—Mini Review—

Peroxisome Proliferator-activated Receptor-γ agonists Prevent Tumor Necrosis Factor-α-mediated Inhibition of FSH-induced Follicle Development and Estradiol Production in A Preantral Follicle Culture System

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Abstract: Although 60–80% of the women with polycystic ovary syndrome (PCOS) ovulate with clomiphene citrate (CC), the rest are CC-resistant. Recently, the use of insulin-sensitizing agents such as metformin and pioglitazone have been proposed for inducing ovulation in CC-resistant women with PCOS, and we have reported that administration of bezafibrate, a lipid-lowering fibrate, in addition to CC, successfully induced ovulation in CC-resistant women with PCOS and dyslipidemia. Both pioglitazone and bezafibrate are peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists. This paper reviews the evidence for the direct effects of the drugs, which are PPAR-γ agonists, on follicle development and steroidogenesis, collected using an *in vitro* mouse preantral follicle culture system. We used the *in vitro* follicle culture system with the addition of tumor necrosis factor-alpha (TNF-α), which plays a role in insulin resistance, as a model for studying follicle development in women with PCOS. TNF-α inhibited FSH-induced follicle development and steroidogenesis in the follicle culture system. Both pioglitazone and bezafibrate prevented TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis through the PPAR-γ-stimulating pathway. Our results suggest that insulin-sensitizing drugs, especially PPAR-γ agonists, may directly influence follicle development and steroidogenesis in women with PCOS.

Key words: Polycystic ovary syndrome, TNF-α, PPAR-γ, Pioglitazone, Bezafibrate

Received: February 5, 2014
Accepted: March 11, 2014
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Introduction

Polycystic ovary syndrome (PCOS), a common ovulatory disorder in reproductive-aged women, affects 5–10% of the population, accounting for more than 75% of anovulatory infertility [1]. Although the pathogenesis of PCOS is still unknown, insulin resistance and compensatory hyperinsulinemia are considered important factors [2]. Insulin resistance is observed in approximately 70% of women with PCOS, irrespective of obesity [3]. Hyperinsulinemia may increase androgen synthesis in the ovaries [4] and decrease the level of sex hormone-binding protein in the liver, resulting in increased levels of free androgen [5]. Moreover, hyperinsulinemia may inhibit hepatic secretion of insulin-like growth factor (IGF) binding protein, resulting in the activation of IGF and an increase in androgen production in theca cells [6]. Hyperinsulinemia may also increase LH secretion, resulting in excessive androgen synthesis by the ovary [7]. It is thought that intraovarian hyperandrogenism inhibits follicular development in women with PCOS.

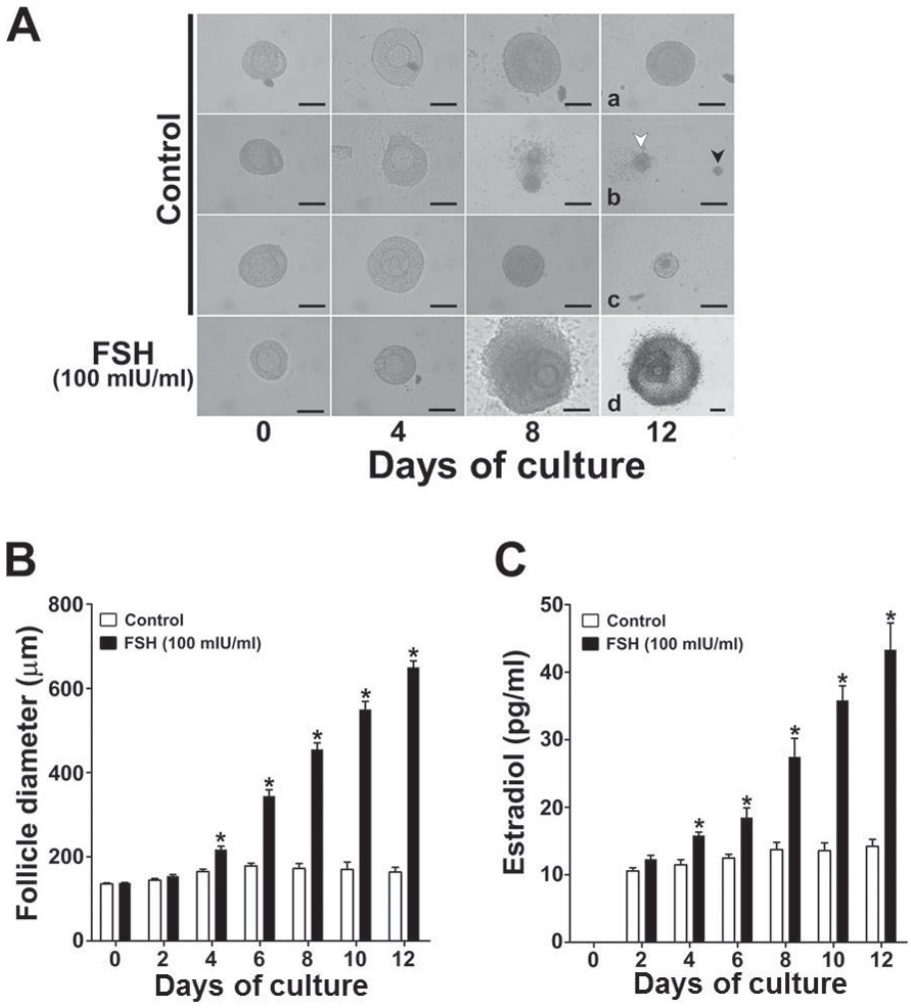

Fig. 1. *The in vitro* mouse preantral follicle culture system and the effects of FSH on follicle development and E2 production.
Follicles were cultured with or without 100 mIU/ml FSH for up to 12 days. The morphology of the follicles was evaluated, and follicle diameter was measured every other day. Media were refreshed every other day, and the E2 concentrations of the collected media were measured. **A:** Representative follicular growth treated with or without FSH during culture. When follicles were cultured without FSH (control), almost none of the follicles survived. All showed signs of degeneration, and did not grow in a multilayered pattern (a). An oocyte (black arrowhead) separated from granulosa cells (white arrowhead) (b), and shrunken follicles (c). FSH treatment induced follicle growth and antral-like cavity formation on the 12th day of culture (d). Bar, 100 μm **B:** Time course of follicle growth, assessed by follicle diameter, in the control and FSH-treated groups during *in vitro* culture. *, $P<0.05$. **C:** Time course of E2 concentrations in the control and FSH-treated groups during culture. *, $P<0.05$. Parts of this figure were originally published in Hara et al., Biol Reprod. 2011 [26].

