J Endocrinol Invest (2017) 40:447–454
DOI 10.1007/s40618-016-0590-x

ORIGINAL ARTICLE

Expression of mRNA and protein of IL-18 and its receptor in human follicular granulosa cells

A. Salmassi[1,2] · A. Fattahi[4] · M. Nouri[1] · J. Hedderich[3] · A. G. Schmutzler[2]

Received: 19 September 2016 / Accepted: 25 November 2016 / Published online: 2 December 2016

Abstract
Purpose There is no information available about the IL-18 receptor in ovarian follicles, so the present study attempts to demonstrate the expression of IL-18 and its receptor in human granulosa cells (GCs).
Methods To evaluate the concentration of IL-18 in serum and follicular fluid (FF), we collected serum and FF from 102 women undergoing oocyte retrieval. Also, to detect expression of IL-18 and its receptor by luteinized GCs, these cells were pooled six times from a total of twenty individual patients with 5–16 follicles each. The IL-18 concentration was determined by ELISA and the expression of IL-18 and its receptor by immunocytochemistry and reverse transcription polymerase chain reaction.
Results Our results showed that the median IL-18 concentration in serum, 159.27 pg/ml (IQR 121.41–210.1), was significantly higher than in FF, 142.1 pg/ml (IQR 95.7–176.5), $p < 0.001$. Moreover, we found that IL-18 and its receptor are expressed by GCs.
Conclusion The presence of IL-18 in FF and the expression of IL-18 and its receptor by GCs suggest an important role for this cytokine in ovarian function.

Keywords Granulosa cells · IL-18 · IL-18 receptor · IVF–ICSI

✉ A. Salmassi
asalmassi@email.uni-kiel.de

[1] Department of Reproductive Biology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Department of Gynaecology and Obstetrics, Center for Reproductive Medicine, Women's Hospital, University Hospitals Schleswig-Holstein, Christian-Albrechts-University, Campus Kiel, Arnold-Heller Str. 3, House 24, 24105 Kiel, Germany

[3] Institute of Medical Informatics and Statistics, University of Kiel, Kiel, Germany

[4] Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Introduction

Cytokines are increasingly recognized as potentially important local regulators of ovarian function [1]. Interleukin 18 (IL-18), which supposed as an IL-1 family member, was originally characterized as interferon-c-inducing factor and is present in the serum of animals with endotoxemia [2]. Like IL-1b, IL-18 is a cytoplasmic protein synthesized as a biologically inactive 24-kDa precursor molecule lacking a signal peptide that requires cleavage into an active and mature 18 kDa molecule by the intracellular cysteine protease called IL-1b-converting enzyme (caspase-1) [3]. Il-18 is primarily produced by hematopoietic cells and also by several nonhematopoietic cell types, such as monocytes, macrophages, osteoblasts, adrenal cortex, keratinocytes, T and B cells, and dendritic cells. Some authors reported the expression of IL-18 in reproductive tissue, e.g., in different compartments of human term placenta, maternal–fetal interface, human chorion, decidua, endometrial tissue, and human testes [4–13].

IL-18 is a multifunctional, proinflammatory cytokine that plays a key role in the host defense against several infectious agents. It is known to participate in the regulation of both Th1 and Th2 responses and it also up-regulates innate and acquired immunity [14]. Also association of IL-18 with low blood glucose [15], insulin resistance [16], hypertension [17], dyslipidemia [18], and atherosclerosis

[19] has been observed in previous studies. Recently, it was reported that high levels of IL-18 in serum were also observed in various complicated pregnancies, including premature rupture of membranes [20]. Furthermore Pacora et al. [10] reported that microbial invasion of the amniotic cavity in either preterm or term parturition was associated with a significant increase in the levels of IL-18 in the amniotic fluid.

Gutman et al. [21] described a high correlation between both pre-ovulatory follicular fluid (FF) and serum levels of IL-18 with the number of retrieved oocytes. Some authors found significantly higher IL-18 levels in patients with ovarian hyperstimulation syndrome (OHSS) and polycystic ovary syndrome (PCOS) on the day of oocyte retrieval and suggest that the serum IL-18 levels on that day may predict OHSS and PCOS. Lower levels of IL-18 have also been found to characterize unexplained infertility [12, 21–27]. However, Nikolaeva et al. [28] have reported that lower concentration of IL-18 in seminal plasma is associated with pregnancy in female patients exposed to seminal plasma during IVF/ICSI treatment. Besides, in a meta-analysis study, a positive association between the −137G/C polymorphism of IL-18 and risk of recurrent pregnancy loss has been demonstrated which shows importance of this cytokine in pregnancy and female fertility [29].

The biological functions of IL-18 are mediated through its receptor (IL-18R). IL-18R resembles IL-1R and shares with it a signal transduction pathway [30]. Expression of IL-18R was confirmed on both Th1 lymphocytes and NK cells, whereas it was not observed on Th2 lymphocytes [31]. The interaction between IL-18R and its ligand could be modified by the presence of IL-18 binding protein (IL18BP) which blocks the binding of IL-18 to IL-18R [32, 33]. Kilic et al. [34] found IL-18 and its binding protein in serum and ovarian follicular fluid. Increased concentrations of IL-18 might be a promising prognostic marker for in vitro fertilization (IVF) success [35, 36].

