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Physical contact with male influences uterovaginal junction and sperm storage tubule development in Japanese quail (*Coturnix japonica*) at the onset of the laying cycle

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ABSTRACT

The sperm storage tubules (SSTs) in the uterovaginal junction (UVJ) of female birds enable long-term sperm storage and are essential for fertility. This study examined how a two-week exposure to a fertile male affects SST morphometry, the incidence of PCNA+ cells (proliferating), and the expression of matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9) in the UVJ of Japanese quail hens. At the beginning of experiment, 8 females with no prior physical or visual contact with a fertile male were selected, along with 8 hens exposed to a fertile male for 14 days. All hens were 42 days old at the beginning of experiment and has begun laying. At 56 days of age, hens from both groups were sacrificed and the UVJ was collected for morphometric, immunohistochemical and gene expression analyses. The results showed that the hens exposed to the male had significantly higher numbers, length and width of SSTs ($p < 0.05$). The luminal epithelium was thicker, and uterovaginal folds were longer in this group ($p < 0.05$). PCNA immunostaining revealed a higher incidence of proliferating cells in male-exposed hens ($p < 0.05$), indicating increased cellular activity within SSTs. Although MMP2 and MMP9 mRNA expression did not differ between groups, immunofluorescence revealed a stronger protein signals in hens exposed to males. The presence of males and mating significantly influences the development of SSTs in quail hens at the beginning of the laying cycle, which can promote a higher capacity for sperm storage.

HIGHLIGHTS

- Exposure to males improves sperm storage tubules development.
- Mating increases cell proliferation in sperm storage tubules.
- Higher capacity for sperm storage may reduce need for mating.

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Sperm storage tubules; morphology; PCNA; MMPs; quail

Introduction

Due to decreasing levels of fertility and hatchability in broiler breeders (Cash et al. 2025), factors associated with fertility rates are a current topic in today's poultry production. Therefore, the influence of factors on reproduction capacity is currently being studied.

One of the main factors influencing this capacity is the ability of hens to store sperm in their reproductive tract (oviduct). Sperm storage tubules (SSTs) are a unique feature of female birds that enables them to store sperm and produce hatching eggs. These tubules are located at the uterovaginal junction (UVJ) of the oviduct. Sperm stored in the SSTs remain fertile for between 2 and 15 weeks, depending on the avian species (Assersohn et al. 2024). Consequently, the sperm can migrate through the oviduct and fertilise eggs in the infundibulum (Matsuzaki et al. 2021; Yoshimura et al. 2025).

The development of SSTs and their sperm storage capacity play a role in overall fertility rates; therefore, factors influencing their development are being studied (Wen et al. 2020; Yang et al. 2020; Chai et al. 2024a; 2024b). Chai et al. (2024a) examined the development and morphology of SSTs in laying hens with high and low sperm storage capacities. A high storage capacity was found to be significantly and positively correlated with the number and surface area of the

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SST, as well as with the proportion of the SSTs containing sperm. Similarly, Kheawkanha et al. (2021) found an association between higher SST capacity and the number of epithelial cells, the height of these cells, the cross-sectional area of the inner lumen and the total cross-sectional area of the SSTs. Fold height and the total area of the UVJ were positively associated with higher fertility rates in younger hens (Yang et al. 2021). The morphological development of SSTs plays an inevitable role in the fertility of the parent flock, and, based on the current literature, significant impacts on their function and morphology are exerted by photo stimulation (Bakst et al. 2010), genotype (Assersohn et al. 2024), and the age of the parent flock (Kheawkanha et al. 2021; Yang et al. 2021). However, little is known about the factors that influence the early development of SSTs, such as the presence of males and the influence of mating on SST development.

