precursors. The GSI was calculated as: (gonad weight/total weight) $\times$ 100. Transport of the animals and exposure of the ovaries took about 2 h in total.

Chromatography

Thin layer chromatography (TLC) and detection of carrier and reference steroids were carried out as described by Schoonen and Lambert (1986a). The following systems were used: I, toluene : cyclohexane (1:1); II, benzene : ethylacetate (3:1); III, chloroform : ethanol (95:5); IV, hexane : acetone (1:1); V, diisopropylether : chloroform : hexane (7:2:1).

Acetylation, recrystallizations and measurement of radioactivity were carried out according to Schoonen and Lambert (1986a).

Incubation procedure

The incubation was carried out with 0.5 g of minced ovarian tissue in 2 ml Leibovitz-15 medium, fortified with 15 m*M* Hepes (pH 7.4), containing [^{3}H]-pregnenolone (2.1 μCi) or [^{3}H]-androstenedione (2.1 μCi) in 70 μl of propylene glycol. No cofactors were added. The incubations were carried out at 30°C in an air atmosphere with continuous shaking. The enzyme reactions were terminated after 2 h by adding 10 ml of ethanol.

Extraction

Before extraction, the following carriers were added:
to incubations with pregnenolone: 25 μg of pregnenolone (P_5), progesterone (P_4), 17α-hydroxypregnenolone (17α-P_5), 17α-hydroxyprogesterone (17α-P_4), 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P_4), androstenedione (A_4), testosterone (T), 11β-hydroxyandrostenedione (11β-A_4), 11β-hydroxytestosterone (11β-T) and 50 μg of estrone (E_1) and estradiol-17β (E_2);
to incubations with androstenedione: 25 μg of androstenedione (A_4), testos-

terone (T), etiocholanolone (ETIO), 5β-androstane-3,17-dione, 5α-androstane-3,17-dione, androsterone, 5β-androstane-3α,17β-diol, 5β-dihydrotestosterone (5β-DHT), 5α-androstane-3α,17β-diol and 50 μg of estrone (E_1) and estradiol-17β (E_2).

The free steroids and steroid conjugates were extracted from the tissue with ethanol (3$\times$10 ml). The ethanol–medium mixture was evaporated and the residue was redissolved in water (2.5 ml) to dissolve the steroid conjugates. Thereafter dichloromethane was added (3$\times$10 ml) to extract the free steroids from the water. The combined dichloromethane extracts were evaporated and the residue was dilipidized overnight at -20°C in hexane : acetone (2:7). The lipids were precipitated by centrifugation (3000 rpm, 10 min) and the supernatant was obtained and evaporated. The remaining residue was dissolved in a few droplets of dichloromethane : methanol (9:1) and subjected to TLC in system I to separate apolar compounds (triglycerides) from steroids. In this system the steroids remain on the baseline, so it is possible to use the same plate for a first separation of the steroids.

Hydrolysis of the steroid conjugates of the water fraction with β-glucuronidase was carried out according to Schoonen and Lambert (1986b).

Histology

Central parts of the ovary were fixed in formol–calcium, dehydrated and embedded in paraffin. Sections of 6 μm were stained with haemalum-eosin.

Statistical analysis

The means of the two groups are given with the standard error of the mean (SEM). Differences between the groups were tested with a Student's *t*-test for statistical significance.

RESULTS

Histology and GSI values

Histological examination showed that in four animals the ovaries were in a postvitellogenic (i.e. prespawning) stage. The tissue mainly consisted of large follicles containing yolk-laden oocytes of approximately 1 mm in diameter with centrally situated nuclei; postovulatory follicles were absent. In the other four animals postvitellogenic oocytes were scarce, but apart from previtellogenic oocytes many postovulatory follicles could be observed, indicating a postovulatory (i.e. a postspawned) stage of the ovaries.

The GSI in the four postvitellogenic and postovulatory animals was 13.6 $\pm$ 7.3 and 6.4 $\pm$ 1.7, respectively.