There is no information available about the IL-18 receptor in ovarian follicles, so the present study attempts to demonstrate the presence of IL-18 and its receptor in human granulosa cells (GCs).

Materials and methods

Patients

Serum and FF on the day of oocyte retrieval were obtained from 102 patients undergoing IVF ($n = 24$) or intracytoplasmic sperm injection (ICSI, $n = 78$) at the Department of Obstetrics and Gynaecology, University of Kiel. We recruited patients with male factor infertility (andrological) or tubal cause of infertility. Patients with other causes of infertility such as endometriosis and PCOS were excluded. Patient who underwent OHSS during ovulation induction also were excluded. The women's age ranged from 20 to 46 years (median 33 years), and the size of the follicles from 19 to 24 mm. The local ethics committee for human research approved the study, and informed consents were obtained from all patients.

Controlled ovarian hyper stimulation

The patients were superovulated with recombinant follicle-stimulating hormone (FSH, Serono or Organon, Munich, Germany) or human menopausal gonadotropin (hMG, Menogon, Ferring, Kiel, Germany) after down-regulation with GnRH agonists. Monitoring of follicle development by real-time ultrasonic scans and detection of serum E2 levels were performed from the sixth day of stimulation to the day of follicular puncture. Once the leading follicle measured >16–18 mm in diameter and the 17-β, E_2 level was adequately increased, but still <3.000 pg/mL in serum, 10,000 IU of hCG (Choragon; Ferring) was administered subcutaneously. Total injected doses of gonadotropins up to the day of hCG injection ranged between 2277.50 ± 563.17 to 3174.96 ± 1318.07 IU according to the ovarian responsiveness. Follicles were aspirated 36 h after administration of hCG and GCs were harvested from the FF after oocyte collection. Patients who their male partners had severe andrological problems and also patients with three times failed in previous IVF cycles underwent ICSI. Patients who their partner had normal spermiogram underwent IVF.

IL-18 assay in serum and follicular fluid

Blood and FF were taken from 102 patients on the day of follicle puncture, processed by centrifuge for 10 min at 350×*g* at 5 °C, shock frozen and kept at −80 °C. After pickup of the oocytes, the FF underwent the same treatment as the blood. IL-18 levels in serum and FF were measured in duplicate by a solid-phase ELISA using the human IL-18 kit (R&D, Wiesbaden, Germany). A fourfold dilution for serum and FF was performed with the Calibrator Diluent RD6P. This assay employs the quantitative sandwich enzyme immunoassay technique. IL-18 levels ranged between 25.6 to 1000 pg/mL, with a sensitivity of 2.5 pg/mL. Precision was 4.93–9.92 (C.V.%) for intra-assay and 5.2–10.7 (C.V.%) for inter-assay. Only those cases were included in this study in which both FF and serum could be simultaneously collected on the day of oocyte retrieval.

Isolation of granulosa cells from follicular fluid

The FF of 20 individual patients (on 6 follicular puncture days, 5–16 follicles per patient) were processed by

centrifuge at 350×*g* for 5 min, and the supernatant was removed. The cells were resuspended in 5–10 mL of HAM's F-10 medium (Sigma, Deisenhofen, Germany). The medium was supplemented with penicillin (100 IU/mL; Sigma) and streptomycin (100 μg/mL; Sigma). The cells were separated over a gradient containing 2 mL 64% and 2 mL 36% of a Percoll solution (Sigma). After centrifugation at 450×*g* for 15 min at room temperature, the interphase cells were pooled and washed twice with Dulbecco's Hanks' balanced salt (D-HBSS) [37, 38]. The cells were resuspended in 3 mL of D-HBSS. To remove the CD 45-positive blood cells (such as lymphocytes), 50 μL of magnetic Dynabeads M-450 CD 45 (DYNAL INC., Sigma) were added. The supernatant of the cell suspension was collected in magnetic rank. These GCs were used for detection of IL-18 and its receptor by immunohistochemical and RT-PCR techniques.

The presence of monocytes/macrophages in separated GCs was tested immunocytochemically by the monoclonal antibody Ki-M_1P as reported by Radzun et al. [39].

Processing for immunocytochemical detection of IL-18R and IL-18 in human GCs

Isolated GCs were suspended in phosphate-buffered saline (PBS), set with 1% (wt/vol) human serum albumin, and used for the preparation of cytospins (8 cytospins per follicular puncture day). Air-dried cytospins (24 h) were fixed in acetone for 10 min. The expression of IL-18 receptor on the GC surface was detected by immunocytochemistry with a monoclonal mouse antihuman IL-18R-alpha antibody (R&D, MAB 840, diluted 1:20 in 5% normal human serum in Tris buffer [TBS]), as secondary biotinylated rabbit anti-mouse immunoglobulins (Dako, E0354) and the avidin biotin complex (ABC)-labeled alkaline phosphatase (Dako). Alkaline phosphatase was visualized by AP-Red substrate chromogen mixture for 20–30 min at room temperature (Zymed, Kiel, Germany). Applying the same method, IL-18 in GCs was detected by the corresponding primary monoclonal antibody of mouse antihuman IL-18 (R&D, D043-3). The slides were washed in distilled water, counterstained with Mayer's hematoxylin (distilled 1:4 vol/vol in water, Merck, Darmstadt, Germany) for 5 min, and then mounted with Histomount solution (Zymed). As negative controls, nonimmune serum was used instead of the primary antibody.

