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Serotonergic and catecholaminergic (dopaminergic) oscillations in the reproductive regulation of Japanese quail

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Running Title: **Phase relation of serotonin and dopamine regulates reproduction of Japanese quail**

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29 **Abstract**

30 Specific temporal phase relation of serotonergic and dopaminergic oscillations alters
31 reproductive responses in many species. Aim of the study was to confirm whether effect
32 of serotonergic drug (5-HTP) and dopaminergic drug (L-DOPA) is due to their
33 conversion into serotonin and dopamine respectively or other products. For this study,
34 PCPA (p-chlorophenylalanine, a long lasting inhibitor of serotonin synthesis), DDC
35 (Diethyldithiocarbamate, which inhibits biosynthesis of nor-adrenaline), α -MT (Methyl-
36 p-tyrosine, an inhibitor for the conversion of tyrosine to DOPA) and DOPS
37 (Dihydroxyphenylserine, a specific precursor for noradrenaline) were used in different
38 groups in addition to 5-HTP and L-DOPA given at specific time interval. Reproductive
39 responses monitored at 10 weeks post treatment indicate that gonadal activity was
40 significantly low in HTP:DOPA (8-hr quail), HTP+PCPA:DOPA and
41 HTP:DOPA+DDC quail compare to control (S:S). However, gonadal activity of
42 HTP:S(HTP control), S:DOPA(DOPA control) and HTP: α -MT+DOPS was not
43 different from S:S control and remained in active condition. These findings indicate
44 that it is not the dose of neurotransmitter precursor drugs (5-HTP and L-DOPA) and the
45 neurotransmitters (serotonin and dopamine itself) that cause the effect, instead it is the
46 function of interval between the drug administration which induces or entrains specific
47 phase relation between serotonergic and dopaminergic oscillations. Further, gonadal
48 suppression observed in HTP:DOPA, HTP+PCPA:DOPA and HTP:DOPA+DDC group
49 three groups is not due to injection of 5-HTP or L-DOPA (alone) but due to conversion
50 of administered 5-HTP into serotonin and conversion of L-DOPA (administered) into
51 dopamine; not due to their further conversion into catecholamines other than dopamine
52 i.e. noradrenaline or adrenaline.

53

54 **Keywords:** 5-HTP; L-DOPA; Specific temporal phase relation; Serotonergic and
55 dopaminergic oscillations

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66 **Introduction**

67 The studies on neurotransmitters proves that the brain levels of nor-epinephrine
68 (Owasoyo and Walker, 1980), dopamine (Owasoyo *et al.*, 1979), acetylcholine (Saito,
69 1971) as well as pineal melatonin (Lynch, 1971) were highest during the dark phase of
70 the light:dark cycle and lowest during the light phase where as serotonin (Quay, 1968;
71 Owasoyo and Walker, 1980) shows an opposite circadian pattern with peak levels
72 occurring during light phase of the light:dark cycle. It has also been shown that brain
73 serotonin and other neurotransmitters (dopamine) exhibit circadian variation in different
74 brain areas including suprachiasmatic nuclei (SCN) (Philo *et al.*, 1977; Héry *et al.*,
75 1981; Wilson and Meier, 1987; 1988). Experimentally it has been demonstrated that the
76 circadian rhythms exist in hormones as well as neurotransmitters viz. 5-HT, NE and DA
77 (Manshardt and Wurtman, 1968; Wilson and Meier, 1987; Forsling, 2000; Tiwari *et al.*,
78 2006). A number of studies also indicate the circadian release of 5-HT in blood and
79 pineal of mammals, birds and fish (Reis and Wurtman, 1968; Reis *et al.*, 1969). Thus,
80 there appears to be considerable synchrony throughout the brain with regards to the
81 rhythms of neurotransmitters content and activity (Reis *et al.*, 1969; Le Bras, 1984;
82 Khan and Joy, 1990).

83 It has been well established from several reports that administration of 5-HTP and L-
84 DOPA at the interval of 12 hrs stimulates gonadal growth and body weight gain
85 whereas administration of these drugs at the interval of 8 hrs results into opposite effect
86 i.e. gonadal suppression in several avian (Red headed bunting- Chaturvedi and Bhatt,
87 1990; Bhatt and Chaturvedi, 1992b; Phillips and Chaturvedi, 1992; Lal munia-
88 Chaturvedi *et al.*, 1994; Japanese quail- Chaturvedi *et al.*, 1991; Bhatt and Chaturvedi,
89 1992a; Phillips and Chaturvedi, 1995; Bhatt and Chaturvedi, 1998; Tiwari and

90 Chaturvedi, 2003; Chaturvedi et al., 2006; Kumar and Chaturvedi, 2008; Indian weaver
91 bird- Chaturvedi et al., 1997; Spotted munia- Chaturvedi and Prasad, 1991; Prasad and
92 Chaturvedi, 1992a, 1992b, 1992c, 2003) and mammalian species whether seasonally
93 breeding (Syrian hamster- Wilson and Meier, 1989; Indian palm squirrel- Chaturvedi
94 and Jaiwal, 1990; Jaiwal and Chaturvedi, 1991; Chaturvedi and Singh, 1992) or
95 continuous breeder (laboratory mouse, *Mus musculus*- Sethi and Chaturvedi, 2009;
96 Sethi et al, 2010).

97 In some of these studies, instead of 5-HTP and L-DOPA given at specific time
98 interval, each drug was given in combination with saline (5-HTP & saline or saline &
99 L-DOPA) (Prasad and Chaturvedi, 2003). Since these combinations did not produce
100 any significant effect or long lasting effect, it was suggested that it is not the effect of
101 either serotonin or dopamine alone, but actually the interval between the
102 administrations of two drugs is important to mimic the seasonal gonadal condition. It
103 was presumed and later on also proved experimentally that, these timed injection of
104 serotonin and dopamine precursor drugs given for a period of 11-13 days may entrain
105 circadian serotonergic and dopaminergic oscillation respectively. Moreover, the two
106 precursor drugs given at different time interval will induce different phase relationship
107 or phase angle between the two oscillations and different physiology (Yadav and
108 Chaturvedi, 2014, 2015). Obviously, in nature also during sexually active and inactive
109 condition these phase relations vary the underlying basis of our hypothesis and all the
110 experimental studies in this direction (Wilson and Meier, 1987, 1988, 1989; Tiwari and
111 Chaturvedi, 2003; Tiwari et al., 2006). In view of fact that neurotransmitters serotonins
112 and dopamine do not cross the blood brain barrier but their precursor do cross
113 (Bianchine, 1980) in all these studies 5-HTP and L-DOPA were used as the precursor
114 of serotonin and dopamine respectively instead of neurotransmitter itself.

