

# Expression of interleukin-1 $\beta$ and its receptor in human granulosa cells and their association with steroidogenesis

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## ABSTRACT

This study aimed to investigate whether interleukin 1 $\beta$  (IL-1 $\beta$ ) and soluble IL-1 receptor 2 (sIL-1R2) are expressed in human granulosa cells (GCs) and relate to ovarian steroidogenesis. Ninety-six women undergoing in vitro fertilization (IVF) were recruited. RT-PCR and immunocytochemistry were used to detect mRNAs and proteins of IL-1 $\beta$  and IL-1R2, respectively. The steroidogenesis of primary cultured GCs was evaluated following treatment with either IL-1 $\beta$  alone or IL-1 $\beta$  and FSH in combination. There were positive correlations between serum IL-1 $\beta$  and serum progesterone ( $r = 0.220$ ,  $p = 0.032$ ) and follicular fluid (FF) estradiol ( $r = 0.242$ ,  $p = 0.018$ ). Additionally, serum and FF sIL-1R2 were negatively and positively correlated with FF estradiol ( $r = -0.376$ ,  $p = 0.005$ ) and FF progesterone ( $r = 0.434$ ,  $p = 0.001$ ), respectively. The mRNA and protein expression of IL-1 $\beta$  and IL-1R2 became evident in GCs. IL-1 $\beta$  alone significantly increased estradiol secretion from GCs, but in the presence of FSH, it could notably promote progesterone secretion in addition to estradiol. In conclusion, IL-1 $\beta$  and sIL-1R2 are expressed in human GCs and substantially contribute to ovarian steroidogenesis, suggesting that the IL-1 $\beta$  system may be a potential target for optimizing ovarian hyperstimulation and steroidogenesis in IVF cycles.

## 1. Introduction

Folliculogenesis consists of an intricate sequence of mutually relevant and extremely orchestrated processes, including the stimulation of primary follicles, the proliferation of follicular cells, the meiotic maturation of the oocyte, the production of gonadal steroids, and ovulation (Sobinoff et al., 2013). Cytokines are fundamental mediators of ovarian physiology, especially with respect to follicular growth and selection, in which they participate in providing a milieu that supports folliculogenesis, steroidogenesis, and ovulation (Field et al., 2014). IL-1 $\beta$ , as one of the important cytokines, is locally produced and accumulates within the ovarian follicular fluid (FF) during folliculogenesis (Loret de Mola et al., 1998; Machelon et al., 1995), wherein mediates several pivotal processes, including the progress of granulosa cells (GCs) toward proliferation or differentiation (Karakji and Tsang, 1995), induction of

vascular endothelial growth factor (VEGF) (Levitas et al., 2000) and nitric oxide (NO) synthesis (Matsumi et al., 2000), and modulation of prostaglandin E (PGE) production (Duffy and Stouffer, 2002), proposing its contribution to folliculogenesis-related processes such as steroidogenesis and ovulation.

IL-1 $\beta$ , a proinflammatory mediator, together with a set of other ligands (IL-1 $\alpha$  and IL-1 receptor antagonist (IL-1Ra)) and receptors (IL-1R1 and IL-1R2) constitute the IL-1 system (Boraschi et al., 2018). IL-1 $\beta$  is a main proinflammatory cytokine, whereas IL-1 $\alpha$  majorly acts as an intracellular mediator, but it is also secreted into extracellular space and exerts proinflammatory effects similar to IL-1 $\beta$  in the state of high inflammation. IL-1Ra represents another ligand of the IL-1 system which is implicated in the competitive inhibition of IL-1 (Dinarello, 2009). There are two major types of ligand-binding receptors for IL-1, namely IL-1R1 and IL-1R2 (Boraschi et al., 2018). IL-1R1 is a receptor that can

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bind to IL-1, initiate signaling transduction, and mediate its biological functions (Sims et al., 1988). IL-1R2, on the contrary, lacks the intact intracellular domain, which makes it unable to initiate signal transduction (McMahan et al., 1991). Hence, IL-1R2 serves as a decoy receptor and an inhibitory mechanism for IL-1 function, in which it captures IL-1 with a high affinity and thereby restrains its interaction with functional IL-1R1 (McMahan et al., 1991). In spite of IL-1R1, which has an identical binding affinity for all of its ligands, i.e. IL-1 $\alpha$ , IL-1 $\beta$ , and IL-1Ra, IL-1R2 strongly binds to IL-1 $\beta$ , has a 10-fold lower affinity for IL-1Ra, and is virtually unable to interact with IL-1 $\alpha$  (Symons et al., 1995). Considering that the affinity of IL-1R2 for antagonist IL-1Ra is low, the probability of reducing IL-1R2 capability to neutralize IL-1 activity by IL-1Ra is insignificant (Boraschi et al., 2018). Different mRNA splicing or proteolytic cleavage of IL-1R2 results in the production of soluble IL-1R2 (sIL-1R2) (Liu et al., 1996; Orlando et al., 1997). While sIL-1R2 and its membrane-bound counterpart are similar in affinity for IL-1 $\beta$ , sIL-1R2 is almost not able to bind antagonist IL-1Ra (Symons et al., 1995), the feature which makes sIL-1R2 a more potent inhibitor of IL-1 even in comparison with membrane-bound IL-1R2 (Boraschi et al., 2018). It has been indicated that almost all members of the IL-1 system are expressed in different compartments of bovine follicles (Passos et al., 2016). Although the transcription of IL-1 $\beta$ , IL-1R1, and IL-1Ra has been evidenced in human GCs (de los Santos et al., 1998; Hurwitz et al., 1992), the protein expression of IL-1 $\beta$  as well as both mRNA and protein expression of IL-1R2, as the major negative regulator of IL-1 $\beta$ , remains to be determined. Furthermore, it is not elucidated if sIL-1R2 exists in FF and is associated with ovarian steroidogenesis and oocyte maturation.

Numerous studies have been carried out to explore the effect of IL-1 $\beta$  on follicular growth and oocyte maturation. It has been demonstrated that IL-1 $\beta$  enhanced the viability and activation of primordial follicles in cattle (Passos et al., 2016). On the contrary, IL-1 $\beta$  hindered the nuclear maturation of oocytes in mares (Caillaud and Gerard, 2009). There is also a report in humans that IL-1 $\beta$  had no association with follicle growth, oocyte maturation, and embryogenesis (Adamczak et al., 2022; Mendoza et al., 1999). Similar contradictions are seen in studies that examined the effect of IL-1 $\beta$  on follicular steroid synthesis. According to in vitro studies, IL-1 $\beta$  suppressed progesterone secretion from GCs of various animal models (Bréard et al., 1998; Fukuoka et al., 1989; Kasson and Gorospe, 1989). By contrast, it displayed a stimulatory effect on progesterone production in cattle GCs (Baratta et al., 1996) as well as rat preovulatory follicles (Brännström et al., 1993). Similarly, there is evidence that IL-1 $\beta$  stimulates the synthesis of progesterone by primary-cultured human GCs in a luteinizing hormone (LH)-independent manner (Chen et al., 2000; Dang et al., 2017). However, some other studies did not find an apparent alteration in progesterone production following IL-1 $\beta$  treatment (Acosta et al., 1998; Barak et al., 1992). Nevertheless, there is relative consistency in the literature that IL-1 $\beta$  decreased the capacity of estradiol synthesis by GCs (Barak et al., 1992; Baratta et al., 1996; Gerard et al., 2004; Ghersevich et al., 2001). Therefore, it is a matter of debate whether IL-1 $\beta$  influences GC steroidogenesis and oocyte meiotic maturation.

Regarding contradictions and/or the lack of evidence on the association of IL-1 $\beta$  and sIL-1R2 with gonadal steroidogenesis and oocyte maturation in humans, the objectives of this study were to explore whether (1) IL-1 $\beta$  and IL-1R2 are expressed in GCs, (2) the levels of serum and FF IL-1 $\beta$  and sIL-1R2 are associated with follicular steroid levels and oocyte maturation, and (3) IL-1 $\beta$  influences steroidogenesis in primary GCs culture.