Extraction of RNA

The GCs were lysed in 1 mL of trizol (GIBCO/BRL, Karlsruhe, Germany) for 10 min. Total cellular RNA was isolated from cell lysates, as described by Chomczynski and Sacchi [40]. The extracted total RNA was mixed with 45 μL of RNA Secure solution (MBI, Fermentas, St. Leon-Rat, Germany) and heated at 60 °C for 10 min. It was then set with 2 μL of DNase (MBI, Fermentas) in 5 μL 10 × DNase buffer (MBI, Fermentas), kept for 30 min at 37 °C, 15 min at 70 °C, and stored at −20 °C. RNA concentration was determined photometrically. All solutions were prepared using diethylpyrocarbonate-treated water (DEPC water).

Reverse transcription polymerase chain reaction analysis

Two micrograms of total cellular RNA was reverse transcribed using the Revert Aid TM H Minus First Strand Synthesis Kit (MBI, Fermentas) for 60 min at 42 °C. Complementary DNA (cDNA) was heated at 70 °C for 10 min. This first-strand synthesized cDNA was used directly for polymerase chain reaction (PCR) amplification.

One-half of each reverse transcription (RT) reaction mixture was used in 40 cycles of PCR amplification in the Minicycler Biozym (Oldentrof, Germany) using 1 U Taq polymerase (Qiagen, Hilden, Germany), the oligonucleotides forward 5′-cca agg aaa tcg gcc tct at-3′ (position 396) and reverse 5′-ccc cca att cat cct ctt tt-3′ for IL-18 (position 764), as well as 5′-gtt gag ttg aat gac aca gg-3′ (position 289, sense primer) and 5′-tcc act gca aca tgg tta ag-3′ (position 711, antisense primer) for IL-18Rα chain [41]. The PCR amplification was performed in 40 cycles, each cycle consisting of 1 min at 95 °C, 1 min at 60 °C (for IL-18), 1 min at 53 °C (for IL-18R), and 1 min at 72 °C.

The primers used for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were: forward 5′-gtc agt ggt gga cct gac ct-3′ and reverse 5′-tga gga ggg gag att cag tg-3′ with a product size of 400 bp. The PCR amplification for GAPDH was performed in 25 cycles, each cycle consisting of 1 min at 94 °C, 1 min at 59 °C, and 1 min at 72 °C. Negative controls consisted of the complete composite of PCR reagents and primers without cDNA. After amplification, 10 μL of the PCR mix was electrophoresed in a 2% agarose gel. DNA molecular weight marker VIII from Roche (Mannheim, Germany) was used as a size marker.

Statistical evaluation and IRB approval

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 20. Based on the Kolmogorov–Smirnov test, normal distribution for the parameters could not be assumed. Therefore, in descriptive statistics, median and interquartile ranges (IQR) were given. Mann–Whitney *U* test was used to compare quantitative parameters. Correlation was measured by Spearman's rank correlation coefficients (r_s). p value <0.05 was considered to be statistically significant throughout the study. Patient

written consent was obtained from all participants. Under the stipulations of the Universitätsklinikum Schleswig–Holstein (UKSH), Kiel Institutional Review Board (IRB), approval did not obtain for a retrospective observational study.

Results

IL-18 levels in serum and FF on the day of oocyte retrieval

On the day of oocyte retrieval, the median IL-18 level in serum, 159.27 pg/ml (121.41–210.1), was significantly higher than that in FF, 142.1 pg/ml (95.7–176.5), $p < 0.001$. On the basis of nonnormal distributed values of IL-18 levels in serum and FF, we found a positive correlation (Spearman's rho $r_s = 0.85$, $p < 0.001$, Fig. 1).

Moreover, we observed significant positive correlations between some clinical parameters with IL-18 levels of serum and/or FF on the day of follicular puncture (FP) as follows: BMI with levels of IL-18 in serum and FF ($r_s = 0.24$ and $r_s = 0.2$, respectively); number of total retrieved oocytes, E2 and progesterone levels only with FF levels of IL-18 ($r_s = 0.23$, $r_s = 0.2$ and $r_s = 0.24$, respectively).

There were no significant statistical differences between IL-18 levels of FF or serum with patients' age and levels of injected total dose from first stimulation day up to the day of hCG injection and the number of transferred embryos. Serum levels of IL-18 in patients who treated with rFSH ($n = 74$) were 169.05 pg/ml (120.2–221.9) and in patients who received hMG ($n = 28$) were 151.1 pg/ml (128.9–182.3) which showed no significant differences between the two groups ($p = 0.258$). Also our result demonstrated that FF levels of IL-18 between patients who treated with rFSH (142.53 pg/ml; 103.8–191.04) and hMG (132.1 pg/ml; 98.9–151) were not statistically different ($p = 0.191$).

