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Expression and tissue distribution of G protein-coupled oestrogen receptor 1 in the reproductive system of the laying hen

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Short communication

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In recent years, G protein-coupled oestrogen receptor 1 (GPER1) has been revealed as an important mediator of oestrogen's action on the reproductive processes in mammals; however, its role in the avian reproductive system is not known. In the present study, it was examined whether GPER1 is present in the hen's reproductive system and if so, its mRNA and protein distribution in the ovary and oviduct. Immunolocalisation of GPER1 in the ovarian follicles and all oviductal parts (i.e. infundibulum, magnum, isthmus, shell gland and vagina) was also investigated. The *GPER1* mRNA transcript and protein were detected in all of the examined tissues by real-time PCR and Western Blot analyses, respectively. The relative expression of *GPER1* depended on the follicle development and the follicular wall layer ($p < 0.001$). It was highest in the white and yellowish follicles and lowest in the granulosa layer of F3-F1 follicles. Immunoreactivity for the GPER1 protein was most prominent in the ovarian stroma with primordial and primary follicles, white follicles and yellowish follicles. Within the oviduct, the highest level of the *GPER1* transcript was in the infundibulum, while the lowest was in the magnum ($p < 0.001$). Moreover, protein abundance was higher ($p < 0.05$) in the shell gland than in the vagina. The strongest immunopositive reaction was observed in the shell gland and vagina, especially in the luminal epithelium. The obtained preliminary results indicate that the oestrogen action in the reproductive systems of female birds also involves GPER1 signalling.

Key words: membrane oestrogen receptor, GPER1, ovary, oviduct, chicken.

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The ovaries of egg-laying hens contain follicles at all stages of development, ranging from slow-growing prehierarchical (primary, white and yellowish; <1-8 mm in diameter) to rapidly growing, yellow preovulatory (small yellow with a diameter >8-12 mm and large yellow with a diameter >12-35 mm, F_n-F1) follicles. The largest follicle (F1) is the most mature and will ovulate to form the first egg. Several prehierarchical atretic follicles and postovulatory follicles (POFs) at different stages of regression are also present in the ovary (for more details, see Hrabia & Sechman 2024). The oviduct engulfs the oocyte released upon the ovulation of the F1 follicle. This organ is divided into five morphologically and functionally different segments: infundibulum, magnum, isthmus, shell gland and vagina, which are responsible for the synthesis of the egg constituents (Hrabia 2022).

The sequence of processes related to the reproductive system's development and functioning, as well

as egg formation, transportation and oviposition in hens, is regulated by numerous hormones, including ovarian steroids, growth factors and neurotransmitters. Oestradiol plays an essential role in various biological events taking place in the ovary and oviduct, including the stimulation of cell proliferation and differentiation, protection against apoptosis, regulation of steroid production and synthesis of the egg components. The action of oestrogen is mediated by two nuclear oestrogen receptors (ERs), ERα and ERβ, which function as ligand-activated transcription factors, as well as transmembrane receptors, mainly G-protein-coupled ER 1 (GPER1, formerly known as GPR30) (Griffin *et al.* 1998; Bernard *et al*. 1999; Revankar *et al*. 2005). Oestrogen binds to the ERα and ERβ receptors, which undergo homo- or heterodimerisation and translocation to the cell nucleus. There, they interact directly with oestrogen regulatory sequences located in the promoter and enhancer regions, or indirectly through interactions with other transcription factors, to regulate the expression of the target genes through the so-called genomic pathway. G protein-coupled oestrogen receptor 1 induces multiple signal transduction pathways. For example, it activates the mitogen activated protein kinases (MAPK) and phosphoinositide 3-kinase (PI3K), phospholipase C (PLC) and adenylate cyclase (AC) (Revankar *et al*. 2005; Filardo *et al*. 2002; Maggiolini *et al.* 2004; Thomas & Dong 2006; Petrie *et al*. 2013). Other findings suggest that the *GPER1* receptor also participates in the formation of inositol-1,4,5-trisphosphate (IP3), calcium ion mobilisation and the activation of protein kinase C (PKC), as well as some ion channels (Sakamoto *et al*. 2007; Ariazi *et al*. 2010; Tica *et al.* 2011). Moreover, this receptor regulates the expression of specific genes, including its own *GPER* gene, in a genomic pathway in a manner independent of the ERα receptor (Kanda & Watanabe 2003; 2004; Ylikomi *et al*. 2004; Albanito *et al*. 2008; Sheng *et al*. 2013; Gaudet *et al*. 2015). G protein-coupled oestrogen receptor 1 possesses structural and functional characteristics shared by members of the G-protein-coupled receptor (GPCR) superfamily, which represent the largest class of plasma membrane receptors. G protein-coupled oestrogen receptor 1 is integral membrane protein synthesised in the rough endoplasmic reticulum, trafficked through the Golgi apparatus, and dynamically shuttled to and from the plasma membrane by means of vesicular transport (Gaudet *et al*. 2015). G protein-coupled oestrogen receptor 1 is widely expressed in different organs, including in the pancreas, liver, heart, arteries, breast, lung, adrenals and reproductive organs (Feldman & Limbird 2017; Lonc *et al*. 2024; Prossnitz & Barton 2023).

