

Androgen effect on connexin expression in the mammalian female reproductive system: A systematic review

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ABSTRACT

The functions of androgen and connexin in the mammalian female reproductive system are suggested to be related. Previous research has shown that androgen affects connexin expression in the female reproductive system, altering its function. However, no definitive conclusion on their cause-effect relationship has been drawn yet. In addition, a high prevalence of women with polycystic ovary syndrome (PCOS), who are characterized by elevated androgen levels and failure of ovulation, has prompted the studies on the relationship between androgen and connexin in the ovaries. This systematic review aims to investigate the effect of androgen on connexin expression in the mammalian female reproductive system. The literature search was conducted using the MEDLINE via EBSCOhost and the Scopus database and the following keywords: "androgen" or "testosterone" or "androgen blocker" or "anti-androgen" or "androstenedione" or "dehydroepiandrosterone" or "flutamide AND connexin" or "gap junction" or "cell junction." We only considered *in vitro* and *in vivo* studies that involved treatment by androgen or androgen receptor blockers and measured connexin expression as one of the parameters. Our review showed that the exposure to androgen or androgen blocker affects connexin expression but not its localization in the mammalian ovary. However, it is not clear whether androgen downregulates or upregulates connexin expression.

KEYWORDS: Androgen; androgen blocker; connexin; ovary; female reproductive system

INTRODUCTION

Androgen in the mammalian female reproductive system

Androgens are male sex hormones that are important for the function of the female reproductive system [1]. In women, androgens are produced in the adrenal glands and the ovaries, and they include dihydrotestosterone (DHT), testosterone, androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone sulphate. Only DHT and testosterone bind to the androgen receptor (AR), while the others act as proandrogens and need to be converted to testosterone for their action. Testosterone is considered as the most potent androgen in women [2].

Fluctuations in plasma testosterone levels occur throughout the menstrual cycle and are related to changes in the

expression of AR in the endometrium [1]. The effects of androgens are mediated through the AR, which belongs to the nuclear receptor subfamily 3 group C and primarily functions as DNA-binding transcription factor to regulate gene expression. The AR also utilizes the non-genomic pathway to exert the effects of androgen, through the modulation of cytoplasmic or cell membrane-bound regulatory proteins [3].

Androgens are involved in both normal and pathological states of the female reproductive system. Testosterone or DHT treatment of postpubertal gilts in the late follicular phase led to an increase in the ovulation rate [4]. The survival of human ovarian tissue *in vitro* was enhanced with DHT treatment [5]. On the other hand, DHT treatment of rats reduces the ovarian weight and oocyte yield [6]. A high concentration of androgens has been reported in women with polycystic ovary syndrome (PCOS), which is related to failure of ovulation [7]. This is because hyperandrogenism disrupts the communication between follicles and stroma, resulting in follicular arrest and disturbed ovulation [8]. Moreover, a high concentration of testosterone administered to rats has been reported to downregulate the endometrial receptivity markers in the endometrium of rats [9].

Connexin expression in the mammalian female reproductive system

Connexins (Cx) are a group of homologous transmembrane proteins that form gap junctions in vertebrates.

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Intercellular gap junctions are channels that directly connect the cytoplasm of adjacent cells, which allows the exchange of small molecules and inorganic ions between the cells, mediating their electrical and metabolic coupling [10,11]. Various types of connexins are expressed in the mammalian female reproductive system, which plays an important role in its physiology. Cx43 and Cx37 are predominantly expressed in the ovarian follicles of humans and mice [12]. In both mice and humans, Cx43 forms gap junctions that connect granulosa cells [13,14]. In mice, Cx37 connects oocytes with the surrounding cumulus cells; while in humans, the location of Cx37 in the granulosa cells of fertile women is not clear [12]. In the endometrium in both rats and humans, Cx26 and Cx43 are the two major connexins [15-17]. In rats, Cx26 was found in the luminal epithelium and Cx43 in the stromal compartment [15]. Additionally, Cx26 and Cx43 were reported to be expressed in the myometrium in rats and humans [18,19].