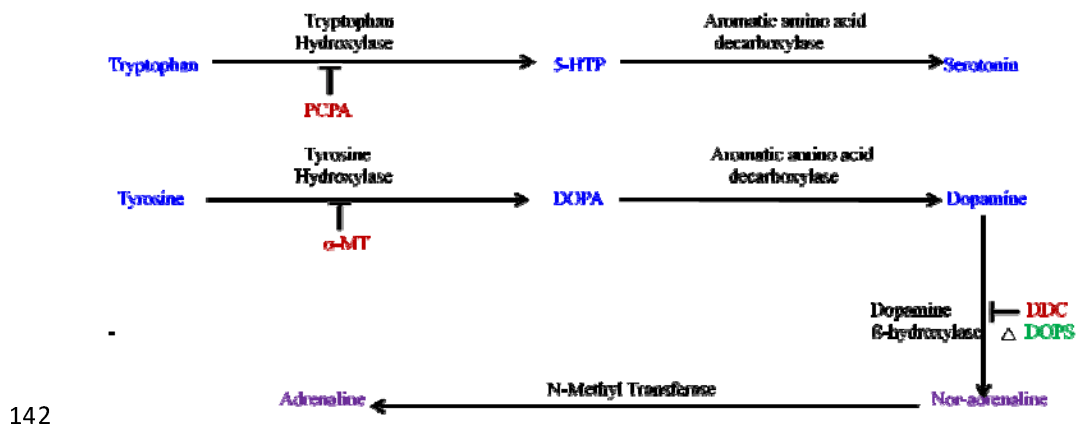
115 In case of Spotted munia, *Lonchura punctulata*, it has been proved that these
116 effects are due to temporal phase relationship of circadian neural oscillations and not
117 due to serotonin and dopamine alone (Prasad and Chaturvedi, 2003). L-DOPA not only
118 gets converted into dopamine in the presence of enzyme tyrosine hydroxylase but the
119 next biosynthetic product is the noradrenaline (by enzyme dopamine hydroxylase) and
120 adrenaline (by enzyme N-methyl transferase) respectively. Further, tryptophan
121 converts into 5-HTP (by tryptophan hydroxylase) and 5-HTP gets converted into
122 serotonin (by enzyme aromatic amino acid decarboxylase) and next product is
123 melatonin in certain tissues. This experiment was conducted to confirm that effects
124 observed in the earlier studied are due to conversion of 5-HTP into serotonin and that of
125 L-DOPA into dopamine only and not their next biosynthetic products. Hence agonist
126 and antagonists of different enzymes were used an addition to 5-HTP and L-DOPA
127 given at specific time interval to study the reproductive response of Japanese quail. The
128 biosynthetic pathway of neurotransmitter serotonin and dopamine, conversion of
129 dopamine into other catecholamines and agonists and antagonists of these biosynthetic
130 products are described below in details along with the line diagram.

131 1. PCPA (p-chlorophenylalanine), a long lasting inhibitor of serotonin synthesis (Koe
132 and Weiseman, 1966). It blocks the conversion of tryptophan into 5-HTP by
133 inactivating the enzyme tryptophan hydroxylase. It does not affects the conversion of 5-
134 HTP into serotonin.

135 2. DDC (Diethyldithiocarbamate), which inhibits the conversion of Dopamine to nor-
136 adrenaline by inducing negative effect on the enzyme Dopamine β -hydroxylase,
137 essential for this biosynthesis (Crevelling et al., 1968).

138 3. α -MT (Methyl-p-tyrosine). An inhibitor for the conversion of tyrosine to DOPA by
139 affecting the enzyme tyrosine hydroxylase.

140 4. DOPS (Dihydroxyphenylserine), a specific precursor for noradrenaline. So it
141 enhances the synthesis of noradrenaline.



142
143 The specific aim of the present study was to prove that stimulation (due to 12 hr
144 phase relation) or regression (due to 8 hr phase relation) of gonadal development is due
145 to specific temporal phase relation of serotonergic and dopaminergic activities or
146 oscillations and not due to i) serotonin/5-HTP or dopamine/L-DOPA alone and ii) that
147 L-DOPA was effective when converted into dopamine and not into noradrenaline or
148 adrenaline. For the above mentioned objectives, some agonist/antagonists of serotonin
149 (PCPA) and Dopamine (α -MT, DDC, DOPS) were used in combination with these
150 neurotransmitters (serotonin and dopamine) precursors (5-HTP and L-DOPA).

151 **Materials and methods**

152 Three week old male Japanese quail purchased from Chuck Gazaria farm,
153 Lucknow, were divided into 7 groups each having 8 birds.

154

- 155 I (Saline control/S:S)- Receiving normal saline (0.9%) twice a day at 8
156 am and 4pm i.e. at the interval of 8 hrs.
- 157 II (HTP control/5-HTP:S)- 5-HTP was administered at 8 am and saline at 4
158 pm i.e.at the interval of 8 hrs.
- 159 III (DOPA control/S:L-DOPA)- Saline was injected at 8 am and L-DOPA at 4 pm
160 i.e.at the interval of 8 hrs.
- 161 IV (HTP:DOPA)- 5-HTP and L-DOPA injections were given 8 hr
162 apart.
- 163 V (HTP+PCPA:DOPA) - 5- HTP and PCPA were injected simultaneously at
164 8 am, and L-DOPA was injected after 8 hrs.
- 165 VI (HTP:DOPA+DDC)- 5-HTP was injected at 8 am and 8 hrs later, L-
166 DOPA and DDC were injected simultaneously.
- 167 VII (HTP: α -MT+DOPS)- 5-HTP was injected at 8 am and 8 hrs later, α -MT
168 and DOPS were injected simultaneously at 4 pm.

