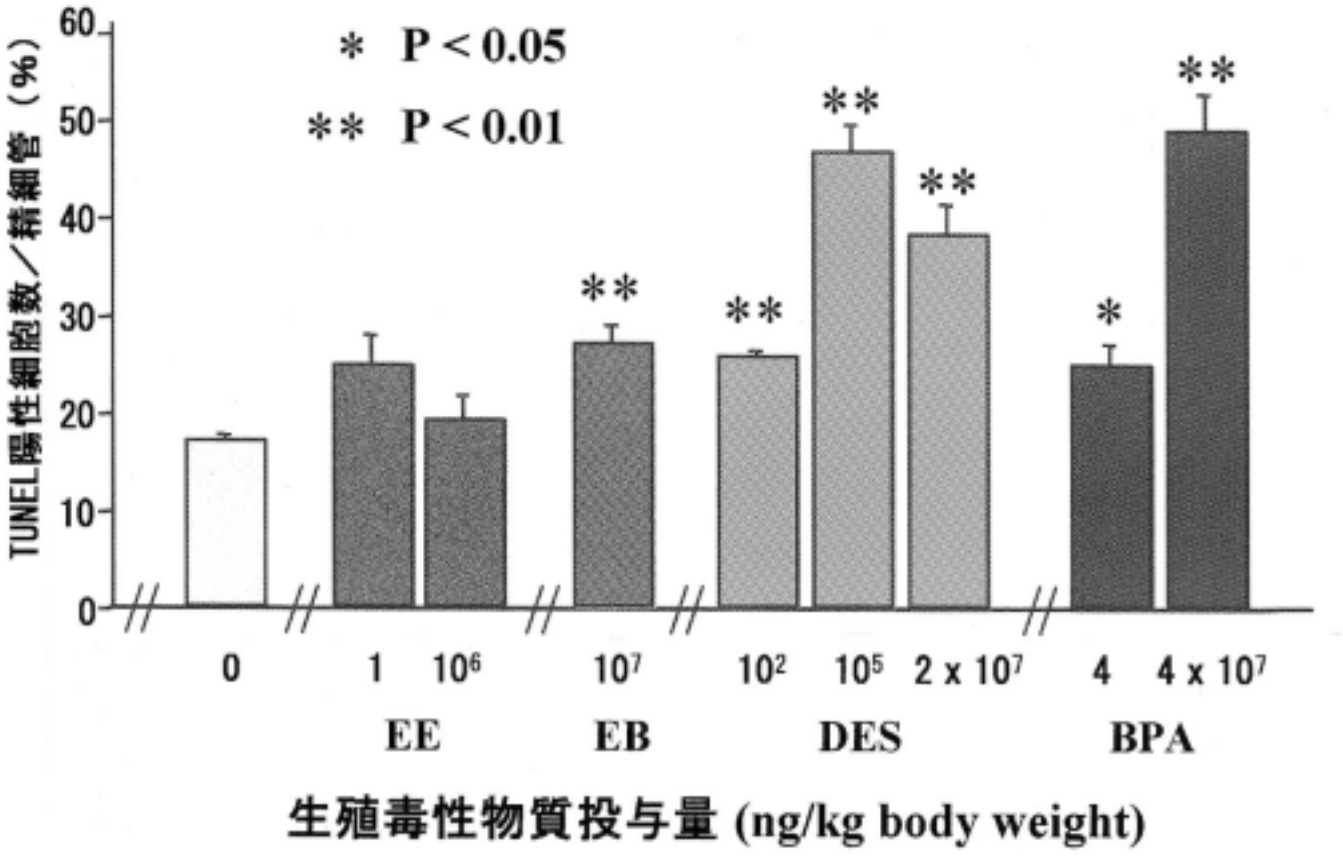

図3　ERKOマウスでの各種生殖毒性物質投与による精巣でのTUNEL陽性細胞数の変化 (n=3)

