Flutamide-induced alterations in *CYP17A1* gene expression and local testosterone synthesis in porcine luteal tissue - a new insight into androgens action during pregnancy

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INTRODUCTION

BACKGROUND: The corpus luteum (CL) is an ephemeral endocrine gland, which primary function is secretion of progesterone (P4). The pig is a particularly interesting model, because porcine CL is required to support pregnancy throughout the entire gestational period and it is the major source of P4. The cytochrome P450 17α-hydroxylase/c17,20-lyase (CYP17A1) was found in small luteal cells from day 50 of gestation, indicating the potential sites of androgen synthesis. To date, it was established that androgens are able to modulate luteal function during pregnancy by stimulation of CL to P4 release.

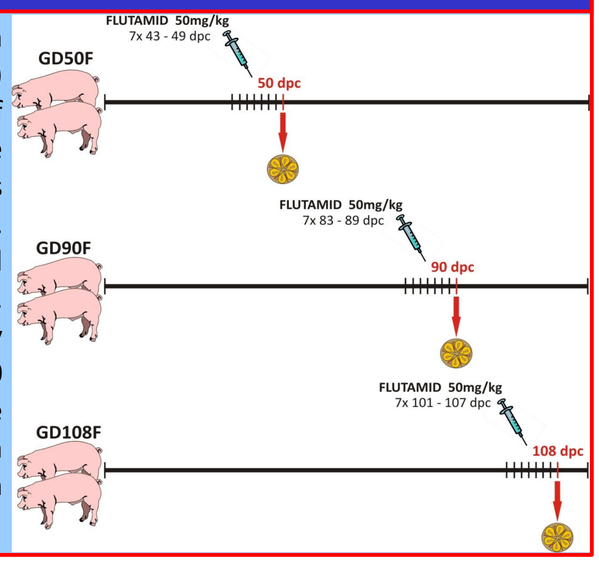
HYPOTHESIS: Androgens are a new important factor, which might be involved directly or indirectly in the CL functioning and maintenance of pregnancy in pigs.

AIM OF THE STUDY: To determine whether mid- and late gestational exposure to the anti-androgen flutamide influences *CYP17A1* gene expression in the luteal tissue and the local testosterone (T) synthesis in pigs.

RATIONALE: Our strong motivation to undertake this endeavor is explained by negative effects of environmental compounds, such as anti-androgens (fungicides, herbicides), which interfere with the action of endogenous hormones and disrupt animal fertility.

MATERIAL

Pregnant gilts (Large White × Polish Landrace) were injected with anti-androgen flutamide (Sigma–Aldrich, St. Louis, Missouri, USA) between days: 43 and 49 of gestation (GD50F), 83 and 89 of gestation (GD90F) or 101 and 107 of gestation (GD108F). Flutamide was suspended in vehicle (corn oil) and delivered as subcutaneous injections daily for seven days at a dose of 50 mg/kg body weight. Respective control groups (GD50C, GD90C, GD108C) were treated with corn oil in a manner similar to the flutamide-treated pigs. Porcine bilateral ovaries were obtained from pregnant gilts by ovariectomy performed under anesthesia with thiopental on day 50 (GD50), 90 (GD90) and 108 (GD108) of gestation. Fresh CLs were excised from right and left ovaries of control (C; n=8-11 for each examined group) and flutamide-treated (F; n=8-11 for each examined group) animals.



METHODS

Real-time PCR : analysis of *CYP17A1* mRNA expression with TaqMan Gene Expression Assay (Applied Biosystems)
Immunohistochemistry: CYP17A1 localization using anti-CYP17A1 antibody (1:100; gift from prof. Dale B. Hales from Southern Illinois University, Carbondale, USA)
Radioimmunoassay (RIA): testosterone (T) concentration in homogenates from CLs