The expression of ERα and ERβ in the ovarian and oviductal compartments of birds has been characterised, with ERα predominating in abundance, and the role of ERα is well established (Hrabia *et al.* 2004, 2008; Hanlon *et al.* 2022; Hrabia 2022). However, information regarding the GPER1 expression in the reproductive system is limited to the detection of the *GPER1* transcript in the inner perivitelline layer of the turkey oocyte (Słowińska *et al.* 2021). In the current study, GPER1 expression and distribution is examined in the chicken ovary in relation to follicle development and in the oviduct of laying hens.

Materials and Methods

Birds and tissue sampling

Animal experiments were conducted according to the research protocol approved by the Local Animal Ethics Committee in Krakow, Poland (Approval No. 218/2015). Hy-Line Brown laying hens at 34 weeks of age were caged individually under a photoperiod of 14L:10D. The birds had free access to commercial feed (11.5 MJ/kg metabolisable energy, 17.5% crude protein) and water. The hens (n = 6 hens) were sacrificed 2-2.5 h after oviposition by stunning, followed by bleeding. The following ovarian follicles were isolated: white, yellowish, small yellow and three of the largest yellow preovulatory F3-F1 (26-35 mm; F3<F2<F1). The theca (T) and granulosa (G) layers were separated from the preovulatory follicles. All oviductal parts, including the infundibulum (I), magnum (M), isthmus (Is), shell gland (Sg) and vagina (V), were also isolated. The tissue samples were stored at −80°C for a further Western Blot analysis, or were placed into RNAlater (Sigma-Aldrich, Saint Louis, MO, USA) and stored at −20°C for a later quantitative real-time PCR (qRT-PCR). The other tissue fragments (n = 3 hens), including the ovarian stroma with primordial and primary follicles, were fixed in a 10% buffered formalin, dehydrated, cleared in xylene and embedded in paraffin wax. Microtome sections (6 µm thickness) were used for the immunohistochemical analysis.

RNA isolation and qRT-PCR

The total RNA extraction, reverse transcription (RT) and qRT-PCR were performed as previously described (Wolak *et al*. 2024). In brief, 2 µg of RNA from each of the samples was reverse-transcribed with a High-Capacity cDNA Reverse Transcription

Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's recommendations. The obtained cDNA was used in duplex qRT-PCR for *GPER1* and *18S rRNA* as an endogenous control, in a 10 µl volume containing 5 µl of TaqMan Gene Expression Master Mix (Applied Biosystems), 0.5 µl TaqMan Gene Expression Assays with a specific TaqMan MGB-probe and one pair of primers (*GPER1* assay ID: Gg07163152_s1, GenBank Accession No. NM_001162405.1, amplicon size: 94 bp; Applied Biosystems), 0.5 µl of Eucaryotic *18S rRNA* Endogenous Control (pair of primers and TaqMan probe-labelled VIC/TAMRA, amplicon size: 187 bp; Applied Biosystems), 3 µl of water and 1 µl of cDNA (10x diluted samples after the RT). Water was used as a negative control in all the reactions. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression level of the *GPER1* gene after its normalisation to *18S rRNA*, and calibration to its expression in the white follicles within the ovary (Fig. 1A) or the infundibulum in the oviduct (Fig. 2A).

Protein extraction and Western Blot

In line with Hrabia *et al*. (2022), supernatants containing 60 µg of total protein from tissues of the pre-recruited follicles and the theca layer of the F3-F1 follicles, as well as 30 µg from the granulosa layer of the F3-F1 follicles, were mixed with loading buffer and warmed at 99.9°C for 7 min. After denaturation, the samples were loaded onto a 12% sodium dodecyl sulphate polyacrylamide gel and the proteins were separated by electrophoresis under reducing conditions using a MiniProtean Tetra Cell apparatus. The resolved proteins were transferred from the gel to a PVDF membrane by employing a semi-dry blotter in the FLASHBlot transfer buffer for 7 min at a constant voltage of 25V. The membranes were then blocked with 5% non-fat milk prepared in Tris-buffered saline with 0.1% v:v Tween 20 (TBST, pH 7.6). After washing, the membranes were incubated overnight at 4 °C with primary rabbit polyclonal antibodies against the GPER1 predicted for chickens (1:500; Cat.# DF2737, Affinity Biosciences, Cincinnati, USA). The membranes were then washed and incubated with a secondary HRP-conjugated goat anti-rabbit antibody (Advansta, USA,1:5000, 60 min). The antibody–antigen reaction sites were detected using enhanced chemiluminescence (WesternBright™ ECL) and were visualised using the ChemiDoc-It 410 Imaging system and VisionWorks Life Science software. The membranes were subsequently stripped and incubated with mouse monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) HRP-conjugated IgG (1:1000; Invitrogen, USA), which served as the loading control. The bands were densitometrically quantified using ImageJ (developed at the National Institutes of Health) and were normalised against the corresponding GAPDH bands.

