EFFECT OF GONADOTROPINS ON THE STEROIDOGENIC CELLS IN THE OVARY OF THE CATFISH, *CLARIAS BATRACHUS* (LINN)

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ABSTRACT

The effect of piscine and mammalian gonadotropins on the steroidogenic cells of the ovary of the catfish, *Clarias batrachus* is investigated during the preparatory and the prespawning periods by histochemical localisation of Δ^5-3β-HSDH and G-6-PDH. The results indicate that Salmon pituitary gonadotropin and human chorionic gonadotropin stimulate i) the thecal cells in stage II and stage III oocytes and ii) the interstitial cells of the ovarian stroma which results in enhanced enzyme activity in these cells as compared to less stimulation brought about by the luteinising hormone and the follicle stimulating hormone. A partial spawning was observed in all the hormone treated fish during the prespawning period which might be due to the enhanced maturation and steroidogenesis of the ovary.

INTRODUCTION

It is known that piscine and mammalian gonadotropins can induce maturation and ovulation in teleosts and restore steroidogenesis in the gonads of hypophysectomised fish[1,2]. But there are no reports on the comparative effects of different gonadotropins on the steroidogenic cells of the ovary of teleosts during different phases of the reproductive cycle. Hence, the present work was undertaken to study the effect of piscine/mammalian gonadotropins on the steroidogenic cells of the ovary of the catfish, *Clarias batrachus* during the preparatory period and the prespawning period, by histochemical localisation of Δ^5-3β-HSDH and G-6-PDH, enzymes involved in steroidogenesis[3].

MATERIALS AND METHODS

Adult catfish, *C. batrachus* collected around Mangalore area (Latitude, 12° 51′, and longitude 74° 60′) were brought to Dharwad during preparatory period (January) and early prespawing period (April)[4]. They were fed with rice bran and oil cake. The first experiment was conducted during preparatory period and the second experiment was conducted during prespawning period. In both the experiments, the regimen of gonadotropin administration was the same as presented in table 1. The hormones were administered through intramuscular route on alternate days for 16 days. In all, 8 injections were given to each fish and the fish were autopsied on 17th day. The ovaries were dissected out and small pieces were quickly frozen over dry ice vapours and 12 μm thick sections were cut on a Pearse-Slee cryostat maintained at -18°C for the histo-chemical demonstration of glucose-6-phosphate dehydrogenase (G-6-PDH) and Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH)[5]. Some frozen sections were stained with haematoxylin eosin for parallel histological study.

RESULTS

During the preparatory period, the ovaries of control and FSH treated fish contained stage I oocytes which were characterized by their small size, presence of large nucleus and surrounded by follicular wall in which the granulosa layer was not clearly demarcated from the thecal layer. The ovaries of fish treated with SG-G 100 (figure 1), HCG and LH contained stage I and some stage II oocytes. The latter showed the appearance of ring of cortical alveoli, an indication of the onset of vitellogenesis and the inner granulosa layer could be distinguished from the outer layer in the follicular wall under high magnification.

The localization of the enzymes is shown in table 2. During the preparatory period, trace activity of G-6-PDH was found in follicular wall of stage I oocytes, whereas, Δ^5-3β-HSDH activity was not found in such oocytes of controls as well as the hormone treated fish. In stage II oocytes of fish treated with SG-G 100 (figure 2), HCG and LH, G-6-PDH and Δ^5-3β-HSDH activity was observed in some thecal cells and also in the interstitial cells of ovarian stroma. However, the thecal cells of stage II oocytes and interstitial cells of fish treated with SG-G 100 and HCG exhibited an

Table 1 *Number of fish used/hormone treatment given*

Groups	Treatment	Dosage/fish/ injection	Preparatory period (Jan–Feb) Number of fish	Preparatory period (Jan–Feb) Av. Wt. (gm)	Prespawning period (May) Number of fish	Prespawning period (May) Av. Wt. (gm)
1	Untreated control	—	5	91.00	5	103 60
2	Saline control	0.01 ml of 0 6 % saline	7	81.80	6	103 17
3	Salmon pituitary gonadotropin (SG-G100)*	50 μg	5	71.14	7	91.80
4	Human chorionic (gonadotropin (HCG) (Ayerst Lab. USA)	50 USP units	6	55.40	5	75.40
5	Luteinizing hormone (LH) (NIH-LH-S18, ovine)	50 μg	7	85.13	5	126.40
6	Follicle stimulating (hormone (FSH) (NIH-FSH-S9 ovine)	50 μg	5	61.20	5	91.50

All the hormones were dissolved in 0.6 % saline and the concentrations were so adjusted as to get the desired dosage in 0.1 ml.
* Free gift of SG-G 100, from Dr. E. M. Donaldson, Fisheries Research Board of Canada, Canada.

