Seminars in Immunopathology (2019) 41:447–459
https://doi.org/10.1007/s00281-019-00737-4

REVIEW

Chronic low-grade inflammation in polycystic ovary syndrome: is there a (patho)-physiological role for interleukin-1?

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Received: 27 February 2019 / Accepted: 15 March 2019 / Published online: 28 May 2019

Abstract

The polycystic ovary syndrome (PCOS) is a frequent endocrine disorder in women of reproductive age. Its main characteristics are the ovarian overproduction of androgens and ovulatory dysfunction which lead to severe symptoms such as hirsutism, acne, insulin resistance, and infertility. Despite the frequency and disease burden of PCOS, its underlying causes remain unknown, and no causal therapeutic options are available. In recent years, several studies have shown that women with PCOS present with chronic low-grade inflammation indicating an overactivity of the pro-inflammatory cytokine interleukin-1 (IL-1). We show here how IL-1 might affect the ovarian physiology and pathophysiology in animals and humans by reviewing experimental studies on ovarian IL-1 system gene expression and on the effects of exogenous IL-1 on ovarian functions. Although IL-1 ligands and receptors are expressed within the ovarian cells, IL-1 seems to negatively affect the delicate balance between the sex hormones and dominant follicle development, as well as fertility. Whether blockade of the IL-1 signaling leads to an improvement of PCOS-related hormonal abnormalities and symptoms remains to be elucidated in future interventional studies.

Keywords Polycystic ovary syndrome · Inflammation · Interleukin-1 · Ovary · Testosterone

Introduction

The polycystic ovary syndrome (PCOS) was first described by Irving F. Stein and Michael L. Leventhal in 1935 [1]. It is a female endocrine disorder that affects approximately 6–10% of women in the reproductive age and not only has an important impact on reproductive and metabolic health [2, 3], but also represents a large economic burden of disease [4]. PCOS is characterized by an overactivity of male sexual hormones and ovulatory dysfunction. Its etiology remains controversially discussed but inflammatory factors seem to play a role [5]. In this review, we give an update on actual knowledge about the role of the pro-inflammatory cytokine interleukin-1 (IL-1) in patients with PCOS and on the ovarian physiology. For better understanding, a brief overview of the physiological mechanisms of a healthy ovary and the most important information on PCOS will be provided in the next two chapters. In chapter 4, experiments investigating the expression of the IL-1 system in the ovary and the effects of exogenous IL-1 on the ovarian physiology will be reviewed. Chapter 5 will cover the studies on IL-1 in actual patients with PCOS. Finally, the significance of the experimental and clinical findings will be put in context for future research.

This article is a contribution to the special issue on Inflammation and Type 2 Diabetes - Guest Editor: Marc Y. Donath

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Overview of the ovarian physiology

The primordial germ cells, which are formed already in the early pregnancy, are surrounded by a single layer of follicular epithelial cells and remain in the ovary until cyclic activation during puberty. This structure is called the primordial follicle and consists of two different structures: the primary oocyte within the follicle which will develop to become a fertilizable germ cell and the surrounding follicle, which will become a

primary, secondary, and tertiary (graafian) follicle before ovulating. The surrounding follicular structure is responsible for the cyclic production of sex hormones and for the timing of the cyclic process of meiotic oocyte maturation. This process is regulated by the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) which are both secreted by the pituitary gland [6]. Hence, the expression of gonadotropin receptors in the follicular cells is crucial for adequate communication between the pituitary and the ovarian follicles [7]. From the secondary follicle stage, the follicular cells can be subdivided into granulosa and theca cells. While a glycoprotein layer (zona pellucida) lies between the oocyte and the granulosa cells, the connective tissue around the follicle develops into the layer of the theca interna cells which start producing steroid hormones. The theca interna is surrounded by the theca externa, which contains smooth muscle cells and is highly vascularized [6]. In the tertiary follicle stage, the theca and granulosa cells proliferate extensively, and the follicle develops an antrum which is filled with fluid produced by the granulosa cells and the oocyte, i.e., the follicular fluid (Fig. 1) [8]. The theca cells possess LH receptors and produce mainly androgens upon the pulsatile stimulation by LH. The granulosa cells in turn express receptors for FSH and for LH in the late follicular phase [9]. Another key enzyme in granulosa cells is aromatase which converts theca cell-derived androgens to estrogens [10]. Every month, only one of a cohort of early tertiary follicles is selected as the dominant follicle via a finely tuned interplay between gonadotropins and granulosa and theca cells. The unselected follicles undergo atresia which is mediated by pro-apoptotic proteins [11]. FSH is obligatory in the selection of the dominant follicle. It stimulates granulosa cell proliferation and modifies gene expression. This results in a substantial increase in aromatase activity with a subsequent increment in estradiol levels and inhibition of substantial androgen increase during the follicular phase of the menstrual cycle [9, 12]. The crossing of a certain estradiol threshold level leads to a LH surge triggering the ovulatory signal cascade in granulosa cells which then suddenly alter their gene expression and start synthesizing progesterone, proteases, and prostaglandins [9]. Prostaglandins are rapidly induced via COX-2 expression and, together with progesterone, are key mediators for ovulation as they induce the formation of a whole in the follicle wall, i.e., the stigma [13, 14]. Before ovulation, the cumulus cells, which are those granulosa cells surrounding the oocyte, respond differently to the LH surge. They create a special form of extracellular matrix containing hyaluronan. This process is called cumulus cell expansion and is a necessary step for the following expulsion of the oocyte around day 14 of the cycle [15]. In the meantime, the oocyte in the preovulatory follicle resumes meiosis (= oocyte maturation), which is mandatory for successful fertilization. After the oocyte is released through the stigma, the remaining follicle wall becomes the corpus luteum indicating the beginning of the luteal phase. The corpus luteum consists of granulosa-luteal cells which are highly active steroidogenic cells [16]. Importantly, they secrete large amounts of progesterone and