169 All the above injections were given intraperitoneally in 100 μ l saline at 8 am
170 and 4 pm so as to establish 8-hr interval/phase relationship between the two injections
171 or set of injections. The dose of 5-HTP and L-DOPA was 5mg/100g body weight per
172 day for 13 days. The doses of antagonists were as follows- PCPA (2mg/100 g body wt.)
173 DDC (10 mg/100g body wt.), α -MT (1 mg/100g body wt.) and agonist of noradrenaline
174 DOPS was (10mg/100 g body wt.). During the treatment of 13 days, all the birds were
175 kept in dim continuous light (2 lux) and after the treatment period, birds of all the
176 groups were transferred to long day condition (LD 16:8).

177 The length and width of the cloacal gland was measured *in situ* with dial
178 calipers weekly; before, during and after the treatment upto the termination of study and
179 cloacal gland volume was calculated in cm^3 (Jaiwal and Chaturvedi, 1991; Chaturvedi

180 *et al.*, 1993). At the terminations of study i.e. 10 weeks post treatment (at the age of 15
181 weeks), blood was collected from the alar vein/wing vein into a heparinized tube and
182 centrifuged at 4000 rpm for 20 min at 4°C to separate plasma and stored at -20° for
183 hormonal assays to be performed later. Plasma testosterone level was measured using
184 EIA kit (DSI s.r.l., Italy) following manufacturer's protocol. The antiserum used in the
185 assay was 100% specific for testosterone (cross reactivity/specificity with testosterone
186 was 100%); the cross reactivity of the assay was 0.056% with progesterone, 0.004%
187 with cortisol, 0.005% with estradiol, 4.8% with dihydrotestosterone, 3.6% with
188 androstenedione, 0.048% with androsterone, 0.004% with cortisone, 0.002% with
189 estriol and 0.007% with estrone. The analytical sensitivity of the assay was 0.0576
190 ng/ml. The intra-assay coefficient of variation (CV) is 5.6% whereas inter-assay CV is
191 7.1%. Accuracy for this assay was 99%. Thereafter, birds from each group were
192 weighed, deeply anaesthetized with thiopentone and then dissected to collect tissues so
193 as to process for measuring testicular volume (cm³) and for calculating gonadosomatic
194 index-GSI (in gram testes/100 gram body weight). Left testis of each quail after
195 measuring its length and width *in situ* for calculating testicular volume was fixed in
196 Zamboni's solution and processed for routine histological study and measurement of
197 seminiferous tubule diameter. For histology, twenty-four hours after fixation, the testes
198 were dehydrated in an ascending series of alcohol, treated with xylene and then
199 embedded in paraffin wax. The 6-µm thick sections were cut by a Weswox rotary
200 microtome (Western Electric and Scientific Works, Ambala Cantt, India), and stained
201 with hematoxylin-eosin. Histological sections were viewed under a microscope
202 (Axioskop 2 Plus; Carl Zeiss AG, Oberkochen, Germany) and images were captured
203 with a digital camera. The diameter of the seminiferous tubules was determined in 10
204 sections from each testis by using the oculometer and micrometer.

205 All the numerical data (cloacal gland and testicular volume, GSI, seminiferous
206 tubule diameter and plasma testosterone concentration) were analyzed by one-way
207 analysis of variance (ANOVA), followed by post hoc Dunnett test for the comparison
208 of group means. Significance was calculated at the level of $p < 0.05$.

209 **Results**

210 Cloacal gland volume of quail of all the groups maintained under LD 16:8
211 remained suppressed during the period of treatment (until 5 weeks of age). Thereafter a
212 sharp increase until 9 weeks of age followed by maintenance of plateau at increased
213 level was observed in saline: saline (S:S) i.e. saline control, HTP: saline i.e. HTP
214 control, saline: DOPA i.e. DOPA control and HTP: α -MT+DOPS group quail.
215 However, those of HTP:DOPA, HTP+PCPA:DOPA and HTP:DOPA+DDC remained
216 at significantly low level throughout the period of study compare to control
217 (saline:saline) (Fig. 1). At the termination of study, testicular volume, GSI and plasma
218 testosterone level of HTP:DOPA, HTP+PCPA:DOPA and HTP:DOPA+DDC group
219 quail were significantly lower in comparison to saline control (S:S) but these
220 parameters of other 3 groups were not different from saline control (Fig. 2, 3 and 4).

221 Histologically, the transverse section of testis of HTP:DOPA, HTP+PCPA:L-
222 DOPA and HTP:DOPA+DDC 8-hr quail group had smaller seminiferous tubules (Fig.
223 5) with decreased spermatogenesis along with vacuolation in HTP:DOPA and
224 HTP:DOPA+DDC quail and atrophic changes with complete degeneration of
225 spermatogenic activity was noted in HTP+PCPA:DOPA quail testis unlike full
226 breeding condition in control (S:S). The interstitial spaces of the seminiferous tubules
227 of these quail testes were reduced and no Leydig cells were evident in triangular spaces.
228 Further, similar to the enlarged seminiferous tubules of saline control quail testis having
229 all the stages of spermatogenesis and bunches of spermatozoa in the lumen, the testis of

230 HTP:S, S:DOPA and HTP: α -MT+DOPS also exhibited full breeding condition (Fig. 5
231 and 6).

232

233 **Discussion**

234

235 Intraperitoneal injections of 5-HTP and L-DOPA (HTP:DOPA) given 8 hrs
236 apart i.e. 8-hr phase relation of serotonergic and dopaminergic oscillations induced by
237 their precursor injections suppressed gonadal activity significantly compared to saline
238 treated control (S:S). On the other hand, reproductive parameters of quail receiving
239 saline 8 hrs after 5-HTP (HTP:S) or 8 hrs before DOPA (S:DOPA) serving as HTP
240 control and DOPA control respectively, did not differ from saline control (S:S). This
241 indicates that suppressive effect of 5-HTP and L-DOPA when given 8 hrs apart (8-hr
242 relation) was neither due to 5-HTP nor L-DOPA alone but is actually the outcome of
243 the interval between the administration of serotonergic and dopaminergic precursor
244 drugs inducing 8-hr temporal relationship between the two neural oscillations. In
245 another group of birds, 5-HTP and PCPA was injected simultaneously followed by L-
246 DOPA injection at interval 8 hr. PCPA blocks the conversion of tryptophan into 5-HTP
247 by inactivating the enzyme tryptophan hydroxylase but it does not affect the conversion
248 of 5-HTP into serotonin. Hence exogenous administration of PCPA blocked the
249 conversion of endogenous tryptophan into 5-HTP, but exogenous 5-HTP was still
250 converted into serotonin. Hence in fact, theoretically 8-hr phase relation of HTP:
251 DOPA should not be different from HTP+PCPA:DOPA and accordingly gonadal
252 response of these two groups are also similar i.e. gonado-suppressive.