Immunohistochemistry and immunofluorescence

Immunohistochemical localisation of the GPER1 protein in tissues was performed according to Lonc *et al.* (2024). Tissue sections were dewaxed in xylene and rehydrated in a graded ethanol solution. Endogenous peroxidase activity was blocked in 0.5% H_2O_2 in methanol for 10 min at room temperature. After washing in TBS, to retrieve antigenicity, the sections were submerged in citric acid-buffered solution (pH 6.0) and heated in a microwave oven (3 × 1 min, 750 W). The slides were then left in the buffer for cooling. Non-specific binding was prevented by the incubating of sections for 10 min in 5% normal goat serum in TBST. After that, the sections were incubated overnight at 4°C in a humidified chamber with primary rabbit polyclonal antibodies against GPER1 (the same as for Western Blot), diluted 1:100 in TBST. The slides were rinsed two times for 5 min in TBS, before incubation with secondary biotin-labelled goat anti-rabbit IgG (1: 300, 40 min, room temperature; Cat # BA-1000, Vector Laboratories, Newark, US), followed by an avidin-biotin-horseradish peroxidase complex – Vectastain ABC kit (30 min; Vector Laboratories). The colour reaction was developed by incubation with a diaminobenzidine and H_2O_2 solution. In addition, the sections were stained with haematoxylin QS (Vector Laboratories). Finally, the slides were washed for 5 min in running water, then dehydrated and mounted in DPX (Sigma-Aldrich). In the case of immunofluorescent staining, after incubation with the fluorescent DyLight 594 secondary anti-rabbit antibody (1:150, 90 min, room temperature; Cat # Di-1594-1.5, Vector Laboratories), the sections were mounted with VECTASHIELD Vibrance Antifade Mounting Medium with DAPI (Vector Laboratories). A negative control was performed by replacing the primary antibody with normal rabbit serum or TBST buffer. The slides were examined under an Axio Scope.A1 light or fluorescent microscope and were photographed using an Axiocam 503 colour camera and Zen 2.3 pro software (Carl Zeiss, Germany). The intensity of the immunoreactivity was estimated as strong, moderate, weak or very weak.

Statistical analysis

The variables were examined for normality using the Shapiro-Wilk test. The data was statistically ana-

lysed by a nonparametric Kruskal-Wallis ANOVA on ranks followed by the Student-Newman-Keuls test (Fig. 1A, B and Fig. 3A). The nonparametric Mann-Whitney *U* test was used to compare the means of the two oviductal parts (Fig. 3B). Differences in values were considered to be significant at $p < 0.05$. The calculations were performed with SigmaPlot_V_13 (Systat Software Inc., San Jose, CA, USA). The results are presented as the mean ± standard error of the mean (SEM).

Results

GPER1 expression in the hen ovary

The qRT-PCR analysis showed the presence of *GPER1* mRNA in all the examined ovarian tissues (Fig. 1A). Within the ovary, the highest relative expression of *GPER1* mRNA was observed in the white and yellowish follicles, as well as in the theca layer of the F2-F1 follicles, with the lowest found in the granulosa layer of the F3 follicle ($p < 0.001$). In the theca layer of the largest follicles ($p > 0.05$), the transcript levels were comparable, but in the granulosa layer of these follicles, there was a slight increase when the follicle developed to ovulation ($p < 0.05$). In the small yellow follicles, mRNA expression was 53% lower, while in the granulosa layer of the F3-F1 follicles, the transcript abundances were 77%-42% lower than in the white follicles ($p < 0.05$). As is shown in Fig. 1B, the Western Blot analysis revealed the presence of the GPER1 protein in all compartments of the chicken ovary. In each tissue, an approximately 42 kDa GPER1 protein band was identified. The protein abundance was comparable in all the ovarian tissues ($p > 0.05$).