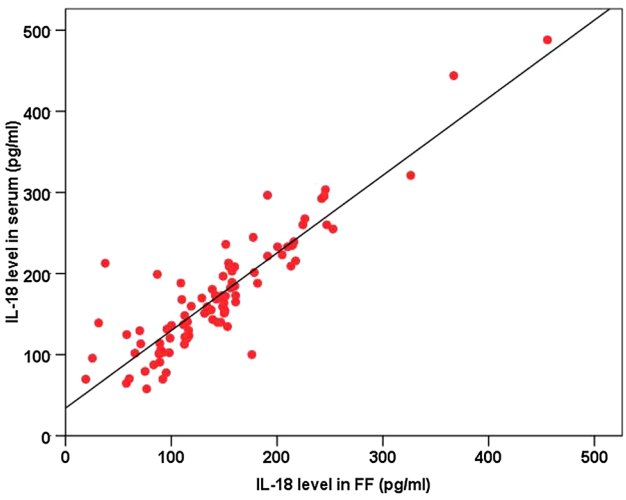

Fig. 1 Correlation between IL-18 concentrations in serum and follicular fluid (FF) on the day of oocyte retrieval ($n = 90$, $r_s = 0.85$, $p < 0.001$)

The median IL-18 levels in FF and serum of patients who underwent ICSI, 140.4 pg/mL (98.6–178.2) and 164.3 pg/ml (123.6–213), respectively, were higher than in those who underwent IVF 147.6 pg/mL (88.2–156.3) and 149.4 pg/mL (101.1–156.3), respectively, but this difference did not meet statistical significance. We found that serum IL-18 levels in patients with tubal cause of infertility ($n = 34$) were 153.6 pg/mL (129–188.6) and in patients with andrological infertility ($n = 68$) were 164.9 pg/mL (121.7–212.9). Also IL-18 levels in follicular fluid of patients with tubal cause and andrological cause of infertility were 128.8 pg/mL (98.8–151.1) and 149 pg/mL (104.5–177.4), respectively. However, we did not find any significant differences in serum and FF levels of IL-18 between the two groups of patients ($p = 0.483$ and 0.225, respectively). From total 102 patients, 32 became pregnant (31.37%). There were no differences in IL-18 levels of serum and FF between patients who became pregnant and those not.

Immunocytochemical detection of IL-18 and its receptor on GCs

By means of immunocytochemistry, we were able to prove that human follicular GCs express IL-18 (Fig. 2a). Figure 2b demonstrates the localization of the IL-18R gene product on the surface of a GC. Figure 2c as a positive control shows the localization of human IL-18R on the surface of monocyte cells isolated from blood and 2d as negative control, GCs without the primary antibody. All of GCs were positive to IL-18 and its receptor. The immunoreactive signals of IL-18 were distributed in the cytoplasm of GCs, whereas its receptor was preferentially localized on the membrane of GCs. The purity of our GC preparations (absence of macrophages) was >99% in all samples, as demonstrated by the immunocytochemical application of the monoclonal antibody Ki-M_1P which recognizes CD 68.

Detection of IL-18 and its receptor on GCs by RT-PCR

By applying RT-PCR on cDNA of IL-18 and its receptor, amplicons of 368 and 422 bp were detected (Fig. 3). The results clearly showed that corresponding amplicons are detectable for IL-18 (Fig. 3, lanes b–d) and IL-18R (Fig. 3, lanes e–g) in samples from GCs, monocytes, and placenta. Amplicons of 400 bp (Fig. 3, lanes h–j) were detected for GAPDH.