ERKO 雄マウスに EE 1ng 及び 1 mg/kg 体重、EB 10 mg/kg 体重、DES 100 ng、100 μg、及び 20 mg/kg 体重、 BPA 4 ng 及び 40 mg/kg 体重を 5 日毎に皮下投与し、初回投与から 20 日目に精巣を採取し、そのパラフィン切片を用いて DNA 二本鎖切断部位を検出する TUNEL 染色を行い、アポトーシス細胞を検出したもの。アポトーシス頻度は、精細管円形断面当たりの TUNEL 陽性細胞数を%として示した。対照としては、5%エタノール/コーンオイルを投与した。EB、DES、BPA 投与によりアポトーシス細胞の増加が認められた。

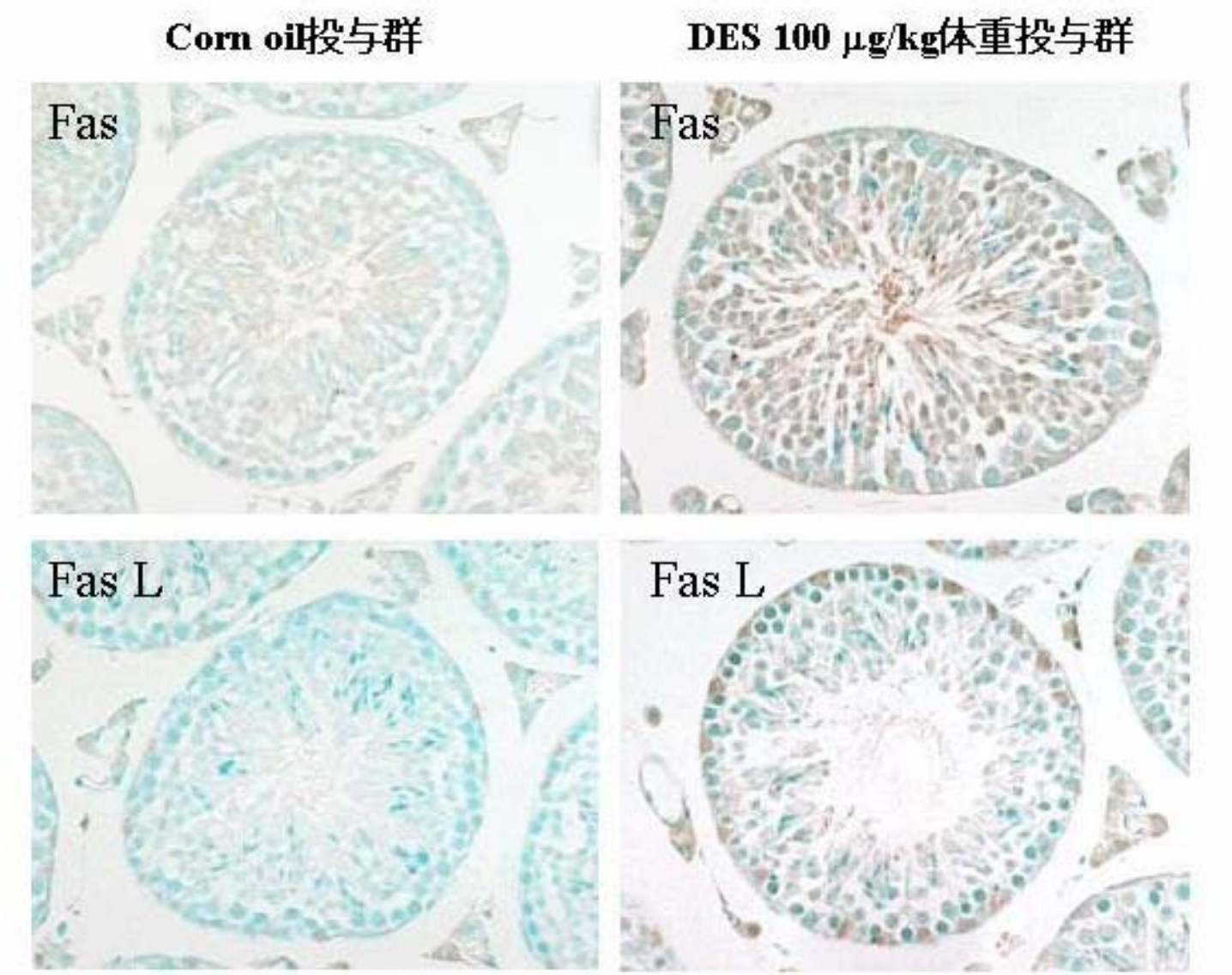

図4　ERKOマウス精巣でのFas及びFas Lの発現

ERKO マウス精巣において、アポトーシス細胞が著明に増加した DES 100 μg/kg 体重投与精巣での Fas 及び Fas リガンド（Fas L）の発現を示す。DES 投与精巣では Fas は Leydig 細胞並びに精子形成細胞に陽性であり、また Fas L の Sertoli 細胞での発現も増強した。

