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Alterations in luteal production of androstenedione, testosterone, and estrone, but not estradiol, during mid- and late pregnancy in pigs: Effects of androgen deficiency

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ABSTRACT

Recently, we have found that flutamide-induced androgen deficiency altered progesterone production in the porcine corpus luteum (CL) during mid- and late pregnancy. Herein, we tested whether flutamide administration subsequently influences androgen and estrogen metabolism in the CL of pregnancy. Pregnant gilts were treated with flutamide between Days 43 and 49 (GD50F), 83 and 89 (GD90F), or 101 and 107 (GD108F) of gestation. Corpora lutea (CLs) were collected from treated and nontreated (control) pigs. The concentrations of androstenedione (A4), testosterone (T), estrone (E1), and estradiol (E2) together with the levels of expression of mRNAs and proteins for cytochrome P450 17α-hydroxylase/c17-20 lyase (CYP17A1), 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1), cytochrome P450 aromatase (CYP19A1), and 17β-hydroxysteroid dehydrogenase type 7 (17β-HSD7) were measured in the CL of control and flutamide-treated animals. Steroidogenic enzymes were also immunolocalized in luteal tissues. The luteal concentrations of A4 and T were higher in the GD50F ($P = 0.006$, $P = 0.03$) and GD108F ($P = 0.005$, $P = 0.035$) groups, but lower in the GD90F ($P = 0.004$, $P = 0.014$) group. The E1 level was greater only in the GD90F ($P = 0.03$) and GD108F ($P = 0.035$) groups, whereas E2 concentration was not affected by flutamide treatment. Increased luteal CYP17A1 mRNA and protein expression was found in the GD50F ($P = 0.002$, $P = 0.03$) and GD108F ($P = 0.0026$, $P = 0.03$) groups, but reduced in the GD90F ($P = 0.002$, $P = 0.03$) group. mRNA of 17β-HSD1 was upregulated in the GD50F ($P = 0.0005$) group, but downregulated in the GD90F ($P = 0.002$) and GD108F ($P = 0.0005$) groups. In contrast, 17β-HSD1 protein expression was higher in the GD50F and GD108F ($P = 0.03$) groups, but lower in the GD90F ($P = 0.03$) group. Both CYP19A1 mRNA and protein levels were greater in the GD90F ($P = 0.001$, $P = 0.028$) and GD108F ($P = 0.005$, $P = 0.03$) groups. Neither 17β-HSD7 mRNA nor protein level were affected by flutamide exposure. Both CYP17A1 and 17β-HSD1 were immunolocalized exclusively in small luteal cells, whereas CYP19A1 and 17β-HSD7 were found in large luteal cells of control and flutamide-treated CLs. Overall, flutamide administration led to the alterations in A4, T, and E1, but not in E2, production in the CL of pregnancy in pigs, probably because of disrupted steroidogenic enzymes expression. These changes suggest that androgens are important modulators of luteal function during pregnancy in pigs.

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1. Introduction

Over the past few years, farm animal species, including pigs, have been increasingly used as experimental models to investigate endocrine disruption related to female reproduction [1]. The rationale for such studies in farm animals is that pigs, sheep, and cows are more appropriate models than rodents for understanding human reproduction [2]. According to the literature, recent attention has been especially focused on environmental factors interfering with endogenous androgen action [3,4]. Keeping in mind the crucial role of androgens in female reproductive organs [5], each animal model that mimics androgen deficiency is useful in elucidating the impact of androgens/antiandrogens on female fertility efficacy.

In pigs, successful pregnancy and parturition depend on the adequate luteal function and secretion of progesterone (P4) [6]. The porcine corpus luteum (CL) is the main source of P4 throughout the entire gestation; however, it is also capable of producing androgens and estrogens [7–9]. These steroids act *via* their cognate receptors as autocrine and paracrine factors and exert intrinsic effects to maintain optimal CL function [10,11]. For example, in pregnant rats androstenedione (A4) has a stimulatory effect on P4 production [12] and prevents luteal regression by inhibition of apoptosis [13], whereas estradiol (E2) induces steroidogenesis and hypertrophy of luteal cells accompanied by proliferation of vascular endothelial cells [14]. To date, limited knowledge exists regarding the direct effects of androgens or the result of their conversion to estrogens in the porcine CL of pregnancy.