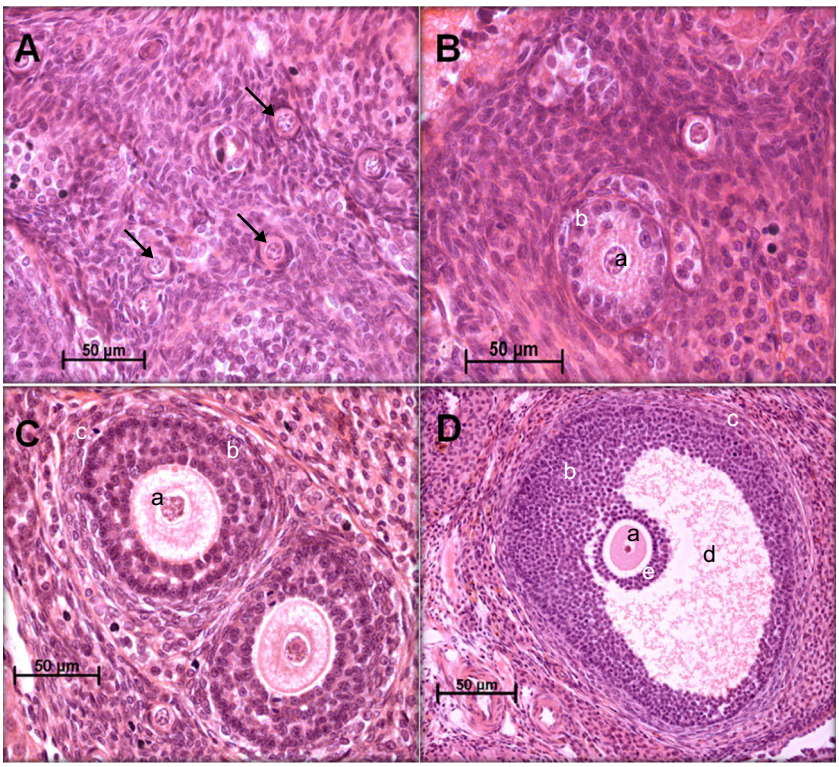

Fig. 1 Representative photographs of hematoxylin and eosin-stained rat ovarian tissue sections. (reprinted from Bernal AB et al. 2010. PLOS ONE 5(12): e15558). (A) primordial (arrows), (B) primary, (C) secondary, and (D) tertiary follicle; (a) Oocyte, (b) granulosa cells, (c) theca cell layer, (d) antrum containing follicular fluid, (e) cumulus cells

estradiol during the first week of the luteal phase. If no fertilization has occurred, the corpus luteum dies 8 days after ovulation and becomes the corpus albicans. The cessation of progesterone production from the corpus luteum triggers the onset of menstruation and allows a re-increase in FSH levels which then again initiates the start of a new menstrual cycle by selection of the new dominant follicle that has previously transitioned from the primordial to the tertiary follicle stage [9].

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a frequent and heterogenous endocrine disorder in women of reproductive age [17]. While the detailed pathophysiological mechanisms are still unknown, the main feature is the ovarian overproduction of androgens, especially testosterone. Women with classical PCOS typically suffer from hirsutism, acne, infertility, oligo/-amenorrhoea, and the metabolic syndrome. Further typical clinical and laboratory features include the presence of high levels of LH, insulin resistance, and polycystic ovarian morphology in ultrasound. Currently, the most commonly used diagnostic criteria for PCOS are a combination of at least two of the following three criteria: hyperandrogenemia (either clinical, biochemical, or both), oligo- or amenorrhoea, and polycystic ovarian morphology in transvaginal ultrasound (so called Rotterdam criteria) [18]. Exclusion of other etiologies for hyperandrogenemia or oligomenorrhoea is mandatory (e.g., late-onset congenital adrenal hyperplasia, hyperprolactinemia, hypothyroidism) [19]. The strict use of the Rotterdam criteria would allow the diagnosis of PCOS without the presence of hyperandrogenemia. It is thus not surprising that women with the diagnosis of PCOS present with various symptoms which interindividually differ in their appearance and severity. By applying the Rotterdam criteria to diagnose PCOS, one might distinguish 4 different phenotypes of PCOS, i.e., "classic" PCOS, fulfilling all three criteria, anovulatory or ovulatory hyperandrogenic PCOS (either without or with polycystic ovarian morphology), and normoandrogenic PCOS [17]. In addition to the heterogenous clinical picture, it is known that women with classical or hyperandrogenic PCOS are at a higher risk for developing dyslipidemia, obesity, hypertension, and gestational diabetes or type 2 diabetes mellitus at a younger age [20, 21]. Furthermore, large observational studies also suggest that women with PCOS might be at a higher risk for developing cardiovascular diseases [22].

Although several clinical trials and investigations have been conducted to better understand the underlying mechanisms and causes leading to PCOS, no substantial progress has been made and the pathophysiological mechanisms leading to hyperandrogenemia are still unclear. On the one hand, it is hypothesized that PCOS results from an intrinsic dysregulation in ovarian steroidogenesis with an increase in androgen production and consequent disruption in folliculogenesis. The local androgen excess would then hinder further development of the dominant follicle, resulting in anovulation [23]. On the other hand, it is possible that other factors interfere with the selection of the dominant follicle. The remaining unselected follicles, in absence of the dominant follicle, produce mainly androgens which would explain hyperandrogenemia and anovulation in these patients [23]. Importantly, at the moment, there is an ongoing debate on whether pro-inflammatory cytokines might play a role in the interference with physiologic folliculogenesis [24].