Intercellular gap junctions between granulosa cells and oocytes facilitate the transfer of ions and molecules between the cells and thus have a crucial role in folliculogenesis and oogenesis [13,20]. For example, Cx37 and Cx43 are important for follicle development and oocyte growth in mammals [21]. In Cx37 or Cx43 deficient mice, folliculogenesis is arrested at the early stage, where oocytes fail to reach the meiotic stage [22,23]. Furthermore, Cx43 expression is increased during follicular development and decreased during follicular atresia [24]. Cx37 is a predominant connexin that makes up gap junctions in oocytes. These gap junctions connect granulosa cells to oocytes and are responsible for the direct transfer of nutrients, such as amino acids and glucose, as well as ions. Besides, Cx37 is important for the regulation of pH in oocytes as well as cyclic guanosine monophosphate that maintains oocytes in meiotic arrest [25,26]. Injection of carbenoxolone - a broad gap junction blocker to mice causes a delay in the implantation of blastocysts [27]. In addition, in cultured primary human endometrial stromal cells treatment with the gap junction blocker results in impaired decidualization [28].

Androgen effect on connexin expression

The functions of androgen and connexin in the mammalian female reproductive system are suggested to be related. Previous research has shown that androgen affects connexin expression in the female reproductive system, altering its function. However, no definitive conclusion on their cause-effect relationship has been drawn yet. In addition, a high prevalence of women with PCOS, who are characterized by elevated androgen levels and failure of ovulation, has prompted the studies on the relationship between androgen and connexin in the ovaries. This systematic review aims to investigate the effect of androgen on connexin expression in the mammalian female reproductive system.

MATERIALS AND METHODS

Literature review

A systematic review of the literature was conducted to identify studies on the effects of androgen on connexin expression in the female reproductive system. The literature search was conducted using the MEDLINE via EBSCOhost (published between 1942 and October 2019) and the Scopus (published between 1941 and October 2019) database. The search was done with the following keywords: androgen* or testosterone* or androgen blocker or anti-androgen or androstenedione or dehydroepiandrosterone or flutamide AND connexin* or gap junction* or cell junction*.

Study inclusion and exclusion criteria

The results were limited to studies that were published in the English language and only academic articles published from 2000 to 2019 were included. For this review, only studies that involved treatment by androgen or AR blockers were included with connexin expression as one of the parameters.

Data extraction and management

The selection of papers involved two phases. In the first phase, the titles and abstracts were screened and any articles that did not match the inclusion criteria were excluded. In the second phase, the remaining papers were retrieved and screened thoroughly by three independent authors (S.F.I., H.M.M., and D.A.). Any differences in the opinion were resolved by the discussion between the authors.

The following data were recorded from the studies: the type and age of used samples, the treatment given to the subjects, the type of analyzed parameters and the method of analysis, and the results and conclusion of the studies.

RESULTS

Search results

The literature search from two databases identified 20 articles. Ten articles were retrieved from MEDLINE and the other

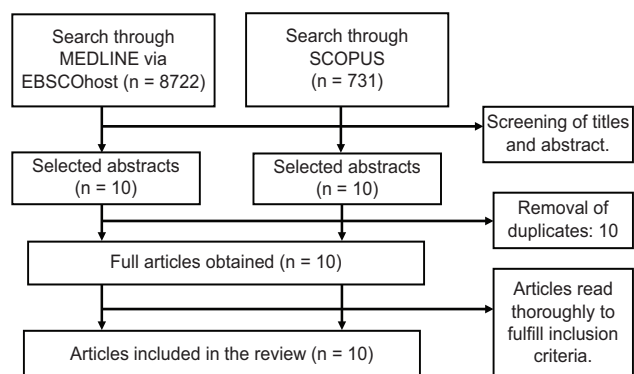


FIGURE 1. Flowchart of the literature selection process.