## 2. Materials and methods

### 2.1. Study population

Ninety-six women who were referred to the Department of Obstetrics and Gynecology, University of Kiel, for in vitro fertilization (IVF)

treatment participated in this study. Serum and FF samples were taken from all participants on the day of oocyte retrieval. Recruited patients had either tubal factor infertility or male factor infertility as inclusion criteria and their follicle size ranged from 19 to 24 mm. Moreover, all patients were of reproductive age (median age 33 years; range 23–44). The informed signed consents were obtained from all participants and the Institutional review board approval was not obtained for this observational study according to the stipulations of the medical faculty.

### 2.2. Controlled ovarian stimulation

The human menopausal gonadotropin (hMG, Menogon, Ferring, Kiel, Germany) or recombinant follicle-stimulating hormone (FSH, Serono or Organon, Munich, Germany) following GnRH agonists protocol was used for ovulation induction. The ovarian follicles were monitored during the IVF cycle by real-time ultrasonic scanning of the ovaries as well as serum estradiol evaluation. Patients have subcutaneously received 10,000 IU human chorionic gonadotropin (hCG, Choragon; Ferring) when a follicle with a diameter of > 16–18 mm was observed and also the serum levels of estradiol reached an acceptable level (< 3000 pg/ml). The follicles were punctured after 36 h and oocytes, GCs, and FF samples were collected. Nuclear maturation and morphological characteristics of oocytes were determined under microscope by an embryologist. Patients were classified as low and high responders according to a scoring system comprising of the increase in estradiol levels, patients' age, the total dose of rFSH, and the number of follicles (< 7 and  $\geq$  7 for low and high responders, respectively) (Salmasi et al., 2019).

### 2.3. Measurement of IL-1 $\beta$ and sIL-1R2 in serum and follicular fluid

Blood and FF samples were collected on the day of oocyte retrieval and after centrifugation were kept at  $-80^{\circ}\text{C}$ . IL-1 $\beta$  and sIL-1R2 levels in serum and FF were measured using commercial ELISA kits (Biosource, Solingen; R&D, Wiesbaden). The detection ranges for IL-1 $\beta$  and sIL-1R2 were 0.10–10 pg/ml (sensitivity 0.05 pg/ml) and 5–2000 ng/ml (sensitivity 2.5 ng/ml), respectively. Intra-assay and inter-assay coefficients of variation (CV) were  $\leq$  8.5% and  $\leq$  8.4% for IL-1 $\beta$  and  $\leq$  3.4% and  $\leq$  5.9% for sIL-1R2, respectively. The average percent of recovery for both IL-1 $\beta$  and sIL-1R2 were 95% in serum according to the manufacturer's specifications. All the tests were conducted in duplicate.

### 2.4. Estradiol and progesterone assessment

The competitive chemiluminescent enzyme immunoassay using Immulite 2000 auto system (DPC-Biermann, Siemens, Bad Mannheim, Germany) was applied for the evaluation of estradiol and progesterone levels. The sensitivity and detection range for estradiol were 5 pg/ml and 5–3000 pg/ml and for progesterone were 0.2 ng/ml and 0.5–40 ng/ml, respectively. Intra-assay CV was < 10% and inter-assay CV was < 12% for both estradiol and progesterone. In order to measure both hormones in FF, 100  $\mu\text{L}$  of samples were diluted at a ratio of 1:500 using physiological serum. The quantification of progesterone in conditioned media of GCs (100  $\mu\text{L}$ ) was also performed at a dilution ratio of 1:500.

### 2.5. Granulosa cells isolation

The FF samples obtained from 10 women under the age of 35 were centrifuged at 350g for 5 min. The resultant cell pellets were pooled and re-suspended in 5–10 ml of HAM's F-10 culture media (Sigma, Deisenhofen, Germany) which was supplemented with streptomycin (100  $\mu\text{g}/\text{ml}$ ; Sigma) and penicillin (100 IU/ml; Sigma). For cell isolation, the Percoll method was applied. For this purpose, a gradient of 2 ml of 64% and 2 ml of 36% of a Percoll solution (Sigma) was prepared and the GCs were collected from the interphase layer of the Percoll and then washed with Dulbecco's Hanks' balanced salt (D-HBSS) two times. The CD 45-

positive blood cells (such as lymphocytes) were removed using the magnetic Dynabeads M-450 CD 45 (DYNAL INC., Sigma) as previously described (Salmassi et al., 2017; Salmassi et al., 2004; Salmassi et al., 2005). To confirm the lack of monocytes/macrophages in the isolated GCs, immunohistochemistry was performed using the monoclonal antibody Ki-M1P based on the previously described method (Radzun et al., 1991). It is noteworthy that the isolation purity of this method was in the range of 95–98% according to our previous study using flow cytometric detection of specific GC and leukocyte markers, i.e. FSH receptor and CD 45, respectively (Aghadavod et al., 2015).

## 2.6. The reverse transcription-polymerase chain reaction of IL-1 $\beta$ and IL-1R2 mRNAs in GCs

Total RNA was extracted by trizol reagent (GIBCO/BRL, Karlsruhe, Germany) based on the previously described method (Chomczynski and Sacchi, 1987). To avoid DNA contamination, extracted RNA (1  $\mu$ g) was dissolved in a solution containing 20 mM Tris-HCl buffer (pH 8.4), 2.0 mM MgCl<sub>2</sub>, 50 mM KCl and 1 U of DNase (MBI, Fermentas) and incubated for 10 min at 37 °C. cDNA synthesis was performed using the Revert Aid TM H Minus First Strand Synthesis Kit (MBI, Fermentas). The RT-PCR was conducted in Peqlab PeqSTAR G-P96Z6G 96-Well Block Thermocycler (Germany) for 40 cycles with the following program: 30 s at 94 °C, 30 s at annealing temperature (60 °C), and 35 s at 72 °C. Forward and reverse primers for IL-1 $\beta$  were 5'-AGCTCGCCA GTGAAATGATG-3' and 5'-ATCTTCCTCAGCTTGTCATGG-3' (product size of 214 bp), respectively. For amplification of IL-1R2, forward 5'-CCATGAAGGCCAGCAATACAAC-3' and reverse 5'-GTTCCAGAAACA CCTTACAG-3' were used (product size of 156 bp). Then, 10  $\mu$ L of the amplicon were electrophoresed on a 3% agarose gel using a size marker of 50 bp DNA Ladder (Yektatajhz, Iran). The specificity of reaction was confirmed by sequencing PCR products.

## 2.7. Immunocytochemistry analysis of IL-1 $\beta$ and IL-1R2 in GCs

The GCs were diluted with 1% human serum albumin (1:1, Sigma, USA) in tris (hydroxymethyl) aminomethane buffered solution (TBS) and then the cytopins slides were prepared. The slides were air-dried (24 h) and fixed with acetone. The protein expression of IL-1 $\beta$  and IL-1R2 in the GC was confirmed by immunocytochemistry using human IL-1 beta antibody (MAB601, R&D Systems, Germany; diluted 1:500 in 5% normal human serum in TBS) and human IL-1 RII antibody (MAB263, R&D Systems, Germany; diluted 1:500 in 5% normal human serum in TBS), respectively. Biotinylated goat anti-Mouse Immunoglobulins (R&D, BAF007) and the avidin-biotin complex (ABC)-labeled alkaline phosphatase (Dako, Hamburg, Germany) were applied for staining. Finally, the AP-Red substrate-chromogen mixture (Zymed, Kiel, Germany) was used for visualization at 25 °C for 20–30 min. Blood monocytes were used as positive control and the primary antibodies were replaced by nonimmune serum for negative control.

**Table 1**  
Comparison of IL-1 beta (IL-1 $\beta$ ) and IL-1 receptor type II (IL-1R2) levels between serum and follicular fluid (FF).

