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The Effect of Pimozide/LHRHa and 17α-Hydroxyprogesterone on Plasma Steroid Levels and Ovulation in the African Catfish, *Clarias gariepinus*

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ABSTRACT

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The effect was studied of a preovulatory gonadotropic hormone (GTH) surge, induced by pimozide/LHRHa treatment, and of 17α-hydroxyprogesterone on oocyte maturation and ovulation and on the plasma levels of 17α-hydroxyprogesterone, 17α-hydroxy-20β-dihydroprogesterone, testosterone and estradiol. GTH and 17α-hydroxyprogesterone initially caused an increase in plasma testosterone levels, followed by an increase in 17α-hydroxy-20β-dihydroprogesterone. Neither treatment affected estradiol levels. All fish treated with pimozide/LHRHa or 17α-hydroxyprogesterone showed oocyte maturation and ovulation.

INTRODUCTION

In female African catfish, *Clarias gariepinus*, under conditions of captivity, oocyte development only procedes up to the postvitellogenic stage. The final stages of development, maturation and ovulation, generally have to be induced by treating the animals with hormones. A variety of techniques, operating at different levels of the hypothalamo-hypophysial-gonadal system, have been used successfully under laboratory and farm conditions. For example, matur-

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ation and ovulation have been induced by combined injections of luteinizing hormone-releasing hormone analogue (LHRHa; Des-Gly^{10} [D-Ala^{6}] LHRH-ethylamide) and pimozide (PIM) (De Leeuw et al., 1985a, b). The LHRH acts by stimulating the release of gonadotropin from the pituitary, and the pimozide by suppressing the action of a natural hypothalamic gonadotropin release-inhibiting factor (GRIF) which has been identified as dopamine (DA) (Peter and Crim, 1978; Peter et al., 1978; Peter and Paulencu, 1980; Chang and Peter, 1983; Chang et al., 1984; De Leeuw et al., 1986). The same combination of compounds had been shown to induce oocyte maturation and ovulation in the goldfish, *Carassius auratus* (Chang and Peter, 1983; Sokolowska et al., 1984), carp, *Cyprinus carpio* (Billard et al., 1983) and loach, *Paramisgurnus dabryanus* (Lin et al., 1985).

Oocyte maturation and ovulation in African catfish has also been induced by direct injection of gonadotropins, either in the form of a carp pituitary homogenate (Hogendoorn and Vismans, 1980) or human chorionic gonadotropin (HCG) (Eding et al., 1982). This technique has been used for many years and been shown to be successful in a wide range of fish species (see review by Donaldson and Hunter, 1983).

Finally, oocyte maturation and ovulation have also been induced in African catfish by injection of the steroid 17α-hydroxyprogesterone (17αP; Richter et al., 1985). Oocyte maturation, but not ovulation, has been induced by 11-deoxycorticosterone (Richter and Van Den Hurk, 1982). It has been well established in cypriniform and salmoniform teleosts that the preovulatory rise in serum gonadotropin, either natural or induced, stimulates the ovarian production of 17α-P and 17α-hydroxy, 20β-dihydroprogesterone (17α, 20β-P) (e.g. carp — Breton et al., 1983; Kime and Dolben, 1985; Levavi-Zermonsky and Yaron, 1986; salmonids — Liley et al., 1986; Scott et al., 1982a, b; Van der Kraak et al., 1985; white sucker, *Catostomus commersonii* — Scott et al., 1984). A similar situation is found in the African catfish, order Siluriformes (Lambert and Van Den Hurk, 1982).

In vitro studies have shown that, of all steroids so far investigated, 17α, 20β-P is generally the most potent in inducing oocyte maturation (but not ovulation) in, e.g., brook trout, *Salvelinus fontinalis* (Duffey and Goetz, 1980), rainbow trout, *Salmo gairdneri*, and pike, *Esox lucius* (Fostier et al., 1973; Jalabert, 1976), yellow perch, *Perca flavescens* and carp (Epler, 1981a, b), ayu, *Plecoglossus altivelis*, amago salmon, *Oncorhynchus rhodurus* and goldfish (Nagahama et al., 1983). In vivo studies have shown that 17α, 20β-P also induces oocyte maturation in the trout (Jalabert et al., 1978), carp (Jalabert et al., 1979) and pike (Montalembert et al., 1978). Ovulation only occurred in these studies if the 17α, 20β-P injections were preceded by a 'priming' injection of hypophysial homogenate.

