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Review

Impact of toxicant exposures on ovarian gap junctions

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ABSTRACT

Ovarian gap junctions function to provide intercellular communication between ovarian cell types and are critical for proper ovarian function. Connexons are communication channels that are comprised of connexin (CX) proteins. Connexins can be regulated through endocrine signals, thus have dynamic expression throughout the estrous cycle. Not surprisingly, ovarian function is negatively affected in mouse models deficient in *Cx* genes; loss of *Gja4* impairs folliculogenesis while ovaries devoid of *Gja1* have reductions in oocyte growth. Chemicals that negatively affect ovarian function, termed ovotoxicants, can directly target *Cx* mRNA or protein abundance. Endocrine disrupting chemicals, medicinal drugs, pesticides, industrial chemicals, polycyclic aromatic hydrocarbons, recreational drugs and dietary components can affect Cx levels and/or function. Also, aging and obesity can impact ovarian Cx's. This review highlights what is currently known about ovotoxicant exposures that impact ovarian gap junction function as well as identifies the many gaps in our knowledge in this area of ovarian biology.

1. Introduction

The ovary is the female reproductive organ responsible for the production of both the female gamete, the oocyte, and two major female sex hormones, 17β-estradiol (E_2) and progesterone (P_4). During embryonic development, oocytes are formed from primordial germ cells and eventually become surrounded by squamous granulosa cells in a follicular structure, termed primordial. The oocyte number encased in primordial follicles is finite at birth and oocytes remain arrested in the diplotene stage of meiosis until ovulation, or they degenerate through atresia [1]. Once the pool of primordial follicles has been depleted, either naturally or chemically-induced, ovarian senescence occurs which is termed as menopause in humans [1]. Premature ovarian failure is defined as ovarian senescence prior to age 40 and affects approximately 1% of women, with often unknown etiology [2].

Gap junction intercellular communication (GJIC) facilitates the exchange of ions, metabolites, Ca^{2+}, inositol phosphates, and/or cyclic nucleotides of up to 1.8 kD in size between cells through contact-dependent mechanisms [3,4]. Oocyte growth and development (as depicted in Fig. 1) depends, at least partly, upon a supply of nutrients, amino acids, glucose metabolites, and nucleotides transmitted from follicle cells *via* gap junctions (GJ). Interconnection of ovarian cells *via* GJ is observed between the innermost layer of cumulus cells and the oocyte, between adjacent cumulus cells, between granulosa cells and also between cumulus and granulosa cells [5]. Gap junctions are made up of six connexin (CX) proteins to form a connexon, a hollow ring in the plasma membrane that enables communication between the cells when coupled with another connexon in an adjacent cell, with the capacity to change function based on protein isoform or post-translational modification, though this mechanism remains vague [6,7].

Localization of CX proteins is used for GJ identification in a variety of tissues [8] and the CX family of proteins is very diverse, with 20 proteins in mice and 21 in humans, each the product of a distinct gene [9]. A total of 8 CX proteins are known to be expressed in the ovary, with expression varying in a species-specific manner. The ovary of the pig expresses *GJB2, GJB4*, *GJB1*, *GJA1,* and *GJA10* [10,11]. In sheep, ovarian *GJB2, GJB1*, *GJA4*, and *GJA1* have been detected [12–14]. Similar to the sheep, the ovary of the cow expresses *GJB2, GJB1, GJA4,* and *GJA1* [15,16]. In mouse and rat, *GJB1*, *GJA4*, *GJA1*, *GJC1,* and *GJA10* have been detected in the ovary [17]. Pannexin genes have also been implicated in GJ channeling, but less is known about their functions [18]. Pannexin1 (Panx1) has been identified in human ovary and placenta, but no further work has been performed to describe Panx1 function in the female reproductive system [19].