Clomiphene citrate (CC) is a first-line drug for induction of ovulation in women with PCOS. Although 60–80% of women with PCOS ovulate after treatment with CC, the remainders are CC-resistant [8, 9]. Alternative treatments for CC-resistant PCOS include gonadotropin therapy and laparoscopic ovarian drilling [10]. Recently, the use of insulin-sensitizing agents such as metformin and thiazolidinedione derivatives have been proposed for inducing ovulation in CC-resistant women with PCOS [11, 12]. Pioglitazone is a thiazolidinedione derivative that is used in the treatment of type 2 diabetes mellitus. Pioglitazone decreases peripheral insulin resistance via the peroxisome proliferator-activated receptor-γ (PPAR-γ) pathway [18]. Pioglitazone-stimulated reduction of pe-

ripheral insulin resistance and its direct effect on the ovaries might induce ovulation in patients with PCOS. However, the direct effects of pioglitazone on ovarian follicular development are still unclear.

Women with PCOS carry a risk of metabolic disorders such as obesity, insulin resistance, glucose intolerance, and dyslipidemia. Dyslipidemia represents the most common metabolic abnormality in women with PCOS, with a prevalence of up to 70% [13–15]. Although weight reduction and increased physical activity constitute the first-line therapy for dyslipidemia, lipid-lowering drugs such as nicotinic acid and fibrates are frequently used to prevent the development of these diseases [16]. We previously reported that administration of bezafibrate, a lipid-lowering fibrate, in addition to CC, successfully induced ovulation in CC-resistant women with PCOS and dyslipidemia [17]. Bezafibrate is a non-selective ligand for PPARs such as PPAR-α, PPAR-δ, and PPAR-γ [18, 19]. Bezafibrate has been reported to improve insulin resistance in obese and non-obese type 2 diabetic patients [20, 21]. Because peripheral insulin resistance did not significantly change in CC-resistant women treated with bezafibrate, we hypothesized that bezafibrate directly affects ovarian follicular development though PPARs. We investigated the direct effects of drugs such as pioglitazone and bezafibrate, which are PPAR-γ agonists, on follicle development and steroidogenesis using an *in vitro* preantral follicle culture system.

The in vitro preantral Follicle Culture System

In addition to the abnormally late phase of follicle development, several studies have been reported that the early phase of follicle development is also impaired in the ovaries of women with PCOS [22, 23]. However, the molecules involved in abnormal follicle development in the polycystic ovary remain unknown. The absence of suitable animal models that mimic PCOS in humans is a barrier to our understanding of follicle development.

In vitro mouse follicle studies provide important information for understanding the early and late stages of folliculogenesis [24, 25]. We previously established an *in vitro* mouse preantral follicle culture system to investigate the direct effects of various compounds on follicle development and steroidogenesis [26]. The detailed method of the *in vitro* preantral follicle culture system was described previously [26]. Early preantral follicles were mechanically isolated from mature female mice ovaries. Follicles with the following characteristics were selected: (1) diameter of 120–150 μm, (2) immature oocytes centrally located within the follicle, (3) intact basal membrane, and (4) surrounding theca cells [27]. The isolated individual preantral follicles were cultured *in vitro* for up to 12 days to study the effects of various compounds on FSH-induced follicle development and steroidogenesis. We assessed the follicle survival rate, antral-like cavity formation rate, and the levels of 17 beta-estradiol (E2) in the culture medium. FSH treatment significantly induced follicle development and E2 secretion in the culture media compared to without FSH treatment (control). Although most follicles in the control group disintegrated and degenerated within 10 days of culture, follicles cultured with FSH grew larger and formed a large antral-like cavity on the 12th day of culture (Fig. 1) [26].

Roles of adipocytokines in the Pathogenesis in PCOS

Adipose tissue plays important roles in insulin resistance [28] and secretes a variety of bioactive cytokines, termed adipocytokines [29]. Adipocytokines have been shown to inhibit insulin signals in skeletal muscle and the liver [7]. The circulating levels of adipocytokines such as tumor necrosis factor-α (TNF-α), resistin, interleukin 6, and free fatty acid, which increase insulin resistance [30–32], are higher in obese and non-obese women with PCOS [33, 34]. These adipocytokines might facilitate abnormal follicle development through direct action, as well as through insulin resistance. Before our study [26], an *in vitro* follicle culture system had not been used to determine whether adipocytokines, which are implicated in the pathogenesis of PCOS, affect follicle development.

Effects of TNF-α on Follicle Development and steroidogenesis in preantral Follicle Cultures

TNF-α is a pro-inflammatory cytokine that is capable of inducing apoptosis in diverse types of cells [35]. Several publications have reported that TNF-α mediates the apoptosis of preantral and antral follicles in murine and human ovaries [36, 37]. TNF-α has also been reported to directly influence *in vitro* ovarian steroidogenesis in granulosa cells [38, 39]. Therefore, we first examined the effects of TNF-α on follicle development and steroidogenesis using our *in vitro* mouse preantral follicle culture system [26]. TNF-α significantly inhibited FSH-induced follicle development and steroidogenesis in a dose-dependent manner with a minimum effective dose of 5 ng/ml. TNF-α significantly inhibited follicle survival and decreased antral-like cavity formation in the FSH-treated group (Fig. 2). We used the *in vitro* follicle culture system, in the presence of TNF-α at 5 ng/ml, as a model for