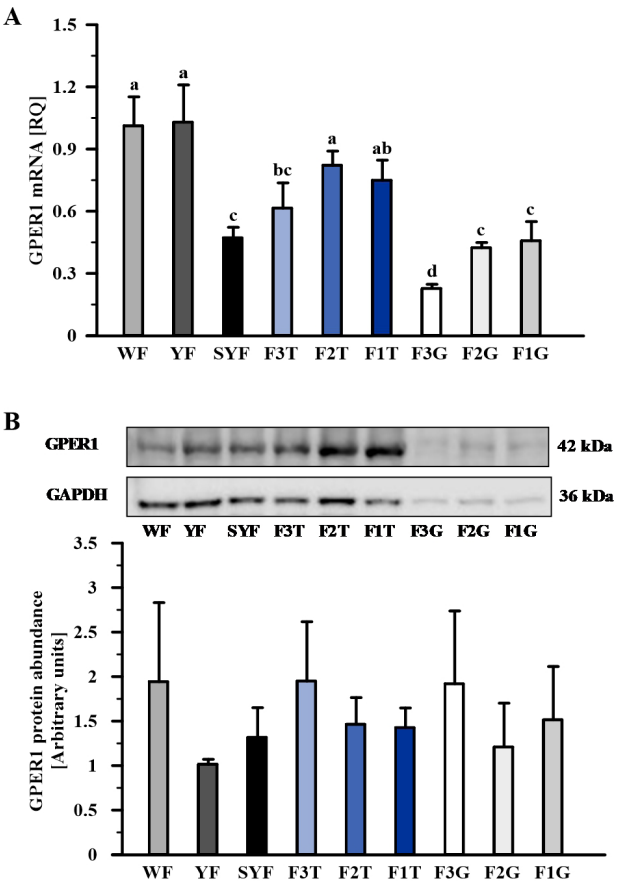

Fig. 1. Expressions of *GPER1* mRNA (A) and protein (B) in chicken ovaries. (A) Data represents the mean of the relative quantity (RQ) ± SEM from six chickens normalised to the expression of *18S rRNA* and standardised to the expression in the white follicles. Values marked with different superscript letters differ significantly ($p < 0.05$). Abbreviations: WF, white follicles (1-4 mm in diameter); YF, yellowish follicles (4-8 mm); F3-F1, yellow pre-ovulatory follicles (20-36 mm, F3<F2<F1); T, theca layer; G, granulosa layer. (B) Protein abundance of GPER1 in chicken ovarian follicles. The images are representative of three independent blots (tissues from six different hens/biological replicates) of the GPER1 protein in the ovarian follicles. The graph shows the relative protein abundance of GPER1 in the ovarian follicles, expressed as the ratio relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Abbreviations are as in Fig. 1A.

The localisation of the GPER1 protein in the ovarian compartments, which was examined by immunohistochemistry and immunofluorescence, is shown in Fig. 2A-D and 2A'-D', respectively. The intensity of the immunoreaction depended on the stage of follicle development and the follicular wall layer. Very strong immunoreactivity was observed in the stroma with primordial follicles (Fig. 2A, A'). A strong immunopositive reaction was found in the granulosa layer, theca interna and theca externa, as well as in the epithelium with loose connective tissue of the white follicles (Fig. 2B, B'). Within the walls of the yellowish follicles, strong immunostaining for GPER1 was localised in the epithelium with loose connective tissue and around the blood vessels (Fig. 2C, C'). The GPER1 immunoreactivity was moderate in the theca interna and theca externa layer, and was weak in the granulosa layer of these follicles (Fig. 2C, C'). A weak or very weak immunoreaction was observed in the entire walls of the yellow follicles (Fig. 2D, D').

GPER1 expression in the hen oviduct

The relative expression of the *GPER1* mRNA transcript in the oviductal segments of the oviduct and the relative abundance of GPER1 protein in the shell gland and vagina are shown in Figs 3A and 3B, respectively. The mRNA expression of *GPER1* was the most prominent in the infundibulum, with the lowest expression being in the magnum ($p < 0.001$). The mRNA transcript abundance was 97%-75% lower in