[^{3}H]-Pregnenolone incubation

After extraction with dichloromethane, the organic fraction contained approximately 90% and 80% of the radioactivity in animals with pre- and postspawned ovaries, respectively. Separation of the steroids of these organic fractions was carried out by TLC in system II (4×) and resulted in six comparable radioactive areas for both groups. These areas corresponded to the following carriers: (A) 11β-T; (B) 17α,20β-P_4; (C) 17α-P_5; 17α-P_4 and T; (D) P_5, A_4 and E_2; (E) P_4; and (F) E_1. After TLC in several systems, derivatization and recrystallization to constant specific activity (Table 1), it could be demonstrated that the tritium activity in area A was not due to 11β-T, but to 5β-pregnane-3α,17α,20β-triol. In area B, besides 17α,20β-P_4, two other steroids, i.e. 5β-pregnane-3α,17α-diol-20-one and 5β-androstane-3α,17β-diol, were also identified, while area C was composed of etiocholanolone and 5α-androstane-3α,17β-diol as well as 17α-P_5, 17α-P_4 and T. In the remaining areas D, E and F the radioactivity belonged to the steroids P_5, A_4, E_2, P_4 and E_1.

After hydrolysis of the water fraction with β-glucuronidase, the free steroids were extracted with dichloromethane. The organic fractions obtained in that way of the animals with pre- and postspawned ovaries contained 4 and 11% of the radioactivity, respectively. After separation of the steroids by TLC in system II (4×), three radioactive areas were distinguished in both groups, corresponding to the carriers: (A) 11β-T; (B) 17α,20β-P_4; and (C) 17α-P_5, 17α-P_4 and T. These ^{3}H-labeled steroids were identified by TLC, derivatization and recrystallizations (Table 1) as (A) 5β-pregnane-3α,17α,20β-triol; (B) 5β-pregnane-3α,17α-diol-20-one and 5β-androstane-3α,17β-diol; and (C) testosterone, 5α-androstane-3α,17β-diol and 5β-dihydrotestosterone.

The quantitative data for these steroids and steroid glucuronides are shown in Fig. 1. It follows that, in the two groups, pregnenolone was converted in very much the same amounts. There were, however, differences in the steroidogenic pathways. Thus, the percentage yields of testosterone were significantly higher in prespawned ovaries than in postspawned ovaries. Likewise, in prespawned ovaries there tended to be higher percentage yields of 17α-P_4 and A_4. The synthesis of 5β-pregnane-3α,17α,20β-tiol, on the other hand, was significantly reduced in prespawned ovaries in comparison with postspawned ovaries. Furthermore, the production of several steroid glucuronides tended to be smaller in prespawned ovaries than in postspawned ovaries. For all the other steroids no significant differences were found.

[^{3}H]-Androstenedione incubation

Extraction with dichloromethane resulted in organic fractions containing approximately 90 and 60% of the radioactivity in ovaries in a pre- and post-

TABLE 1

Identification of incubation products from ovaries of the African catfish, *Clarias gariepinus*, in vitro

