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SOME ASPECTS OF OOCYTE MATURATION IN CATFISH

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SUMMARY

Plasma levels of cortisol, testosterone and oestradiol increase in the gravid catfish and reach peak values at 15, 90 and 120 min, respectively, following administration of ovine luteinizing hormone (LH) or partially-purified salmon gonadotropin (SG-G100). Of the three steroids, cortisol is of interrenal origin, whereas testosterone and oestradiol are contributed by the ovaries. Further, the ovarian tissue of the gravid catfish, under the influence of gonadotropin, can also synthesize pregnanolone (3α-hydroxy-5β-pregnan-20-one). These findings suggest that the injected gonadotropin stimulates the interrenal to produce cortisol and also promotes steroidogenesis in the ovary of the gravid catfish. Experiments were designed to evaluate the interactions between gonadotropin, cortisol and ovarian steroids during oocyte maturation.

In vitro culture of catfish oocytes with cortisol acetate or LH alone and in various combinations revealed that not only is cortisol acetate a much more potent maturation-inducing agent than LH but that the two hormones act synergistically over a wide range of dosages. Prior exposure of oocytes to LH or pregnanolone for as short a duration as 90 min sensitized them to subsequent cortisol action on maturation, thereby indicating that possibly LH sensitizes oocytes through the formation of pregnanolone. Addition of testosterone or oestradiol to the culture medium simultaneously with cortisol, LH or pregnanolone markedly inhibited oocyte maturation.

On the basis of our data a new hypothesis has been proposed to explain the hormonal regulation of oocyte maturation in the catfish. The gonadotropin injected to induce ovulation, or released spontaneously in the gravid catfish at the time of ovulation, acts at two sites, the ovarian tissue and the interrenal; the former produces pregnanolone, testosterone and oestradiol, while the latter produces cortisol. Pregnanolone sensitizes oocytes so that the maturation response to cortisol is enhanced. The significance of the increase in the plasma levels of testosterone and oestradiol is not properly understood. Further work will be necessary to elucidate this problem.

INTRODUCTION

In the catfish, *Heteropneustes fossilis*, vitellogenesis is initiated and completed during the prespawning period (May–June) under the influence of gonadotropin and oestrogen [1], so that at the onset of the spawning period (July–August) the ovaries are packed with yolky oocytes. The catfish spawns but once in a year during the monsoon season (July–August), when the stimulus for oocyte maturation is provided by a combination of environmental factors prevailing at this time. In the catfish kept under laboratory conditions, oocyte maturation remains indefinitely blocked but can be induced by administration of gonadotropins or adrenocorticosteroids, principally cortisol and deoxycorticosterone [2, 3]. Therefore, the hormonal requirements for oocyte growth and vitellogenesis seem to be different from those necessary for oocyte maturation; the former involves principally the operation of gonadotropin–ovarian axis, whereas the latter requires the activation of not only the gonadotropin–ovarian axis but also the gonadotropin–inter-renal axis. Corticosteroids have little role to play in oocyte growth and vitellogenesis [1], but the contribution of ovarian steroids in catfish oocyte maturation is not adequately understood. The present paper deals with the hormonal profile in the gravid catfish prior to and during oocyte maturation as well as interactions between gonadotropin, cortisol and ovarian steroids during oocyte maturation.

EXPERIMENTAL

The experimental procedures as well as materials and methods used in this report have been described previously [2, 4–6].

RESULTS AND DISCUSSION

Gonadotropin–inter-renal axis in oocyte maturation

Gonadotropins as well as C_{21} corticosteroids can induce oocyte maturation in the hypophysectomized catfish [2, 3], indicating that the action of corticosteroids on oocyte maturation is independent of the pituitary. Several lines of evidence, however, suggest that the maturation-inducing action of gonadotropins is mediated through a steroidogenic relay [2, 7–9]. Consequently, hypophysectomized gravid catfish, in which steroidogenesis has been chemically blocked by metopirone, do not spawn optimally after receiving gonadotropin but do so following corticosteroid administration [7]. This clearly suggests that steroids are the terminal hormones that act on the yolky oocytes to induce maturation. This appears to be true for all the teleost species so far studied [10–19]. The source

Table 1. Effect of ovine luteinizing hormone (LH) or partially-purified salmon gonadotropin (SG-G100) on *in vitro* maturation of catfish oocytes in ovary–head kidney co-culture

S. No.	LH* (40 μg/ml)	SG-G100 (20 μg/ml)	Head kidney pieces (40 mg/ml)	Number of† mature oocytes (%)	n‡
1	–	–	–	0	15
2	+	–	–	14 ± 5	15
3	–	–	+	9 ± 5	15
4	+	–	+	50 ± 7	15
5	–	+	–	13 ± 7	6
6	–	–	+	19 ± 6	6
7	–	+	+	54 ± 8	6

* NIH-LH-S18.