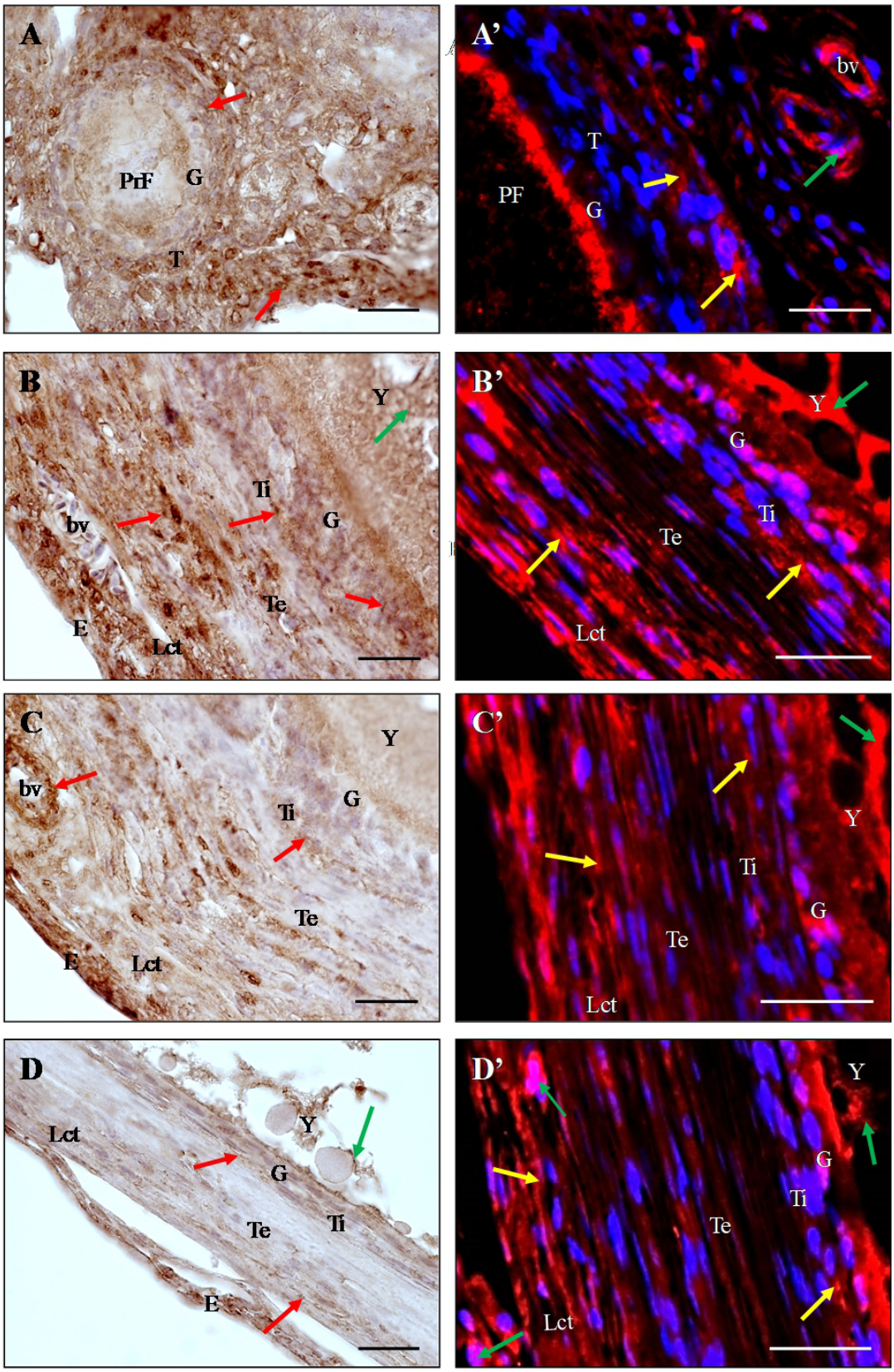

Fig. 2. Representative micrographs of immunohistochemical (A-D) and immunofluorescent (A'-D') localisation of the GPER1 protein in paraffin-embedded sections of the chicken ovary. A strong immunoreaction (red and yellow arrows) for GPER1 was observed in the stroma with primordial and primary follicles (A, A') and in the white follicles (B, B'). In the yellowish follicles, strong staining was observed in the epithelium with loose connective tissue and around the blood vessels, while being moderate in the theca layer and in the granulosa layer (C, C'). A less intense GPER1 immunoexpression was observed in the wall of the yellow hierarchical follicle (D, D'). Green arrows show nonspecific fluorescence in the red blood cells and yolk. Abbreviations: G – granulosa cells; T – theca cells; Ti – theca interna; Te –theca externa; Y – yolk; E – epithelium; bv – blood vessels; Lct – loose connective tissues; PrF – primordial follicle; PF – primary follicle. Scale bars represent 20 µm.