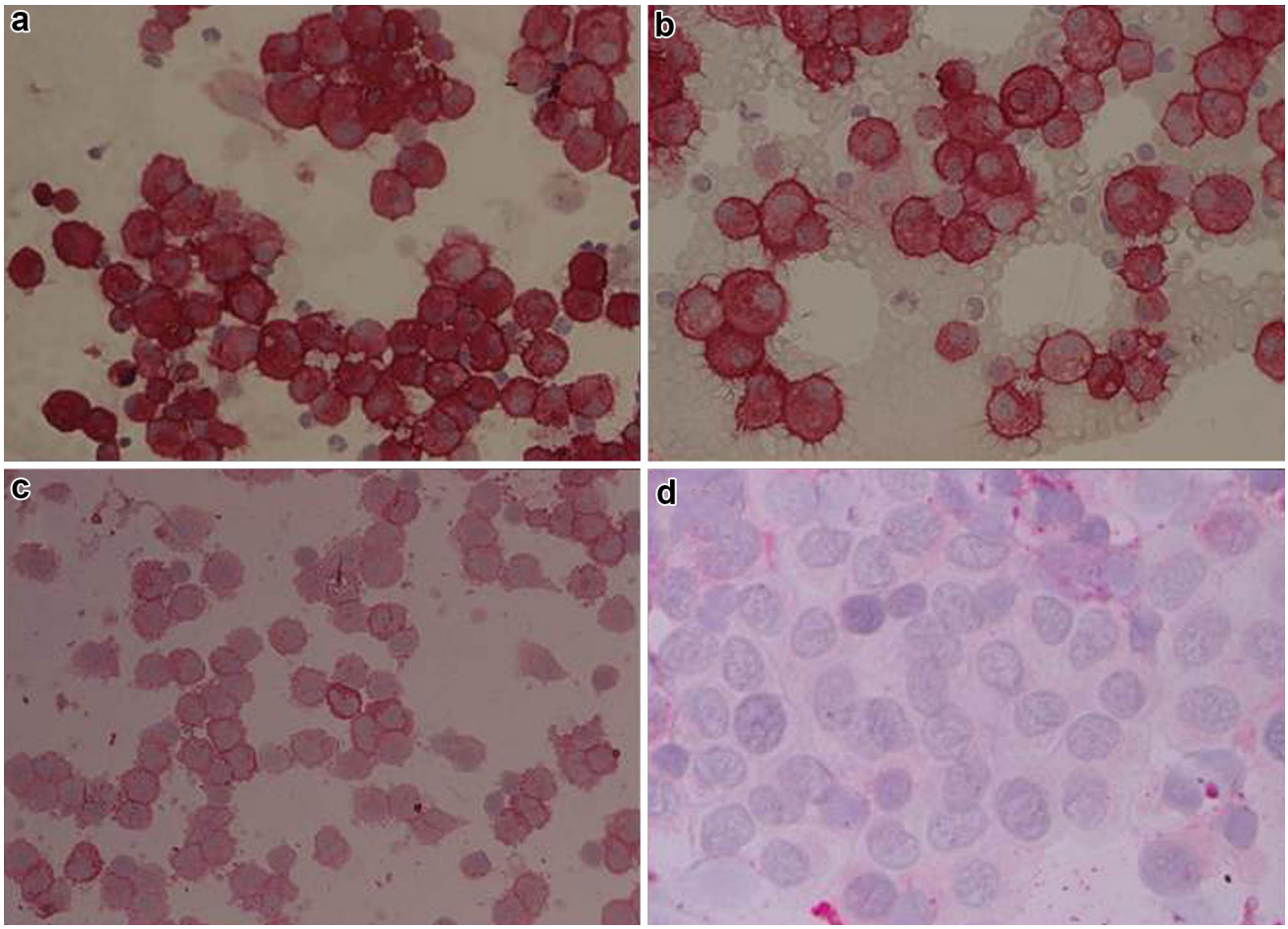

Fig. 2 Immunocytochemical demonstration of IL-18 and its receptor (IL-18R). **a** IL-18 in the cytoplasm and also its localization on the cell membrane surface of human follicular granulosa cells (GCs). **b** Localization of IL-18R on the surface of GCs. **c** Localization of IL-18 on the surface of monocytes, isolated from blood as positive control. **d** GCs without the primary antibody as negative control. Immunoalkaline phosphatase reaction. Magnification, 600

Discussion

By applying immunocytochemical methods, we could show the expression of human IL-18 in follicular luteal GCs and the localization of its receptor on the surface of GCs, a finding so far not reported. Detection of IL-18 in this study is well in line with the findings of Sarapick et al. [36]. They recently described the presence and local production of mRNA IL-18 in the human mural and cumulus granulosa cells. Kilic et al. [34] found IL-18 and IL-18 binding protein in serum and ovarian follicular fluid. The expression of its receptor on GCs, which is important to clarify the mechanism of IL-18, is now described in this study. The biological functions of IL-18 are mediated through its receptor (IL-18R). IL-18R resembles IL-1R and shares same signal transduction pathway [30]. Expression of IL-18R was confirmed on both Th1 lymphocytes and NK cells, whereas it was not observed on Th2 lymphocytes [31, 42]. As monocytes, in addition to placenta, may express IL-18 and its receptor [4–7], these samples were used as positive controls throughout this study. The purity of our GC preparations (absence of macrophages) based on the immunocytochemical test was >99% which allows us to conclude that the obtained results are reliable.

Using RT-PCR, a 368-bp amplicon for IL-18 and a 422-bp amplicon, which characterizing the amplification of IL-18R, could be detected. This result is supported by findings of previous studies for expression of the IL-18R on human dendritic cells in peripheral blood and epidermis, as well as monocyte-derived dendritic cells and lymphocytes and placenta [4, 41].

The mammalian ovulation event can be considered from the perspective of an inflammatory reaction, with proinflammatory cytokines producing and functionally interacting throughout the process [36, 43]. IL-18 is known to induce cytokines that are important for both folliculogenesis and ovulation, including IL-1β, TNF-α, and IFN-γ [44, 45].

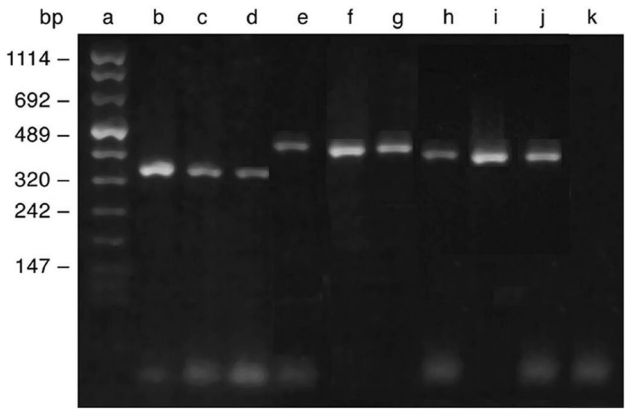

Fig. 3 In vitro reverse transcription (RT) polymerase chain reaction (PCR) of IL-18 and its receptor (IL-18R). *Lane a* separation of a molecular weight marker; *lanes b–d* IL-18 amplicon in human granulosa cells (GCs), in human monocytes and in human placenta as positive control, respectively; *lanes e–g* IL-18R amplicon in human GCs, in human monocytes and in human placenta as positive control, respectively; *lanes h–j* glyceraldehyde 3-phosphate dehydrogenase (GAPDH) amplicon in human GCs, human monocytes and placenta, respectively; *lane k* negative control omitting RT