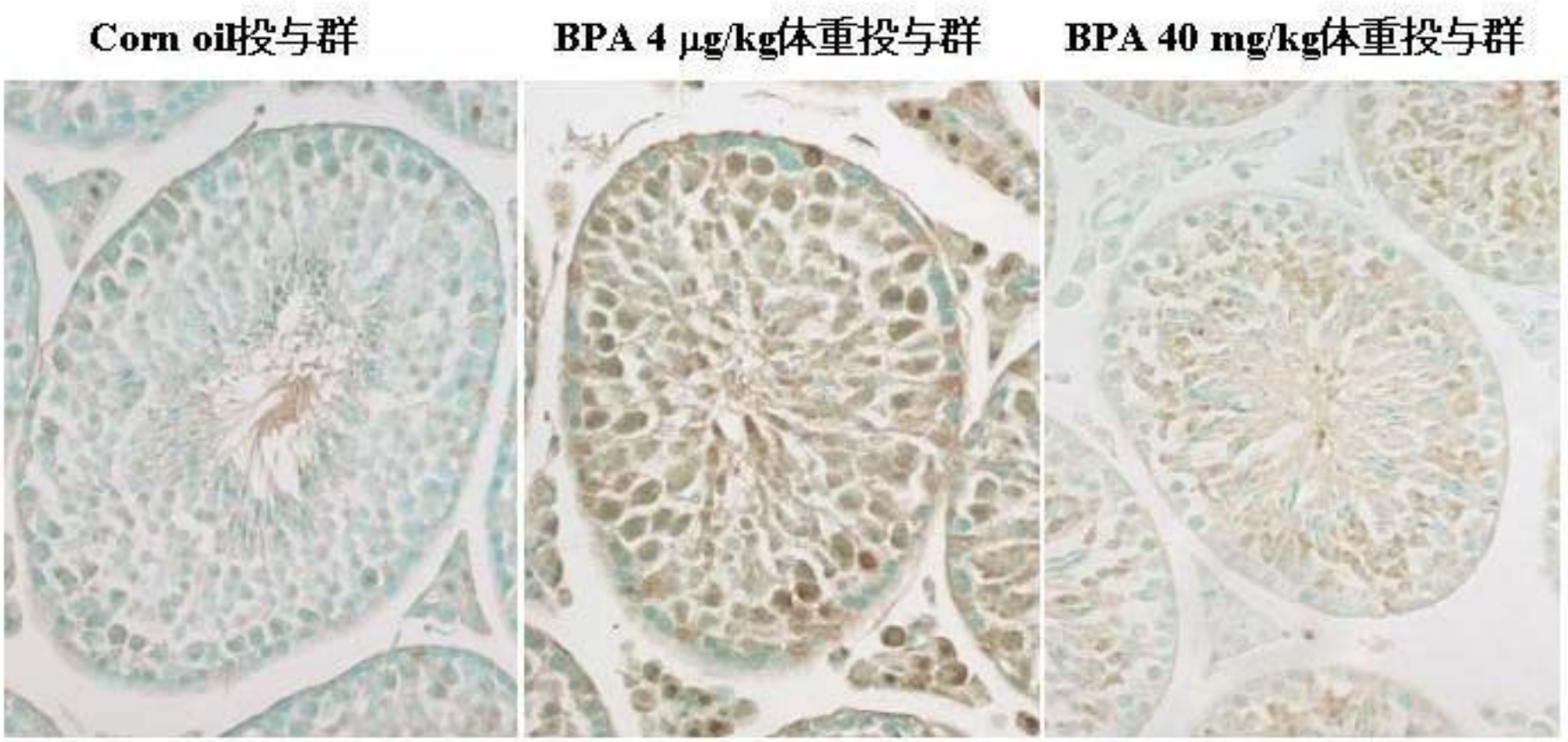

図5　BPA投与精巣におけるBcl-2の発現

BPA 投与精巣での Bcl-2 発現を免疫組織化学的に検討した。生殖細胞死阻害が認められた BPA 4 μg/kg 体重投与精巣において、精母細胞及び精子細胞に顕著な Bcl-2 の発現の増加が認められた。

