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Steroidogenesis in the Testes and Seminal Vesicles of Spawning and Non-Spawning African Catfish, *Clarias gariepinus*

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ABSTRACT

Schoonen, W.G.E.J., Granneman, J.C.M., Lambert, J.G.D. and Van Oordt, P.G.W.J., 1987. Steroidogenesis in the testes and seminal vesicles of spawning and non-spawning African catfish, *Clarias gariepinus*. *Aquaculture*, 63: 77–88.

The in vitro biosynthesis of steroids was studied in testes as well as seminal vesicles of non-spawning pond and spawning feral African catfish, collected during the breeding season. In testes of non-spawners the conversion of [^{3}H]-pregnenolone was directed towards 11-oxygenated androgens and 5β-pregnane-3α,17α,20α-triol, whereas in testes of spawners the production of progesterone and 17α-hydroxyprogesterone prevailed. The seminal vesicles, on the other hand, produced mainly pregnenolone ester, androstenedione and testosterone in non-spawning catfish and 5β-reduced steroids in spawning catfish. Furthermore, in these seminal vesicles, steroid glucuronide synthesis dominated much more than in testes. There was, however, no difference in synthesizing capacity between the two groups. Spawning was accompanied by elevated plasma gonadotropin levels.

INTRODUCTION

In teleosts, as in many animals, successful breeding depends on adequate male and female breeding behaviour and the simultaneous expulsion of ripe sperm cells and oocytes. In captivity African catfish, *Clarias gariepinus*, do produce ripe gametes, but fail to show breeding behaviour, sperm release and oviposition. There are even no signs of spontaneous spermiation, oocyte maturation and ovulation.

With regard to the role of the male, there are indications that certain testicular steroids can induce spermiation and that certain steroid glucuronides

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function as sex pheromones. Thus, in the sockeye salmon, *Oncorhynchus nerka* (Idler et al., 1961), and the rainbow trout, *Salmo gairdneri* (Billard et al., 1981), the androgens 11-ketotestosterone and testosterone, respectively, are needed for stimulation of spermiation. In hypophysectomized goldfish, *Carassius auratus* (Billard, 1976), and intact pike, *Esox lucius* (De Montalembert, 1978), on the other hand, progesterone was more effective in spermiation induction than were some other steroids.

Steroid glucuronides of testicular origin have been described for the teleosts *Gobius paganellus* (Colombo et al., 1970), *G. jozo* (Colombo et al., 1977), the rainbow trout (Hews and Kime, 1978), the goldfish (Kime, 1980), *Sarotherodon mossambicus* (Kime and Hyder, 1983) and the zebrafish, *Brachydanio rerio* (Lambert et al., 1986; Van Oordt, 1986; Van Den Hurk et al., 1987b). In *G. jozo* etiocholanolone-glucuronide attracts ovulated females (Colombo et al., 1980, 1982). This compound is synthesized in a compartment of the testes, named the mesorchial gland (Colombo et al., 1977). Similarly, holding water of zebrafish males (Chen and Martinich, 1975) and more particularly, a testis fraction containing steroid glucuronides, can induce ovulation in female conspecifics (Van Den Hurk et al., 1987b). This fraction looses its pheromonal activity after treatment with β-glucuronidase, which points to the possible pheromonal function of steroid glucuronides.

Male sex pheromones attracting ovulated females have also been demonstrated in the African catfish, and are in all probability formed in the so-called seminal vesicles (Resink et al., 1987b). In some groups of teleosts, i.e. the Blennidae, Gobiidae (Eggert, 1931; Weisel, 1949), Ictaluridae (Sneed and Clemens, 1963) and Siluridae (Van Tienhoven, 1983), the male gonads are composed of two separate organs, the testes and the seminal vesicles. In the Siluridae, histological and enzyme-cytochemical investigations of the Indian catfish, *Heteropneustes fossilis* (Sundararaj and Nayyar, 1967; Nayyar and Sundararaj, 1969), and the African catfish (Resink et al., 1987c; Van Den Hurk et al., 1987a) point to interstitial cells in the seminal vesicles as well as the testes as possible sources of steroids.

