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Immunolocalisation and mRNA expression of selected sirtuins in the avian liver

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Original article

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Sirtuins are evolutionary conserved enzymes that function as NAD+ -dependent deacetylases and ribosyltransferases. Seven sirtuins (SIRT1-7) with specific distributions and functions in the cell have been detected in mammals. They have also been detected in birds. Sirtuins regulate the DNA repair function, the cell cycle and metabolism. Many studies concerning anti-aging factors are currently focused on the potential of sirtuins. The aim of the study was to determine the expression of four sirtuin genes (*SIRT1, SIRT3, SIRT6* and *SIRT7*) and to immunolocalise SIRT1 and SIRT7 protein in chicken and quail liver tissue at the ages of embryonic development to 32 weeks old. The *SIRT1* mRNA expression was found to be significantly higher in the embryonic livers than in the posthatch and older bird livers, whereas the *SIRT3* and *SIRT7* mRNA expression was significantly lower in the embryonic than in the older bird livers. The *SIRT3*, *SIRT6* and *SIRT7* mRNA expression was highest in the livers isolated from chicken at the age of 32 weeks. The immunopositive expression of SIRT1 and SIRT7 in the liver has been demonstrated in the cytoplasm of hepatocytes, both in embryonic and in adult birds. It may indicate these enzymes play an important role in the liver's development and functions. They may be key regulators of glucose and lipid metabolism. Thus, the avian model may be very helpful in gaining knowledge about the mechanism of action of sirtuins, which can be used in anti-aging medicine and in the treatment of metabolic diseases.

Key words: SIRT, birds, expression, embryonic development

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Recently, there has been a high increase in interest in anti-aging medicine. The role of maintaining metabolic homeostasis, limiting oxidative stress, preventing lifestyle diseases, strengthening natural immunity and reducing the incidence of inflammation have been emphasised. Many regulatory mechanisms and signalling pathways are currently being analysed, the modification of which could affect the aging process and ensure longevity in the future. One of the factors that are associated with longevity processes are proteins from the sirtuin family (SIRTs) (Ji *et al.* 2022). Sirtuins are enzymatic proteins that belong to the deacetylhistone group. They are dependent on nicotinamide adenine dinucleotide – NAD+. Initially, they were detected in yeast (*Saccharomyces cerevisiae*), and then in a number of organisms belonging to Prokaryota and Eukaryota. In primitive organisms, they were called silent information regulator 2 (Sir-2) (Haigis & Sinclair 2010), and the Sir2 orthologs found in vertebrates are marked with

the abbreviations SIRT1 to SIRT7 (Ye *et al.* 2017). Sirtuins participate in the post-translational processing of proteins, which is usually associated with their deacetylation, or less frequently, with demalonylation, polyADP-ribosylation or lipoamidation (Schiedel *et al.* 2018). Therefore, sirtuins affect various cellular processes, including DNA repair, lipid and glucose metabolism, apoptosis, carcinogenesis and the cell aging process (Mei *et al.* 2016). Research on the mechanism of aging and the factors regulating this process is a very promising field of science. A research model is being sought, preferably from the group of so-called alternative models, to assess the aging mechanisms as quickly and inexpensively as possible. Therefore, an interesting choice of an alternative animal model for the aging process may be Japanese quail and chicken, which are species that have been used in scientific research for many years. However, there is little data in the literature on sirtuins in chickens. Many groups have conducted research on rodents, mainly rats, but studies on the expression and activity of sirtuins using Japanese quail embryos have not been conducted so far. The aim of this study was to determine the gene expression of sirtuins *(SIRT1, SIRT3, SIRT6* and *SIRT7)* and the localisation of SIRT family proteins in the tissue of chicken livers at ages ranging from Day 14 of embryogenesis to 32 weeks, and in Japanese quails on Day 14 of their embryonic development.

Materials and Methods

Ethics approval

Experiments and procedures with chicken and quail embryos do not require the approval of a Local Ethics Committee (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals used for Scientific Purposes). The chicken tissue (D0, D1, D14 and 32 weeks) used in the molecular analyses was derived from previous experiments carried out in the Department of Physiology and Endocrinology of the University of Agriculture in Krakow, which meets the assumptions of the 3R Principle. All the people in contact with animals had the authority to plan, perform procedures and to sacrifice the animals.

Avian embryos and chicks

The research was conducted on tissues from 15 fertilised Japanese quail (*Coturnix japonica*) eggs obtained from a local breeder (Komorniki, Poland) and 30 fertilised chicken *(Gallus gallus domesticus)* eggs (Tarnów, Poland). The incubation process took place in a Brinsea 190 Advance incubator under standard conditions (relative humidity 55-60% and temperature 37.8°C). On Day 14 of incubation (ED14), as well as on the day of hatching (0D) and Day 1 of life (1D), the embryos/birds were decapitated and liver tissues were collected, then placed in Eppendorf tubes with RNALater™ (Sigma, MO, USA) for the gene expression analyses, or in freshly prepared 10% buffered formalin for the histological preparations.

In the older birds (Day 14 (14D) and 32 weeks of age (32W)): chicken (n=6 for each age point) breeding was carried out in accordance with the principles of breeding and feeding, using commercially available feed in cages with an area and equipment consistent with the EU requirements.