† Mean ± SEM. *P* values calculated by Student's *t*-test between groups: 2 and 4 <0.001; 3 and 4 <0.001; 5 and 7 <0.001; 6 and 7 <0.005.

‡ Figures indicate number of catfish donors from which ovarian pieces were obtained for culture.

of these maturation-inducing steroids has, however, been debated in recent years.

In the catfish, the available evidence suggests that the maturation-inducing steroids are produced principally by the inter-renal. Employing *in vitro* techniques [4], which isolate the ovarian tissue from other hormonal influences, we have shown that ovine LH, a potent maturation-inducing agent *in vivo* [2], is only marginally effective *in vitro* even at very high concentrations [4, 5]. Similarly, partially-purified salmon gonadotropin (SG-G100) which is about 2.5 times more potent than LH in inducing *in vivo* catfish oocyte maturation [6] loses much of its effectiveness under *in vitro* conditions [20]. Data presented in Table 1 for co-culture experiments show that the ability of both LH as well as SG-G100 to evoke *in vitro* oocyte maturation is considerably enhanced by introducing inter-renal tissue into ovarian cultures. These data permit us to conclude that gonadotropins *per se* cannot evoke optimal oocyte maturation in the absence of another suitable steroidogenic relay and that in the catfish the ovary itself is not capable of contributing adequate amounts of the appropriate maturation-inducing steroid(s). Therefore, the site of production of major maturation-inducing steroids is most likely to be the inter-renal. The data also show that gonadotropins, such as ovine LH and SG-G100, can stimulate the inter-renal of the catfish to release potent maturation-inducing steroids.

As in all teleost fishes investigated thus far [21], cortisol is the major corticosteroid in the catfish [22]. Table 2 shows that, under *in vitro* conditions, cortisol is much more effective in inducing oocyte maturation than ovine LH. That cortisol is the terminal hormone acting on the oocytes to induce maturation, even *in vivo*, is supported by the observation that plasma cortisol levels increase significantly within 15 min following LH injection in the gravid catfish [22]. Our earlier studies have shown that the catfish ovary does not have the ability to synthesize 21-hydroxylated steroids [23]. Obviously, therefore, the site of gonadotropin-stimulated cortisol synthesis is the inter-renal.

Our recent studies suggest that while the gonadotropin-inter-renal axis plays a major role in catfish

Table 2. *In vitro* catfish oocyte maturation response following treatment with various dosages of ovine luteinizing hormone or cortisol acetate

Treatment	Dosage (μg/ml)	Number of† mature oocytes (%)	n‡
Ovine luteinizing hormone*	1	0	5
Ovine luteinizing hormone*	2.5	0	2
Ovine luteinizing hormone*	5	11 ± 5	6
Ovine luteinizing hormone*	10	13 ± 2	6
Ovine luteinizing hormone*	20	14 ± 2	6
Ovine luteinizing hormone*	40	36 ± 10	8
Cortisol acetate (Sigma)	0.05	7 ± 7	2
Cortisol acetate (Sigma)	0.1	10 ± 4	6
Cortisol acetate (Sigma)	0.25	18 ± 6	6
Cortisol acetate (Sigma)	0.5	31 ± 12	5
Cortisol acetate (Sigma)	1	68 ± 10	6
Cortisol acetate (Sigma)	5	89 ± 3	6

* NIH-LH-S18.

† Mean ± SEM.

‡ Figures indicate number of catfish donors from which ovarian pieces were obtained for culture.

Table 3. Effect of ovine luteinizing hormone (LH) or cortisol acetate (FA) alone and in combination on *in vitro* maturation of catfish oocytes

Treatment (Dosage: μg/ml) LH*	FA†	Number of‡ mature oocytes (%)
5	—	11 ± 5
10	—	13 ± 2
20	—	14 ± 2
—	0.1	10 ± 4
5	0.1	50 ± 7
10	0.1	48 ± 11
20	0.1	51 ± 11

* NIH-LH-S18.
† Cortisol acetate (Sigma).
‡ Mean ± SEM. Number of catfish donors from which ovarian pieces were obtained for culture = 6.