Biochemical experiments by Schoonen and Lambert (1986a,b) and Schoonen et al. (1987a,b) have shown that both the testes and the seminal vesicles of African catfish are able to produce C_{21}- and C_{19}-steroids, and steroid glucuronides. Considerable qualitative and quantitative differences in steroidogenesis were, however, observed between the two organs. Furthermore, steroidogenesis in the testes and seminal vesicles was different between spawning and non-spawning fish. For these experiments, spawning male African catfish were collected during the breeding season in the Hula Nature Reserve, and non-spawning conspecifics from ponds of the nearby Ginosar Fish Breeding Station in Northern Israel. Minced tissue of testes and seminal vesicles respectively was incubated with tritiated pregnenolone. Following a qualitative and quantitative determination of the steroids formed under these conditions, the

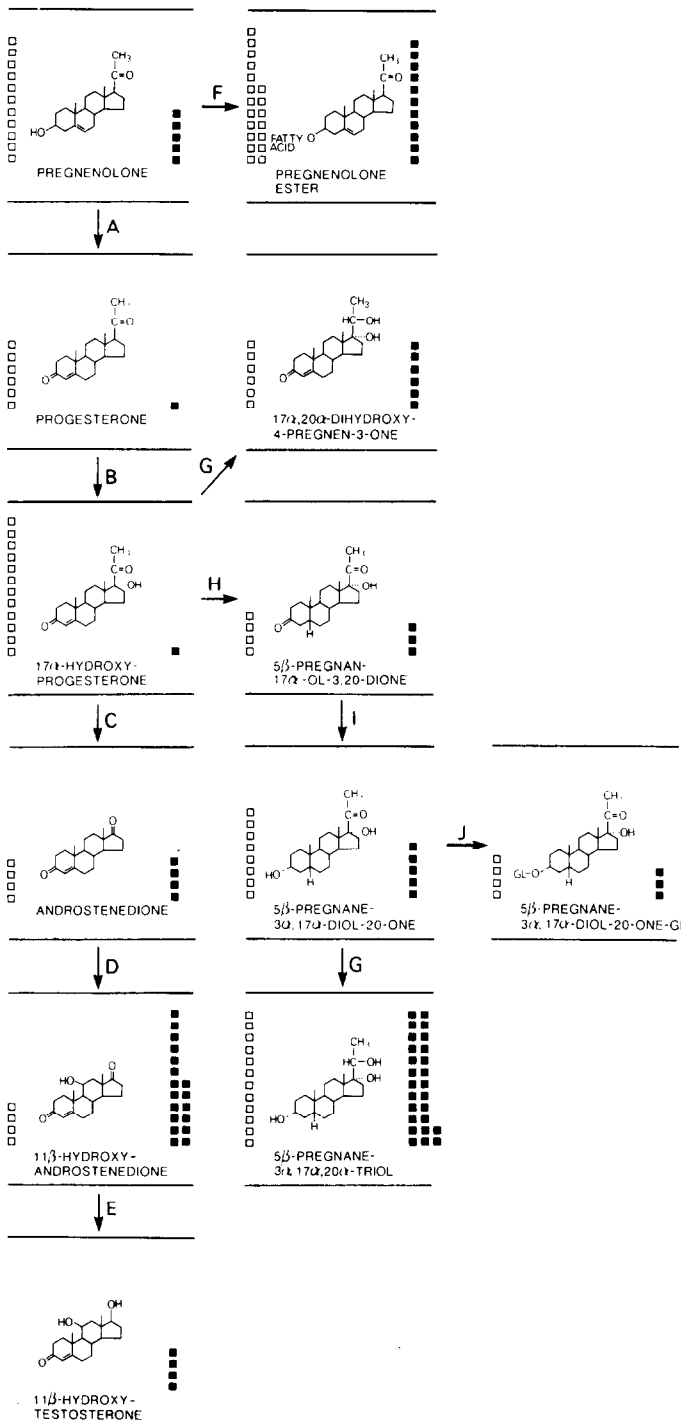
relative percentage contribution of various enzymes to the steroidogenic capacity of testes and seminal vesicles was calculated. The results of these experiments are reviewed and discussed with regard of the question of the role of male sex steroids in successful breeding of the African catfish.

