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EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON METHALLIBURE INHIBITED SPERMATOGENESIS IN *RANA TIGRINA* DURING THE PREPARATORY PERIOD

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THE antigonadotrophic property of methallibure (MB) is well known in mammals[1-3]. The present work was undertaken to determine the gonadotrophin-dependent/sensitive spermatogenetic stages in the Indian bullfrog *Rana tigrina* during the preparatory period when rapid spermatogenetic activity occurs[4]. Since human chorionic gonadotrophin (hCG) is known to induce spermatogenesis in *R. tigrina*[5], it was administered to MB-treated frogs to examine its effect on spermatogenesis in the frogs deprived of endogenous gonadotrophins.

Adult male frogs were obtained from Karwar in the first week of April. They were acclimated to the laboratory conditions for 5 days. Five frogs were killed to serve as start controls. The remaining frogs were kept in cement tanks containing little water. They were fed with minced beef on alternate day. The treatment groups were as follows:

Group 1. 0.25 ml distilled water (controls)
2. 1 mg MB in 0.25 ml distilled water
3. 1 mg MB in 0.25 ml distilled water + 20 IU hCG in 0.25 ml distilled water

Injections (ip) were given on alternate days for 30 days. The frogs were autopsied a day after the last injection. Five frogs from each group were used. The testes were fixed in Bouin's fluid and processed for histological observations. The paramcters used for the evaluation of spermatogenetic activity are the cell nest countings and frequency distribution of cells in the sectioned spermatocysts as described previously[4-6].

The seminiferous tubules of start control frogs contained cell nests of stages 0 to V (table 1). Cell nests of stages II to V in distilled water injected frogs increased significantly over the start controls (table 1). However, cell nests of stages 0 and I were numerically reduced.

MB treatment significantly reduced the mean number of cell nests of stages II, IV and V compared to controls. There was a corresponding increase in the cell nests of stage 0 (table 1). Cell nests of stages 0 to V in MB+hCG-treated frogs did not differ from those of the controls (table 1). However, when compared to MB-treated frogs, there was a significant reduction in the cell nests of stage 0 and corresponding increase in stage II. Further, there was a numerical increase in the mean number of cell nests of stages III to V.

Frequency distribution studies revealed that the secondary spermatogonial cell nests containing 13–18 cells were the highest in start controls, MB and MB+hCG groups. In controls the peak was in the cell nests that contained 19–24 cells (table 2A).

With regard to primary spermatocytic cell nests, start controls, MB and MB+hCG groups showed the peaks in the cell nests containing 19–24 cells. In controls the peak occurred in the cell nests with 31–36 cells (table 2B).

Secondary spermatocytic cell nests containing 37–48 cells were the highest in start controls (table 2C). In MB-treated frogs the peak occurred in the cell nests that contained 49–60 cells while cell nests having 61–72 cells were the highest in both controls and MB+hCG groups (table 2C).

The antigonadotrophic effect of MB has been reported in some amphibians[7-10]. In *R. tigrina* MB inhibited the spermatogenetic activity as revealed by the quantitaive analysis of spermatogenetic stages. In intact *Rana esculenta*, Rastogi *et al*[7] observed an increase in the spermatogonial cysts and a notable decrease in the primary spermatocytic cysts due to MB while secondary spermatocytes and spermatids were hardly affected. In *R. tigrina* also MB increased the primary spermatogonia and reduced the primary spermatocytic cysts but the number of spermatids and sperm bundles decreased significantly.

It has been suggested that the action of MB is at

Table 1 *Effects of MB and MB+hCG on the spermatogenetic stages of R. tigrina during the preparatory period*

Spermatogenetic stages	Start control (5)	Control (5)	1 mg MB (5)	1 mg MB + 20 IU hCG (5)
0 Primary spermatogonia	1.53 ± 0.54	0.32 ± 0.07 ns	1.87 ± 0.37 $P < 0.001$	0.35 ± 0.02 ns
I Secondary spermatogonia	5.24 ± 0.15	4.73 ± 0.54 ns	5.21 ± 0.39 ns	4.23 ± 0.93 ns
II Primary spermatocytes	2.22 ± 0.21	3.94 ± 0.34 $P < 0.001$	2.00 ± 0.25 $P < 0.001$	4.01 ± 0.47 ns
III Secondary spermatocytes	0.35 ± 0.08	0.83 ± 0.08 $P < 0.001$	0.69 ± 0.12 ns	0.72 ± 0.18 ns
IV Spermatids	0.17 ± 0.07	1 16 ± 0.20 $P < 0.001$	0.61 ± 0.10 $P < 0.05$	0.91 ± 0.12 ns
V Sperm bundles attached to Sertoli cells	0.36 ± 0.12	1.33 ± 0.24 $P < 0.001$	0.37 ± 0.07 $P < 0.001$	0.77 ± 0.18 ns

Mean number of spermatogenetic stages/tubule cross section ± SE. SE = Standard error; ns = nonsignificant; figures in parantheses indicate the number of animals; P values were calculated by student's t test; controls were compared with start controls while other groups were compared with the controls.

