EFFECT OF THIOUREA ON HUMAN *CHORIONIC GONADOTROPHIN (hCG)* INDUCED SPERMATOGENESIS IN THE FROG *RANA TIGERINA* DURING THE POSTBREEDING REGRESSION PHASE

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It is well known that goitrogens interfere with the synthesis of thyroid hormones[1]. Hyperplastic changes are known to occur in the thyroid gland after thiourea treatment in a few amphibians[2,3]. However, there are no reports regarding its effect on spermatogenetic activity in amphibians. Therefore, in the present work the effect of thiourea on human chorionic gonadotrophin (hCG) induced spermatogenesis[4] was studied in *Rana tigerina*.

Adult male *R. tigerina* obtained from Karwar during the 2nd week of September was used. After acclimation they were separated into 3 groups and placed in cement tanks containing some water. They were force-fed with minced beef every alternate day. Treatments were given as follows:

Group 1. 0.2 ml distilled water (controls)
2. 20 IU hCG in 0.2 ml distilled water
3. 20 IU hCG in 0.1 ml distilled water + 25 μg thiourea in 0.1 ml distilled water

Injections (i.p.) were given on alternate days for 30

days. On the 31st day 5 frogs from each group were weighed and killed by decapitation. The testes were weighed and the gonadosomatic index (GSI) was calculated. They were processed for histological and histometric studies as described earlier[5-7]. The data were analysed using student's t test. The differences were judged as significant if $P < 0.05$.

In the distilled water injected controls the GSI and the diameters of testis and testis tubules were in a highly reduced state (table 1). The mean number of cell nests of stage 0 was greater while those of stage I and II were very few (table 2).

Administration of 20 IU hCG induced a significant increase in the GSI value and the diameters of testis and testis tubules over the control values (table 1). Cell nests of all stages (0–V) were present in the testis tubules (table 2). The mean number of stage 0 declined significantly ($P < 0.001$), while subsequent stages increased (table 2).

In hCG + thiourea-treated frogs GSI and diameter of testis tubules were significantly lower as compared to the hCG treated frogs (table 1). Spermatogenetic stages III to V were absent (table 2) whereas the cell nests of stage 0 were significantly greater and those of stage II were smaller (table 2).

Studies on the frequency distribution of cell numbers in the spermatocysts revealed that, in the control frogs, the peak occurred in the secondary spermatogonial cell nests that contained < 7 cells. Thirty seven percent of the cell nests in hCG-treated frogs contained 25–30 cells. A small percentage of cell nests contained as many as 43–48 cells (table 3A). In the hCG + thiourea-treated frogs the peak occurred in the cell nests that contained only 7–12 cells (table 3A).

With regard to primary spermatocytes, in the control frogs the peak occurred in the cell nests that contained 7–12 cells (table 3B). In hCG-alone-treated frogs, 24 % of the cell nests contained 31–36 cells with

Table 1 *Effects of hCG and hCG + thiourea on the testis of R. tigerina during the post breeding regression period*

Group		Mean Testis wt. (mg)/ 100 g body wt. ± SE	Mean diameter (μm) ± SE	
			Testis	Testis tubule
Control	(5)	12 ± 3	1126 ± 69	86 ± 10
20 IU hGC	(5)	55 ± 6	1182 ± 89	148 ± 8
		$P < 0.001$	$P < 0.001$	$P < 0.01$
20 IU hCG +		27 ± 1	1874 ± 167	119 ± 8
25 μ thiourea	(5)	$P < 0.01$	ns	$P < 0.05$

SE = Standard error; ns = nonsignificant; figures in paranthesis indicate the number of animals; P values were calculated by student's t test; hCG treated group was compared with controls while hCG + thiourea treated group was compared with hCG treated group.

Table 2 *Effects of hCG and hCG + thiourea on the spermatogenetic stages of R. tigerina during the postbreeding regression period*

	Control	20 IU hCG	20 IU hCG + 25 μg thiourea
0 Primary spermatogonia	9.27 ± 0.61	1.21 ± 0.13	3.52 ± 0.85
		$P < 0.001$	$P < 0.05$
I Secondary spermatogonia	1.01 ± 0.17	6.19 ± 0.52	5.53 ± 0.75
		$P < 0.001$	ns
II Primary spermatocytes	0.07 ± 0.04	3.71 ± 0.39	1.18 ± 0.40
		$P < 0.001$	$P < 0.01$
III Secondary spermatocytes	—	0.57 ± 0.24	—
IV Spermatids	—	0.34 ± 0.12	—
V Sperm bundles attached to Sertoli cells	—	0.51 ± 0.14	—

Mean number of spermatogenetic stages/tubule cross section ± SE.

Table 3 *Effects of hCG and hCG + thiourea on the frequency distribution of cell numbers in the sectioned cysts of (A) secondary spermatogonia, (B) primary spermatocytes and (C) secondary spermatocytes of R. tigerina during the postbreeding regression period*

Group	Number of cells per sectioned cyst A: Secondary spermatogonia								
	<7	7–12	13–18	19–24	25–30	31–36	37–42	43–48	>48
Control	52	46	2	–	–	–	–	–	–
20 IU hCG	0	1	7	28	37	17	7	3	–
20 IU hCG + 25 μg thiourea	9	36	34	18	3	–	–	–	–
	B: Primary spermatocytes								
Control	25	60	15	–	–	–	–	–	–
20 IU hCG	0	0	8	20	23	24	15	8	2
20 IU hCG + 25 μg thiourea	1	24	33	18	16	8	–	–	–
	C: Secondary spermatocytes								
	<13	13–24	25–36	37–48	49–60	61–72	73–84	85–96	>96
Control	–	–	–	–	–	–	–	–	–
20 IU hCG	0	0	8	19	17	31	10	10	5
20 IU hCG + 25 μg thiourea	–	–	–	–	–	–	–	–	–

Figures represent the percentages of spermatocysts in the cross-section.

few nests having more than 48 cells (table 3B). But the peak in hCG + thiourea-treated group was in the cell nests having only 13–18 cells (table 3B).

In hCG-treated frogs the peak occurred in the cell nests of secondary spermatocytes that contained 61–72 cells (table 3C). The secondary spermatocytes were absent in the controls and hCG + thiourea treated frogs.

The tests in *R. tigerina* remain regressed during the prolonged postbreeding regression phase extending between August and March when the hypophysial gonadotrophs (B_2 cells) are nonsecretory[8]. However, it has been shown in *R. tigerina* that administration of hCG during the postbreeding period induces complete spermatogenesis[4]. Therefore, this experimental model was used in the present study to assess the involvement of thyroid gland, if any, in the spermatogenetic process of the frog. The present study shows that thiourea affects spermatogenesis so that stages III to V fail to develop in spite of stimulation by the hCG (table 2). Further, the rate of mitotic activity in the spermatogonial cell nests is also reduced by thiourea which is evidenced by the fact that the cell nests in hCG + thiourea treated frogs contained fewer cells (table 3A & 3B). Thus the present studies show that goitrogens severely affect spermatogenesis in the frog. The present work apppears to be the first report to show that goitrogens impair spermatogenesis in amphibians. It is suggested that the normal functioning of thyroid gland is essential for spermatogenetic activity in the frog.

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