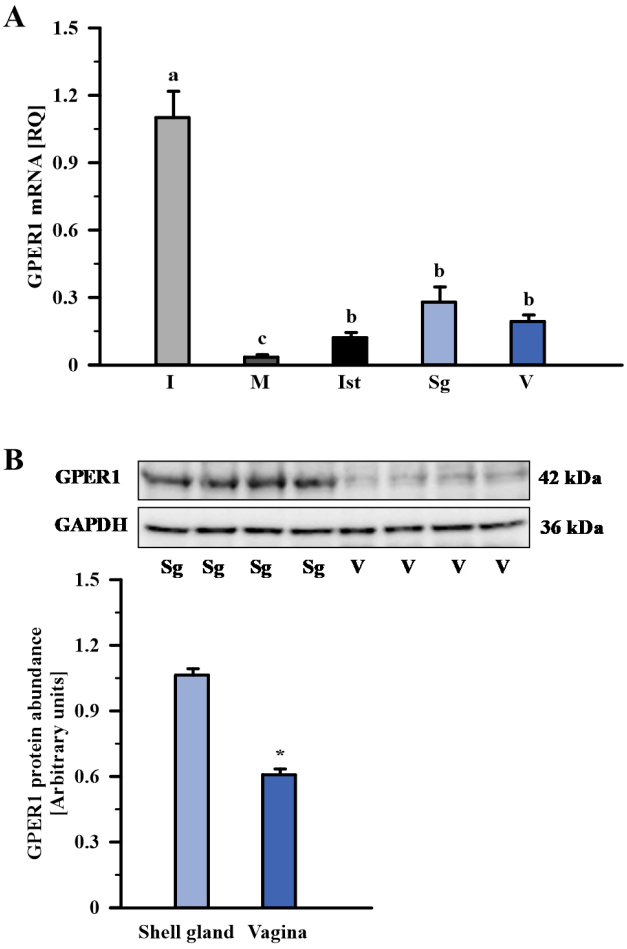

Fig. 3. Expression of the *GPER1* mRNA (A) and protein (B) in the chicken oviduct. (A) Each value represents the mean of the relative quantity (RQ) ± SEM from six chickens normalised to the expression of *18S rRNA* and standardised to the expression in the infundibulum. Values marked with different letters differ significantly ($p < 0.05$). Abbreviations: I, infundibulum; M, magnum; Is, isthmus; Sg, shell gland; V, vagina. (B) Western Blot analysis of the GPER1 protein in the shell gland and vagina. The images are representative of four independent blots (tissues from six different hens/biological replicates). The graph shows the relative protein abundance of GPER1 in the shell gland and vagina. Values are the mean ± SEM of the ratios of GPER1 to GAPDH (*$p < 0.05$).

the magnum, isthmus, shell gland and vagina than in the infundibulum. Due to the stronger immunoreactivity for the GPER1 protein observed in the shell gland and vagina than in other oviductal parts, as well as low or undetectable levels of the GAPDH protein (control protein) in the magnum and isthmus, the results of densitometry of the Western Blot analysis were shown only for the shell gland and vagina. The protein abundance in the shell gland was higher by 43% ($p < 0.05$) than in the vagina (Fig. 3B).

The localisation of the GPER1 protein in the wall of the oviductal segments is shown in Fig. 4A-F and Fig. 4A'-G. Specific immunoreactivity for the GPER1 protein was found in the wall of all oviductal segments (Fig. 4A-D', F, F'). A very strong positive reaction for GPER1 was found in the membranes of the luminal epithelium and blood vessel cells in the shell gland (Fig. 4D, D'). A strong immunoreaction was observed in the cells of the epithelium, stromal muscles and blood vessels of the vagina (Fig. 4F, F'). A moderate staining intensity was found in the luminal epithelium and in the stromal muscles of the infundibulum, magnum and isthmus (Fig. 4A-C'). Weak or very weak immunoreactivity for the GPER1 protein was present in the cell membranes of the tubular glands in the magnum, isthmus and shell gland (Fig. 4B-D'). Replacing the primary antibody with normal rabbit serum or the TBST buffer abolished the staining (Figs 4E and G).

Discussion

This is the first report to demonstrate the unique distribution of the *GPER1* mRNA transcript and protein in the ovaries and the oviducts of laying hens. GPER1 was present in all the ovarian follicles throughout the chicken' development until ovulation, as well as in all parts of the oviduct. Thus, this finding implies a possible involvement of GPER1 signalling in the oestrogen mediation of ovarian folliculogenesis and oviductal functions.

Numerous studies have highlighted the role of GPER1 in the ovaries of different animal species. For example, the involvement of GPER1 in folliculogenesis in hamster ovaries has been suggested, and it was shown that the expression of GPER1 is regulated by gonadotropins (Wang *et al*. 2007). Moreover, oestrogen, via GPER1, regulates the maturation of oocytes in striped danio (*Danio rerio*) and micuna (*Micropogonias undulatus*). Oestrogen, acting through GPER1, can arrest meiosis by transactivating the epidermal growth factor receptor (EGFR) and phosphorylating MAPK3/1 kinase to regulate the oocyte maturation induced by 17ß-estradiol through non-genomic mechanisms (Thomas 2012). In our experiment, the highest amount of the *GPER1* mRNA transcript within the ovary was found in the pre-recruited, white and yellowish follicles. In these follicles, oestradiol is the main secreted steroid and is responsible for regulating follicle development by stimulating proliferation (for more details, see Hrabia 2022). This process may require rapid signalling pathways, which are identified in the pre-recruited follicles of hens, including the MAPK/ERK signalling and PKC pathways, as well as Smad1/5/8 signalling (Johnson 2011). It