Consequently, there is no option to treat underlying causes, and no drug is primarily licensed for the use in PCOS [25]. The therapeutic recommendations depend on the symptoms, psychological strain, and therapeutic preferences of affected women. Combined oral contraceptives are used for suppression of hyperandrogenemia and subsequent amelioration of hirsutism and acne [26]. Furthermore, they help re-establishing menstrual regularity. However, this is no option for patients pursuing pregnancy. In patients with infertility, initial therapy aims at developing mature follicles by different forms of either direct or indirect gonadotropic stimulation. If these therapies fail, patients may be offered in vitro fertilization (IVF) [27].

Interleukin-1 and the ovary

Expression of the IL-1 system in the ovary

The interleukin-1 (IL-1) system plays an important role in the immune system and in inflammatory processes, and has been extensively reviewed elsewhere [28, 29]. Briefly, the pro-inflammatory cytokines IL-1α and IL-1β and their physiological antagonist IL-1 receptor antagonist (IL-1RA) constitute the ligands that bind to the IL-1 receptors (IL-1R) type 1 (IL-1R1) and 2 (IL-1R2), which can be membrane-bound or soluble. While IL-1R1 plays a role in the transmission of inflammatory effects of IL-1, IL-1R2 might counterbalance IL-1-effects by competing for IL-1 as a decoy receptor not capable for transmembrane signaling. IL-1 activity via IL-1R1 is hence counterbalanced by two endogenous systems, the IL-1RA and the IL-1R2 [30]. IL-1α, as well as IL-1β, is known to be mainly secreted by monocytes and macrophages, whereas IL-1α is also produced by many other cell types, especially those with barrier function (e.g., epi-/endothelial cells). The anti-inflammatory IL-1RA is secreted by various cell types, binds to the IL-1 receptor, and thus blocks pro-inflammatory IL-1 signaling by both IL-1α and IL-1β.

Many studies have confirmed that the IL-1 system is not only localized in immune and barrier cells but that the ovary as well is a site of IL-1 expression.

Animal data

Most of the investigations on IL-1 system gene expression in the ovary of animals were performed in rodents [31–36]. These studies reported the presence of IL-1α, IL-1β, IL-1R1, and IL-1RA mRNA in theca, as well as in granulosa cells, and oocytes of developing follicles. However, no signal for IL-1R2 was detected. Interestingly, IL-1 system gene expression peaked around the time of ovulation. In accordance with these findings, Brännström et al. showed that the enhanced IL-1 system gene expression is accompanied by increased IL-1 bioactivity in ovarian perfusates around the time of ovulation [37]. After ovulation, the granulosa-luteal cells produced IL-1α, IL-1β, and IL-1R1 [31]. The presence of IL-1 system ligands/receptors was also found in other animals (e.g., mares). Furthermore, consistently with data on rodents, there was a rapid increase in granulosa cell-derived IL-1β during ovulation, which was followed by an increment in IL-1RA [38, 39].

Altogether, these data demonstrate ovarian IL-1 system gene expression in various mammals and an increase in transcriptional activity around the time of ovulation.

Human data

Data in humans are scarce. There are some data on local IL-1 gene expression and production in the human ovary. IL-1β (and less IL-1α) gene expression was confirmed in preovulatory follicular fluid of women undergoing assisted reproduction [40–43]. Nevertheless, in dispersates from early (day 4 of the menstrual cycle) and late (day 12 of the menstrual cycle) not artificially stimulated follicles, no IL-1α or IL-1β transcripts were detected [40]. In the same study, IL-1R1 was expressed in both types of follicles (early and late unstimulated) and in preovulatory follicular fluid. The anti-inflammatory IL-1RA was detected in granulosa cells and preovulatory follicular fluid [40]. In three studies, IL-1β activity was detectable after ovulation in postovulatory granulosa-luteal cell cultures. Nevertheless, only one study could confirm that the granulosa-luteal cells themselves were responsible for IL-1β production; the other two could not exclude contamination with macrophages [41–43]. Studies on human gametes showed no IL-1 transcripts in oocytes, whereas cumulus cells had IL-1α, IL-1β, IL-1R1, and IL-1RA mRNA. The authors did not seek for IL-1R2 [44].

In summary, many studies have confirmed that not only the immune system but also the animal and human ovary is a site of IL-1 system gene expression. Of note, the study with the highest impact for human physiology was the above-mentioned study by Hurwitz et al. [40] which showed in non-stimulated, developing human follicles no IL-1α or IL-1β, but IL-1R1 and IL-1RA expression. The fact that follicular cells express IL-1R1 suggests that these cells are receptive for the actions of exogenous IL-1. However, the lack of IL-1α and IL-1β but presence of the IL-1RA indicates that in normal human physiology, anti-inflammatory components prevail the pro-inflammatory effects of IL-1α and IL-1β during follicle development. Nevertheless, a consistent finding among all studies was the increase in IL-1 expression until and at the time of ovulation which could imply a physiological role of IL-1 in ovulatory mechanisms. This hypothesis will be reviewed in chapter 4.2.2.

Effects of exogeneous IL-1 on the normal ovary

Many experimental studies have investigated the effects of IL-1 on steroidogenesis and on ovulation-associated mechanisms. An overview of the main effects is given in Table 1.