Chemicals

The chemicals for the RNA isolation and RT-PCR were purchased from the following companies: TRI-Reagent (ThermoFisher Scientific, USA), DEPC – diethylpyrocarbonate (Sigma, USA), 1-bromo-2-chloropropane (Sigma, USA), ethyl alcohol 75% (Chempur, Poland), isopropyl alcohol (ICN Biomedical, USA), RNAase-free water (Fermentas, Lithuania), High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, USA), oligonucleotide primers (IBB PAN, Poland), 5 x HOT FIREPol® EvaGreen Master Mix (Solis BioDyne, Estonia) and RNAlater™ (ThermoFisher Scientific, USA). The chemicals used for the immunohistochemical analyses were purchased from the following companies: ethyl alcohol (POCH, Poland), xylene (POCH, Poland), sodium citrate (BioShop, Canada), citric acid (Warchem, Poland), paraformaldehyde (POCH, Poland), Tris (BioShop, Canada), normal goat serum (Vector Laboratories, USA); Antibodies: rabbit polyclonal antibody against SIRT1 (1:250, Bethyl Laboratories, USA, Cat. A300-687A-T), rabbit polyclonal antibody against SIRT7 (1:100, Invitrogen, Cat. PA5-13226), goat anti-rabbit secondary antibody labelled with DyLight 594 (Vector Laboratories, USA) and VECTASHIELD®VibranceTM Antifade

Mounting Medium with DAPI (Vector Laboratories, USA).

All other reagents were obtained from Sigma (USA), POCH (Poland) or Warchem (Poland).

Total RNA isolation, cDNA synthesis and qPCR analysis

The RNA from the liver samples was isolated as described previously (Grzegorzewska *et al.* 2020), a reverse transcription was conducted and the EvaGreen real-time PCR analyses were performed according to the manufacturer's instructions. Briefly, the total RNA (2 µg from each liver extracted with TRI-reagent) was reverse transcribed with a High-Capacity cDNA Reverse Transcription Kit including random primers. The samples were incubated in a thermocycler (Mastercycler Gradient; Eppendorf, Hamburg, Germany), in accordance with the following thermal profile: 25°C for 10 min, 37°C for 120 min and 85°C for 5 min. The obtained cDNA was used in a qPCR for sirtuins (*SIRT1, SIRT3, SIRT6, SIRT7*) and for glyceraldeyde-3-phosphate dehydrogenase (*GAPDH*) as a housekeeping gene. The amplifications were performed in a 96-well thermocycler (StepOnePlus; Applied Biosystems, USA), according to the following recommended cycling programme: 15 min at 95°C, 40 cycles of 15 s at 95°C, 20 s at 62°C and 20 s at 72°C. The primer sequences are presented in Table 1. Sirtuins are very conservative enzymes and the mRNA sequence is almost identical in the quail and the chicken. Therefore, the primer sequences designed according to the mRNA sequences of the chicken (*Gallus domesticus*) can be used also for quail tissue. *SIRT1*, *SIRT6* and *SIRT7* are 100% identical, while the primers for *SIRT3* differ only in 1 nucleotide; therefore, we used the same primers for the quail and chicken samples. The singleplex qPCRs for the examined genes were performed according to the manufacturer's protocol, in a 10 µl volume containing: 2 µl 5x HOT FIREPol EvaGreen qPCR Mix Plus, 0.12 µl Primer Forward (10 pmol/µl), 0.12 µl Primer Reverse (10 pmol/µl), 1 µl cDNA (10 x diluted sample after the RT reaction) and PCR grade H_2O. Each sample was analysed in duplicate. A no-template control was included in each run. A relative quantification of the examined genes was calculated after a normalisation with the GAPDH transcript, taking into account the reaction efficiency for individual genes and by employing the expression in the liver tissue of the chicken embryos as the calibrator by using the $2^{-\Delta\Delta Ct}$ method

Table 1

GenBank Accession Numbers, localisation, sequences of amplified gene primers and product size

Gene	GenBank Accession Number	Primer sequence (5'-3')	Product size [bp]
SIRT1 (Transcript variant X1)	XM_015866377.2	F: TAGCCAATGGTTTCCACTCC R: AAGAATTGTCCGTGGGTCTG	149
SIRT3 (Transcript variant X1)	XM_015863369.2	F: AGGAAATTCCCAGGAGAGGA R: TGTCATGTGCAGGAAGAAGC	141
SIRT6	XM_015886640.2	F: TCTTCCAATGTGGTGTTCCA R: TTTCAGGATGCCGACTCTCT	195
SIRT7	XM_015879897.2	F: GCCAGAGGAGCTGAAGAGAA R: CTTCTGCAGCAATGTCCAAA	151
GAPDH	NM_204305	F: GTGTGCCAACCCCCAATGTCTCT R: GCAGCAGCCTTCACTACCCTCT	97

GAPDH – Glyceraldehyde 3-phosphate dehydrogenase (housekeeping gene); *SIRT1* – Sirtuin 1, *SIRT3* – Sirtuin 3, *SIRT6* – Sirtuin 6, *SIRT7* – Sirtuin 7.

