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The effects of omentin on estradiol and progesterone release in human granulosa cell culture

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Abstract

Adipokines serve as a link between energy metabolism and reproduction, helping to explain infertility related to polycystic ovary syndrome (PCOS). Granulosa cells are known to produce adipokines such as omentin; however, research on the potential effects of omentin on hormone levels in PCOS patients is lacking. Thus, the aim of our study was to evaluate the effect of different concentrations of omentin, FSH, and FSH + omentin on estradiol and progesterone secretion from human granulosa cells of PCOS patients. Granulosa cells were obtained from follicular fluid of 12 women undergoing IVF/ICSI treatment. Then they were treated with different doses of omentin (1 and 10 ng/ml), FSH (10 and 100 ng/ml), and with combinations of omentin and FSH for 48 h. The levels of estradiol and progesterone secreted by granulosa cells into the culture supernatants were measured by ELISA. Progesterone levels were increased when 10 ng/ml omentin was administered together with high-dose FSH (100 ng/ml), and were also increased at 1 and 10 ng/ml omentin doses compared to the control. Estradiol levels increased with 1 and 10 ng/ml omentin, and this effect was enhanced when combined with high-dose FSH; however, the combination of 1 ng/ml omentin with high-dose FSH resulted in reduced estradiol secretion. These findings demonstrated that omentin treated with different doses changed the secretion of estradiol and progesterone in granulosa cells in a dose-dependent manner.

Keywords Estradiol · Granulosa cells · Omentin · PCOS · Progesterone

Introduction

The regulation of ovarian function is closely linked to metabolic status, which modulates reproductive processes primarily through adipokines, a group of peptide hormones secreted by adipose tissue (Estienne *et al*. 2019; Warzych and Lipinska 2020). Omentin is a recently discovered adipokine produced predominantly by visceral adipose tissue (Silvestris *et al*. 2018). It has two isoforms, omentin-1 and omentin-2 (de Souza Batista *et al*. 2007). Omentin-1 is the primary circulating form of omentin (Schäffler *et al*. 2005; Silvestris *et al*. 2018). Omentin was initially discovered in mouse Paneth cells, where it functions as a lectin contributing to intestinal defense against pathogenic bacteria. Subsequently, it was shown to be expressed in several cell types, such as endothelial, mesothelial, vascular, and granulosa cells, exerting paracrine, autocrine, and endocrine effects (Tsuji *et al*. 2001; Cloix *et al*. 2014; Watanabe et al. 2017; Zhao *et al*. 2022). It is a secretory adipokine with anti-diabetic and anti-cancer properties. It is important in conditions such as inflammation, endothelial dysfunction,

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adipocyte differentiation, energy metabolism, and insulin resistance (Reverchon *et al*. 2014; Franik *et al*. 2020; Recinella *et al*. 2020; Hussein *et al*. 2024).

Granulosa cells are one of the basic functional units of ovarian follicles and produce follicular fluid, which has many functions such as regulation of oocyte maturation and control of follicular growth (Orisaka *et al*. 2009; Su *et al*. 2021; Cavalcanti *et al*. 2023). By the steroids (estradiol and progesterone) and proteins secreted from the granulosa cells in the follicular fluid, oocyte maturation, oocyte quality, and embryo development in later periods are supported (Wen *et al.* 2010; Peluso 2022; Yu *et al*. 2022). As an adipokine, omentin may affect steroidogenesis steps by regulating the release of estradiol and progesterone from human granulosa cells (Cloix *et al*. 2014). A specific receptor for omentin has not been identified yet. However, Cloix *et al*. demonstrated its expression in human granulosa cells. Omentin expression has also been investigated at the ovarian level. Bongrani *et al*. reported that omentin concentration in follicular fluid and its mRNA expression in granulosa cells were elevated in obese women (Bongrani *et al*. 2019).

The role of adipokines in the development of PCOS has not been established. Studies are investigating the relationship between omentin and PCOS, which is known to be associated with insulin resistance and obesity. Several studies have demonstrated that omentin levels are significantly lower in the plasma of women with PCOS compared to healthy controls, particularly in those with insulin resistance (Choi *et al*. 2011; Yang *et al*. 2015; Tang *et al*. 2017). However, in a different study, no significant difference was found between serum omentin levels in the non-obese PCOS group compared to the control group (Franik *et al*. 2020). Serum omentin levels tend to decline as free testosterone levels rise (de Souza Batista *et al*. 2007; Choi *et al*. 2011). In obese adolescent girls with PCOS, lower omentin-1 levels have similarly been linked to enhanced free testosterone concentrations (Özgen *et al*. 2019). Furthermore, weight loss has been shown to increase serum omentin levels in obese women with PCOS (Mahde *et al*. 2009; Moreno-Navarrete *et al*. 2010; Choi *et al*. 2011; Orlik *et al*. 2014). Therefore, there are conflicting findings regarding the effects of omentin in PCOS patients.