Variable	Total (n = 96) Mean $\pm$ SD Median (IQR)		p-value
Age (year)	33.49 $\pm$ 4.71 33 (30–37)		-
No. of metaphase II oocytes	5.35 $\pm$ 3.14		-
	<i>Serum</i>	<i>Follicular fluid</i>	
IL-1 $\beta$ (pg/ml)	0.879 $\pm$ 1.761 0.191 (0.11–0.49)	0.274 $\pm$ 0.34 0.175 (0.118–0.317)	0.004 <sup>§</sup>
IL-1R2 (ng/ml)	17.42 $\pm$ 5.32 16.26 (14.0–19.67)	11.678 $\pm$ 4.15 11.78 (8.36–14.40)	< 0.001 <sup>†</sup>
E2 (pg/ml)	981.22 $\pm$ 660.07	577,432.65 $\pm$ 347,030.68	< 0.001 <sup>§</sup>
P4 (ng/ml)	8.93 $\pm$ 6.15	135,24.47 $\pm$ 5988.08	< 0.001 <sup>§</sup>

<sup>§</sup>Wilcoxon and <sup>†</sup>paired t-test were used for the statistical analyses.

E2, estradiol; IQR, interquartile range; P4, progesterone; SD, standard deviation

## 2.8. Time course experiments

As preincubation, the GCs were cultured in Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-10 Ham (DMEM/F10; Sigma) supplemented with 10% fetal bovine serum (Gibco), streptomycin (100  $\mu$ g/ml; Sigma), and penicillin (100 IU/ml; Sigma) within 24-well plastic dishes (2  $\times$  10<sup>4</sup> viable cells per well; Cat No.: CLS351147, Corning® Falcon®, Corning, NY) for 36 h. Then, the supernatant was removed and the cells were treated with IL-1 $\beta$  (Sigma) at different concentrations (0, 1, 5, 10, and 50 ng/ml) in the presence of testosterone (300 ng/ml; Sigma) and levels of estradiol and progesterone were measured in the supernatant after 72 h. For the time-course study, FSH-stimulated GCs (75 IU/ml; Serono, Munich, Germany) were treated with IL-1 $\beta$  (Sigma) at different concentrations (0, 1, and 10 ng/ml) in the presence of testosterone (300 ng/ml; Sigma) and the estradiol and progesterone levels were evaluated after 24, 48, and 72 h. The culture was performed at 37 °C in a humidified tissue culture incubator gassed with 95% air and 5% CO<sub>2</sub>. All in vitro experiments were performed in triplicate.

## 2.9. Statistical analysis

The Kolmogorov-Smirnov test was used to check the distribution of the data. In the cases with abnormal distribution, median values and interquartile ranges (IQR) were reported in addition to the means and SDs. To compare factors between serum and FF, paired t-test or Wilcoxon were applied based on the distribution of data. The correlations among factors were analyzed by Pearson's or Spearman's correlation coefficients. Variables were compared between low- and high-responders using independent t-test or Mann-Whitney U test. One-way analysis of variance (ANOVA) followed by post hoc Tukey's test was used to compare the means of steroid hormones among the groups treated with different IL-1 $\beta$  doses. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 20. A p-value of  $p \leq 0.05$  was considered to be statistically significant.

## 3. Results

### 3.1. IL-1 $\beta$ and sIL-1R2 level in serum and follicle fluid

The demographic and clinical characteristics of the study population are shown in Table 1. The mean age of participants was 33.49  $\pm$  4.7 years. The levels of IL-1 $\beta$  and its sIL-1R2 were significantly higher in serum than in FF, whereas the levels of estradiol and progesterone were inversely higher in FF than in serum ( $p < 0.05$ , Table 1). However, there was a significantly positive correlation between serum and FF levels of IL-1 $\beta$  ( $r = 0.206$ ,  $p = 0.044$ ), but not sIL-1R2 (Table 2). Moreover, our results showed a significant correlation between serum levels of IL-1 $\beta$  and progesterone ( $r = 0.220$ ,  $p = 0.032$ ) but there was no such correlation in the FF ( $p > 0.05$ ). Serum levels of IL-1 $\beta$  were also positively

**Table 2**Correlation between IL-1 $\beta$  and sIL-1R2 with hormones and number of mature (MII) oocytes.

	Serum IL-1 $\beta$		FF IL-1 $\beta$		Serum sIL-1R2		FF sIL-1R2		Serum E2		FF E2		Serum P4		FF P4	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
FF IL-1 $\beta$	<b>0.206</b>	<b>0.044</b>														
Serum sIL-1R2	-0.228	0.093	0.058	0.676												
FF sIL-1R2	<b>-0.434</b>	<b>0.001</b>	-0.143	0.294	<b>0.306</b>	<b>0.023</b>										
Serum E2	0.090	0.348	0.112	0.278	-0.111	0.424	-0.164	0.232								
FF E2	<b>0.242</b>	<b>0.018</b>	-0.019	0.849	<b>-0.376</b>	<b>0.005</b>	-0.109	0.430	<b>0.232</b>	<b>0.023</b>						
Serum P4	<b>0.220</b>	<b>0.032</b>	0.175	0.087	0.031	0.823	-0.181	0.181	<b>0.494</b>	<b>0.001</b>	0.048	0.643				
FF P4	0.011	0.920	0.070	0.495	0.080	0.566	<b>0.434</b>	<b>0.001</b>	<b>-0.245</b>	<b>0.018</b>	0.120	0.248	-0.008	0.939		
MI Oocytes	0.045	0.660	0.014	0.887	-0.210	0.124	<b>-0.284</b>	<b>0.034</b>	<b>0.547</b>	<b>0.001</b>	0.090	0.381	<b>0.468</b>	<b>0.001</b>	-0.206	0.440

Based on the distribution of data, Pearson's or Spearman's rank correlation coefficients were used for the statistical analyses. The number in bold indicates the presence of a significant correlation.

E2, estrogen; FF, follicular fluid; P4, progesterone

correlated with those of FF E2 ( $r = 0.242$ ,  $p = 0.018$ ). Serum levels of sIL-1R2 were negatively correlated with FF E2 levels ( $r = -0.376$ ,  $p = 0.005$ ). More interestingly, there were positive and negative correlations between FF levels of sIL-1R2 with FF progesterone levels ( $r = 0.434$ ,  $p = 0.001$ ) and the number of mature oocytes ( $r = -0.282$ ,  $p = 0.034$ ), respectively (Table 2). There was no significant correlation between serum or FF levels of IL-1 $\beta$  and sIL-1R2 with the age of patients (data not shown,  $p > 0.05$ ).

Additionally, we found no significant difference in serum and FF levels of IL-1 $\beta$  and serum levels of sIL-1R2 between low and good ovarian responders ( $p > 0.05$ , Table 3). However, FF levels of sIL-1R2 were significantly higher in the low responders compared to the good responder patients ( $p = 0.042$ ).

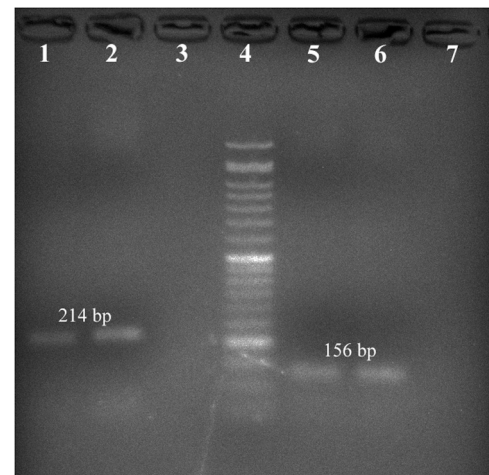
### 3.2. mRNA and protein expression of IL-1 $\beta$ and IL-1R2 in GCs

By applying RT-PCR on the total RNA of GCs, amplicons of 214 bp and 156 bp were detected for IL-1 $\beta$  and membrane-bound IL-1R2 (Fig. 1). The results clearly show that corresponding amplicons are detectable for IL-1 $\beta$  (Fig. 1, lanes 1 and 2) and membrane-bound IL-1R2 (Fig. 1, lanes 5 and 6) in samples from GCs and monocytes, respectively.

By means of immunocytochemistry, we were able to demonstrate that human follicular GCs express IL-1 $\beta$  (Fig. 2a). Fig. 2b exhibits the localization of membrane-bound IL-1R2 on the surface of GCs with reference to the negative control (Fig. 2d). As the positive controls, IL-1 $\beta$  and membrane-bound IL-1R2 are demonstrated on the surface of monocytes isolated from blood in the Fig. 2e and f, respectively.