(Livak & Schmittgen 2001). A quantification was performed using StepOne integrated software.

Statistical analysis

The results were analysed statistically using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered significant at $p<0.05$ (SigmaStat, V4.0 software, USA). The data is presented as the means ± SEM.

Immunohistochemical localisation of SIRT1 and SIRT7

The fragments of the liver were fixed in freshly prepared 10% buffered formalin (pH=7.4), then processed and embedded in paraffin wax. Immunocytochemical staining was performed, according to Grzegorzewska *et al.* (2022) with a small modification. The localisation of the SIRT1 and SIRT7 proteins in the livers of the Japanese quail embryos and 32-week old chickens was performed using the immunohistochemical method. The liver sections were deparaffinised in xylene and then rehydrated in alcohol: absolute, 96%, 70% and 50%. The preparations were rinsed twice in distilled water, and were then heated in a citrate buffer for 20 minutes at 75°C, which allowed the epitopes to be exposed. In the next step, they were incubated with 5% normal goat serum in TBST at room temperature for 30 minutes. Then, the sections were incubated overnight at 4°C with the appropriate polyclonal rabbit antibodies against SIRT1 (dilution according to the manufacturer's recommendations 1:250, Bethyl Laboratories, USA, Cat. A300-687A-T) or against SIRT7 (dilution 1:100, Invitrogen, Cat. PA5-13226). Both antibodies against human sirtuins are specific enough to detect Sirtuin1 and Sirtuin7 in quail or chicken tissue. The amino acid sequences in the region of the antibody-antigen interaction are very conservative. The slides were washed with TBS and incubated with the anti-rabbit secondary antibody labelled with the fluorescent dye DyLight 594 (Vector Laboratories, USA) for two hours in darkness. The stained sections were mounted using VECTASHIELD® Hardset™ with DAPI (Vector Laboratories, USA). A negative control was incubated without a primary antibody. Additionally, to analyse the specific localisation of sirtuins in the cell and to compare it between the liver and another tissue, we decided to also analyse the expression of sirtuins in the ovarian stroma of laying hens. The obtained preparations were observed, analysed and documented under an AxioScope fluorescence microscope with an Axiocam 503 colour camera and with ZEN 2.3 pro software (Carl Zeiss, Jena, Germany).

Results

Expression of the mRNA encoding *SIRT1*, *SIRT3, SIRT6* and *SIRT7*

The mRNA expression of all the tested sirtuins, i.e. *SIRT1, SIRT3, SIRT6* and *SIRT7,* was demonstrated in the avian livers isolated from the embryos, as well as from the older chickens (Fig. 1). The *SIRT1* mRNA expression was significantly higher in the embryonic livers of the quail and chicken than in the posthatch and older birds (Fig. 1A); whereas the *SIRT3* (Fig. 1B) and *SIRT7* (Fig. 1D) mRNA expression was significantly lower in the embryonic livers than in the livers isolated from older birds. The *SIRT3*, *SIRT6* (Fig. 1C) and *SIRT7* mRNA expression was the highest in the 32-week old chicken livers.

Immunolocalisation of SIRT1 and SIRT7 in the avian liver

The immunohistochemical staining analysis allowed for the detection of the SIRT1 and SIRT7 protein expression in the livers of the Japanese quail embryos. An immunopositive signal for both sirtuins – SIRT1 (Fig. 2A) and SIRT7 (Fig. 2B) – was expressed in the cytoplasm of selected hepatocytes, but not in the nuclei.

In the livers of the 32-week old chickens, the expression of SIRT1 (Fig. 3A) and SIRT7 (Fig. 3B) was more intensive than in the embryonic liver and it was localised in the cytoplasm of the hepatocytes, not in the nucleus of the cell.

In contrast to the liver, the positive signal for both sirtuins in the ovarian stroma was very intensive and it was localised in the cell nucleus, not in the cytoplasmatic compartments (SIRT1 – Fig. 4A, SIRT7 – Fig. 4B). In this case, the localisation in the cell was completely different in the ovary than in the liver. The intensities of the immunopositive reactions for both sirtuins were more intensive in the ovarian stroma of the laying hens than in the livers of the birds in all the examined stages of development and age. A negative control (Fig. 3C) was performed with