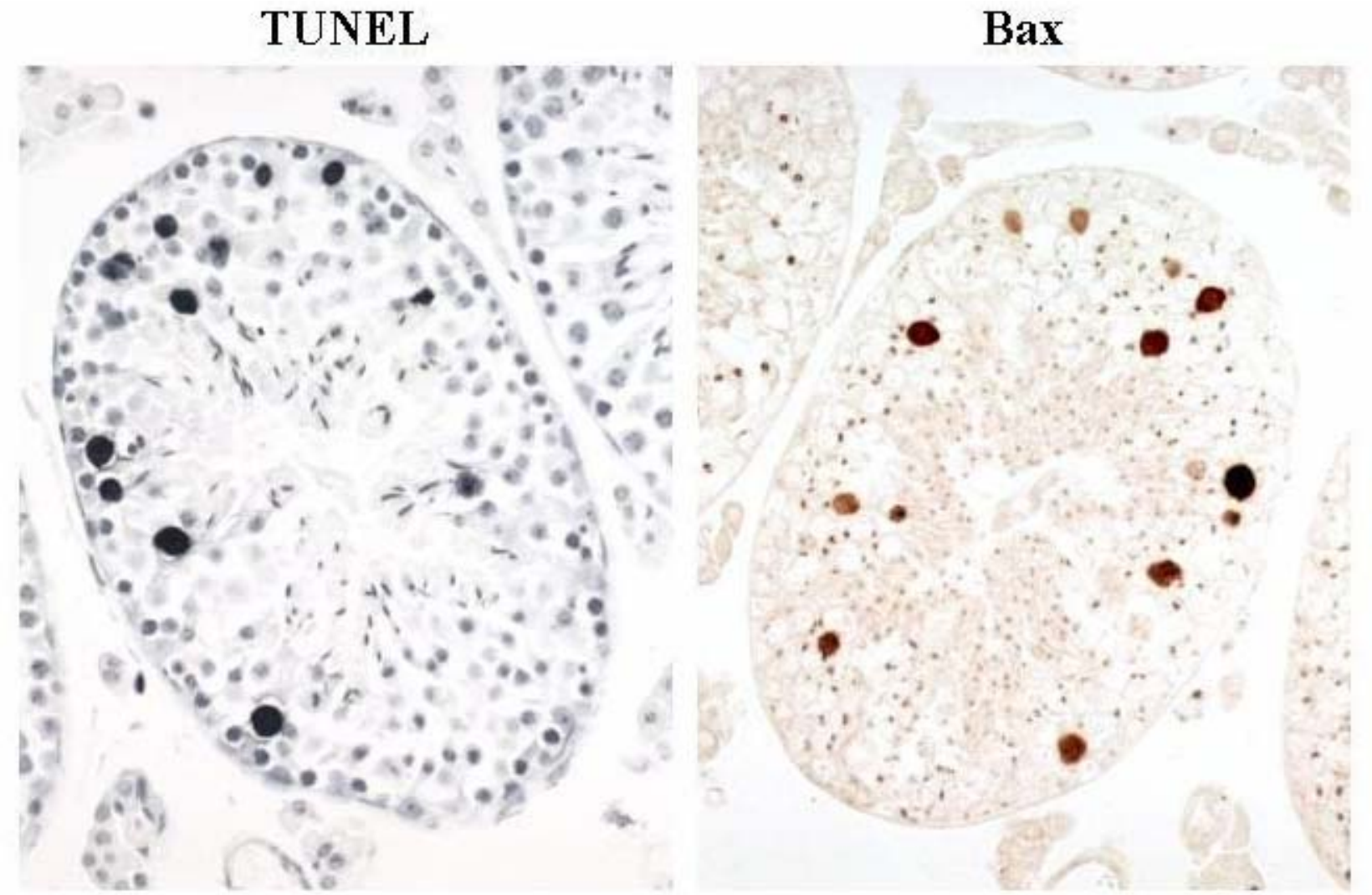

図6　DES 100 ng/kg体重投与でのTUNELとBaxの発現

ミラー切片を用いて、DES 100 ng/kg 体重投与に於けるアポトーシス細胞と Bax 陽性細胞の発現分布の直接的検討を行った。本投与量に於いては TUNEL 陽性細胞数の有意な増加が認められ、また TUNEL 陽性細胞はほぼ Bax 陽性細胞と一致していた。