Development of tissues involves cell proliferations and remodelling of the extracellular matrix (ECM). One of the crucial markers of cell proliferation is the proliferating cell nuclear antigen (PCNA), which is a polymerase delta cofactor essential for DNA damage repair during DNA synthesis (Bravo and Macdonald-Bravo 1987). In turn, a key factor responsible for ECM remodelling are matrix metalloproteinases (MMPs), a large family of endopeptidases that are zinc- and calcium-dependent and responsible for degrading ECM components, including collagen, laminin, and fibronectin. Through this activity, MMPs contribute to various physiological processes, including angiogenesis and cell proliferation, death, migration, and adhesion. MMP2 (gelatinase A) and MMP9 (gelatinase B) are particularly important in degrading type IV collagen, which is a major component of basement membranes. This facilitates structural reorganisation and cellular migration (Visse and Nagase 2003; Murphy and Nagase 2008). In the chicken oviduct, MMPs activity has been linked to tissue regression, repair, development, and differentiation (Leśniak-Walentyn and Hrabia 2016a, 2016b; Hrabia 2021). As SST formation and maintenance require the remodelling of epithelial and stromal tissues, MMP2 and MMP9 may play a crucial role in this process and in creating an environment that is conducive to sperm storage, survival, and subsequent release. However, the information about these enzymes in UVJ and/or SST development and their regulation in response to male stimulation remains unknown.

We hypothesised that physical contact with males and mating stimulate early SST development by enhancing epithelial proliferation and tissue remodelling in the UVJ. The aim of this study was therefore, to evaluate the impact of physical contact with male on SST development in meat-type Japanese quail (*Coturnix japonica*) at the start of the laying cycle. Accordingly, the histological characteristics, cell proliferation, and expression and localisation of key gelatinases (MMP2 and MMP9) were examined in UVJ tissues of quails exposed and not exposed to males.

Materials and methods

Animals and treatments

The experiment was carried out at the Department of Animal Breeding, Faculty of AgriSciences, Mendel University in Brno. For the experiment the meat type Japanese quails (*Coturnix japonica*) were used. The animals used in the experiment were obtained from the university's breeding facility. A total of 60 hatching eggs were placed in an incubator to produce the animals required for the experiment. After hatching, the quails were kept in cages with *ad libitum* access to water and feed. Immediately after gender recognition at 21 days of age based on different colouration of the breast, the males were separated from the females and kept without visual contact. For the experiment, clinically healthy females and males were chosen. All laying hens included in the experiment have already started laying eggs. At 42 days of age, eight females were put into four cages (two females per cage). Another four cages contained two females and one male each. In total, 16 females and 4 males were used in the experiment. After the groups were formed, it was visually verified that mating was taking place between males and females. During the experimental period, eggs were collected and then put in the incubator and after 7 days were evaluated the fertility rates to ensure that all males used in the experiment were fertile. The fertility rate of the eggs collected from the hens with the males was 95%, which confirmed that the used males are fertile. For a period of 14 days, all the animals were kept with no visual contact between the cages of both groups. Both groups had unlimited access to water provided *via* nipple drinkers and a commercial feed mixture. Lighting regime was 8 h of darkness and 16 h of light with 20 lx intensity. After 14 days, all the females (eight with no physical and visual access to the male and eight with physical contact with the male) were sacrificed by cervical dislocation, after which their reproductive systems were carefully removed. The uterovaginal junction, where the uterus meets the vagina, was identified in the oviduct and a 5 mm width section of this junction was

obtained from each hen. The subsequent processing of the tissues is described in the following sections.

Histological and morphometry characteristics of uterovaginal junction (UVJ) tissue

UVJ tissue fragments were fixed in 10% formalin in phosphate-buffered saline (PBS) for a period of 24 h, after which they were embedded in paraffin. Subsequent to this, the UVJ sections (6 μm thick) were cut on a microtome and stained with haematoxylin and eosin. The characteristics of the UVJ were then subjected to microscopic evaluation using Olympus BX51 microscope with PROMICAM 3-5CP digital camera (5Mpx SONY PREGIUS C MOUS USB 3). Morphometric analyses were performed according to methodology described by Kheawkanha et al. (2021), with small modification, using software Quick PHOTO MICRO 3.2. (Promicra s.r.o., Prague, Czech Republic). As part of the evaluation of morphometric analyses, the researcher was not informed about which group was involved, i.e. the samples were blinded. The evaluation encompassed the following: the number of folds (the number of folds visible from the cross section of the UVJ); the length of folds (the length of 10 randomly selected folds measured from the bottom to the top); the thickness of the luminal epithelium (measured in 10 randomly selected folds from each hen); the number of SSTs (the number of SSTs visible on the cross section of the UVJ); the length of SSTs (the length of 10 randomly selected SSTs measured from the bottom to the upper edge, including the lumen); and the width of SSTs (the width of 10 randomly selected SSTs measured from each hen).