TABLE 1. *In vitro* studies related to androgen effects on connexin expression in the mammalian female reproductive system

Reference	Cell type/age	Treatment administered	Parameter and method of analysis	Findings	Conclusion
Lee et al. [30]	Granulosa cell culture from immature female Sprague Dawley rats	Granulosa cells were treated with different doses of testosterone (0.01, 0.1, and 1 µg/ml) in the presence or absence of 0.1 µM 1,25-dihydroxyvitamin D ₃	Parameter: Cx43 Method: western blot, qPCR, and immunofluorescence	Immunofluorescence: Cx43 protein expression was markedly decreased when treated with a high dose of testosterone (1 µg/ml) and treatment with 1,25-dihydroxyvitamin D ₃ reversed this effect. qPCR: Cx43 mRNA was significantly decreased when treated with a high dose of testosterone (1 µg/ml) and treatment with 1,25-dihydroxyvitamin D ₃ reversed this effect. Western blot: Cx43 protein expression was significantly decreased when treated with a high dose of testosterone (1 µg/ml) and treatment with 1,25-dihydroxyvitamin D ₃ reversed this effect.	High doses of testosterone (1 µg/ml) reduce Cx43 expression and 1,25-dihydroxyvitamin D ₃ abolishes this effect.
Talhok et al. [37]	Cultured granulosa cells derived from Sprague Dawley rats	Rats received daily s.c. injections of DES 1 mg/day for three consecutive days. Ovaries were then excised and granulosa cells were harvested for <i>in vitro</i> studies. Granulosa cell groups: 1) Control (EHS-drip only) 2) Androstenedione 10 ⁻⁷ M 3) Androstenedione 10 ⁻⁵ M 4) Androstenedione 10 ⁻⁷ M + 18αGA 5) Androstenedione 10 ⁻⁵ M + 18αGA	1) Assessment of the ability of granulosa cells to transfer Lucifer yellow through gap junctions using the SL/DT technique 2) Progesterone assay	IHC: Cx43 distribution was not altered in any group. Western blot: Cx43 expression was increased in a dose dependent manner of androstenedione. Enhanced phosphorylation of Cx43 was also noted in androstenedione-treated cells. SL/DT technique: An increase in Lucifer yellow transfer was seen when granulosa cells were cultured with 10 ⁻⁵ M androstenedione. Progesterone assay: There was a significant increase in progesterone production in androstenedione-treated granulosa cell culture with 18αGA, a gap junction inhibitor that reverses this effect.	Androstenedione induces luteinization of cultured granulosa cells together with the enhancement of intercellular gap junctions and Cx43 expression. These results suggest a role of gap junctions in partially mediating the effects of androstenedione on progesterone production.
Wu et al. [31]	Human granulosa cell line HO-23	EHS growth factor reduces matrix. 18αGA is a gap junction inhibitor. 1) Control group 2) DHT at 0, 1, 10, or 100 ng/ml 3) 8-Br-cAMP (0.1 mM) 4) 8-Br-cAMP + (DHT) at 10 ng/ml 5) 8-Br-cAMP (0.1 mM) + flutamide (100 ng/ml) 6) 8-Br-cAMP (0.1 mM) + DHT (10 ng/ml) + flutamide (100 ng/ml) 8-Br-cAMP, an analogue of cyclic AMP, is known to activate adenyl cyclase and increase the cAMP pool in granulosa cells and upregulate the Cx43 expression. Treatment duration was 24 hours.	1) Cx43 protein expression using western blot analysis 2) Assessment of the ability of HO-23 granulosa cells to transfer Lucifer yellow through gap junctions using the SL/DT technique	Western blot analysis: Granulosa cell line treated with DHT at 0, 1, 10, or 100 ng/ml showed a statistically significant decrease in the expression of Cx43 in a dose dependent manner of DHT. Flutamide significantly blocked the inhibitory effects of DHT. SL/DT technique: The number of dye-coupled cell layers was statistically significantly reduced in HO-23 granulosa cells grown in 8-Br-cAMP with DHT compared to cells grown in 8-Br-cAMP and this was prevented with flutamide treatment.	High levels of androgen reduce Cx43 expression in human granulosa cells, hence impairing the communication between granulosa cells or communication with oocytes that may compromise folliculogenesis and oogenesis in PCOS patients. This study also demonstrated that androgen regulates Cx43 expression in cultured human granulosa cells by an AR dependent process, considering that the suppressive effect of DHT on Cx43 is blocked by the AR antagonist flutamide.

