Expression of Prostaglandin Metabolising Enzymes COX-2 and 15-PGDH and VDR in Human Granulosa Cells

MARC THILL[1], STEFFI BECKER[1], DOROTHEA FISCHER[1], TIM CORDES[1], AMADEUS HORNEMANN[1], KLAUS DIEDRICH[1], DARIUS SALEHIN[2] and MICHAEL FRIEDRICH[2]

[1]*Department of Obstetrics and Gynecology, University Schleswig-Holstein, Campus Luebeck, 23538 Luebeck;*
[2]*Department of Gynecology and Obstetrics, Helios Hospital Krefeld, 47805 Krefeld, Germany*

Abstract. *Background: Prostaglandins (PGs) within the periovulatory follicle are essential for various female reproductive functions such as follicular development and maturation. In animal models, granulosa cells express the PG synthesizing enzyme cyclooxygenase-2 (COX-2) and the PG inactivating enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH). First references suggest a correlation between vitamin D and prostaglandin metabolism through the impact of 1,25(OH)$_2$D$_3$ (calcitriol) on the expression of COX-2 and 15-PGDH. Materials and Methods: The expression of COX-2, 15-PGDH and the vitamin D receptor (VDR) in human granulosa cells (COV434, hGC and HGL5), which were originally isolated from different stages of follicular maturation, was determined by real-time PCR (RT-PCR) and Western blot analysis. Results: A positive correlation of COX-2 and VDR protein was found in the COV434 and HGL5 cells and an inverse correlation of 15-PGDH and VDR protein levels in all the investigated cell types. Conclusion: There may be a link between VDR, associated target genes and prostaglandin metabolism in human follicular maturation and luteolysis.*

Prostaglandins (PGs) are members of a large group of hormonally active fatty acids synthesised from arachidonic acid by the action of cyclooxygenases (COX), prostaglandin G (PGG)/prostaglandin H (PGH) synthases. COX is the rate-limiting enzyme in the synthesis of PGs and exists in two isoforms, COX-1 and COX-2. These two isoforms are encoded by two separate genes and show distinct cell type-specific expression. The expression of COX-1 is generally constitutive, whereas COX-2 is induced by inflammatory stimuli, including cytokines and growth factors (1). PGs are produced in many tissues with a manifold impact in the human organism. They are produced, among others, by the ovarian follicle and are essential for ovulation to occur. PGs produced within the periovulatory follicle are involved in various female reproductive functions of such essential processes as steroidogenesis (2-4), tissue remodelling (5, 6), and neovascularization of the luteinizing follicle (7) as well as being novel mediators of follicular development and maturation, including cell proliferation, hormone synthesis, maturation of the oocyte and ovulation (2, 8-10). They are essential for follicle rupture and oocyte release in primates and rats (11, 12).

Gonadotropins are able to induce granulosa cell expression of phospholipase A_2 and PGE synthase, which leads to the synthesis of PGs (13, 14). The induction of COX-2 by the ovulatory gonadotropin surge might be the rate-limiting step in follicular PGE_2 production and ovulation (15, 16). Duffy *et al.* (8) found that COX-2 expression by granulosa cells of primate periovulatory follicles was also induced by the ovulatory gonadotropin surge.

Gene targeting studies in mice have revealed the impact of PGs in female reproduction and the distinct functions of the isozymes COX-1 and -2. COX-1-deficient female mice are mostly fertile and have specific parturition defects, whereas COX-2-deficient mice are mostly infertile (17). The infertility is caused by the lack of the key prostaglandin, E_2 (PGE_2) with consequent, severely impaired ovulation and fertilization. As the decidualisation of the endometrium is disturbed, so is implantation (17-19). The effects of PGE_2 are usually mediated through the G protein-coupled cell-surface receptors EP_{1-4} (20).

The key enzyme that regulates the prostaglandin levels by converting them to the corresponding 15-keto derivatives is 15 hydroxyprostaglandin dehydrogenase (15-PGDH), which is responsible for the biological inactivation of PGs (21).

Correspondence to: Marc Thill, MD, Department of Obstetrics and Gynecology, University Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany. Tel: +49 04515002134, Fax: +49 04515002139, e-mail: marc.thill@uk-sh.de

Key Words: Granulosa cells, cyclooxygenase-2, 15-hydroxyprostaglandin dehydrogenase, vitamin D receptor, prostaglandin metabolism.

Duffy *et al*. (22) detected 15-PGDH in granulosa, but not in thecal cells of periovulatory follicles of adult monkeys. Thus the follicular granulosa cells are the site of PGE_2 inactivation in the ovary and for the various grades of cell differentiation (2, 23-25).

The molecular mechanisms by which COX-2 and 15-PGDH regulate human ovarian function is have not been fully elucidated. Periovulatory follicles of primates (8, 26) and rats (27) express COX-2 both in the follicle-surrounding theca and in the follicle-lining mural granulosa cell layer. However, the expression of 15-PGDH within monkey periovulatory follicles is restricted to the granulosa cells (22).

Currently, published studies suggest that intrafollicular increased PGE_2 levels due to an increased COX-2 expression of the mural granulosa cell layer and the cumulus cells promote the expansion of the cumulus cells. The cumulus cells build the cumulus oophorum and ensure the nutritional supply of the oocyte. Cumulus cells may be associated with the maturation of the oocyte, as direct effects of prostaglandins are mediated by EP-receptors that oocytes do not have (28).