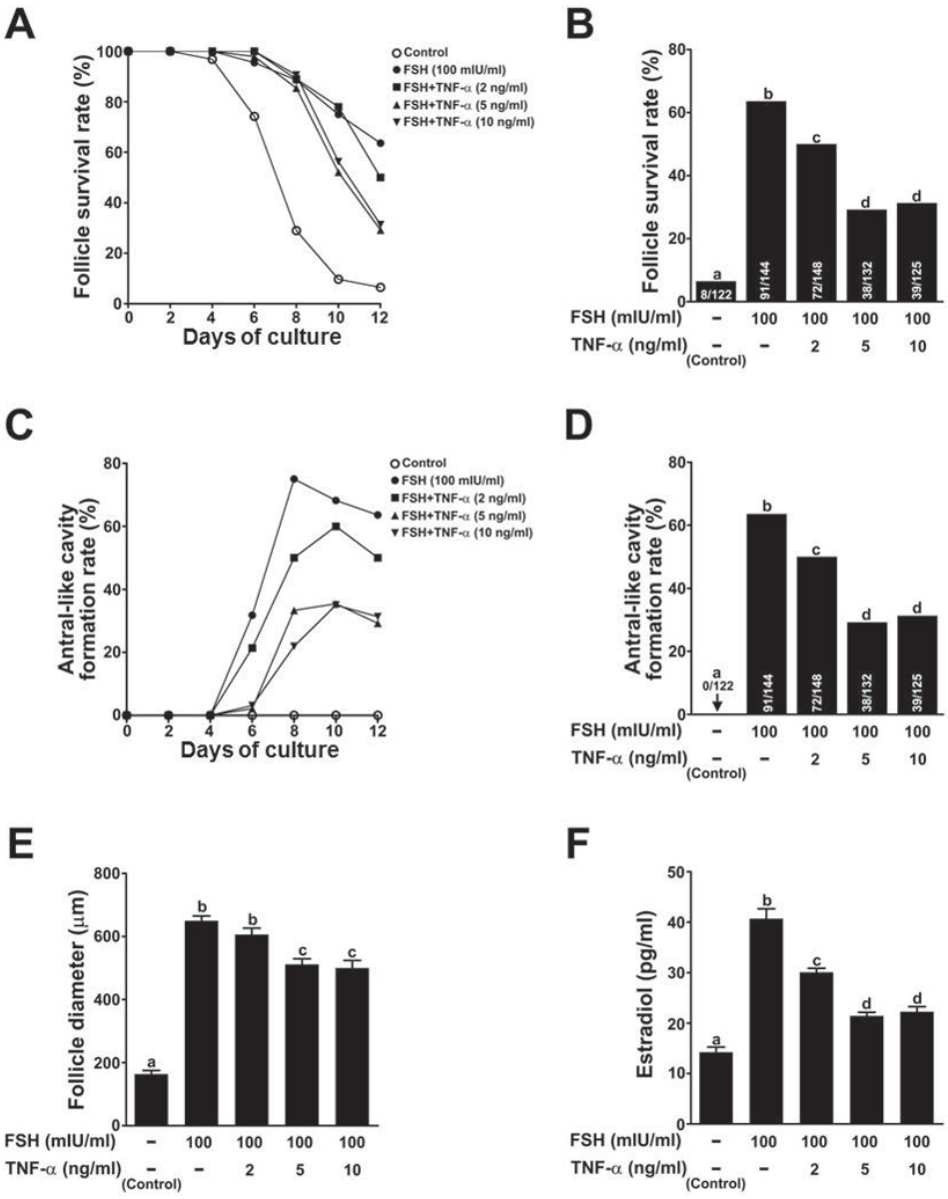

Fig. 2. Effects of TNF-α on FSH-induced follicle development and steroidogenesis.
Follicles were cultured with 2, 5, and 10 ng/ml TNF-α in the presence or absence of FSH. The morphology of the follicles was evaluated under a microscope and the survival rate and antral-like cavity formation rate were determined. Follicle diameters were measured every other day. Media were refreshed every other day, and E2 concentrations in the collected media were measured. **A:** Time course of dose-dependent inhibition by TNF-α of average (n=5) FSH-induced follicle survival during culture. **B:** Dose-dependent inhibition by TNF-α of FSH-induced follicle survival at 12 days of culture. Numbers inside the bars indicate the numbers of surviving follicles/total tested number of follicles. **C:** Time course of dose-dependent inhibition by TNF-α of average (n=5) FSH-induced antral-like cavity formation rate during culture. **D:** Dose-dependent inhibition by TNF-α of antral-like cavity formation rate at 12 days of culture. Numbers inside bars indicate the number of follicles with antral-like cavity/total tested number of follicles. **E:** Dose-dependent inhibition by TNF-α of FSH-induced follicle growth at 12 days of culture. Data are expressed as mean ± SEM. **F:** Dose-dependent inhibition by TNF-α of FSH-induced E2 production at 12 days of culture. Data are expressed as mean ± SEM. All experiments consisted of at least five independent experimental runs. A group treated without FSH and TNF-α was used as a control. Bars with different letters indicate a significant difference ($P<0.05$). Parts of this figure were originally published in Hara et al., Biol Reprod. 2011 [26].