11-Deoxycorticosterone has also been shown to be fairly effective in inducing oocyte maturation in vitro in a number of species, e.g., zebrafish, *Brachy-*

danio rerio (Van Ree et al., 1977), Indian catfish, *Heteropneustes fossilis* (Goswami and Sundararaj, 1971, 1974), brook trout and yellow perch (Goetz and Theofan, 1979; Duffey and Goetz, 1980), goldfish and pike (Jalabert, 1976; Jalabert et al., 1973). In the last four species, however, 17α, 20β-P has been shown to be more potent, and so it is not clear whether 11-deoxycorticosteroids play a natural role in oocyte maturation in any of these species. The production of these steroids requires the presence of a 21-hydroxylase. This enzyme has been identified positively in the ovaries of certain marine teleosts (Colombo et al., 1978) but not in the ovary of the zebrafish (Lambert, 1978) and the African catfish (Lambert and Van Den Hurk, 1982). Goswami and Sundararaj (1971, 1974) have hypothesized that the interrenal is a source of oocyte-maturation inducing steroid(s) in the Indian catfish.

The aim of the present study was to investigate the effect of PIM/LHRHa and 17α-P treatments on the levels of gonadotropin and sex steroids in the plasma of female African catfish. Special attention was given to (1) whether oocyte maturation and ovulation induced by PIM/LHRHa treatment were, as in other species, preceded by a surge in 17αP and 17α, 20β-P levels, (2) whether the production of testosterone and estradiol (E_2) was also affected (which might give us some clues about the steroid pathways involved) and (3) in what way 17α-P exerts its effect on oocyte maturation and ovulation (directly via conversion to 17α, 20β-P or by stimulation of GTH release?).

MATERIALS AND METHODS

Experimental animals

Clarias gariepinus were reared from eggs to maturity in the hatchery of the Department of Fish Culture and Inland Fisheries, Agricultural University, Wageningen, The Netherlands. At the time of the experiments the fish were one year old, and their weight was about 500 g. Hatchery conditions and the method of checking the maturity of the animals have been reported in previous papers (Hogendoorn and Vismans, 1980; Richter and Van Den Hurk, 1982). Only animals with postvitellogenic eggs were selected for the experiments. The fish were transferred from the hatchery to the laboratory, where they were kept individually in 45-l aquaria at 25 °C; they received no food for one day prior to the experiments.

Administration of pimozide/LHRHa and 17α-hydroxyprogesterone

Pimozide, which was a gift from Janssen Pharmaceutica Ltd. (Beerse, Belgium) and LHRHa, Des-Gly^{10} [D-Ala^6] LHRH-ethylamide (kindly provided by Dr. Gielen, Intervet, Boxmeer, The Netherlands), were suspended and dissolved respectively, in a vehicle consisting of 0.8% NaCl with 0.1% sodium

metabisulphite and 0.25% bovine serum albumin (BSA, fraction V, Sigma, St. Louis, MO, U.S.A.). Pimozide and LHRHa were administered together in a single intraperitoneal injection, containing 5 and 0.05 mg/ml of vehicle per kg body weight of the drugs, respectively. Control fish received a placebo injection, consisting of an equivalent volume of the PIM/LHRHa vehicle.

17α-Hydroxyprogesterone (Intervet International B.V., Boxmeer, The Netherlands) was dissolved in a vehicle consisting of dimethylisosorbide, containing 450 mg pyrrolidone, 20 mg plasdone C_{15} and 10 mg benzyl alcohol per ml respectively. 17α-P was injected intramuscularly, near the dorsal fin. Two successive injections, containing 3 and 5 mg/0.75 ml of vehicle per kg body weight respectively, were given with a time interval of 4 h according to Richter et al. (1985). Placebo-treated fish received corresponding dosages of solvents without hormone.