### 3.3. Time course experiments

In vitro experiments showed that IL-1 $\beta$  at a concentration of 5 ng/ml could significantly increase estradiol secretion from GCs after 72 h culture compared to the control group ( $p = 0.003$ ). However, high concentrations of IL-1 $\beta$  (10 and 50 ng/ml) did not show the same effect on estradiol secretion ( $p > 0.05$ , Fig. 3a). IL-1 $\beta$  had no stimulatory effect on the secretion of progesterone by GCs ( $p > 0.05$ , Fig. 3b). Co-treatment of GCs with IL-1 $\beta$  and FSH (75 IU/ml) demonstrated that IL-1 $\beta$  at a concentration of 1 ng/ml could significantly increase secretion of estradiol and progesterone by GCs after 24 ( $p = 0.015$  and  $p = 0.032$ ),



**Fig. 1.** Reverse transcription (RT)-polymerase chain reaction (PCR) of beta (IL-1 $\beta$ ) and IL-1 receptor type II (IL-1R2) in human granulosa cells (GCs). The lanes show the following: lanes 1 and 2, IL-1 $\beta$  amplicon in human GCs and monocytes (positive control), respectively; lane 3, negative control omitting RT; lane 4, molecular weight marker (50 bp); lanes 5 and 6, IL-1R2 amplicon in human GCs and monocytes, respectively; lane 7: negative control omitting RT.

48 ( $p = 0.005$  and  $p = 0.023$ ), and 72 h ( $p = 0.001$  and  $p = 0.017$ ) compared to treatment with FSH alone; however, a higher concentration of IL-1 $\beta$  (10 ng/ml) could not significantly induce secretion of the hormones in the same time points ( $p > 0.05$ , Fig. 4a and b). Moreover, in each treatment group, the release of estradiol and progesterone by GCs revealed a significant hierarchical elevation by increasing the time of incubation from 24 to 48 h and from 48 to 72 h (Fig. 4a and b).

## 4. Discussion

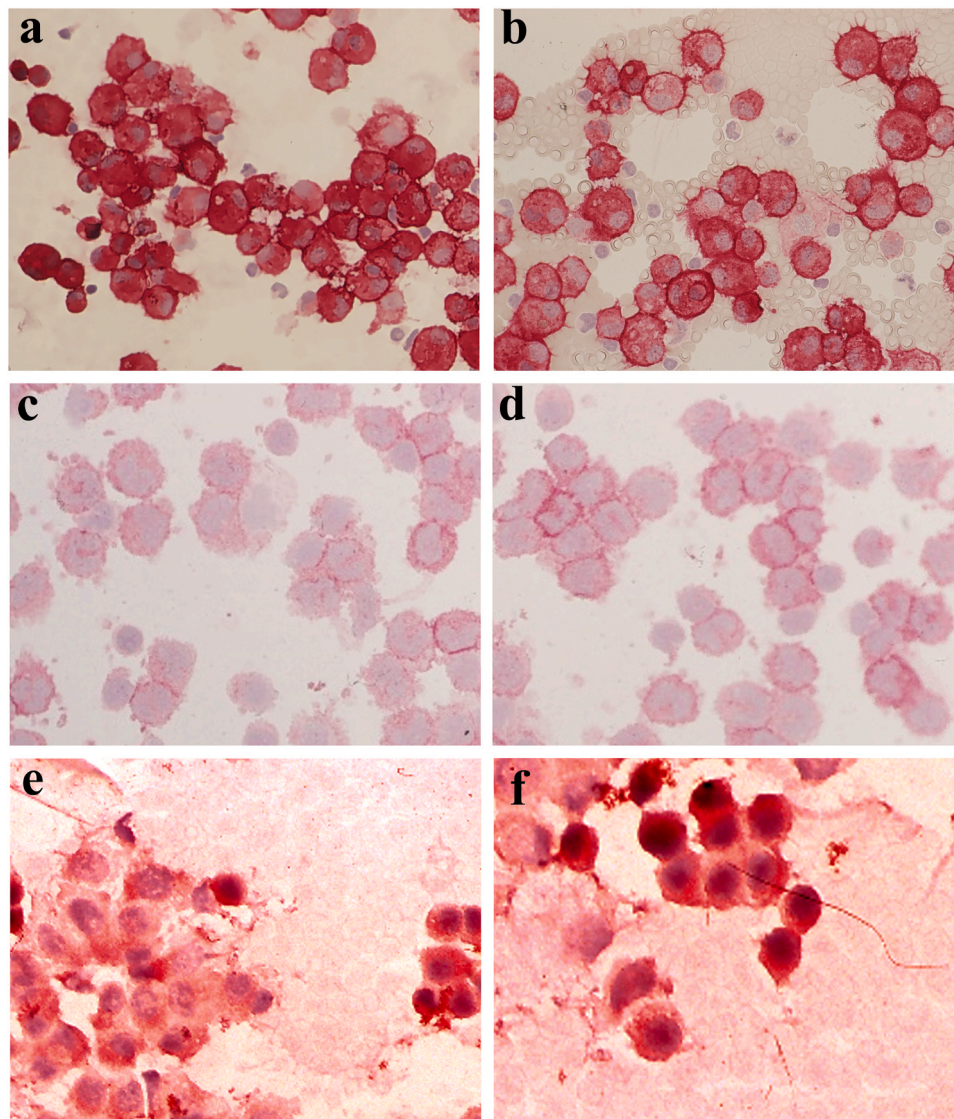
According to evidence mainly obtained from studies on animal models, IL-1 $\beta$  plays a key role in the regulation of ovarian physiology, especially in terms of follicular growth, development, and ovulation (Field et al., 2014). In this regard, IL-1 $\beta$  attenuates the expression of luteinizing hormone receptors (Gottschall et al., 1988) and stimulates GCs to shift transitionally from proliferation to differentiation (Karakji and Tsang, 1995), indicating that it is involved in making the decision whether follicles continue growing or be subjected to atresia. By increasing the biosynthesis of NO in GCs and intrafollicular macrophages, IL-1 $\beta$  also participates in the decision about progress to growth or apoptosis (Matsumi et al., 2000). In addition, IL-1 $\beta$  seems to take a part in ovulation, as its transcription is upregulated in FF macrophages and GCs with approaching follicular rupture (Loret de Mola et al., 1998; Machelon et al., 1995). Furthermore, IL-1 $\beta$  modulates the production of VEGF and PGE in GCs and thereby assists in promoting the flow of blood

**Table 3**Comparison of serum and follicular fluid (FF) levels of IL-1 $\beta$  and sIL-1R2 between ovarian responder groups.

	Low responders (n = 60)	Good responders (n = 36)	p-value
Serum IL-1 $\beta$	0.92 $\pm$ 1.71	0.79 $\pm$ 1.89	0.432 <sup>s</sup>
FF IL-1 $\beta$	0.28 $\pm$ 0.39	0.24 $\pm$ 0.17	0.767 <sup>s</sup>
Serum IL-1R2	18.06 $\pm$ 5.75	15.98 $\pm$ 3.99	0.183 <sup>f</sup>
FF IL-1R2	12.42 $\pm$ 4.23	9.98 $\pm$ 3.53	0.042 <sup>f</sup>

Data are presented as mean  $\pm$  SD. <sup>s</sup> Mann-Whitney U test and <sup>f</sup> independent t-test were used.





**Fig. 2.** Immunocytochemical demonstration of IL-1 $\beta$  and its receptor IL-1R2. (a) IL-1 $\beta$  in the cytoplasm and also its localization on the cell membrane surface of human follicular granulosa cells (GCs). (b) Localization of receptor IL-1R2 on the surface of GCs. (c, d) GCs without the primary antibodies of IL-1 $\beta$  and IL-1R2 as negative controls, respectively. (e, f) Localization of IL-1 $\beta$  and IL-1R2 on the surface of monocytes isolated from blood as positive controls, respectively. Immunohistochemical reaction. Magnification,  $\times 600$ .