(Contd...)

TABLE 1. (Continued)

Reference	Cell type/age	Treatment administered	Parameter and method of analysis	Findings	Conclusion
Zhang et al. [29]	Cultured COCs from 6- to 8-week-old ICR mice ovaries	1) Control blank group 2) 10 ⁻¹¹ mol/l testosterone (low concentration) 3) 10 ⁻⁹ mol/l testosterone (low concentration) 4) 10 ⁻⁷ mol/l testosterone (physiological concentration) 5) 10 ⁻⁵ mol/l testosterone (high concentration) 6) 10 ⁻⁷ mol/l testosterone + 10 ⁻⁶ mol/l flutamide Treatment duration for testosterone was 24 hours.	Parameter: protein expression of Cx37 Analysis method: western blot	The expression of Cx37 in COCs increased with elevated testosterone concentrations. There were significant differences between four groups treated with testosterone. The expression of Cx37 in COCs treated with 10 ⁻⁷ mol/l testosterone + 10 ⁻⁶ mol/l flutamide was significantly lower than in the groups treated with 10 ⁻⁷ mol/l testosterone.	This study suggested that testosterone via the AR pathway improves the expression of Cx37 located at COCs in a dose dependent manner. This can strengthen the communication between oocytes and somatic cells, which can improve ovarian response in patients with poor ovarian response.

s.c.: Subcutaneous; DES: Diethylstilbestrol; qPCR: quantitative PCR; 18αGA: 18α-glycyrrhetic acid; EHS: Engelbreth-Holm-Swarm tumor; SL/DT: Scrape-loading/dye transfer; DHT: Dihydrotestosterone; 8-Br-cAMP: 8-Bromoadenosine 3',5'-cyclic adenosine monophosphate; PCOS: Polycystic ovary syndrome; Cx: Connexin; IHC: Immunohistochemistry; AR: Androgen receptor; COCs: Cumulus oocyte cells; ICR: Institute of Cancer Research

10 from Scopus. Ten articles were removed due to duplication. For the remaining 10 articles, full texts were obtained for data extraction. The flowchart of the selection process is shown in Figure 1.

Study characteristics

The characteristics of all studies are summarized in Table 1-3. Of the 10 eligible articles, three were *in vitro* studies that involved culture of oocytes derived from the Institute of Cancer Research (ICR) mice [29], Sprague Dawley rats [30], or humans [31]. Six articles were *in vivo* studies involving mice [24] or pigs [32-36]. One article combined both *in vitro* and *in vivo* studies on Sprague Dawley rats [37]. Nine studies were on ovaries or ovaries related tissue and cells, while one study involved the uterus and the placenta [36].

All studies measured the expression of Cx43 as the parameter, except for Zhang et al. [29] who assessed Cx37 expression. Testosterone was used as treatment in three studies [24,29,30], one study used DHT [31], and one study used androstenedione [37]. The remaining five studies used the AR blocker flutamide as treatment [32-36].

Treatment by androgen decreased the expression of connexin in all studies except in the studies by Zhang et al. [29] and Talhouk et al. [37], which reported an increase in Cx37 after testosterone treatment and in Cx43 after androstenedione treatment, respectively. With flutamide treatment, connexins were reported to fluctuate according to the different gestation and postpartum period. The methods used to determine the expression of connexins included western blot, quantitative PCR, immunofluorescence, or immunohistochemistry. Two studies [31,37] used the scrape-loading/dye transfer (SL/DT) technique to assess the ability of granulosa cells to transfer Lucifer yellow through gap junctions.