Substrate	Area	Metabolite	TLC systems[a]				Crystallizations (dpm/mg)			
							Original	1	2	3
Pregnenolone	A	5β-pregnane-3α,17α,20β-triol	II (4×)	III (2×)	A^+,II (2×)		34751	34790	32332	32622
	B	17α,20β-dihydroxy-4-pregnen-3-one	II (4×)	III (2×)	IV	A^+,II (2×)	4853	871	855	833
		5β-pregnane-3α-,17α-diol-20-one	II (4×)	III (2×)	A^+,II (2×)		25340	24990	23767	24732
		5β-androstane-3α,17β-diol	II (4×)	III (2×)	A^+,II (2×)		3299	1422	1458	1412
	C	17α-hydroxypregnenolone	II (4×)	III	A^+,II (2×)		3645	383	386	388
		17α-hydroxyprogesterone	II (4×)	III	A^-,II (2×)		3947	2707	2604	2557
		testosterone	II (4×)	III	A^+,II (2×)		38672	29586	29052	28460
		etiocholanolone	II (4×)	III	A^+,II (2×)		12409	12304	12650	12686
		5α-androstane-3α,17β-diol	II (4×)	III	A^+,II (2×)		2100	1221	1127	1200
	D	estradiol-17β	II (4×)	III	V(3×)	A^+,II (2×)	10165	7427	7702	7293
		pregnenolone	II (4×)	III	A^+,II (2×)		39166	39606	38338	37806
		androstenedione	II (4×)	III	A^-,II (2×)		8845	8713	8678	8891
	E	progesterone	II (4×)	III	A^-,II (2×)		2966	1299	1219	1401
	F	estrone	II (4×)	III	V (3×)	A^+,II (2×)	4490	3676	3496	3474
	A	5β-pregnane-3α,17α,20β-triol-gl	II (4×)	III (2×)	A^+,II (2×)		5370	4196	3817	3987
	B	5β-pregnane-3α,17α-diol-20-one-gl	II (4×)	III (2×)	A^+,II (2×)		7516	6609	6859	6468
		5β-androstane-3α,17β-diol-gl	II (4×)	III (2×)	A^+,II (2×)		5340	4025	4058	4062
	C	testosterone-gl	II (4×)	III	A^+,II (2×)		4927	4608	4732	4416
		5α-androstane-3α,17β-diol-gl	II (4×)	III	A^+,II (2×)		1191	1094	1051	1061
		5β-dihydrotestosterone-gl	II (4×)	III	A^+,II (2×)		6015	2721	2561	2446
Androstenedione	A	5β-androstane-3α,17β-diol	II (4×)	III (2×)	A^+,II (2×)		19992	17305	16685	16223
	B	testosterone	II (4×)	III	A^+,II (2×)		47769	40196	39358	37944
		etiocholanolone	II (4×)	III	A^+,II (2×)		16895	14087	13875	13733
	C	estradiol-17β	II (4×)	III	A^+,II (2×)		15319	11141	10901	10674
		androstenedione	II (4×)	III	A^-,II (2×)		4967	4949	5028	4906
	D	estrone	II (4×)	III	A^+,II (2×)		4177	2476	2551	2563
	A	5β-androstane-3α,17β-diol-gl	II (4×)	III (2×)	A^+,II (2×)		18825	14909	14516	14233
	B	testosterone-gl	II (4×)	III	A^+,II (2×)		32771	27711	28669	28843
		5α-androstane-3α,17β-diol-gl	II (4×)	III	A^+,II (2×)		1841	1845	1729	1726
		5β-dihydrotestosterone-gl	II (4×)	III	A^+,II (2×)		6538	6150	6274	6142

[a]For explanation of Roman numerals, see Materials and Methods. A^+ = positive acetylation; A^- = negative acetylation; gl = glucuronide.