There are few studies investigating the potential link between adipokines such as omentin with PCOS in human granulosa cells derived from follicular fluid. A study investigated the effects of omentin on hormone secretion from granulosa cells in the presence of metabolic regulators such as IGF-1 and insulin (Cloix *et al*. 2014). However, to date, no study has investigated the effects of varying doses of omentin in combination with FSH on estradiol and progesterone secretion in primary granulosa cells derived from individuals with PCOS. Thus, the aim of this study was to investigate the effects of different doses of omentin on estradiol and progesterone release from granulosa cells in the context of PCOS.

Materials and methods

Patients Follicular fluid samples were obtained from PCOS patients undergoing IVF/ICSI treatment during OPU at the Mikrogen Genetic Diagnosis Centre (Gen-ART IVF Center). Ethical approval for this study was obtained from the Ethics Committee of Yuksek Ihtisas University (approval no.: 2023/02/04, date: 31/03/2023). All participants in the study signed informed consent. This study was conducted in accordance with the Helsinki Declaration. Human primary granulosa cells were isolated from 12 PCOS patients included in this study. Patients with obesity, hyperthyroidism, and hypothyroidism were excluded from the study. Data on the characteristics about the patients are presented in Table 1.

Hormones and reagents Recombinant human omentin (cat no. 228–20167-2) was purchased from RayBiotech (Peachtree Corners, GA). Recombinant human FSH alpha/beta protein (cat no. 228–12609-2) was also purchased from RayBiotech.

Follicular fluid sample collection and processing Following oocyte selection, the remaining follicular fluid was used. After adding 3 ml Histopaque solution (Histopaque®−1077, Sigma, St. Louis, MO) to the bottom of the tube, follicular fluid (12 ml) was added gently to prevent mixing. Then the samples were centrifuged at 2500 rpm for 30 min. After centrifugation, the cells remaining in the buffy coat were taken into another sterile tube. Basal medium (DMEM:F12, Capricorn Scientific, Ebsdorfergrund, Germany) was added onto these cells and centrifuged at 1200 rpm for 5 min. The supernatant was discarded and the pellet was dispersed in complete medium containing 10% FBS (fetal bovine serum,

Table 1. Demographic and clinical characteristics of women with PCOS

$n = 12$	
Age, year	
Median (minimum–maximum)	29 (24–37)
Body mass index (BMI), kg/m^2	
Median (minimum–maximum)	23.47 (20.80–27.2)
Oocytes retrieved, number	
Median (minimum–maximum)	19.5 (8–42)
Cigarettes, *n* (%)	
Smoker	4 (33.33)
Non-smoker	8 (66.67)

Serana, Pessin, Germany) and 1% antibiotic/antimycotic solution (Capricorn Scientific, Ebsdorfergrund, Germany).

Human granulosa cell culture After seeding into 24-well plates ($3–4 \times 10^4$ cells/ml), the cells were incubated in 5% CO_2 at 37°C. The medium of the cells was replaced with fresh medium every 2–3 days. When the cells reached 70% confluency, their medium was replaced with omentin (1, 10 ng/ml), FSH (10, 100 ng/ml), and omentin + FSH (10 ng/ml FSH + 1 ng/ml omentin, 10 ng/ml FSH + 10 ng/ml omentin, 100 ng/ml FSH + 10 ng/ml omentin, 100 ng/ml FSH + 1 ng/ml omentin) prepared in serum-free medium. FSH and omentin concentrations were determined based on previously published in vitro studies in human granulosa cell models (Sirotkin *et al*. 2024; Messini *et al*. 2019). The cultures were incubated at 37 °C in 5% CO_2 for 48 h. At the end of incubation, the supernatants were collected and stored at −20°C.

ELISA The release of estradiol and progesterone levels in culture supernatants was analyzed using Human Estradiol ELISA Kit (cat no. EA0030Hu, Btlab, Shanghai, China) and Human Progesterone ELISA Kit (cat no. EA0024Hu, Btlab, Shanghai, China), according to the manufacturer's protocol. The optical density was measured at 450 nm using the ELISA reader (Biotek Epoch 2, Agilent, Santa Clara, CA).

