EFFECTS OF PRENATAL ANTIANDROGEN EXPOSURE ON HSD3B EXPRESSION IN THE FETAL PORCINE GONADS

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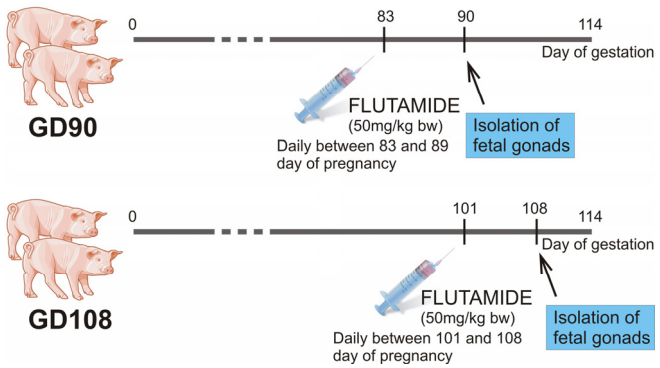
INTRODUCTION

Hormonal disruption during fetal period induces abnormalities in the developing reproductive system, which may have far-reaching consequences. 3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (HSD3B) is a key enzyme catalyzing an essential step in the formation of all classes of steroid hormones. There is growing evidence that steroid hormones modulate HSD3B expression. Previously, we have reported the presence of androgen receptors in the fetal porcine gonads denoting the role of androgens during gonadal development. Thus, the aim of the present study was to determine the effect of androgen deficiency during late prenatal period on HSD3B expression in the fetal porcine gonads.

MATERIALS & METHODS



For each flutamide-exposed group, a respective control group was used and control animals were treated with corn oil in a manner similar to the flutamide treated pigs. HSD3B immunolocalization was performed using rabbit polyclonal anti-mouse HSD3B antibody (provided by prof. A. H. Payne from Stanford University). To assess HSD3B mRNA expression real-time PCR was carried out using the TaqMan Gene Expression Assay (Applied Biosystems).

RESULTS

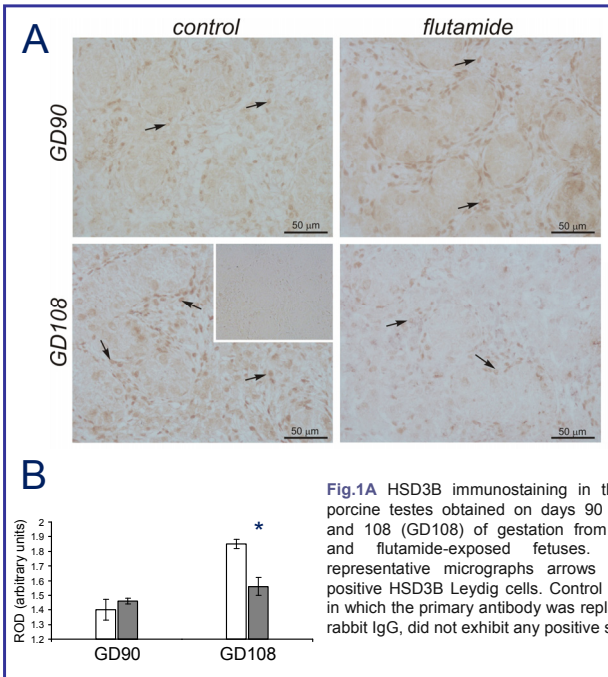


Fig.1A HSD3B immunostaining in the fetal porcine testes obtained on days 90 (GD90) and 108 (GD108) of gestation from control and flutamide-exposed fetuses. In all representative micrographs arrows indicate positive HSD3B Leydig cells. Control section, in which the primary antibody was replaced by rabbit IgG, did not exhibit any positive staining.

Fig.1B Charts represent the intensity of HSD3B immunostaining expressed as a relative optical density (ROD) in the fetal porcine testes in all examined days (white bars, control groups; grey bars, flutamide-treated groups). Bars express means ± SEM. Asterisks denote significant differences (Mann-Whitney test; *P<0.05).