oocyte maturation, gonadotropin may be performing functions other than merely increasing cortisol output from the inter-renal. For instance, ACTH, which is even more effective than LH in stimulating cortisol production from the inter-renal of the catfish [22], is almost totally ineffective in inducing oocyte maturation either *in vivo* [2] or *in vitro* [4, 5]. It is likely that gonadotropin and cortisol may be acting synergistically on oocyte maturation. Jalabert[24] has reported that steroid levels (particularly of cortisol and cortisone) which by themselves are inactive on *in vitro* trout oocyte maturation increase the effectiveness of gonadotropic extracts 2–6 times, thereby indicating a synergism between corticosteroid and gonadotropin. Culture of catfish oocytes in the presence of both LH and cortisol acetate revealed the existence of a well-marked synergism between the two hormones in evoking oocyte maturation (Table 3). Subsequent experiments showed that the continuous presence of LH in the culture medium is not necessary and that a prior exposure of oocytes to LH for only 90 min greatly enhances the subsequent cortisol action on oocyte maturation (see Table 4). These results suggest that the gonadotropin, in addition to stimulating cortisol release from the inter-renal, also acts on the ovarian tissue and sensitizes the oocytes to cortisol action or causes follicular secretion of a synergistic steroid. This points to the existence of a gonadotropin–ovarian axis in addition to the gonadotropin–inter-renal axis.

Gonadotropin–ovarian axis in oocyte maturation

While the role of ovarian oestrogens in synthesis of vitellogenin and its deposition as yolk in the developing oocytes is unanimously acknowledged [1, 19], considerable difference of opinion exists regarding the contribution of ovarian steroids in maturation of oocytes. Instances where gonadotropins can stimulate *in vitro* maturation in intact oocytes but lose this ability in oocytes devoid of the follicular tissue, afford the most convincing evidence in favour of the ovarian follicular origin of the maturation-inducing steroids [10–13, 25–28]. In other cases, the ability of ovarian tissue to synthesize maturation-inducing steroids is indicated by the demonstration in this tissue of some of the important steroidogenic enzymes such as Δ^5-3β-hydroxysteroid dehydrogenase and 11β-, 17α- and 21-hydroxylases [29–35], and also by the fact that in these fishes the most potent maturation-inducing steroids (progesterone and its 17- and 20-hydroxy derivatives) are presumably of ovarian origin [12].

On the other hand, in several teleosts, evidence exists against the ovary being the only source of maturation-inducing steroids. For instance, in the goldfish, *Carassius auratus*, carp pituitary extract, carp pituitary gonadotropin and SG-G100 are only marginally effective in inducing oocyte maturation *in vitro* [25]. Similarly, in the yellow perch, *Perca flavescens*, both LH and hCG produce only a very slight maturational response under *in vitro* conditions [18]. Similar findings have also been reported for the catfish, *Heteropneustes fossilis* [3–5, 9]. Further, Wallace and Selman[19] have indicated that the inter-renal gland may play a role in *Fundulus heteroclitus* oocyte maturation.

Table 4. Effect of ovine luteinizing hormone (LH) pretreatment on cortisol acetate (FA)-induced *in vitro* maturation of catfish oocytes. After 90-min pretreatment with LH, oocytes were washed with fresh culture medium and then transferred to fresh medium containing FA for 16 h

S. No.	90-min pretreatment with LH* (μg/ml)	16 h treatment with FA† (μg/ml)	Number of‡ mature oocytes (%)	n§
1	—	0.01	0	6
2	—	0.1	11 ± 5	2
3	—	0.25	17 ± 5	6
4	20	—	1 ± 1	6
5	20	0.01	3 ± 2	6
6	20	0.1	55 ± 12	2
7	20	0.25	78 ± 6	6

* NIH-LH-S18.
† Cortisol acetate (Sigma).
‡ Mean ± SEM. P values calculated by Student's t-test between groups: 2 and 6 <0.05; 3 and 7 <0.001.
§ Figures indicate number of catfish donors from which ovarian pieces were obtained for culture.

Table 5. Effect of pregnanolone (3β-hydroxy-5β-pregnan-20-one) pretreatment on cortisol acetate-induced *in vitro* maturation of catfish oocytes. After 90-min pretreatment with pregnanolone, oocytes were washed with fresh culture medium and then transferred to fresh medium containing FA for 16 h

S. No.	90-min pretreatment with pregnanolone* (5 μg/ml)	16-h treatment with cortisol acetate* (μg/ml)	Number† of mature oocytes (%)	n‡
1	–	0.01	0	6
2	–	0.1	11 ± 5	6
3	–	0.25	17 ± 5	6
4	+	—	10 ± 4	6
5	+	0.01	22 ± 15	6
6	+	0.1	56 ± 6	6
7	+	0.25	68 ± 9	6

* Steraloids (pregnanolone); Sigma (cortisol acetate).

