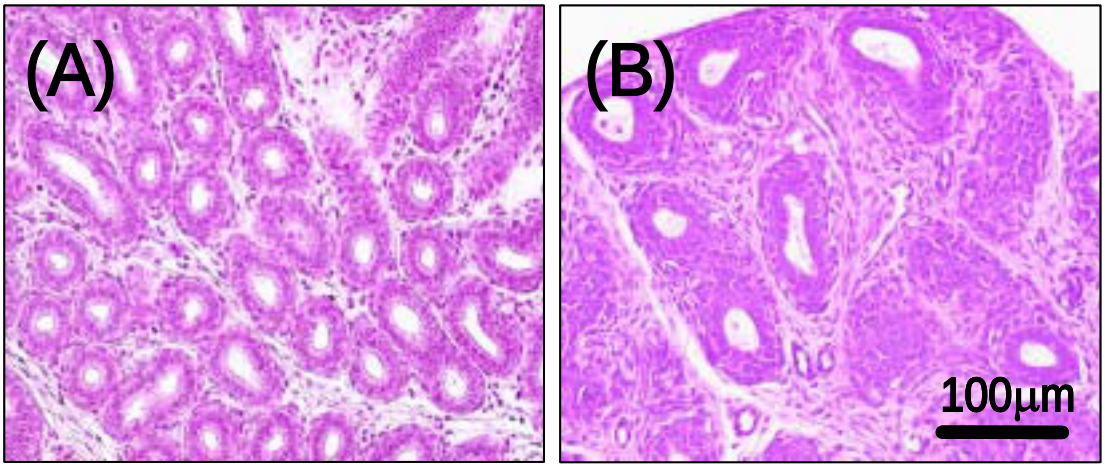


**図 17　精巣上体の Caput の組織像(HE 染色)(2 週齢)**
コントロール群と比べ、DES 群において間質の増加、管腔の拡張や上皮高の低下が観察された。(A) コントロール群、(B) DES 投与群、バーは 100 μm を示す。

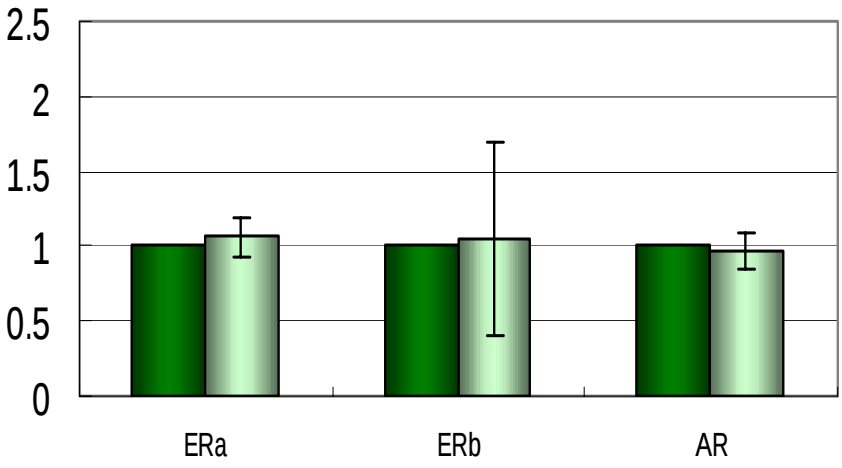


**図 18　3 週曝露モデルにおける PND31 での仔の精巣での性ホルモン受容体遺伝子の mRNA 発現レベル**
mRNA 発現レベルはリアルタイム RT-PCR で測定し、IF free 群 (左) を 1 として 0.05% IF 群 (右) を相対値で示した。ERa: estrogen receptor α, ERb: estrogen receptor β, AR: androgen receptor