$1,25(OH)_2D_3$ (calcitriol), the biologically active form of vitamin D, mediates antiproliferative and differentiating effects *via* the nuclear vitamin D receptor (VDR). It has an important but still unclear role in reproduction. Vitamin D-deficient female rats have reduced fertility and a reduction in the numbers of fetuses delivered per litter compared with normal vitamin D-replete rats (29, 30). Calcitriol is also critical for the maintenance of normal reproduction in male rats. Most of the effects of calcitriol are mediated by the VDR (31).

First studies have shown a correlation between prostaglandin and vitamin D metabolism (32, 33). Moreover Moreno *et al*. (32) have shown a synergistic action of $1,25(OH)_2D_3$, and COX-2 selective as well as non-selective non-steroidal anti-inflammatory drugs (NSAIDs) in the reduction of prostate cancer cell growth.

The available data have predominantly been collected in animal models, thus the results allow only suggestions of their function in human female reproduction. The aim of this study was to determine the expression of COX-2, 15-PGDH and VDR in human granulosa cells which were originally isolated from different stages of follicular maturation.

Materials and Methods

Cell culture. Non-luteinized COV434 cells (a gift from Dr. Sonntag, University of Muenster) were grown in DMEM (Invitrogen, Karlsruhe, Germany) supplemented with 4,500 mg/l glucose, 1% penicillin/streptomycin and 10% fetal bovine serum at 5 % CO_2, 37˚C.

Human granulosa cells (hGC) were obtained from women undergoing IVF (*in vitro* fertilization)/ICSI (intracytoplasmatic sperm injection) treatment and were separated from aspirated follicular fluid by sedimentation. After washing steps with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin, the cells were suspended in PBS and layered over Ficoll-paque™ Plus (Amersham Biosciences, Freiburg, Germany) density gradient separation solution and centrifuged at 800 × g for 20 min at room temperature. The interphase layer was removed and washed twice with PBS with 0.1% bovine serum albumin. To remove resting erythrocytes, the cells were incubated with hemolyse buffer (155 mmol/L NH_4Cl, 10 mmol/L $KHCO_3$, 1 mmol/L EDTA, pH 7.2) for 5 min at room temperature. After an additional washing step, the cells were maintained in growth media (RPMI-1640; Invitrogen) supplemented with 10% Ultroser G (PALL BIOSEPRA, Cergy-Saint-Christophe, France), 1% penicillin/streptomycin and 10% fetal bovine serum at 37˚C, 5% CO_2. The cell viability and cell counts were assessed by trypan blue exclusion. For the stimulation and inhibition experiments, the granulosa cells of 3-4 patients were pooled and only the second cell passage was used.

Immortalized human granulosa cells, HGL5 cells (a gift from Dr. Sonntag, University of Muenster) were grown in the same media as hGC under standard conditions.

RNA isolation. The total RNA from the COV434, hGC and HGL5 cells was isolated with RNeasy (Qiagen, Hilden, Germany) according to manufacturer's instructions. The integrity of the isolated RNA was verified by 1% agarose gel electrophoresis and the total RNA was spectrophotometrically quantified at OD260/280.

Reverse transcription and real-time PCR. Prior to utilization, the RNA was DNase I (Invitrogen, Karlsruhe, Germany) treated. The synthesis of cDNA from 1 μg total RNA of granulosa cells was performed using Super Script-II reverse transcriptase (Invitrogen) and oligo-$d(T)_{15}$ primer (Invitrogen). Reverse transcription reaction mixtures were diluted 1:10 and quantitative real-time PCR (RT-PCR) was performed using Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen), primers for human *COX-2, 15-PGDH* and *VDR* (Qiagen) and 2 μl of RT reaction mixture as a template. A melting curve was generated after 50 cycles for the final PCR product of all the investigated genes by reducing the temperature to 65˚C for 15 s followed by a slow increase in temperature to 95˚C. The fluorescence was measured at 0.2˚C increments during the slow heating process. The obtained threshold cycles (Ct) were normalized to *TBP* (TATA-binding protein) and *PBDG* (porphobilinogen deaminase) as housekeeping genes and MCF-7 cDNA as a calibrator. The fold change was determined with the formula: efficiency target gene^(calibrator gene to investigated gene–sample gene to investigated gene)/efficiency housekeeping gene^(calibrator housekeeping gene–sample housekeeping) (34). The experiments were performed in triplicate for each gene and were repeated twice.

Western blotting. The harvested cells were lysed in sample buffer (125 mM Tris, 30% glycerine, 8% SDS, pH 6.8) and 40 μg of isolated proteins were subjected to 10% SDS PAGE under reducing conditions and the nitrocellulose membranes (Schleicher Schuell, Dassel, Germany) were blocked after transfer in 5% non-fat dry milk in PBST for 1 h at room temperature. The membranes were incubated with the primary antibodies for human COX-2 and 15-PGDH (IBL International GmbH, Hamburg, Germany) and VDR (Dianova, Hamburg, Germany), at a dilution of 1:1,000 in blocking reagent overnight at 4˚C with gentle shaking. The secondary antibodies conjugated to horseradish peroxidase (New England Biolabs, Frankfurt Main, Germany) were used at a dilution of 1:2,000 (COX-2 and 15-PGDH) and 1:5,000 (VDR) and the membranes were incubated for 1