THE TESTES

The main steroids, identified by Schoonen and Lambert (1986a) and Schoonen et al. (1987b) after in vitro incubation with [^{3}H]-pregnenolone of minced testis tissue of spawning feral catfish and non-spawning pond catfish, are given in Fig. 1. The bioconversion of the precursor was more pronounced in the non-spawning pond group than in the spawning feral fish. This led to a higher percentage yield of 5β-pregnane-3α,17α,20α-triol, 11β-hydroxyandrostenedione and 11β-hydroxytestosterone in the non-spawning fish. In the spawners, on the other hand, the production of pregnenolone ester, and of the progestins progesterone and 17α-hydroxyprogesterone was relatively great. Other steroids, including 17α,20α-dihydroxy-4-pregnen-3-one, 5β-pregnan-17α-ol-3,20-dione, 5β-pregnane-3α,17α-diol-20-one, 5β-pregnane-3α,17α-diol-20-one-glucuronide, and androstenedione, were synthesized in about equal amounts by the testes fragments of both experimental groups.

In Table 1 the enzymes are indicated that are involved in testicular steroidogenesis of the African catfish. Each of these enzymes contributes to the conversion rate of the steroids. The percentage contribution of each enzyme to this conversion rate is given by the percentage yield of the steroid formed by its activity plus the percentage yield of all subsequent steroids. For example: the percentage contribution of the enzyme 3β-Δ^5-hydroxysteroid dehydrogenase (3β-HSD), involved in the conversion of pregnenolone into progesterone, to the total conversion of the precursor is given by the sum of the percentage yields of progesterone and all its derivatives, shown in Fig. 1. Thus, it appears that the contributions of the enzymes 3β-HSD, 17α-hydroxylase, C_{17-20}-lyase, 11β-hydroxylase, 5β-reductase, 20α-HSD, and 17β-HSD were considerably greater in the non-spawning than in the spawning animals. The percentage contribution of the enzyme UDP-glucuronosyltransferase was almost the same for both groups, and in the spawning catfish the percentage contribution of the enzyme acyl-transferase was relatively high.

These differences in enzyme contribution were accompanied by differences in gonadotropin concentration in the general circulation. The plasma gonadotropin level in the non-spawning pond fish was 1 ng·ml^{-1}, and in the spawning feral fish somewhat higher, i.e. 3 ng·ml^{-1} (Schoonen et al., 1987b). It could be argued that a prespawning rise in pituitary gonadotropin release will lead to a decrease in activity of certain steroidogenic enzymes. Thus, in many fish species, gonadotropins are able to induce spermiation (for review: Donaldson and Hunter, 1983), and in male African catfish at the same time suppress the pro-


PREGNENOLONE
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FATTY ACID
PREGNENOLONE ESTER
A
PROGESTERONE
17α,20α-DIHYDROXY-4-PREGNEN-3-ONE
B
G
17α-HYDROXY-PROGESTERONE
H
5β-PREGNAN-17α-OL-3,20-DIONE
C
I
ANDROSTENEDIONE
5β-PREGNANE-3α,17α-DIOL-20-ONE
J
5β-PREGNANE-3α,17α-DIOL-20-ONE-GI
D
G
11β-HYDROXY-ANDROSTENEDIONE
5β-PREGNANE-3α,17α,20α-TRIOL
E
11β-HYDROXY-TESTOSTERONE

TABLE 1

Contribution (as percentage of total activity) of the enzymes involved in steroidogenesis in the testes and seminal vesicles of the African catfish, *Clarias gariepinus*, calculated from the percentage yields of steroids after incubation of these organs with ^{3}H-pregnenolone