Experiments

Pimozide/LHRHa experiment. Ten fish were injected with PIM/LHRHa and five with the PIM/LHRHa vehicle (controls). At the time of injection and 4, 8, 12, 16 and 24 h later, blood samples were taken for hormone measurement. To prevent oversampling of the animals, the blood samples were taken as follows: all animals were bled at $t=0$ and $t=8$; half of the fishes were bled additionally at $t=4$ and $t=16$, the other half at $t=12$ and $t=24$. Since the hormone levels at $t=8$ in the fish bled at 4 and 16 h did not differ from those in the fish bled at 12 and 24 h, all experimental animals were considered as one group. The control fish were sampled throughout the experiment.

17α-Hydroxyprogesterone experiment. Ten fish were injected with 17α-P and five with the 17α-P vehicle. At $t=0$, the time of the first injection, and 4, 8, 12, 16 and 24 h later blood samples were taken. The sampling schedule was as in the PIM/LHRHa experiment.

Hormone measurements. Gonadotropic hormone levels were determined with the homologous catfish-GTH radioimmunoassay according to Goos et al. (1986). 17α, 20β-P, testosterone, 17α-P and estradiol were measured by radioimmunoassays as described by Scott et al. (1984). The 17α, 20β-P assay was carried out on plasmas extracted with diethyl ether. The other assays were carried out on plasmas diluted 1/100 with buffer and heated to 85 °C for 1 h.

Checking of oocyte maturation and ovulation

At the time of each blood sampling, the fish were checked for ovulation by gently pressing the abdomen. Fish that yielded a copious stream of green-brown eggs were rated as ovulated. Fertilizability of the eggs was used as a parameter

for egg quality or final maturation. Egg samples were fertilized artificially and incubated at 27°C for 24 h.

Statistical analysis

The data were statistically analysed with the Student's t test; $P<0.05$ was used as the threshold value for significance.

RESULTS

The effect of PIM/LHRHa (Fig. 1)

Injection with PIM/LHRHa caused a sharp increase in the plasma GTH level at 4 h (from 0.9 ± 0.1 ng/ml at $t=0$ to 54.5 ± 9.6 ng/ml) and a maximum at 8 h (72.1 ± 13.0 ng/ml). Thereafter, the level gradually decreased but was still different from control values at 24 h (6.8 ± 1.0 ng/ml versus 0.58 ± 0.09 ng/ml). GTH plasma levels at all sampling times, except $t=0$, were higher in the PIM/LHRHa-treated animals than in the PIM/LHRHa-vehicle-treated ones.

PIM/LHRHa treatment also elevated testosterone levels. The maximum level was measured at 4 h (from 32.3 ± 3.3 ng/ml at $t=0$ to 117 ± 7.9 ng/ml), declined to 70.8 ± 14.2 ng/ml at 8 h and dropped under the $t=0$-level at 12, 16 and 24 h after injection. No differences were measured in the testosterone levels of the controls at any time.

PIM/LHRHa treatment also elevated 17α, 20β-P plasma levels. A rise was first detected at 4 h (9.0 ± 1.6 ng/ml), the maximum was measured at 8 h ($46.6\pm4,6$ ng/ml) and levels gradually declined at 12, 16 and 24 h. Vehicle treatment had no effect on 17α, 20β-P levels.

With regard to the estradiol levels in the PIM/LHRHa-treated animals, at none of the sampling times was there a significant difference compared to $t=0$. However, at 4, 8, 12, 16 and 24 h, E_2 levels were higher in PIM/LHRHa-treated than in control animals.

The effect of 17α-hydroxyprogesterone (Fig. 1)

17α-P caused a slight but significant increase in plasma GTH levels at 4, 8 and 12 h ($t=0$, 0.9 ± 0.1 ng/ml; $t=4$, 2.4 ± 0.6 ng/ml; $t=8$, 1.6 ± 0.25 ng/ml; $t=12$, 1.42 ± 0.34 ng/ml).

17α-P caused an increase in testosterone levels at 4 h after the first injection ($t=0$, 41 ± 3.5 ng/ml; $t=4$, 64 ± 6.1 ng/ml). Thereafter the level declined and dropped under the $t=0$-value at 12, 16 and 24 h respectively. The testosterone plasma level of the placebo-treated fish at $t=0$ was 49.25 ± 12.3 ng/ml. The values were lower at all other sampling times.

