

**From:** Dr. K. Dabrowski and Dr. R.E.Ciereszko  
School of Natural Resources,  
Dr. J.S. Ottobre  
Department of Animal Sciences  
Ohio State University, Columbus, OH 43210

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**To:** Dr. J.L. Busch  
Lake Erie Protection Fund  
Toledo, OH 43604

### **Final report**

**Project title:** Effect of organochlorine xenobiotics on ovarian steroidogenesis in yellow perch.

**Small Grant:** AGRMNT DTDF 10/2/95

**Period reported:** from 10/2/1995 to 10/2/1996

**1. Experiment 1:** The effect of organochlorine xenobiotics on ovarian steroidogenesis during the postvitellogenic phase of oocyte development

This experiment has been designed to address the objective 1 b (see project goals).

Postvitellogenic ovaries and blood plasma were collected (April, n=3) from two year old female yellow perch, maintained in the Piketon Research and Extension Center. GSI has been estimated. Ovarian tissue (100mg) was cultured in 1 ml of Cortland salt solution at 15°C for 24 h in the presence or absence of human chorionic gonadotropin

(hCG, 10 IU, Sigma). In addition to hCG, the effect of four doses (0.01, 0.1, 1, 10 ppm) of DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane), PCBP (3,3',4,4',5-pentachlorobiphenyl), and HCBP (3,3',4,4',5,5'-hexachlorobiphenyl) on steroid production *in vitro* was examined. Toxicants were dissolved in DMSO (dimethyl sulfoxide). DMSO (<1% in total incubation volume) did not affect cultured tissue. Testosterone (T) and estradiol 17- $\beta$  (E) concentrations in media and blood plasma were determined by radioimmunoassay (RIA) procedures (Ottobre et al., Biol.Reprod. 1989, 41: 393-400; Dabrowski et al., Fish Physiol. Biochem., 1995, ).

Table 1 presents the mean weight, GSI, plasma concentrations of E and T and diameter of oocyte of the fish at the time of sampling. Figures 1-3 show the mean concentrations of E and T in the control and hCG-treated ovarian follicles *in vitro* exposed to DDT, PCBP and HCBP *in vitro* during the postvitellogenic phase of ovarian development.

Neither hCG nor the toxicants affected estradiol concentrations in the media. Basal production of T was not affected by any of the xenobiotics. hCG stimulated T production and the hCG-stimulated production of T was inhibited by three doses of HCBP and one dose of PCBP.

**2. Experiment 2:** The effect of organochlorine xenobiotics on ovarian steroidogenesis during the vitellogenic phase of oocyte of development.

This experiment has been designed to address the objective 1a (see project goals).

At this time two year old females during the vitellogenic phase of ovarian development (November) were used (n=5; PCBP experiment: n=4). The entire experiment was performed as described above (Experiment 1). Briefly, blood plasma was collected and stored at -20°C. Ovaries were dissected and weighed. Ovarian slices (100 mg)

were incubated in Cortland salt solution at 15°C for 24 h. hCG (10 IU) and the four doses (0.01, 0.1, 1, 10 ppm) of the following treatments were tested: DDT, PCBP, HCBP, TriCBP (trichlorobiphenyl), and Tri(OH)CBP (trichlorobiphenylol). T and E concentrations in media and blood plasma were determined by RIA.

Tables 2 (DDT, PCBP and HCBP studies) and 3 (TRICBP and TRIOHCBP studies) present the mean weight, GSI, plasma concentrations of E and T and diameter of oocyte of the fish at the time of sampling. Figures 4-8 show the mean concentrations of E and T in the control and hCG-treated ovarian follicles in vitro exposed to DDT, PCBP, HCBP, TRICBP and TRIOHCBP in vitro during the vitellogenic phase of ovarian development.