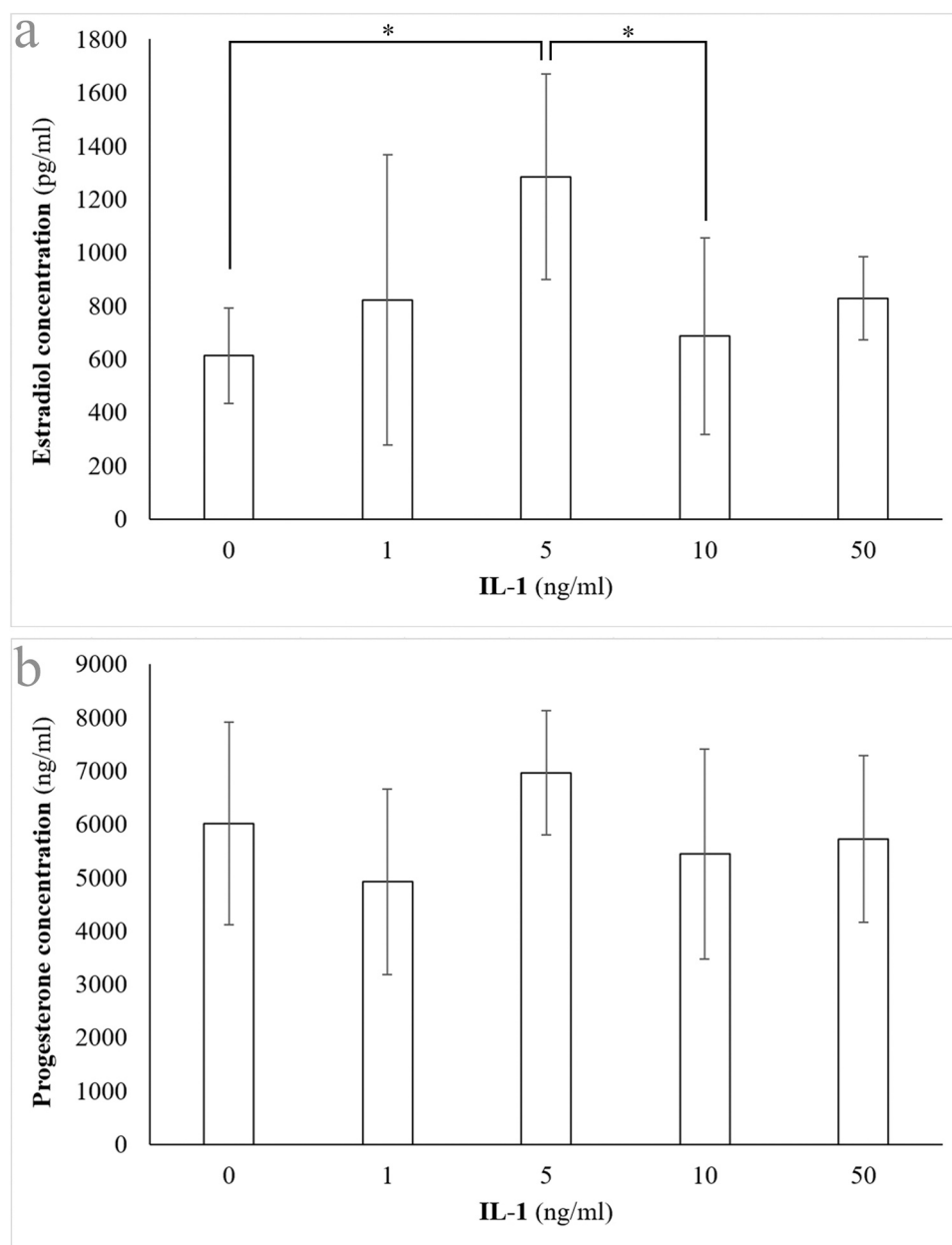
into the follicle and determining the position of follicle rupture, respectively (Levitas et al., 2000).

Given that the acquisition of oocyte competence during folliculogenesis requires a paracrine interaction between the oocyte and surrounding GCs (Alam and Miyano, 2020), IL-1 $\beta$ , and thus its regulators, can be linked to oocyte maturation and developmental competence directly by acting as signals between GCs and oocyte and/or indirectly by altering the development and function of GCs. Nevertheless, insights into the association of IL-1 $\beta$  and its potent inhibitor, i.e. sIL-1R2, with folliculogenesis and oogenesis are limited, particularly in human beings. Hence, we designed the current study to delineate the expression of IL-1 $\beta$  and sIL-1R2 in the human follicular environment with respect to oocyte developmental competence and gonadal steroidogenesis.

In this study, we showed that levels of IL-1 $\beta$  and sIL-1R2 were significantly higher in serum than in FF. This finding suggests that blood plasma is probably the major source of intrafollicular IL-1 $\beta$  and sIL-1R2 and the blood-follicle barrier acts as a semipermeable membrane against the infiltration of IL-1 $\beta$  and sIL-1R2 from the blood into FF. In support of this, we observed positive correlations between serum and FF in terms of IL-1 $\beta$  and sIL-1R2 values. In fact, FF is formed as a consequence of the

permeation of blood plasma through the follicular basement membrane in conjunction with the secretory activity of the oocyte, GCs, and theca cells (Rodgers and Irving-Rodgers, 2010). However, a proportion of intrafollicular IL-1 $\beta$  and sIL-1R2 might stem from GCs (Caillaud et al., 2005; Martoriati et al., 2003). In support of this, we demonstrated the expression of IL-1 $\beta$  and IL-1R2 at transcriptional and translational levels in human GCs.

The expression of the IL-1 system members in the ovarian follicles of several species has previously been reported. Accordingly, equine and rat GCs have been found to express IL-1 $\beta$  transcript (Gerard et al., 2004; Martoriati and Gerard, 2003). Human GCs have also been reported to express some members of the IL-1 system genes including IL-1 $\beta$ , IL-1R1, and IL-1Ra (de los Santos et al., 1998; Hurwitz et al., 1992). Similarly, our results indicated the mRNA and protein expression of IL-1 $\beta$  in human GCs. Furthermore, we revealed for the first time that sIL-1R2 is expressed at both transcriptional and translational levels in human GCs. In agreement with our results, Passos et al. (2016) have discerned the mRNAs and proteins of different members of the IL-1 system such as IL-1 $\beta$  and IL-1R2 in bovine GCs and oocytes throughout all stages of follicular development. Interestingly, the authors observed an increase



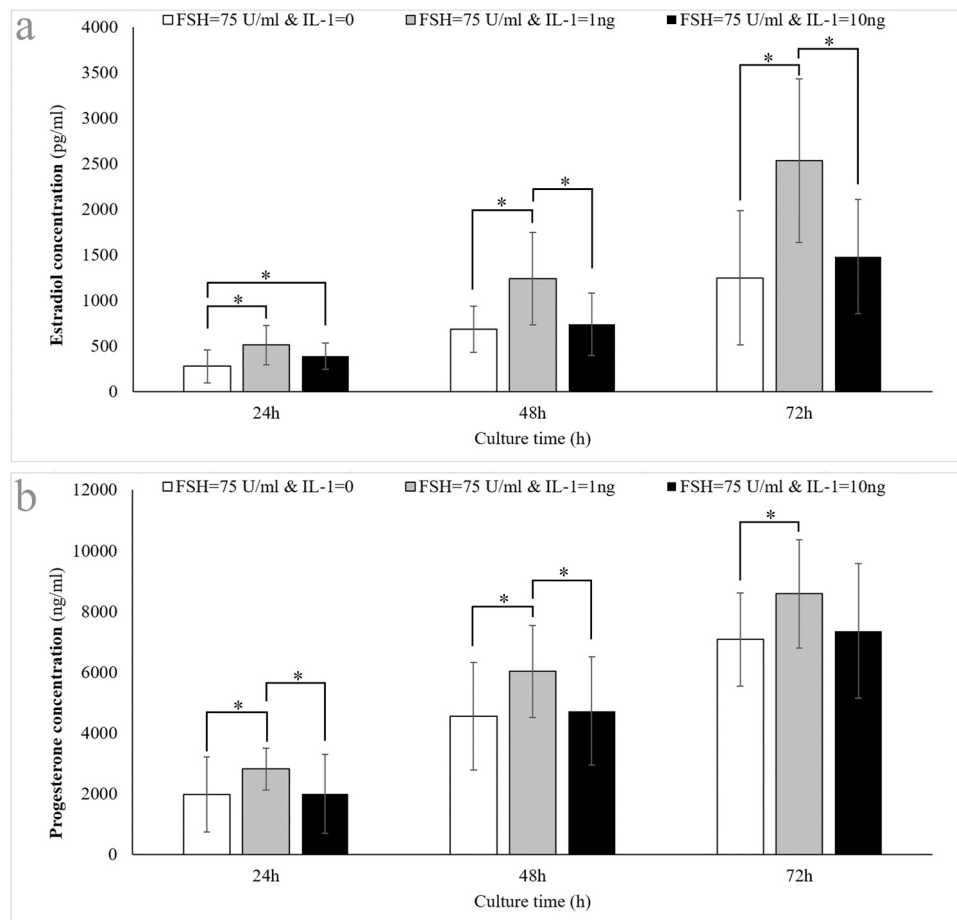
**Fig. 3.** Effect of increasing doses of recombinant human IL-1 $\beta$  on (a) estradiol and (b) progesterone secretion in human granulosa cells after 72 h culture. The experiments were performed in triplicate and all measurements were conducted in duplicate ( $n = 6$ ). Number of cultured cells was  $2 \times 10^4$ . Data are presented as mean  $\pm$  SD. \*significant difference ( $p < 0.05$ ).

