**FOLIA HISTOCHEMICA ET CYTOBIOLOGICA**
Vol. 46, No. 4, 2008
pp. 423-428

# Effects of transferrin on aromatase activity in porcine granulosa cells *in vitro*

**Małgorzata Durlej, Małgorzata Duda, Katarzyna Knapczyk, Maria Słomczyńska**

Department of Endocrinology and Tissue Culture, Institute of Zoology, Jagiellonian University, Krakow, Poland

**Abstract:** Proliferating cells have an absolute requirement for iron, which is delivered by transferrin with subsequent intracellular transport via the transferrin receptor. Recent studies have reported that transferrin plays a crucial role in the local regulation of ovarian function, apart from its iron-binding characteristic. Therefore, the present study was undertaken to explore the possible role of transferrin in porcine granulosa cells function by examining its influence on aromatase activity, the most important indicator of follicular cell differentiation. In the first series of studies, pig granulosa cells isolated from small, immature follicles were cultured in the presence of transferrin alone (10 µg/ml or 100 µg/ml) or with the addition of FSH (100ng/ml). The second series of studies was undertaken to determine transferrin-stimulated granulosa cells ability to aromatize exogenous testosterone ($1\times10^{-7}$M). One hour after the establishment of cultures an aromatase inhibitor CGS16949A was added to test its influence on estradiol production. After 48 hours, cultures were terminated and cells were processed for immunocytochemical staining of aromatase. Media were frozen for further estradiol level analysis. Positive immunostaining for aromatase was found in all granulosa cell cultures. The intensity of immunostaining was always stronger in cultures supplemented with FSH whereas the addition of transferrin had no effect. Granulosa cells *in vitro* synthesized the highest amount of estradiol after the addition of FSH and exogenous testosterone as measured radioimmunologically. Concomitant treatment with FSH and transferrin caused an inhibition of FSH-stimulated aromatase activity. The production of estradiol also declined in the presence of FSH, testosterone and transferrin. This study demonstrates that transferrin had a dose-dependent inhibitory effect on FSH-stimulated aromatase activity, which was confirmed by radioimmunoassay. Our results indicate that transferrin may be an important factor in the regulation of granulosa cell diferentiation.

**Key words:** granulosa cells, transferrin, FSH, aromatase, pig

## Introduction

The development of ovarian follicles requires endocrine regulation by the pituitary gonadotropins such as luteinizing hormone (LH) and follicle stimulating hormone (FSH) as the main factors. The complex regulatory network also includes some steroids, growth factors and peptides present in the follicular fluid and acting via autocrine and paracrine pathways [1,2]. The essence of the follicular growth is the proliferation and differentiation of the granulosa cells [3], which mainly synthesize estrogens due to the expression of cytochrome P450 aromatase [4] and perform a crucial role in controlling oocyte maturation [5]. Aromatase activity is primarily under the control of FSH [3], which binds to specific receptors on granulosa cells [1] and induces estrogen biosynthesis by triggering cAMP-dependent signaling cascades to regulate transcription of the *CYP19* gene [6]. Interestingly, porcine ovarian follicles differ from other animals because their theca interna cells have some aromatising ability [5]. Cytochrome P450 aromatase is a microsomal, highly conserved enzyme encoded in humans by a single-copy gene (*CYP19*) [3,4,7]. Unlike to other mammals, the aromatase produced by the porcine tissues exists as three distinct isoforms encoded by three distinct genes [8,9].