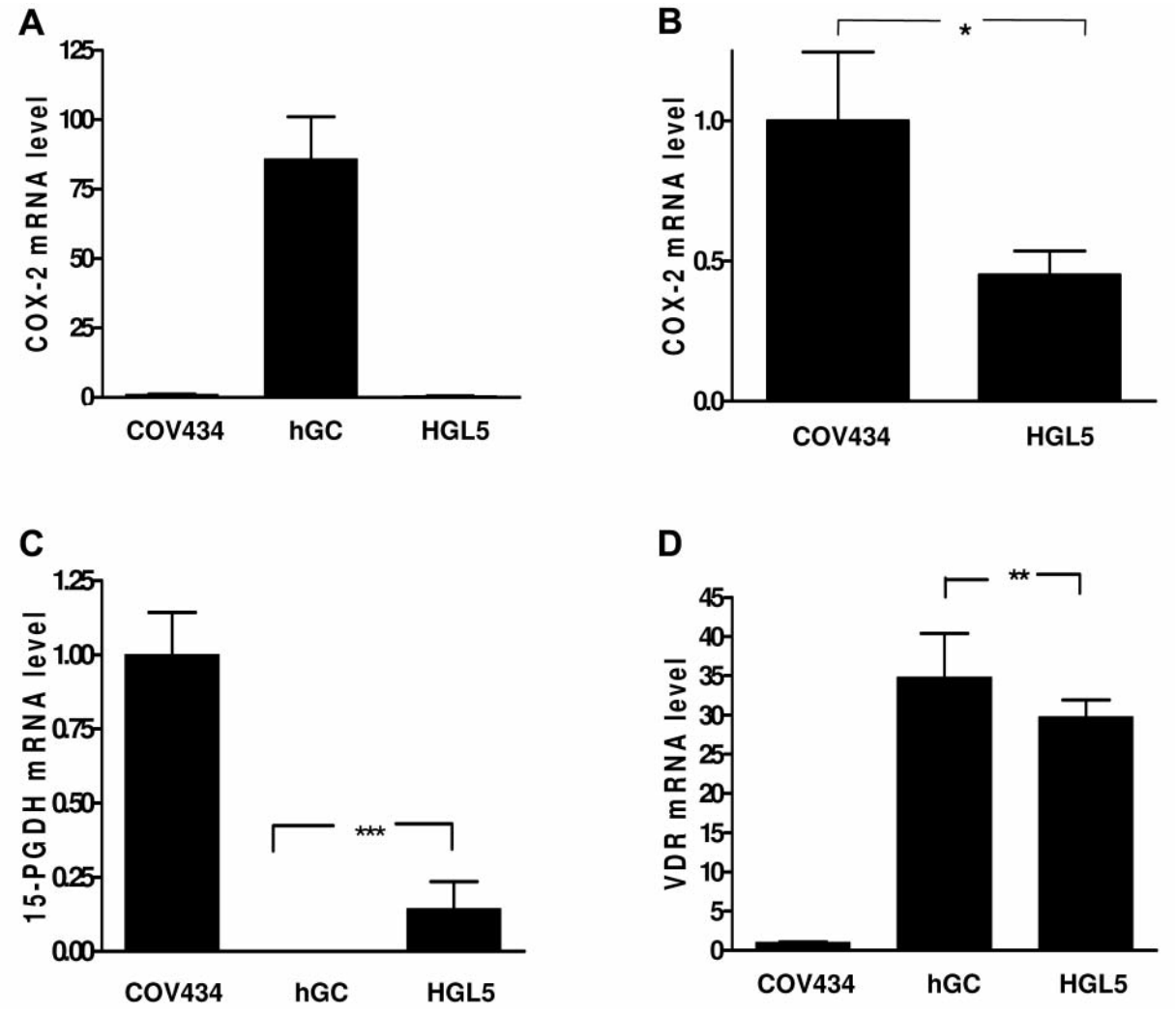

Figure 1. *Real-time PCR (RT-PCR) analysis of COX-2 (A and B), 15-PGDH (C) and VDR (D) mRNA expression in human granulosa cells (N=8). *p<0.05, **p<0.01, ***p<0.001.*

h at room temperature. The obtained signals were visualized using an enhanced chemiluminescence (ECL) detection system (Amersham Biosciences) and compared to β-actin as loading control.

Statistical significance. The statistical analysis of the RT-PCR and Western blots results was performed using Student's *t*-test.

Results

Immortalised granulosa cell line isolated from preovulatory follicles. In the context of IVF treatment, it is possible to obtain granulosa cells from the follicular fluid. These hGC cells from FSH and LH stimulated female patients in the early luteal phase can be cultivated. HGL5 is an established luteinised granulosa cell line that was extracted from postovulatory follicles in the middle luteal phase.

As it is difficult to isolate hGC from pre- and postovulatory follicles, these cell models offer the opportunity to examine COX-2 and 15-PGDH expression in the different stages of follicular maturation.

COX-2 expression in COV434, hGC and HGL5 cells. As shown by RT-PCR, the *COX-2* mRNA expression in the hGC cells was 82% higher as compared to that in the luteinized COV434 and HGL5 cells (Figure 1 A), and the expression in the HGL5 cells was 55% ($p<0.05$) lower than that in the COV434 cells (Figure 1 B). The expression of COX-2 protein levels was contrary to the results of the RT-PCR. As compared to the COV434 cells, the COX-2 protein expression of the HGL5 cells was double (Figure 2 A; 3 A, C) while in the hGC cells it was only weakly detected (Figure 2 A; 3 B).