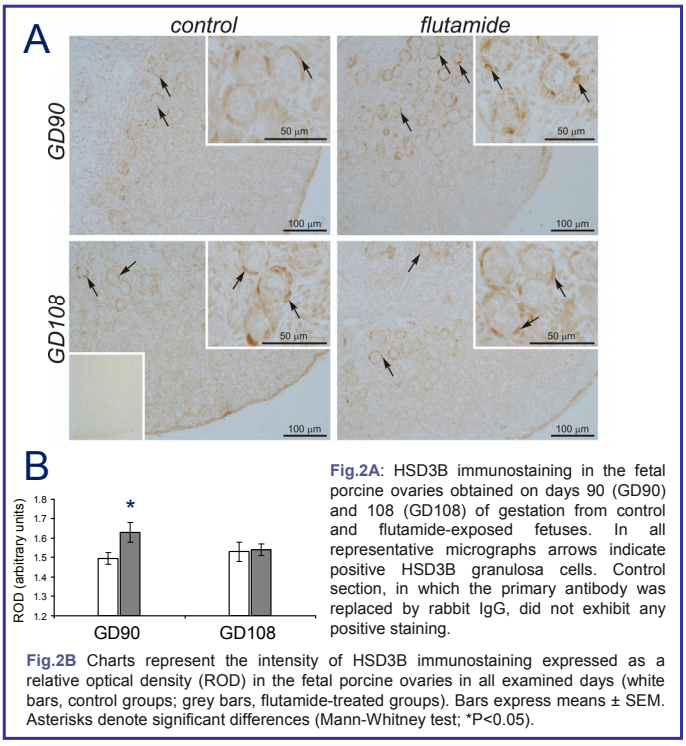


Fig.2A: HSD3B immunostaining in the fetal porcine ovaries obtained on days 90 (GD90) and 108 (GD108) of gestation from control and flutamide-exposed fetuses. In all representative micrographs arrows indicate positive HSD3B granulosa cells. Control section, in which the primary antibody was replaced by rabbit IgG, did not exhibit any positive staining.

Fig.2B Charts represent the intensity of HSD3B immunostaining expressed as a relative optical density (ROD) in the fetal porcine ovaries in all examined days (white bars, control groups; grey bars, flutamide-treated groups). Bars express means ± SEM. Asterisks denote significant differences (Mann-Whitney test; *P<0.05).

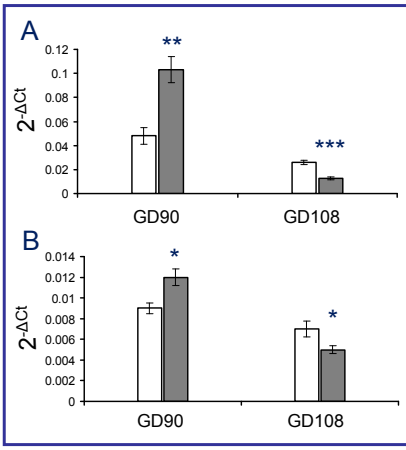


Fig. 3 HSD3B mRNA expression in porcine testes (**A**) and ovaries (**B**) obtained on days 90 and 108 of gestation from control (white bars) and flutamide-treated (grey bars) fetuses. As an intrinsic control, GAPDH mRNA level was measured in the same samples. Ct value of the reference gene GAPDH was subtracted from the Ct value of HSD3B (ΔCt) and relative expression was expressed as $2^{-\Delta Ct}$ ± SEM. Asterisks indicate statistically significant differences between control and experimental groups (Mann-Whitney test; *<0.05, **P<0.01, ***P<0.001).

SUMMARY OF RESULTS

- ✓ In testes from control and flutamide-exposed fetuses, HSD3B was immunolocalized in Leydig cells (Fig.1A).
- ✓ Following flutamide treatment, the intensity of immunostaining was lower on GD108 vs. control (Fig.1B).
- ✓ Flutamide administration resulted in increased HSD3B mRNA expression on GD90 and decreased HSD3B mRNA expression on GD108 vs. respective controls (Fig.3A).
- ✓ In ovaries from control and flutamide-exposed fetuses, HSD3B was immunolocalized in granulosa cells of forming follicles (Fig.2A).
- ✓ Following flutamide administration, increased expression of HSD3B mRNA and protein were observed on GD90 (Fig.2B and Fig.3B). However on GD108, flutamide treatment led to decreased HSD3B mRNA expression (Fig.3B), while no changes in the intensity of immunostaining were observed (Fig.2B).

CONCLUSIONS

Diminished androgen action during late gestation induces changes in HSD3B expression in porcine fetal gonads, which may result in functional changes in Leydig and granulosa cells. However, it seems that androgens exert diverse biological effects depending on the gestational period.

Supported by Iuventus Plus grant (IP2011 024571) of the Polish Ministry of Science and Higher Education.