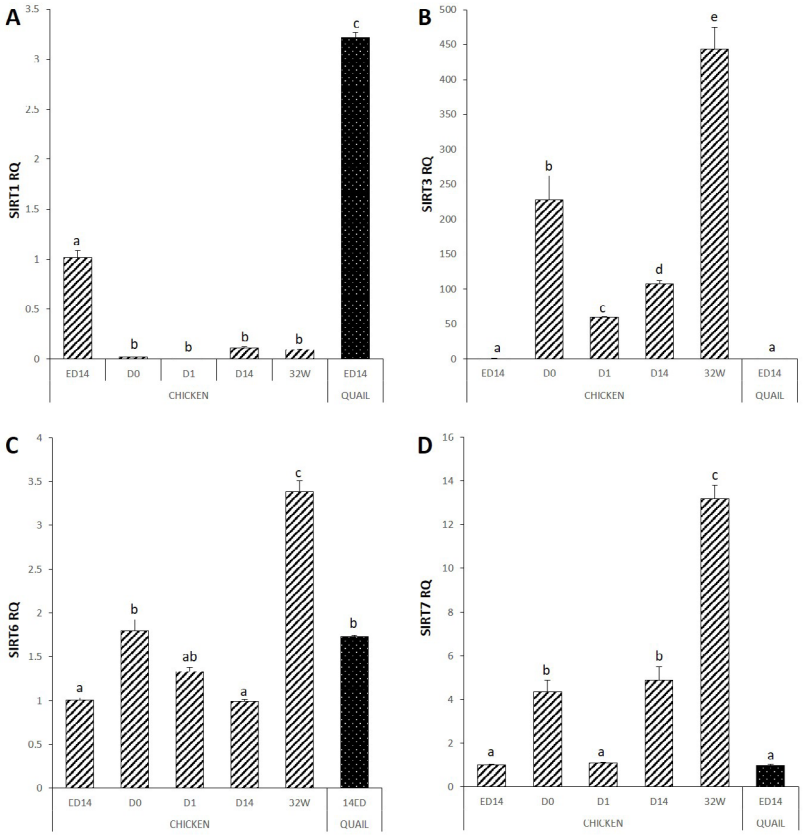

Fig. 1. mRNA expression of the genes encoding the selected sirtuins *SIRT1, SIRT3, SIRT6* and *SIRT7*, with *GAPDH* as a reference gene, in the liver tissues of quail and chicken embryos on Day 14 of embryogenesis (ED14), newly-hatched chickens (D0), 1-day old chickens (D1), 14-day old chickens (D14) and 32-week old chickens (32W). Each value represents the mean ± SEM from n = 6 livers (embryonic, D0, D1) or liver lobes (D14, 32W). The gene expression data represents the mean relative quantity (RQ) standardised to the control expression in the liver (RQ = 1). Values marked with different letters differ significantly ($p<0.05$).

TBST instead of the primary antibody and did not show any positive immunofluorescent reaction.

Discussion

Sirtuins play a key role in the regulation of metabolism in many organisms. First discovered in yeast, they play a pivotal role in the regulation of physiological processes in many animals including humans. The most significant factors influencing the activity of sirtuins are NAD+, AMP-activated kinase (AMPK), forkhead box transcription factors (FOXO) and peroxisome proliferator-activated receptor proteins (PPARs). The interactions of these molecules with sirtuins may exert different effects depending on the tissue, and may also contribute to inhibiting or stimulating the development and progression of metabolic disorders (Maissan *et al.* 2021). Sirtuins are also known as novel and promising factors that may be responsible for delaying and slowing down the aging process.

Hens and quails are becoming more popular experimental animal models in tissue engineering (Dos Santos *et al.* 2018), physiological disorders detection (Vilches-Moure 2019), telomere biology analyses (Swanberg & Delany 2005) and toxicological studies *in vitro* and *in vivo* (Wu *et al.* 2018; Grzegorzewska *et al.* 2024). Avian embryos are currently used as models in developing anti-cancer therapies (Meta *et al.* 2023), toxicological studies (Grzegorzewska *et al.* 2020) and research concerning the angiogenesis and metastasis of cancer cells (Ribatti 2014).

In this study we decided to analyse and compare the mRNA and protein expression of sirtuins in the livers of chicken and quail embryos, as well as in hatched and older chickens. Expressions of sirtuin mRNA were detected in all the analysed samples. The expression of *SIRT1* was

