

Figure 1: HPLC-MS Chromatograms showing the disappearance of target compounds before and after ozone treatment. Initial concentrations of hormones were: EE2: 0.42 $\mu\text{g/L}$; E2: 2.22 $\mu\text{g/L}$; E1: 6.71 $\mu\text{g/L}$ and E3 : 11 $\mu\text{g/L}$. Concentration of ozone was 500 $\mu\text{g/L}$.

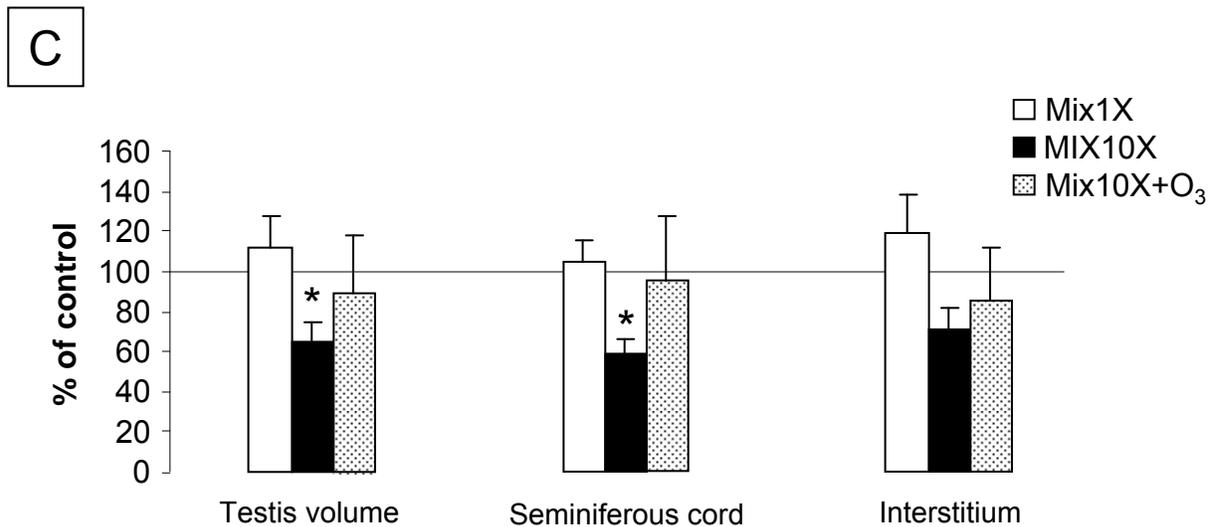
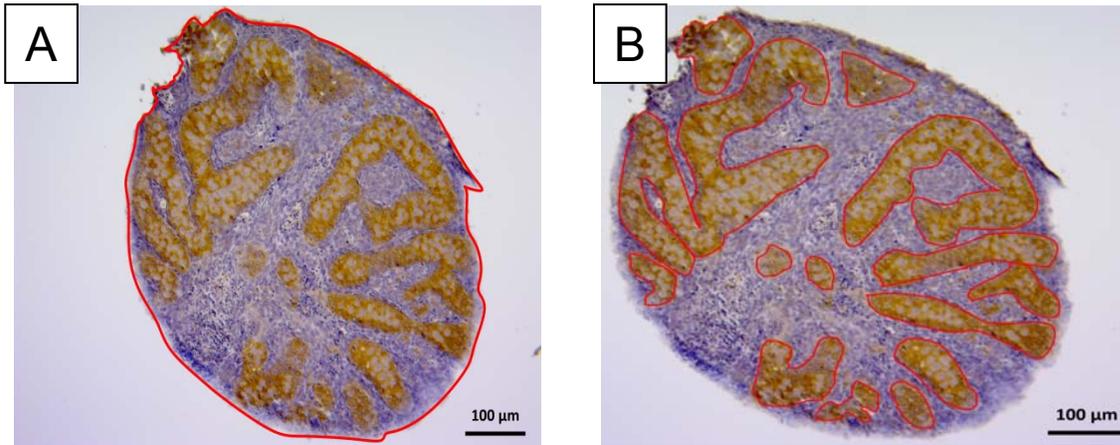


Figure 2: Effect of the MIX before or after ozonation on rat fetal testis development. Testis were explanted from 15.5-day-old rat fetuses cultured for 3 days. Cross sectional areas of the whole testis (A) and seminiferous cords (B) were measured for all sections. The interstitium volume was calculated as the difference between the total testis and the seminiferous cords. Quantitative data are expressed as % of the respective control cultures (C) and are represented as the mean \pm SEM from 3 to 4 cultures. * $p < 0.05$ in the paired statistical comparison with the corresponding control values.