DISCUSSION

Based on our review, the results of studies investigating the effect of androgen or AR blocker treatment on connexin expression are inconsistent. About half of the included studies reported direct effects of androgens on connexin expression, while another half reported the effects of AR blockers on connexin expression. The studies involving AR blockers were in the prenatal period, postnatal period, or during gestation.

In vitro studies

Four *in vitro* studies investigated androgen effects on connexin expression, with three studies using animal and one study using human cells. The three studies on animal cells showed contradictory results. Zhang et al. [29] showed increased expression of Cx37 in cultured cumulus-oocyte-cells (COCs)

TABLE 2. *In vivo* studies related to androgen effects on connexin expression in the mammalian female reproductive system

Reference	Cell type/age	Treatment administered	Parameter and method of analysis	Findings	Conclusion
Talhouk et al. [37]	21- to 24-day-old Sprague Dawley rats	1) Control group received s.c. injections of 100 µl/day corn oil for 3 days. Ovaries were subsequently harvested one day after the last injection. 2) S.c. injections of 1 mg/day DES for 3 days. Rats were then either sacrificed or subsequently received i.p. injections of 3) 15 IU eCG. Forty-eight hours later, the animals were either sacrificed or administered i.p. 4) 15 IU hCG and sacrificed after 8 hours. This protocol was used to mimic the different follicular development stages in the rat ovary. 5) Injection of 6 mg/100 g body weight androstenedione dissolved in 200 µl corn oil for 21 days (route of injection not stated), to induce PCOS in rats.	Parameter: Cx43 Method: 1) IHC 2) Western blot	IHC: Cx43 was localized in granulosa cells in every group. Western blot: Cx43 was upregulated in PCOS ovary compared to normal ovary.	This study was able to show modulation of Cx43 in androstenedione-induced PCO in rats.
Yang et al. [24]	Ovarian tissues of 6-week-old ICR mice	1) Control group treated with bean oil 2) 1.3 mg/kg testosterone group All mice were induced to super ovulate by administering 10 IU PMSG followed by 10 IU hCG 48 hours later. Testosterone and bean oil injected i.p. for 7 days.	Parameter: 1) Cx43 2) AR 3) Metaphase II (MII) oocyte number Method: 1) IHC 2) Western blot	IHC: Control group: Cx43 was observed between granulosa cells as well as between granulosa cells and oocytes in large antral follicles and preovulatory follicles. Testosterone-treated group: Cx43 immunostaining was notably weakened in the whole ovary, whereas AR expression was enhanced in the whole ovary. Western blot analysis: Following testosterone administration, Cx43 expression markedly decreased and AR expression significantly increased.	Exposure to testosterone resulted in reduced Cx43 protein expression and increased AR expression using both IHC and western blot, with concomitant reduction of MII oocytes. Changes in Cx43 and AR expression in mouse ovary disturb normal ovarian functions and ovulation. This may be involved in the disorder of follicular development or dysfunction of oocyte meiotic maturation.

PCOS: Polycystic ovary syndrome; Cx: Connexin; s.c.: Subcutaneous; i.p.: Intraperitoneal or intraperitoneally; ICR: Institute of Cancer Research; PMSG: Pregnant mare's serum gonadotropin; hCG: Human chorionic gonadotropin; IHC: Immunohistochemistry; AR: Androgen receptor

TABLE 3. Studies related to AR blocker flutamide effects on connexin expression in the mammalian female reproductive system