15-PGDH expression in COV434, hGC and HGL5 cells. By RT-PCR, the detection of *15-PGDH* mRNA expression of the hGC cells was very low as compared to the non-luteinized COV434 cells, and in the HGL5 cells it was about 80% lower ($p<0.01$) as compared to the COV434 cells (Figure 1 C). A correlation was found between the mRNA and the Western blot analysis of protein levels in the COV434 and HLG5 cells. The 15-PGDH protein expression of the HGL5 cells was 50% of that in the COV434 cells. However, there was no correlation between the *15-PGDH* mRNA and protein expression in the hGC cells (Figures 1 C and 2 B). In these cells, the 15-PGDH protein level was 25% lower compared to that of the COV434 cells (Figure 2 B). Furthermore, heterogeneity of the bands in Western blot analysis was conspicuous (Figure 3 A, B, C).

VDR expression in COV434, hGC and HGL5 cells. The *VDR* mRNA expression was only slightly detectable in the COV434 cells, while the HGL5 cells showed a significantly lower *VDR* mRNA expression ($p<0.01$) as compared to the hGC cells (Figure 1 D). In the Western blot analysis, the VDR protein level in the hGC cells was significant higher ($p<0.05$) as compared to that of the COV434 cells and it was higher still in the HGL5 cells ($p<0.01$) as compared to the COV434 cells (Figure 2 C; 3 A, B, C). An inverse correlation between the 15-PGDH and VDR protein levels was found in all the investigated cell types.

Discussion

In the present study, the COX-2 protein expression was contrary to the results of RT-PCR. A strong expression of *COX-2* mRNA and only weakly detected COX-2 protein expression was found in the hCG cells. This was in line with the results of Narko *et al*. (35) who also found inconsistent data for COX-2 expression. The reason might be posttranscriptional and posttranslational modifications that are described in detail in the literature (36). These modifications are related to the mRNA stability, as 12-18 AUUUA sequences are located in the *COX-2* gene which mediate quick and selective mRNA degradation (36). These sequences have an effect on the level and, if applicable, on the stability of the COX-2 proteins and might explain the different results for mRNA and protein expression. COX-2 protein levels in the HGL5 cells were twofold higher compared to that in the COV434 cells. The enhanced COX-2 levels were associated with lower 15-PGDH protein expression. The present results suggested that 15-PGDH is variably expressed in the granulosa cells in the follicular and the luteal phases in the female cycle.

Moreover, a positive correlation of COX-2 and VDR protein was observed in the COV434 and HGL5 cells and an inverse correlation of 15-PGDH and VDR protein levels in

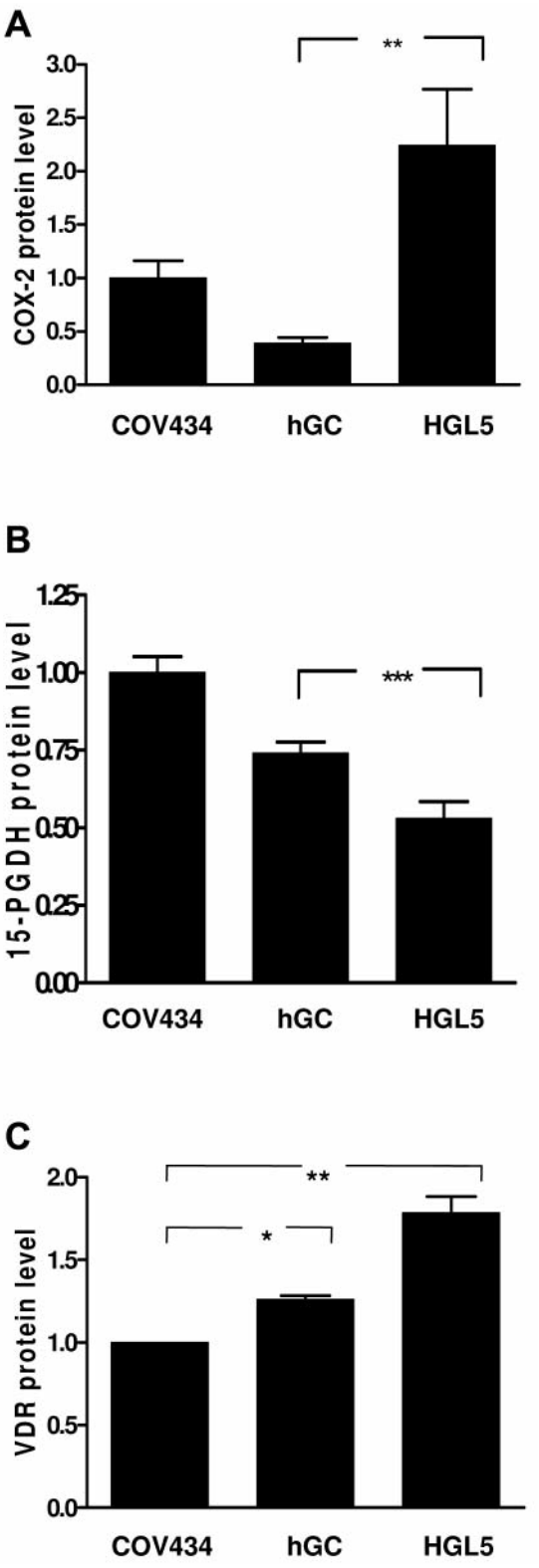

Figure 2. *Western-blot analysis of COX-2 (A), 15-PGDH (B) and VDR (C) protein levels in human granulosa cells (N=8). The obtained signals were densitometrically evaluated and normalised to COV434 cells. *p<0.05, **p<0.01, ***p<0.001.*