Evaluation of cell proliferation by proliferating cell nuclear antigen (PCNA) immunohistochemistry

Immunohistochemical staining of PCNA protein was performed as described by Socha and Hrabia (2019), Grzesiak et al. (2020), Grzegorzewska et al. (2020), and Gumułka et al. (2022). Initially, UVJ tissue sections were deparaffinized in xylene and rehydrated by passing through a series of graded ethanol dilutions. Following this, the sections were rinsed in water and treated with 0.5% (v:v; 10 min) H_2O_2 in methanol to block endogenous peroxidase activity. After washing in water, the slides were subjected to heat-induced epitope retrieval by microwaving them in a 0.01 M citric acid buffer solution (pH 6.0). Non-specific binding of the secondary antibody was then blocked by incubating the slides in a solution of 5% normal goat serum in Tris-buffered saline containing 0.1% Tween 20 (TBST, pH 7.4). The sections were then incubated for 60 min at 37 °C with a monoclonal mouse antibody (clone PC10) against rat PCNA (dilution 1:150), followed by incubation with a secondary biotinylated goat anti-mouse IgG (dilution 1:300). The clone PC10 recognises PCNA in all the vertebrate and insect species that have been tested. Rat and chicken PCNA proteins share 94.3% identity. The colour reaction was developed through incubation with a solution of 3,3′-diaminobenzidine (DAB) and H_2O_2. In addition, the sections were stained with haematoxylin QS. After washing, they were dehydrated and mounted with Dibutylphthalate Polystyrene Xylene (DPX). Negative control slides were prepared by replacing the primary antibody with non-immune mouse IgG. The slides were examined using an Axio Scope A1 light microscope and photographed using an Axiocam 503 colour camera and ZEN 2.3 Pro software (Carl Zeiss, Germany). Moreover, UVJ sections were viewed using a 40x objective and PCNA-positive cell frequencies were analysed using a computerised image analysis system (Multi ScanBase v.14.02, Computer Scanning System). PCNA+ cells were counted in SSTs per two cross-sections of UVJ tissue and counts were averaged per bird and bird-level means ($n = 8$/group) were used for statistical analysis. Every SST was examined in a blinded manner. In total, 63 SSTs were examined in the group without males and 106 SSTs in the group with males. The results are expressed as the number of proliferating cells per SST.

Evaluation of MMP2 and MMP9 protein localisation by immunofluorescence

Immunofluorescence assay for MMP2 and MMP9 proteins was performed according to Hrabia et al. (2019) and Gumułka et al. (2022) with minor modification. Firstly, UVJ tissue sections were treated in the same way as described for PCNA localisation. After that, sections were incubated overnight in 4 °C in the presence of polyclonal rabbit antibody against MMP2 or MMP9 (1:100; Abcam, Cambridge, UK), followed by 90 min incubation with the fluorescent secondary antibody DyLight 594 goat anti-rabbit (1:150; Vector Laboratories) and mounted with VECTASHIELD® Vibrance™ Antifade Mounting Medium with DAPI (Vector Laboratories). Negative control slides were prepared by replacing the primary antibody with non-immune rabbit IgG. The sections were analysed using an Axio Scope.A1 fluorescent microscope, photographed with an Axiocam 503 colour camera, and

analysed using ZEN 2.3 Pro software (all from Carl Zeiss). The intensity of immunopositive signals was assessed as strong, moderate, weak, and very weak. The resulting micrographs shows a composite image where blue fluorescence from DAPI staining indicates cell nuclei, while red fluorescence represents an immunopositive reaction specific to MMP2 and MMP9.

Total RNA extraction, reverse transcription, and quantitative real time polymerase chain reaction (qRT-PCR)