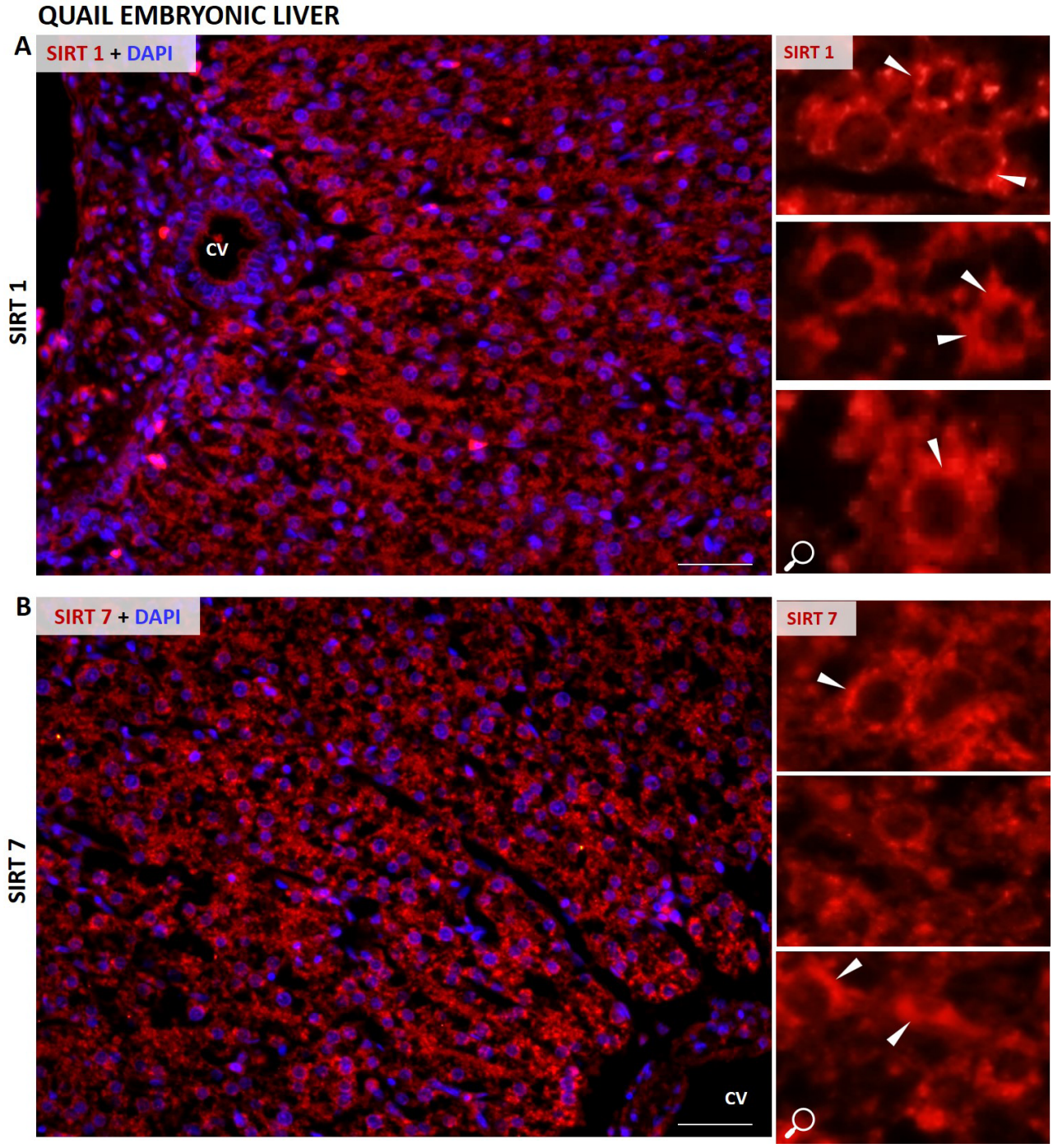

Fig. 2. Immunolocalisation of SIRT1 (A) and SIRT7 (B) in the livers of quail embryos (Day 14 of embryogenesis): SIRTs – red fluorescence, arrowheads; DAPI – blue fluorescence of the cell nuclei; SIRT1/SIRT7 + DAPI – merge, images of red and blue fluorescence; CV – central vein. Magnification 40x, scale bars represent 100 µm.

significantly higher in the embryonic livers than in the livers isolated from older birds. However, we obtained opposite results during analyses of the *SIRT3* mRNA expression. In the embryonic livers (quail and chicken) a trace expression was observed, whereas in the older chicken livers it increased significantly. The *SIRT6* and *SIRT7* expression was the highest in the 32-week old chickens. This may suggest that *SIRT1* plays a crucial role in the physiological function of the liver during embryogenesis, and later, after hatching, the activity of other sirtuins becomes more important.

Sirtuin expression patterns at various stages of development were previously analysed in the chicken's liver by Ren *et al.* (2017). Their results