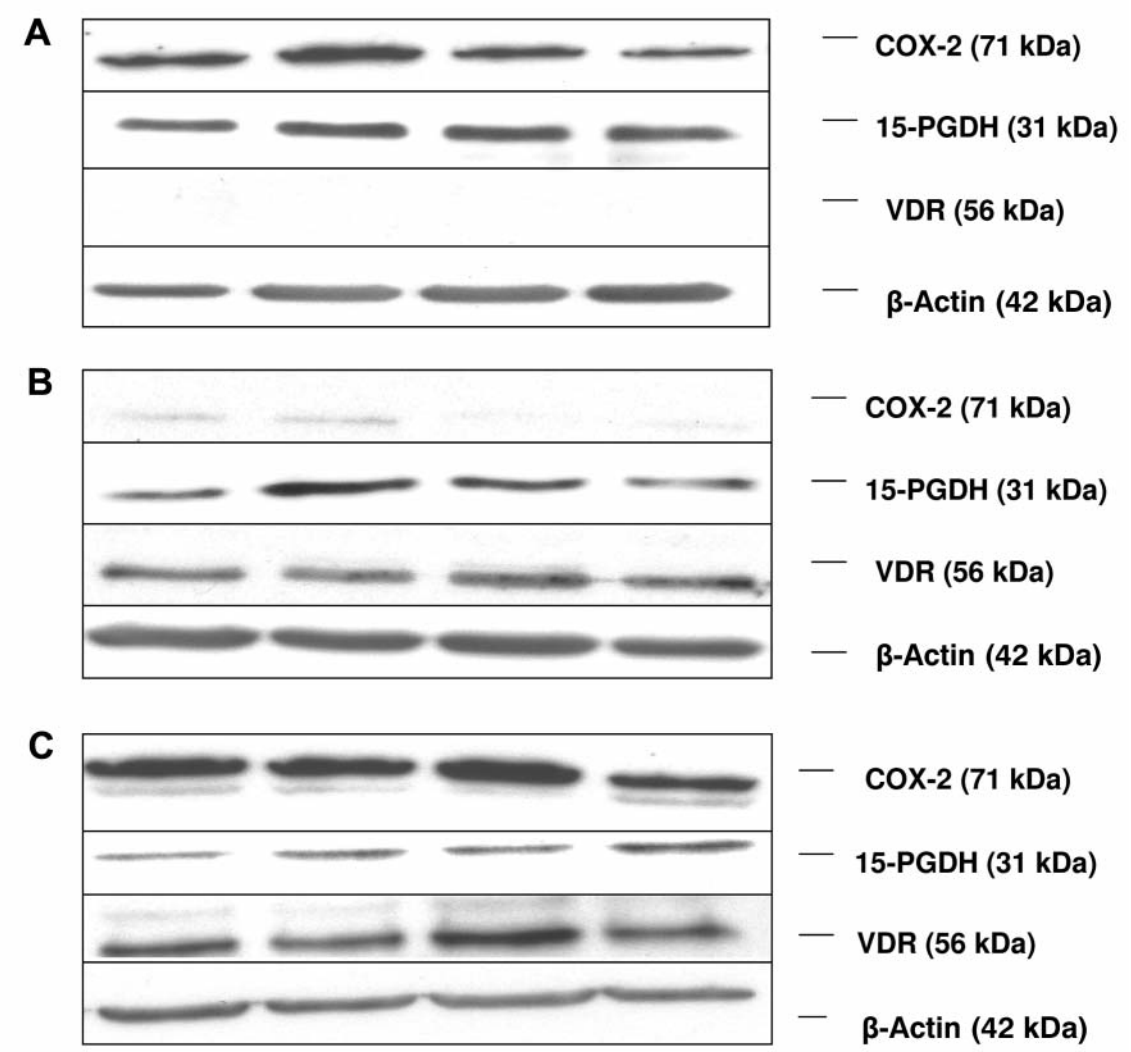

Figure 3. *Detection of COX-2, 15-PGDH and VDR proteins in human granulosa cells (Western blot) (N=8). Protein lysates of COV434 (A), hGC (B) and HGL5 cells (C).*

all the investigated cell types. The differences of the hormone receptor status (*e.g.* LH, FSH) of the COV434, hGC and HGL5 cells seemed to be associated with a differential expression of COX-2, 15-PGDH and VDR in the distinct hormonally regulated phases of the female cycle.

The corpus luteum is a temporary endocrine structure and its major task is the synthesis of progesterone. This hormone is essential for establishing and successfully maintaining pregnancy. Examinations in patients with corpus luteum insufficiency support the importance of progesterone for a successful development of the embryo as these patients have a higher incidence of abortions (37, 38). Studies with rabbits showed that prostaglandin metabolizing enzymes are even expressed from corpora lutea (39, 40). Although the function of prostaglandins in the corpus luteum is ambiguous some studies showed that PGE_2 was able to stimulate preovulatory intrafollicular progesterone synthesis which is important for the induction of ovulation (37, 38). It is presumed that prostaglandins take part in luteolysis but no evidence is yet available. The possibility to block these effects with non-selective or COX-2 selective inhibitors might be suggested.