RESULTS

Analysis of *CYP17A1* mRNA expression of by real-time PCR

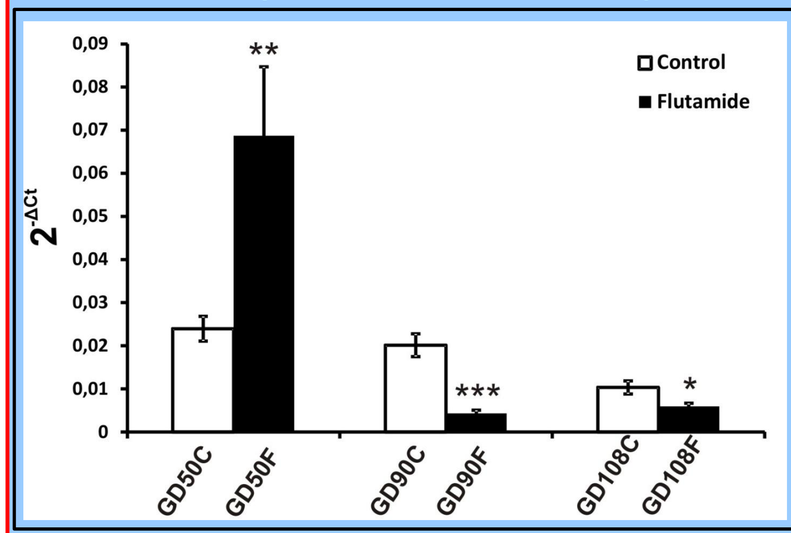


Fig. 1. Expression of *CYP17A1* mRNA in the corpora lutea (CLs) retrieved from control (**C**) and flutamide-treated (**F**) gilts on days 50 (**GD50**), 90 (**GD90**) and 108 (**GD108**) of gestation. Relative expression of *CYP17A1* mRNA was determined with the use of quantitative real-time PCR analysis and expressed as the mean ± S.E.M. of ratios relative to *GAPDH* (open bars, control groups; shaded bars, flutamide-treated groups). *GAPDH* was used as an internal control. $2^{-\Delta Ct}$ values were used to find statistical differences by the Mann-Whitney U test. Asterisks denote significant differences (*P < 0.05, **P < 0.01, ***P < 0.001).

Radioimmunological analysis of T level

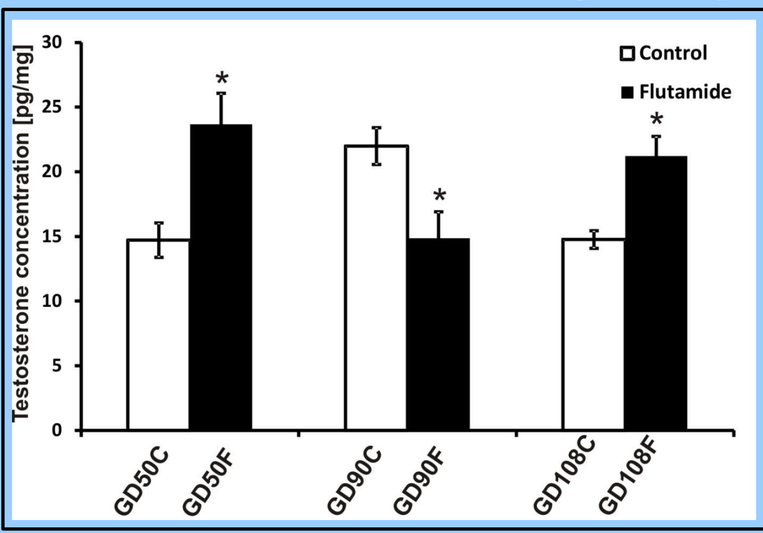
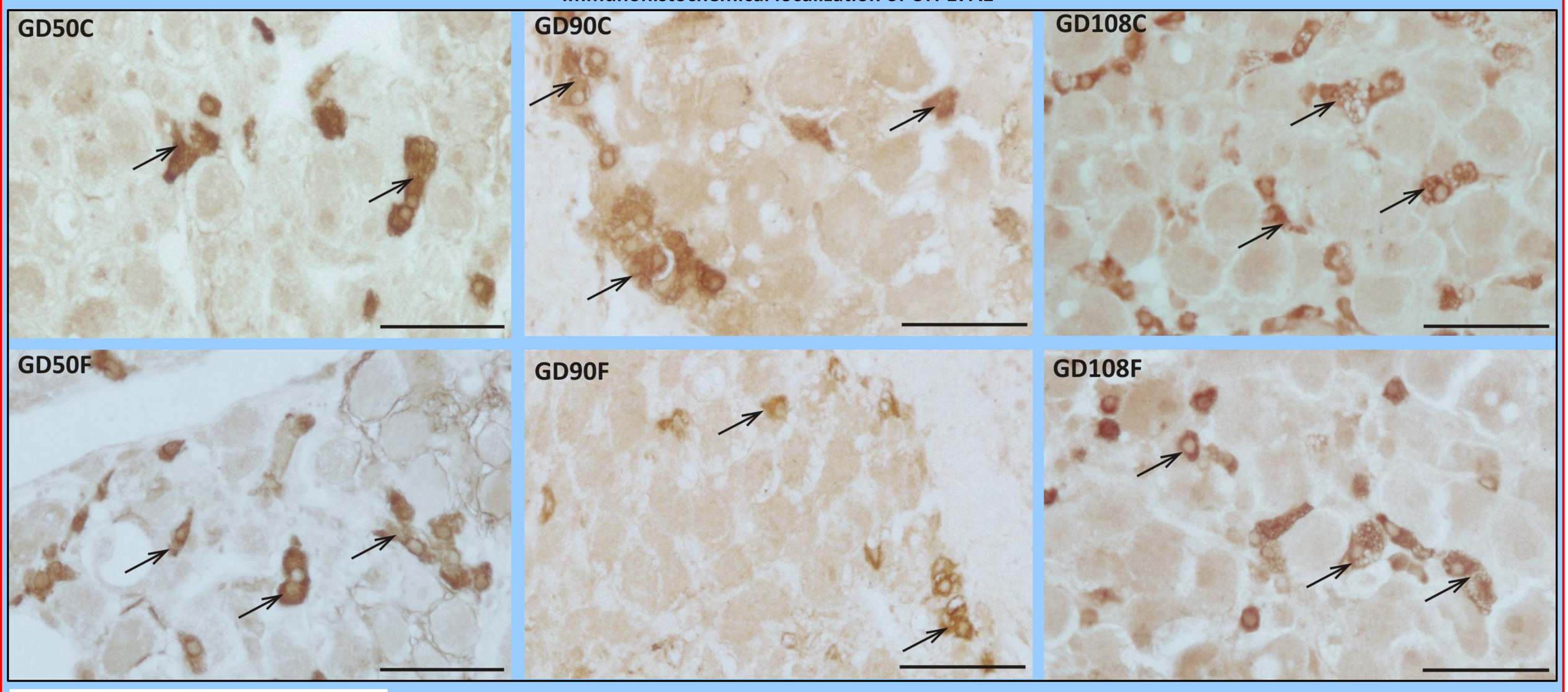