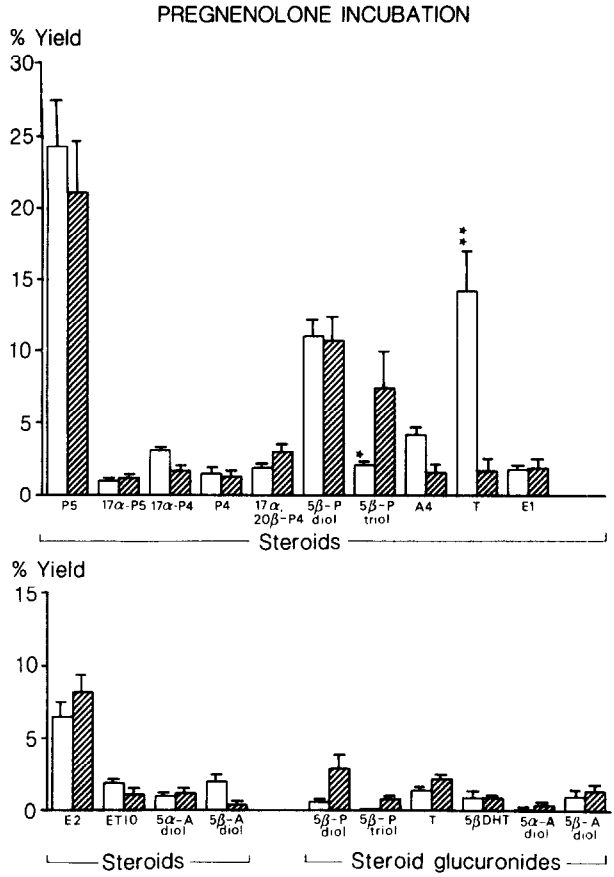

Fig. 1. Percentage yields of steroids (mean ± SEM, $n=4$) obtained by incubating ovarian tissue of feral African catfish, *Clarias gariepinus*, with [^{3}H]-pregnenolone during prespawning (open columns) and postspawning (cross-hatched columns). $^*P<0.05$, $^{**}P<0.01$. P_5 = pregnenolone, 17α-P_5 = 17α-hydroxypregnenolone, 17α-P_4 = 17α-hydroxyprogesterone, P_4 = progesterone, 17α,20β-P_4 = 17α,20β-dihydroxy-4-pregnen-3-one, 5β-P-diol = 5β-pregnane-3α,17α-diol-20-one, 5β-P-triol = 5β-pregnane-3α,17α,20β-triol, A_4 = androstenedione, T = testosterone, E_1 = estrone, E_2 = estradiol-17β, ETIO = etiocholanolone, 5α-A-diol = 5α-androstane-3α,17β-diol, 5β-A-diol = 5β-androstane-3α,17β-diol, 5β-DHT = 5β-dihydrotestosterone.

spawned stage, respectively. After TLC in system II (4×) the steroids of these organic fractions in both groups were separated into four radioactive areas. These areas corresponded with the following carriers: (A) 5β-androstane-3α,17β-diol; (B) T, ETIO, 5β-DHT and 5α-androstane-3α,17β-diol; (C) A_4 and E_2; and (D) E_1.

After TLC in system III, derivatization, rechromatography in system II (2×) and finally recrystallizations to constant specific activity (Table 1), it could be demonstrated that the areas represent (A) 5β-androstane-3α,17β-diol; (B) T and ETIO; (C) A_4 and E_2; and (D) E_1.

After β-glucuronidase treatment of the water fraction, and extraction with

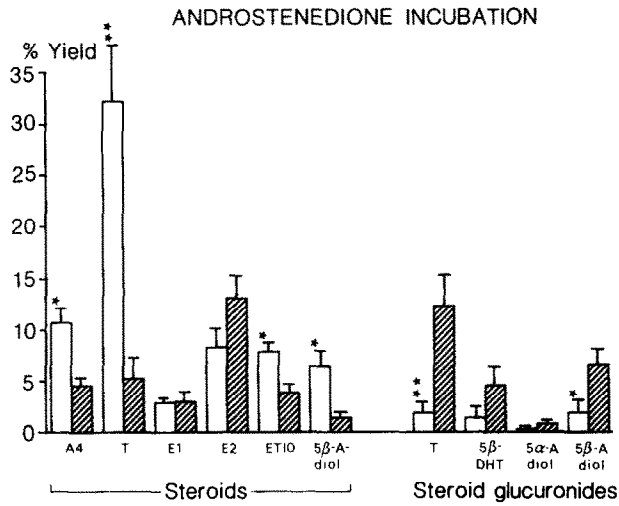

Fig. 2. Percentage yields of steroids (mean ± SEM, $n=4$) obtained by incubating ovarian tissue of feral African catfish, *Clarias gariepinus* with [^{3}H]-androstenedione during prespawning (open columns) and postspawning (cross-hatched columns). $^*P<0.05$, $^{**}P<0.01$. A_4 = androstene-dione, T = testosterone, E_1 = estrone, E_2 = estradiol-17β, ETIO = etiocholanolone, 5β-A-diol = 5β-androstane-3α,17β-diol, 5α-A-diol = 5α-androstane-3α,17β-diol, 5β-DHT = 5β-dihydrotestosterone.

dichloromethane, the organic fractions of the pre- and postspawned ovaries contained approximately 5 and 30% of the radioactivity, respectively. The separation by TLC in system II (4×) resulted in two radioactive areas in both groups, corresponding to the carriers: (A) 5β-androstane-3α,17β-diol and (B) T, ETIO, 5β-DHT and 5α-androstane-3α,17β-diol. From these two radioactive areas the steroids 5β-androstane-3α,17β-diol, T, 5β-DHT and 5α-androstane-3α,17β-diol could be identified in the same way as described above (Table 1).