in the transcript levels of IL-1 $\beta$  and IL-1R2 as follicles moved to higher stages of development, suggesting the active involvement of these compounds in the physiological events of ovarian follicles.

The literature is contradictory with respect to the effect of IL-1 $\beta$  on follicular growth and oocyte maturation. IL-1 $\beta$  has been found to interfere with follicular survival and cytoplasmic integrity of organelles in oocytes of the bovine secondary follicles (Paulino et al., 2018) and inhibit nuclear maturation of oocytes from equine cumulus-oocyte complexes (COCs), which was counteracted by IL-1Ra (Caillaud and Gerard, 2009). Moreover, serum and FF levels of IL-1 have been shown to be higher in women with premature ovarian failure than in their healthy counterparts (Yang et al., 2018). On the contrary, there are some reports in which IL-1 $\beta$  did not influence oocyte growth, germinal vesicle breakdown, and oocyte maturation (mare: (Caillaud et al., 2008); cattle: (Lima et al., 2018); human: (Mendoza et al., 1999)). Additionally, FF concentrations of IL-1 $\beta$  were correlated with none of the cumulus-oocyte

complexes, metaphase II oocytes, and top-quality embryos according to a recent study. (Adamczak et al., 2022). Although our results did not indicate a direct association between serum or FF levels of IL-1 $\beta$  and the number of mature oocytes, we found that FF levels of sIL-1R2 were negatively correlated with oocyte maturation rate. Since IL-1R2 is an inhibitor of IL-1 $\beta$  function (Boraschi et al., 2018), the latter finding might imply the participation of intrafollicular IL-1 $\beta$  in human oocyte growth and development. In support of this assumption, IL-1 $\beta$  has been demonstrated to improve bovine primordial follicles' survival and activation and promote cumulus cell expansion and oocyte developmental competence from buffalo COCs (Chaubey et al., 2018; Passos et al., 2016).

Similar to oocyte maturation, there are conflicting results in the literature with respect to the effect of IL-1 $\beta$  on follicular steroidogenesis. Multiple studies have found that the basal production of progesterone was elevated by IL-1 $\beta$  in human luteal or GCs in vitro (Chen et al., 2000;



**Fig. 4.** Effect of increasing culture time on (a) estradiol and (b) progesterone secretion by FSH-stimulated (75 IU/ml) granulosa cells in the absence or presence of recombinant human IL-1 $\beta$  (1 and 10 ng/ml). The experiments were performed in triplicate and all measurements were conducted in duplicate ( $n = 6$ ). Number of cultured cells was  $2 \times 10^4$ . Data are presented as mean  $\pm$  SD. \*significant difference ( $p < 0.05$ ).

Dang et al., 2017; Miceli et al., 2003). IL-1 $\beta$  was similarly reported to promote progesterone secretion in bovine GCs (Baratta et al., 1996). Conversely, there is also evidence that progesterone production was reduced by IL-1 $\beta$  in human (Tobai and Nishiya, 2001) and rat (Gottschall et al., 1987) GCs. However, consistent with our findings, Best and Hill (1995) observed no change in the basal progesterone secretion from cultured human GCs. Under FSH-stimulated situation, we observed an increase in progesterone synthesis in human GCs by a low concentration of IL-1 $\beta$ . From the mechanistic perspective, the reinforcement of FSH-induced progesterone production by IL-1 $\beta$  might result from the fact that IL-1 $\beta$  activates extracellular signal regulated-kinases (ERK) 1/2 signaling pathway and thereby increases cAMP regulatory element-binding protein (CREB) phosphorylation (Dang et al., 2017). On the other hand, FSH exerts its effects on the steroidogenic activity of GCs primarily through cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) signaling pathway (Andersen and Ezcurra, 2014). Consequently, the concurrent presence of IL-1 $\beta$  and FSH might trigger maximum phosphorylation and activation of CREB by ERK1/2 and cAMP/PKA cascades, leading to the increase of progesterone biosynthesis in GCs. Conversely, it has been reported that IL-1 $\beta$  either decreased (Baratta et al., 1996) or at least did not affect gonadotropin-induced progesterone production in GCs (Best and Hill, 1995; Chen et al., 2000; Dang et al., 2017). Of note, methodological variations, including species, cell type, culture medium, and treatment dose could not explain the reason(s) behind the disparity between the findings of studies regarding the effect of IL-1 $\beta$  on GC progesterone synthesis. Therefore, further studies are needed to clarify the role of

IL-1 $\beta$  in human GC steroidogenesis using multiple biological replicates and additional treatments with and without various gonadotropins and growth factors.

With respect to basal estradiol production, IL-1 $\beta$  alone was mostly found to have either an inhibitory (Baratta et al., 1996; Tobai and Nishiya, 2001) or no effect (Best and Hill, 1995; Gottschall et al., 1989) on GCs irrespective of species type. Our findings also demonstrated that various concentrations of IL-1 $\beta$  did not influence basal estradiol production, with the exception of the concentration of 5 ng/ml, which increased the basal estradiol synthesis. Similarly, IL-1 $\beta$  promoted the estradiol production of GCs subsequent to their stimulation by FSH in our study. This observation is also in conflict with the results obtained from other studies, in which IL-1 $\beta$  either reduced (Baratta et al., 1996; Chen et al., 2000; Gottschall et al., 1989) or did not affect (Best and Hill, 1995; Spicer and Alpizar, 1994) gonadotropin-induced estradiol secretion from cultured GCs.

Our results also demonstrated positive correlations between the levels of IL-1 $\beta$  and those of estradiol and progesterone in serum and/or FF in vivo. This observation concurs with our in vitro findings due to the simultaneous existence of IL-1 $\beta$  and FSH in the follicular milieu during the ovarian hyperstimulation process. However, we paradoxically noted a strong association between the FF levels of sIL-1R2 and progesterone.

To the best of our knowledge, our study is the first that demonstrates the expression of IL-1R2 in human GCs at transcriptional and translational levels and reveals the association of its soluble form in serum and FF with ovarian steroidogenesis and also oocyte meiotic maturation. Nevertheless, the lack of data regarding the association of IL-1R2 in



particular but also IL-1 $\beta$  with IVF outcomes other than oocyte maturation such as fertilization rate, the number of good embryos, pregnancy rate, etc. is the major limitation of the present study.

## 5. Conclusion

The gene and protein expression of IL-1 $\beta$  and sIL-1R2 were evidenced in human GCs. Moreover, there was a negative correlation between FF sIL-1R2 and oocyte maturation rate and a positive one between serum IL-1 $\beta$  and serum or FF gonadal steroids. Finally, IL-1 $\beta$  demonstrated the ability to promote the steroidogenic activity of GCs, especially in the presence of the FSH. Current findings suggest that The IL-1 $\beta$  system is involved in ovarian follicular development and may be a potential target for optimizing ovarian stimulation and gonadal steroids in IVF cycles.