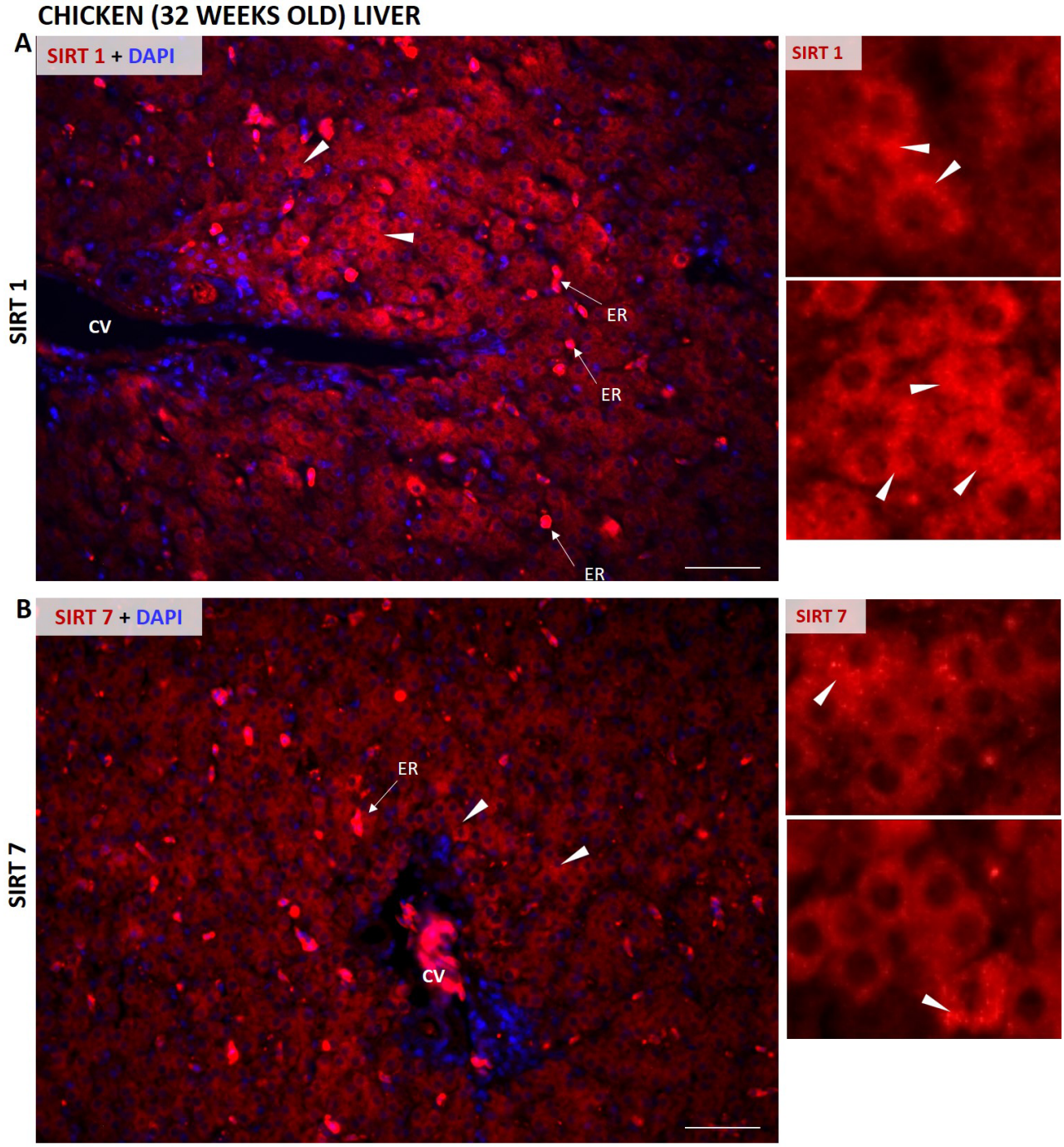

Fig. 3. Immunolocalisation of SIRT1 (A) and SIRT7 (B) in the chicken livers (32-week old): SIRTs – red fluorescence, arrowheads; DAPI – blue fluorescence of the cell nuclei; SIRT1/SIRT7 + DAPI – merge, images of red and blue fluorescence; CV – central vein; ER – erytrocytes (autofluorescence). Magnification 40x, scale bars represent 100 µm.

showed that the expression levels of all sirtuins genes tended to increase with sexual maturation. An age-related regulation of the *cSIRT1, cSIRT2,* c*SIRT4*, *cSIRT6* and *cSIRT7* genes was observed in the liver. There were no differences between the sirtuin expression levels in 30- and 35-week old chickens. However, changes were evident in the mRNA expression levels of *cSIRT1, cSIRT2, cSIRT4, cSIRT6* and *cSIRT7* in 30-week old chickens, which increased compared to 10-week old chickens. In our study, an increase in the mRNA expression in *SIRT6* and *SIRT7* was also observed when we compared newly-hatched, D1, D14 chickens and 32-week old chickens.