The quantitative data are summarized in Fig. 2. It is noteworthy that androstenedione was converted significantly less in prespawned ovaries than in postspawned ones. Furthermore, significantly higher percentage yields of testosterone, etiocholanolone and 5β-androstane-3α,17β-diol were observed in the prespawned stage, while the percentage yields of E_1 and E_2 did not differ significantly between the two groups. The production of steroid glucuronides, on the other hand, was significantly higher in the postspawned stage than in the prespawned stage. Especially synthesis of testosterone-glucuronide and 5β-androstane-3α,17β-diol-glucuronide had increased significantly in postspawned ovaries, and there was a tendency for an increased formation of 5β-DHT-glucuronide and 5α-androstane-3α,17β-diol-glucuronide in the latter group.

DISCUSSION

The present results demonstrate that under in vitro conditions tissue fragments of both pre- and postspawned ovaries of *Clarias gariepinus* can convert pregnenolone and androstenedione into a number of other steroids, and that there are hardly any qualitative differences in steroidogenic capacity between the two experimental groups. In both pregnenolone was converted into androstenedione and testosterone partly via the $\Delta 4$ route, i.e. via progesterone and 17α-hydroxyprogesterone, and partly via the $\Delta 5$-$\Delta 4$ route, i.e. via 17α-hydroxypregnenolone and 17α-hydroxyprogesterone. Since dehydroepiandrosterone was not synthesized, it seems that the $\Delta 4$ route prevailed. Other products of pregnenolone incubation were the C_{21}-steroids 17α,20β-dihydroxy-4-pregnen-3-one, 5β-pregnane-3α,17α-diol-20-one and 5β-pregnane-3α,17α,20β-triol, the C_{19}-steroids 5α-androstane-3α,17β-diol, etiocholanolone and 5β-androstane-3α,17β-diol, and the C_{18}-steroids estrone and estradiol-17β. A number of steroid glucuronides were derived from pregnenolone, namely 5β-pregnane-3α,17α-diol-20-one-, 5β-pregnane-3α,17α,20β-triol-, testosterone-, 5β-dihydrotestosterone-, 5α-androstane-3α,17β-diol-, and 5β-androstane- 3α,17β-diol-glucuronide. The androstenedione incubations yielded the same androgens, estrogens and C_{19}-steroid glucuronides.

The above results, obtained with tissue incubations, and the results of Lambert and Van Den Hurk (1982), obtained with homogenate incubations, differ in the production of several 5β-reduced steroids. This difference may be due to the fact that in tissue incubations the bioconversion of steroid precursors into end products including 5β-reduced steroids, is a faster process than in tissue homogenates. It should, however, also be kept in mind that following cell destruction and decompartimentalization, steroid biosynthesis will follow less normal routes than in intact tissue, incubated in the presence of nutrients (Hall, 1984; Schoonen and Lambert, 1986a,b).

Quantitative differences were restricted to the formation of testosterone, and the conversion of pregnenolone into 5β-pregnane-3α,17α,20β-triol and of androstenedione into etiocholanolone, 5β-androstane-3α,17β-diol and the glucuronides of testosterone and 5β-androstane-3α,17β-diol. In ovaries filled with postvitellogenic follicles the production of testosterone was relatively strong. A similar situation has been described for prespawned ovaries of *Tilapia aurea* (Eckstein and Katz, 1971), ayu, *Plecoglossus altivelis* (Suzuki et al., 1981), and the carp, *Cyprinus carpio* (Colombo et al., 1982a). Likewise, cell-free homogenates of prespawned ovaries of laboratory-reared African catfish have a strong capacity to synthesize testosterone (Lambert and Van Den Hurk, 1982). This probably results from a reduction in the aromatase activity necessary for the production of estrogens that during ovarian recrudescence stimulate vitellogenin formation in the liver (Van Bohemen et al., 1982; Wiegand, 1982; Ng and Idler, 1983). At any rate, a decrease in the plasma concentration