It is known that proliferating granulosa cells have an absolute requirement for iron, which is delivered by transferrin [10]. Transferrin is a metal-binding glycoprotein with molecular weight of 76-81 kDa, responsible for iron supply to various vertebrate cells [11]. A single polypeptide chain of approximately 680 amino acid residues is folded into two homologous globular domains by intragenic duplication, each domain hav-

**Correspondence**: M. Duda, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Krakow, Poland; tel.: (+4812) 66324 66, fax.: (+4812) 6340785, e-mail: duda.m@op.pl


Folia Histochem Cytobiol. 2008:46(4): 423 (423-428)
doi: 10.2478/v10042-008-0070-z

ing one high-affinity binding site for iron [12]. Intake of iron is mediated by the transmembrane transferrin receptor [13]. Until recently, only one type of human transferrin receptor 1 had been identified. However, in 1999, a second human transferrin receptor 2 was cloned [14]. Both transferrin receptors assist iron transport into cells and undergo multiple rounds of clathrin-mediated endocytosis [13,15]. The principal place of transferrin production is liver, but some extrahepatical sites are also known, including Sertoli cells and granulosa cells [12].

Although both transferrin and transferrin receptor are present throughout human and mice follicle maturation, their exact role in the differentiation of the granulosa cells has not been properly investigated [12]. Moreover, transferrin exists in the follicular fluid at a relatively high level suggesting an important role in the local regulation of ovarian function, apart from its iron-binding characteristic [2,10,12].

Therefore, the aim of the present study was to indicate the potential relevance of transferrin in the porcine ovary by examining its influence on aromatase activity, the most important indicator of follicular cells differentiation.

## Materials and methods

**Animals.** Porcine ovaries from Polish landrace sows were obtained at a local slaughterhouse and placed in a cold phosphate- buffered saline (PBS; Laboratory of Sera and Vaccines, Lublin, Poland), pH 7.4, containing penicillin (100 IU/ml; POLFA Tarchomin SA, Tarchomin, Poland). Ovaries were transported to the laboratory and rinsed once with PBS supplemented with antibiotics.

**Cell culture conditions.** Follicles were classified according to their size as small, immature (2-4 mm in diameter) and 10-20 follicles in this stage were used for each experiment. Cells from freshly excised follicles from 3-5 animals were pooled. Granulosa cells were collected by syringe aspiration, washed with PBS and centrifuged twice at room temperature using low-speed centrifugation (90×g for 10 min). The cells were seeded in multiwell culture dishes (Nunc, Kalmstrup, Denmark) at an initial density of $3\times10^5$ cells/ml and cultured in M199 medium supplemented with 5% calf serum and penicillin (100 IU/ml), in a humidified atmosphere of 95% air: 5% $CO_2$ at 37°C. In the first series of studies granulosa cells were cultured with the addition of transferrin (Sigma – Aldrich, USA) at a concentration of 10 µg/ml or 100 µg/ml; FSH (100 ng/ml, a gift from NIH, Bethesda, MD, USA); transferrin (10 µg/ml) plus FSH (100 ng/ml) and transferrin (100 µg/ml) plus FSH (100 ng/ml). In the second series of studies $1x10^{-7}$ M testosterone were added to all types of cultures as a substrate for estradiol production. One hour after the establishment of cultures $1x10^{-3}$M aromatase inhibitor CGS16949A (Ciba – Geigy GA, Basel) was added for the next 47 hours. All culture media were collected after 48 hours and stored at -20°C for further estradiol level analysis. Cell viability was tested by the trypan blue exclusion test and was > 90%. All the experiments described above were performed in duplicate in three separate cultures. For immunocytochemistry, each well was equipped with a round coverslip.

**Steroid hormone measurement.** Estrogens were assessed according to Hotchkiss *et al.* [16] using 2,4,6,7,-$^3$H-estradiol (New England Nuclear), spec. act. 140 Ci/mmol, as a tracer and an antibody raised in a rabbit against estradiol-17β-6-oxime-BSA (a generous gift from Professor Brian Cook, University Glasgow, Scotland). The specificity of the antiserum for estrogen was high. It cross-reacted with estradiol (100%), with estrone (66%) and with estriol (only 2.1%). Series of other steroids were tested for cross-reactivity but they showed less than 0.1%. The lower limit of sensitivity of the assay was of the order of 5 pg/tube. Coefficients of variation within and between assay were 3.5% and 7.5%, respectively.