Effect of IL-1 on ovarian steroidogenesis

Androgens Reports on the influence of IL-1α or IL-1β on androgen secretion are very sparse. To our knowledge, no data on the effects of IL-1 on testosterone or other androgens in *human* ovaries have been reported. Moreover, no data are available on the effects of IL-1β per se on testosterone in any animal. Nevertheless, two studies have investigated the effects of IL-1α on testosterone, and of IL-1β on androstenedione (another androgen with weak biological activity) secretion in rodents. Thereby, IL-1α stimulated basal, but not gonadotropin-induced testosterone secretion in theca cell cultures derived from preovulatory follicles. However, when investigating cultures of the whole follicle, IL-1α was without effect on testosterone. Molecular mechanisms on the stimulatory effect of IL-1α remain to be elucidated [45]. Concerning the effect of IL-1β on androstenedione, IL-1β did not lead to a statistically significant increased secretion from theca cells [46].

Due to the lack of comparable and mechanistic data, it is difficult to conclude whether IL-1 has any effects on androgen secretion from theca cells.

Estradiol In *humans*, two studies investigated the effect of IL-1 on estradiol production in postovulatory granulosa-luteal cells, whereas no studies have explored the effect of IL-1 on estradiol production in developing or preovulatory human follicles. The two studies showed that IL-1β *inhibited* basal and gonadotropin-stimulated estradiol production in cultures of granulosa luteal cells [41, 50].

Animal studies investigating granulosa cell cultures from *preovulatory* follicles suggested an inhibitory effect of IL-1α and IL-1β on gonadotropin-dependent estradiol production [47–49], which is in line with the human data found in

Table 1 Summary of the effects of IL-1 on ovarian physiology

Target group	Target	Type of cell	Effect*	References**	
				Animal studies	Human studies
Androgens	Testosterone	Theca cells (preov.)	+	[45]	n.a.
		Preovulatory follicles	=	[45]	n.a.
	Androstenedione	Preovulatory follicles	=	[45]	n.a.
		Theca cells (preov.)	=	[46]	n.a.
Estrogens	Estradiol	Granulosa cells (preov.)	–	[47–49]	n.a.
		Granulosa-luteal cells	–	n.a.	[41, 50]
		Preovulatory follicles/ovary	=/+	[45, 46, 51, 52]/ [53, 54]	n.a.
Gestagens	Progesterone	Granulosa-luteal cells	+/(=)	n.a.	[55–57]/ [50]
		Granulosa cells (preov.)	–	[47, 49, 58–63]	n.a.
		Preovulatory follicles/ovary	+	[52, 53, 64]	n.a.
		Theca cells (preov.)	+/−	[46]/ [60]	n.a.
Steroidogenic enzymes/proteins	StAR protein	Granulosa-luteal cells	+	n.a.	[55]
	Aromatase	Granulosa cells	–	[49, 61, 65]	n.a.
	17β-Hydroxysteroid dehydrogenase	Granulosa cells	–	[65]	n.a.
Gonadotropin receptors	FSH receptor	Granulosa cells	–	[36, 66]	n.a.
	LH receptor	Granulosa cells	–	[58, 62, 67]	n.a.
		Theca cells	?	n.a.	n.a.
Ovulatory mechanisms	Ovulatory trigger	In vivo/in vitro perfused ovary	+	[52, 68–70]	n.a.
	Prostaglandin production	Granulosa (luteal) cells/whole ovary	+	[51, 52, 54, 64, 71–73]	[56, 74]
	Plasminogen activator activity	Theca and granulosa cells/whole preovulatory follicles	–	[73, 75, 76]	n.a.
	Fertility measures	In vivo/in vitro ovary	–	[36, 64, 66, 69, 70]	n.a.

*("+" stimulatory; "- "inhibitory; "=" no effect; "?" unknown); ** n.a. not available

granulosa-*luteal* cells. However, in the presence of other cell types, i.e., in investigations using cultures of whole preovulatory follicles or ovarian perfusates, IL-1 had no effects on basal and/or gonadotropin-stimulated estradiol production [45, 46, 51, 52]. Nevertheless, although IL-1α was without effect on basal estradiol secretion in one of these studies, it led to an increase in gonadotropin-stimulated estradiol levels after 24 h of incubation [45]. Another study showed a stimulatory effect of IL-1β. However, this effect was not dose-dependent, as estradiol levels did not increase, but rather decrease with higher doses of IL-1β. Interestingly, the increase and subsequent decrease in estradiol levels was accompanied by a proportional change in cAMP levels, which suggests that low doses of IL-1β might stimulate estradiol production through activation of second-messenger systems. Furthermore, the stimulatory effect on estradiol and cAMP secretion was inhibited by the addition of IL-1RA, suggesting an IL-1 receptor dependent effect [53].

In sum, the inhibitory effects of IL-1α and IL-1β on estradiol production by granulosa and granulosa-luteal cells per se were consistent. Estradiol secretion by the emerging dominant follicle is thought to have two important effects in the late follicular phase. First, it leads to a partial reduction of FSH levels in order to suppress further stimulation of the unselected follicles [77]. Second, estradiol might be the agent responsible for the LH surge through a positive feedback [78]. Consequently, IL-1-mediated inhibition of granulosa cell-derived estradiol secretion could lead to an inhibition of dominant follicle emergence and of ovulatory signals. Nevertheless, the inhibitory effect could not be confirmed in cultures of preovulatory follicles or in in vitro perfused ovaries.

Progesterone In *humans*, most investigations on progesterone production were performed again in granulosa-luteal cells. IL-1β was shown to dose-dependently stimulate progesterone mRNA expression and protein production in vitro in postovulatory granulosa-luteal cells [55, 56]. In contrast, in two other in vitro studies of granulosa-luteal cells from women undergoing IVF, IL-1β had no effect neither on basal nor

on gonadotropin-stimulated progesterone production [41, 50]. Surprisingly, although IL-1β per se had no effect on progesterone in these studies, the addition of a non-selective IL-1 antagonist inhibited progesterone production, which could imply either endogenous IL-1β or IL-1α signaling that would intrinsically stimulate progesterone production [41].

