Domestic Animal Endocrinology 59 (2017) 81–89

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

The impact of flutamide on prostaglandin $F_{2\alpha}$ synthase and prostaglandin $F_{2\alpha}$ receptor expression, and prostaglandin $F_{2\alpha}$ concentration in the porcine corpus luteum of pregnancy

M. Grzesiak [a,b,*], K. Knapczyk-Stwora [a], M. Slomczynska [a]

[a] Department of Endocrinology, Institute of Zoology, Jagiellonian University in Krakow, Gronostajowa 9, 30-383 Krakow, Poland
[b] Department of Animal Physiology and Endocrinology, University of Agriculture in Krakow, Al. Mickiewicza 24/28, 30-059 Krakow, Poland

ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 23 November 2016
Accepted 1 December 2016

Keywords:
Flutamide
PGFS
PTGFR
Corpus luteum
Pregnancy
Pig

ABSTRACT

Recently, we have indicated that flutamide-induced androgen deficiency diminished progesterone production in the porcine corpus luteum (CL) during late pregnancy and before parturition, as a sign of functional luteolysis. In pigs, the main luteolytic factor is prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which acts via specific receptors (PTGFRs), and its biosynthesis is catalyzed by prostaglandin $F_{2\alpha}$ synthase (PGFS). The present study investigated the impact of flutamide on luteal PGFS and PTGFR expression, as well as intraluteal $PGF_{2\alpha}$ content during pregnancy in pigs. Flutamide (50 mg/kg BW per day, for 7 d) or corn oil (control groups) were administered subcutaneously into pregnant gilts (n = 3 per group) between 83 and 89 (GD90) or 101–107 (GD108) days of gestation (GD). On GD90 and GD108 ovaries were collected and CLs were obtained. Real-time PCR and Western blot analyses were conducted to quantify PGFS and PTGFR mRNA and protein expression, respectively. In addition, immunohistochemical localization of both proteins was performed and the concentration of $PGF_{2\alpha}$ was analyzed by enzyme immunoassay method. Flutamide caused upregulation of PGFS mRNA and protein in GD90F ($P = 0.008$; $P = 0.008$, respectively) and GD108F ($P = 0.041$; $P = 0.009$, respectively) groups. The level of PTGFR mRNA increased only in the GD90F ($P = 0.007$) group, whereas PTGFR protein expression was greater in both gestational periods ($P = 0.035$; $P = 0.038$, respectively). On GD90 PGFS was immunolocalized in the cytoplasm of large luteal cells only, whereas on GD108, sparse small luteal cells also displayed positive staining. PTGFR showed membranous localization within large luteal cells on both days of pregnancy. In luteal tissue, $PGF_{2\alpha}$ concentration was greater after flutamide exposure on both days ($P = 0.041$; $P = 0.038$, respectively), when compared with control groups. Overall, the enhanced luteal $PGF_{2\alpha}$ content due to increased PGFS expression after flutamide administration might contribute to premature CL regression. Moreover, higher PTGFR protein levels indicate enhanced sensitivity of luteal cells to $PGF_{2\alpha}$ under androgen deficiency.

1. Introduction

A growing body of evidence suggests that exposure to environmental substances with hormonal activity (estrogenic, antiestrogenic, androgenic, antiandrogenic) designated as endocrine disrupting chemicals (EDCs)

* Corresponding author. Tel.: +48 12 662 40 43; fax: +48 12 633 33 07.
E-mail address: m.grzesiak@ur.krakow.pl (M. Grzesiak).

0739-7240/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.domaniend.2016.12.001

contributes to problems with animal fertility [1]. Recently, we have developed the pig model mimicking physiological androgen deficiency using specific antiandrogen flutamide [2–4]. Disruption of androgen signaling during critical periods of gestation in pigs influenced the expression of many genes related to reproductive processes within fetal and maternal ovaries [5,6]. Importantly, we have revealed alterations in luteal production and metabolism of progesterone in the porcine corpus luteum (CL) during late pregnancy and before parturition [4].

In many mammals, the establishment of pregnancy is dependent on the presence of functional CLs [7]. In pigs, the CL of pregnancy is the major source of progesterone throughout the entire gestational period due to insufficient production of this hormone in the placenta [8]. Notably, ovariectomy at any time during the gestation causes immediate abortion of the fetuses [9]. This is evidence that a functional CL is essential for the continuation of normal pregnancy in sows.