253 In case of HTP:DOPA+DDC quail also, gonadal suppression was observed
254 similar to those quail receiving only two drugs HTP:DOPA 8 hrs apart. The injection of
255 DOPA (dopamine precursor) increases the synthesis of dopamine (DA) and enzyme

256 dopamine β -hydroxylase (DBH) converts DA into noradrenaline (NA). The drug DDC
257 selectively inhibits NA synthesis because it inhibits the enzyme DBH therefore
258 inhibiting the synthesis of NA. Hence simultaneous injection of DOPA and DDC is
259 expected to stimulate dopamine synthesis but inhibits synthesis of NA. Obviously effect
260 of this combination of drugs (HTP:DOPA+DDC) was also similar to that of HTP:
261 DOPA combination indicating that inhibitory effect of 8-hr temporal relation of
262 precursor drugs was only due to their conversion into serotonin and dopamine and not
263 any further conversion into noradrenaline.

264 In the HTP: α -MT+DOPS group, quail received α -MT and DOPS simultaneously
265 8 hrs after 5-HTP administration and the effect was not different from control. The drug
266 α -MT inhibits the conversion of the amino acid tyrosine to DOPA, whereas the drug
267 DOPS is a specific precursor for NA. Hence, simultaneous injections these two drugs
268 (α -MT i.e. antagonist of DOPA synthesis and DOPS i.e. agonist of NA) should
269 selectively inhibit synthesis of DOPA (and hence also the dopamine synthesis) and
270 increase NA synthesis respectively. Because in this treatment of HTP: α -MT+DOPS,
271 there is no exogenous DOPA (precursor of dopamine) and internal source of DOPA is
272 also blocked by α -MT, the DA synthesis is expected to be blocked completely but NA
273 is still available synthesized from its selective precursor DOPS. Although natural
274 precursor of adrenaline/NA i.e. dopamine is not available (as endogenous dopamine
275 synthesis have been blocked by α -MT, and exogenous DOPA is not available), but NA
276 will be still available because of injecting its selective precursor DOPS. Thus this
277 combination of drugs is equivalent to 8-hr relationship between 5-HTP/serotonin and
278 NA (noradrenaline) and not the 5-HTP/ serotonin and L-DOPA /dopamine. But, in
279 terms of gonadal response, effect is similar to HTP control which in turn is similar to
280 saline control.

281 These various combinations of serotonergic and nor adrenergic drugs instead of
282 classical serotonin: dopamine combination indicates that reproductive effect of specific
283 phase relation of neural oscillations occurs due to serotonin and dopamine; and not due
284 to serotonin and noradrenaline (Fig. 1 and 6). These experimental findings following
285 the use of various agonists and antagonist of monoamine (serotonin) and catecholamine
286 (dopamine and noradrenaline) suggest that reproductive effect of 5-HTP and L- DOPA
287 given 8 hrs apart apparently result from their conversion into serotonin and dopamine
288 and not due to other monoamine/catecholamine. Selective potentiation of dopaminergic
289 activity by DOPA and DDC injections did suppress reproductive development whereas
290 specific inhibition of dopaminergic activity by α -MT and stimulation of noradrenaline
291 by DOPS injection did not. Therefore, HTP/ /serotonergic or L-DOPA/dopaminergic
292 activity independently is unable to induce any change in gonadal activity which is
293 actually the effect/function of interval between the administration of neurotransmitter
294 precursor drugs 5-HTP and L- DOPA. Moreover, it is not the dose of neurotransmitter
295 precursor drugs (5-HTP and L-DOPA) that cause the effect, instead it is the specific
296 time interval between the drug administrations, otherwise the effects could have been
297 similar in all cases and not the one observed in all the reports consistently. Invariably in
298 many species, 8-hr phase relation is gonado-suppressive, 12-hr is gonado-stimulatory
299 and other relations are ineffective (Chaturvedi and Bhatt, 1990; Chaturvedi and Yadav,
300 2013).

301 Based on these findings it is restated that effects observed in the experiments of
302 present study, are due to specific phase relation of serotonergic and dopaminergic
303 oscillations induced by systemic injections of 5-HTP and L- DOPA given at different
304 interval. Present findings also suggest that the observed effect are due to conversion of
305 DOPA into dopamine and not into the next biosynthetic product (NA).