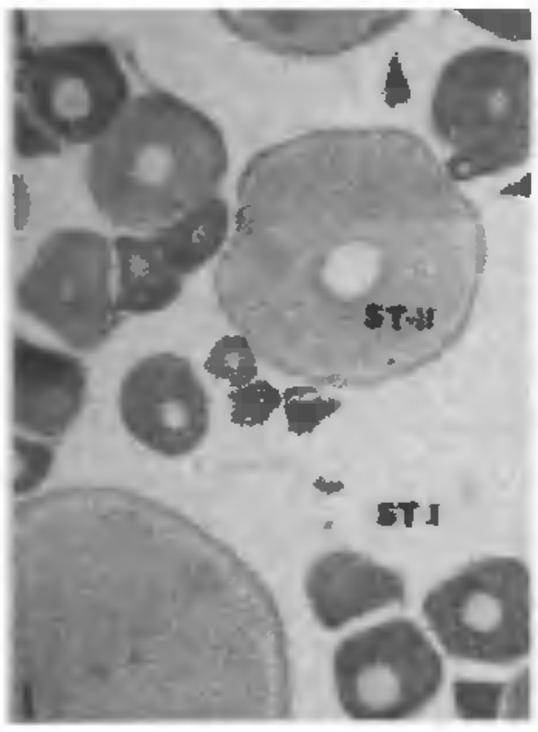
Table 2 *G-6-PDH and Δ^5-3β-HSDH activities in the ovary of catfish, C. batrachus during preparatory and prespawning period*

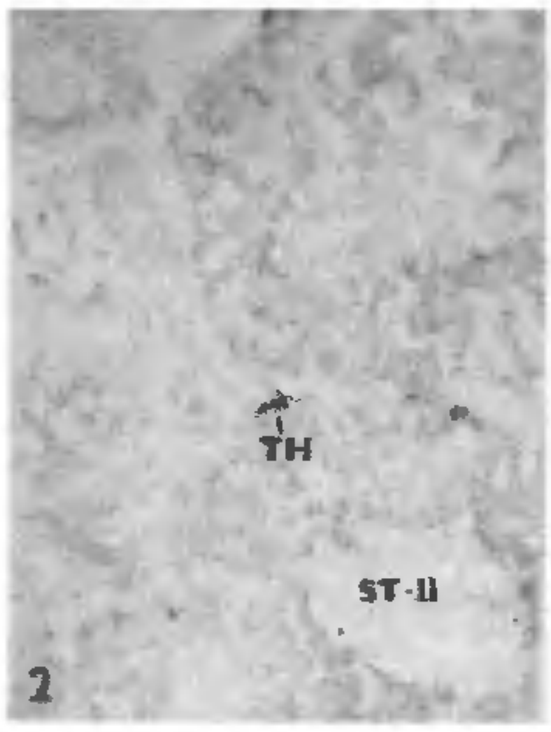
Group	Enzyme activities	Preparatory period: Stage I Follicular cells	Preparatory period: Stage II Oocyte thecal cells	Preparatory period: Stage II Inter-stitium	Prespawning period: Stage II Oocyte thecal cells	Prespawning period: Stage III Oocyte thecal cells	Prespawning period: Stage III Inter-stitium
Saline intact control	G-6-PDH	±	Stage II oocytes were absent	++	++	+++	+++
	Δ^5-3β-HSDH	−		+	+	+	+
SG-G 100	G-6-PDH	±	+++	+++	++	++++	++++
	Δ^5-3β-HSDH	−	++	++	++	++	++
HCG	G-6-PDH	+	+++	+++	++	++++	++++
	Δ^5-3β-HSDH	−	++	++	+	++	++
LH	G-6-PDH	±	++	++	++	+++	+++
	Δ^5-3β-HSDH	−	+	+	+	+	+
FSH	G-6-PDH	±	Stage II oocytes were absent	++	++	+++	+++
	Δ^5-3β-HSDH	−		+	+	+	+

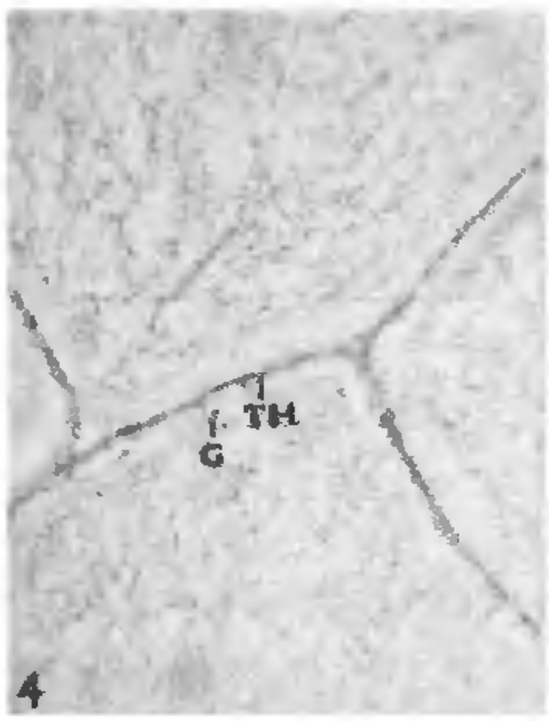
Intensity of reaction is graded from minimum (+) to maximum (++++); (−) denotes absence of reaction and (±) denotes trace activity.