**Immunocytochemistry.** The cells were fixed in 2% paraformaldehyde, permeabilized with 0.1% TritonX-100 in Tris-buffered saline (TBS; 0.05 M Tris-HCl plus 0.15 M NaCl, pH=7.6). To quench endogenous peroxidase activity cells were treated with 0.1% $H_2O_2$ for 30 min. Non specific binding was blocked by an incubation with 20% normal goat serum for further 30 min. Cells were then incubated overnight at 4°C with a primary rabbit polyclonal antibody against human placental P450 aromatase (dilution 1:100; a generous gift from Dr Yoshio Osawa; Hauptman-Woodward, Medical Resesrch Institute, Buffalo, NY, USA). Afterwards, the cells were intensively washed in TBST (TBS plus 0.1% Tween 20) and incubated for 1.5 h at room temperature (RT) with biotinylated goat anti-rabbit antibody (1:400; Vector Lab., Burlingame CA, USA) followed by washing with TBST and incubated at RT for 1 h with avidin-biotin-peroxidase complex (1:1000; Strept ABC complex/HRP, DAKO/AS, Glostrup, Denmark). The color reaction was performed using Stable DAB solution (Research Genetics, Inc., Huntsville AL, USA) for 4 min. For negative control the primary antibody was omitted.

**Statistical analysis.** All data were expressed as mean ± SEM of measurements derived from at least three different experiments. Differences between steroid concentrations in control and treated cultures were assayed by Duncan test. $p<0.05$ were considered statistically significant.

## Results

Granulosa cells isolated from small, immature porcine follicles produced only minor amounts of estradiol (7.39±0.53 pg/$10^5$ cells). Addition of FSH (100 ng/ml) resulted in an almost 5-fold increase in estradiol secretion (34.67±4.72 pg/$10^5$ cells), when compared to basal granulosa cells production, whereas transferrin at doses 10 µg/ml and 100 µg/ml had no effect. Concomitant treatment with FSH and transferrin caused an inhibition of FSH-stimulated aromatase activity, although surprisingly lower dose of transferrin was found to be more inhibitory (20.0±2.90 pg/$10^5$ cells) than the higher one (31.0±2.40 pg/$10^5$ cells). Testosterone added as a substrate increased significantly the amount of estradiol secreted by granulosa cells. However, only cell cultures supplemented with FSH and testosterone together, released substantial amount of estradiol (102±1.76 pg/$10^5$ cells). On the other hand, simultaneous addition of FSH, testosterone and transferrin to the culture medium caused a decrease in estradiol production. Lower ability to aromatize exogenous testosterone was observed (52.78±2.22 pg/$10^5$ cells) after treatment with transferrin the at a dose of 100 µg/ml. The second series of studies was undertaken to determine the influence of an aromatase inhibitor


Folia Histochem Cytobiol. 2008:46(4): 424 (423-428)
doi: 10.2478/v10042-008-0070-z

