PTX and TPTX rats, duodenal Mg disappearance was significantly reduced - which is in accordance with the present observation and earlier reports[14,15] that in PTX rats Mg absorption is increased by the administration of PTH. However, the effect of PTH seems not to be a direct one, but to be mediated by the intestinally highly active 1,25-D_3, as it has been shown that PTH stimulates the renal synthesis of 1,25-D_3 in vitro[20] and in vivo[21], and as the effect of PTH on intestinal Mg absorption of PTX rats can be mimicked by the administration of 1,25-D_3 (this study). Administration of b-PTH 1-34 and 1,25-D_3 in this study and others[14,15] increases Mg absorption not only in the duodenum but also in the ileum, a bowel segment whose Mg absorption seems not to be controlled by endogenous PTH (fig.). However, this effect may be the expression of supraphysiologic doses of b-PTH 1-34 and 1,25-D_3, and a final interpretation would necessitate the determination of the blood values achieved by the exogenous administration of each substance. Nevertheless, our results support the view of a differential influence of endogenous PTH on small intestinal Mg absorption depending on the anatomical region under study.

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Interrelationship between plasma and ovarian cholesterol in a teleost fish

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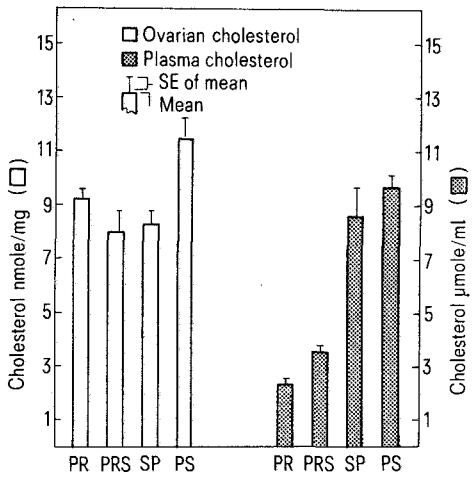
Summary. The profiles of plasma and ovarian cholesterol altered similarly as a result of seasonal influence. Fish pituitary extract and LH significantly depleted plasma and ovarian free cholesterol only, esterified cholesterol remaining unaltered. Findings indicated that plasma cholesterol was the primary source of sterol for ovarian steroidogenesis.

Circulatory cholesterol is the potential source for ovarian steroidogenesis and sterol ester storage by the mammalian ovary[2-4]. Considerable insight into the relationship between circulatory and ovarian cholesterol in steroidogenic tissues has also been gained from studies with mammals[3-7]. Again, investigation with mammals have clearly demonstrated the regulatory role of gonadotropin hormone (GtH) in the utilization of cholesterol as a substrate during steroidogenesis[8-10]. For fish, no such information is yet available. Recently we have reported that the dynamics of ovarian free (f) and esterified (e) cholesterol in a teleostean fish differ markedly from that of the mammal[11,12]. Information regarding the relationship between circulatory and ovarian cholesterol is essential in order to comprehend the pattern of cholesterol utilization in ovarian steroidogenesis. Therefore, the present work was designed to follow the profiles of circulatory and ovarian cholesterol at different stages of the reproductive cycle of a seasonally breeding teleost and after treatment with GtH and GnRH.

Materials and methods. To determine the plasma and ovarian cholesterol in different stages of the reproductive cycle, specimens of *Channa punctatus*, a commonly available murrel, were collected from ponds near this University. Prior to sacrifice within 24 h after collection, blood from each fish was drawn from the caudal vein with a heparinized syringe. After sacrifice, the ovary of each fish was dissected out, homogenized and subjected to low speed centrifugation (1000 × g). The supernatant was saved for cholesterol assay.

For the hormonal treatments pituitaries were collected from *C. punctatus* (at the preparatory stage; weight of the freshly collected pituitary being between 350 and 400 µg) and stored in cold acetone. Each pituitary (p) was separately homogenized with 1 ml of 0.6% saline and centrifuged at 1000 × g. The supernatant was injected i.m. Each fish received 1 p/day and the treatment was continued for 7 days. Other treatments included a) ovine LH (NIAMDD-oLH-22) at a dose of 1 µg/100 g/day for 7 days; and b) LH/FSHRH (GnRH, 21-103-DH-NIAMDD) at a dose of 1 µg/100 g, for 10 days. Injections were always given in the morning to the MS 222 (Sandoz Ltd) anesthetized fishes in order to minimize handling and injection shock. The sample size in the experiments varied from 7 to 10.