Taken together, IL-1 seems to stimulate progesterone production in granulosa-luteal cells, but this stimulatory effect might be counterbalanced in granulosa-luteal cells from gonadotropin-stimulated IVF-cycles by an undefined mechanism. As previously mentioned, postovulatory progesterone production is vital for maintenance of the pregnancy and IL-1 could have a supportive effect.

The preovulatory rise in progesterone is a necessary mediator for ovulation [79]. As human data on the IL-1 effects on preovulatory progesterone increase are lacking, it is interesting to look at *animal data*. Many experiments investigating the effects of IL-1 on progesterone secretion in animal ovaries have been performed. Overall, the results are conflicting, as the experimental setting varied in the different studies (e.g., dosage of IL-1, cell type, species, sexual maturity of the animals, etc). When investigating preovulatory animals, IL-1β seemed to have either no or stimulatory effects on progesterone secretion. The experiments that showed a stimulatory effect were performed either in cultures of the whole follicle [53], or in the ex vivo perfused ovary [52], or in vivo by aspirating follicular fluid [64]. However, when looking at isolated granulosa cell cultures, IL-1 consistently *decreased* basal, as well as gonadotropin-mediated progesterone levels [47, 49, 58–63].

In summary, IL-1β seems to inhibit progesterone production from preovulatory granulosa cell cultures but stimulate progesterone release in investigations of the whole follicle/ovary. Theoretically, it could be that IL-1β has a stimulatory effect on theca cell-derived progesterone. This hypothesis was confirmed by one study in the rat [46], but disproved by another study in the rabbit [60]. These discrepancies and the lack of human data do not allow a clear conclusion about the effects of IL-1β on the preovulatory rise in progesterone.

Steroidogenic activity Only few studies reported effects of IL-1α or IL-1β on steroidogenic protein/enzyme gene expression and/or activity. IL-1β stimulated mRNA expression and protein production of the steroidogenic acute regulatory (StAR) protein in human granulosa-luteal cells in vitro. The StAR protein is responsible for the mitochondrial transport of cholesterol which is the first and necessary, rate-limiting step for steroidogenesis [55]. This could explain why progesterone levels increase when granulosa-luteal cells are treated with IL-1. Inhibitory effects of IL-1α and IL-1β were shown for FSH-induced aromatase activity in rats, which would normally convert theca-cell-derived testosterone to estradiol [49, 61, 65]. Importantly, no human data are available on the effects of IL-1 on aromatase. As mentioned previously, the aromatization of androgens to estrogens is key to limit androgen secretion, and IL-1 seems to interfere with this process. Interestingly, in one of these studies, the authors observed a decrease of progesterone which was accompanied by an increase in 20α-hydroxyprogesterone (-OHP). This suggests an increase in metabolism of progesterone by enhanced activity of the species-specific 20α-hydroxysteroid dehydrogenase. The 20α-hydroxysteroid dehydrogenase converts progesterone to an inactive form [49]. This might serve as an explanation for the inhibition of progesterone secretion in granulosa cell cultures. Furthermore, IL-1β inhibited FSH-induced 17β-hydroxysteroid dehydrogenase in the cultures of rat granulosa cells which results in inhibition of estradiol generation [65].

Overall, IL-1α and IL-1β are likely to initiate steroidogenesis by their ability to activate StAR protein expression and translation. However, in a healthy ovary, steroidogenesis should lead to an increase in estradiol levels, and a preovulatory increase in progesterone levels which is in turn inhibited by IL-1. Hence, IL-1 might have disturbing effects on steroidogenic enzymes.

Gonadotropin receptors As indicated in the second chapter, the acquisition of gonadotropin receptors is another pivotal event in folliculogenesis. Generation of IL-1α knockout mice demonstrated increased FSH receptor-mRNA expression [36, 66]. From these observations, it can be concluded that IL-1α has an inhibitory effect on FSH receptor expression. Consistently with this hypothesis, treatment of wild-type mice with anakinra (a recombinant IL-1RA, blocking both IL-1α and IL-1β signaling) stimulated FSH receptor-mRNA expression in granulosa cells [66]. With the latter findings, it could be possible that IL-1β also has a negative role on FSH receptor expression. It is known that IL-1β inhibits LH-receptor formation in animal granulosa cell cultures [58, 62, 67]. LH receptor formation (=luteinization) of granulosa cells is a mandatory preliminary step for successful ovulation as the LH binds to LH receptors on granulosa cells during the LH surge and thereby initiates the ovulatory process [9]. No human data are available.

In sum, the results on gonadotropin receptor expression are very consistent showing that IL-1 inhibits FSH and LH receptor expression in granulosa cells. It would be very interesting to know whether IL-1 has divergent effects on LH receptor expression in theca cells as LH is a potent stimulator of androgen formation in the theca layer, but here, data are lacking.