Gene targeting experiments have shown that EP1- and EP3-deficient female mice have normal fertility, suggesting their nonessential roles in fertilisation, ovulation and implantation (41, 42). In contrast, EP2-deficient female mice have similar defects to those in COX-deficient mice (43), and EP4 deficiency in embryos results mostly in perinatal lethality (44). Thus, the EP2 and EP4 receptors are essential for successful ovulation and fertilisation in mice. Similar results have also been found in studies with adult monkeys.

In these studies the non-selective COX-1/2 inhibitor indomethacin was injected into the follicular fluid of the pre-ovulatory follicle and successfully prevented ovulation (11). Restoring ovulatory function by co-injection of indomethacin and PGE_2 supported the hypothesis that the synthesis of PGE_2 is essential for ovulation in primates (11).

Systemic administration of non-selective (45) and selective COX-2 inhibitors (46) in rats caused delayed ovulation and failure of the follicle to rupture (45). Besides these animal studies, Pall *et al*. (47) gave the selective COX-2 inhibitor rofecoxib (n=6) or placebo (n=7) to women in a randomized double-blind fashion. Four of the six women who received rofecoxib demonstrated delayed follicle rupture. These results suggested that selective COX-2 inhibition had a negative, local effect on human ovulation resulting in delayed follicular rupture, without affecting peripheral hormonal cyclicity. Therefore it could be possible that the effects of prostaglandins in human follicular maturation and luteolysis might be inhibited by NSAIDs. Hopefully, further research will shed light on this.

Ligand binding assays in which homogenates of tissues are tested for their ability to bind radiolabelled calcitriol have demonstrated the presence of the VDR in ovarian homogenates (48). Johnson *et al*. (45) studied male and female rat tissues with a specific and sensitive polyclonal antibody directed against VDR and found widespread distribution of VDR in both male and female rat reproductive tissue by using immunohistochemical techniques. VDR was found in both nuclei and cytoplasm of granulosa cells of ovarian follicles, weaker staining was present in thecal cells of follicles and intense VDR staining was seen in corpora lutea. Calcitriol has also been found in human follicular fluid (49) and may act with the VDR in follicle granulosa cells to regulate some of their functions. Ahonen *et al*. (50) studied the immunohistochemical distribution of the VDR in rat ovaries and human ovarian cancer. The VDR was observed in rat ovaries, mainly in granulosa and thecal cells (50). In cases of human ovarian cancer, VDR was found in 43% (50). The authors concluded that the growth of ovarian tissue might be regulated by calcitriol. In the present study, the VDR protein level in the hGC cells was the highest as compared to the COV434 and the HGL5 cells. Granulosa cells have an aromatase activity that converts androstendione to estradiol (51). The synthesis of estradiol is necessary for normal folliculogenesis and, thus, ovulation (45). Calcitriol treatment was found to increase the rate of conversion of androstendione in estrone by increasing aromatase activity in human skin fibroblasts (52). Calcitriol, through VDR, may exert similar effects in ovarian follicle granulosa cells. Kinuta *et al*. (53) observed in VDR null mutant female mice uterine hypoplasia and impaired folliculogenesis. The aromatase activities in these mice were low in the ovary (24%). The supplementation of estradiol normalised histological abnormality and of calcium increased aromatase activity and partially corrected the hypogonadism. Moreover the aromatase activity increased in the ovary. The authors concluded that calcitriol plays a role in estrogen biosynthesis, however, a direct regulation of the aromatase gene was also considered (53).

In conclusion, an inverse correlation between 15-PGDH and VDR protein levels in all the investigated cell lines suggests a possible link between the VDR, associated target genes and prostaglandin metabolism during human follicular maturation and luteolysis. Thus, the hormonally regulated expression of the VDR and prostaglandin metabolizing enzymes COX-2 and 15-PGDH might be involved in the regulation of the different cellular responses of human granulosa cells, which are important for female reproduction.

Acknowledgements

We thank Birte Münchow for her technical assistance.

Conflict of Interest Statement

The authors declare no conflict of interest relevant to this article.

References

1 Smith W, Garavito M and DeWitt D: Prostaglandin endoperoxide H synthase (cyclooxygenase)-1 and -2. J Biol Chem *271*: 33157-33160, 1996.

2 Duffy DM and Stouffer RL: Luteinizing hormone acts directly at granulosa cells to stimulate periovulatory processes: modulation of luteinizing hormone effects by prostaglandins. Endocrine *22(3)*: 249-256, 2003.

3 Polan ML, Seu D and Tarlatzis B: Human chorionic gonadotropin stimulation of estradiol production and androgen antagonism of gonadotropin-stimulated responses in cultured human granulosa-luteal cells. J Clin Endocrinol Metab 62: 628-635, 1996.

4 Rodriguez-Rigau LJ, Steinberger E, Atkins BJ and Lucci JA: Effect of testosterone on human corpus luteum steroidogenesis *in vitro*. Fertil Steril *31*: 448-450, 1979.

5 Curry TE Jr, Clark MR, Dean DD, Woessner JF Jr and Lemaire WJ: The preovulatory increase in ovarian collagenase activity in the rat is independent of prostaglandin production. Endocrinology *118*: 1823-1828, 1986.

6 Mann JS, Kindy MS, Edwards DR and Curry TE Jr: Hormonal regulation of matrix metalloproteinase inhibitors in rat granulosa cells and ovaries. Endocrinology *128*: 1825-1832, 1991.

7 Murdoch WJ, Hansen TR and McPherson LA: A review-role of eicosanoids in vertebrate ovulation. Prostaglandins *46*: 85-115, 1993.