At the end of each treatment, collection of plasma and ovarian homogenization was done as described above. Determination of the cholesterol content and separation of (f) and (e) cholesterol by TLC was carried out as described by us recently[11,12]. The data were analyzed by Student's t-test[13].



Ovarian and plasma cholesterol profiles in relation to 4 different stages of the reproductive cycle, preparatory (PR), prespawning (PRS), spawning (SP) and postspawning (PS).

Results and discussion. This fish is a seasonal breeder and its reproductive cycle can be divided into 4 stages; preparatory (PR), prespawning (PRS), spawning (SP) and postspawning (PS)[12]. We measured total cholesterol to determine plasma and ovarian cholesterol at different stages of the murrel's reproductive cycle. It can be seen from the figure that the cholesterol content of the plasma is higher than that of the ovary. Again, plasma cholesterol level fluctuated prominently at different stages of the reproductive cycle, and was remarkably lower during the preparatory and prespawning stages. In contrast, the fluctuations of ovarian cholesterol were less prominent, the lowest being recorded during PRS. This shows that utilization of ovarian cholesterol was highest during PRS, indicating active steroidogenesis. Earlier reports with Indian teleosts demonstrated that recrudescence of ovarian activity started at PR stage after refractory PS, then significantly increased during PRS, and ovarian steroidogenic activity also increased following a similar pattern[14-17]. Since cholesterol is the substrate for steroidogenesis, decrease of ovarian cholesterol content during PRS in comparison with PS also supports the above contention. During the PR and PRS stages plasma cholesterol has also reduced remarkably in comparison with the PS stage. Highest cholesterol content in PS (both in plasma and ovary) indicates a decrease in cholesterol utilization in regressed ovaries.

The effect of hormones on ovarian and plasma cholesterol was studied by chromatographing the extract in each case on silica gel G for separating (f) and (e) cholesterol. The table shows that in both ovaries and plasma (f) cholesterol content was higher than that of (e) cholesterol. LH and fish pituitary extract significantly reduced (f) cholesterol in both cases, whereas (e) cholesterol remained unchanged. This is a striking difference from the mammal, where (e) cholesterol content was greater than (f) cholesterol and GtH induced depletion of (e) cholesterol only[18,19]. In fish, since (f) cholesterol concentration was higher in plasma and ovary and since this is the precursor in mitochondrial steroidogenesis, this moiety of cholesterol has possibly been readily utilized in response to GtH stimulation. Identification of immunoreactive LHRH in fish hypothalamus has recently been reported[20]. Reduction of plasma and ovarian cholesterol during PRS may be mediated by the hypothalamic stimulation of pituitary fraction. Hence an experiment was conducted with GnRH. Plasma and ovarian (f) cholesterol was significantly depleted in response to GnRH (table), suggesting hypothalamic role in seasonal influence.

The present work has elucidated an important aspect of the interrelationship of plasma and ovarian cholesterol in fish. Utilization of circulatory cholesterol pool for ovarian steroidogenic function is clearly indicated in this fish, as was found for mammals[3,5,6], but a major difference from the mammal is the reduction of (f) cholesterol in both plasma and ovary after hormonal treatment. GtH possibly induces rapid utilization of ovarian (f) cholesterol resulting in depletion of plasma (f) cholesterol since gonadotropin was found to have no direct effect on the plasma (f) cholesterol level in ovariectomized fish (unpublished observation).

Influence of gonadotropin and GnRH on plasma and ovarian cholesterol

Treatment	Plasma cholesterol (μmole/ml)		Ovarian cholesterol (nmole/mg)	
	Free	Esterified	Free	Esterified
Saline control	5.78±0.25	3.85±0.15	7.8 ±0.29	2.8 ±0.12
Fish pituitary extract	3.25±0.38*	3.76±0.15	6.21±0.11*	2.67±0.25
LH	3.93±0.12*	3.83±0.12	6.47±0.18**	2.6 ±0.13
GnRH	4.03±0.11*	3.89±0.07	6.8 ±0.25***	2.85±0.28

*$p<0.001$; **$p<0.005$; ***$p<0.025$; in comparison to saline control.

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