Enzymes		Testes		Seminal vesicles	
		Spawning feral	Non-spawning pond	Spawning feral	Non-spawning pond
A	3β-hydroxysteroid dehydrogenase	60	73	62	40
B	17α-hydroxylase	54	72	59	38
C	C_{17-20}-lyase	8	26	28	29
D	11β-hydroxylase	4	22	—	—
E	17β-hydroxysteroid dehydrogenase	—	4	21	21
F	acyl-transferase	19	11	8	17
G	20α-hydroxysteroid dehydrogenase	18	32	—	—
H	5β-reductase	28	39	38	9
I	3α-hydroxysteroid dehydrogenase	24	36	27	5
J	UDP-glucuronosyl-transferase	4	3	8	9

duction of testicular androgens (Schoonen et al., unpublished results). Likewise, the testes of male rats, treated with high doses of human chorionic gonadotropin, show a decline in androgen production due to a reduced activity of the cytochrome-P-450 enzymes 17α-hydroxylase and C_{17-20}-lyase (Purvis et al., 1973; Cigorraga et al., 1978; Nozu et al., 1982; Kühn-Velten and Staib, 1984).

The relatively small production of androgens and large production of progestins has also been observed in the testes of rainbow trout during the spermiation period, and may be related to a spermiation-inducing capacity of progestins (for review: Donaldson and Hunter, 1983). Thus, a decrease or even the absence of 11β-hydroxyandrostenedione and a concomittant increase of the 17α,20β-dihydroxy-4-pregnen-3-one has been described for spermiating

Fig. 1. Aspects of the pathway of pregnenolone bioconversion in the testes of the African catfish, *Clarias gariepinus*. The steroids represent the main intermediates and end products. The mean percentage yields of these steroids in testes of spawning feral animals ($n=5$) and non-spawning pond animals ($n=5$) are represented by open and closed squares, respectively. Each square represents 1%. The various steroidogenic enzymes are indicated as follows: A = 3β-hydroxysteroid dehydrogenase (3β-HSD), B = 17α-hydroxylase, C = C_{17-20}-lyase, D = 11β-hydroxylase, E = 17β-HSD, F = acyl-transferase, G = 20α-HSD, H = 5β-reductase, I = 3α-HSD and J = UDP-glucuronosyltransferase.

rainbow trout (Arai and Tamaoki, 1967; Suzuki and Tamaoki, 1972; Kime, 1979; Depêche and Sire, 1982). Scott and Baynes (1982) found high levels of the progestin in the blood plasma of rainbow trout during spermiation, and Ueda et al. (1984) suggested that the hormone might be synthesized by sperm cells. It is not certain whether in African catfish 17α,20β-dihydroxy-4-pregnen-3-one is involved in spermiation induction. Indeed, this steroid could not be identified in the present incubation experiments, and the related form, 17α,20α-dihydroxy-4-pregnen-3-one, was produced in equal amounts by testis fragments of spawning and non-spawning fish. If, in African catfish, progestins have anything to do with the induction of spermiation, 17α-hydroxyprogesterone, being one of the main end products of testicular steroidogenesis in spawning animals, seems to be a good candidate. Simultaneously with the higher synthesis of progestins in feral spawning catfish, the synthesis of a pregnenolone ester increased. The acylation of pregnenolone might be part of a regulatory system in steroidogenesis as the synthesis of the ester results in a decreased bioconversion of 3β-hydroxy-Δ5-steroids by protecting pregnenolone from dehydrogenation.

