

**IDENTIFICATION OF CRITICAL RAT TESTICULAR GENES ALTERED AFTER FETAL ANDROGENIC DISRUPTION BY FLUTAMIDE: USE OF DNA MICROARRAY
ACRONYM: ENDISRUPT**

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• **OBJECTIVES:**

This project aimed at strengthening scientific approach concerning the effects on male fertility of exposure to environmental factors such as endocrine disrupters. The objective was to use DNA microarray approach to improve the current approach for assessing the endocrine (androgen) disrupting effects of chemicals such as flutamide on testicular development and function.

Specific aims were to:

- (i) identify among thousands of testicular genes those affected by antiandrogenic (flutamide and finasteride) disruption using the DNA microarray approach;
- (ii) understand the specificity and the function(s) of the proteins encoded by these genes in the activity of testicular germ, Sertoli and Leydig cells.

As this is a genomic approach to identify the transcripts, the levels of which are affected in the different testicular major cell types, the doses of flutamide were chosen in order to avoid or minimise germ cell loss that may confound the interpretation of effects of flutamide on testicular (germ cell) gene expression. For these reasons, the doses of the antiandrogen selected were 0.4, 2 and 10 mg/kg/day.

The project was subdivided in 5 work packages (WP) that were conducted in parallel as follows: WP01 was devoted to the project coordination
WP02 corresponded to the production of DNA microarrays, mainly from rat testis cDNA libraries. By using these DNA microarrays and the testis transcripts obtained from male rats treated during their foetal life with antiandrogens at different concentrations (WP03), the

plan was to identify among the thousands of genes the largest number possible affected. Once these genes of interest were identified, (i) their expression was localised in the three major testicular cell types: germ cells (WP04), Sertoli cells (WP05) and Leydig cells (WP 06); (ii) the function(s) of the encoded proteins were delineated in each testicular cell types after manipulation of these genes in the different testicular cell types.

- **RESULTS OBTAINED:**

Over a period of 4 years, the consortium succeeded in achieving the aims of the project. The first aim was to assess the validity of our experimental model, since we used low doses of anti-androgens. Indeed, morphological studies displayed no (at 0.4, 2 mg/kg•d) or low cellular alterations (10 mg/kg•d) nor were hormonal alterations evidenced in pubertal or adult rats exposed in utero to flutamide. In contrast, in utero exposure to flutamide induced chronic apoptosis in adult germ cells. This cell death process is associated to functional abnormalities in the different cell types of the testis. Indeed, we observed alteration in steroidogenesis of foetal Leydig cells, linked to decreased expression of steroidogenic enzymes and modifications in the hedgehog signalling pathway, shown here to be involved in the regulation of steroidogenesis. In adult male, germ cells displayed chronic apoptosis related to a long-term activation of the apoptotic cascade (activation of effector caspase-3; overexpression of pro-apoptotic factor of the mitochondrial pathway) and a decrease in the inhibitor of apoptosis (IAPs). The chronic apoptosis of testicular germ cells was devoted to modifications in the Sertoli cell functions: detoxification through GST, TGF β signalling, lactate metabolism and hematotesticular barrier formation.

- **BENEFITS AND BENEFICIARIES:**

Endocrine disrupters are widespread chemicals in the environment due to their many applications in manufacturing, agriculture and healthcare. Establishing whether these chemicals are safe for humans is a responsibility shared by the chemical industry together with scientists and regulators. However, confidence in a prediction that chemicals are safe for humans will only come from an understanding of how such chemicals interact with organisms and /or cells. This is usually determined by tests in laboratory animals such as rats and mice depending on the chemical tested. In these models, diversion from normal physiology is accompanied by a panoply of histological and biochemical changes and fundamental to all of these methods is the fact that toxicity is preceded by, and results in, alterations in gene expression. The present project aimed at improving the strategy to identify the potential endocrine disruption activity of chemicals in the male reproductive function. The identification in flutamide-treated rats of altered testicular genes with key function(s) evidenced by a new powerful methodology (DNA microarray approach) was to allow to generate biomarkers useful to detect and evaluate the deleterious effects of endocrine disruptors on health. As such, this project should contribute to the improvement of the quality of life and health as by helping to prevent the consumer from exposure to harmful factors.

- **CONCLUSIONS:**

We demonstrated that in utero exposure to flutamide induced molecular alterations in the different testicular cell types, whereas no morphological alterations were observed. In the Leydig cells, in utero exposure to flutamide induced defects in the Dhh signalling system. Furthermore, the anti-androgen decreased the expression of SF1 and Insl3 during testicular development that might disturb the testicular descent and explain the cryptorchidism observed at dose 10 and above. In the germ cell, the foetal androgen disruption triggered chronic apoptosis. This apoptotic process is related to a long term activation of the apoptotic cascade through the mitochondrial pathway, the activation of effector caspases and the decreased expression of their inhibitors. Finally, the androgen disruption induced

different defects in key Sertoli cell functions such as hematotesticular barrier modeling, TGF β signalling, detoxification process and energy metabolism. These data suggest that the long-term consequences of foetal androgen disruption are related to foetal malprogramming.