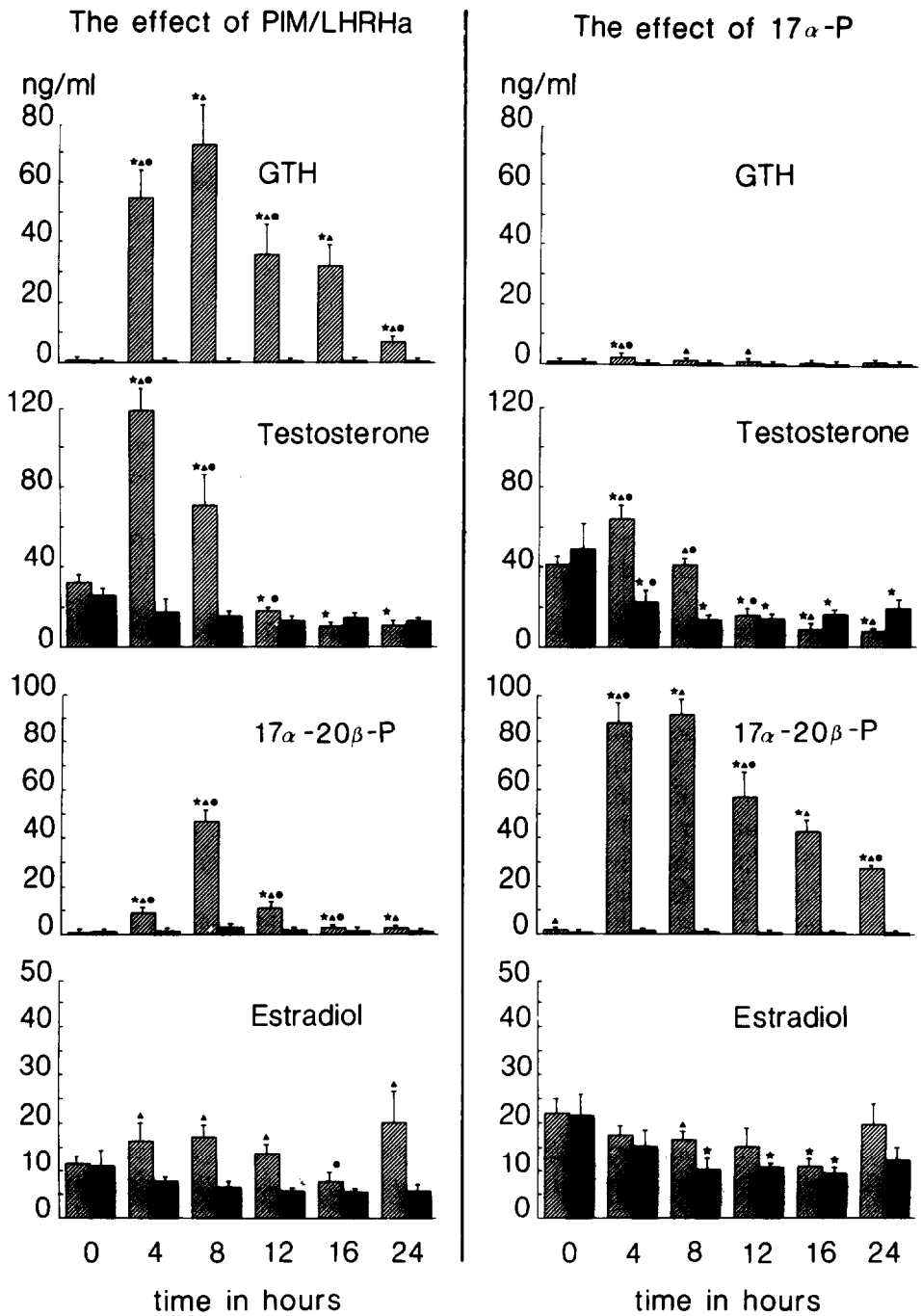

Fig. 1. The effect of PIM/LHRHa and 17α-hydroxyprogesterone on plasma levels of GTH, testosterone, 17α-hydroxy-20β-dihydroxyprogesterone and estradiol in female African catfish. Shaded columns indicate the values for PIM/LHRHa and 17α-P-injected animals, black columns the values for the placebo-treated animals.

*Significantly different compared to $t=0$, $P<0.05$; ▲ significantly different from control values at the same sampling time, $P<0.05$; ● significantly different from the previous sampling, $P<0.05$.