Gonadotropin stimulated both estradiol and testosterone production by ovarian follicle of yellow perch collected during the vitellogenic phase of ovarian development. The basal production of T was inhibited by the highest doses of DDT and HCBP as well as by two of the PCBP doses. TRICBP stimulated basal production of T (two doses) and E (all doses). Basal E production was also stimulated by TRIOHCBP (all four doses). hCG-stimulated production of T was inhibited by DDT (all four doses), PCBP (three doses), HCBP (all four doses), TRICBP (two doses) and TRIOHCBP (one dose). DDT (three doses), PCBP (one dose), TRICBP (one dose) and TRIOHCBP (two doses) inhibited hCG-stimulated production of E.

In summary, it appears that one day incubations of perch ovaries may provide a good model for studying the mechanisms of the organochlorine xenobiotics effect on reproductive processes in yellow perch. Ovarian response to these toxicants is more pronounced during the vitellogenic phase of ovarian development.

Table 1. Examined parameters of the female yellow perch collected during the postvitellogenic phase of oocyte development.

| Parameters                    | Mean<br>$\pm$ SEM    |
|-------------------------------|----------------------|
| Fish weight (g)               | 78.7 $\pm$ 7.7       |
| GSI (%)                       | 33.8 $\pm$ 0.21      |
| Plasma E (ng/ml)              | 0.374 $\pm$ 0.0<br>4 |
| Plasma T (ng/ml)              | 21.8 $\pm$ 5.4       |
| Oocyte diameter<br>( $\mu$ m) | 1281 $\pm$ 8.1       |

Table 2. Examined parameters of the female yellow perch collected during the vitellogenic phase of oocyte development (DDT, PCBP and HCBP studies).

| Examined parameters           | Mean<br>$\pm$ SEM    |
|-------------------------------|----------------------|
| Fish weight (g)               | 60.8 $\pm$ 5.7       |
| GSI (%)                       | 7.9 $\pm$ 0.18       |
| Plasma E (ng/ml)              | 0.962 $\pm$ 0.2<br>1 |
| Plasma T (ng/ml)              | 1.16 $\pm$ 0.16      |
| Oocyte diameter<br>( $\mu$ m) | 538 $\pm$ 16.3       |

Table 3. Examined parameters of the female yellow perch collected during the vitellogenic phase of oocyte development (TRICBP and TRIOHCBP studies).

| Examined parameters        | Mean $\pm$ SEM       |
|----------------------------|----------------------|
| Fish weight (g)            | 51.2 $\pm$ 2.0       |
| GSI (%)                    | 7.3 $\pm$ 0.29       |
| Plasma E (ng/ml)           | 0.630 $\pm$ 0.0<br>6 |
| Plasma T (ng/ml)           | 0.902 $\pm$ 0.0<br>4 |
| Oocyte diameter ( $\mu$ m) | 553 $\pm$ 31.0       |

## Figure captions

Figure 1. Effect of DDT on basal and hCG-stimulated estradiol and testosterone production by ovarian follicles of yellow perch collected during the postvitellogenic phase of oocyte development. Ovarian tissue (100mg) was cultured in Cortland salt solution for 24 h at 15°C. Data are expressed as a mean  $\pm$ sem (n=3). Mean GSI was  $33.8\% \pm 0.2\%$ . \* Significantly ( $p < 0.05$ ) different from cultures without hCG.

Figure 2. Effect of PCBP on basal and hCG-stimulated estradiol and testosterone production by ovarian follicles of yellow perch collected during the postvitellogenic phase of oocyte development. Ovarian tissue (100mg) was cultured in Cortland salt solution for 24 h at 15°C. Data are expressed as a mean  $\pm$ sem (n=3). Mean GSI was  $33.8\% \pm 0.2\%$ . \* Significantly ( $p < 0.05$ ) different from cultures without hCG; <sup>b</sup> ( $p < 0.05$ ) and <sup>c</sup> ( $p < 0.06$ ) indicate means different from hCG-treated cultures without PCBP.