In the present study, we could show that IL-18 and its receptor were localized in human luteinized follicular GCs. GCs can synthesize both IL-18 and IL-18R. GCs seem to represent one of the sources and targets of IL-18. The expression of IL-18 and its receptor in follicular GCs suggests that IL-18 may play a role as an inflammatory factor involved in ovulation. The concomitant occurrence of IL-18 and IL-18R in GCs implies that IL-18 may exert an effect on GCs via autocrine or paracrine mechanisms. IL-18 which is secreted from other cell types in the ovaries (such as granulocytes or macrophages) may affect the biological activity of GCs via the IL-18 receptor expressed on these cells.

In the present study, we measured the concentration of IL-18 in FF and serum on the day of oocyte retrieval. IL-18 concentration in serum was higher than in FF. Higher levels of IL-18 in serum compared with FF were described by Gutman et al. [21]. As we detected for the first time, the follicular granulosa cells are the major source of IL-18 and its receptors expression sites. This means that IL-18 in FF implies an intrafollicular production and a potential autocrine or paracrine role of IL-18 within the follicular microenvironment. Furthermore, there was a significant positive correlation between IL-18 levels in serum and FF. Moreover, there were significant positive correlations between IL-18 levels of serum and FF and BMI on the day of FP. Other authors have also documented increased levels of IL-18 in serum of patients with higher BMI, and it was reported that the polycystic ovary syndrome and obesity increase IL-18 levels [46]. Another study has showed that weight loss in obese women leads to a reduction in IL-18 levels [47]. The median IL-18 levels in serum and FF of patients who underwent ICSI were higher than in those who underwent IVF. The possible reason for higher levels of IL-18 in ICSI patients is that in candidate couples for ICSI, women has healthy ovaries and men has pathologic spermiogram, so the ovaries produces more IL-18 than IVF patients who have female factor infertility. This is in accordance with our earlier finding relating to granulocyte [48] and macrophage colony-stimulating factors [49].

In conclusion, we found that IL-18 is synthesized and secreted in GCs prior to ovulation. Therefore, we speculate that IL-18 is produced locally in the ovary in response to gonadotropins and gonadal hormones as a consequence of an inflammatory reaction and plays an important role in folliculogenesis and mechanism of ovulation. With regard to IL-18 concentrations in serum and FF, follicles are one of the major production sites of IL-18 and one of the contributors to the level of IL-18 in serum during folliculogenesis and ovulation.

Acknowledgements The authors thank Dawn Ruether for her editorial assistance, Parisa Ahani for graphical work and Kerstin Koch for her assistance in the laboratory experiments at the Department of Obstetrics and Gynaecology, University Hospitals Schleswig–Holstein, Campus Kiel, Germany.

Compliance with ethical standards

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

Ethical approval Under the stipulations of the Universitätsklinikum Schleswig–Holstein (UKSH), Kiel Institutional Review Board (IRB), approval did not obtain for a retrospective observational study.

Informed consent Patient written consent was obtained from all participants.

References

1. Erickson GF, Danforth DR (1995) Ovarian control of follicle development. Am J Obstet Gynecol 172(2 Pt 2):736–747
2. Dinarello CA (1999) Interleukin-18. Methods 19(1):121–132. doi:10.1006/meth.1999.0837
3. Dinarello CA (1998) Interleukin-1 beta, interleukin-18, and the interleukin-1 beta converting enzyme. Ann N Y Acad Sci 856:1–11
4. Amash A, Huleihel M, Eyal S, Maor E, Myatt L, Holcberg G (2007) The expression of interleukin-15 and interleukin-18 by human term placenta is not affected by lipopolysaccharide. Eur Cytokine Netw 18(4):188–194. doi:10.1684/ecn.2007.0102
5. Udagawa N, Horwood NJ, Elliott J, Mackay A, Owens J, Okamura H, Kurimoto M, Chambers TJ, Martin TJ, Gillespie MT (1997) Interleukin-18 (interferon-gamma-inducing factor) is produced by osteoblasts and acts via granulocyte/macrophage colony-stimulating factor and not via interferon-gamma to inhibit osteoclast formation. J Exp Med 185(6):1005–1012