Summary of the effects of IL-1 on ovarian steroidogenesis IL-1α, as well as IL-1β, was shown to interfere with steroidogenesis on multiple levels. First, IL-1 is able to stimulate testosterone production from theca cells. In ovarian physiology, androgen production is a feature of the remaining, unselected follicles and should be kept at a low level. Second, IL-1 was

shown to inhibit FSH receptor and LH receptor expression. FSH receptor expression is mandatory throughout the follicular phase as FSH initiates dominant follicle selection in the early, and stimulates the further development of the dominant follicle in the late follicular phase. Luteinization is required for successful ovulation to mediate ovulatory LH signaling during the LH surge. Third, both cytokines inhibited the FSH-induced estradiol and progesterone increase from granulosa cells from preovulatory follicles. Estradiol secretion of the dominant follicle might have an important role in suppressing the further growth of the unselected follicles. Furthermore, a preovulatory rise in progesterone is a necessary event for ovulation. This implies an inhibitory role of IL-1 on dominant follicle steroidogenesis and preovulatory steps. Nevertheless, these findings could not be replicated in all studies, and some studies reported even a stimulatory effect. On the one hand, IL-1β was shown to be able to initiate steroidogenesis by increase in StAR protein. On the other hand, it prevented aromatase-induced conversion of androgens to estrogens which could explain why estradiol levels were unchanged with IL-1 treatment in whole follicles. A schematic way how the IL-1 system might affect steroidogenesis during the follicular phase is shown in Fig. 2.

Effect of IL-1 on ovulation, associated mechanisms, and fertility

Ovulations rates/fertility Several groups have examined *animal* data on the role of IL-1 on the ovulatory cascade and consequent fertilization rates. IL-1β is able to induce ovulation partially or to the same amount as the LH surge either in vivo or in the in vitro perfused ovary in different animals [52, 68–70]. These data propose a pro-ovulatory function of IL-1β, meaning that ovulations can be triggered by administration of IL-1β without LH. One study in the perfused rat ovary showed no LH-independent ovulation upon IL-1β administration but a reduction of ovulation rates if IL-1RA was added [51]. This implies that either endogenous IL-1α or IL-1β signaling is one of the required mediators for successful ovulation, as IL-1RA blocks the activity of both cytokines. However, the ovulation rates (=number of ovulated follicles) in the above-mentioned studies were not accompanied by an accordant rate in the number of fertilized oocytes and pregnancies (=fertility rate). For instance, although the intrafollicular injection of IL-1β was able to induce ovulation in mares, it lowered their fertility rates and inhibited nuclear maturation of the oocyte [64, 69]. Moreover, in the rabbit, despite IL-1β achieving similar number of ovulations as a LH analogue, the rates of successful oocyte fertilization and maturation were largely decreased in the IL-1β group compared to LH-analogue-treated controls [70]. Consistently with the data showing a negative impact of IL-1 on fertility, blocking the IL-1 pathway with the IL-1RA anakinra in wild-type mice resulted in *higher* oocyte fertilization rates compared with saline-treated controls, although this finding was not statistically significant due to low sample size [66].

Interestingly, mice that lack the gene for IL-1α had an increased number of growing follicles compared with their wild-type controls, despite having the same number of primordial follicles. Accordingly, pregnancy rates were increased in IL-1α knockout mice. The generation of IL-1β knockout mice showed an increase in the number of ovulations and a consequent increase in litter size [36].

These recent findings contradict the previous studies that showed a stimulatory role of IL-1β on ovulation. Nonetheless, if IL-1β was necessary for ovulation, then IL-1β knockout mice would not display an increased but a reduced number of ovulations. A possible explanation is that exogenous IL-1β administration on a preovulatory follicle is able to activate similar mechanisms as the LH surge (e.g., prostaglandin production, see next paragraph) but in turn has a negative impact on fertility by impacting oocyte quality.

After all, the data on fertility measures are consistent, showing that both IL-1α and IL-1β (a) are not required for successful ovulation in mice, and (b) lead to decreased

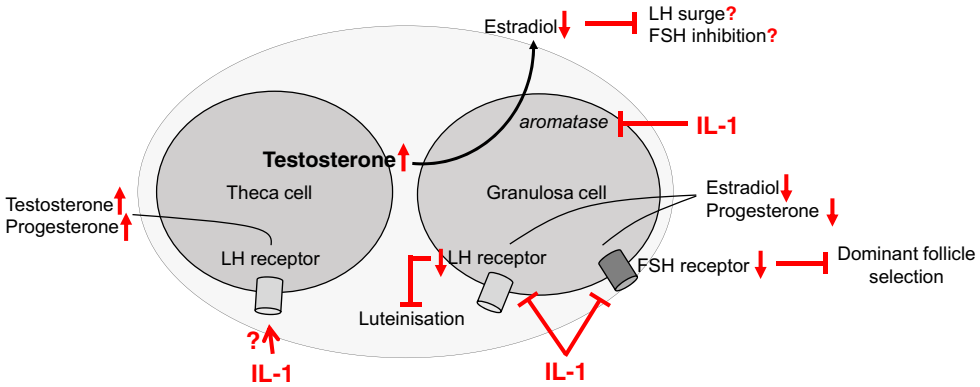

Fig. 2 Schematic representation of the interaction of IL-1 with the theca and granulosa cells during the follicular phase

fertilization/pregnancy rates in different animals. From an evolutionary perspective, IL-1 might play a role in limiting the litter size to bearable numbers for the animals.

Human data are sparse. Interestingly, and somehow contradictory with the previous findings on fertility measures in animals, IL-1 activity was higher in follicular fluid of women undergoing assisted reproductive technology in those cases where embryo implantation was successful [80]. Furthermore, IL-1β was significantly higher in follicular fluid from mature oocytes [81]. However, these studies investigated only an association between IL-1β activity and oocyte maturity and could not prove any causality.

Overall, to some extent, IL-1α and IL-1β seem to have the ability to mimic the effect of the LH surge and trigger ovulation. However, the pro-ovulatory role of IL-1β is not accompanied by an expected increase in fertilized oocytes and pregnancies. In in vivo studies, IL-1α and IL-1β had a negative impact on oocyte maturation and fertilization rates in animal studies.