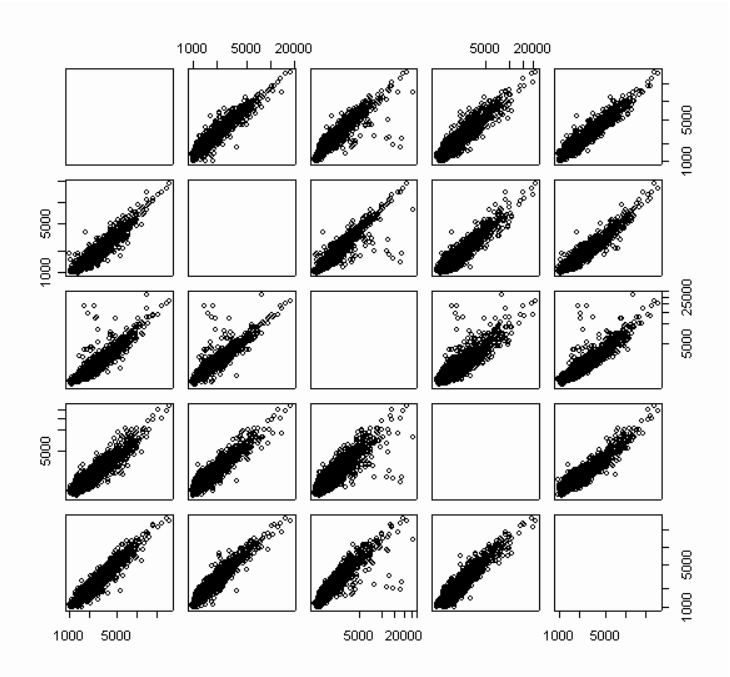


**図** 19　DNA **マイクロアレイのプロット**

In-house cDNA マイクロアレイ (3704 遺伝子) を用い、発現強度をグローバルノーマリゼション後,それぞれについてプロットした。

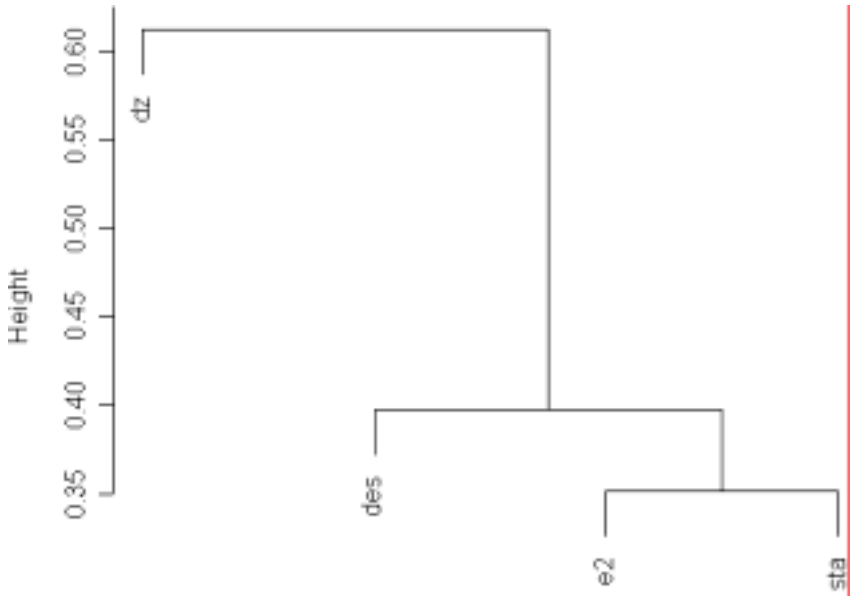


**図** 20　cDNA **マイクロアレイの結果によるクラスター解析**

統計学的解析はピアソン相関係数を用い、シングル リンケージクラスタリングを用いてクラスター リング解析を行った。

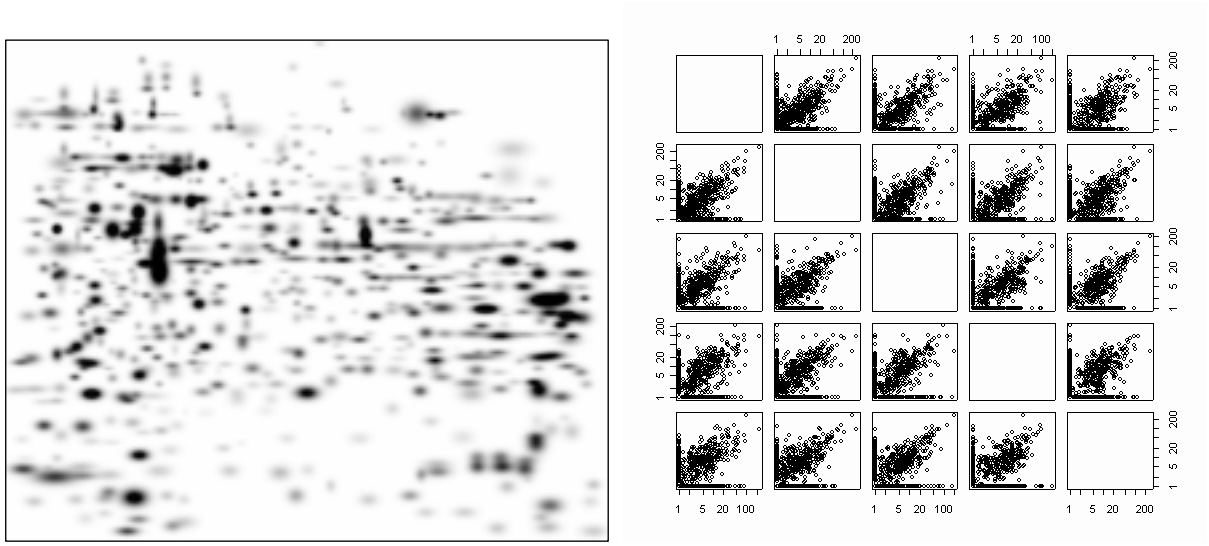

**図 21　二次元電気泳動像(左)とタンパク質スポットのプロット(右)**

スポットのタンパクの強度を数値化し、発現強度をグローバルノーマリゼション後,それぞれについてプロットした。

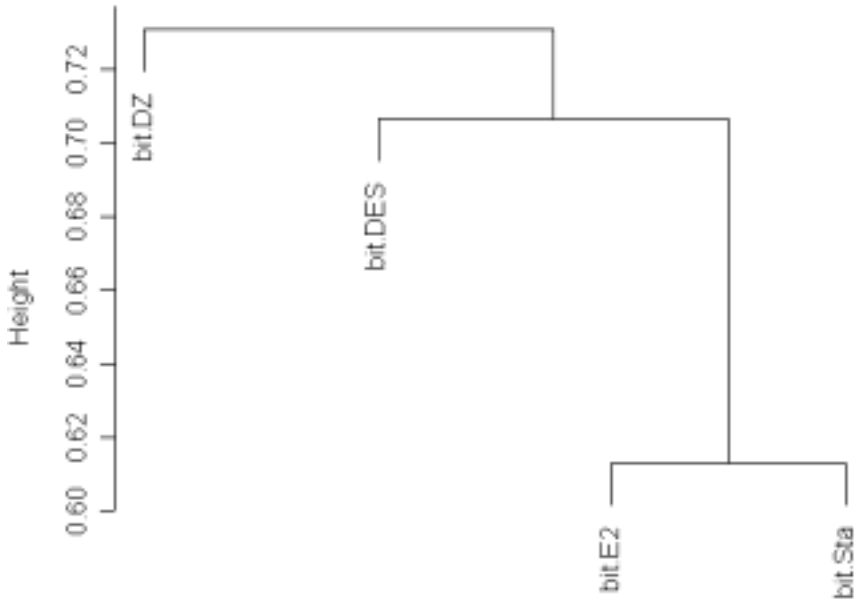


**図 22　二次元電気泳動のスポットによるクラスター解析**

化学物質投与によって出現あるいは消滅したスポットに解析を行った。統計学的解析はピアソン相関係数を用い、シングルリンケージクラスタリングを用いてクラスターリング解析を行った。

**表 1　仔の発育ランドマーク**

PND 1, 7, 14, 21, 30 における体重および肛門生殖突起間距離が IF-free 群に対して 0.05% IF 群がどうであったかを相対的にし示した。．　↑: 0.05% IF 群 > IF-free 群, ↓: 0.05% IF 群 < IF-free 群, →: 両者に差がなかった　矢印 2 つは $p<0.01$ (Student's *t*-test)であった。

| 曝露モデル: | 3w | | 5w | |
|---|---|---|---|---|
| PND | BW | AGD | BW | AGD |
| 1 | ↑ | → | → | → |
| 7 | ↑↑ | → | → | → |
| 14 | ↑ | → | → | → |
| 21 | → | → | → | → |
| 30 | → | → | ↓ | → |