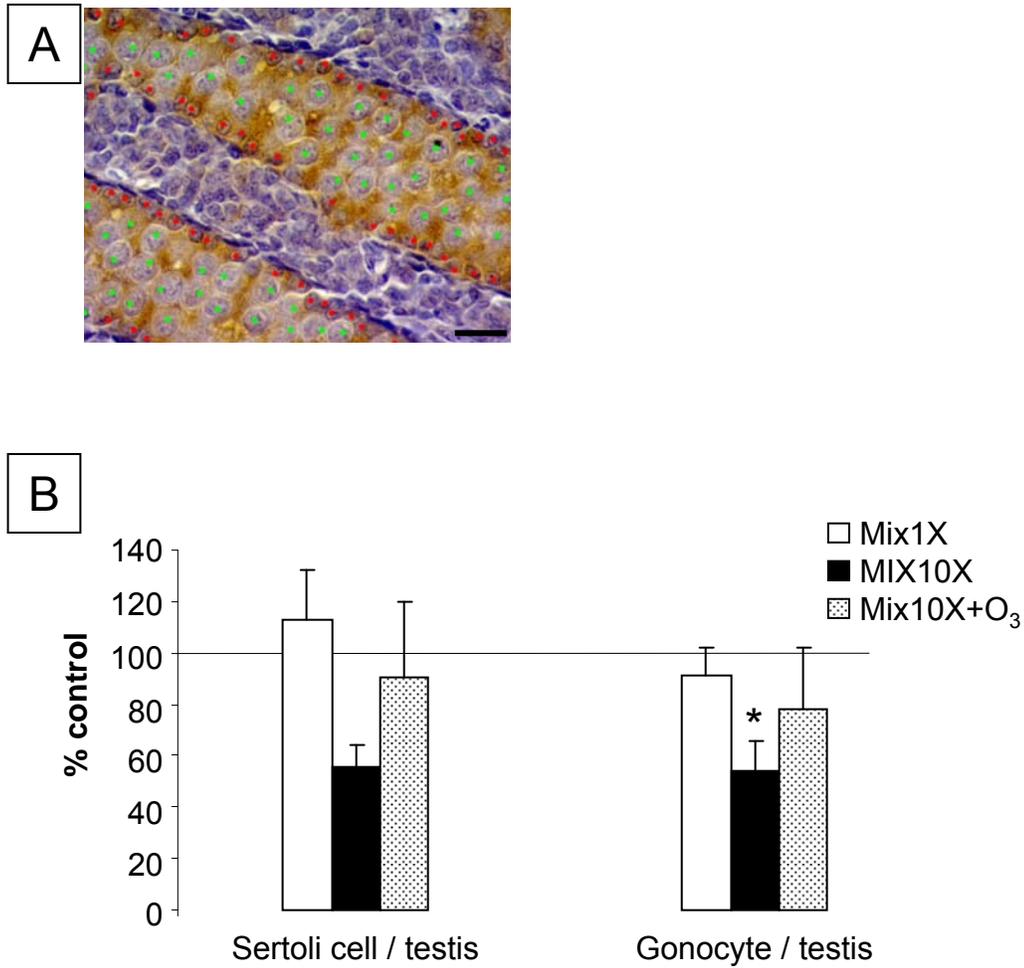


Figure 3: Effect of the MIX before or after ozonation on the total numbers of Sertoli cells and gonocytes in the 15.5-day-old rat fetal testis cultured for 3 days. Sertoli cells (red dots) and Gonocytes (green dots) were counted on histological sections after immunohistochemical detection of AMH (A). Scale bar represents 20 μ m. Quantitative data are expressed as % of the control cultures (B) and are represented as the mean \pm SEM from 3 to 4 cultures. * $p < 0.05$ compared with the corresponding control testis using a paired t-test.