RNA isolation, reverse transcription (RT), and qRT-PCR analyses were performed according to Wolak and Hrabia (2020). In brief, total RNA was extracted from the tissues using TRI reagent and 2 μg of total RNA from each sample was reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), which included random primers. The obtained cDNA (after 10 X dilution) was used in qRT-PCR for *MMP2* (Genebank accession no. UO7775.1; TaqMan probe FAM5′ →3′NFQ: CCTGGCCCTGGTCCTG; amplicon size of 69 bp), *MMP9* (assay ID: Gg03338324_g1; GenBank accession no. AF222690.1; amplicon size of 78 bp), and *18S ribosomal RNA* (*18S rRNA*; housekeeping gene) in a 96–well thermocycler (QuantStudio 3 Real Time PCR; Applied Biosystems) according to the recommended cycling program: 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s. Duplex PCR was performed for the genes under investigation and the reference gene (*18S rRNA*) in a 10 μL volume containing 5 μL of TaqMan Gene Expression Master Mix, 0.5 μL of TaqMan Gene Expression Assay containing a specific TaqMan MGB probe and a pair of primers designed by Applied Biosystems, 0.5 μL of Eukaryotic *18S rRNA* Endogenous Control containing a pair of primers and a TaqMan probe labelled with VIC/TAMRA, 3 μL of water, and 1 μL of cDNA. Each sample was run in duplicate. A no-template control was included in each run. Relative quantification of the examined genes was calculated after normalisation with the *18S rRNA* transcript (commonly used reference gene for tissues of the reproductive system of female and male birds), and by employing expression in the UVJ tissue of quails kept without a male as the calibrator using the $2^{-\Delta\Delta}$Ct method.

Statistical analysis

The Shapiro-Wilk test was used to determine whether the data followed a normal distribution. The homogeneity of variances was verified using Levene's test. Since the data passed the Shapiro-Wilk test and Levene's test, the Student's t-test was used to compare the means of the two groups (no male vs. with male). A $p < 0.05$ level was identified as statistically significant. Calculations were performed using GraphPad Prism 8.0.1. (GraphPad Software, Inc., La Jolla, CA, USA). Results are presented as the mean ± standard deviation (SD). One quail represented the experimental unit for all considered parameters.

Results

Because the experimental group experienced physical contact, mating behaviour, and sperm transfer simultaneously study setup does not permit to discriminate between these distinct variables. Consequently, the results attributed to mating, male presence or physical contact should be interpreted as a collective effect of these combined stimuli rather than a specific response to any single cue.

Effect of male on morphological and histological characteristics of UVJ tissues

Figure 1A–D shows representative micrographs of histological staining of UVJ of hens caged with males and without males. The number of folds in UVJs (Figure 1E) did not differ significantly between the groups ($p > 0.05$); however, the mean fold length was significantly greater in hens with males than in those without males ($p < 0.05$; Figure 1F). Similarly, the luminal epithelium of the UVJ folds was thicker in the group exposed to males ($p < 0.05$; Figure 1G). A significant difference was also observed in the organisation of SSTs. There were more frequent SSTs in hens with males than in hens without males ($p < 0.05$; Figure 1H). Furthermore, the male-exposed group had SSTs that were both longer and wider ($p < 0.05$; Figure 1I,J).

Cell proliferation

Immunohistochemical staining of proliferating cells (PCNA-positive) showed their presence in the SST epithelium in the UVJ sections (Figure 2). The presence of male had a significant effect on the number of proliferating cells in the SSTs. The number of PCNA+ cells in SSTs was higher ($p < 0.05$) in group of hens with males (1.90 ± 0.69) than in group without males (0.85 ± 0.31).