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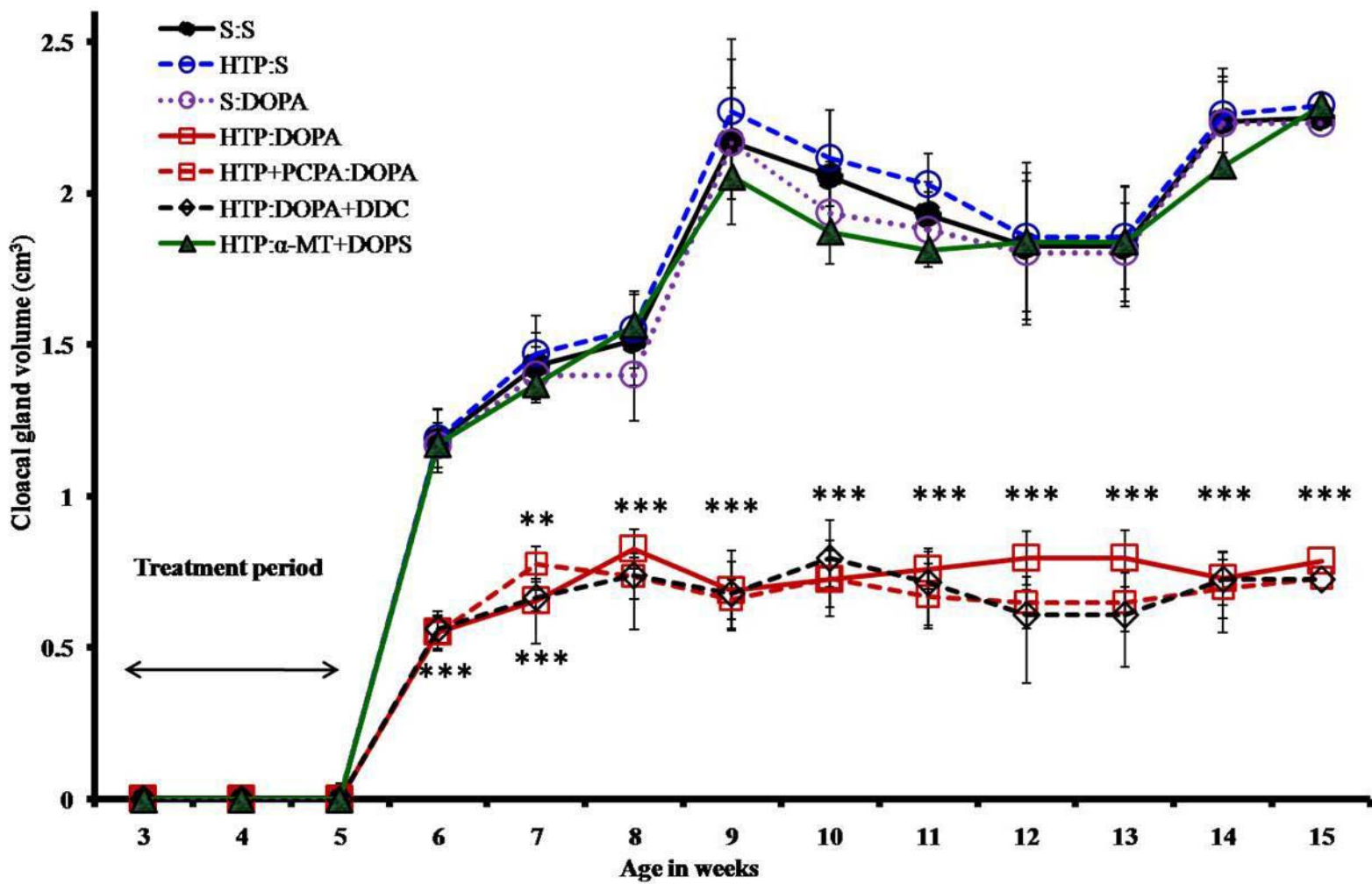


Fig. 1. Cloacal gland responses of Japanese quail receiving 5-HTP and L-DOPA in combination with saline and agonist and/or antagonist of serotonin and catecholamines 8 hrs apart.

Data is presented as means \pm SEM. **p<0.01, ***p<0.001, Significance of difference from the control group.

Group details:

- | | |
|------------------------|--|
| Saline control/S:S | - Received normal saline (0.9%) twice a day at 8am and 4pm i.e. at the interval of 8 hrs. |
| HTP control/HTP:S | - 5-HTP was administered at 8 am and saline at 4 pm i.e. at the interval of 8 hrs. |
| DOPA control/S:DOPA | - Saline was injected at 8 am and L-DOPA at 4 pm i.e. at the interval of 8 hrs. |
| HTP:DOPA | -5-HTP and L-DOPA injections were given 8 hr apart. |
| HTP+PCPA:DOPA | -received 5- HTP and PCPA simultaneously at 8 am, and L-DOPA was injected after 8 hrs. |
| HTP:DOPA+DDC | -5-HTP was injected at 8 am and 8 hrs later, L-DOPA and DDC were injected simultaneously. |
| HTP: α -MT+DOPS | -5-HTP was injected at 8 am and 8 hrs later, α -MT and DOPS were injected simultaneously at 4 pm. |