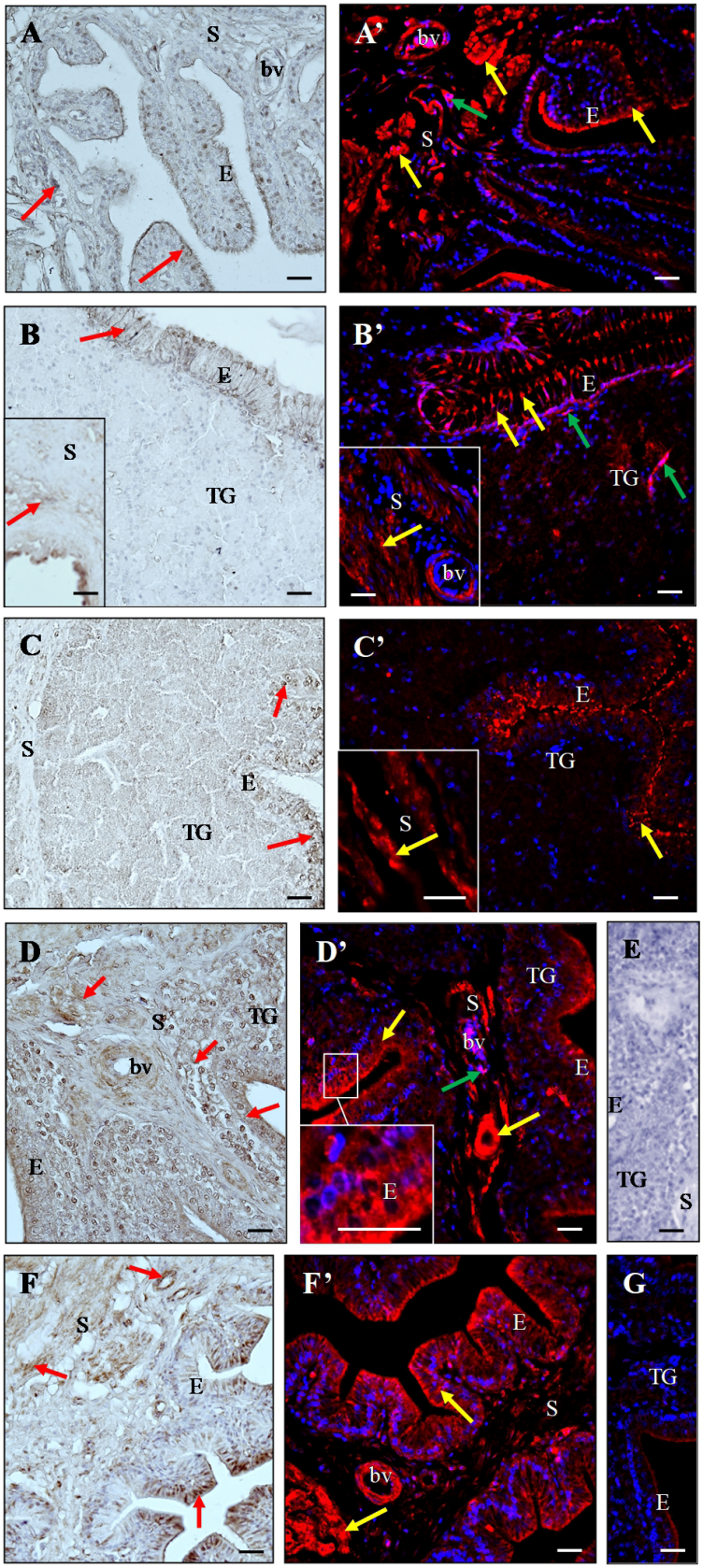

Fig. 4. Representative micrographs of the immunohistochemical (A-F) and immunofluorescent (A'-F') localisation of the GPER1 protein in the infundibulum (A, A'), magnum (B, B'), isthmus (C, C'), shell gland (D, D') and vagina (F, F') of chickens. E and G show the negative control. Note, the strongest immunopositive reaction (red and yellow arrows) for the GPER1 protein is observed in the luminal epithelium of the shell gland and vagina, as well as in the blood vessels. The insert in B, B' and C' shows the stroma with muscles. The insert in D' shows the higher magnification of the area indicated by a small frame. Green arrows show nonspecific fluorescence in the red blood cells. Abbreviations: S – stroma (connective tissue + muscles); E – luminal epithelium; TG – tubular glands; bv – blood vessels. Scale bars represents 20 µm.

is plausible that the GPER1 protein, localised to the granulosa and theca layers in high abundance, participates in the initiation of the abovementioned signalling pathways in the pre-recruited follicles. It is important to note that after recruitment, the abundance of the *GPER1* mRNA transcript in the small yellow follicles decreases significantly. After the entrance into the fast-growing stage of development, cyclic adenosine monophosphate (cAMP) signalling is initiated, with a subsequent differentiation of the granulosa cells and the production of progesterone (Johnson 2011). Although oestradiol is involved in the regulating of progesterone secretion by yellow preovulatory follicles (Sasanami & Mori 1999), our results might suggest that the role of GPER1 is not crucial in this process, but its cooperation with ERα cannot be excluded. GPER1 can also interact with integrins and receptor tyrosine kinases (RTKs), including the epidermal growth factor receptor (EGFR), further promoting signal transduction through the MAPK/ERK and PI3K/Akt pathways. In turn, activation of the PI3K/Akt signalling pathway promotes the granulosa cell's survival, proliferation and differentiation. Additionally, this pathway protects the ovarian cells from apoptosis (Johnson, 2011). In studies conducted on another species such as humans and monkeys, membrane activation of GPER1 was shown to induce a phosphorylation cascade, in particular involving ERK1/2, PKA and PI3K (Perian & Vanacker, 2020). The PKA-dependent mechanism was proposed to mediate the actions of ghrelin in avian ovary. It is known that ghrelin is a hormone involved in the modulation of chicken ovarian cell proliferation, apoptosis and hormone secretion (Sirotkin *et al*. 2006; Sirotkin & Grossmann 2007). Therefore, a signalling pathway activated by ghrelin may interact with oestrogen signalling via GPER1, affecting follicular development and atresia in hens.