Gap junction proteins are subject to hormonal regulation in various tissue types. Both E_2 and P_4 regulate GJIC in the reproductive system, heart, brain, and liver *via* complementary or opposing actions dependent on physiological context and tissue phenotype [20]. An increase in *Gja1* mRNA and GJ formation is stimulated by E_2 while, in contrast, an inhibitory effect is mediated by P_4 in the female reproductive system

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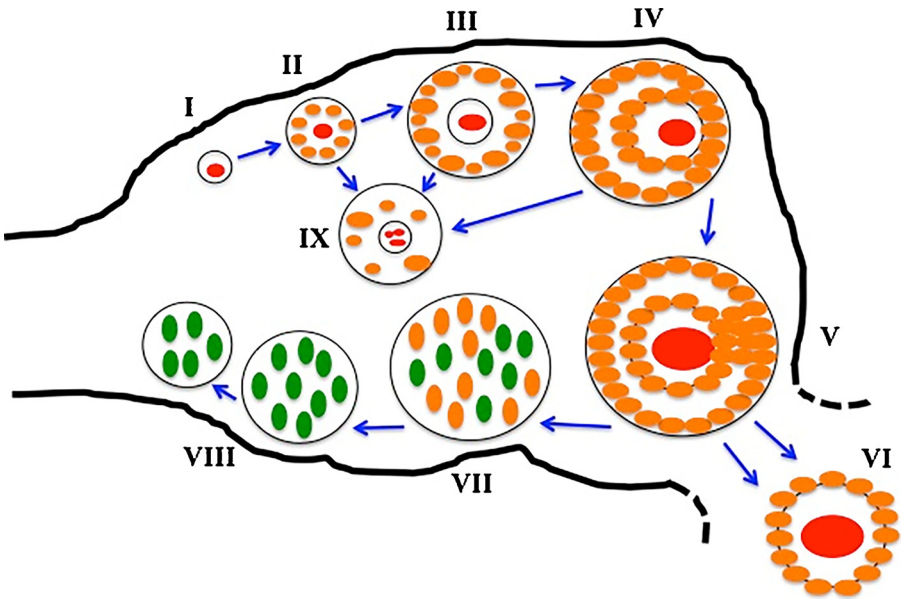

Fig. 1. Stages of ovarian follicular development. The stages of folliculogenesis are depicted with red indicating the oocyte, orange indicating granulosa cells and green indicating luteal cells. The stages of follicle maturation are indicated by roman numerals: I - Primordial, II - Primary, III - Secondary, IV - Antral, V - Pre-ovulatory, VI - Ovulated oocyte, VII - Early corpus luteum, VIII - Mature and regressing corpus luteum, IX – Atretic. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

[21,22]. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) also have similar effects on GJIC in reproductive tissues, where FSH stimulates upregulation of *Gja1* mRNA and protein, and *Gja1* expression is elevated as follicle size increases in response to FSH [23–26]. In contrast, LH stimulates a reduction in *Gja1* mRNA and protein [27–30]. Alterations of CX expression are also detected throughout the stages of the estrous cycle. In sheep, *GJB2* mRNA expression in the corpus luteum (CL) is at its highest at d10 of the estrous cycle and decreases in PGF-induced luteal regression [13]. In cows, *GJB2* mRNA is highest during the second half of the estrous cycle and after luteal regression [31]. *GJB1* mRNA and protein expression remains relatively stable throughout the estrous cycle [13,14]. In contrast, *GJA4* mRNA expression is increased after hCG treatment in sheep, and expression of GJA4 protein and mRNA is greatest on d5 of the estrous cycle with a gradual decrease thereafter [12]. *GJA1* mRNA expression in sheep decreases in the granulosa and theca cells after hCG treatment and in the luteal tissue, but is observed to increase in the CL on d5 of the estrous cycle [13,14]. The expression of *GJA1* mRNA decreases after injection of GnRH and after luteal regression in the ovary of the cow, with higher *GJA1* levels observed in the CL during the early luteal phase [31]. In addition to changes during the estrous cycle, high levels of *GJB2* and *GJA1* mRNA are detected throughout pregnancy in cows [31].