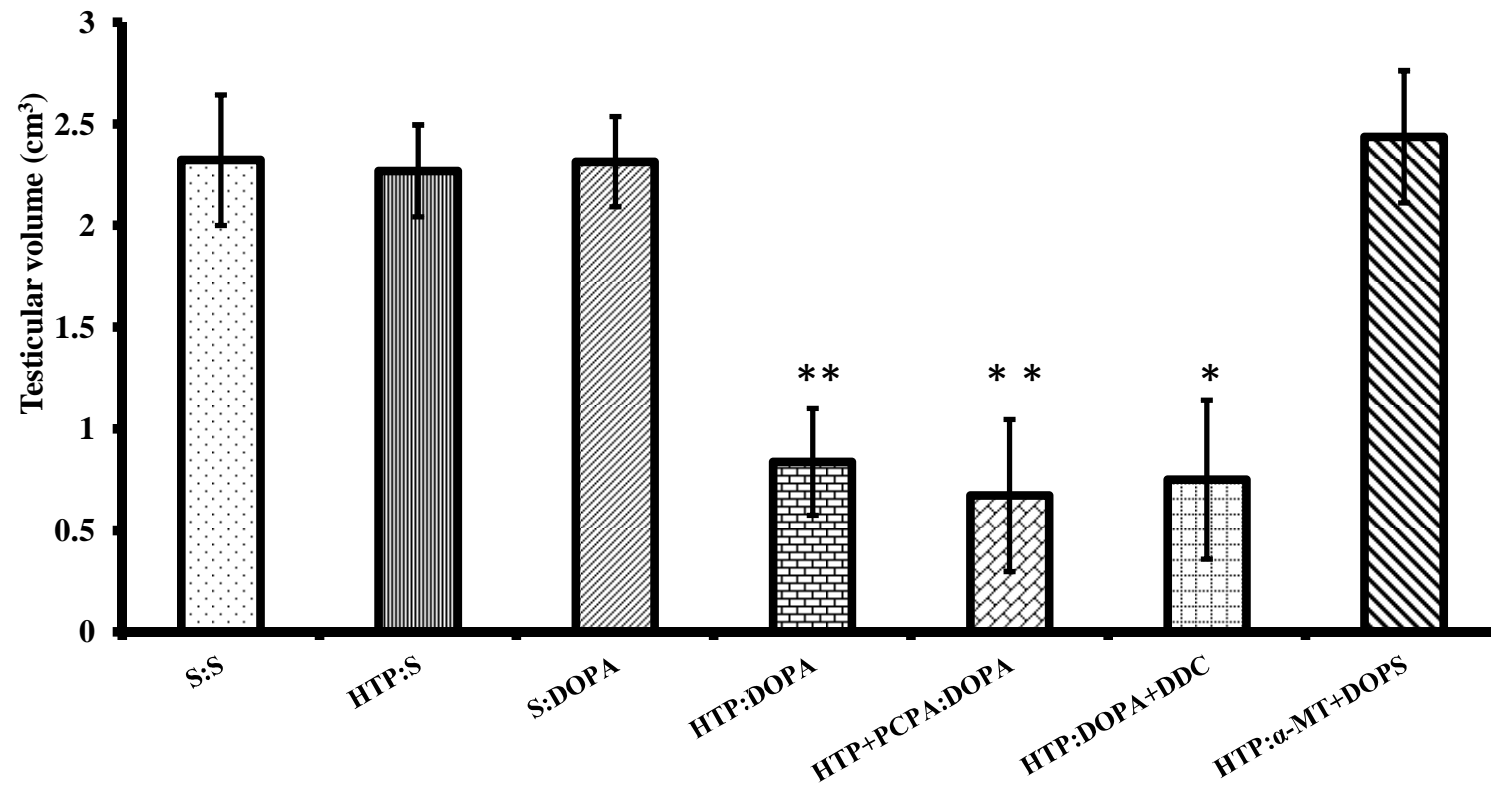


Fig. 2. Testicular volume of Japanese quail receiving 5-HTP and L-DOPA in combination with saline and agonist and/or antagonist of serotonin and catecholamine 8 hrs apart. For group details, see Fig. 1. Data is presented as mean \pm SEM. * p <0.05, ** p <0.01; Significance of difference from the control (S:S) group.

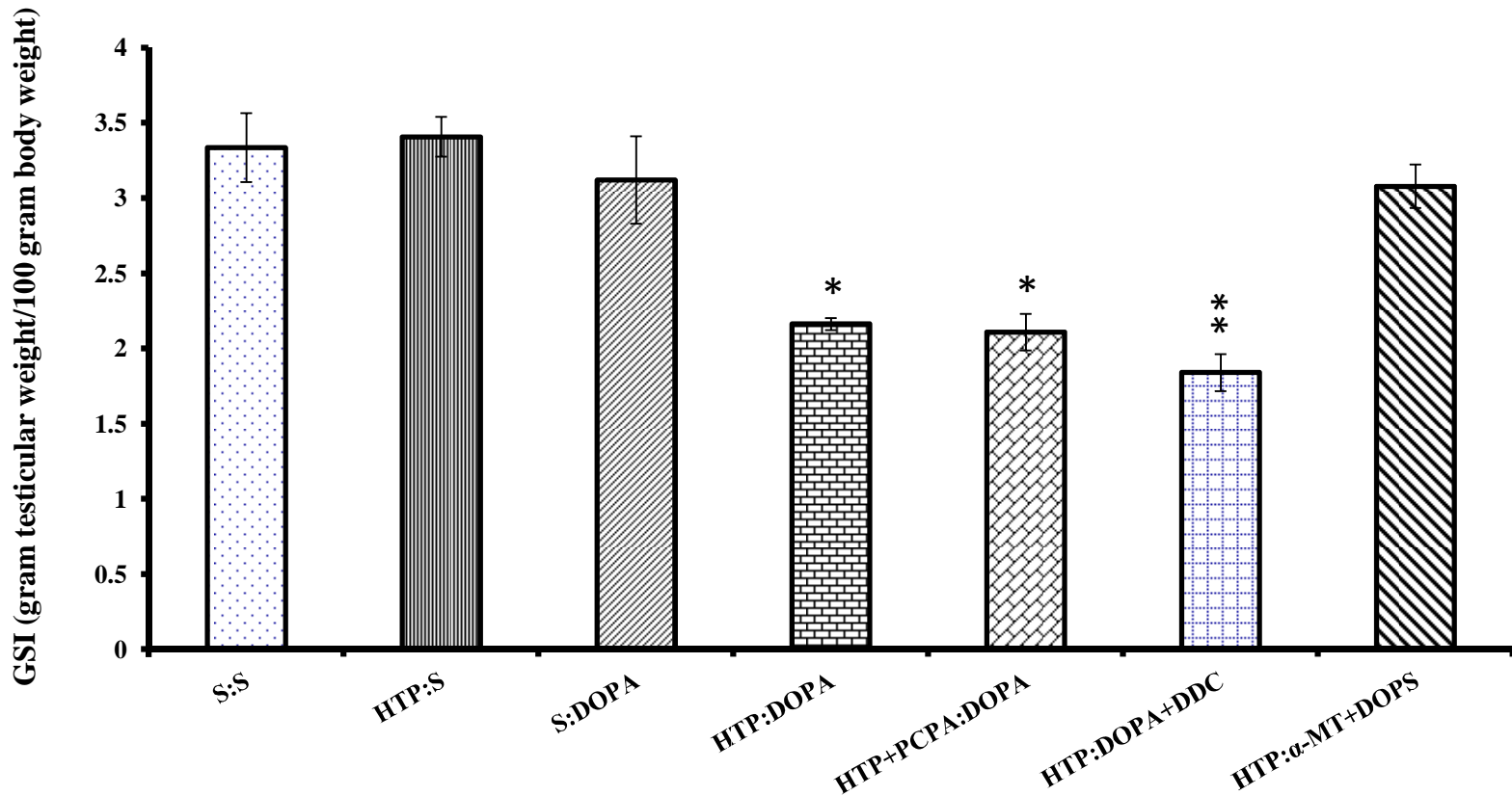


Fig. 3. GSI (Gonadosomatic index) of Japanese quail receiving 5-HTP and L-DOPA in combination with saline and agonist and/or antagonist of serotonin and catecholamine 8 hrs apart. For group details, see Fig. 1.