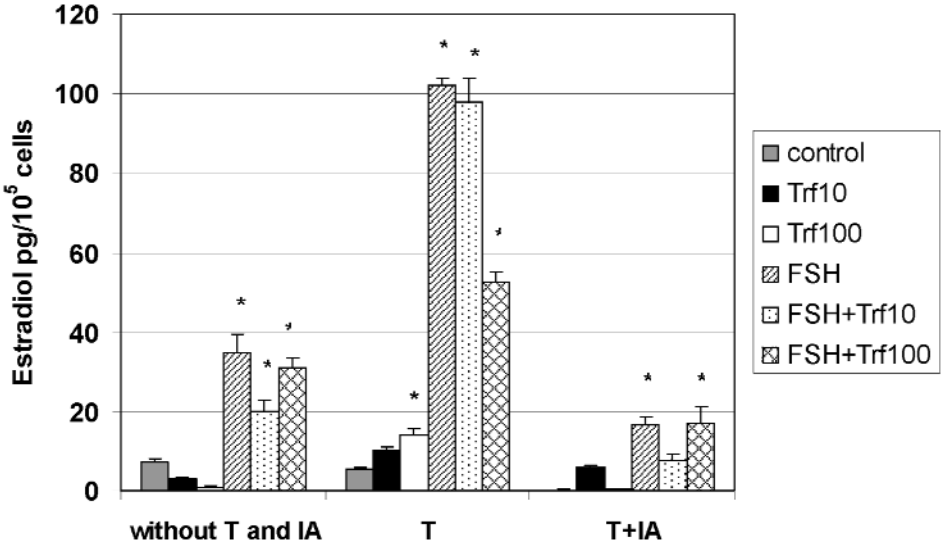


**Fig. 1.** Estradiol secretion (pg/$10^5$ cells) by granulosa cells isolated from small, immature porcine follicles during 48 hours cultures. The first series represents cell cultures without addition of testosterone and aromatase inhibitor CGS16949A, the second series was stimulated by testosterone ($10^{-7}$M), the third series was under the effect of testosterone and aromatase inhibitor. Each error bar represents the mean ± SEM from 3 independent experiments. The differences between estradiol concentration in control and treated cultures were assayed by Duncan test. Asterisks indicate statistically significant differences (* $p<0.05$).

CGS16949A on estradiol secretion by granulosa cells. The addition of that inhibitor to the culture medium confirmed its inhibitory effect on estradiol production. Subsidiarily, transferrin in both doses showed the synergistic impact, resulting in a minimal synthesis of estradiol by porcine granulosa cell *in vitro* (Fig. 1).

Immunostaining of cytochrome P450 aromatase was detected in all granulosa cell cultures isolated from small, immature porcine follicles. Immunoreaction was localized in the cytoplasm, whereas the nuclei were negative. The intensity of immunostaining for aromatase varied among the cells and was considered very weak, weak, moderate or strong based on the visual examination of cytoplasmic localization of the antigen (Table 1).

In FSH-stimulated granulosa cells cultured without either, testosterone or aromatase inhibitor, a moderate immunoreaction was observed. The addition of transferrin to the culture medium caused a decline in the intensity of immunoreaction. More precisely, the intensity of immunostaining was negatively correlated with the transferrin dose used in the experiment. The strongest positive immunostaining of aromatase was observed in granulosa cell cultures supplemented with both testosterone and FSH. The addition of testosterone and an aromatase inhibitor resulted in a moderate immunostaining for aromatase in FSH-stimulated granulosa cell cultures what was not the case in cultures grown without FSH (Fig. 2).

In general, transferrin clearly diminished the intensity of immunostaining for aromatase, what was also manifested by a decrease in estradiol secretion.

**Table 1.** Evaluation of the immunocytochemical reaction's intensity.

| | Without T and IA | T | T+IA |
|---|---|---|---|
| Control | +/+++ | +/- | +/- |
| Trf10 | +/- | +/- | +/+++ |
| Trf100 | +/- | +/+++ | +/+++ |
| FSH | ++/+++ | +++ | ++/+++ |
| FSH+Trf10 | +/+++ | +/+++ | +/+++ |
| FSH+Trf100 | +/- | +/+++ | +/- |

Aromatase immunoreactivity was designated as very weak (+/-), weak (+/+++), moderate (++/+++) and strong (+++); Trf10 – transferrin 10 μg/ml; Trf100 – 100 μg/ml; T – testosterone; IA – aromatase inhibitor.

## Discussion

The presence of transferrin and its receptor has been shown previously in rat [17], human and mouse ovary [12]. More specifically, transferrin mRNA was detected in granulosa cells of follicles, but not in the oocytes. The aim of our study was to explore the potential relevance of transferrin in the maturation of follicles, apart from its role in iron transport. However, there is little data supporting this hypothesis and our study is the first one describing the effects of transferrin on porcine granulosa cells.