† Mean ± SEM. *P* values calculated by Student's *t*-test between groups: 2 and 6 <0.001; 3 and 7 <0.001.

‡ Figures indicate number of catfish donors from which ovarian pieces were obtained for culture.

Our recent experiments have shown that following intramuscular administration of ovine LH at dosages (200 μg/fish) which normally induce oocyte maturation, ovulation and spawning in the gravid catfish, plasma levels of cortisol, testosterone and oestradiol show peak values at 15, 90 and 120 min, respectively as estimated by radioimmunoassay. In view of the fact that the catfish ovary lacks the ability to synthesize cortisol or any other 21-hydroxylated steroid [23], the observed increases in the levels of the three steroids suggest gonadotropin-mediated steroidogenesis in the inter-renal (cortisol) as well as in the ovary (testosterone and oestradiol).

Our earlier studies have shown that the catfish ovary, in addition to producing testosterone and oestradiol, can convert pregnenolone into 3α-hydroxy-5β-pregnan-20-one (pregnanolone) and another steroid tentatively identified as 5β-pregnane-3α-20α-diol (pregnanediol) [23]. Also *in vivo* pretreatment with SG-G100, but not ACTH, enhanced the conversion rate to pregnanolone [23]. When the various catfish ovarian steroids were tested for their ability to induce *in vitro* oocyte maturation, oestradiol and pregnanediol were found to be totally ineffective [4], while testosterone and 3β-hydroxy-5β-pregnan-20-one could induce *in vitro* oocyte maturation although they were much less effective than cortisol. It is quite likely that the slight maturational response observed when catfish oocytes are cultured with mammalian or piscine gonadotropins is due to the production of testosterone and/or pregnanolone by the ovarian tissue. Obviously, therefore, gonadotropin acts on at least two different loci, the inter-renal to produce potent maturation-inducing 21-hydroxylated steroids such as cortisol [22, 23], and the ovary to produce steroids such as oestradiol, testosterone, pregnanolone and pregnanediol, some of which possess a slight maturation-inducing activity. In view of our earlier observations that gonadotropin sensitizes the oocytes to subsequent cortisol action, it was

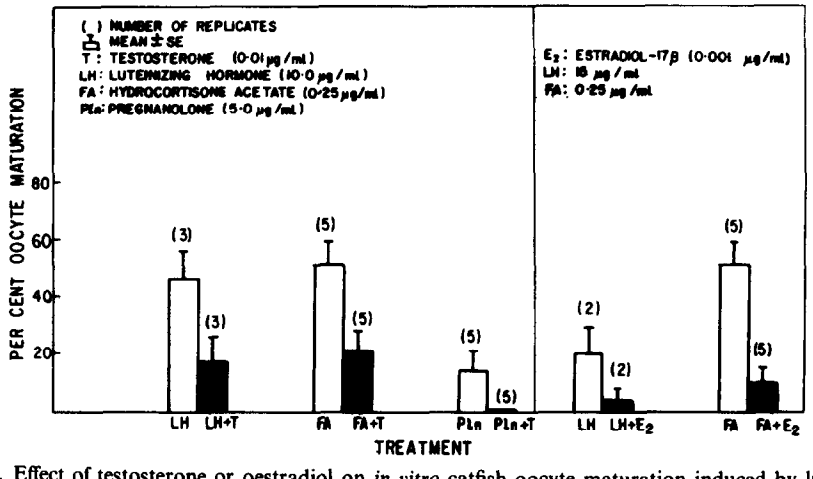

Fig. 1. Effect of testosterone or oestradiol on *in vitro* catfish oocyte maturation induced by luteinizing hormone, hydrocortisone acetate or pregnanolone (3β-hydroxy-5β-pregnan-20-one). Testosterone or oestradiol was added to the culture medium simultaneously with luteinizing hormone (NIH-LH-S 18), hydrocortisone acetate (Sigma), or pregnanolone (Steraloids).

thought worthwhile to investigate the interaction, if any, between the inter-renal and the ovarian steroids.

Interestingly, pretreatment of catfish oocytes for 90 min with 5 μg/ml of 3β-hydroxy-5β-pregnan-20-one, which by itself brings about only marginal oocyte maturation (10%), significantly enhances the maturational action of cortisol acetate (see Table 5). For instance, cortisol acetate at concentrations of 0.1 and 0.25 μg/ml is able to induce maturation in only 11 and 17% of the oocytes, respectively; this response to the two dosages of cortisol acetate increases to 56 and 68%, respectively, when the oocytes are pretreated with 3β-hydroxy-5β-pregnan-20-one for the first 90 min of the culture period.

