Theriogenology 89 (2017) 9–19

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Disorders in barrier protein mRNA expression and placenta secretory activity under the influence of polychlorinated biphenyls *in vitro*

A. Wojciechowska, J. Mlynarczuk*, J. Kotwica

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form 6 October 2016
Accepted 7 October 2016

Keywords:
Polychlorinated biphenyls
Placenta
Connexins
Prostaglandins
Oxytocin
Cow

ABSTRACT

Pregnancy disorders are often correlated with the presence of organic pollutants in the tissues of living bodies. The aim of this study was to investigate the effects (over 24 and 48 hours) of polychlorinated biphenyls (PCBs) 153, 126, and 77 at doses of 1, 10, and 100 ng/mL on barrier function and secretory activity in cow placentome sections collected during the second trimester of pregnancy. None of the PCBs affected the viability of the sections ($P > 0.05$). Polychlorinated biphenyl 153 decreased ($P < 0.05$) connexin 26 (*Cx 26*) mRNA expression, and all three PCBs reduced ($P < 0.05$) *Cx 43* mRNA expression. *Cx 32* mRNA expression showed a downward trend ($P > 0.05$) under the influence of PCBs 126 and 77. Moreover, PCBs 153 and 126 increased keratin 8 (*KRT8*) mRNA expression, whereas all PCBs decreased ($P < 0.05$) placenta specific protein 1 (*PLAC-1*) mRNA expression without changing ($P > 0.05$) hypoxia inducible factor 1α (*HIF1α*) mRNA expression. Concomitantly, PCBs 153 and 126 stimulated ($P < 0.05$) *cyclooxygenase 2* (*COX-2*) mRNA expression, all PCBs increased ($P < 0.05$) prostaglandin E2 synthase (*PGES*) mRNA expression, and PCBs 126 and 77 increased prostaglandin E2 (PGE2) secretion. All three PCBs decreased ($P < 0.05$) prostaglandin F2α synthase (*PGFS*) mRNA expression and prostaglandin F2α (PGF2α) secretion. In addition, all three PCBs increased ($P < 0.05$) neurophysin I/oxytocin (*NP-I/OT*) mRNA expression and OT secretion but did not affect peptidyl-glycine-α-amidating monooxygenase (*PGA*) mRNA expression ($P > 0.05$). Moreover, the PCBs increased ($P < 0.05$) estradiol (E2) secretion, whereas progesterone (P4) secretion remained unchanged ($P > 0.05$). These changes could affect trophoblast invasion and uterine contractility and thus impact the course of gestation and/or fetal development in the cow.

1. Introduction

Losses associated with embryo mortality and miscarriages in farm animals significantly reduce the profitability of livestock production [1]. Although the greatest risk of pregnancy loss occurs in the first trimester, the loss rate remains high throughout pregnancy in cows [2,3]. One cause for disorders during the course of pregnancy [4,5], including those in humans [6], is increased environmental pollution by xenobiotics (XBs). This group includes a number of polychlorinated biphenyls (PCBs) that have been detected in the umbilical cord, amniotic and allantoic fluids, maternal blood, and breast milk [7,8]. These PCBs disrupt the function of the reproductive system in animals and humans [9,10], probably also by affecting the placental barrier. Population studies and the application of *in vitro* models, that is, cell lines [11], chorion membranes [12], and endometrial slices [13], have not generated substantial insights into how placental function responds to XBs. We believe that this problem can be more thoroughly investigated using bovine placentome sections, which contain functional maternal–fetal connections [14]. This model

* Corresponding author. Tel.: +48 89 539 31 18; fax: +48 89 539 31 46.
E-mail address: j.mlynarczuk@pan.olsztyn.pl (J. Mlynarczuk).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.10.007

enables assessment of the impact of various factors, including PCBs, on genes involved in the stabilization of placental connections *in vitro*.

In ruminants, the spuria cotyledonary placenta consists of placentomes that contain fetal trophectodermal cotyledons and uterine mother-originated endometrial caruncles [15,16]. Most of the fetal surface of the placenta is created by mononuclear cells, which participate in the exchange of nutrients between the mother and fetus [17]. Binucleate cells (BNCs), which represent approximately 15% to 20% of trophectodermal cells, migrate across the maternal–fetal border and fuse with the endometrial cells to form syncytial plates [15]. This process leads to gradual transformation of chorionic epithelial connections and the creation of a placenta with some syndesmochorial characteristics [15,16]. Both types of trophoblast cells join with endometrial cells to form the placental barrier. The most common type of connection between cells within the barrier is the gap junction, which is composed of connexins (Cxs) [18]. This group of transmembrane proteins determines the integrity of the placental barrier [19]. In particular, Cx 26 is responsible for the migratory properties of BNCs [15], whereas Cx 32 is involved in villous differentiation and placental growth [20], and Cx 43 is an important regulator of cell growth [21]. Moreover, both Cx 26 and Cx 43 may be involved in the fusion of BNCs with endometrial cells [22] and in regulating the depth of trophoblast invasion [19,23]. Keratin 8 (KRT8) also maintains the integrity of maternal–fetal connections and is a marker for placenta barrier function [24,25]. The significance of KRT8 is confirmed by the high embryo mortality rate observed in *KRT8* gene-knockout mice at midgestation [26].