of estradiol, accompanied by an increase in the plasma testosterone level, has been described for postvitellogenic plaice (Wingfield and Grimm, 1977), rainbow trout (Zohar et al., 1982; Scott et al., 1983), white-spotted char (Kagawa et al., 1981), amago salmon (Kagawa et al., 1983) and Indian catfish (Lamba et al., 1983). Also in laboratory-reared African catfish with postvitellogenic ovaries, the plasma levels of testosterone are relatively high (30 $ng \cdot ml^{-1}$) and those of estradiol relatively low (11 $ng \cdot ml^{-1}$) (Richter et al., 1987). Cell-free homogenates of such ovaries, likewise, yield much more testosterone than estradiol (Lambert and Van Den Hurk, 1982). Thus it seems that the high postvitellogenic level of testosterone results from a reduced aromatase activity in the ovaries. The androgen might be involved in GTH synthesis and storage prior to the prespawning GTH surge (Peter and Crim, 1979; Gielen and Goos, 1983; Kagawa et al., 1983; Van Den Hurk et al., 1984; De Leeuw et al., 1986).

A relatively strong production of the 5β-reduced pregnanes, 5β-pregnane-3α,17α-diol-20-one and 5β-pregnane-3α,17α,20β-triol was observed in the postspawned ovaries. The percentage yield of the former product had remained high since the prespawned stage; the relative production of the latter had increased. On the other hand, the conversion of androstenedione into testosterone and the 5β-reduced androgens etiocholanolone and 5β-androstane-3α,17β-diol had significantly changed to the synthesis of steroid glucuronides. A similar high yield of 5β-reduced pregnanes and low yield of testosterone has also been described for postovulatory ovaries of the ayu (Suzuki et al., 1981), and a reduced testosterone synthesis was mentioned for postspawning *Tilapia aurea* (Eckstein and Katz, 1971). The synthesis of 5β-reduced steroids has been described for ovaries of *Centropristes striatus* (Reinboth et al., 1966), *H. fossilis* (Ungar et al., 1977), *Spicara maena, Serranus cabrilla* (Reinboth, 1979), *Coris julis* (Reinboth and Becker, 1984) and *Poecilia latipinna* (Kime and Groves, 1986). Up to this moment, however, the biological function of these compounds remains unknown.

The enzyme UDP-glucuronosyltransferase seems to have increased in activity following ovulation, leading to a higher percentage yield of steroid glucuronides, especially after incubation of ovarian fragments with androstenedione. These steroid conjugates have also been described for the testes of *Gobius paganellus* (Colombo et al., 1970), *G. jozo* (Colombo et al., 1977), *S. gairdneri* (Hews and Kime, 1978), *Carassius auratus* (Kime, 1980), *Clarias gariepinus* (Schoonen et al., 1987b) and *Brachydanio rerio* (Van Den Hurk et al., 1987), the seminal vesicles of *C. gariepinus* (Schoonen and Lambert, 1986b; Schoonen et al., 1987a), and for the ovary of *C. gariepinus* (Lambert and Van Den Hurk, 1982), and *Poecilia latipinna* (Kime and Groves, 1986). Recent investigations indicate that in teleosts such steroid glucuronides may function as sex pheromones (Colombo et al., 1982b; Van Den Hurk and Lambert, 1983; Lambert et al., 1986; Resink et al., 1987; Van Den Hurk et al., 1987). If so, it seems likely that these water-soluble steroid derivatives are excreted, following ovulation

and during oviposition. A free steroid may also be excreted, i.e. the highly polar and water-soluble 5β-pregnane-3α,17α,20β-triol. The high percentage yield of this pregnane in postspawned ovaries might indicate that it has a role, together with steroid glucuronides, as a sex pheromone during the spawning period.

It should, however, be kept in mind that the material used in the present experiments reflects the situation some time before and shortly after ovulation, and that the changes in steroidogenesis leading to the production of sex attractants and ovulation-synchronizing pheromones were not studied in these experiments. Likewise, the material did not allow any conclusions to be drawn about the changes in steroid biosynthesis immediately preceding oocyte maturation and ovulation, or the nature of the steroids inducing these processes. In order to enlarge the present data regarding the situation in the ovaries of feral *C. gariepinus* during the spawning season, experiments have been carried out with ovaries of laboratory-reared specimens treated with pimozide and LHRHa that induce an endogenous GTH surge and ovulation.

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