Statistical analysis Statistical analyses were performed using GraphPad Prism 9.5.1 (GraphPad Software, Boston, MA). Data are presented as mean ± SEM (standard error of the mean). One-way ANOVA followed by Tukey's multiple comparisons test was conducted to evaluate differences among multiple treatment groups. In addition, ordinary two-way ANOVA was used to assess the overall effects of different FSH and omentin treatment combinations. A p value < 0.05 was considered statistically significant.

Results

Culture of granulosa cells Granulosa cells isolated from the remaining follicular fluid of each patient were taken into cell culture. The cells exhibited epithelial characterization. It was observed that cells make contact with each other through their extensions and show less growth in areas where they are distant. Primary granulosa cells completely adhered to the flasks in approximately 24 h (Fig. 1).

Effect of omentin on progesterone and estradiol secretion by human granulosa cells The levels of estradiol and progesterone in cell supernatants treated with different FSH, omentin, and FSH + omentin concentrations were analyzed by ELISA. Progesterone levels increased in all omentin-treated groups, with the highest increase in 100 ng/ml FSH + 10 ng/ml omentin. However, this increment was not significant ($p > 0.05$) (Fig. 2*a*).

All doses of omentin-treated groups increased the release of estradiol except the 100 ng/ml FSH + 1 ng/ml omentin dose when compared with the control. When treated with the 10 ng/ml dose of FSH, omentin (10 ng/ml) additionally increased the secretion of estradiol, even though not significantly ($p > 0.05$) (Fig. 2*b*). The omentin treated with FSH caused variable effects on estradiol levels; particularly, the combination of 100 ng/ml FSH + 1 ng/ml omentin led to a decrease in estradiol levels compared to the application of omentin alone. Overall, FSH and omentin treatments had a significant effect on progesterone and estradiol levels

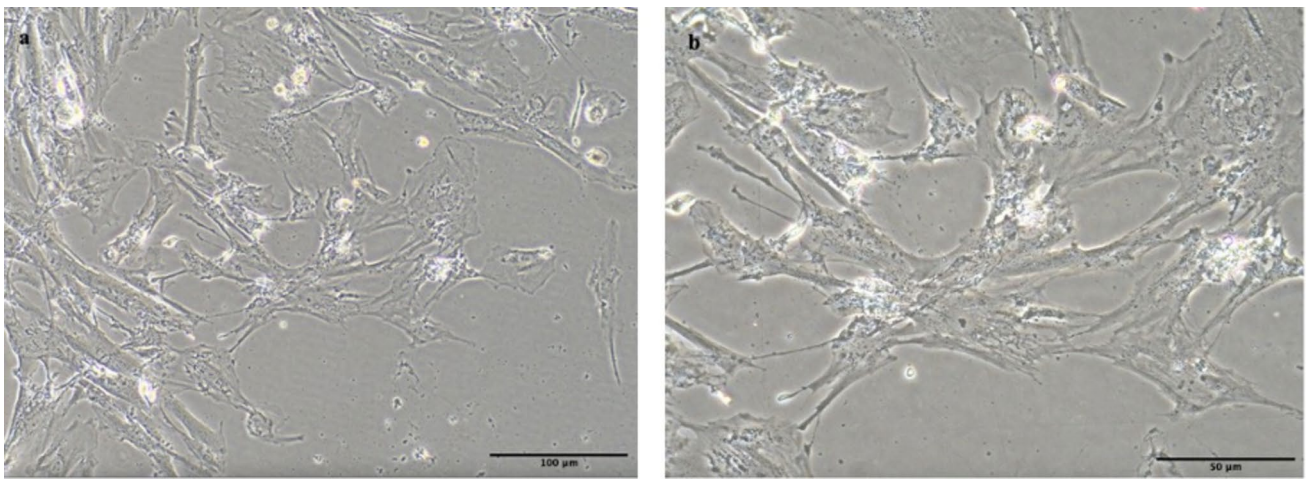

Figure 1. Primary culture micrographs of granulosa cells. Granulosa cells are seen at lower magnification (20×) in *a*, and at higher magnification (40×) in *b*.