Figure 3. Effect of HCBP on basal and hCG-stimulated estradiol and testosterone production by ovarian follicles of yellow perch collected during the postvitellogenic phase of oocyte development. Ovarian tissue (100mg) was cultured in Cortland salt solution for 24 h at 15°C. Data are expressed as a mean  $\pm$ sem (n=3). Mean GSI was  $33.8\% \pm 0.2\%$ . \* Significantly ( $p < 0.05$ ) different from cultures without hCG; <sup>b</sup> ( $p < 0.05$ ) and <sup>c</sup> ( $p < 0.06$ ) indicate means different from hCG-treated cultures without HCBP.

Figure 4. Effect of DDT on basal and hCG-stimulated estradiol and testosterone production by ovarian follicles of yellow perch collected during the vitellogenic phase of oocyte development. Ovarian tissue (100mg) was cultured in Cortland salt solution for 24 h at 15°C. Data are expressed as a mean  $\pm$ sem (n=5). Mean GSI was  $7.9\% \pm 0.2\%$ . \*

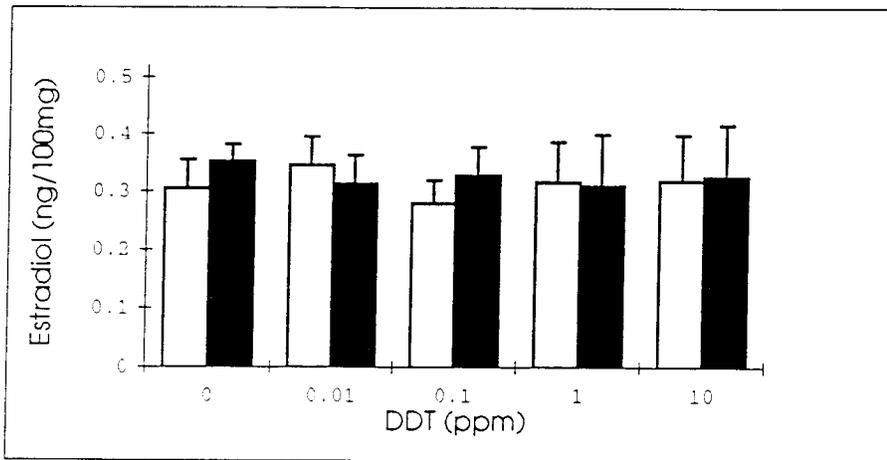
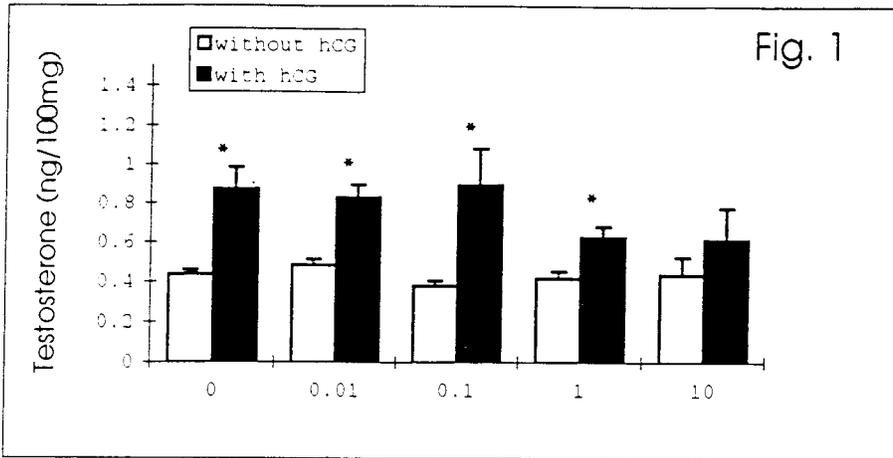
Significantly ( $p < 0.05$ ) different from cultures without hCG; <sup>a</sup> ( $p < 0.05$ ) indicate means different from cultures without DDT; <sup>b</sup> ( $p < 0.05$ ) indicate means different from hCG-treated cultures without DDT.