Reference	Cell type/age	Treatment-administered	Parameter and method of analysis	Findings	Conclusion
Durlej et al. [32]	Ovaries of sexually mature gilts	Pregnant pigs were divided into three groups: 1) First group - treated with vehicle. 2) Second group - treated with flutamide 50 mg/kg starting at day 80 post coitum (GD80). 3) Third group - not treated but their newborn female offspring were treated with flutamide 50 mg/kg starting at day 2 postpartum (PD2). All newborn female offspring of the pregnant pigs were kept until they were sexually mature; they were then sacrificed to obtain the ovaries.	mRNA expression of AR and Cx43 by qPCR Cx43 localization by IHC	AR and Cx43 mRNA expression: In preantral follicles, AR and Cx43 mRNA decreased in GD80 and PD2 groups. In large antral follicles, AR mRNA increased in both GD80 and PD2 but Cx43 mRNA expression increased only in PD2 group. In corpus luteum, AR expression increased in PD2 group. IHC: In preantral follicles, Cx43 expression decreased in GD80 and PD2 groups. In large antral follicles, the highest expression of Cx43 was in PD2 group, lower in GD80 group, and the lowest in control group. In luteal cells, Cx43 immunostaining decreased in PD2 group.	Exposure to flutamide during important developmental stages (GD80 or PD2) influences Cx43 mRNA and protein expression in adult porcine ovaries. This may affect the development of ovarian follicles or the formation and function of porcine corpus luteum. Although the data demonstrated an association between AR and Cx43 mRNA expression, the results did not fully support the hypothesis of Cx43 regulation by androgens and further studies are needed to determine the molecular mechanism of this regulation.
Durlej et al. [33]	Two-day-old pig ovaries (Large White x Polish Landrace)	1) Group 1 (GD20) - flutamide treatment on gestational days 20–28 (every second day). 2) Group 2 (GD80) - flutamide treatment on gestational days 80–88 (every second day). 3) Group 3 - control group injected with corn oil. Flutamide injected s.c. 50 mg/kg body weight. Ovaries were removed on postnatal day 2 from neonatal piglets.	Parameter: Cx43 Method: 1) IHC 2) Western blot 3) qPCR	IHC: The intensity of Cx43 staining was moderate to strong in all groups. A stronger signal was observed between granulosa cells of primary follicles and among the interstitial cells surrounding clusters of oocytes in the ovarian cortex of GD80 individuals, and it was found significantly different from the respective controls. Western blot analysis: A single band, 43kDa in size, was observed in the ovaries of both flutamide-treated pigs and the respective controls. qPCR: Cx43 mRNA expression detected in the ovaries. However, due to the PCR method used, the exact amount of the transcript was not determined.	In newborn piglets, flutamide does not affect the presence of Cx43 protein and mRNA in the ovaries, which suggests that androgens acting through ARs are not involved in the control of Cx43 gene expression in neonates.
Knapczyk-Stwora et al. [34]	Porcine fetal ovaries (Large White x Polish Landrace)	1) Group 1: injected with 50 mg/kg flutamide between days 83 and 89 of gestation (surgery done at GD90 to get fetus). 2) Group 2: injected with 50 mg/kg flutamide between days 101 and 107 of gestation (surgery done at GD108 to get fetus). 3) Group 3: control group injected with corn oil between days 83 and 89 of gestation (surgery done at GD90 to get fetus). 4) Group 4: control group injected with corn oil between days 101 and 107 of gestation (surgery done at GD108 to get fetus). Treatment via s.c. injection for 7 days.	Parameter: Cx43 Method: 1) IHC 2) qPCR	GD90 group: in the ovaries, flutamide treatment caused Cx43 expression to decrease compared to control. GD108 group: in the ovaries, flutamide treatment caused Cx43 to increase compared to control. IHC: GD90 group: flutamide treatment decreased Cx43 expression compared to control group. GD108 group: flutamide treatment increased Cx43 expression compared to control group.	Cx43 expression is altered by antiandrogen treatment during fetal ovarian development in pigs.

(Contid...)

TABLE 3. (Continued)