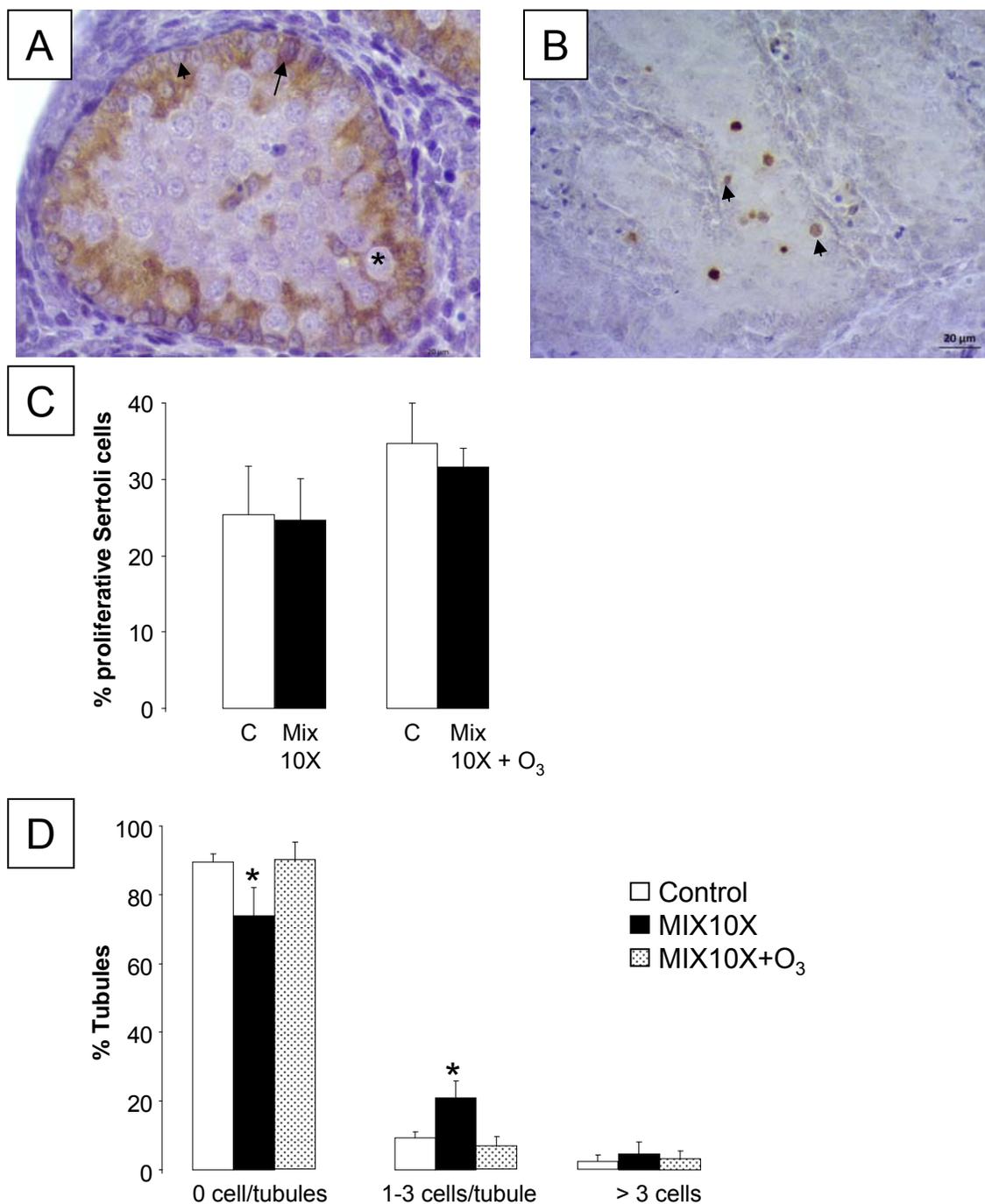


Figure 4: Effect of the MIX before or after ozonation on cell proliferation and apoptosis in testes from 15.5 days old rat fetuses in organ culture for 3 days. A: Proliferative Sertoli cells and Gonocytes were identified using PCNA and AMH double staining. Only PCNA-negative gonocytes (asterix) could be observed, but PCNA-positive (arrowhead) and PCNA-negative (arrow) Sertoli cells (positive for AMH) were counted. B: Apoptotic cells were identified as cleaved-caspase 3- positive (arrowheads). C: the percentage of PCNA-positive Sertoli cells was measured in at least 400 cells and is represented as the mean \pm SEM from 3 cultures. D: Apoptosis was quantified as the percentage of tubules containing none, 1-3 cells or more cleaved-caspase 3 positive cells in at least 60 tubules per sample (n=6 and 3 for the Mix 10X and the Mix 10X +O₃ respectively)