6. Stoll S, Muller G, Kurimoto M, Saloga J, Tanimoto T, Yamauchi H, Okamura H, Knop J, Enk AH (1997) Production of IL-18 (IFN-gamma-inducing factor) messenger RNA and functional protein by murine keratinocytes. J Immunol 159(1):298–302
7. Matsumoto S, Tsuji-Takayama K, Aizawa Y, Koide K, Takeuchi M, Ohta T, Kurimoto M (1997) Interleukin-18 activates NF-κB in murine T helper type 1 cells. Biochem Biophys Res Commun 234(2):454–457. doi:10.1006/bbrc.1997.6665
8. Conti B, Jahng JW, Tinti C, Son JH, Joh TH (1997) Induction of interferon-gamma inducing factor in the adrenal cortex. J Biol Chem 272(4):2035–2037
9. Rothe H, Jenkins NA, Copeland NG, Kolb H (1997) Active stage of autoimmune diabetes is associated with the expression of a novel cytokine, IGIF, which is located near Idd2. J Clin Invest 99(3):469–474. doi:10.1172/JCI119181
10. Pacora P, Romero R, Maymon E, Gervasi MT, Gomez R, Edwin SS, Yoon BH (2000) Participation of the novel cytokine interleukin 18 in the host response to intra-amniotic infection. Am J Obstet Gynecol 183(5):1138–1143. doi:10.1067/mob.2000.108881
11. Menon R, Lombardi SJ, Fortunato SJ (2001) IL-18, a product of choriodecidual cells, increases during premature rupture of membranes but fails to turn on the Fas–FasL-mediated apoptosis pathway. J Assist Reprod Genet 18(5):276–284
12. Komsky A, Huleihel M, Ganaiem M, Kasterstein E, Komorovsky D, Bern O, Raziel A, Friedler S, Ron-El R, Strassburger D (2012) Presence of IL-18 in testicular tissue of fertile and infertile men. Andrologia 44(1):1–8. doi:10.1111/j.1439-0272.2010.01090.x
13. Ledee N, Petitbarat M, Rahmati M, Dubanchet S, Chaouat G, Sandra O, Perrier-d'Hauterive S, Munaut C, Foidart JM (2011) New pre-conception immune biomarkers for clinical practice: interleukin-18, interleukin-15 and TWEAK on the endometrial side, G-CSF on the follicular side. J Reprod Immunol 88(2):118–123. doi:10.1016/j.jri.2011.01.007
14. Okamura H, Tsutsui H, Kashiwamura S, Yoshimoto T, Nakanishi K (1998) Interleukin-18: a novel cytokine that augments both innate and acquired immunity. Adv Immunol 70:281–312
15. Yamashita H, Ishikawa M, Inoue T, Usami M, Usami Y, Kotani J (2016) Interleukin-18 reduces blood glucose and modulates plasma corticosterone in a septic mouse model. Shock. doi:10.1097/shk.0000000000000747
16. Fischer CP, Perstrup LB, Berntsen A, Eskildsen P, Pedersen BK (2005) Elevated plasma interleukin-18 is a marker of insulin-resistance in type 2 diabetic and non-diabetic humans. Clinical Immunology 117(2):152–160
17. Rabkin SW (2009) The role of interleukin 18 in the pathogenesis of hypertension-induced vascular disease. Nat Clin Pract Cardiovasc Med 6(3):192–199
18. Evans J, Collins M, Jennings C, van der Merwe L, Söderström I, Olsson T, Levitt NS, Lambert EV, Goedecke JH (2007) The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease. Eur J Endocrinol 157(5):633–640
19. Yamagami H, Kitagawa K, Hoshi T, Furukado S, Hougaku H, Nagai Y, Hori M (2005) Associations of serum IL-18 levels with carotid intima-media thickness. Arterioscler Thromb Vasc Biol 25(7):1458–1462
20. Ida A, Tsuji Y, Muranaka J, Kanazawa R, Nakata Y, Adachi S, Okamura H, Koyama K (2000) IL-18 in pregnancy; the elevation of IL-18 in maternal peripheral blood during labour and complicated pregnancies. J Reprod Immunol 47(1):65–74
21. Gutman G, Soussan-Gutman L, Malcov M, Lessing JB, Amit A, Azem F (2004) Interleukin-18 is high in the serum of IVF pregnancies with ovarian hyperstimulation syndrome. Am J Reprod Immunol 51(5):381–384. doi:10.1111/j.1600-0897.2004.00172.x
22. Espey LL (1994) Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. Biol Reprod 50(2):233–238
23. Escobar-Morreale HF, Botella-Carretero JI, Villuendas G, Sancho J, San Millan JL (2004) Serum interleukin-18 concentrations are increased in the polycystic ovary syndrome: relationship to insulin resistance and to obesity. J Clin Endocrinol Metab 89(2):806–811. doi:10.1210/jc.2003-031365
24. Wilson R, Moor J, Jenkins C, Miller H, Walker JJ, McLean MA, Norman J, McInnes IB (2004) Abnormal first trimester serum interleukin 18 levels are associated with a poor outcome in women with a history of recurrent miscarriage. Am J Reprod Immunol 51(2):156–159
25. Yang Y, Qiao J, Li R, Li MZ (2011) Is interleukin-18 associated with polycystic ovary syndrome? Reprod Biol Endocrinol 9:7. doi:10.1186/1477-7827-9-7
26. Kaya C, Pabuccu R, Berker B, Satiroglu H (2010) Plasma interleukin-18 levels are increased in the polycystic ovary syndrome: relationship of carotid intima-media wall thickness and cardiovascular risk factors. Fertil Steril 93(4):1200–1207. doi:10.1016/j.fertnstert.2008.10.070
27. Ebejer K, Calleja-Agius J (2013) The role of cytokines in polycystic ovarian syndrome. Gynecol Endocrinol 29(6):536–540. doi:10.3109/09513590.2012.760195
28. Nikolaeva MA, Babayan AA, Stepanova EO, Smolnikova VY, Kalinina EA, Fernández N, Krechetova LV, Vanko LV, Sukhikh GT (2016) The relationship of seminal transforming growth factor-β1 and interleukin-18 with reproductive success in women exposed to seminal plasma during IVF/ICSI treatment. J Reprod Immunol 117:45–51
29. Chen H, Yang X, Du J, Lu M (2015) Interleukin-18 gene polymorphisms and risk of recurrent pregnancy loss: A systematic review and meta-analysis. J Obstet Gynaecol Res 41(10):1506–1513
30. Akira S (2000) The role of IL-18 in innate immunity. Curr Opin Immunol 12(1):59–63
31. Malamitsi-Puchner A, Sarandakou A, Baka SG, Tziotis J, Rizos D, Hassiakos D, Creatsas G (2001) Concentrations of angiogenic factors in follicular fluid and oocyte-cumulus complex culture medium from women undergoing in vitro fertilization: association with oocyte maturity and fertilization. Fertil Steril 76(1):98–101
32. Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M (1999) Interleukin-18 binding protein: a novel modulator of the Th1 cytokine response. Immunity 10(1):127–136
33. Tsuji Y, Tamaoki TH, Hasegawa A, Kashiwamura S, Iemoto A, Ueda H, Muranaka J, Adachi S, Furuyama J, Okamura H, Koyama K (2001) Expression of interleukin-18 and its receptor in mouse ovary. Am J Reprod Immunol 46(5):349–357
34. Kilic S, Moraloglu O, Yuksel B, Ozaksit G, Tasdemir N, Dogan M (2009) IL-18 and IL-18 binding protein concentrations in serum and ovarian follicular fluid do not affect in vitro fertilization outcomes in obese, overweight, and non-obese patients. J Reprod Immunol 79(2):211–214. doi:10.1016/j.jri.2008.10.002
35. Ledee-Bataille N, Olivennes F, Kadoch J, Dubanchet S, Frydman N, Chaouat G, Frydman R (2004) Detectable levels of interleukin-18 in uterine luminal secretions at oocyte retrieval predict failure of the embryo transfer. Hum Reprod 19(9):1968–1973. doi:10.1093/humrep/deh356
36. Sarapik A, Velthut A, Haller-Kikkatalo K, Faure GC, Bene MC, de Carvalho Bittencourt M, Massin F, Uibo R, Salumets A (2012) Follicular proinflammatory cytokines and chemokines as markers of IVF success. Clin Dev Immunol 2012:606459. doi:10.1155/2012/606459