The placental barrier is responsible for the maintenance of proper gas exchange and nutrient supplementation for the growing fetus, as well as disposal of its metabolic waste. Moreover, many hormones are synthesized in the placental barrier, such as placental lactogen [27], estradiol (E2) [28], P4 [29], and prostaglandins (PGs) [30], which are involved in fetal growth. Prostaglandins F2α (PGF2α) and E2 (PGE2) are produced by midgestation bovine BNCs from PGH2 by the PGF and PGE synthases (PGFS and PGES, respectively). The precursor of PGH2 is transformed from arachidonic acid (AA) by cyclooxygenase (COX), which is the rate-limiting step in PG biosynthesis. PGs are involved in implantation, early embryonic development, placental blood flow regulation, and parturition [31,32]. Placental PGF2α mediates the stimulation of myometrial contractions, whereas PGE2 causes myometrium excitation or relaxation [33,34]. In cows, the placenta also synthesizes oxytocin (OT) [35]. The precursor of OT is encoded by neurophysin I/oxytocin (NP-I/OT), and active OT is released by peptidyl glycine-α-amidating mono-oxygenase (PGA), which is the terminal enzyme in posttranslational OT processing [36]. OT also participates in uterine contraction, but its influence on myometrium activity during pregnancy is abolished by P4 block until parturition [37]. Thus, both PGs and OT are essential regulators of uterine motility and, hence, pregnancy maintenance.

Polychlorinated biphenyls, due to their physical and chemical properties, were widely used in industry until the 1970s [38]. However, their high resistance to degradation [39] and their lipophilic nature have resulted in their widespread distribution in the environment, and accumulation in the tissues of living bodies [40]. Depending on the number of chlorine atoms and their locations on the biphenyl structure, PCBs affect the reproductive system in various ways. *Ortho*-substituted congeners may mimic estradiol (E2) [41], whereas non–*ortho*-substituted congeners, which are similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, can act via the aryl-hydrocarbon receptor (AhR) and activate cytochrome P450 enzymes (CYP450) involved in the metabolism of exogenous and endogenous compounds [42]. Some PCBs can be bound by both AhR and estradiol receptor (ER) [43]. As endocrine disruptors, PCBs can block or mimic the actions of hormones and neurotransmitters or alter the metabolism of some regulatory factors; therefore, they affect many physiological processes associated with the reproductive system [44–46]. When present at detectable concentrations in the reproductive tract, XBs [47,48] disturb the secretory function of the ovary, uterus, and oviduct [49,50] and the motor activity of the uterus and oviduct [51] in cows both during the estrous cycle and in pregnancy. Furthermore, the presence of XBs in maternal and fetal blood and fetal fluids [52] can lead to changes within maternal–fetal connections and, consequently, pregnancy disorders [53,54]. This is particularly important for the cow, in which the trophoblast has a limited degree of invasiveness [19].

Therefore, the aim of this study was to investigate the effects of PCBs 153, 126, and 77 on the mRNA expression levels of connexins (*Cx 26*, *Cx 32* and *Cx 43*) and *KRT8*, which are responsible for placental barrier function, as well as on the synthesis and secretion of PGE2, PGF2α, and OT from placentome sections. Moreover, the following additional parameters were measured: E2 and P4 secretion from placental sections; mRNA expression of placenta specific protein 1 (*PLAC-1*) [55], which is indicative of proliferative activity; and mRNA expression of hypoxia indicible factor 1α (*HIF1α*) [56], which is responsible for the state of tissue oxygenation.

2. Materials and methods

2.1. Preparation and incubation of placentome sections

Uteri were collected from 12 cows in the second trimester of pregnancy 15 to 20 minutes after slaughter. The period of pregnancy was classified based on morphologic observations of the reproductive tract and fetus [57,58] and the Keller formula calculation: x (x + 2) = length of fetus, where x is the gestational age. The uteri were placed in ice-cold 0.9% NaCl supplemented with 10-IU/mL penicillin, 100-μg/mL streptomycin, 2-μg/mL amphotericin, and 100-μg/ml L-glutamine and transported to the laboratory within 1 hour. The placentomes were cut into 1- to 2-mm-thick slices using a razor blade, and they were divided into 60- to 80-mg sections with complete fetal–maternal connections, as described by Wojciechowska et al. [14]. The placentome sections were incubated in M-199 medium supplemented with 2% fetal calf serum (FCS) and 10% amniotic fluid (AF) under a controlled atmosphere (95% O_2 + 5% CO_2) at 37.5 °C. PCBs (purity 98%)