The immunocytochemical and molecular findings suggest that granulosa cells can produce *de novo* their own transferrin, which is translocated across the oocyte membrane by endocytosis [12]. However, the contribution of this local synthesis to the overall transferrin concentration in the follicular fluid is unknown.

In the present study transferrin had no effect on the basal, but inhibited FSH-stimulated aromatase activity. Thus, our results indicating the requisite presence of FSH to reveal transferrin inhibiting abilities are in accordance with an earlier work by Li *et al.* [2], who


Folia Histochem Cytobiol. 2008:46(4): 425 (423-428)
doi: 10.2478/v10042-008-0070-z

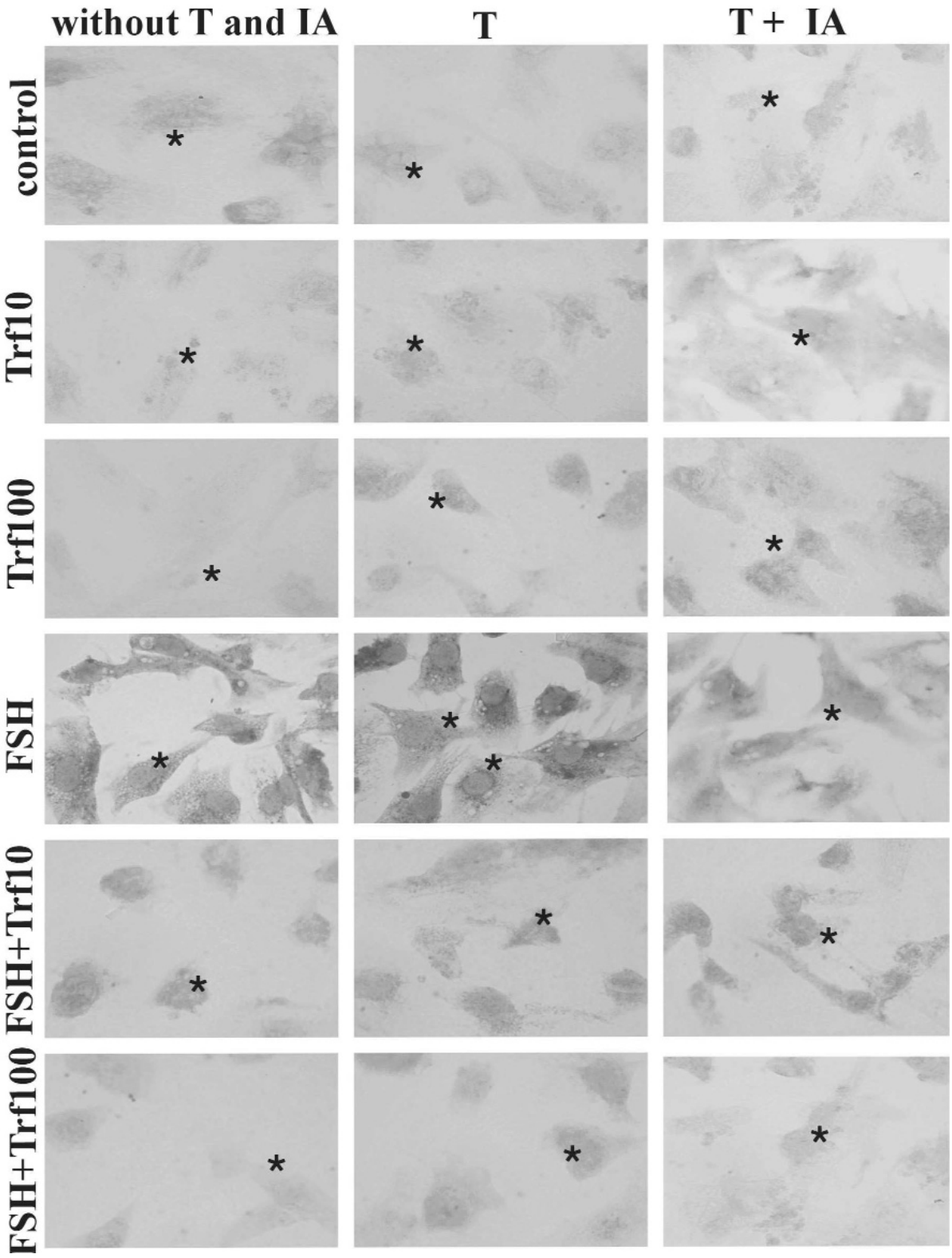


**Fig. 2.** Immunocytochemical localization of aromatase in porcine ganulosa cells *in vitro*, isolated from small, immature follicles. The first series shows cell cultures without addition of testosterone and aromatase inhibitor CGS16949A, the second series was stimulated by testosterone ($10^{-7}$ M), the third series was under the effect of testosterone and aromatase inhibitor. Granulosa cells were incubated in culture medium as a control and respectively with Trf10, Trf100, FSH (100 ng/ml), FSH + Trf10 and FSH + Trf100. Asterisks indicate positive staining (magnification × 100 – all photos). IA – aromatase inhibitor CGS16494A ($10^{-3}$ M); T – testosterone; Trf10 – transferrin 10 µg/ml; Trf100 – transferrin 100 µg/ml.


Folia Histochem Cytobiol. 2008:46(4): 426 (423-428)
doi: 10.2478/v10042-008-0070-z

focused on rat granulosa cells. They additionally suggested that a higher dose of transferrin is more suppressing. On the contrary, our work showed much more inhibitory action of transferrin at a dose of 10 μg/ml. These differences are presumably connected with the amount of transferrin receptors present on porcine granulosa cells and their affinity to transferrin [10]. There is no data concerning the affinity of the transferrin receptors in the pig ovary, however it is worthy of further examination.

The cellular mechanism associated with the inhibitory action of transferrin on aromatase activity remains unclear [2,10]. The fact that transferrin suppresses prostaglandin E2 (second messenger), 8-bromo-cAMP- and forskolin-induced aromatase activity leads to the assumption that it may act at sites distal to cAMP generation [2]. According to Yu *et al.