Reference	Cell type/age	Treatment administered	Parameter and method of analysis	Findings	Conclusion
Kopera et al. [35]	90- to 100-day-old pig ovaries (Large White x Polish Landrace)	1) Group 1: flutamide treatment on gestational days 20–28 (GD20). 2) Group 2: flutamide treatment on gestational days 80–88 (GD80). 3) Group 3: flutamide treatment on days 2–10 after birth (PD2). 4) Group 4: control group treated only with corn oil. Ovaries were obtained from 90- to 100-day-old pigs. Flutamide was given in five doses of 50 mg/kg every second day.	Parameter: localization and expression of Cx43 Method: 1) IHC 2) Western blot 3) qPCR	IHC: Cx43 was localized in the adjacent granulosa cells of preantral and antral follicles. A significant decrease was observed in Cx43 expression in the granulosa cells of group 1 and group 3 compared to the control group. In theca cells, there was an increase in staining intensity of Cx43 in group 2 and 3, while a decrease was observed in group 1 compared to control group. Western blot: The intensity of immunoblots was slightly higher in the ovaries of flutamide-treated groups compared to control groups. qPCR: Cx43 mRNA was detected in the ovaries. However, due to the PCR method used the exact amount of the transcript was not determined.	The findings revealed that androgen is somehow involved in the regulation of Cx43 gap junction communication in the ovaries of prepubertal pigs.
Wieciech et al. [36]	Uterine and placenta tissues of sexually mature crossbred gilts aged 10–11 months (Large White x Polish Landrace)	1) Group 1: 50 mg/kg flutamide injected daily between days 43–49 of gestation (50 dpc). 2) Group 2: 50 mg/kg flutamide injected daily between days 83–89 of gestation (90 dpc). 3) Group 3: 50 mg/kg flutamide injected daily between days 101–107 of gestation (108 dpc). 4) Respective control for each group, treated with corn oil. Tissue collection divided to placenta and non-placenta uterus. Placenta tissues further divided into FP and MP of the placenta.	Parameter: Cx43 mRNA and protein expression Method: PCR, western blot, and IHC	qPCR: Flutamide caused a significant increase in Cx43 mRNA expression in FP on 90 and 108 dpc. Flutamide resulted in a significant decrease in Cx43 mRNA levels on day 50 dpc in non-placental uterus. Western blot: A significant decrease of Cx43 protein expression in MP on 108 dpc and in non-placental uterus on 90 and 108 dpc. IHC: Flutamide treatment resulted in a significantly higher intensity of Cx43 staining at FP stroma cells on 50 dpc, but in lower on 90 and 108 dpc. In MP, flutamide caused a decrease in Cx43 staining in glandular epithelium on 108 dpc. The intensity of Cx43 staining after flutamide treatment in the myometrium of the placenta increased on 90 dpc but decreased on 108 dpc. In non-placental uterus, flutamide treatment caused Cx43 staining intensity to decrease in glandular epithelium on 108 dpc and in the myometrium on 50 and 108 dpc.	Androgens regulate Cx43 expression in the porcine placenta and non-placental uterus especially during late pregnancy (90 dpc) and periparturient period (108 dpc). This may affect the cells involved in cell communication and feto-maternal exchange. A decrease in Cx43 levels in the myometrium of the uterus may result in delayed parturition in androgen deficient pigs.

Cx: Connexin; s.c.: Subcutaneous or subcutaneously; IHC: Immunohistochemistry; AR: Androgen receptor; d.p.c.: Days post coitum; FP: Fetal part; MP: Maternal part; qPCR: quantitative PCR

from ICR mice treated with testosterone. Similar findings were reported by Talhouk et al. [37], where Cx43 expression in the granulosa cells of rats was upregulated by androstenedione treatment. On the other hand, Lee et al. [30] showed that testosterone downregulated the expression of Cx43 in cultured granulosa cells derived from rats. In addition, the *in vitro* study using human granulosa cell lines revealed that Cx43 expression was decreased following DHT treatment and treatment with flutamide blocked this inhibitory effect on Cx43 [31].