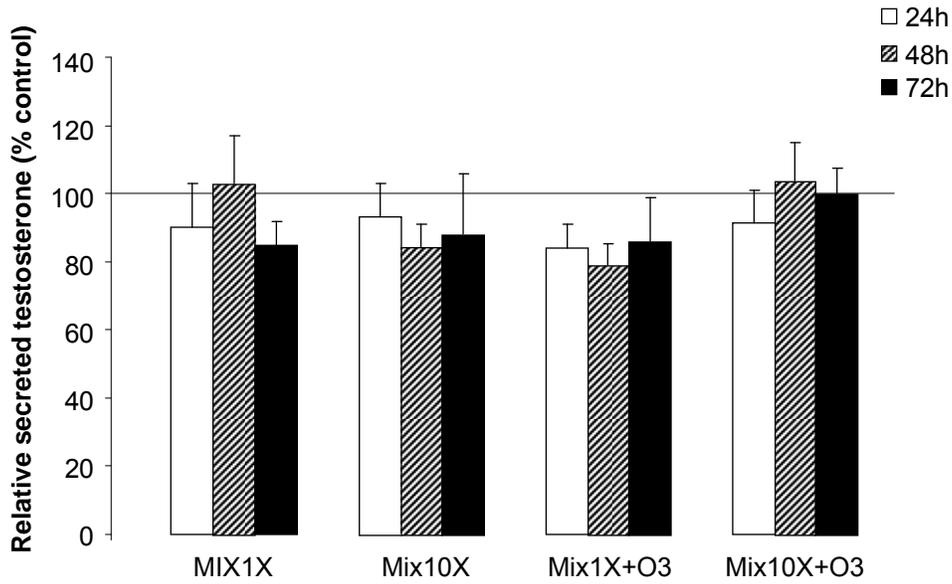


Figure 5: Effect of the MIX before or after ozonation on testosterone secretion from 15.5 days old rat fetal testes in organ culture for 3 days. Media were changed every 24 h and their testosterone content was measured by ELISA. Values (mean \pm SEM) are the relative testosterone secretion, expressed as the percentage of the control value from 6 to 9 cultures.

Supplementals

Supplemental Table 1: Summary of experimental conditions for the organ culture
EtOH: anhydrous ethyl alcohol