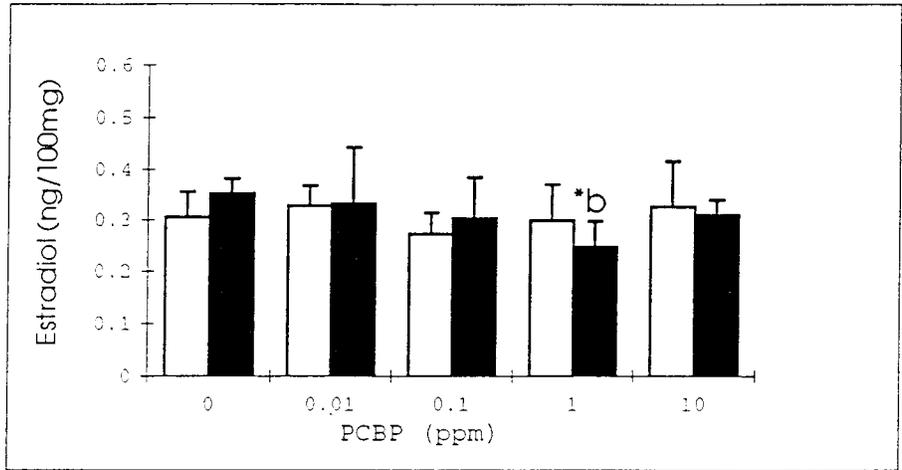
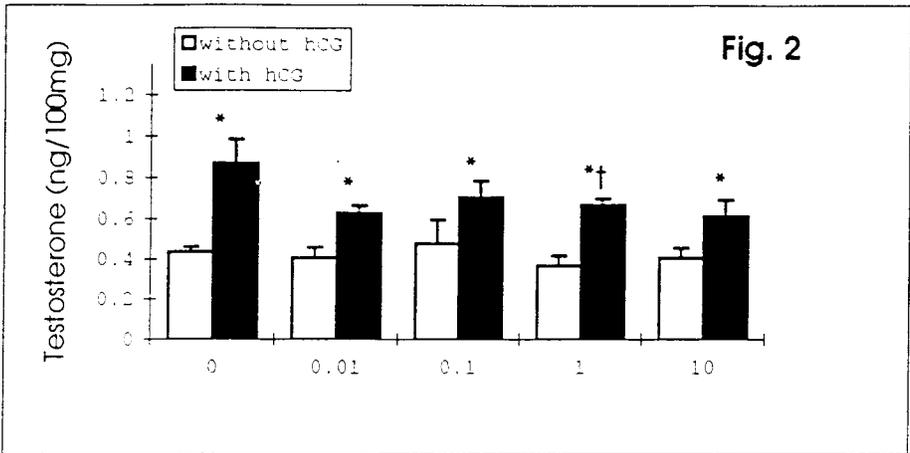
Figure 5. Effect of PCBP on basal and hCG-stimulated estradiol and testosterone production by ovarian follicles of yellow perch collected during the vitellogenic phase of oocyte development. Ovarian tissue (100mg) was cultured in Cortland salt solution for 24 h at 15°C. Data are expressed as a mean  $\pm$ sem (n=4). Mean GSI was 7.9% $\pm$ 0.2%. \* Significantly ( $p < 0.05$ ) different from cultures without hCG; <sup>a</sup> ( $p < 0.05$ ) and <sup>t</sup> ( $p < 0.06$ ) indicate means different from cultures without PCBP; <sup>b</sup> ( $p < 0.05$ ) indicates means different from hCG-treated cultures without PCBP.