Figure 1 shows that *in vitro* maturation of catfish oocytes induced by LH, cortisol acetate or 3β-hydroxy-5β-pregnan-20-one is significantly inhibited by simultaneous addition of testosterone to the culture medium. Similarly, oestradiol also prevents LH- or cortisol acetate-induced *in vitro* oocyte maturation. The exact mechanism of testosterone inhibition of oocyte maturation is not known at present. It is possible that the three steroids may be competing for common receptor sites on the oocytes. One of the functions of gonadotropin may be to displace testosterone or oestradiol from some of the binding sites which would then be occupied by cortisol which is a more potent maturation-inducing agent than testosterone. Experimental support for this hypothesis is not yet available. Preliminary experiments, however, show that incubation of catfish oocytes with LH and an antiserum to testosterone increases the maturational response to LH. Further work is in progress in our laboratory to elucidate the role of testosterone and oestradiol in oocyte maturation.

CONCLUSION

The yolky oocytes in the gravid catfish are maintained in a viable state through a tonic release of gonadotropin [6]. These yolky oocytes, however, do not mature spontaneously but do so only after the onset of monsoon season. Consequently, it is logical to assume the existence of inhibitory mechanisms that prevent premature maturation and ovulation of oocytes in the gravid catfish. While the information presented in this paper does not explain the nature of this inhibition, some tentative conclusions can be drawn regarding hormonal interactions prior to and during oocyte maturation (see Fig. 2).

Gonadotropin, exogenously administered or even released endogenously under the influence of suitable environmental factors, acts at two sites—the inter-renal and the ovary. In time sequence, plasma levels of cortisol increase within 15 min of gonadotropin administration, while testosterone and oestradiol levels rise much later. We also have reason to believe that pregnanolone is synthesized in the ovaries following gonadotropic stimulation, at about the same time as cortisol in the inter-renal. Thus, oocytes sensitized by pregnanolone, which is produced locally in the ovary, are acted upon by cortisol to promote maturation. The significance of the increase in plasma levels of testosterone and oestradiol following gonadotropin

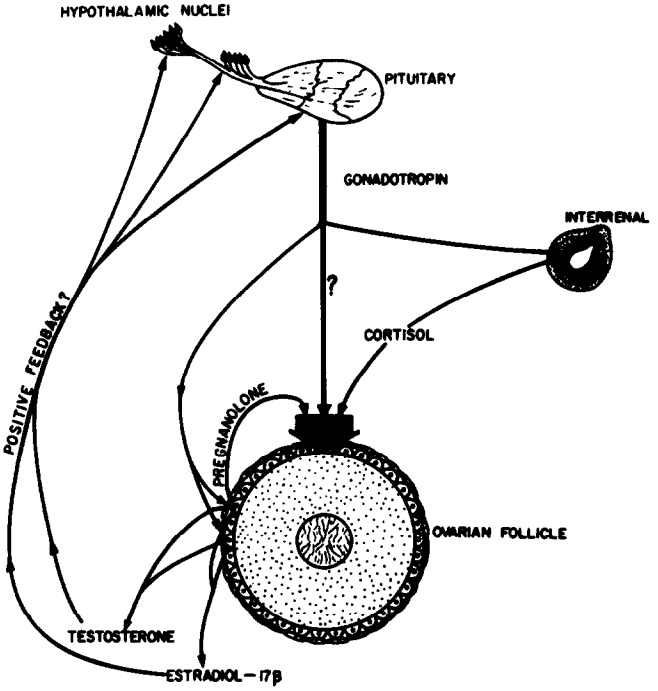

Fig. 2. Schematic diagram showing possible interactions between gonadotropin, cortisol and ovarian steroids during oocyte maturation in the catfish.

administration is not completely understood and at present remains a matter for speculation. Preliminary studies suggest that in the catfish, testosterone and cortisol share common binding sites on the oocyte; increased levels of testosterone after gonadotropic stimulation might reflect its displacement from the binding sites in favour of cortisol, which is a much more potent inducer of maturation than testosterone. The observed increase in oestradiol levels may be due to its synthesis or to its conversion from testosterone. Presumably, the elevated plasma levels of these two steroids might exert a positive feedback action on the hypothalamo–hypophyseal system for further release of gonadotropin or may be involved in courtship and spawning behaviour.

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