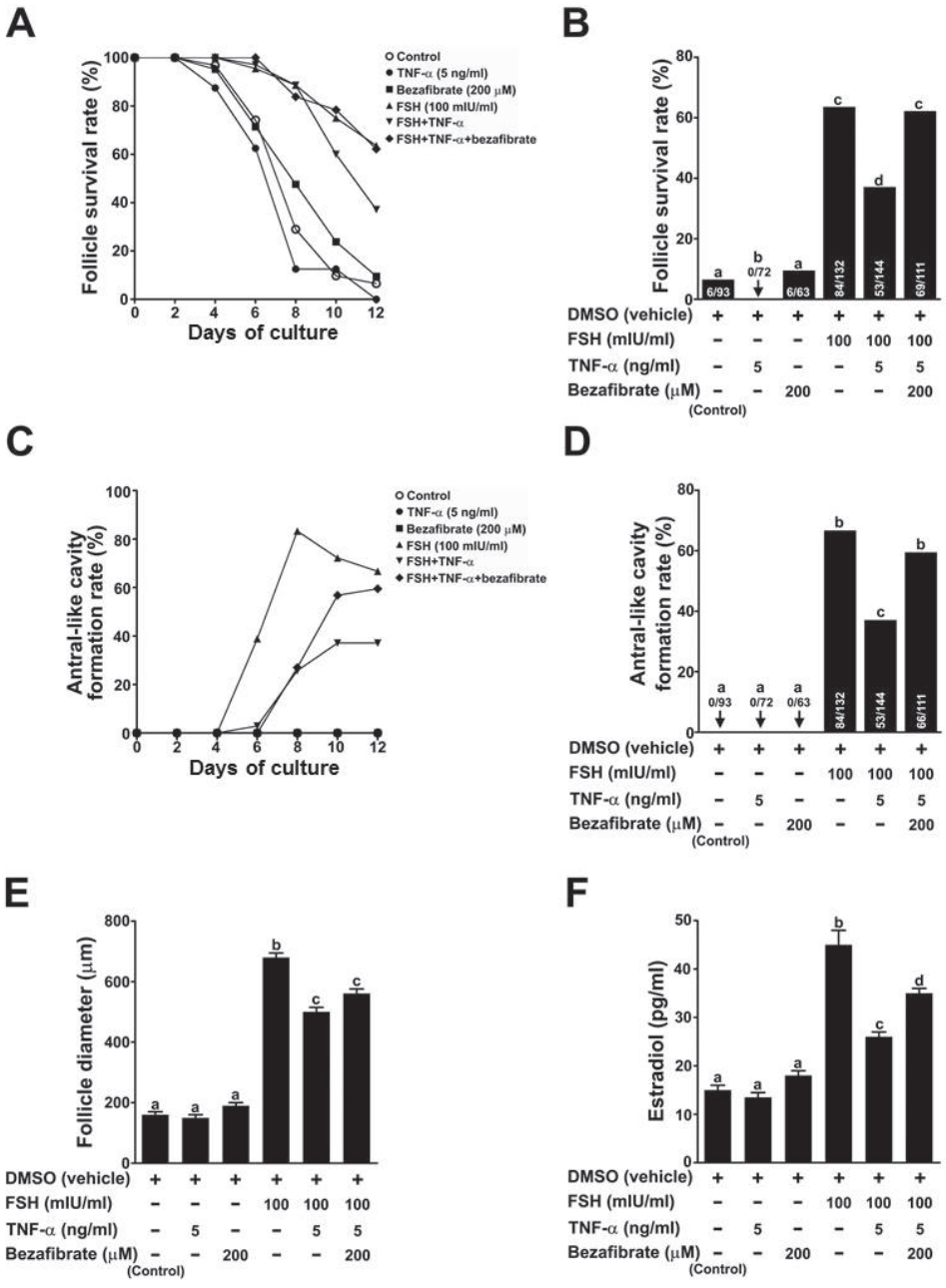

Fig. 3. Suppression by bezafibrate of TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis.

Follicles were cultured with TNF-α (5 ng/ml) and/or bezafibrate (200 μM) in the presence or absence of FSH. **A:** Follicle survival rates at 12 days of culture of the various treatments. Numbers inside bars indicate the number of surviving follicles/total tested number of follicles. **B:** Antral-like cavity formation rates at 12 days of culture of the various treatments. Numbers inside bars indicate the number of follicles with antral-like cavity/total tested number of follicles. **C:** E2 concentrations in the media at 12 days of culture of the various treatments. Data are expressed as mean ± SEM. **D:** Follicle growth at 12 days of culture of the various treatments. Data are expressed as mean ± SEM. All experiments consisted of at least 5 independent experimental runs. A group treated only with dimethyl sulfoxide (DMSO) was used as a control. Bars with different letters indicate a significant difference (P<0.05). Parts of this figure were originally published in Hara et al., Biol Reprod. 2011 [26].