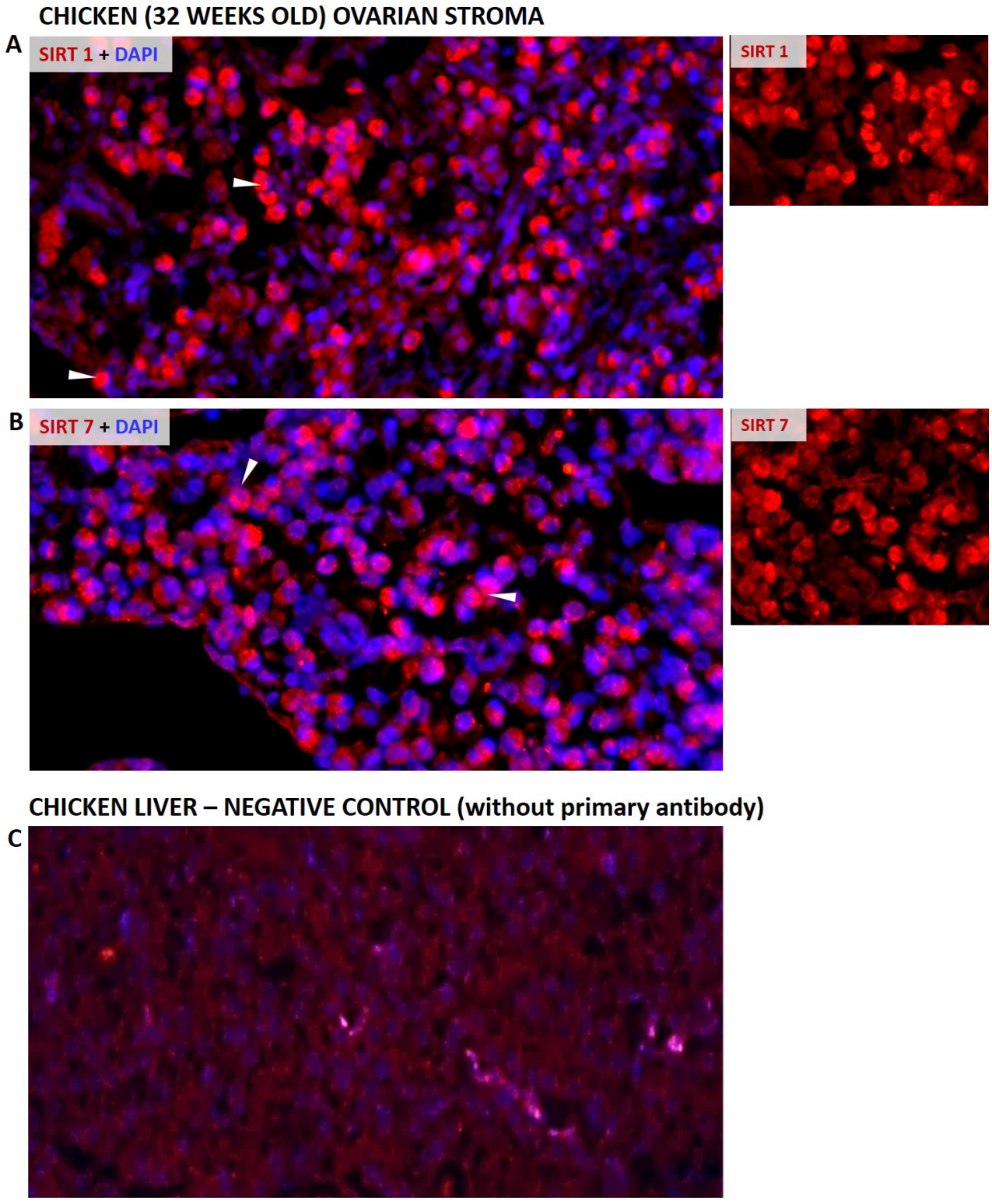

Fig. 4. Immunolocalisation of SIRT1 (A) and SIRT7 (B) in the ovaries of the chickens (32-week old laying hens), negative control (C) – staining with a TBST buffer instead of a primary antibody: SIRTs – red fluorescence, arrowheads; DAPI – blue fluorescence of the cell nuclei; SIRT1/SIRT7 + DAPI – merge, images of red and blue fluorescence. Magnification 40x, scale bars represent 100 µm.

In the studies of Ren *et al.* (2017), the levels of *cSIRT3* and *cSIRT5* mRNA expression in the liver did not show significant changes throughout the entire period of the chicken's development (Ren *et al.* 2017). Conversely, in our study the mRNA expression of *SIRT3* decreased on D1 in

comparison with D0, then increased on D14 and next significantly increased in the livers of the 32-week old chickens.

Although hatching is perhaps the most abrupt and profound metabolic challenge that a chicken must undergo, there have been no attempts to functionally map out the metabolic pathways induced in the liver during the embryo-to-hatchling transition. Cogburn *et al.* (2018) analysed the metabolic and lipid metabolism regulatory factors in late embryos or newly-hatched chicks. They found that several transcription factors and their coactivators/co-inhibitors appear to exert opposing actions on the lipid metabolism, leading to the predominance of lipolysis in embryos and lipogenesis in hatchlings. Some papers concerning the metabolic function of sirtuins have focused on SIRT1 as a key lipolysis regulating molecule, mainly in adipocytes (Khan *et al.* 2015; Maleki *et al.* 2024). Perhaps the visible increase in the *SIRT1* mRNA expression during embryogenesis is related to the lipolysis process. In the liver, SIRT1 may act as a detector of nutrient availability via NAD+, promoting gluconeogenesis and increasing the glucose production during fasting, i.e. caloric deficit, while decreasing the level of glycolysis by deacetylating PGC1α. An increased glucose level results in a decrease of SIRT1 protein levels, while pyruvate increases them. The SIRT1 protein levels are likely regulated in a post-translational manner, because the *SIRT1* mRNA levels do not change during this process (Hallows *et al*. 2012). In addition, SIRT1 deacetylates FOXO1 which then interacts with PGC1α (Park *et al.* 2010). PPARγ is also likely involved in the SIRT1-dependent regulation of glucose homeostasis (Yu *et al.* 2016). Moreover, SIRT1 activates AMPK and PPARα to reduce the fatty acid synthesis and stimulate fatty acid oxidation, thus playing a role in hepatic lipid metabolism. Adiponectin is an important intermediary in the interaction between SIRT1, AMPK and PPARα (Iwabu *et al.* 2010), and AMPK regulates the PI3K/AKT/mTOR pathway, which acts through FOXOs to regulate lipogenesis (Laplante & Sabatini 2009). The SIRT1 levels increase in healthy livers during a high-fat diet, in a manner similarly to those maintained in a caloric deficit. Studies in rodents have shown that calorie restriction increases the *SIRT1* expression in various tissues such as the brain, kidney, liver, white adipose tissue and the skeletal muscle. The first study using a SIRT1 knock-in mouse model showed that mice overexpressing *SIRT1* are leaner, more glucose tolerant, and show reduced blood cholesterol, adipokines and insulin levels compared to wild-type control mice (Bordone *et al.* 2007). Another study showed that SIRT1 transgenic mice are resistant to hepatic steatosis and insulin resistance (Pfluger *et al.* 2008). The next detected sirtuin – *SIRT3* mRNA expression – was very low in the embryonic livers. This sirtuin plays key role in the protection of the liver against lipotoxicity (Zhang *et al.* 2020). SIRT3, which is a mitochondrial NAD+-dependent deacetylase, regulates the acetylation status and activity of many substrates involved in energy metabolism during fasting, as well as fatty acid oxidation while a caloric deficit is maintained. The activation of AMPK by SIRT3 inhibits lipogenesis and protects against lipotoxicity. The *SIRT6* mRNA expression was significantly higher in the oldest analysed chicken livers, but in this case the differences were not so visible as with another sirtuins. SIRT6 negatively regulates glycolysis, triglyceride synthesis and fat metabolism, which provides protection for the liver (Ka *et al.* 2017). An overexpression of *SIRT6* protects against the disruption of glucose homeostasis and increases insulin sensitivity (Naiman *et al.* 2019). *SIRT6* also activates PPARα to promote fatty acid β-oxidation, thereby reducing the liver's fat content. *SIRT6* is regulated by *SIRT1* and *FOXO3a*. The interaction between *FOXO3a* and *SIRT6* may result in a lowering of LDL levels (Anderson *et al.* 2015). In our study, the *SIRT7* mRNA expression was lower in the embryonic livers than in the D0, D14 and 32W livers, and the expression in the 32W chicken livers was significantly higher than the others. This sirtuin positively affects the pathways that are involved in fatty acid uptake and triglyceride synthesis. Nonetheless, the role of *SIRT7* is still under debate in studies on the development and progression of non-alcoholic fatty liver disease (NALFD) (Shin *et al.* 2013).