Ovulatory mediators In humans, as mentioned earlier, prostaglandins and other local tissue factors (such as plasminogen activator) are essential mediators for ovulation. IL-1β dose-dependently stimulated the secretion of prostaglandins E2 and F2α in human granulosa and granulosa luteal cells which was dependent on the IL-1 receptor [56, 74].

Findings in animal studies were consistent with the data on human ovarian cells. IL-1β increased the secretion of prostaglandins E2 and F2α in different animals [51, 52, 54, 64, 71–73].

The effect of IL-1β on plasminogen activator (PA) was also investigated. Plasminogen activators, through their fibrinolytic activity, are jointly responsible for the breakdown of the follicle wall, thereby enabling ovulation [9]. By examining the effect of IL-1 on prostaglandins where IL-1 acts pro-ovulatory, one would expect that IL-1 triggers a rise in PA activity. Contrarily, IL-1β inhibited basal and LH-induced PA activity in theca cells and whole preovulatory follicles, and stimulated PA inhibitor 1. Both of these effects were reversed by addition of IL-1RA [73, 75, 76].

In total, the findings on IL-1-mediated effects on prostaglandin production are very consistent, showing stimulatory effects on prostaglandin production. As prostaglandins are necessary mediators for ovulation, this could explain why exogenous IL-1 is able to induce ovulation. Despite having pro-ovulatory effects on prostaglandin, IL-1 inhibits PA activity which in turn interferes with normal ovulation and follicular remodeling.

Summary of the effects of IL-1 on ovulatory mechanisms and fertility Ex vivo experimental data on artificially perfused ovaries suggest that IL-1 signaling in the dominant follicle could represent an alternative pathway to the LH surge to trigger ovulations. This pro-ovulatory effect could be mediated by the IL-1-induced production of prostaglandins which are necessary for the ovulatory process. However, IL-1 inhibited plasminogen activators, which in turn is not beneficial for ovulation. Furthermore, it was consistently shown that IL-1 has negative effects on different fertility measures, for instance on the number of fertilized oocytes, oocyte maturation, and pregnancy rates. Accordingly, the knockout of the genes for IL-1α and IL-1β in mice and the treatment with the IL-1RA anakinra in wild-type mice led to increased fertility rates. Nevertheless, the number of studies is very small and it is not clear whether and how these data can be translated onto humans.

Studies on IL-1 activity and inflammatory markers in patients with PCOS

IL-1-related inflammatory markers in PCOS

The concentrations of IL-1β in the blood are extremely low and the injection of only a few nanograms suffices to induce an acute systemic inflammatory reaction. This is why IL-1β itself is difficult to measure with regular assays, as its concentration in the peripheral blood is mostly below the detection limit, especially in the setting of chronic low-grade inflammation [82]. A marker for IL-1β activity is elevated levels of C-reactive protein (CRP). CRP is formed in the liver and can be easily measured in the blood by high-sensitivity assays. It is known to reflect IL-1β activity in acute as well as in chronic low-grade inflammatory disease states [83].

In 2001, Kelly et al. reported for the first time that C-reactive protein (CRP) levels are elevated in patients with PCOS compared with weight-matched controls, consistent with a state of chronic low-grade inflammation [84]. In the following years, several studies investigating inflammatory markers, especially CRP, in PCOS women have been performed. The presence of chronic low-grade inflammation in PCOS was confirmed by a meta-analysis that showed CRP elevation in women with PCOS as compared to weight-matched controls and after adjusting for BMI [85]. Another marker which is able to induce IL-1β and vice versa is nuclear factor κB (NFκB). Intracellular NFκB activity was shown to be elevated after a glucose challenge in women with PCOS compared with their weight-matched controls [86]. Moreover, it was shown that secretion of IL-1β from mononuclear cells was higher in patients with PCOS compared with controls, independently of obesity [87].

Genetic studies on IL-1 polymorphisms in PCOS

Genetic polymorphisms of the gene coding for IL-1β (*IL1B*) might lead to an increment in IL-1β signaling activity. Four

studies investigating the presence of the genetic polymorphism *IL1B*-511 C/T in patients with PCOS and control groups have been summarized in a meta-analysis in 2015 [88]. Thereby, no association between PCOS and this specific polymorphism of *IL1B* was found. However, this analysis holds some caveats: First, 3 out of 4 studies were performed in Asian patients which does not allow a direct translation to women of European descent. Second, sample sizes were small and the studies did not provide sufficient clinical data. Last but not least, the authors of the above mentioned declared significant heterogeneity between the studies which remained unexplained. In one of these studies performed in a cohort of 105 Caucasian PCOS patients and 102 control women, Kolbus et al. have demonstrated that a polymorphism of the IL-1α gene (*IL1A*) was more frequent in the PCOS group, especially in those patients with anovulation [89]. Concerning polymorphisms of the IL-1RA gene (*IL1RN*), no association between *IL1RN* polymorphisms and PCOS with respect to their clinical characteristics was shown [90].

Lastly, there is currently not sufficient evidence that genetic variations of the IL-1 system genes might account for chronic low-grade inflammation in PCOS.

Metabolic effects of IL-1

In addition to the many publications on the effects of IL-1β on the immune system, there have been several investigations concerning the detrimental effects of IL-1β on glucose/insulin metabolism and the cardiovascular system. IL-1β was not only shown to have a physiological role in postprandial glucose metabolism [91], it was also shown to have a detrimental effect on the pancreas in the setting of diabetes [92], and to induce insulin resistance [93]. Consequently, the antagonism of the IL-1 pathway led to an improvement in glucose metabolism [94]. A recent study has demonstrated that IL-1β antagonism reduced mortality in patients with cardiovascular disease and chronic low-grade inflammation [95]. There is an overlap of IL-1β induced pathological mechanisms and findings in patients with PCOS: Interestingly, insulin resistance was suggested to be present in PCOS patients irrespective of obesity [96]. Furthermore, the early development of cardiovascular risk might also be mediated by chronic low-grade IL-1β activity PCOS. Therefore, in PCOS, IL-1β might not only be a key player in dysregulation of ovarian steroidogenesis, but also contribute to the increased likelihood to develop features of the metabolic syndrome, i.e., insulin resistance with consequent development of type 2 diabetes, and eventually cardiovascular disease. However, this is speculative at the moment and remains to be shown in future studies.

