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Immunohistochemical localization of CD31, NOTCH1 and JAGGED1 proteins in experimentally induced polycystic ovaries of immature rats

Anita Skrtic [a,*], Lea Sokolic [b], Ana Borovecki [a], Jagoda Rosa [c], Vanja Fenzl [a]

[a] Department of Gynaecological Pathology and GY & OB, University Hospital Merkur, Zajceva 19, 10000 Zagreb, Croatia
[b] Department of Laboratory Medicine, University Hospital Vuk Vrhovac, University of Zagreb, Dugi dol 4a, 10000 Zagreb, Croatia
[c] Department of Physiology, School of Dental Medicine, University of Zagreb, Salata 3, 10000 Zagreb, Croatia

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ABSTRACT

We analyzed histomorphometrical changes and blood vessel immunohistochemical staining of CD31, NOTCH1 and JAGGED1 in induced polycystic ovaries of immature female Wistar rats, as well as serum hormone levels. The rats were randomly divided into control (n=18) and treated (n=18) groups. Treated animals received intramuscularly testosteronenantat weekly (0.1 mg/g). Controls received the same amount of ricinus oil. Rats were weighed daily. Control and treated subgroups (6 rats per subgroup) were subsequently sacrificed after 21, 28 and 35 days of treatment. In ovaries of treated rats we found large cystic follicles, thick stromal tissue, many atretic preantral follicles, no ovulation and a thinner granulosa cell layer. CD31 stained blood vessels in the theca layer were reduced, with reduced JAGGED1 and NOTCH1 immunostaining. In controls, preantral and antral follicles were larger than in the treated group. Treated animals showed statistically significant lower progesterone and higher testosterone levels. They gained more weight than controls. Reduced immunostaining for NOTCH1 and JAGGED1 of reduced blood vessels of the theca layer was found in all stages of folliculogenesis with a distinct reduction in cystic and atretic follicles. Our results provide evidence of intrinsic abnormality during all stages of folliculogenesis in polycystic ovaries and this may result from crosstalk between circulating gonadotropins and follicular angiogenesis.

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulation in woman of reproductive age (Dunaif, 1997) and may occur in 5–10% of women of reproductive age (Aherne, 2004). The criteria defining the syndrome include clinical and/or biochemical evidence of hyperandrogenism, oligo-/anovulation and polycystic ovaries with exclusion of other etiologies (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). The syndrome exists either as a minimal biochemical disorder with minor clinical disturbances of the menstrual cycle or it can induce numerous major metabolic and reproductive disorders such as insulin resistance, type 2 *Diabetes mellitus*, cardiovascular disease and infertility (Tsilchorozidou et al., 2004). The disorder is often biochemically present soon after menarche or even at the time of adrenarche, but the clinical symptoms of the syndrome can appear at any time during the reproductive period (Legro, 2002). Moreover, adolescent hyperandrogenemia of any origin seems to be associated with the syndrome in reproductive age and results in lower fertility rates (Apter and Vihko, 1990). Hyperandrogenemia as a hallmark of the syndrome has direct effects on neuroendocrine secretion, not only by deregulation of the secretory function of hypothalamic and hypophyseal cells, but also by affecting the expression of the progesterone receptors in that area (Foecking and Levine, 2005). The "steady state" concentrations of gonadotropins and sex steroids are associated with persistent and chronic anovulation. PCOS is characterized by the onset of elevated serum values of androgens, almost exclusively from the ovary (Dunaif and Thomas, 2001). Regardless of whether hyperandrogenemia is due to this or any other origin, it results in cessation of normal follicular development as the luteinizing hormone (LH) ovulatory surge is disabled and normal patterns of both LH and follicle-stimulating hormone (FSH) secretion are disrupted (Urbanek et al., 2005).

In experimental animal models, morphologic, metabolic, endocrine and molecular changes can be induced that mimic PCOS. The most widely held viewpoint is that the syndrome has a multifactorial origin and that current animal models do not

Abbreviations: AF, antral follicle; CF, cystic follicle; CL, corpora lutea; FFPE, formalin fixed paraffin embedded; FSH, follicle-stimulating hormone; HE, hematoxylin and eosin; LH, luteinizing hormone; PAF, preantral follicle; PCO(S), polycystic ovary (syndrome); PF, primary follicle

* Corresponding author. Rebar 115, 10000 Zagreb, Croatia.
Tel.: +385 1 242 00 33.
E-mail address: skrtic.anita@gmail.com (A. Skrtic).

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completely represent the actual "vicious circle" of complex clinical effects of the syndrome, despite the benefit of these experimental models in evaluation of diverse systems or organs (Dunaif and Thomas, 2001; Tsilchorozidou et al., 2004; Urbanek et al., 2005). In several experimental animal models, steroids were used to induce hyperandrogenemia, anovulation and polycystic morphology of the ovaries (Allon et al., 2005; Beloosesky et al., 2004; Foecking and Levine, 2005; Henmi et al., 2001; Perello et al., 2003; Singh, 2005; Tamura et al., 2005). Such experiments were based on prenatal or early postnatal androgen exposure of the animals. Morphologic changes of the animal ovaries appeared in all of these experiments with development of multiple large cystic formations. Absence of ovulation, lack of corpora lutea and full cystic transformation of ovaries in these studies were achieved in time- and dose-dependent treatments.