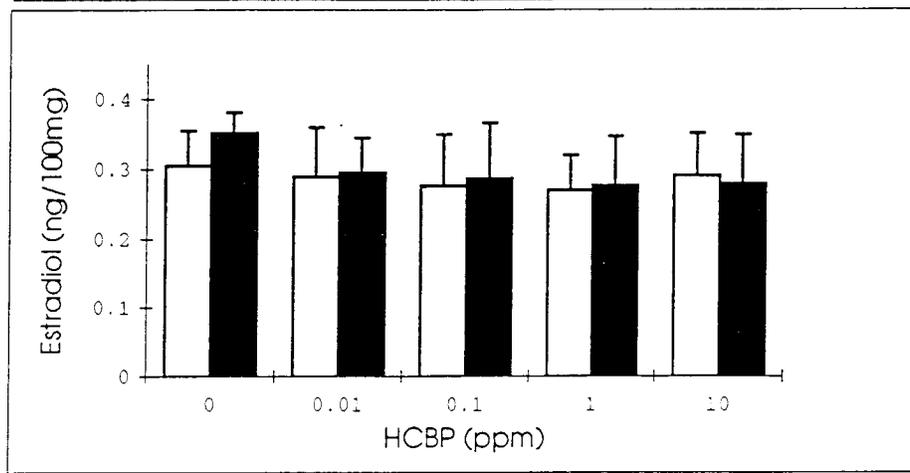
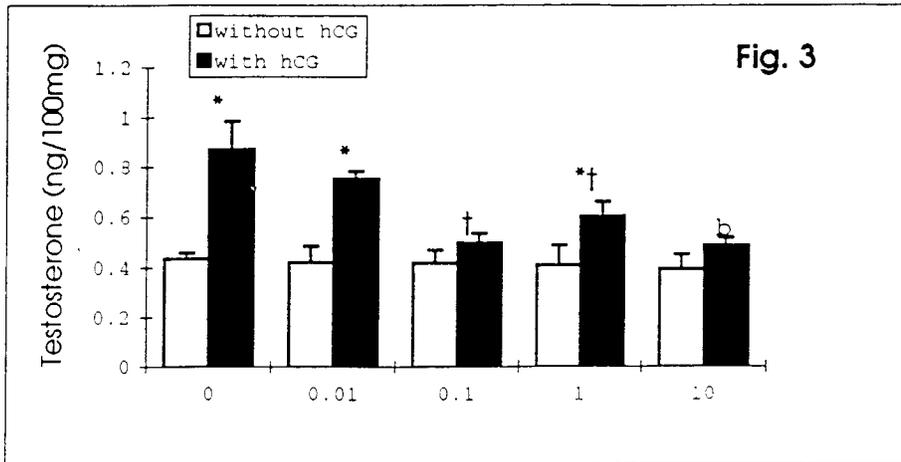
Figure 6. Effect of HCBP on basal and hCG-stimulated estradiol and testosterone production by ovarian follicles of yellow perch collected during the vitellogenic phase of oocyte development. Ovarian tissue (100mg) was cultured in Cortland salt solution for 24 h at 15°C. Data are expressed as a mean  $\pm$ sem (n=5). Mean GSI was 7.9% $\pm$ 0.2%. \* Significantly ( $p < 0.05$ ) different from cultures without hCG; <sup>a</sup> ( $p < 0.05$ ) indicates means different from cultures without HCBP; <sup>b</sup> ( $p < 0.05$ ) and <sup>t</sup> ( $p < 0.06$ ) indicate means different from hCG-treated cultures without HCBP.