17α-Hydroxy-20β-dihydroxyprogesterone levels increased sharply after the first 17α-P injection ($t=0$, 1.67 ± 0.14 ng/ml; $t=4$, 88.0 ± 8.8 ng/ml). A similar level was measured at 8 h and, thereafter, a gradual decrease occurred; the level at 24 h still differed from $t=0$ ($t=24$, 27.6 ± 1.24 ng/ml). The estradiol level appeared not to be affected by the 17α-P treatment. It was noticeable, however, that like testosterone, the levels tended to decrease during the experiment. This was also significant in the controls at $t=8$, $t=12$ and $t=16$.

The effect of PIM/LHRHa and 17α-hydroxyprogesterone injections on plasma 17α-P levels (Fig. 2)

The effect of PIM/LHRHa treatment on 17α-P plasma levels was similar to that on 17α, 20β-P levels. At 4 h it was different from the corresponding control sample (34.25 ± 8.64 versus <10 ng/ml) and at 8 h the level was at a maximum, with a gradual decrease up to 24 h.

As expected, in response to 17α-hydroxyprogesterone injections, levels of this steroid reached high values in plasma. At 4 h after the first 17α-P injections the level increased from <10 (10 ng/ml was the lower detection level in the assay) to 273 ± 35.7 ng/ml. Almost the same level was maintained by the second injection (at $t=4$) so that at $t=8$ 293 ± 16.25 ng/ml could be measured. Thereafter, there was a gradual decrease, although the level was still 125 ± 9.4 ng/ml at 24 h.

Vehicle treatments did not cause any measurable changes in 17α-P levels.

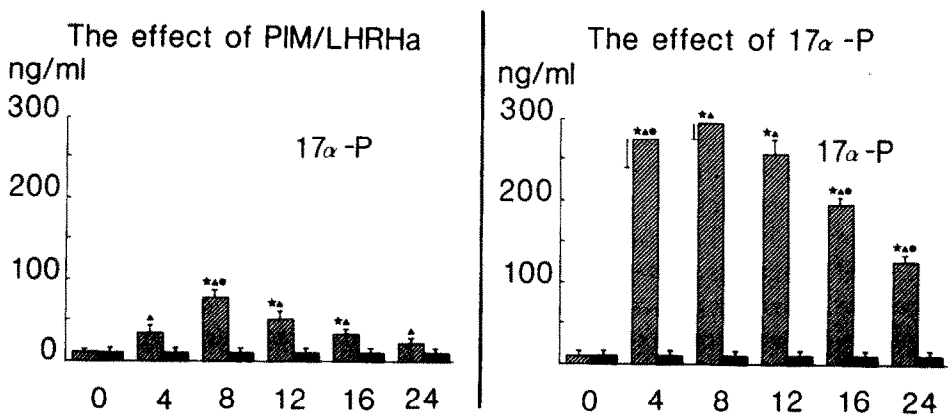

Fig. 2. The effect of PIM/LHRHa and 17α-hydroxyprogesterone on plasma levels of 17α-P in female African catfish. Shaded columns indicate the values for PIM/LHRHa and 17α-P-injected animals, black columns the values for the placebo-treated animals.
*Significantly different compared to $t=0$, $P<0.05$; ▲ significantly different from control values at the same sampling time, $P<0.05$; ● significantly different from the previous sampling, $P<0.05$.

Maturation and ovulation

All animals treated with PIM/LHRHa or 17α-P had ovulated at $t=16$ after injection or the first injection, respectively. The percentage of fertilized eggs was between 80 and 90, indicating that oocyte maturation had occurred normally as a result of the treatment. None of the placebo-treated fish showed ovulation.

DISCUSSION

It has been hypothesized that pituitary gonadotropins induce final oocyte maturation indirectly by stimulating the synthesis of maturational steroids in the ovarian follicles (Hirose, 1976; Jalabert, 1976; Sundararaj and Goswami, 1977; Iwamatsu, 1978). Our experiments have shown that PIM/LHRHa treatment enhances the production of GTH, testosterone, 17α-P and 17α, 20β-P in the African catfish, and that the pattern of secretion of steroids, particularly a testosterone peak followed by a progestagen peak, is very similar to that found in cypriniform and salmoniform teleosts (see references in Introduction). Some of the testosterone seems to be aromatized, however, since there is a slight but overall increase in estradiol levels. This contrasts to the situation in most fish species studied, where estradiol levels decline during the periovulatory period (Scott et al., 1982b; Kime and Dolben, 1985; Van der Kraak et al., 1985; Levavi-Zermonsky and Yaron, 1986; Liley et al., 1986).