It is worth mentioning that in the largest yellow follicles, the *GPER1* mRNA transcript was more abundant in the theca layer than in the granulosa layer. These results are in contrast to the expression of nuclear ERα and ERβ, of which the mRNA transcript abundance was low in the theca layer and high in the granulosa layer of the largest follicles in quails and chickens (Hrabia *et al.* 2004, 2008). The observed results might indicate a key role of membrane GPER1 in the theca layer, where they may be involved in testosterone and oestradiol synthesis in the theca interna and externa, respectively. The observation that GPER1 signalling, in cooperation with ERs, is involved in oestrogen's actions on oestrogen secretion by mouse Leydig cells, supports this hypothesis (Kotula-Balak *et al*. 2018). The same mechanism may exist in the avian ovary. A functional cross-talk between ERα and GPER1 in mediating the apoptotic effect was observed in primary cultures of adult rat round spermatids (Chimento *et al.* 2010). GPER1 physically interacts with ERα and the p65 subunit of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). This complex is found both in the cytoplasm and the nucleus, and is related to the oestrogen inhibitory NFκB expression of interleukin-6 (IL-6) and tumour necrosis factor (α) (TNFα) (Pelekanou *et al.* 2016). Moreover, the interaction of GPER1 and ERα and ERβ activates protein kinase cascades that result in the phosphorylation of transcription factors, as well as ERs themselves, that can then interact with DNA sequences to regulate transcription (Björnström & Sjöberg 2005). Taken together, the results of the present and previous studies showing a nuclear receptor abundance in the ovarian tissues of hen ovaries (Hrabia *et al.* 2004, 2008) indicate that in the theca layer of the avian ovarian follicles, rapid non-genomic signalling of oestrogen dominates, while in granulosa cells, classical genomic signalling of oestrogen is predominant.

The next novel finding of this study was the detection of the *GPER1* mRNA transcript and protein in the oviductal sections: infundibulum, magnum, isthmus, shell gland and vagina. We found an oviductal part-dependent expression of GPER1. Generally, the highest mRNA expression level of *GPER1* was in the infundibulum in comparison to other parts, but the GPER1 protein was most abundant in the shell gland and vagina. Observed discrepancies in the mRNA and protein amounts may be due to regulatory mechanisms at the post-transcriptional and translational levels, as well as to the stability of mRNA and differences in the sensitivity between methods. An interesting observation was that the GPER1 protein is localised to the luminal epithelium of the chicken oviducts. It cannot be excluded that oestradiol acting by GPER1 may regulate egg component secretion by the epithelial cells, e.g. molecules required for eggshell formation. Another important observation was the GPER1 protein localisation in the smooth muscles located in the stroma. That may be attributable to the regulation of oviduct contractility. In studies conducted on monkey kidney fibroblasts, the activation of GPER1 by oestradiol results in, among other things, intracellular calcium mobilisation. In turn, calcium ions are essential for proper muscle contractility. This may be especially important during the oviposition process. Moreover, a strong or moderate immunoreaction for the GPER1 protein was observed in the walls of the blood vessels. This

may suggest the involvement of GPER1 in the modification of the blood flow through the oviduct, thereby participating in the processes taking place in the oviduct during egg formation, or this receptor may have a role in oviductal angiogenesis. Further studies are necessary to clarify the processes mediated by the GPER1 signalling pathways and cooperation of the membrane GPER1 with nuclear ERs in the hen ovary and oviduct.

Conclusions

In conclusion, our results provide evidence that *GPER1* mRNA transcripts and proteins are presented in the hen ovary and oviduct. The abundance of GPER1 depends on the stage of follicle development and the follicular wall layer, as well as on the oviductal segments. This suggests that GPER1 signalling may be engaged in the effect of oestradiol on follicle development and oviduct functioning. Further studies clarifying the regulation of GPER1 expression and the specific role of the GPER1 receptor in the chicken's reproductive system are required.

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Author Contributions

Research concept and design: D.W.; Collection and/or assembly of data: K.F., M.K.; Data analysis and interpretation: D.W.; Writing the article: D.W.; Critical revision of the article: A.H.; Final approval of article: D.W., K.F., M.K., A.H.

Conflict of Interest

The authors declare no conflict of interest.

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