The primary luteolytic factor in several domestic species, including pigs, is uterine-derived prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) [10]. It is produced predominantly by terminal $PGF_{2\alpha}$ synthase (PGFS) within the endometrium and acts via specific seven-transmembrane G protein-coupled receptor (PTGFR) localized in large luteal cells [11]. Intraluteal action of $PGF_{2\alpha}$ causes a decrease in steroidogenic capacity and diminished production of progesterone [12]. In addition, an autoamplification pathway in which $PGF_{2\alpha}$ stimulates its own luteal production has been found [13]. In sheep [14] and pigs [15], $PGF_{2\alpha}$ analog treatment markedly increased the production of $PGF_{2\alpha}$ by luteal cells in vitro. Furthermore, Waclawik et al [16] confirmed the expression of prostaglandin synthesis pathway enzymes in the porcine CL during the estrous cycle and early pregnancy. Since intraluteal production of $PGF_{2\alpha}$ is considered to be an important part of the luteolytic machinery [13], the examination of new factors that might regulate $PGF_{2\alpha}$-mediated regression in the CL is warranted.

Previously, we have found that experimentally induced androgen deficiency led to reduced luteal progesterone concentration and decreased expression of related steroidogenic enzymes, CYP11A1, and 3β-HSD, during the late gestational period in pigs [4]. These findings suggest the occurrence of functional luteolysis. To our knowledge, there is no information concerning the role of androgens in luteal regression during pregnancy in pigs. Therefore, the aim of the present study was to examine whether anti-androgen flutamide treatment affected the expression of PGFS and PTGFR in the porcine CL, as well as intraluteal $PGF_{2\alpha}$ concentration on days 90 and 108 of pregnancy.

2. Materials and methods

2.1. Animals and experimental design

Twelve sexually mature crossbred gilts (Large White × Polish Landrace) of similar age (~10 mo), BW (109.5 ± 7.5 kg) and genetic background were kept under the same farm conditions. Detection of estrus was based on typical estrous symptoms (swelling of the vulva and enlargement of the clitoris, the presence of a sticky discharge, increased activity and vocalization, elevation of the ears and immobilization in response to manual back pressure). Animals with at least one estrous symptom were checked daily for other estrous signs. After 2 consecutive estrous cycles, gilts were mated with a fertile boar at the onset of estrus and again 12 and 24 h later. The day on which mating took place was considered the first gestation day (GD). Pregnant animals were fed according to nutritional recommendations for pregnant pigs. Randomly assigned pregnant gilts were allotted into 2 experimental groups (n = 3/group) and injected with the anti-androgen flutamide (Sigma-Aldrich, St. Louis, MO, USA) between days 83 and 89 of gestation (GD90F) or 101 and 107 of gestation (GD108F). Flutamide (2-methyl-N-[4-nitro-30-(trifluoromethyl)-phenyl]propamide) was suspended in corn oil and injected subcutaneously daily for 7 d at a dose of 50 mg/kg BW. The dose and frequency were chosen based on data published by our group [3] to antagonize androgen action via androgen receptors (ARs) without exerting toxic effects on the gilts or their offspring. For each flutamide-treated group, a respective control group (n = 3/group) given a vehicle only (corn oil) was established (GD90C, GD108C). The days of pregnancy chosen for flutamide treatments reflect the periods of late pregnancy (GD90) and around parturition (GD108).

All experiments were approved by the Local Ethics Committee at the Jagiellonian University in Krakow (approval no. 122/2009) and the surgical procedures were performed by a veterinarian.

2.2. CL collection

Pregnant gilts were fasted for 12 h before surgery but had free access to water. For premedication, the gilts received atropine (0.05-mg/kg BW i.m.; Biowet, Gorzow Wielkopolski, Poland) and azaperone (2-mg/kg BW i.m.; Stresnil, Janssen Pharmaceutica, Beerse, Belgium). Once the sow was sedated (20–30 min later), anesthesia was induced by injecting thiopental (10 mg/kg BW; SandozGmbH, Austria) into an ear vein, and a silastic catheter (o.d. 2.4 mm; 1.d. 1.8 mm) was aseptically placed through the desensitized skin into the external jugular vein for continuous infusion of thiopental until deep anesthesia. Bilateral porcine ovaries were obtained on day 90 (GD90) or 108 (GD108) of gestation. Fresh CLs were excised from the right and left ovaries of control (C; n = 8–11/group) and flutamide-treated (F; n = 8–11/group) animals. Each CL was cut into four pieces, which were either snap frozen in liquid nitrogen for real-time PCR, Western blot and enzyme immunoassay (EIA) analyses or fixed in 10% (v/v) neutral buffered formalin for immunohistochemistry.

2.3. RNA isolation and TaqMan real-time PCR analysis

Total cellular RNA from CLs samples was extracted with TRI Reagent solution (Ambion, Austin, TX) following the manufacturer's instructions. RNA quality and quantity was determined by the A260/A280 ratio using the NanoDrop ND-2000 Spectrophotometer (Thermo Scientific, Wilmington, DE), and the RNA integrity was evaluated through the observation of 18S and 28S ribosomal bands after electrophoresis on 1% (v/v) formaldehyde-agarose gel. The first