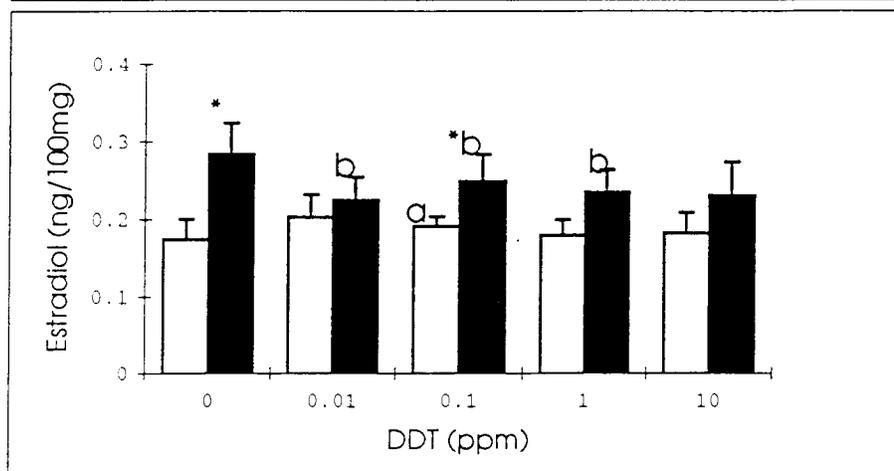
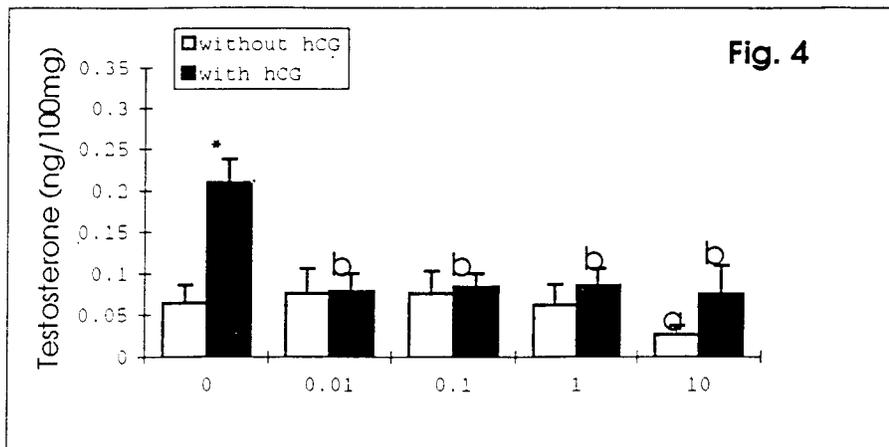
Figure 7. Effect of TRICBP on basal and hCG-stimulated estradiol and testosterone production by ovarian follicles of yellow perch collected during the vitellogenic phase of oocyte development. Ovarian tissue (100mg) was cultured in Cortland salt solution for 24 h at 15°C. Data are expressed as a mean  $\pm$ sem (n=5). Mean GSI was 7.3% $\pm$ 0.3%. \* Significantly ( $p < 0.05$ ) different from cultures without hCG; <sup>a</sup> ( $p < 0.05$ ) and <sup>s</sup> ( $p < 0.06$ ) indicate means different from cultures without TRICBP; <sup>b</sup> ( $p < 0.05$ ) and <sup>t</sup> ( $p < 0.06$ ) indicate means different from hCG-treated cultures without TRICBP.