Injection of 17α-P in our experiments caused significant increases in plasma GTH and testosterone levels. At present there is no explanation for these rises. Exogenous 17α-P may have served as a direct precursor for testosterone synthesis or acted via GTH.

The injection of 17α-P also caused a dramatic increase in 17α, 20β-P levels. This was entirely unexpected as most studies (for review, see Young et al., 1982; Goetz, 1983) have suggested that 20β-hydroxysteroid dehydrogenase, which is responsible for the reduction of the 20-ketone group of 17α-P, is induced by GTH. The rapidity of the rise in 17α, 20β-P levels in response to the 17α-P injections, however, would suggest that 20β-hydroxysteroid dehydrogenase is already present in non-GTH-stimulated fish and that GTH acts mainly on the production of 17α-P, rather than on the conversion of 17α-P to 17α-20β-P. It is more likely that GTH acts on the enzyme C17–20 lyase, which converts 17α-P to androstenedione. C17–20 lyase is a cytochrome P-450-dependent enzyme, known to be under control of gonadotropins (for review, see Schoonen et al., 1987). They suggested a down-regulation of the enzyme under the influence of high levels of GTH, resulting in an accumulation of 17α-P, which in turn will be converted to 17α, 20α-P.

The conversion of 17α-P to 17α, 20β-P in the African catfish in the present

experiments makes it impossible to say whether 17α-P acts directly, or via 17α, 20β-P, on oocyte maturation.

As mentioned in the Introduction, it has been found in most teleost species that steroids by themselves will not induce ovulation. The principal factor which has been found to induce ovulation both in vitro (Jalabert and Szölösi, 1975; Epler, 1981b) and in vivo (Stacey and Pandey, 1975) is prostaglandin F2α (PGF2α). The evidence suggests that under natural conditions, an ovulation-inducing wave of gonadotropin directly or indirectly stimulates the synthesis and/or release of PGF2α in the ovaries (for review, see Goetz, 1983). If PGF2α also has this function in the African catfish, then the synthesis of PGF2α is probably not directly stimulated by GTH; the 17α-P-injected animals all showed ovulation but only a slight increase in GTH secretion. It could be argued that the rise in GTH, caused by the 17α-P injection, may be large enough to explain why this steroid induces ovulation, as well as oocyte maturation, in the African catfish. However, De Leeuw et al. (1987) found that a LHRHa treatment usually would not cause ovulation in the African catfish, although the induced GTH levels are much higher than in the present experiment after 17α-P injection.

Although a direct pituitary–ovarian relay has been demonstrated in many fish, in at least one species, the Indian catfish (*Heteropneustes fossilis*), a pituitary–interrenal–ovarian relay has been proposed: pituitary gonadotropin stimulates the interrenals to produce a maturational steroid which, in turn induces final maturation. The basic evidence in support of this hypothesis has been thoroughly reviewed by Sundararaj and Goswami (1977). Richter and Van Den Hurk (1982) could induce oocyte maturation in the African catfish in vivo with 11-deoxycorticosterone and Van Ree et al. (1977) also found 11-deoxycorticosterone to be a potent steroid in inducing maturation in vitro in oocytes from the zebrafish. Thus, for oocyte maturation, the pituitary–interrenal–ovarian axis might also be of importance in fish other than the Indian catfish.

Our experiments were not designed to study the role of the interrenal tissue in oocyte maturation and ovulation. However, in view of the close similarity between the identity and profile of steroids found in the African catfish and those in salmoniform and cypriniform teleosts one might postulate that the interrenal only has a subsidiary role in the periovulatory process. But if so, it is unlikely that the action of corticosteroids is by stimulating the synthesis of 17α-P and 17α-20β-P, since Richter and Van Den Hurk (1982) found only oocyte maturation after administration of 11-deoxycorticosterone and no ovulation, while in the present experiment both events occurred after 17β-P treatment.

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