* [18,19] transferrin partially blocks the binding of FSH with its receptors and reduces the formation of intracellular cAMP, and therefore inhibits the expression of FSH receptors. Thus, diminished secretion of estradiol is observed. To the contrary, Yu and Findlay [10] examining the influence of transferrin on inhibin production, suggested cAMP-independent pathways of its action. Moreover, the earlier results ruled out the possibility that the inhibitory effect of transferrin was a consequence of its toxicity, reducing granulosa cell number [2,10]. Just the opposite, transferrin in the culture medium may improve cell vitability [2] and is considered an autocrine growth factor and mitogen-like factor [20]. Consequently, an alternative mechanism of transferrin action might be related to the inverse relationship between differentiation and proliferation of granulosa cells [10]. Granulosa cells proliferation stimulating factors usually inhibit its differentiation, as measured by estradiol synthesis.

The explanation mentioned above is consistent with our data provided by the radioimmunoassay procedures. It was observed that the addition of transferrin to the culture medium decreased the amount of estradiol secreted by FSH-stimulated porcine granulosa cells. The production of estradiol also declined in the presence of FSH, testosterone and transferrin. These results were positively correlated with the intensity of aromatase immunostaining.

Proliferation and differentiation of granulosa cells are the essence of follicular development. Previous research confirmed an increase in iron and transferrin concentration in the follicular fluid with the degree of follicular maturity [2,12]. The average level of transferrin in the follicular fluid of healthy follicles is about 2000 μg/ml [2], which is much higher than we applied in the present work (10 and 100 μg/ml) and that used by Li *et al.* [2] (3-300 μg/ml). Interestingly, this high concentration of transferrin implies its important role in ovarian functions. Based on our findings, there is some critical concentration of transferrin and above it might act anti-apoptotic. The lower dose of transferrin demonstrated stronger suppression of aromatase activity and resulted in the reduction of estradiol synthesis and attenuated the response of granulosa cells to FSH. The raised level of FSH is an absolute requirement for the recruitment and selection of ovarian follicles, otherwise they undergo degeneration [21]. In order to substantiate the role of transferrin in folliculogenesis, we suggest that the capacity concentration of transferrin in the follicular fluid increases with advancing follicular development and weaker inhibitory action at a dose of 100 μg/ml, indicates its anti-apoptotic function, counteracting atresia.