Interestingly, Lee et al. [30] found that 1,25-dihydroxyvitamin D₃ treatments of cultured granulosa cells reversed the inhibitory effects of testosterone on Cx43 expression. This finding suggests the use of 1,25-dihydroxyvitamin D₃ as a treatment to prevent downregulation of Cx43. Wu et al. [31] and Talhouk et al. [37] used the SL/DT technique to assess the ability of granulosa cells to transfer Lucifer yellow through gap junctions. The Wu study showed that granulosa cells grown with DHT had a reduced ability to transfer Lucifer yellow dye, corresponding with Cx43 downregulation [31]. On the other hand, Talhouk et al. [37] showed an increase in Lucifer yellow transfer with the upregulation of Cx43 expression in androstenedione-treated cells. These studies confirmed the specific relationship between androgens and gap junctions composed of connexins. Furthermore, Talhouk et al. measured the production of progesterone by the cultured granulosa cells to determine the degree of cell differentiation. Their results showed that the progesterone production in androstenedione-treated cells increased parallel with the increase in Cx43 expression and Lucifer yellow transfer rate; whereas treatment with gap junction inhibitor 18 α glycyrrhetic acid (18 α GA) reduced the progesterone yield. This suggests the role of gap junctions in mediating the effects of androstenedione on progesterone production [37].

With regards to androgen doses, all *in vitro* studies showed that the androgen effect on connexin expression is dose-dependent, except the study by Lee et al. [30], which showed that only high concentrations of testosterone (1 μ g/ml) downregulated Cx43.

In vivo studies

Only two *in vivo* studies involving direct androgen treatment were included in this review, showing contradictory results. Yang et al. [24] reported that testosterone reduced Cx43 expression in the ovaries of mice, while Talhouk et al. [37] showed that androstenedione increased Cx43 expression in the ovaries of rats. Moreover, in the Yang study, while Cx43 expression in the ovaries was reduced by testosterone treatment, the AR expression was upregulated [24]. This indicates that the effect of testosterone to downregulate the expression of Cx43 is mediated through the AR. Interestingly, this study also measured the number of metaphase II (MII) oocytes in

the oviductal ampulla, showing a significantly reduced number of MII oocytes in testosterone-treated mice compared to control group. These results suggest that androgen affects connexin expression and results in follicular development disorder or dysfunction of oocyte meiotic maturation [24]. The Yang study considered 1.3 mg/kg testosterone as high-dose testosterone mimicking clinical feature of PCOS.

Talhouk et al. [37] induced PCOS by administering androstenedione to rats and compared the level of connexin between the ovaries of normal rats and PCOS-induced rats. They showed that the expression of connexin was upregulated in the PCOS ovary compared to control. However, since androstenedione can be aromatized to estrogen, it is not clear whether the observed effect on connexin was induced by androstenedione or estrogen. Furthermore, androstenedione must be converted to testosterone or DHT to exert its effects [2].

AR blocker

The five remaining studies [32-36] investigated the effects of flutamide, a potent AR blocker, on connexin expression. All five studies were *in vivo* studies using pigs as a model organism and the same concentration of flutamide (50 mg/kg). The studies considered prenatal, postnatal, neonatal, or during gestation exposure to flutamide. All studies were on the ovaries, except the study by Wiecech et al. [36], which involved the placenta and the uterus. These studies reported that the expression of Cx43 or AR varied in the ovaries, the placenta or the uterus depending on different stages (prenatal, postnatal, or neonatal), gestational days, or periods of exposure to flutamide. Four studies concluded that Cx43 expression is affected and regulated by anti-androgen flutamide treatment, while the study by Durlej et al. [33] indicated that Cx43 is not regulated by flutamide. None of the studies, however, explained the details on 50 mg/kg flutamide dose used.

This review shows that androgen or flutamide treatment does not affect the localization of Cx43. In addition, all included studies were focused on the effects of Cx37 or Cx43 downregulation on the ovary or uterus physiology, but none of the studies investigated the upregulation of Cx43 or Cx37 above the physiological expression levels, which may also disrupt the physiology of the female reproductive system.

CONCLUSION

This review showed that the exposure to androgen or AR blockers affects the connexin expression but not its localization in the mammalian ovary. However, it is not clear whether androgen downregulates or upregulates connexin expression. In addition, most of the studies on the effects of androgen or AR blockers on connexin expression were conducted on

the ovaries. Future studies should include other parts of the female reproductive system, especially the uterus, as women with PCOS that are characterized by high levels of androgens have shown defects in the uterus.

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