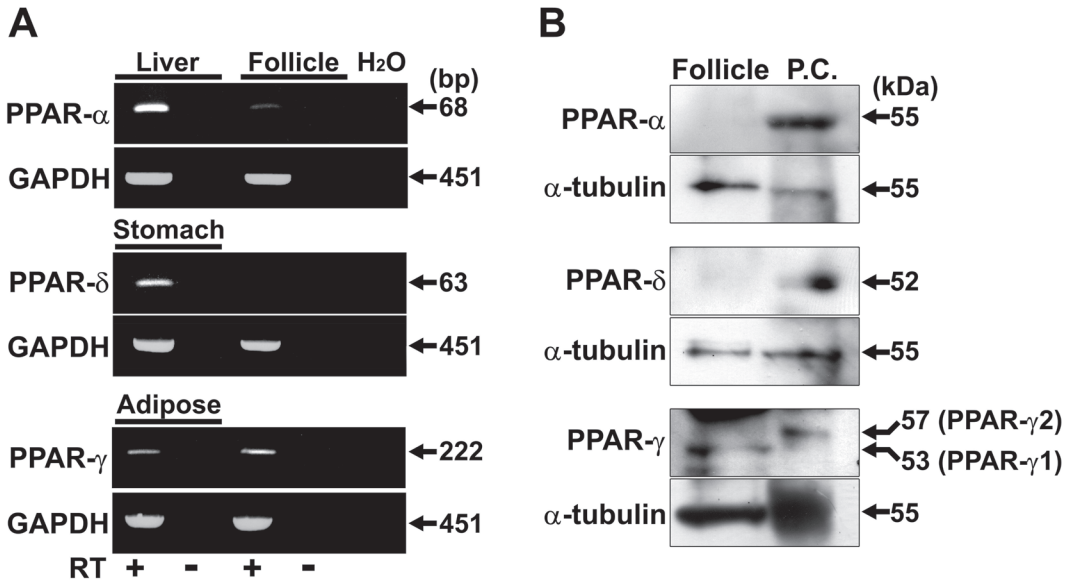

Fig. 4. Expression of PPAR subtypes in mouse preantral follicles.
The mRNA (A) and protein (B) expression of PPAR subtypes in mouse preantral follicles. Preantral follicles were mechanically isolated from mouse ovaries. One hundred preantral follicles were collected and subjected to RT-PCR and western blot analysis. **A:** Total RNA was isolated from preantral follicles and various mouse tissues: liver, stomach, and adipose tissues. RT-PCR was performed to detect the mRNA expression of PPAR-α (upper panel), PPAR-δ (middle panel), and PPAR-γ (lower panel). Positive controls for PPAR-α, PPAR-δ, and PPAR-γ were the liver, stomach, and adipose tissue, respectively. GAPDH was used as an internal control. **B:** Protein expressions of PPAR-α (upper panel), PPAR-δ (middle panel), and PPAR-γ (lower panel) were examined by western blot analysis using their respective antibodies. Alpha-tubulin was used as an internal control. Positive controls for PPAR-α and PPAR-δ were Hep G2 cell lysate and Jurkat nuclear extract, respectively. Mouse adipose tissue was used as a positive control for PPAR-γ. Experiments were repeated in triplicate, and representative results are shown. +, –, Presence or absence of RT in the RT-PCR reaction. GAPDH, glyceraldehyde-3-phosphate dehydrogenase, P.C., positive control. Parts of this figure were originally published in Hara et al., Biol Reprod. 2011 [26].

studying follicle development in women with PCOS.

Effects of bezafibrate on TNF-α-mediated Inhibition of FSH-induced Follicle Development and steroidogenesis in preantral Follicle Cultures

We examined whether bezafibrate could suppress TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis (Fig. 3) [26]. Bezafibrate significantly suppressed the TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis. Bezafibrate completely restored the TNF-α-mediated inhibition of FSH-induced follicle survival and antral-like cavity formation. Partial, but significant, suppression of TNF-α-mediated inhibition of FSH-induced E2 secretion was noticed after bezafibrate treatment. Moreover, our data showed that a selective PPAR-γ agonist (GW1929) similarly suppressed TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis. Furthermore, a selective PPAR-γ antagonist (GW9662) reversed the restorative effects of bezafibrate on TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis [26].

Role of PPAR-γ in the ovary

The PPARs are members of a nuclear receptor superfamily of ligand-dependent transcription factors. After binding of ligands to PPAR-responsive regulatory elements, PPARs heterodimerize with the retinoid X receptor. The PPARs play roles in the regulation of inflammation and metabolic processes, especially lipid and glucose homeostasis. In mammals, there are three PPAR subtypes, PPAR-α, PPAR-δ and PPAR-γ. Because PPAR-γ is highly expressed in adipose tissue and plays a key role in adipogenesis, PPAR-γ ligands, such as a thiazolidinedione derivative, mediate various cellular effects, such as the regulation of adipocyte differentiation,