## Ethical approval

This research study was conducted retrospectively from data obtained for clinical purposes. The study was in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Universitätsklinikum Schleswig-Holstein (UKSH), Kiel Institutional Review Board (IRB) has confirmed that no ethical approval is required.

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## CRediT authorship contribution statement

**A.F.** Data analysis, Manuscript writing; **R.Z.** Manuscript writing; **Y.R.** Data collection; **L.M.** Data collection; **M.N.** Manuscript editing; **A.G.S.** Project development, Data management, Manuscript editing; **A.S.** Project development, Data management, Manuscript editing.

## Data Availability

Data will be made available on request. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Declaration of interest

None.

## References

- Acosta, T.J., Miyamoto, A., Ozawa, T., Wijayagunawardane, M.P., Sato, K., 1998. Local release of steroid hormones, prostaglandin E<sub>2</sub>, and endothelin-1 from bovine mature follicles *In vitro*: effects of luteinizing hormone, endothelin-1, and cytokines. *Biol. Reprod.* 59, 437–443.
- Adamczak, R., Ukleja-Sokolowska, N., Lis, K., Bartuzi, Z., Dubiel, M., 2022. Progesterone-induced blocking factor 1 and cytokine profile of follicular fluid of infertile women qualified to in vitro fertilization: the influence on fetus development and pregnancy outcome. *Int. J. Immunopathol. Pharm.* 36, 3946320221111134.
- Aghadavod, E., Zarghami, N., Farzadi, L., Zare, M., Barzegari, A., Movassaghpour, A.A., Nouri, M., 2015. Isolation of granulosa cells from follicular fluid; applications in biomedical and molecular biology experiments. *Adv. Biomed. Res.* 4, 250.
- Alam, M.H., Miyano, T., 2020. Interaction between growing oocytes and granulosa cells *in vitro*. *Reprod. Med Biol.* 19, 13–23.
- Andersen, C.Y., Ezcurra, D., 2014. Human steroidogenesis: implications for controlled ovarian stimulation with exogenous gonadotropins. *Reprod. Biol. Endocrinol.* 12, 128.
- Barak, V., Yanai, P., Treves, A.J., Roisman, I., Simon, A., Laufer, N., 1992. Interleukin-1: local production and modulation of human granulosa luteal cells steroidogenesis. *Fertil. Steril.* 58, 719–725.
- Baratta, M., Basini, G., Bussolati, S., Tamanini, C., 1996. Effects of interleukin-1 beta fragment (163–171) on progesterone and estradiol-17 beta release by bovine granulosa cells from different size follicles. *Regul. Pept.* 67, 187–194.
- Best, C.L., Hill, J.A., 1995. Interleukin-1 alpha and -beta modulation of luteinized human granulosa cell oestrogen and progesterone biosynthesis. *Hum. Reprod.* 10, 3206–3210.
- Boraschi, D., Italiani, P., Weil, S., Martin, M.U., 2018. The family of the interleukin-1 receptors. *Immunol. Rev.* 281, 197–232.
- Brännström, M., Wang, L., Norman, R.J., 1993. Effects of cytokines on prostaglandin production and steroidogenesis of incubated preovulatory follicles of the rat. *Biol. Reprod.* 48, 165–171.
- Bréard, E., Delarue, B., Benhaim, A., Féral, C., Leymarie, P., 1998. Inhibition by gonadotropins of interleukin-1 production by rabbit granulosa and theca cells: effects on gonadotropin-induced progesterone production. *Eur. J. Endocrinol.* 138, 328–336.
- Caillaud, M., Dell'aquila, M.E., De Santis, T., Nicassio, M., Lacalandra, G.M., Goudet, G., Gerard, N., 2008. In vitro equine oocyte maturation in pure follicular fluid plus interleukin-1 and fertilization following ICSI. *Anim. Reprod. Sci.* 106, 431–439.
- Caillaud, M., Duchamp, G., Gerard, N., 2005. In vivo effect of interleukin-1beta and interleukin-1RA on oocyte cytoplasmic maturation, ovulation, and early embryonic development in the mare. *Reprod. Biol. Endocrinol.* 3, 26.
- Caillaud, M., Gerard, N., 2009. In vivo and in vitro effects of interleukin-1beta on equine oocyte maturation and on steroidogenesis and prostaglandin synthesis in granulosa and cumulus cells. *Reprod. Fertil. Dev.* 21, 265–273.
- Chaubey, G.K., Kumar, S., Kumar, M., Sarwalia, P., Kumaresan, A., De, S., Kumar, R., Datta, T.K., 2018. Induced cumulus expansion of poor quality buffalo cumulus oocyte complexes by Interleukin-1beta improves their developmental ability. *J. Cell Biochem* 119, 5750–5760.
- Chen, H.F., Shew, J.Y., Chao, K.H., Chang, L.J., Ho, H.N., Yang, Y.S., 2000. Luteinizing hormone up-regulates the expression of interleukin-1 beta mRNA in human granulosa-luteal cells. *Am. J. Reprod. Immunol.* 43, 125–133.
- Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem* 162, 156–159.
- Dang, X., Zhu, Q., He, Y., Wang, Y., Lu, Y., Li, X., Qi, J., Wu, H., Sun, Y., 2017. IL-1beta upregulates STAR and progesterone production through the ERK1/2- and p38-mediated CREB signaling pathways in human granulosa-lutein cells. *Endocrinology* 158, 3281–3291.
- de los Santos, M.J., Anderson, D.J., Racowsky, C., Simon, C., Hill, J.A., 1998. Expression of interleukin-1 system genes in human gametes. *Biol. Reprod.* 59, 1419–1424.
- Dinarello, C.A., 2009. Immunological and inflammatory functions of the interleukin-1 family. *Annu. Rev. Immunol.* 27, 519–550.
- Duffy, D.M., Stouffer, R.L., 2002. Follicular administration of a cyclooxygenase inhibitor can prevent oocyte release without alteration of normal luteal function in rhesus monkeys. *Hum. Reprod.* 17, 2825–2831.
- Field, S.L., Dasgupta, T., Cummings, M., Orsi, N.M., 2014. Cytokines in ovarian folliculogenesis, oocyte maturation and luteinisation. *Mol. Reprod. Dev.* 81, 284–314.
- Fukuoka, M., Yasuda, K., Taii, S., Takakura, K., Mori, T., 1989. Interleukin-1 stimulates growth and inhibits progesterone secretion in cultures of porcine granulosa cells. *Endocrinology* 124, 884–890.
- Gerard, N., Caillaud, M., Martoriati, A., Goudet, G., Lalmanach, A.C., 2004. The interleukin-1 system and female reproduction. *J. Endocrinol.* 180, 203–212.
- Ghersevich, S., Isomaa, V., Vihko, P., 2001. Cytokine regulation of the expression of estrogenic biosynthetic enzymes in cultured rat granulosa cells. *Mol. Cell Endocrinol.* 172, 21–30.
- Gottschall, P.E., Katsuura, G., Arimura, A., 1989. Interleukin-1 suppresses follicle-stimulating hormone-induced estradiol secretion from cultured ovarian granulosa cells. *J. Reprod. Immunol.* 15, 281–290.
- Gottschall, P.E., Katsuura, G., Hoffmann, S.T., Arimura, A., 1988. Interleukin 1: an inhibitor of luteinizing hormone receptor formation in cultured rat granulosa cells. *Faseb J.* 2, 2492–2496.
- Gottschall, P.E., Uehara, A., Hoffmann, S.T., Arimura, A., 1987. Interleukin-1 inhibits follicle stimulating hormone-induced differentiation in rat granulosa cells *in vitro*. *Biochem Biophys. Res Commun.* 149, 502–509.
- Hurwitz, A., Loukides, J., Ricciarelli, E., Botero, L., Katz, E., McAllister, J.M., Garcia, J.E., Rohan, R., Adashi, E.Y., Hernandez, E.R., 1992. Human intraovarian interleukin-1 (IL-1) system: highly compartmentalized and hormonally dependent regulation of the genes encoding IL-1, its receptor, and its receptor antagonist. *J. Clin. Invest.* 89, 1746–1754.
- Karakji, E.G., Tsang, B.K., 1995. Regulation of rat granulosa cell plasminogen activator system: influence of interleukin-1 beta and ovarian follicular development. *Biol. Reprod.* 53, 1302–1310.
- Kasson, B.G., Gorospe, W.C., 1989. Effects of interleukins 1, 2 and 3 on follicle-stimulating hormone-induced differentiation of rat granulosa cells. *Mol. Cell Endocrinol.* 62, 103–111.
- Levitas, E., Chamoun, D., Udoff, L.C., Ando, M., Resnick, C.E., Adashi, E.Y., 2000. Periovarian and interleukin-1 beta-dependent up-regulation of intraovarian vascular endothelial growth factor (VEGF) in the rat: potential role for VEGF in the