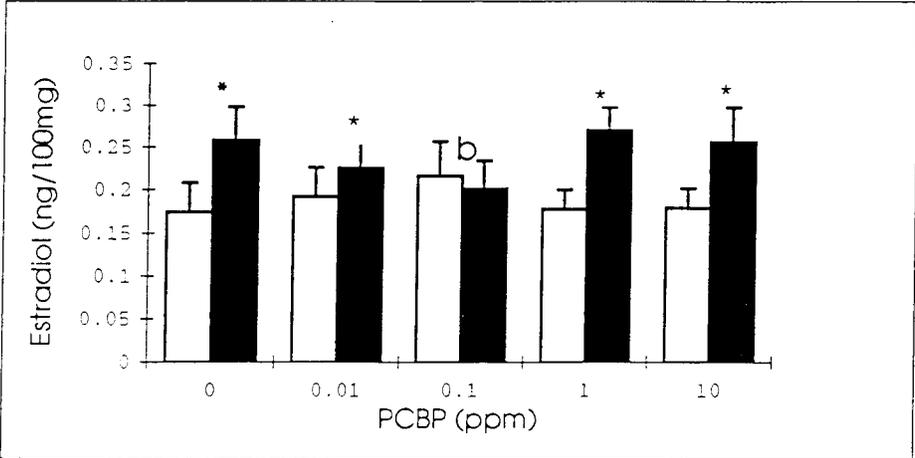
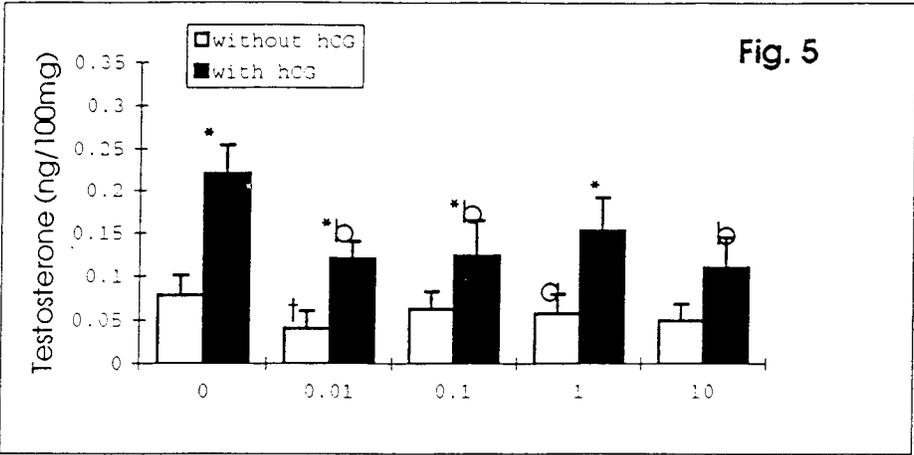
Figure 8. Effect of TRIOHCBP on basal and hCG-stimulated estradiol and testosterone production by ovarian follicles of yellow perch collected during the vitellogenic phase of oocyte development. Ovarian tissue (100mg) was cultured in Cortland salt solution for 24 h at 15°C. Data are expressed as a mean  $\pm$ sem (n=5). Mean GSI was 7.3% $\pm$ 0.3%. \* Significantly (p<0.05) different from cultures without hCG; <sup>a</sup> (p<0.05) indicates means different from cultures without TRIOHCBP; <sup>b</sup> (p<0.05) indicates means different from hCG-treated cultures without TRIOHCBP.

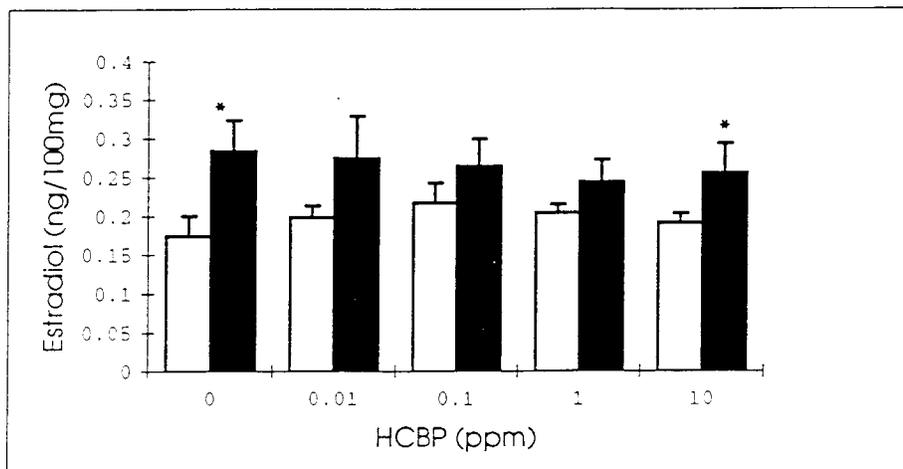
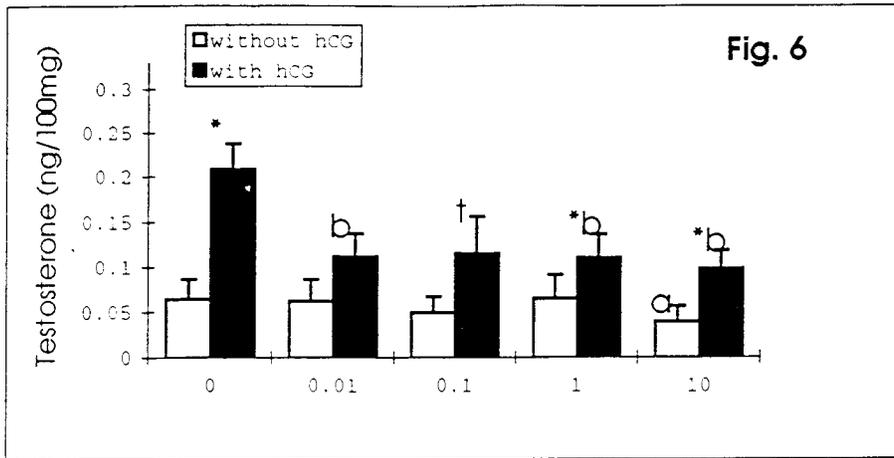