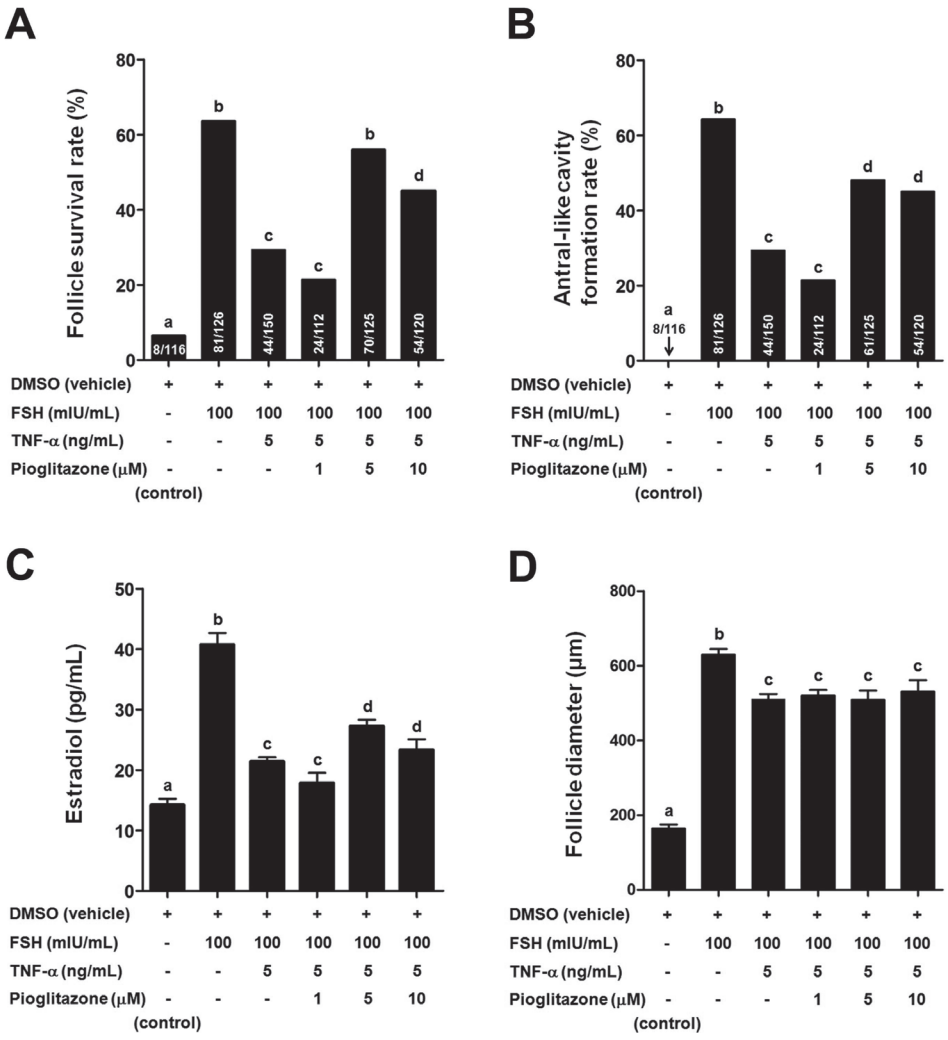

Fig. 5. Restorative effects of pioglitazone on TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis.
Follicles were cultured with 1, 5, or 10 μM of pioglitazone in the presence or absence of 5 ng/ml of TNF-α or 100 mIU/ml of FSH. **A:** Follicle survival rates at 12 days of culture of the various treatments are shown. Numbers inside bars indicate the number of surviving follicles/total tested numbers of follicles. **B:** Antral-like cavity formation rates at 12 days of culture of the various treatments are shown. Numbers inside bars indicate the number of follicles with antral-like cavity/total tested number of follicles. **C:** E2 concentrations in the culture media at 12 days of culture of the various treatments are shown. **D:** Follicle diameters at 12 days of culture of the various treatments are shown. Data are expressed as mean ± SEM. The group treated only with dimethyl sulfoxide (DMSO) was used as a control. Bars with different letters indicate a significant difference (P<0.05). Parts of this figure were originally published in Hara et al., J Ovarian Res. 2013 [46].

lipid metabolism, and glucose homeostasis [40].

It has been reported that PPAR subtypes are expressed in various ovarian cells. In the rat ovary, both PPAR-α and PPAR-δ mRNA expression were observed in thecal and stromal cells, whereas PPAR-γ mRNA was localized to granulosa cells [41, 42]. We examined the expression of PPAR subtypes in mouse preantral follicles [26]. PPAR-α and PPAR-γ mRNA expression were detected by RT-PCR, whereas only PPAR-γ protein expression was observed in preantral follicles. PPAR-γ2 was expressed in mouse adipose tissue [43], whereas PPAR-γ1 was expressed in preantral follicles (Fig. 4). By studying the ovarian-specific disruption of the PPAR-γ model using cre/loxP technology, Cui et al. found that

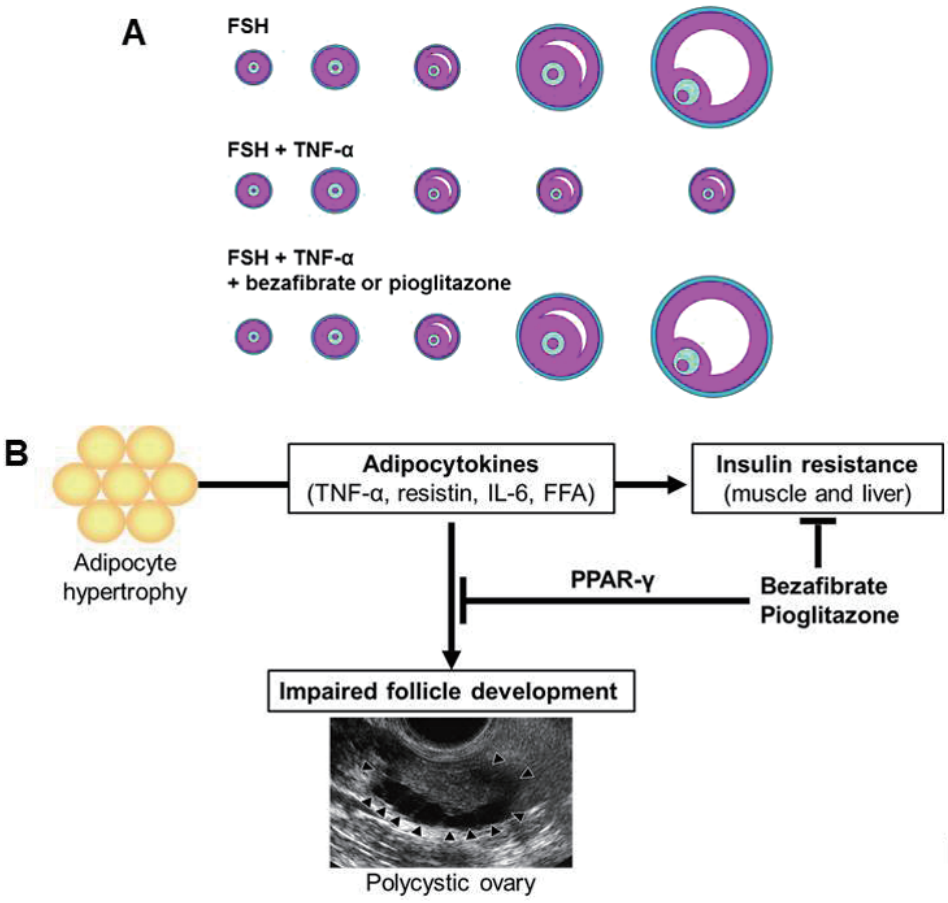

Fig. 6 Scheme of the action of both bezafibrate and pioglitazone in the rescue of TNF-α-impaired follicle development.
A: Summary of the experiments. **B:** Scheme of the action of both bezafibrate and pioglitazone in the rescue of TNF-α-impaired follicle development through the PPAR-γ pathway. IL-6, interleukin 6; FFA, free fatty acid.

PPAR-γ plays an important role in normal ovarian function [44]. Seto-Young et al. reported that PPAR-γ stimulates the expression of steroidogenic acute regulatory protein in the human ovary [45]. These reports suggest that PPAR-γ may play an important role in follicle development and steroidogenesis.

Effects of pioglitazone on TNF-α-mediated Inhibition of FSH-induced Follicle Development and steroidogenesis in the Cultures

We examined whether pioglitazone directly affects follicle development, in a manner similar to bezafibrate, using the *in vitro* follicle culture system supplemented with TNF-α [46]. Pioglitazone also significantly suppressed the TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis (Fig. 5). Although 1 μM of pioglitazone failed to show effects, both 5 and 10 μM of pioglitazone significantly suppressed the TNF-α-mediated inhibition of FSH-induced follicle survival, antral-like cavity formation rate, and E2 production. These results were similar to those observed with bezafibrate.