The more diverse enzyme activity in the testes of non-spawning pond catfish led to the formation of several androgens; the main end products were 11β-hydroxyandrostenedione, and to a lesser extent 11β-hydroxytestosterone. A large production of 11β-hydroxyandrostenedione has also been found during the spermatogenic stage of the reproductive cycle of the eel, *Anguilla anguilla* (Eckstein et al., 1982) and the rainbow trout (Depêche and Sire, 1982). In other species, including the pike, the goldfish, *Salvelinus fontinalis* and *Sparus aurata* (for review: Fostier et al., 1983), however, 11β-hydroxytestosterone, 11-ketotestosterone and testosterone were the prevalent androgens during spermatogenesis. Since 11-ketotestosterone and testosterone were hardly produced, and 11β-hydroxytestosterone only to a relatively low extent during testicular recrudescence, the period of strong spermatogenic activity and the prespawning period (Resink et al., 1987a; Schoonen et al., 1987b), it seems that none of these androgens, but rather the much more abundant 11β-hydroxyandrostenedione, may stimulate the germinal epithelium and the development of the secondary sex characters of male African catfish during the prespawning season.

The production of steroid glucuronides by testis fragments of African catfish seems to be restricted to 5β-pregnane-3α,17α-diol-20-one-glucuronide, and to be relatively small in both experimental groups. In contrast, the production of the highly polar steroid 5β-pregnane-3α,17α,20α-triol was much more pronounced, especially in the non-spawning pond catfish. It is not impossible that, upon excretion with the testis fluid, this water-soluble steroid, together with the steroid glucuronide, may likewise have a pheromonal function.