Table 2 *Effects of MB and MB+hGC on the frequency distribution of cell numbers in the sectioned cysts of (A) secondary spermatogonia, (B) primary spermatocytes and (C) secondary spermatocytes of R. tigrina during the preparatory period*

A: Secondary spermatogonia

	Number of cells per sectioned cyst								
Group	<7	7–12	13–18	19–24	25–30	31–36	37–42	43–48	>48
Start control	0	18	26	24	18	11	2	1	0
Control	0	5	14	29	27	15	5	3	2
1 mg MB	3	30	41	12	9	3	1	1	0
1 mg MB + 20 IU hCG	1	23	32	20	19	2	3	0	0

B: Primary spermatocytes

	<13	13–18	19–24	25–30	31–36	37–42	43–48	48–54	>54
Start control	3	14	23	22	20	9	7	1	1
Control	0	9	13	20	23	13	13	2	7
1 mg MB	4	31	38	12	12	3	0	0	0
1 mg MB + 20 IU hCG	2	16	27	23	15	8	6	2	1

C: Secondary spermatocytes

	<25	25–36	37–48	49–60	61–72	73–84	85–96	97–108	>108
Start control	2	16	30	20	17	13	1	1	0
Control	0	0	10	15	24	14	9	13	11
1 mg MB	0	7	16	33	23	10	7	2	2
1 mg MB + 20 IU hCG	1	14	19	13	22	12	11	5	3

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

the hypothalamohypophysial axis[1-3]. The fact that exogenous administration of hCG overcomes MB inhibited spermatogenetic activity in the frog supports the above view. However, in hypophysectomized as well as in intact *R. esculenta* homologous pars distalis homogenate failed to overcome the inhibitory effects of MB on spermatogenesis[8]. In the present work, as hCG could induce proliferation of spermatogonia and their transformation into primary spermatocytes in MB-treated frogs, it is suggested that early stages of spermatogenesis require gonadotrophin.

Frequency distribution studies provide additional clues with regard to the possible role of gonadotrophin in the regulation of spermatogenesis. For instance, tables 2A and 2B show a decreased mitotic activity in the early stages of spermatogenesis due to MB treatment. These findings suggest that proliferation of spermatogonia and their subsequent transformation into primary spermatocytes in the frog are gonadotrophin dependent.

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CURLY WING MUTANT IN *MANSONIA UNIFORMIS* (DIPTERA : CULICIDAE)

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MUTATIONS affecting the wing in mosquitoes are rare and there are only two reports on the heritable curly wing mutant in them[1,2]. This paper reports two curly wing mutants (females) discovered in the laboratory colony of *Mansonia uniformis* during a screening programme for mutant forms. Of the two mutants, one had bilateral curly wings, where the right one was always held upwards (figure 1), this wing was found to be rolled from either side to form a scroll-shaped structure, whose apex was again bent forwards. The left wing of this mutant was almost normal but its apex was bent sharply upwards. Interestingly, the mutant was pentapodite and the hind legs were exceptionally long due to the abnormal size of the tibia and tarsae (figure 1). Also, the middle and the hind legs of the mutant were sharply curved especially at the joints between femur and tibia, tibia and tarsa I and between the tarsae, almost similar to the condition reported in *Culex tritaeniorhynchus*[3].

Because of the aberrant development of the wings and legs, the mutant mosquito was unable to fly, and dragged itself around. Normally, *Ma. uniformis* exhibits mating even in test tubes during day time, preferably in the evening. Attempts to mate the mutant female with a normal male failed. However, she fed when held on the human hand. The mutant oviposited 3 days after the blood meal, not as the usual spherical-shaped egg cluster on the under-side of the *Pistia* leaf. The eggs were found dispersed individually on the water surface; this characteristic behaviour of the mutant mosquito may be due to the aberrant development of the middle and hind legs. The number of eggs deposited by this individual was 35 as against 160–200, which is usual for a normal female. The deposited eggs did not hatch. The attempt for second blood meal was not successful and the mutant individual died a week after its emergence.

The second specimen (figure 2) had unilateral curly wing, i.e. the mutant possessed a normal left wing whereas the right one was a curly wing similar to the one reported for *Cx. tritaeniorhynchus*[2]. However, she was not able to fly, and was making quick