Conclusion

In the present review, we discussed the evidence that both bezafibrate and pioglitazone suppress TNF-α-mediated inhibition of FSH-induced follicle development and steroidogenesis through the PPAR-γ-stimulating pathway in a preantral follicle culture system. We summarize the scheme in Fig. 6 Our results suggest that insulin-sensitizing drugs, especially PPAR-γ agonists, directly influence follicle development and steroidogenesis in women with PCOS. However, the precise mecha-

nisms by which follicle development and steroidogenesis are restored by these drugs remain unknown. Further investigations are needed to elucidate the mechanisms by which PPAR-γ agonists influence follicle development and steroidogenesis in normal and PCOS ovaries.

Acknowledgement

This study was supported by a Grant-in-aid for General Science Research No. 22591815 to Toshifumi Takahashi, No. 24791681 to Shuichiro Hara, No. 22390308 and 24659723 to Hirohisa Kurachi, and the Global COE Program for Medical Sciences from the Japan Society for the Promotion of Science.

References

1) Asunción, M., Calvo, R. M., San Millan, J. L., Sancho, J., Avila, S. and Escobar-Morreale, H. F. (2000): A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab. 85, 2434–2438.
2) ACOG Committee on Practice Bulletins--Gynecology. (2009): ACOG Practice Bulletin No. 108: Polycystic ovary syndrome. Obstet Gynecol. 114, 936–949.
3) DeUgarte, C. M., Bartolucci, A. A. and Azziz, R. (2005): Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertil Steril. 83, 1454–1460.
4) Adashi, E. Y., Resnick, C. E., D'Ercole, A. J., Svoboda, M. E. and Van Wyk, J. J. (1985): Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr Rev. 6, 400–420.
5) Nestler, J. E., Powers, L. P., Matt, D. W., Steingold, K. A., Plymate, S. R., Rittmaster, R. S., Clore, J. N. and Blackard, W. G. (1991): A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. J Clin Endocrinol Metab. 72, 83–89.
6) Palomba, S., Falbo, A., Zullo, F. and Orio, F., Jr. (2009): Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. Endocr Rev. 30, 1–50.
7) Diamanti-Kandarakis, E. and Dunaif, A. (2012): Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev. 33, 981–1030.
8) Moll, E., Bossuyt, P. M., Korevaar, J. C., Lambalk, C. B. and van der Veen, F. (2006): Effect of clomifene citrate plus metformin and clomifene citrate plus placebo on induction of ovulation in women with newly diagnosed polycystic ovary syndrome: randomised double blind clinical trial. BMJ. 332, 1485.
9) Palomba, S., Orio, F., Jr., Falbo, A., Russo, T., Tolino, A. and Zullo, F. (2007): Clomiphene citrate versus metformin as first-line approach for the treatment of anovulation in infertile patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 92, 3498–3503.
10) Amin, M., Abdel-Kareem, O., Takekida, S., Moriyama, T., Abd el-Aal, G. and Maruo, T. (2003): Minireview: Up-date management of non responder to clomiphene citrate in polycystic ovary syndrome. Kobe J Med Sci. 49, 59–73.
11) Katsiki, N., Georgiadou, E. and Hatzitolios, A. I. (2009): The role of insulin-sensitizing agents in the treatment of polycystic ovary syndrome. Drugs. 69, 1417–1431.
12) Tang, T., Lord, J. M., Norman, R. J., Yasmin, E. and Balen, A. H. (2012): Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. Cochrane Database Syst Rev. 5, CD003053.
13) Carmina, E., Chu, M. C., Longo, R. A., Rini, G. B. and Lobo, R. A. (2005): Phenotypic variation in hyperandrogenic women influences the findings of abnormal metabolic and cardiovascular risk parameters. J Clin Endocrinol Metab. 90, 2545–2549.
14) Apridonidze, T., Essah, P. A., Iuorno, M. J. and Nestler, J. E. (2005): Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 90, 1929–1935.
15) Legro, R. S. (2003): Polycystic ovary syndrome and cardiovascular disease: a premature association? Endocr Rev. 24, 302–312.
16) Rizzo, M., Berneis, K., Carmina, E. and Rini, G. B. (2008): How should we manage atherogenic dyslipidemia in women with polycystic ovary syndrome? Am J Obstet Gynecol. 198, 28 e21-25.
17) Hara, S., Takahashi, T., Amita, M., Igarashi, H. and Kurachi, H. (2010): Usefulness of bezafibrate for ovulation induction in clomiphene citrate-resistant polycystic ovary syndrome patients with dyslipidemia: a prospective pilot study of seven cases. Gynecol Obstet Invest. 70, 166–172.
18) Tenenbaum, A., Fisman, E. Z., Boyko, V., Benderly, M., Tanne, D., Haim, M., Matas, Z., Motro, M. and Behar, S. (2006): Attenuation of progression of insulin resistance in patients with coronary artery disease by bezafibrate. Arch Intern Med. 166, 737–741.
19) Tenenbaum, A., Motro, M., Fisman, E. Z., Schwammenthal, E., Adler, Y., Goldenberg, I., Leor, J., Boyko, V., Mandelzweig, L. and Behar, S. (2004): Peroxisome proliferator-activated receptor ligand bezafibrate for prevention of type 2 diabetes mellitus in patients with coronary artery disease. Circulation. 109, 2197–2202.
20) Taniguchi, A., Fukushima, M., Sakai, M., Tokuyama, K., Nagata, I., Fukunaga, A., Kishimoto, H., Doi, K., Yamashita, Y., Matsuura, T., Kitatani, N., Okumura, T., Nagasaka, S., Nakaishi, S. and Nakai, Y. (2001): Effects of bezafibrate on insulin sensitivity and insulin secretion in non-obese Japanese type 2 diabetic patients. Metabolism. 50, 477–480.
21) Tenenbaum, A., Motro, M., Fisman, E. Z., Adler, Y., Shemesh, J., Tanne, D., Leor, J., Boyko, V., Schwammenthal, E. and Behar, S. (2005): Effect of bezafibrate on incidence of type 2 diabetes mellitus in obese patients. Eur Heart J. 26, 2032–2038.
22) Oktay, K., Briggs, D. and Gosden, R. G. (1997): Ontogeny