Of particular interest, porcine luteal tissue is a potential target for androgens and estrogens, because their receptors are localized there during pregnancy. The expression of androgen receptors (ARs) was observed until late pregnancy in pigs. Interestingly, luteal cells showed a nuclear pattern of staining by Day 90 of gestation and cytoplasmic subsequently [8]. Estrogens influence porcine luteal tissue only *via* estrogen receptor β. This receptor is found throughout the entire gestation. Until Day 70 of gestation, estrogen receptor β is expressed exclusively by small luteal cells, whereas later in pregnancy this receptor was observed in both small and large luteal cells [15]. On the basis of these findings, we hypothesize a prominent action of androgens and estrogens in the CL during late pregnancy, which in turn may be essential for the maintenance of porcine luteal function.

Steroidogenesis in the CL of pregnancy begins with the production of P4, which is subsequently metabolized to androgens. The enzyme cytochrome P450 17α-hydroxylase/c17-20 lyase (CYP17A1) is involved in A4 synthesis, whereas 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) converts A4 to testosterone (T), which can be aromatized to E2 directly. In luteal tissue, A4 is the major substrate for cytochrome P450 aromatase (CYP19A1); it is converted to estrone (E1) and then to E2 by 17β-hydroxysteroid dehydrogenase type 7 (17β-HSD7) [16]. It is currently thought that small luteal cells produce the most androgens, whereas large luteal cells are responsible for converting androgens to estrogens [17].

Following our previous results obtained using *in vivo* animal model generated for studying androgen deficiency, we have found that the antiandrogen flutamide alters the luteal production and metabolism of P4 during mid- and late pregnancy in pigs [18]. Therefore, the question arises whether exposure to flutamide affects further steroidogenic steps within the porcine CL of pregnancy? Along this issue, the specific objectives of the present research were to determine (1) luteal concentrations of A4, T, E1, and E2; (2) luteal expression of mRNAs and proteins for CYP17A1, 17β-HSD1, CYP19A1, and 17β-HSD7; and (3) immunolocalization of CYP17A1, 17β-HSD1, CYP19A1, and 17β-HSD7 in CLs on Days 50, 90, and 108 of pregnancy in pigs.

2. Materials and methods

2.1. Ethics experimentation

The animal protocols were conducted in accordance with the national guidelines and approved by the Local Ethics Committee at the Jagiellonian University in Krakow, Poland (approval no. 122/2009). Surgical procedures were performed by a veterinarian.

2.2. Experimental design

Porcine CLs of pregnancy used in the present investigation were derived from the same experimental animals that were examined in our previous study [18]. Briefly, 12 sexually mature crossbred gilts (Large White × Polish Landrace; weighing 109.5 ± 7.5 kg) of similar age (~10 months) and genetic background were housed at the same farm conditions with food and water *ad libitum*. Animals with at least one normal estrus symptom were checked daily for other estrus signs. After two consecutive estrous cycles, gilts were mated to a fertile boar at the onset of estrus and again 12 and 24 hours later. The first day of estrus was designated as Day 0. Pregnant animals were fed according to the nutritional recommendations for pregnant pigs. Randomly assigned pregnant gilts were treated with the antiandrogen flutamide (Sigma-Aldrich, St. Louis, MO, USA) between Days (1) 43 and 49 of gestation (GD50F), (2) 83 and 89 of gestation (GD90F), or (3) 101 and 107 of gestation (GD108F). Flutamide was suspended in corn oil and injected subcutaneously daily for 7 days at a dose of 50 mg/kg body weight (bw). For each flutamide-treated group, a respective control group given a vehicle only (corn oil) was established (GD50C, GD90C, and GD108C). The days of pregnancy chosen for flutamide treatments reflect the periods of midpregnancy (GD50), late pregnancy (GD90), and around parturition (GD108).

2.3. Corpora lutea collection

Pregnant gilts were fasted for 12 hours before surgery, but had free access to water. For premedication, the gilts received atropine (0.05 mg/kg bw, im; Biowet, Gorzow Wielkopolski, Poland) and azaperone (2 mg/kg bw, im; Stresnil, Janssen Pharmaceutica, Beerse, Belgium). Once the sow was sedated (20–30 minutes later), anesthesia was induced by injecting thiopental (10 mg/kg bw; Sandoz GmbH, Vienna, Austria) into an ear vein, and a silastic catheter (o.d. 2.4 mm; i.d. 1.8 mm) was aseptically placed