- promotion of periovulatory angiogenesis and vascular permeability. *J. Soc. Gynecol. Invest.* 7, 51–60.
- Lima, F., Bezerra, F., Souza, G.B., Matos, M., van den Hurk, R., Silva, J., 2018. Influence of interleukin 1 beta and tumour necrosis factor alpha on the in vitro growth, maturation and mitochondrial distribution of bovine oocytes from small antral follicles. *Zygote* 26, 381–387.
- Liu, C., Hart, R.P., Liu, X.J., Clevenger, W., Maki, R.A., De Souza, E.B., 1996. Cloning and characterization of an alternatively processed human type II interleukin-1 receptor mRNA. *J. Biol. Chem.* 271, 20965–20972.
- Loret de Mola, J.R., Goldfarb, J.M., Hecht, B.R., Baumgardner, G.P., Babbo, C.J., Friedlander, M.A., 1998. Gonadotropins induce the release of interleukin-1 beta, interleukin-6 and tumor necrosis factor-alpha from the human preovulatory follicle. *Am. J. Reprod. Immunol.* 39, 387–390.
- Machelon, V., Nome, F., Durand-Gasselin, I., Emilie, D., 1995. Macrophage and granulosa interleukin-1 beta mRNA in human ovulatory follicles. *Hum. Reprod.* 10, 2198–2203.
- Martoriati, A., Duchamp, G., Gerard, N., 2003. In vivo effect of epidermal growth factor, interleukin-1beta, and interleukin-1RA on equine preovulatory follicles. *Biol. Reprod.* 68, 1748–1754.
- Martoriati, A., Gerard, N., 2003. Interleukin-1 (IL-1) system gene expression in granulosa cells: kinetics during terminal preovulatory follicle maturation in the mare. *Reprod. Biol. Endocrinol.* 1, 42.
- Matsumi, H., Yano, T., Osuga, Y., Kugu, K., Tang, X., Xu, J.P., Yano, N., Kurashima, Y., Ogura, T., Tsutsumi, O., Koji, T., Esumi, H., Taketani, Y., 2000. Regulation of nitric oxide synthase to promote cytotaxis in ovarian follicular development. *Biol. Reprod.* 63, 141–146.
- McMahan, C.J., Slack, J.L., Mosley, B., Cosman, D., Lupton, S.D., Brunton, L.L., Grubin, C.E., Wignall, J.M., Jenkins, N.A., Brannan, C.I., et al., 1991. A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. *Embo J.* 10, 2821–2832.
- Mendoza, C., Cremades, N., Ruiz-Requena, E., Martinez, F., Ortega, E., Bernabeu, S., Tesarik, J., 1999. Relationship between fertilization results after intracytoplasmic sperm injection, and intrafollicular steroid, pituitary hormone and cytokine concentrations. *Hum. Reprod.* 14, 628–635.
- Miceli, F., Tropea, A., Minici, F., Navarra, P., Lanzone, A., Apa, R., 2003. Interleukin-1 beta stimulates progesterone production by in vitro human luteal cells: evidence of a mediatory role of prostaglandins. *J. Clin. Endocrinol. Metab.* 88, 2690–2694.
- Orlando, S., Sironi, M., Bianchi, G., Drummond, A.H., Boraschi, D., Yabes, D., Mantovani, A., 1997. Role of metalloproteases in the release of the IL-1 type II decoy receptor. *J. Biol. Chem.* 272, 31764–31769.
- Passos, J.R., Costa, J.J., da Cunha, E.V., Silva, A.W., Ribeiro, R.P., de Souza, G.B., Barroso, P.A., Dau, A.M., Saraiva, M.V., Goncalves, P.B., van den Hurk, R., Silva, J., 2016. Protein and messenger RNA expression of interleukin 1 system members in bovine ovarian follicles and effects of interleukin 1beta on primordial follicle activation and survival in vitro. *Domest. Anim. Endocrinol.* 54, 48–59.
- Paulino, L., Cunha, E.V., Barbalho Silva, A.W., Souza, G.B., Lopes, E.P.F., Donato, M.A.M., Peixoto, C.A., Matos-Brito, B.G., van den Hurk, R., Silva, J.R.V., 2018. Effects of tumour necrosis factor-alpha and interleukin-1 beta on in vitro development of bovine secondary follicles. *Reprod. Domest. Anim.* 53, 997–1005.
- Radzun, H.J., Hansmann, M.L., Heidebrecht, H.J., Bodewadt-Radzun, S., Wacker, H.H., Kreipe, H., Lumbeck, H., Hernandez, C., Kuhn, C., Parwaresch, M.R., 1991. Detection of a monocyte/macrophage differentiation antigen in routinely processed paraffin-embedded tissues by monoclonal antibody Ki-MIP. *Lab Invest* 65, 306–315.
- Rodgers, R.J., Irving-Rodgers, H.F., 2010. Formation of the ovarian follicular antrum and follicular fluid. *Biol. Reprod.* 82, 1021–1029.
- Salmassi, A., Fattahi, A., Nouri, M., Hedderich, J., Schmutzler, A.G., 2017. Expression of mRNA and protein of IL-18 and its receptor in human follicular granulosa cells. *J. Endocrinol. Invest* 40, 447–454.
- Salmassi, A., Fattahi, A., Simon, N., Latifi, Z., Ghasemnejad, T., Nouri, M., Schmutzler, A.G., 2019. Messenger RNA and protein expression of tumor necrosis factor alpha and its receptors in human follicular granulosa cells. *J. Cell Physiol.* 234, 20240–20248.
- Salmassi, A., Schmutzler, A.G., Huang, L., Hedderich, J., Jonat, W., Mettler, L., 2004. Detection of granulocyte colony-stimulating factor and its receptor in human follicular luteinized granulosa cells. *Fertil. Steril.* 81 (Suppl 1), 786–791.
- Salmassi, A., Zhang, Z., Schmutzler, A.G., Koch, K., Buck, S., Jonat, W., Mettler, L., 2005. Expression of mRNA and protein of macrophage colony-stimulating factor and its receptor in human follicular luteinized granulosa cells. *Fertil. Steril.* 83, 419–425.
- Sims, J.E., March, C.J., Cosman, D., Widmer, M.B., MacDonald, H.R., McMahan, C.J., Grubin, C.E., Wignall, J.M., Jackson, J.L., Call, S.M., et al., 1988. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science* 241, 585–589.
- Sobinoff, A.P., Sutherland, J.M., McLaughlin, E.A., 2013. Intracellular signalling during female gametogenesis. *Mol. Hum. Reprod.* 19, 265–278.
- Spicer, L.J., Alpizar, E., 1994. Effects of cytokines on FSH-induced estradiol production by bovine granulosa cells in vitro: dependence on size of follicle. *Domest. Anim. Endocrinol.* 11, 25–34.
- Symons, J.A., Young, P.R., Duff, G.W., 1995. Soluble type II interleukin 1 (IL-1) receptor binds and blocks processing of IL-1 beta precursor and loses affinity for IL-1 receptor antagonist. *Proc. Natl. Acad. Sci. USA* 92, 1714–1718.
- Tobai, H., Nishiya, I., 2001. Nitric oxide mediates inhibitory effect of interleukin-1beta on estrogen production in human granulosa-luteal cells. *J. Obstet. Gynaecol. Res* 27, 53–59.
- Yang, H., Pang, H., Miao, C., 2018. Ovarian IL-1alpha and IL-1beta levels are associated with primary ovarian insufficiency. *Int. J. Clin. Exp. Pathol.* 11, 4711–4717.