of follicle-stimulating hormone receptor gene expression in isolated human ovarian follicles. J Clin Endocrinol Metab. 82, 3748–3751.
23) Wright, C. S., Hovatta, O., Margara, R., Trew, G., Winston, R. M., Franks, S. and Hardy, K. (1999): Effects of follicle-stimulating hormone and serum substitution on the in-vitro growth of human ovarian follicles. Hum Reprod. 14, 1555–1562.
24) Nayudu, P. L. and Osborn, S. M. (1992): Factors influencing the rate of preantral and antral growth of mouse ovarian follicles in vitro. J Reprod Fertil. 95, 349–362.
25) Hartshorne, G. M. (1997): In vitro culture of ovarian follicles. Rev Reprod. 2, 94–104.
26) Hara, S., Takahashi, T., Amita, M., Igarashi, H., Tsutsumi, S. and Kurachi, H. (2011): Bezafibrate restores the inhibition of FSH-induced follicular development and steroidogenesis by tumor necrosis factor-alpha through peroxisome proliferator-activated receptor-gamma pathway in an in vitro mouse preantral follicle culture. Biol Reprod. 85, 895–906.
27) Lenie, S. and Smitz, J. (2009): Functional AR signaling is evident in an in vitro mouse follicle culture bioassay that encompasses most stages of folliculogenesis. Biol Reprod. 80, 685–695.
28) Heilbronn, L. K. and Campbell, L. V. (2008): Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. Curr Pharm Des. 14, 1225–1230.
29) Chen, X., Jia, X., Qiao, J., Guan, Y. and Kang, J. (2013): Adipokines in reproductive function: a link between obesity and polycystic ovary syndrome. J Mol Endocrinol. 50, R21-37.
30) Hotamisligil, G. S. (1999): The role of TNFalpha and TNF receptors in obesity and insulin resistance. J Intern Med. 245, 621–625.
31) Roden, M., Price, T. B., Perseghin, G., Petersen, K. F., Rothman, D. L., Cline, G. W. and Shulman, G. I. (1996): Mechanism of free fatty acid-induced insulin resistance in humans. J Clin Invest. 97, 2859–2865.
32) Brown, J. E., Onyango, D. J. and Dunmore, S. J. (2007): Resistin down-regulates insulin receptor expression, and modulates cell viability in rodent pancreatic beta-cells. FEBS Lett. 581, 3273–3276.
33) Carmina, E., Orio, F., Palomba, S., Cascella, T., Longo, R. A., Colao, A. M., Lombardi, G. and Lobo, R. A. (2005): Evidence for altered adipocyte function in polycystic ovary syndrome. Eur J Endocrinol. 152, 389–394.
34) González, F. (2012): Inflammation in Polycystic Ovary Syndrome: underpinning of insulin resistance and ovarian dysfunction. Steroids. 77, 300–305.
35) Cleveland, J. L. and Ihle, J. N. (1995): Contenders in FasL/TNF death signaling. Cell. 81, 479–482.
36) Hussein, M. R. (2005): Apoptosis in the ovary: molecular mechanisms. Hum Reprod Update. 11, 162–178.
37) Morrison, L. J. and Marcinkiewicz, J. L. (2002): Tumor necrosis factor alpha enhances oocyte/follicle apoptosis in the neonatal rat ovary. Biol Reprod. 66, 450–457.
38) Basini, G., Mainardi, G. L., Bussolati, S. and Tamanini, C. (2002): Steroidogenesis, proliferation and apoptosis in bovine granulosa cells: role of tumour necrosis factor-alpha and its possible signalling mechanisms. Reprod Fertil Dev. 14, 141–150.
39) Montgomery Rice, V., Limback, S. D., Roby, K. F. and Terranova, P. F. (1999): Tumor necrosis factor alpha inhibition of follicle-stimulating hormone-induced granulosa cell estradiol secretion in the human does not involve reduction of cAMP secretion but inhibition at post-cAMP site(s). Endocrine. 10, 19–23.
40) Ahmadian, M., Suh, J. M., Hah, N., Liddle, C., Atkins, A. R., Downes, M. and Evans, R. M. (2013): PPARgamma signaling and metabolism: the good, the bad and the future. Nat Med. 19, 557–566.
41) Komar, C. M., Braissant, O., Wahli, W. and Curry, T. E., Jr. (2001): Expression and localization of PPARs in the rat ovary during follicular development and the periovulatory period. Endocrinology. 142, 4831–4838.
42) Puttabyatappa, M., Vandevoort, C. A. and Chaffin, C. L. (2010): hCG-induced down-regulation of PPARgamma and liver X receptors promotes periovulatory progesterone synthesis by macaque granulosa cells. Endocrinology. 151, 5865–5872.
43) Kim, J., Sato, M., Li, Q., Lydon, J. P., Demayo, F. J., Bagchi, I. C. and Bagchi, M. K. (2008): Peroxisome proliferator-activated receptor gamma is a target of progesterone regulation in the preovulatory follicles and controls ovulation in mice. Mol Cell Biol. 28, 1770–1782.
44) Cui, Y., Miyoshi, K., Claudio, E., Siebenlist, U. K., Gonzalez, F. J., Flaws, J., Wagner, K. U. and Hennighausen, L. (2002): Loss of the peroxisome proliferation-activated receptor gamma (PPARgamma) does not affect mammary development and propensity for tumor formation but leads to reduced fertility. J Biol Chem. 277, 17830–17835.
45) Seto-Young, D., Avtanski, D., Strizhevsky, M., Parikh, G., Patel, P., Kaplun, J., Holcomb, K., Rosenwaks, Z. and Poretsky, L. (2007): Interactions among peroxisome proliferator activated receptor-gamma, insulin signaling pathways, and steroidogenic acute regulatory protein in human ovarian cells. J Clin Endocrinol Metab. 92, 2232–2239.
46) Hara, S., Takahashi, T., Amita, M., Matsuo, K., Igarashi, H. and Kurachi, H. (2013): Pioglitazone counteracts the tumor necrosis factor-alpha inhibition of follicle-stimulating hormone-induced follicular development and estradiol production in an in vitro mouse preantral follicle culture system. J Ovarian Res. 6, 69.