Data is presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$; significance of difference from the control group

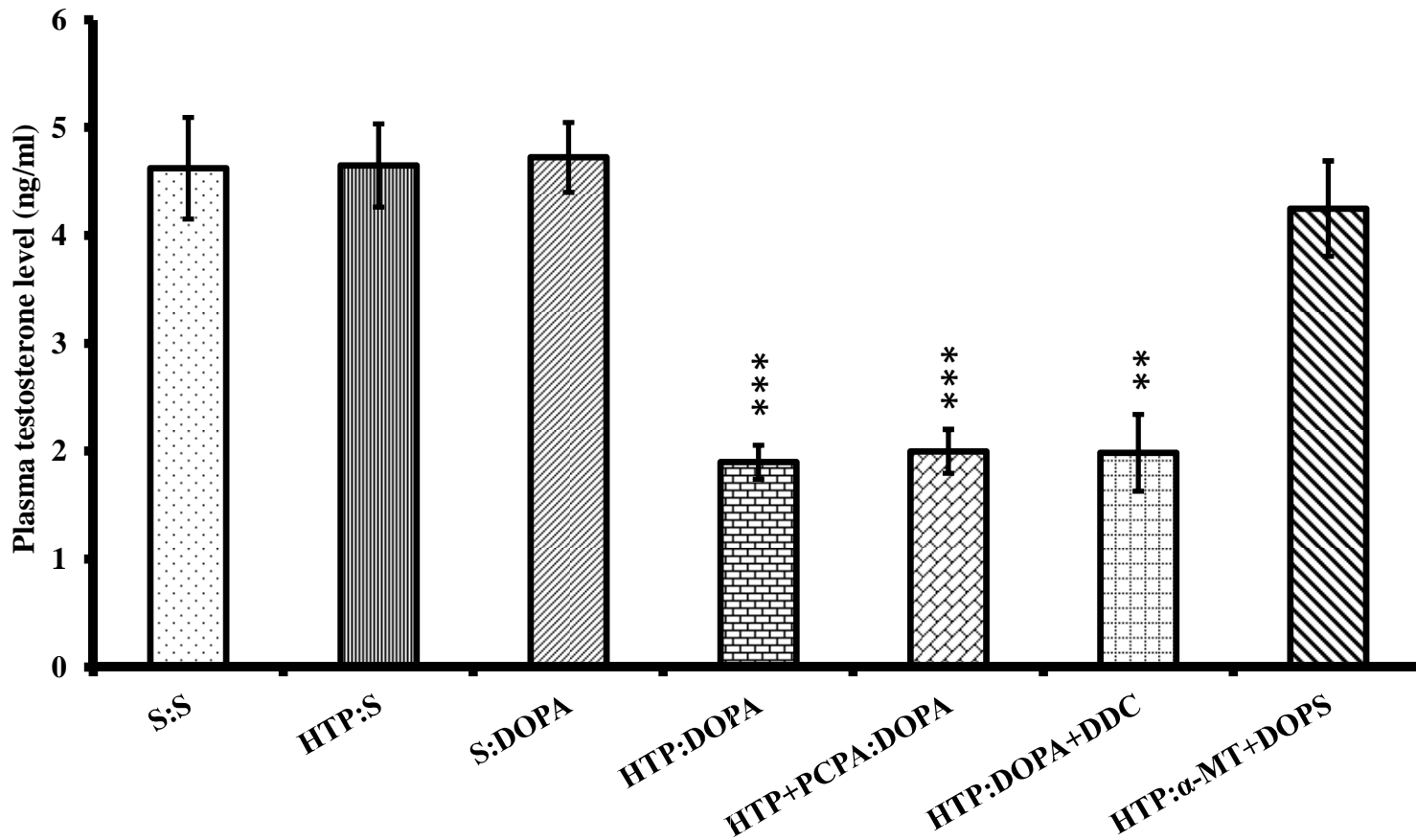


Fig. 4. Plasma testosterone level of Japanese quail receiving 5-HTP and L-DOPA in combination with saline and agonist and/or antagonist of serotonin and catecholamine 8 hrs apart. ** $p < 0.01$, *** $p < 0.001$; Significance of difference from the control group.