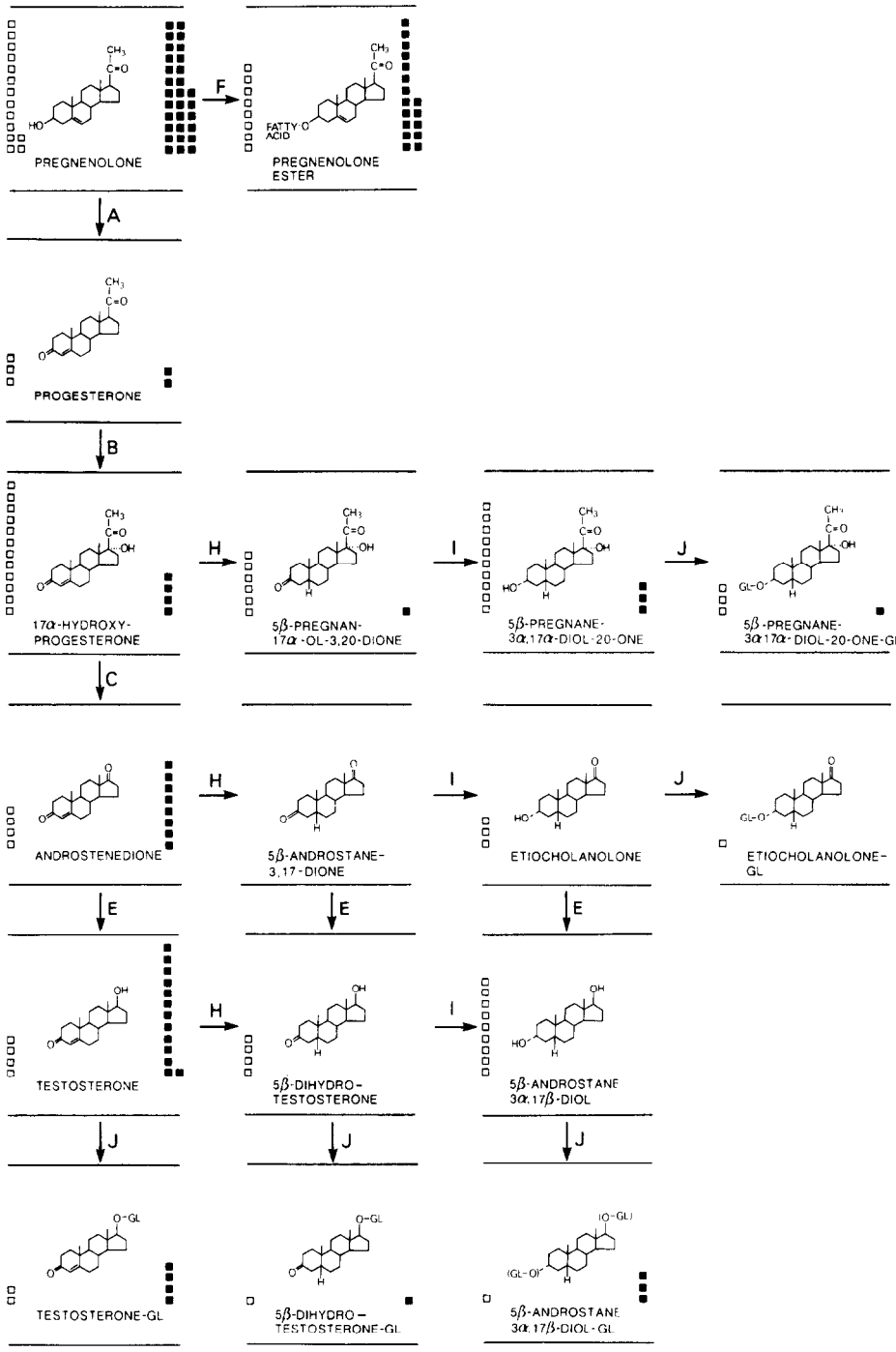
THE SEMINAL VESICLES

Incubating minced fragments of seminal vesicles of African catfish with [^{3}H]-pregnenolone led to the production of many different steroids and steroid glucuronides (Schoonen and Lambert, 1986b; Schoonen et al., 1987a). The main ones are given in Fig. 2, together with the percentage yield of each compound. The enzymes involved in steroid biosynthesis, and the percentage contribution of each of these enzymes to the total steroid conversion rate are given in Table 1.

Contrary to the situation in testis tissue of the same animals, the bioconversion of the precursor was much less complete in the seminal vesicle tissue of the non-spawning pond fish than in that of the spawning feral fish. Another difference with the situation in the testes was the relatively strong conversion of pregnenolone into pregnenolone ester by seminal vesicle tissue of non-spawning pond animals, and the relatively small production of pregnenolone ester in incubations of seminal vesicle tissue of spawning feral fish. In other words, the contribution of the enzyme acyl-transferase was considerably lower in seminal vesicles of spawning than in those of non-spawning catfish.

Apart from pregnenolone ester, the prevailing products of pregnenolone in incubation of seminal vesicle tissue of non-spawning pond fish were androstenedione and testosterone. The percentage yield of these products was considerably higher than in seminal vesicles of spawning feral fish, even though the contributions of the enzymes C_{17-20}-lyase and 17β-HSD were almost equal in both groups (Table 1). The androgens of the seminal vesicles may assist the testicular androgens in stimulating the development of the male reproductive organs during the months preceding the nuptial period (Resink et al., 1987a). Elucidation of the physiological significance of the various androgens awaits both steroid biochemical analysis of the blood plasma, and the study of steroid receptors in the potential target organs.

Steroidogenic enzymes more active in seminal vesicle of spawning than in those of non-spawning catfish were 3β- and 3α-HSD, 17α-hydroxylase and 5β-reductase. This situation is very different from that in the testes; for instance, the cytochrome-P-450 enzymes 17α-hydroxylase, C_{17-20}-lyase and 11β-hydroxylase did not decrease in activity with a rise in the plasma gonadotropin concentration, as was suggested for the testes. Maybe, the intracellular effect of gonadotropin in the two target organs is different, or the seminal vesicles are not only activated by gonadotropin but also by other pituitary hormones or testicular hormones. In this respect it is of interest that in a related species, the Indian catfish, a hypersecretion of the seminal vesicles was found in hypophysectomized and castrated males after treatment with gonadotropin, prolactin, growth hormone, and some androgens, and with a combination of these hormones (Sundararaj and Goswami, 1965; Nayyar and Sundararaj, 1969; Sundararaj et al., 1971).