肛門生殖突起間距離 (AGD) は Gallavan R H *et al.* 1999 の方法で標準化した。

**表 2　PND31 の仔の相対生殖器重量**

| | 相対生殖器重量 (mg/gBW) | |
|---|---|---|
| 臓器 | IF-free | 0.05% IF |
| 3 週曝露モデル | | |
| 精巣 | 2.88 ± 0.21 | 3.14 ± 0.21* |
| 精巣上体 | 0.70 ± 0.03 | 0.73 ± 0.16 |
| (仔の数/リター) | (3/1) | (6/2) |
| 5 週曝露モデル | | |
| 精巣 | 2.26 ± - | 2.83 ± 0.21 |
| 精巣上体 | 0.71 ± - | 0.66 ± 0.07 |
| (仔の数/リター) | (1/1) | (4/2) |

データは、平均 ± SD で示した。

*印: 0.05% IF 群は IF-free 群に対し有意差があった。$p<0.05$ (Student 's *t*-test)

# Studies on the molecular and cellular biological mechanisms on male reproductive system

## affected by endocrine disruptors

Chisato MORI, Chiba University, Professor

Key Word:
mouse, testis, epididymis, TM4, spermatogenesis, ectoplasmic specializations, diethylstilbestrol,
flutamide, 17・-estradiol, ・-estradiol 3-benzoate, bisphenol A, cyproterone acetate, ICI 182,780,
microarray, GFP mouse

Abstract: (1)
In this study, it is indicated that flutamide (Flu) has estrogen-like effect on mouse testes (Anahara R et al. 2004 in press), as previously reported (Toyama Y et al. 2001). The effects of Flu+・-estradiol 3-benzoate (E2B) and Flu+ cyproterone acetate (CA) were found to be more severe than those of the single Flu, and the Flu+ICI 182,780 (ICI) treatment tended to recover the damages. These results suggested that E2B and CA worsened the adverse effects of Flu on spermatogenesis. On the other hand, ICI repressed the effects of Flu. This study also suggests that the relative concentration of estrogens to androgens in spermatogenesis is fixed, and the exogenous chemicals disrupted the balance of the relative concentration. Our present study may be a key in preventing the endocrine disrupting chemical's effects.
(2) Testes of two lines of infertile knockout mice were observed by electron microscopy. The phenotype showed abnormalities in acrosomes and nuclei of spermatids in addition to abnormalities in ectoplasmic specialization. The phenotype was the same as that of E2-, E2B-, and bisphenol A (BPA)-treated mice. Specific localization of an actin binding protein in ectoplasmic specialization was not observed, as of these estrogenic chemical-treated mice. Mutant azoospermic rats, which show impaired blood-testis barrier, were treated with these estrogenic chemicals. No changes in phenotype were observed, suggesting that the chemicals act on the same point(s).
(3) Although it is thought that disturbance of spermatogenesis by diethylstilbestrol (DES) cause decrease of sperm counts disturbed, it is not clear which is damaged by DES, germ cells or Sertoli cells. Therefore, we adapted transplantation technique to the question. When germ cells prepared from GFP (Green Fluorescent Protein) mouse (donor) were transplanted into seminiferous tubules (recipient) of non-DES-treated mouse from which germ cells were eliminated by busulfan treatment before transplantation, the germ cells colonized in the recipient tubules and spermatogenesis was observed. While in the DES-treated recipient,

spermatogenesis was disordered. These suggested that DES disorders at least Sertoli cells rather germ cells.

(4) In in-house cDNA microarray analysis, we isolated some genes whose expression was altered in epididymis after neonatal treatment of mice with DES or E2 at 2, 4 and 8 weeks of age. These genes appear to be related with the deterioration of epididymal function by neonatal treatment with DES or E2.

(5) The results of chronic exposure to low-dose isoflavone, especially the period before gestation to the end of lactation and after weaning, dose not affect to male mice on reproductive development. On the other hand, transcriptome analysis and proteome analysis of in vitro exposure experiments using TM4 testicular cells to estrogenic/non-estrogenic compound including isoflavones revealed that chemicals were classified into some groups by the response of genes and proteins.