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ScienceDirect
Theriogenology 75 (2011) 877–886
Theriogenology
www.theriojournal.com

Expression and functional implications of Peroxisome Proliferator—Activated Receptor Gamma (PPARγ) in canine reproductive tissues during normal pregnancy and parturition and at antiprogestin induced abortion

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Received 1 June 2010; received in revised form 19 October 2010; accepted 19 October 2010

Abstract

PPARγ is a nuclear hormone receptor of the PPAR family of transcription factors closely related to the steroid hormone receptors serving multiple roles in regulating reproductive function. Endogenous factors from the arachidonic acid metabolites group serve as ligands for PPARs. PPARγ modifies the steroidogenic capacity of reproductive tissues and has been defined as a key mediator of biological actions of progesterone receptor in granulosa cells; it modulates biochemical and morphological placental trophoblast differentiation during implantation and placentation. However, no such information is available for the dog. Hence, the expression and possible functions of PPARγ were assessed in corpora lutea (CL) and utero/placental (Ut/Pl) compartment collected from bitches (n = 3 to 5) on days 8 to 12 (pre-implantation), 18 to 25 (post-implantation), 35 to 40 (mid-gestation) of pregnancy and at prepartal luteolysis. Additionally, 10 mid-pregnant bitches were treated with the antiprogestin Aglepristone [10mg/Kg bw (2x/24h)]; ovariohysterectomy was 24h and 72h after the 2nd treatment. Of the two PPARγ isoforms, PPARγ1 was the only isoform clearly detectable in all canine CL and utero/placental samples. The luteal PPARγ was upregulated throughout pregnancy, a prepartal downregulation was observed. Placental expression of PPARγ was elevated after implantation and at mid-gestation, followed by a prepartal downregulation. All changes were more pronounced at the protein-level suggesting that the PPARγ expression may be regulated at the post-transcriptional level. Within the CL PPARγ was localized to the luteal cells. Placental expression was targeted solely to the fetal trophoblast cells; a regulatory role of PPARγ in canine placental development possibly through influencing the invasion of fetal trophoblast cells is suggested. Treatment with Aglepristone led to downregulation of *PPARγ* in either compartment, implying the functional interrelationship with progesterone receptor.

Keywords: PPARγ; Corpus luteum; Placenta; Dog

1. Introduction

Dogs are monoestrus, predominantly nonseasonal breeders, with up to several months of anoestrus between active reproductive phases. The endocrine mechanisms controlling the reproductive function in dog are

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doi:10.1016/j.theriogenology.2010.10.030

still unclear. As the placenta does not secrete progesterone (P4), the Corpora lutea (CL) are required for maintenance of pregnancy throughout gestation being the only source of P4. Interestingly the functional life span is almost identical in pregnant and non-pregnant animals [1,2]. The CL are independent of gonadotropic support during the first 20 to 30 days of the dioestrus [3,4,5]; afterwards prolactin is an essential luteotropic factor [6,7]. Intriguingly, the luteal regression occurs in spite of an increased availability of gonadotropic support [8,9]. Based on the hypothesis that CL-derived $PGF_{2\alpha}$ might act as a luteolytic agent in a para/autocrine manner we have addressed the role of luteal prostaglandins in luteal regression in the non-pregnant and pregnant bitch in a number of studies [10–14]. However, the results obtained were not in support of this hypothesis, since $PGF_{2\alpha}$-synthase (PGFS), the main downstream enzyme leading to the formation of $PGF_{2\alpha}$ is either absent or present at very low level in canine CL [11,13]. Moreover, from the spatio-temporal expression pattern of the Cycloogygenase 2 (COX2; PTGS2) and PGE_2-synthase (PGES) in canine CL during the dioestrus [10,12,13], luteotropic effects of the locally produced PGE_2 have been suggested. This would explain the above-mentioned independency of luteal function from gonadotropic support during this period of time.

Furthermore, the potential role of placenta derived prostaglandins in regulating the CL function in pregnant bitches has been investigated [14]. It has been clearly shown that all important components of the prostaglandin system are expressed in the utero/placental unit of the pregnant dog. The data obtained strongly suggest that the prepartal $PGF_{2\alpha}$ increase in maternal blood plasma that has been shown in conjunction with the onset of parturition [15], originates from the upregulated PGTS2 expression in the fetal trophoblast. The withdrawal of progesterone, acting within a feto-maternal feedback loop, seems to have a signalling function in this process [14]. This resembles the situation in cattle, where placental PTGS2 is strongly upregulated during parturition, primarily in the uninucleated trophoblast cells, implying its contribution to the prepartal $PGF_{2\alpha}$ output [16].

Classically prostaglandins act through their G protein coupled receptors designated as PTGFR in case of $PGF_{2\alpha}$ and PRGER1-PGTER4 for PGE_2. Upon the activation their signals are transduced through different intracellular signalling pathways including phospholipase C -induced second messengers inositol triphosphate and diacyl glycerol, an activator of protein kinase C (PTGER1, PTGER3, PTGFR) [17], or are coupled to cAMP formation and hence activation of protein kinases A (PTGER2 and PTGER4) [18]. Various endogenous factors, however, including eicosanoids, fatty acids and cyclooxygenase-, lipooxygenase-, and epoxygenase- derived metabolites of arachidonic acid, which are known to regulate function of reproductive tissues, can alternatively exert their role via the peroxisome proliferator-activated receptor gamma (PPARγ) (for review, see [19]). In other words, PPARγ can serve as an alternative, intracellular receptor for those factors, including prostaglandins. Like the progesterone- and estrogen- receptors, similarly PPARγ is a nuclear hormone receptor. It belongs to the PPAR family of transcription factors that have essential roles in controlling cellular differentiation, development and metabolism (for review see [19,20]).

The expression of PPARγ in reproductive tissues of different species and its involvement in regulation of gonadal function are well documented [19,21]. Acting mostly in agonist-dependent mechanisms, PPARγ uses different intracellular pathways regulating the function of reproductive organs, e.g. by modulating the expression of their steroidogenic enzymes. Those include both the direct and indirect effects on genes expression [19]. Hence, the cooperation and/or interaction of different signalling pathways is a part of the PPARγ-mediated cellular events in reproductive tissues. The critical role of PPARγ in regulating ovarian function became obvious after Kim et al [22] showed that the process of ovulation is impaired due to a defective follicular rupture in mice with a granulosa-specific deletion of PPARγ. Kim et al [22] also specified the action of PPARγ as a crucial downstream molecular target that conveys progesterone receptor (PR) action.

Recently the expression and endocrine function of PPARγ has been shown also in relation to the Leydig cells function [23]; an indirect effect of PPARγ on the expression and function of steroidogenic acute regulatory protein (StAR) and hence the steroidogenesis, acting probably through the AP-1 family member c-JUN has been demonstrated. Thus, PPARγ became a new player in regulating not only ovarian, but potentially also testicular steroidogenic function [23].

A further important role of PPARγ relates to the processes of implantation and placentation where it acts to modulate the biochemical and morphological differentiation of placental trophoblast [24,25]. In human term placenta PPARγ is localized predominantly in the feto-maternal contact zone and targeted to the fetal trophoblast cells [26,27], an observation that further