Fig. 2. Testosterone (T) concentration in homogenates of corpora lutea (CLs) retrieved from control (**C**) and flutamide-treated (**F**) gilts on days 50 (**GD50**), 90 (**GD90**) and 108 (**GD108**) of gestation. To determinate T concentrations, radioimmunoassay (RIA) was performed. Results are expressed in pg/mg tissue as the mean ± S.E.M. (open bars, control groups; shaded bars, flutamide-treated groups). Asterisks denote significant differences (Mann-Whitney U test; *P < 0.05).

Immunohistochemical localization of CYP17A1



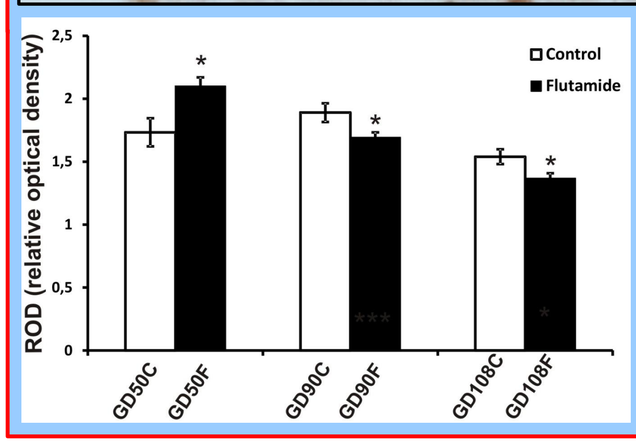


Fig. 3. CYP17A1 immunostaining in corpora lutea (CLs) retrieved from control (**C**) and flutamide-treated (**F**) gilts on days 50 (**GD50**), 90 (**GD90**) and 108 (**GD108**) of gestation. In the representative microphotographs positive CYP17A1 staining was found in the cytoplasm of small luteal cells (*arrows*). Chart represents the intensity of CYP17A1 immunostaining expressed as a relative optical density (ROD) in CLs within all examined days of pregnancy (open bars, control groups; shaded bars, flutamide-treated groups). Bars express mean ± S.E.M. *P < 0.05 indicates statistically significant differences (Mann-Whitney U test). All the scale bars represent 50 µm.

CONCLUSIONS

In conclusion, androgen deficiency during mid- and late pregnancy in pigs following antiandrogen flutamide administration affects luteal T synthesis in CLs. The observed changes in T production are probably the consequences of altered *CYP17A1* gene expression. However, we found different regulation depending on the day of pregnancy. Because T undergoes further conversion to estradiol, a steroid hormone importan before parturition, the altered T level might subsequently affect estradiol concentration.

Obtained results confirm our hypothesis that androgens are important factors regulating the maintenance of pregnancy in pigs.

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