8 Duffy DM and Stouffer RL: The ovulatory gonadotrophin surge stimulates cyclooxygenase expression and prostaglandin production by the monkey follicle. Mol Hum Reprod *7(8)*: 731-739, 2001.

9 Takahashi T, Morrow JD, Wang H and Dey SK: Cyclooxygenase-2-derived prostaglandin E(2) directs oocyte maturation by differentially influencing multiple signaling pathways. J Biol Chem *281(48)*: 37117-37129, 2006.

10 Tokuyama O, Nakamura Y, Musoh A, Honda K, Ozaki K and Ishiko O: Expression and distribution of cyclooxygenase-2 in human ovary during follicular development. Osaka City Med J *49(1)*: 39-47, 2003.
11 Duffy DM and Stouffer RL: Follicular administration of a cyclooxygenase inhibitor can prevent oocyte release without alteration of normal luteal function in rhesus monkeys. Hum Reprod *17*: 2825-2831, 2002.
12 Sogn JH, Curry TE Jr, Brannstrom M, Lemaire WJ, Koos RD, Papkoff H and Janson PO: Inhibition of follicle-stimulating hormone-induced ovulation by indomethacin in the perfused rat ovary. Biol Reprod *36*: 536-542, 1987.
13 Ben-Shlomo I, Kol S, Ando M, Altman KR, Putowski LT, Rohan RM and Adashi EY: Ovarian expression, cellular localization, and hormonal regulation of rat secretory phospholipase A2: increased expression by interleukin-1 and by gonadotropins. Biol Reprod *57*: 217-225, 1997.
14 Kurusu S, Iwao M, Kawaminami M and Hashimoto I: Involvement of cytosolic phospholipase A2 in the ovulatory process in gonadotropin-primed immature rats. Prostaglandins Leukot Essent Fatty Acids *58*: 405-411, 1998.
15 Sirois J and Dore M: The late induction of prostaglandin G/H synthase-2 in equine preovulatory follicles supports its role as a determinant of the ovulatory process. Endocrinology *138*: 4427-4434, 1997.
16 Dozier BL, Watanabe K and Duffy DM: Two pathways for prostaglandin F2 alpha synthesis by the primate periovulatory follicle. Reproduction *136(1)*: 53-63, 2008.
17 Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM and Dey SK: Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell *91(2)*: 197-208, 1997.
18 Dinchuk JE, Car BD, Focht RJ, Johnston JJ, Jaffee BD, Covington MB, Contel NR, Eng VM, Collins RJ, Czerniak PM, Gorry SA and Trzaskos JM: Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature *378*: 406-409, 1995.
19 Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, Kim HS and Smithies O: Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell *83*: 438-492, 1995.
20 Negishi M, Sugimoto Y and Ichikawa A: Molecular mechanisms of diverse actions of prostanoid receptors. Biochim Biophys Acta *1259(1)*: 109-119, 1995.
21 Anggard E: The biological activities of three metabolites of prostaglandin E1. Acta Physiol Scand *66*: 509-10, 1966.
22 Duffy DM, Dozier BL and Seachord CL: Prostaglandin dehydrogenase and prostaglandin levels in periovulatory follicles: implications for control of primate ovulation by prostaglandin E_2. J Clin Endocrinol Metab *90(2)*: 1021-1027, 2005.
23 Patwardhan VV and Lanthier A: Prostaglandins PGE and PGF in human ovarian follicles: endogenous contents and *in vitro* formation by theca and granulosa cells. Acta Endocrinol (Copenh) *97*: 543-550, 1981.
24 Satoh K, Mitsuhashi N, Kawai Y, Kinoshita K and Sakamoto S: Bioconversion of arachidonic acid to prostaglandins by Graafian follicles and stroma from the human ovary. Prostaglandins Med *7*: 515-525, 1981.
25 Davis JS: Mechanisms of hormone action: luteinizing hormone receptors and second-messenger pathways. Curr Opin Obstet Gynecol *6*: 254-261, 1994.
26 Duffy DM, Dozier BL and Seachord CL: Prostaglandin dehydrogenase and prostaglandin levels in periovulatory follicles: implications for control of primate ovulation by prostaglandin E_2. J Clin Endocrinol Metab *90(2)*: 1021-1027, 2005.
27 Wong WYL and Richards JS: Evidence for two antigenically distinct molecular weight variants of prostaglandin H synthase in the rat ovary. Mol Endocrinol *5*: 1269-1279, 1991
28 Richards JS: Ovulation: new factors that prepare the oocyte for fertilization. Mol. Cell. Endocrinol *234*: 75-79, 2005.
29 Halloran BP and Deruca HF: Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. J Nutr *110*: 1573-1580, 1980.
30 Kwiecinski GG, Petrie GI and DeLuca HF: 1,25-Dihydroxyvitamin D_3 restores fertility of vitamin D-deficient female rats. Am J Physiol *256*: E483-E487, 1989.
31 Johnson JA, Grande JP, Roche PC and Kumar R: Immunohistochemical detection and distribution of the 1,25-dihydroxyvitamin D_3 receptor in rat reproductive tissues. Histochem Cell Biol *105(1)*: 7-15, 1996.
32 Moreno J, Krishnan AV, Swami S, Nonn L, Peehl DM and Feldman D: Regulation of prostaglandin metabolism by calcitriol attenuates growth stimulation in prostate cancer cells. Cancer Res *65(17)*: 7917-7925, 2005.
33 Swami S, Krishnan AV, Moreno J, Bhattacharyya RB, Peehl DM and Feldman D: Calcitriol and enistein actions to inhibit the prostaglandin pathway: potential combination therapy to treat prostate cancer. J Nutr *137*: 205S-210S, 2007.
34 Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res *29(9)*: e45, 2001.
35 Narko K, Ritvos O and Ristimäki A: Induction of cyclooxygenase-2 and prostaglandin F2α receptor expression by interleukin-1β in cultured human granulosa-luteal cells. Endocrinology *138(9)*: 3638-3644, 1997.
36 Oyesanya RA, Lee ZP, Wu J, Chen J, Song Y, Mukherjee A, Dent P, Kordula T, Zhou H and Fang X: Transcriptional and post-transcriptional mechanisms for lysophosphatidic acid-induced cyclooxygenase-2 expression in ovarian cancer cells. FASEB J *22(8)*: 2639-2651, 2008.
37 Chaffin CL, Hess DL and Stouffer RL: Dynamics of periovulatory steroidogenesis in the rhesus monkey follicle after controlled ovarian stimulation. Hum Reprod *14*: 642-649, 1999.
38 Greenland KJ, Jantke I, Jenatschke S, Bracken KE, Vinson C and Gellersen B: The human NAD-dependent 15-hydroxyprostaglandin dehydrogenase gene promoter is controlled by Ets and activating protein-1 transcription factors and progesterone. Endocrinology *141*: 581-597, 2000.
39 Okita JR, Robertson SJ and Okita RT: Changes in ovarian NAD-dependent 15-hydroxyprostaglandin dehydrogenase activity in pregnant and pseudopregnant rabbits. Prostaglandins Leukot Essent Fatty Acids *46*: 93-98, 1992.
40 Boiti C, Maranesi M, Dall'aglio C, Pascucci L, Brecchia G, Gobbetti A and Zerani M: Vasoactive peptides in the luteolytic process activated by PGF2α in pseudopregnant rabbits at different luteal stages. Biol Reprod *77(1)*: 156-164, 2007.