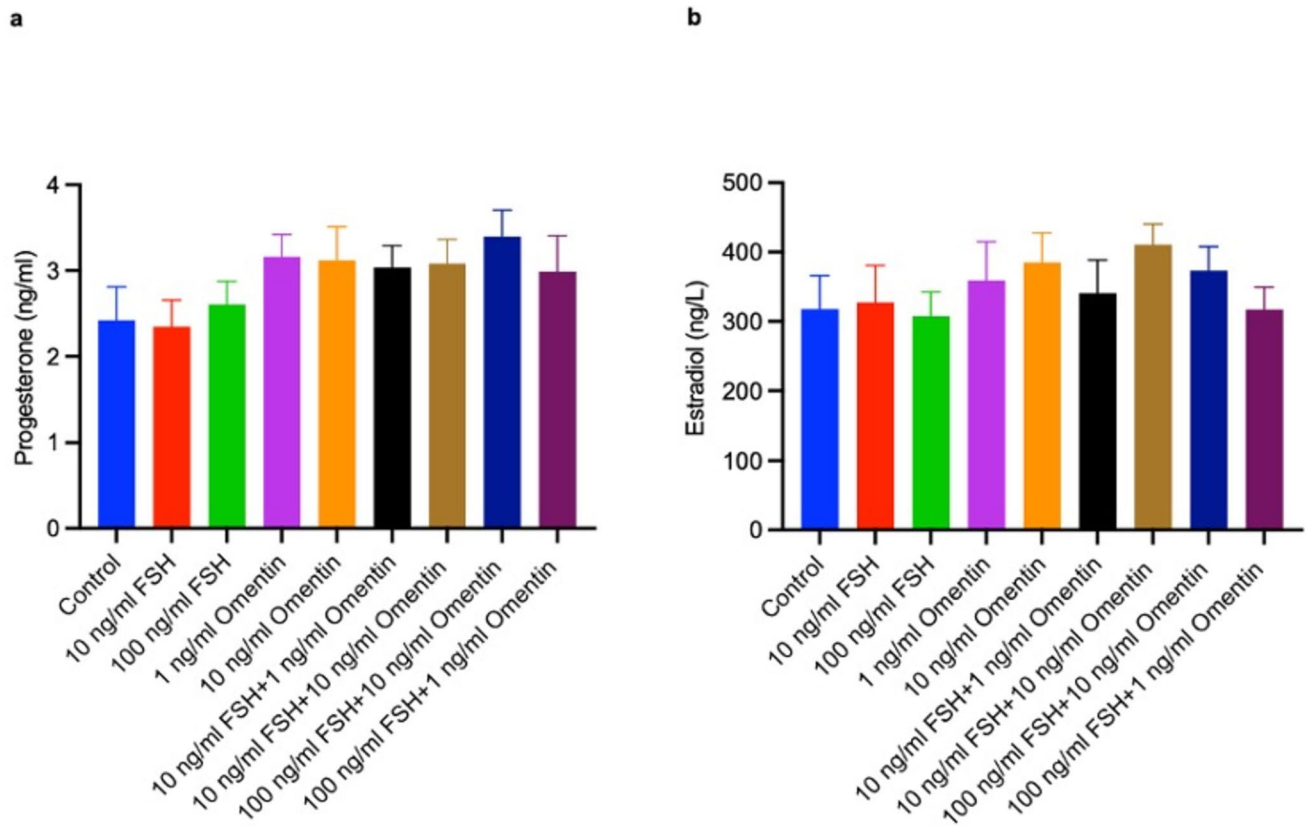

Figure 2. Effect of various doses of omentin on progesterone and estradiol secretion from human granulosa cell cultures. Progesterone (*a*) and estradiol (*b*) concentrations in the culture media of human granulosa cells treated with different concentrations of FSH, omentin, or their combinations for 48 h. Overall, FSH and omentin treatments significantly affected hormone levels ($p < 0.0001$), although no significant differences were observed between individual treatment groups. Data are presented as mean ± SEM.

($p < 0.0001$). However, no statistically significant differences were found between individual treatment groups.

Discussion

Adipokines produced by the ovary can regulate folliculogenesis, ovulation, and steroidogenesis in a paracrine and autocrine manner. For this reason, omentin may be important for understanding the pathophysiology of PCOS. PCOS is associated with risk factors such as genetic factors, neuroendocrine changes, environmental factors, and obesity (Witchel *et al*. 2019). When omentin levels were examined in the plasmas of women with PCOS, they were found to be lower than those of healthy women (Franik *et al*. 2020). Additionally, while the levels of omentin in plasma and follicular fluid were similar in healthy women, it was found to be at higher levels in the follicular fluid than in the plasma of women with PCOS (Cloix *et al*. 2014). When the expression of omentin in the granulosa cells of PCOS patients was investigated, it was found to be higher compared to the control group in the ovaries (Cloix *et al*. 2014; Bongrani *et al*. 2019). This situation suggests that omentin might be a compensatory response at the local cellular level to modulate the function of granulosa cells (Bongrani *et al*. 2019). However, the role of omentin in the pathogenesis of PCOS has not been fully elucidated. Therefore, treating granulosa cells obtained from PCOS patients with omentin allows for the investigation of this molecule's effects on the secretion of key steroid hormones, particularly estradiol and progesterone. This approach not only reveals omentin's potential regulatory role in hormone production but also contributes to a better understanding of PCOS pathogenesis.