Aromatase was localized in the cytoplasm of cultured granulosa cells *in vitro* obtained from small, immature porcine follicles. The immunoreaction was positive in each culture however there were significant differences in the intensity of staining. We were able to show that the intensity of immunostaining reached its maximum in the FSH-stimulated granulosa cells and was comparatively weak in nonstimulated cultures. These findings are in agreement with our previous study [22]. We demonstrated that the expression of P450 aromatase mRNA is dependent on follicular development stage, being at a very low level in the small follicles and increased in the medium and large ones, respectively. Also further findings [23] clearly showed a weak aromatase immunostaining in the small porcine follicles and strong intensity of immunoreaction in the granulosa cells of the preovulatory follicles, either mural or antral layers. Our present study provides immunocytochemical and radioimmunological evidence that incubation of granulosa cells with FSH and testostosterone induced aromatase activity that resulted in the most intensive staining for aromatase and consecutively higher secretion of estradiol. Consequently, synergistic action of androgens and FSH enhanced the increase of aromatase expression and conversion of androgens to estrogens. Thus, androgens are not only substrates for P450 aromatase, but also act at the level of the FSH receptor [24].

Additionally, our work showed that granulosa cells cultured in the presence of an aromatase inhibitor secreted less estradiol and demonstrated a weak intensity of immunoreaction for aromatase. Subsequent addition of transferrin intensified this effect, leading to the attenuation of FSH-stimulated aromatase activity.

In summary, our study demonstrates that transferrin had a dose-dependent inhibitory effect on FSH-stimulated aromatase activity, which was confirmed by both radioimmunoassay and immunocytochemistry. Granulosa cells *in vitro* synthesized the major amount of estradiol after the addition of FSH and testosterone. Our data support the idea that transferrin might be a significant regulator of ovarian function in the pig. The


Folia Histochem Cytobiol. 2008:46(4): 427 (423-428)
doi: 10.2478/v10042-008-0070-z

anti-apoptotic action of transferrin in relatively high concentration is likely to protect granulosa cells from atresia.

**Acknowledgements:** The authors wish to thank Professor Barbara Bilińska for her valuable comments. Research was supported by DS/IZ/BiNoZ/770/2008.