37. Salmassi A, Schmutzler AG, 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. doi:10.1016/j.fertnstert.2003.09.039
38. Salmassi A, Zhang Z, Schmutzler AG, 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(2):419–425. doi:10.1016/j.fertnstert.2004.06.072
39. Radzun HJ, Hansmann ML, Heidebrecht HJ, Bodewadt-Radzun S, Wacker HH, Kreipe H, Lumbeck H, Hernandez C, Kuhn C, Parwaresch MR (1991) Detection of a monocyte/macrophage differentiation antigen in routinely processed paraffin-embedded tissues by monoclonal antibody Ki-M1P. Lab Invest 65(3):306–315
40. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162(1):156–159. doi:10.1006/abio.1987.9999
41. Gutzmer R, Langer K, Mommert S, Wittmann M, Kapp A, Werfel T (2003) Human dendritic cells express the IL-18R and are chemoattracted to IL-18. J Immunol 171(12):6363–6371
42. Radwan P, Radwan M, Polac I, Lewkowicz P, Wilczynski JR (2013) IL-18 and IL-18 binding protein concentration in ovarian follicular fluid of women with "tubal factor" subjected to in vitro fertilization. Postepy Hig Med Dosw (Online) 67:463–470. doi:10.5604/17322693.1050036
43. Gerard N, Caillaud M, Martoriati A, Goudet G, Lalmanach AC (2004) The interleukin-1 system and female reproduction. J Endocrinol 180(2):203–212
44. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001) Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. Cytokine Growth Factor Rev 12(1):53–72
45. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001) Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 19:423–474. doi:10.1146/annurev.immunol.19.1.423
46. Escobar-Morreale HF, Botella-Carretero JI, Villuendas G, Sancho J, San Millán JL (2004) Serum interleukin-18 concentrations are increased in the polycystic ovary syndrome: relationship to insulin resistance and to obesity. J Clin Endocrinol Metab 89(2):806–811
47. Esposito K, Pontillo A, Ciotola M, Di Palo C, Grella E, Nicoletti G, Giugliano D (2002) Weight loss reduces interleukin-18 levels in obese women. J Clin Endocrinol Metab 87(8):3864–3866
48. Salmassi A, Schmutzler A, Schaefer S, Koch K, Hedderich J, Jonat W, Mettler L (2005) Is granulocyte colony-stimulating factor level predictive for human IVF outcome? Hum Reprod 20(9):2434–2440
49. Salmassi A, Mettler L, Jonat W, Buck S, Koch K, Schmutzler AG (2010) Circulating level of macrophage colony-stimulating factor can be predictive for human in vitro fertilization outcome. Fertil Steril 93(1):116–123