increased enzyme activity when compared to the activity found in these cells of LH treated fish.

During the prespawning period, the ovaries of control fish contained stage III oocytes, (figure 3) characterised by extensive deposition of yolk. The fish treated with each of the four gonadotropins showed a partial spawning, as indicated by the presence of postovulatory follicles resulting in the decrease of fully mature follicles in the ovary when compared to that of ovary of control fish which did not spawn. Previously Ramaswamy and Sundararaj[6] have reported spawning in *C. batrachus* after administering HCG during prespawning period. The maximum response of ovulation/spawning was seen with SG-G 100 but a







Figures 1–4. 1. T.S. of ovary of SG-G 100 treated *C. batrachus* during preparatory period containing stage II oocytes with yolk vesicles. **2.** T.S. of ovary of *C. batrachus* treated with SG-G 100 during preparatory period, showing Δ^5-3β-HSDH activity. **3.** T.S. of ovary of *C. batrachus* treated with saline during prespawning period. Showing packed yolky oocytes. **4.** T.S. of ovary of *C. batrachus* treated with HCG during prespawning period showing G-6-PDH activity in the thecal cells (TH) of stage III oocytes. *Abbreviations*: ST.I. Stage I oocyte; ST II. Stage II oocyte; ST III. Stage III oocyte; TH. Theca; G. Granulosa

relatively less response was seen with HCG, LH and FSH, in decreasing order.

With regard to enzyme activity (table 2), some of the thecal cells of stage III oocytes showed the presence of Δ^5-3β-HSDH activity whereas granulosa cells did not. All the thecal cells of stage III oocytes showed an intense G-6-PDH activity (figure 4) but granulosa cells showed a trace activity. There was more enzyme activity (G-6-PDH and Δ^5-3β-HSDH) in the thecal cells of stage III oocytes and the interstitial cells of the ovary of fish treated with SG-G 100 and HCG compared to the activity observed in these cells of the ovary of controls and fish treated with FSH and LH.

DISCUSSION

Though there are some reports on the effect of individual gonadotropin on the steroidogenic cells of fish ovary, there are no reports on the relative effects of different gonadotropins to the best of our knowledge. Funk *et al*[7] have observed a moderate Δ^5-3β-HSDH activity in the ovaries of SG-G 100 treated juvenile *Onchorhynchus gorbuscha*, whereas, there was only a monoformazon deposit in the ovary of untreated juveniles indicating a trace activity. van Ree[8] has observed enhanced activities of G-6-PDH and Δ^5-3β-HSDH in the ovaries of LH treated zebra fish, *Brachydanio rerio*. van den Hurk and Richter[9] have observed increased Δ^5-3β-HSDH activity in the post-ovulatory follicles of ovaries of carp pituitary gonadotropin treated catfish, *Clarias lazera*. The present work shows that SG-G 100, HCG and LH induces the formation of stage II oocytes and steroidogenesis as indicated by the presence of Δ^5-3β-HSDH and G-6-PDH in some thecal cells and interstitial cells of the ovarian stroma and further the absence of stage II oocytes in the FSH treated fish ovary suggests that FSH at the given dose level is not capable of stimulating the growth of the stage I oocyte during the preparatory period. The ovary in the control as well as the hormone treated fish attains active steroidogenic potential indicated by the presence of the enzymes in some thecal cells of stage II and stage III oocytes as well as the interstitial cells during the prespawning period. However, the enzyme activity was more in these cells in fish treated with SG-G 100 and HCG compared with that of LH, FSH treated fish and of control fish. The present work suggests that during the preparatory as well as the prespawning period, SG-G 100 and HCG have a higher potency to enhance maturation and steroidogenesis in the ovary of *C. batrachus* as compared to that of LH or FSH at the given dose level. It is known that steroids act as terminal agents in ovulation and spawning in teleosts[10]. The partial spawning observed in the fish treated with gonadotropins and the absence of spawning in the control fish during the prespawning period also provides an additional evidence, albeit indirect, for the enhanced steroidogenesis in the ovary of gonadotropin treated *C. batrachus*.

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