PREGNENOLONE
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5β-PREGNANE-3α,17α-DIOL-20-ONE
J
5β-PREGNANE-3α17α-DIOL-20-ONE-GL
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5β-DIHYDRO-TESTOSTERONE
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5β-ANDROSTANE 3α,17β-DIOL
J
J
J
TESTOSTERONE-GL
5β-DIHYDRO-TESTOSTERONE-GL
5β-ANDROSTANE 3α,17β-DIOL-GL

In the seminal vesicles, as in the testes, the biosynthesis of C_{21}-steroids, especially 17α-hydroxyprogesterone, was more pronounced in spawning than in non-spawning fish. It is not likely, however, that these steroids of the seminal vesicles have a function in spermiation induction, as they do in the testes. The activity of the enzyme 5β-reductase led to the production of a number of 5β-reduced steroids, i.e. the C_{21}-steroids 5β-pregnan-17α-ol-3,20-dione and 5β-pregnane-3α,17α-diol-20-one, and the C_{19}-steroids etiocholanolone, 5β-dihydrotestosterone and 5β-androstane-3α,17β-diol. All of these showed considerably higher percentage yields in the seminal vesicles of spawning feral fish than in those of non-spawning pond fish. This situation contrasts with the relatively strong production of 5β-pregnane-3α,17α,20α-triol in the testes of non-spawning fish. Such differences in production of 5β-reduced steroids point to some – as yet unknown – physiological role of these compounds.

Apart from the steroid glucuronide 5β-pregnane-3α,17α-diol-20-one-glucuronide, also found in incubations of testis, the fragments of the seminal vesicles also yielded testosterone-glucuronide, 5β-dihydrotestosterone-glucuronide, 5β-androstane-3α,17β-diol-glucuronide, and etiocholanolone-glucuronide. The differences between the two experimental groups were limited to a somewhat more pronounced synthesis of testosterone-glucuronide and 5β-dihydrotestosterone-glucurone in the seminal vesicles of the non-spawning fish, and of 5β-pregnane-3α,17α-diol-20-one-glucuronide and etiocholanolone-glucuronide in the seminal vesicles of the spawning animals. However, these differences were not reflected by a difference in activity of the enzyme UDP-glucuronosyltransferase. This means that steroid glucuronides are available before as well as during the spawning period (Resink et al., 1987a). Recent experiments by Resink et al. (1987b) – see also Lambert et al. (1986) and Van Oordt (1986) – have shown that the fluid produced by the seminal vesicles of African catfish is involved in the attraction of females after ovulation. Preliminary experiments have made it possible to identify three steroid glucuronides in this fluid, namely 5β-pregnane-3α,17α-diol-20-one-glucuronide, 5β-androstane-3α,17β-diol-glucuronide and etiocholanolone-glucuronide (Schoonen, in preparation). The identification of other steroid glucuronides and the quantification of these substances in the seminal vesicle fluid of spawning and non-spawning animals are in progress. The results may help in identifying the pheromones stimulating vitellogenesis (Henken et al., 1987), spawning behaviour and ovulation (Lambert et al., 1986; Resink et al., 1987b).

Fig. 2. Aspects of the pathway of pregnenolone bioconversion in the seminal vesicles of the African catfish, *Clarias gariepinus*. The steroids represent the main intermediates and end products. The mean percentage yields of these steroids in seminal vesicles of spawning feral animals ($n=5$) and non-spawning pond animals ($n=5$) are represented by open and closed squares, respectively. Each square represents 1%. The various steroidogenic enzymes are indicated as follows: A = 3β-hydroxysteroid-dehydrogenase (3β-HSD), B = 17α-hydroxylase, C = C_{17-20}-lyase, E = 17β-HSD, F = acyl-transferase, H = 5β-reductase, I = 3α-HSD and J = UDP-glucuronosyltransferase.

Knowing the composition of the male sex pheromones may help to overcome the absence of spontaneous reproduction of African catfish under husbandry conditions.

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