41 Ushikubi F, Segi E, Sugimoto Y, Murata T, Matsuoka T, Kobayashi T, Hizaki H, Tuboi K, Katsuyama M, Ichikawa A, Tanaka T, Yoshida N and Narumiya S: Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. Nature *395*: 281-284, 1998.

42 Markosyan N, Dozier BL, Lattanzio FA and Duffy DM. Primate granulosa cell response *via* prostaglandin E_2 receptors increases late in the periovulatory interval. Biol Reprod *75(6)*: 868-876, 2006.

43 Matsumoto H, Ma W, Smalley W, Trzaskos J, Breyer RM and Dey SK: Diversification of cyclooxygenase-2-derived prostaglandins in ovulation and implantation. Biol Reprod *64(5)*: 1557-1565, 2001.

44 Nguyen M, Camenisch T, Snouwaert JN, Hicks E, Coffman TM, Anderson PA, Malouf NN and Koller BH: The prostaglandin receptor EP_4 triggers remodeling of the cardiovascular system at birth. Nature *390*: 78-81, 1997.

45 Hedin L, Gaddy-Kurten G, Kurten R, Dewitt W and Richards J: Prostaglandin endoperoxide synthase in rat ovarian follicles: content, cellular distribution, and evidence for hormonal induction preceding ovulation. Endocrinology *121*: 722-731, 1987.

46 Gaytán M, Bellido C, Morales C, Sánchez-Criado JE and Gaytán F: Effects of selective inhibition of cyclooxygenase and lipooxygenase pathways in follicle rupture and ovulation in the rat. Reproduction *132(4)*: 571-577, 2006.

47 Pall M, Fridén BE and Brännström M: Induction of delayed follicular rupture in the human by the selective COX-2 inhibitor rofecoxib: a randomized double-blind study. Hum Reprod *16(7)*: 1323-1328, 2001.

48 Dokoh S, Donaldson CA, Marion SL, Pike JW and Haussler MR: The ovary: a target organ for 1,25-dihydroxyvitamin D_3. Endocrinology *112*: 200-206, 1983.

49 Potashnik G, Lunenfeld E, Levitas E, Itskovitz J, Albutiano S, Yankowitz N, Sonin Y, Levy J, Glezerman M and Shany S: The relationship between endogenous oestradiol and vitamin D_3 metabolites in serum and follicular fluid during ovarian stimulation for *in vitro* fertilization and embryo transfer. Hum Reprod *7*: 1357-1360, 1992.

50 Ahonen MH, Zhuang YH, Aine R, Ylikomi T and Tuohimaa P: Androgen receptor and vitamin D receptor in human ovarian cancer: growth stimulation and inhibition by ligands. Int J Cancer *86(1)*: 40-46, 2000.

51 Erickson GF, Magoffin DA, Cragun JR, and Chang RJ: The effects of insulin and insulin-like growth factors I and II on estradiol production by granulosa cells of polycystic ovaries. J Clin Endocrinol Metab *70*: 894-902, 1990.

52 Hodgins MB and Murad S: 1,25-Dihydroxycholecalciferol stimulates conversion of androstenedione into oestrone by human skin fibroblasts in culture. J Endocrinol *110*: R1-4, 1986.

53 Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S and Seino Y: Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinology *141(4)*: 1317-1324, 2000.

Received January 29, 2009
Revised May 27, 2009
Accepted May 28, 2009