Ovarian folliculogenesis and formation of corpora lutea are characterized by the development of an angiogenic network localized in the theca layer, which is required to enable physiologic progress of a follicle to a corpus luteum. Angiogenesis participates in most of the pathologic processes of the ovary, cystic formations, benign and malignant tumors, PCOS and hyperstimulation syndrome. Recent studies have shown a critical role of Notch signaling in vascular development in mice (Hrabe de Angelis et al., 1997; Krebs et al., 2000; Uyttendaele et al., 2001; Xue et al., 1999). Endothelial-expressed protein CD31 is a marker of angiogenesis and mediates cell-to-cell adhesion. NOTCH1 and JAGGED1 protein expression is restricted to a subset of ovarian vessels in mature vasculature as well as in neoangiogenic vessels. This expression suggests a complex role of Notch signaling in mouse ovarian folliculogenesis and angiogenesis (Vorontchikhina et al., 2005). Notch signaling receptor–ligand interaction has a highly conserved mechanism and comprises a group of four Notch receptors (Notch 1–4), transmembrane proteins that function as surface receptors, as well as direct regulators of gene transcription. There are two families of ligands (JAGGED1, 2 and Delta-like (Dll) 1, 3, 4), also transmembrane proteins. Binding of ligands on adjacent Notch receptors induces extracellular conformational change of receptors and activating signaling pathway. Notch signaling pathway determines proliferation, differentiation, and apoptosis and thus controls the fate of individual cells (Iso et al., 2003; Weinstein and Lawson, 2002).

Ovarian formation of follicular cysts induced by hyperandrogenemia is still poorly understood. Contact dependent cell-to-cell interactions appear to be significant for ovarian structure and function. Notch pathway components are distributed in the vascular tissues controlling vasculogenesis and angiogenesis. Their role is not only present in normal vascular development, but it is also presumed that these control mechanisms are present in pathological conditions (Iso et al., 2003).

The aim of this study was to follow morphological changes and determine the immunohistochemical localization of CD31 and protein NOTCH1 and JAGGED1 in experimentally androgenized ovaries of immature rats. We followed changes in values of progesterone, testosterone, LH and FSH in blood serum as well as changes in weight gain in the treated and control groups of rats.

Materials and methods

Animals

Female Wistar rats 21 days old from 8 different broods were used from the animal facility of the Physiology department, School of Dental Medicine, University of Zagreb, where the study took place. The rats were weaned between days 23 and 28 post-partum, but were separated from other pups immediately on the first day of the experiment. They were housed in wire cages in a temperature-controlled room (21 ± 1 °C), on a 12 h light–dark cycle, with free access to rat chow and water.

Animals aged 21 days were randomly divided into two experimental groups: a control group (n=18) and a treated group (n=18). On a weekly basis at the same time of the day, animals were given an intramuscular injection with an oil suspension of testosteronenantat (Testoviron®, -Depot-250, Schering, Germany)

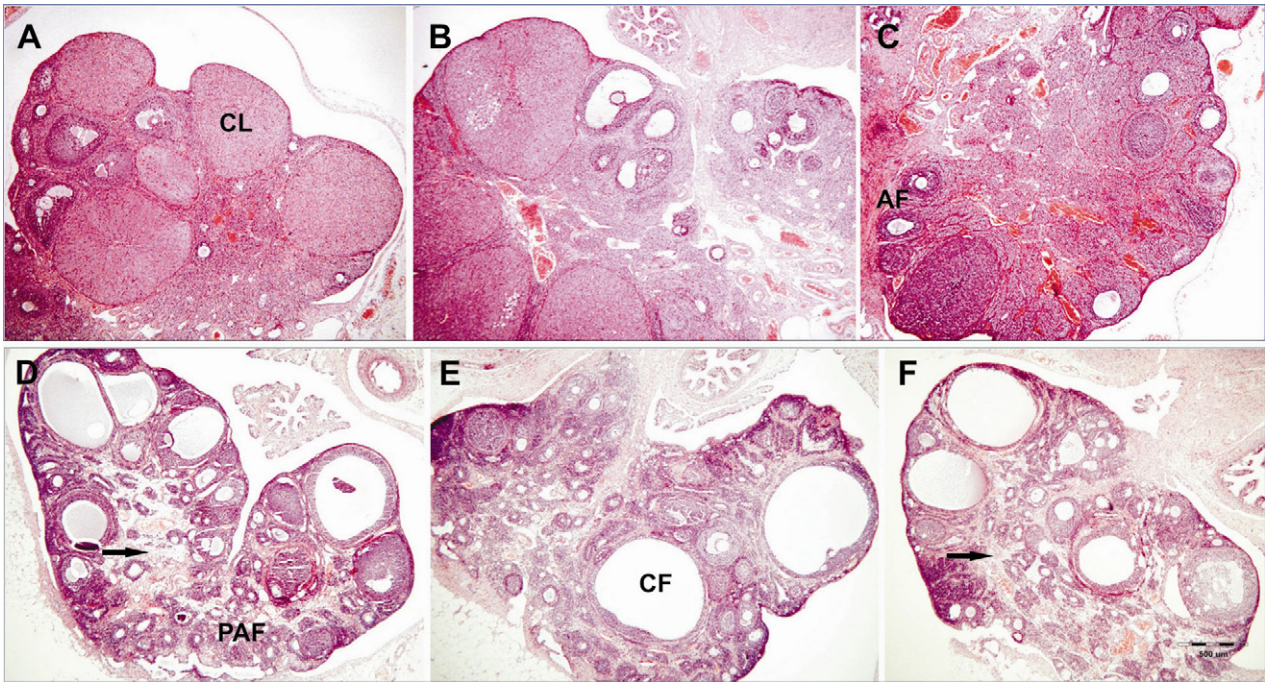

Fig. 1. Histological features of ovaries 21 (A, D), 28 (B, E) and 35 (C, F) days after treatment. Control group (A–C). There are mainly small and medium large antral follicles (AF) and corpora lutea (CL). Treated group (D–F). The ovaries are loaded with atretic preantral follicles (PAF), with large cystic follicle (CF). No evidence of corpora lutea. Ovarian stroma is enlarged (arrow). Scale bar, 500 μm.