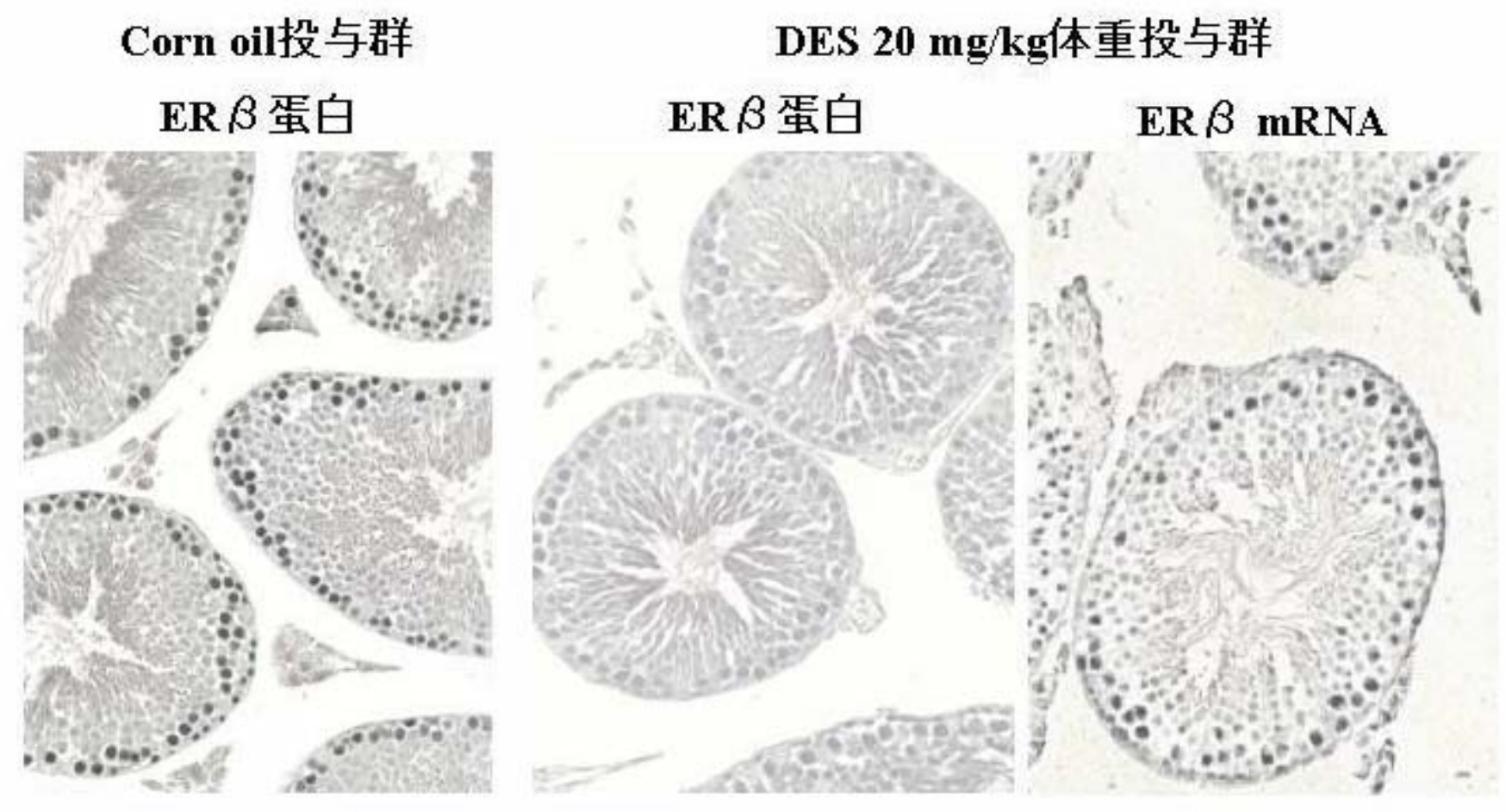

図7　DES投与精巣におけるERβの発現

成熟雄マウスにDES 20 mg/kg体重投与した精巣に於けるERβの発現について蛋白並びにmRNAレベルでの検討を行った。ERβ蛋白の発現は、対照群では精粗細胞並びに精母細胞に発現した。DES高濃度投与によりER蛋白発現の著明な減少を認めたが、in situ hybridizationを用いたmRNAレベルの検討ではERβ mRNAは精粗細胞並びに精母細胞に検出された。

Effects of environmental endocrine disruptors upon mouse spermatogenic cell death and their possible molecular mechanisms

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Key words: endocrine disruptors, germ cell death, Fas/Fas ligand, Bcl-2/Bax, estrogen receptors, mouse testis

Abstract:

For a better understanding of effects of various environmental endocrine disruptors (ED) on mammalian spermatogenesis, a systematic study with an experimental rodent model on germ cell death would be beneficial. In the project of this year, we focused on the effects of lower and wide-ranging concentrations (1 ng - 1 mg/kg BW) of ethynylestradiol (EE), estradiol-3-benzoate (EB), diethylstilbestrol (DES), bisphenol A (BPA) and dichlorodiphenyl dichloroethene (DDE) upon germ cell apoptosis in a short term protocol, where ICR male mice were injected subcutaneously with a compound dissolved in 5% ethanol/corn oil in every 5 days and killed on 20 days after the first injection. When germ cell apoptosis was examined by TUNEL, EE had no effect on the number of TUNEL positive cells at any dose investigated. On the other hand, germ cell apoptosis was increased in a dose-dependent fashion with EB and DDE. In the case of DES, the number of TUNEL positive cells had two peaks at doses of 100 ng/kg BW and 20 mg/kg BW. Very interestingly, BPA inhibited the autonomous germ cell apoptosis by 50-60% at doses of 4 – 40 μg/kg BW, while a significant increase in the number of TUNEL positive cells was found at lower and higher doses. These results indicate that ED