The immunolocalisation of SIRT1 and SIRT7 in the embryonic and 32-week old birds livers show that both sirtuins are expressed in the liver tissue and that the localisation in the hepatocytes is cytoplasmatic, not nuclear. Surprisingly, in the ovarian stroma of the 32-week old chickens, we observed a nuclear localisation of SIRT1 and SIRT7. Sirtuins are localised in specific compartments of the cell. According to data from the literature, SIRT1 and SIRT2 are localised

in the nucleus and cytoplasm. Furthermore, SIRT3, SIRT4 and SIRT5 are mitochondrial, while SIRT6 and SIRT7 are nuclear (Kida & Goligorsky 2016). We need more results from other tissues to explain this phenomenon, but it seems that the localisation of sirtuins in the cell may be tissue-specific.

Glucose and lipid metabolism are regulated by sirtuins. In ruminants, gluconeogenesis is the main biochemical pathway through which glucose is obtained. Ghinis-Hozumi *et al.* (2011) determined the expression patterns of SIRT1 and SIRT3 in the bovine liver, muscles and adipose tissue. They found that the highest expression of *SIRT1* mRNA was observed in the liver, whereas the lowest was in muscles. *SIRT3* was highest in the muscles, while their was lowest in the adipose tissue (Ghinis-Hozumi *et al.* 2011). The detection and localisation of SIRT1 is a part of many studies concerning the pathogenesis of liver damage and disfunctions. There is increasing evidence showing that SIRT1 significantly contributes to the protection against hepatitis and related injuries (Zhou *et al.* 2020). For example, Yin *et al.* (2014) found that mice with a liver-specific deletion of SIRT1 were hypersensitive to ethanol. The removal of SIRT1 from the liver may lead to steatosis, inflammation or fibrosis (Yin *et al.* 2014). Another study showed that hepatic stellate cells (HSCs) in SIRT1 knockout mice were more susceptible to long-term and repeated ethanol-induced liver fibrosis (Ramirez *et al.* 2017). Isaacs-Ten *et al.* (2022) found that SIRT1 may contribute to liver inflammation and liver damage after an LPS/GaIN and bile duct ligation, which is associated with the activation of inflammasomes in macrophages (Isaacs-Ten *et al.* 2022). These conflicting results suggest that SIRT1 may be a promising target for a therapeutic intervention in inflammatory liver diseases, but further research is needed. Sirtuins act as regulators of many metabolic processes, as SIRT1 stimulates gluconeogenesis, reducing glycolysis similarly to SIRT6. In turn, SIRT2 can stimulate the glucose uptake and tolerance (Watanabe *et al.* 2018) and also increase insulin sensitivity in a manner similarly to SIRT1 and SIRT6 (Lemos *et al.* 2017). Triglyceride synthesis is reduced by SIRT6 and SIRT7 (Ding *et al.* 2017). SIRT6, along with SIRT1, SIRT3 and SIRT5, also affects fatty acid β-oxidation through the action of PPARα (Naiman *et al.* 2019). In addition, SIRT4 inhibits its fatty acid oxidation by repressing PPARα (Laurent *et al.* 2013). Furthermore, SIRT4 reduces the mitochondrial gene expression, while SIRT3 is involved in maintaining mitochondrial integrity by protecting against ischemic injury (Xie *et al.* 2017).

To summarise, the results obtained in this study indicate that during development the expression of sirtuins changes, and they are also localised in different regions of the cells in different organs. Thus, the avian model may be very helpful in gaining knowledge about the mechanism of action of sirtuins, which can be used in anti-aging medicine and in the treatment of metabolic diseases.

Author Contributions

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

Conflict of Interest

The authors declare no conflict of interest.

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