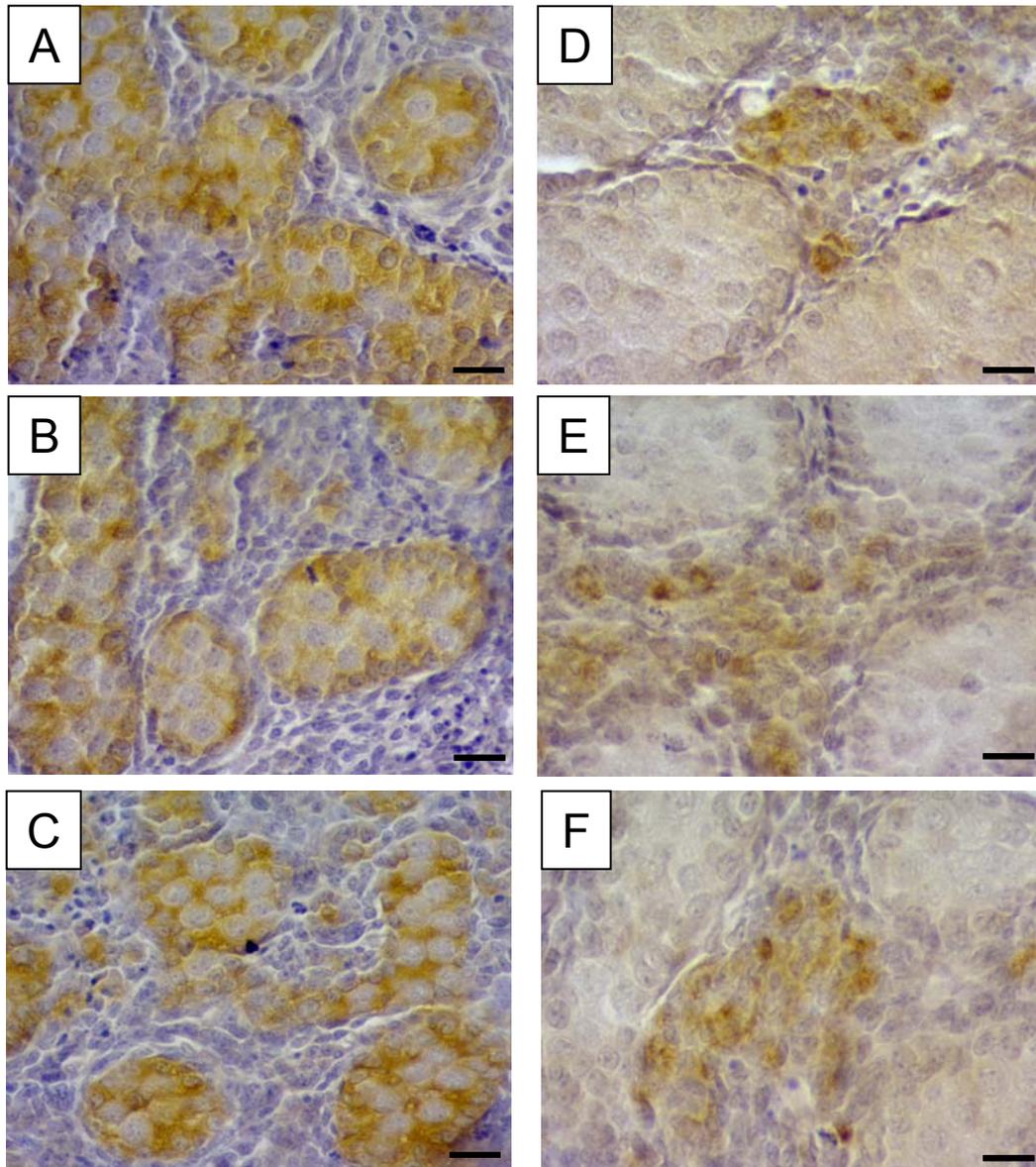
Control media	Treatment media
EtOH (0.01%)	MIX 1X
EtOH (0.01%)	MIX 10X
EtOH (0.01%) +50 µg/L O ₃	MIX 10X + 50 µg/L O ₃

Supplemental Table 2. Retention times and molecular ion mass to charge ratios of target compounds and their labelled surrogates
LOD: Limit of detection

Compound	[M-H] ⁻	Retention time (min)	HPLC-MS LOD (µg/L)	Method LOD (ng/L)
Estrone	269.16	4.4	0.13	6.5
13C2- Estrone	271.16			
-Estradiol	271.18	3.3	0.15	7.5
13C2-β-Estradiol	273.18			
Estriol	287.17	1.2	0.05	2.5
17α-ethinylestradiol	295.18	4.2	0.24	12
13C2- 17α-ethinylestradiol	297.18			

Supplemental Table 3: Sertoli cells and Gonocytes average diameters measured out of at least 100 cells

	Sertoli cells	Gonocytes
Controls	7.93 ± 0.13	12.06 ± 0.40
10X MIX	8.06 ± 0.13	11.93 ± 0.35
10X MIX + O ₃	7.74 ± 0.45	12.55 ± 0.30



Supplemental Figure 1: Effect of the MIX before or after ozonation on 15.5 days old rat fetal testes development in organ culture for 3 days. Immunostaining for AMH (A-C) was done to visualize Sertoli cell cytoplasm and seminiferous cord structure. Immunostaining for P450 Side-chain cleavage (D-F) was done to visualize Leydig cells (Asterix). Analysis of successive sections showed that the compared to controls (A and D), neither the the 10X MIX (B and E) nor the 10X MIX + O3 (C and F) had an effect on seminiferous cord organization (A-C), AMH expression in Sertoli cells (A-C) and P450scc expression in Leydig cells (D-F). Scale bar represents 20µm (A-C) or 15µm (D-F).