## References

[ 1] Bonnet A, Frappart PO, Dehais P, Tosser – Klopp G, Hatey F. Identification of differential gene expresion in *in vitro* FSH treated pig granulosa cells using suppression substractive hybridization. *Reprod Biol Endocrinol*. 2006;4:1-11.
[ 2] Li YD, Zhang ZW, Li WX. Transferrin inhibits aromatase activity of rat granulosa cells *in vitro*. *J Endocrinol.* 1991; 131:245-250.
[ 3] Simpson ER, Clone C, Rubin G *et al.* Aromatase – a brief overview. *Annu Rev Physiol.* 2002;64:93-127.
[ 4] Kamat A, Hinshelwood MM, Murry BA, Mendelson CR. Mechanism in tissue – specific regulation of estrogens biosynthesis in humans. *Trends Endocrinol Matab.* 2002;13: 122-127.
[ 5] Conley AJ, Howard HJ, Slanger WD, Ford JJ. Steroidogenesis in the preovulatory porcine follicle. *Biol Reprod.* 1994;51: 655-661.
[ 6] Parakh TN, Hernandez JA, Grammer JC *et al.* Follicle – stimulating hormone/cAMP regulation of aromatase gene expression requires β-catenin. *Proc Natl Acad Sci USA*. 2006;103: 12435-12440.
[ 7] Conley A, Hinshelwood M. Mammalian aromatase. *Reproduction.* 2001;121:685-695.
[ 8] Conley A, Corbin J, Smith T, Hinshelwood M, Liu Z, Simpson E. Porcine aromatases: studies on tissue – specific, functionally distinct isozymes from a single gene? *J Steriod Biochem Molec Biol.* 1997;61:407-413.
[ 9] Graddy LG, Kowalski AA, Simmen FA, Davis SLF, Baumgartner WW, Simmen RCM. Multiple isoforms of porcie aromatase are encoded by three distinct genes. *J Steriod Biochem Molec Biol.* 2000;73:49-57.
[10] Yu JH, Findlay JK. An inhibitory effect of transferrin on differentiation of rat granulosa cells *in vitro*. *Endocrinology.* 1991;128:1841-1848.
[11] McGraw TE, Greenfield L, Maxfield FR. Functional expression of the human transferrin receptor cDNA in Chanise Hamster Ovary cells deficient in endogenous transferrin receptor. *J Cell Biol.* 1987;5:207-214.
[12] Briggs DA, Sharp DJ, Miller D, Gosden RG. Transferrin in the developing ovarian follicle: evidence for de-novo expression by granulosa cells. *Mol Hum Reprod.* 1999;5:1107-1114.
[13] Ponka P, Lok ChN. The transferrin receptor: role in health and disease. *Int J Biochem Cell Biol.* 1999;31:1111-1137.
[14] Trinder D, Baker E. Transferrin receptor 2: a new molecule in iron metabolism. *Int J Biochem Cell Biol.* 2003;35:292-296.
[15] Lawrence CM, Ray S, Babyonyshev M, Galluser R, Borhani DW, Harrison SC. Crystal structure of the ectodomain of human transferrin receptor. *Science.* 1999;286:779-782.
[16] Hotchkiss J, Atkison LE, Knobil E. Time course of serum estrogen and luteinizing hormone (LH) concentration during the menstrual cycle of the rhesus monkey. *Endocrinology.* 1971;89:177-183.
[17] Aleshire SL, Osteen KG, Maxon WS, Entman SS, Bradley CA, Parl FF. Localization of transferrin and its receptor in ovarian follicular cells: morphologic studies in relation to follicular development. *Fertil Steril.* 1989;51:444-449.
[18] Yu JH, Guo J, Guo J. Inhibitory effect of transferrin on the binding and maintanance of FSH receptors in rat granulosa cells. *Sheng Li Xue Bao.*1992;44:496-501.
[19] Yu JH, Guo J, Guo J, Zeng FX, Tang GH. The inhibitory effect and its mechanism of transferrin on FSH-induced differentiation of granulosa cells. *Sheng Li Xue Bao*.1994;46: 209-216.
[20] Vanderstichele H, Delaey B, de Winter J *et al.* Secretion of steroids, growth factors and cytokines by immortalized mouse granulosa cells lines. *Biol Reprod.* 1994;50:1190-1202.
[21] Knox RV. Recrutiment and selection oh ovarian follicle for determination of ovulation rate in pig. *Domest Anim Endocrinol.* 2005;29:385-397.
[22] Słomczyńska M, Duda M, Slęzak K. The expression of androgen receptor, cytochrome P450 aromatase and FSH receptor mRNA in the porcie ovary. *Folia Histochem Cytobiol.* 2001; 39:9-13.
[23] Słomczyńska M, Tabarowski Z. Localization of androgen receptor and cytochrome P450 aromatase in the follicle and corpus luteum of the porcine ovary. *Anim Reprod Sci.* 2001; 65:127-134.
[24] Słomczyńska M, Szołtys M, Duda M, Sikora K, Tabarowski Z. Androgens and FSH affect androgen receptor and aromatase distribution in the porcine ovary. *Folia biol (Krakow).* 2003;51:63-67.

*Submitted: 15 February, 2008*
*Accepted after reviews: 16 April, 2008*