The granulosa cells used in this study were obtained from women undergoing IVF and were partially luteinized with the capacity to produce progesterone. Therefore, to evaluate the direct effects of omentin, serum containing steroid precursors that could influence the results was not used. Several previous studies have successfully measured estradiol and progesterone production in granulosa cells under serum-free conditions and without androgen supplementation (Pich *et al*. 2025a, b; Messini *et al*. 2019; Cloix *et al*. 2014). While examining the effects of omentin treatment on

estradiol and progesterone secretion, we found that progesterone production tended to increase at 1 ng/ml and 10 ng/ml omentin treatment compared to other doses in the granulosa cells from PCOS patients. Especially, 10 ng/ml omentin, when treated together with high dose of FSH (100 ng/ml), increased progesterone secretion. In the studies conducted, it has been shown that other adipokines such as chemerin and resistin decrease progesterone levels (Reverchon *et al*. 2012; Messini *et al*. 2019). In these studies, the induction of cells with IGF-1 may have caused the results to differ from our study. This may also be explained by the fact that chemerin and resistin have pro-inflammatory roles, in contrast to the anti-inflammatory effects of omentin in metabolic diseases (Reverchon *et al*. 2014; Clemente-Suárez *et al*. 2023).

PCOS patients have low FSH levels, resulting in inadequate aromatase expression by granulosa cells, leading to impaired follicular maturation and decreased estradiol production (Desforges-Bullet *et al*. 2010; Tanbo *et al*. 2018). Estradiol levels increased with 1 ng/ml and 10 ng/ml omentin compared to the control, and this effect was further enhanced when 10 ng/ml omentin was combined with the same dose of FSH. When low doses of omentin (1 ng/ml) were treated with high doses of FSH (100 ng/ml), an opposite effect was observed, reducing estradiol secretion. In a study conducted with another adipokine, resistin, it was found that resistin, unlike omentin, eliminated the stimulatory effect of FSH. This suggests that different adipokines may have varying effects on the inductive action of FSH. These findings suggest that the stimulatory effect of FSH may increase when used with omentin, and this condition could regulate folliculogenesis in PCOS. Therefore, our study is important for a better understanding of the pathophysiology of PCOS. The ovulation disorders and hormonal imbalances frequently observed in PCOS suggest that they can be regulated not only by gonadotropins but also by locally produced adipokines. Considering that the granulosa cells themselves produce omentin, it is understood that these cells can regulate their own hormone production through autocrine and paracrine signals.

Conclusion

In conclusion, we showed that omentin treated in different doses altered the secretion of estradiol and progesterone release in granulosa cells. Also it has been shown that the stimulatory effect of FSH on granulosa cells can be dose-dependently regulated by omentin. Therefore, omentin may play a role in regulating the altered steroid hormone production observed in patients with PCOS, and changes in its levels appear to be associated with hormonal conditions such as PCOS. However, further studies are required to elucidate this relationship by using the molecular methods.

Limitations Although this study may contribute to unravelling the potential pathophysiological link between omentin and PCOS, it has some limitations. The most significant limitations are the differences in the number of cells obtained from the follicular fluid of each patient, their adhesion properties, and their growth rates. Since the samples were obtained via the vaginal route, patient-related infection might have caused cell loss. For this reason, increasing the number of patients will strengthen the results of the study. We also studied only women requiring IVF and hence under controlled ovarian stimulation, a condition that surely affects normal functions of the female reproductive system. These women generally consist of individuals with PCOS who are referred to assisted reproductive methods due to endocrine disorders or genetic diseases. Therefore, granulosa cells were collected only from women with PCOS, and the findings were interpreted in relation to this disease. Therefore, including a non-PCOS control group to evaluate potential differences in omentin sensitivity would make the study more valuable.

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Author contribution PT: writing—review and editing, writing—original draft, validation, data curation. BB: data curation, supervision, methodology, conceptualization. LÖ: writing—review and editing, formal analysis. TE: methodology, data curation. ÇZK: writing—review and editing, writing—original draft, methodology, supervision, project administration.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author.

Declarations

Ethics approval Ethical approval for this study was obtained from the Ethics Committee of Yuksek Ihtisas University (approval no.: 2023/02/04, date: 31/03/2023). All participants in the study signed informed consent. This study was conducted in accordance with the Helsinki Declaration.

Conflict of interest The authors declare no competing interests.

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