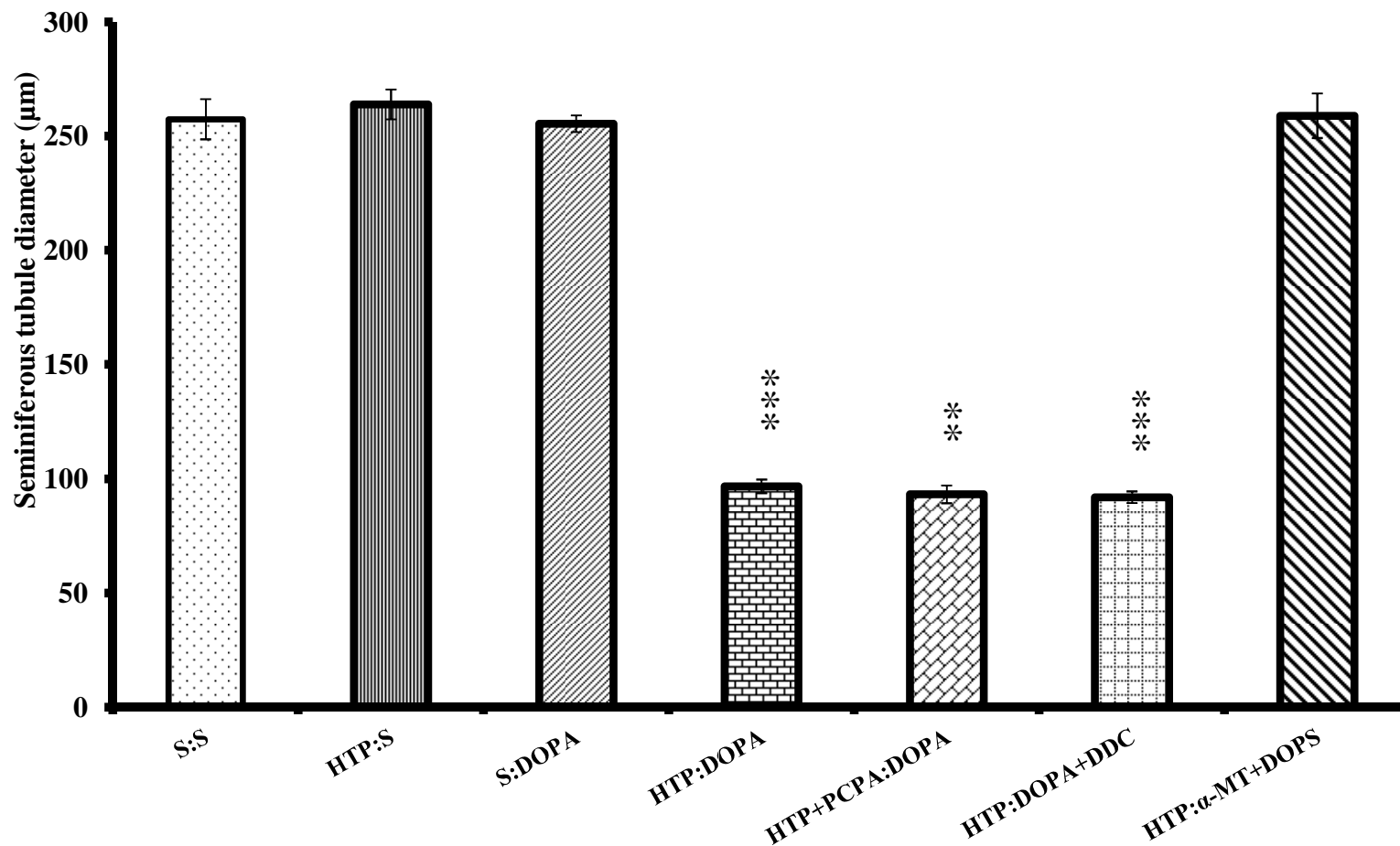


Fig. 5. Seminiferous tubule diameter of Japanese quail receiving 5-HTP and L-DOPA in combination with saline and agonist and/or antagonist of serotonin and catecholamine 8 hrs apart. ** $p < 0.01$, *** $p < 0.001$; Significance of difference from the control group.

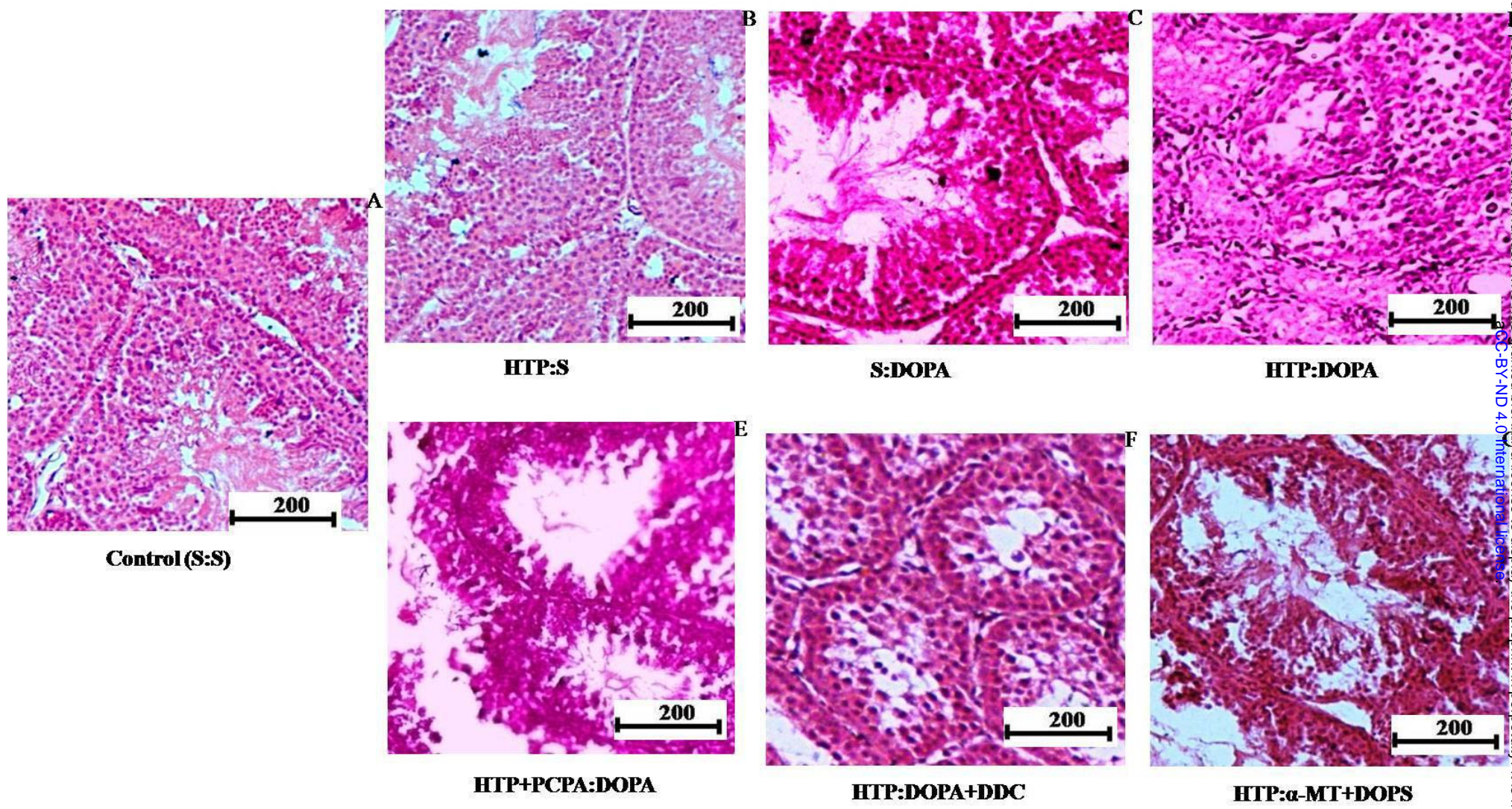


Fig. 6. T.S. of testis of Japanese quail receiving 5-HTP and L-DOPA in combination with saline and agonist and/or antagonist of serotonin and catecholamines 8 hrs apart.

- A. Control (S:S)- Note full breeding condition of testis having enlarged seminiferous tubules with all the stages of spermatogenesis and spermatozoa in the lumen.
- B. HTP control (HTP:S)- showing full breeding condition as in S:S quail testis.
- C. DOPA control (S:DOPA)- showing full breeding condition as in S:S quail testis.
- D. HTP:DOPA- Note non breeding condition with smaller seminiferous tubules containing only inactive spermatogonial cells and some vacuolation and debris in the lumen of few tubules
- E. HTP+PCPA:DOPA- Note smaller seminiferous tubules with suppression of stages of spermatogenic activity and empty lumen or some debris in lumen .
- F. HTP:DOPA+DDC- Note non-breeding and spermatogenetically inactive condition having smaller seminiferous tubules containing inactive spermatogonial cells and vacuolation.
- G. HTP: α -MT+DOPA group receiving 5-HTP and α -MT +DOPA at the interval of 8 hr. This section shows normal breeding condition like control.