have a wide variety of effects on germ cell apoptosis. To analyze the molecular mechanism underlying the induction of germ cell apoptosis by ED, we investigated the expression of Fas/Fas ligand and Bcl-2/Bax immunohistochemically. As a result, we found a close association between Fas expression in germ cells and TUNEL positive cells at high doses of ED, while at 100 ng/kg BW of DES no expression of Fas was detected in germ cells. Also, we found that the redistribution of Bax in germ cells was tightly associated with TUNEL positive cells in any cases. Moreover, a marked increase in Bcl-2 expression, which is supposed to protect cells from apoptosis, was detected at 4-40 μg/kg BW of BPA. These results strongly indicate that the roles of the Fas system in germ cell apoptosis may be limited to the case of severe and acute damages of testis by high doses of ED, and rather the Bcl-2 and Bax system can be more important in the induction of germ cell apoptosis by ED. Since estrogenic compounds are supposed to affect cells primarily through estrogen receptor (ER) α and β, we examined their expression by immunohistochemistry. Our results revealed that ERα is expressed in only Leydig nuclei, while ERβ is in the nuclei of spermatogonia and

spermatocytes, suggesting the direct action of these comopounds upon germ cells through ERβ. In accord with the findings, a similar apoptosis-inducing effect of ED was observed in ERα knockout mice. Therefore, we propose that ED may act on mammalian male germ cells through ERβ, mimicking the expression and subcellular distribution of Bcl-2 and/or Bax at the relatively lower concentrations. Moreover, at the higher concentrations of ED, spermatogenic cell death may be induced by the activation of the Fas system in combination with the changes in Bax expression.