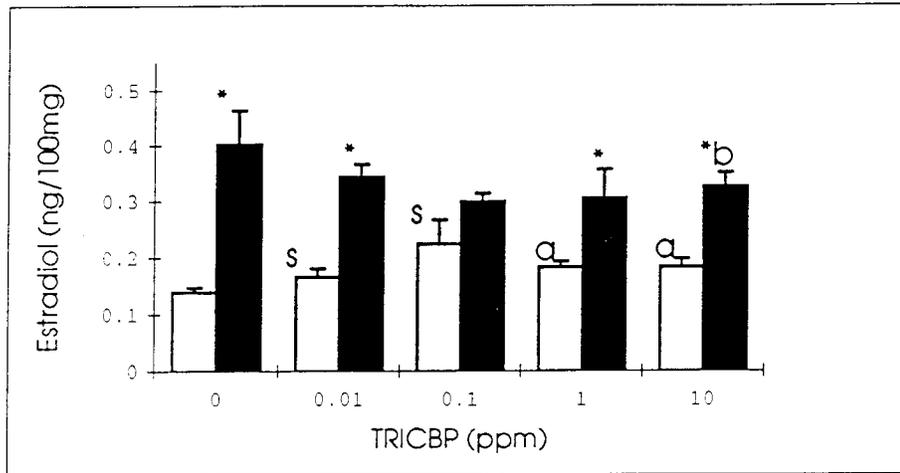
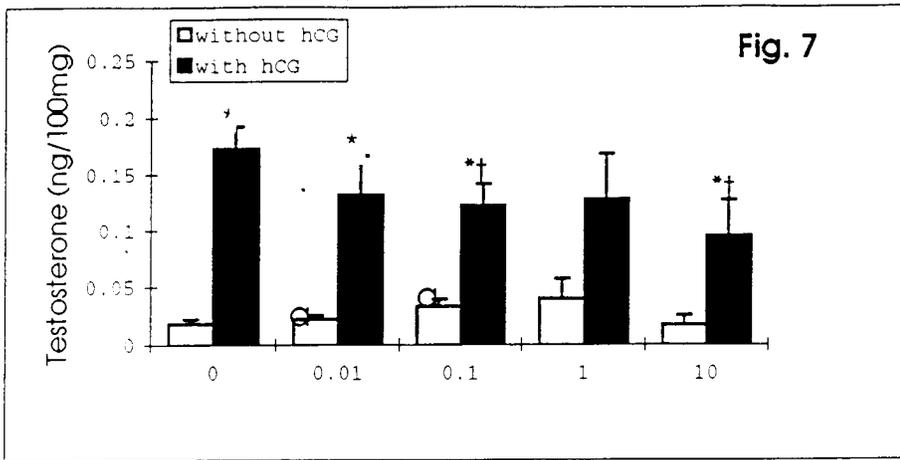


Fig. 8