Source and cause of elevated IL-1 activity in PCOS

Studies on mononuclear cells from women with PCOS have demonstrated an increased production of IL-1β and NFκB upon glucose challenge, especially in obese but also in lean subjects without excess of abdominal adipose tissue, proposing an overactivity of the immune system to metabolic triggers in patients with PCOS [87]. Nonetheless, not only the immune system but a local ovarian inflammation might play a role in the inflammatory state. Such investigations require histologic examinations of the polycystic ovary which are sparse. It is known that macrophages are present in the ovary and contribute to folliculogenesis, as it was shown that macrophages infiltrate the theca layer during folliculogenesis. Nevertheless, the available histologic studies do not report a significant infiltration by macrophages, arguing against a local ovarian inflammatory reaction [97]. On the other hand, a recent report showed that there is a disbalance between the pro-inflammatory M1 and the anti-inflammatory M2 macrophages, in favor of the M1 type, secreting pro-inflammatory cytokines [98]. Nonetheless, one older study showed that IL-1β levels in ovarian culture media were not significantly different between women with and without PCOS [99].

Although chronic low-grade inflammation was demonstrated to be present also in lean subjects, a significant contributor to chronic low-grade inflammation in PCOS is the adipose tissue. The hypertrophy of the adipocytes leads to a hypoxic state which initiates the generation of reactive oxygen species and pro-inflammatory cytokines [24]. Obesity comes along with a more pronounced inflammatory state (e.g., higher CRP levels) and further aggravates the clinical features of PCOS [85, 100, 101].

The question is whether IL-1 causally contributes to the disturbances in ovarian steroidogenesis and follicle development seen in PCOS or whether the increase in IL-1 is a consequence of hyperandrogenemia. On the one hand, experimentally induced hyperandrogenemia in healthy, reproductive-aged women led to a significant increase in postprandial NFkB activity from mononuclear cells [102]. Considering this finding, one would hypothesize that chronic low-grade inflammation arises due to the hyperandrogenemia in PCOS. Two studies have addressed this question by reducing testosterone levels for 6 months and investigating outcomes of inflammatory markers, i.e., CRP. In one study, women with PCOS were treated with a gonadotropin releasing hormone agonist. CRP levels were not reduced, but rather increased at the end of testosterone-reducing treatment, especially in obese subjects [103]. In another study that included PCOS patients without elevation of CRP levels, it was shown that androgen suppression by ethinylestradiol combined with spironolactone increased CRP levels [104]. Taken together, androgen suppression does not ameliorate inflammation thereby rendering the hypothesis of inflammation being secondary to hyperandrogenism unlikely.

Summary and outlook

Taken all the results together, it appears that IL-1 has physiological, as well as pathophysiological, effects on the reproductive axis. What argues for a physiological role is, first, the occurrence of IL-1 ligands and receptors in ovarian cells, which is most pronounced around ovulation. Second, IL-1 stimulates prostaglandins that enable ovulation. Third, IL-1 increased postovulatory progesterone production which is pivotal for maintenance of the pregnancy. On the contrary, IL-1 seems to play a pathophysiological role by interfering with steroidogenesis in the follicular phase of the estrous cycle and by decreasing fertility rates. Moreover, IL-1 gene deficiency in mice did not result in reduced but rather increased fertility. This fact argues against a major mandatory role of IL-1 for successful reproduction in mice. The results of studies in PCOS patients argue for the presence of IL-1 overactivity, making it interesting to look at experiments investigating the effects of exogenous IL-1 on ovarian physiology. Consistent findings were the inhibitory effects on FSH receptor expression and luteinization of the granulosa cell layer, and the inhibition of aromatase activity and estradiol secretion by granulosa cells. All of these factors could contribute (a) to the suppression of dominant follicle selection, mostly by reducing FSH signaling in the follicular phase, or possibly by inhibiting the estradiol increase, (b) to high androgen levels by inhibition of aromatase, and (c) to anovulation by inhibiting luteinization. This could explain why PCOS patients with chronic low-grade inflammation fail to develop a dominant follicle and have high androgen levels. Nonetheless, very few, and particularly no human data are available on the direct effects of IL-1 on testosterone. Despite the paucity of reports on testosterone (only 1 study in animals showing a stimulatory effect of IL-1), it can be assumed that IL-1 leads to an elevation of testosterone (a) through a direct stimulation of testosterone production from theca cells, (b) through its inhibitory effect on aromatase activity, and (c) through the suppression of dominant follicle selection, as unselected follicles produce mainly androgens. Therefore, IL-1 might be a potential target in PCOS. However, the source of IL-1β remains to be elucidated. Although IL-1 was suggested to originate from an overactivity of immune cells, the most recent report shows a prevail of pro-inflammatory macrophages in the PCOS ovary, proposing ovarian inflammation as a source of IL-1.

To further investigate a possible interplay between the IL-1 system and PCOS, an interventional study blocking the IL-1 pathway in vivo in patients with PCOS is required, by administration of either a direct antibody or an IL-1RA, with the subsequent examination of the effects of IL-1 blockade on clinical and laboratory parameters in affected patients.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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