Ovarian GJ investigations have primarily focused on defining the function and role of ovarian GJA4 and GJA1. The generation of a *Gja4*-null mouse demonstrated arrest of folliculogenesis at the early antral follicular stage and oocytes that do not reach meiotic competence [32,33]. Interestingly, a genetic variant of *Gja4* is associated with primary ovarian insufficiency in women [34] while another *Gja4* gene variant is associated with polycystic ovarian syndrome in women [35]. Deficiency of *Gja1* is postnatally lethal in mice, so *Gja1*$^{-/-}$ prenatal ovaries have been cultured *ex vivo* or *via* transplant, and these ovaries have retarded oocyte growth and arrested folliculogenesis [36,37]. Further supporting the roles of ovarian GJA4 and GJA1 are chimeric ovary studies which paired wild-type (WT) oocytes with *Gja1*-deficient somatic cells; *Gja1*-deficient oocytes with WT granulosa cells; WT oocyte with *Gja4*-deficient granulosa cells; or *Gja4*-deficient oocytes with WT granulosa cells [38,39]. In ovaries containing WT granulosa cells with *Gja1*$^{-/-}$ oocytes or WT oocytes and *Gja4*$^{-/-}$ granulosa cells, meiosis occurred and fertilization could be achieved. In contrast, ovaries with WT granulosa cells and *Gja4*$^{-/-}$ oocytes could not proceed with meiosis and did not achieve fertilization. In ovaries with *Gja1*$^{-/-}$ granulosa cells and WT oocytes, follicles remained in the early preantral stages and contained smaller oocytes [38]. Thus, both oocyte and granulosa cell GJIC is important for follicular development.

Regulation of *Gja4* involves a member of the wingless-type MMTV integration site family, WNT4. Mice deficient in *Wnt4* had reduced (30%) expression of GJA1 compared to WT mice [40]. It is thought that WNT signaling regulates GJA1 expression and GJIC in granulosa cells by modulating beta-catenin stability and localization. Furthermore, knockdown of the beta-catenin gene altered FSH-mediated mobilization of GJA1 into GJ's [41]. Thus, WNT signaling is likely involved in control of GJA1 function, but whether other GJ proteins are regulated similarly remains unclear.

In addition to *Gja4* and *Gja1*, knockout of *Gjc1* and *Gjb2* in mice has been accomplished but leads to embryonic death with cardiovascular defects and insufficient embryonic development, respectively [42–45]. *Gja10*$^{-/-}$ mice have no ovarian defects although they do have deficiencies in the visual system and *Gjb1*$^{-/-}$ mice remain fertile [46,47]. Ovarian findings for these genetic mice models lacking *Gja4* and *Gja1* support that ovarian CX proteins have important roles in relation to follicle and oocyte survivability, quality, and growth.

In conjunction with the developmental functions of GJ in the ovary, they are also targets for reproductive toxicants and may represent conduits for toxicants to reach the oocyte. Exposure to chemicals during development or in adulthood can have serious implications for female fertility *via* disruption of normal ovarian function (Fig. 2). Ovotoxicants can selectively affect a follicle population in the ovary, resulting in either temporary or permanent infertility [48]. The breakdown of GJIC due to ovotoxicant exposure is a potential etiology underlying ovarian dysfunction. In this review, we describe the studies that have investigated the impacts of ovarian toxicants on ovarian GJ and we identify gaps in our understanding of this area of reproductive biology including the dearth of information on the underlying mechanisms that impair GJIC.

2. Ovotoxicants that impact gap junctions

Endocrine disrupting chemicals (EDC) are exogenous substances or mixtures (natural or synthetic) that disrupt normal actions of endogenous hormones such as their synthesis, secretion, transport, metabolism, binding, and elimination in various organ systems [49]. In the ovary, EDC's have been shown to affect fertility through impacting follicle growth [50–54], alteration of ovarian steroidogenesis [50–52], mimicry of receptor signaling [55,56], and impaired oocyte