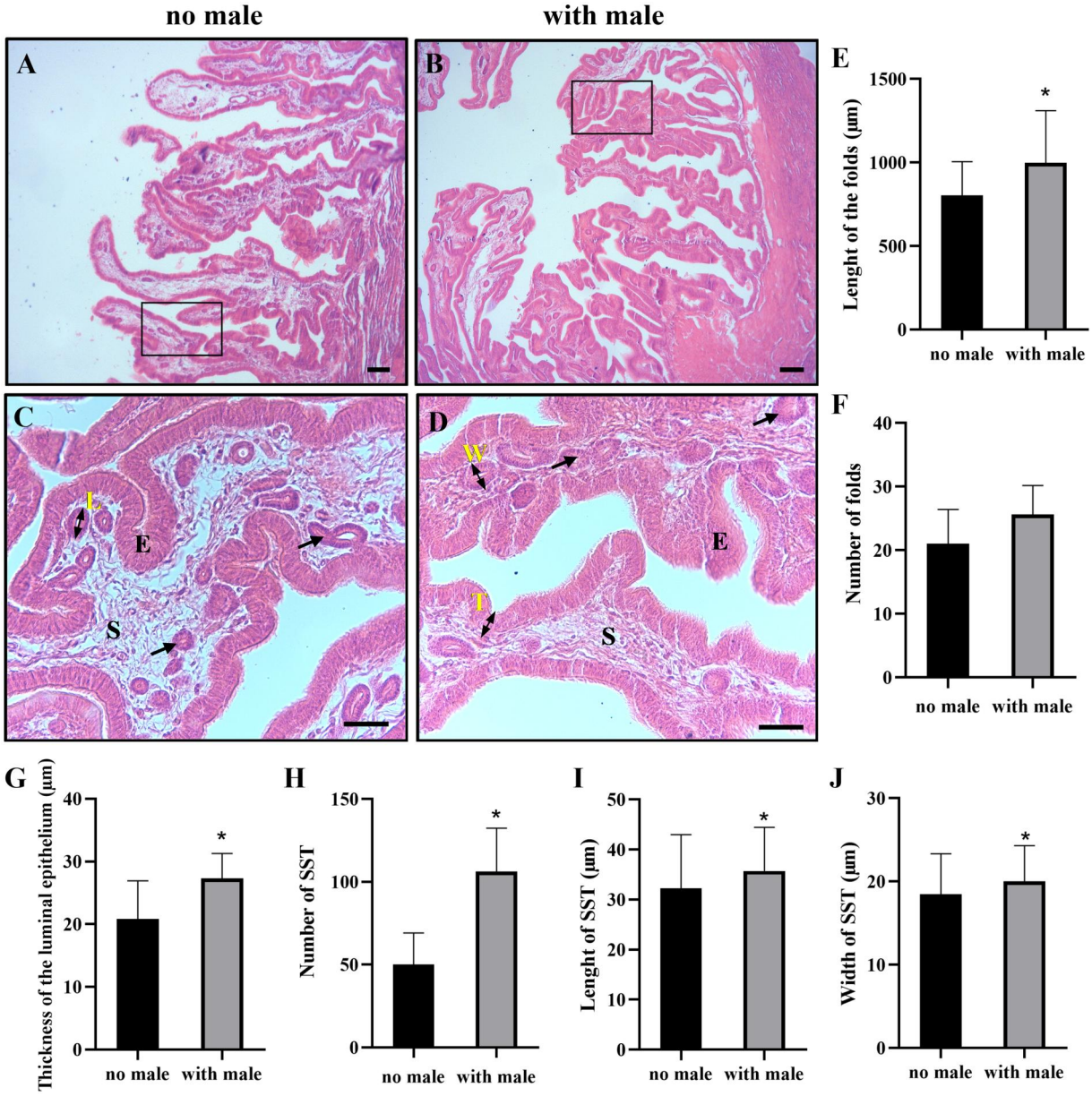

Figure 1. Histoarchitecture (haematoxylin and eosin staining) of representative sperm storage tubules (SST; black arrows) in utero-vaginal junction of quails (A–D). S: stroma (muscles + connective tissue); E: luminal epithelium; L: length of SST (black double-headed arrow); W: width of SST (black double-headed arrow); T: thickness of luminal epithelium (black double-headed arrow). Scale bars = 50 µm and 20 µm. (E–J) represent the quantitative measurements of histomorphometry parameters. Asterisks (*) indicate statistically significant differences between groups ($p < 0.05$).

Expression of mRNA and localisation of MMP2 and MMP9 proteins in UVJ

The relative expression [RQ] of MMP2 and MMP9 mRNA transcript in the UVJ of hens of both groups is shown in Figure 3A,B. There were no differences and trends ($p > 0.05$) in MMP transcript abundances between the hens caged with males and without males.

Immunofluorescent staining of MMP2 protein in the UVJ (Figure 3C,D) revealed positive reaction in cells and/or in the ECM of the luminal epithelium of the UVJ folds and in SSTs. As estimated subjectively, strong immunoreactivity was observed in the luminal epithelium of UVJ in birds of both groups (Figure 3C,D), as well as in SSTs of quails exposed to males (Figure 3D). Moderate or weak immopositive signals for MMP2 protein were seen in SSTs of quails kept without males (Figure 3C). Strong immunopositive reaction for MMP9 protein was localised in the ECM of the UVJ stroma, particularly around SSTs in quails of both groups (Figure 3F,G). A negative control sections incubated without primary antibodies

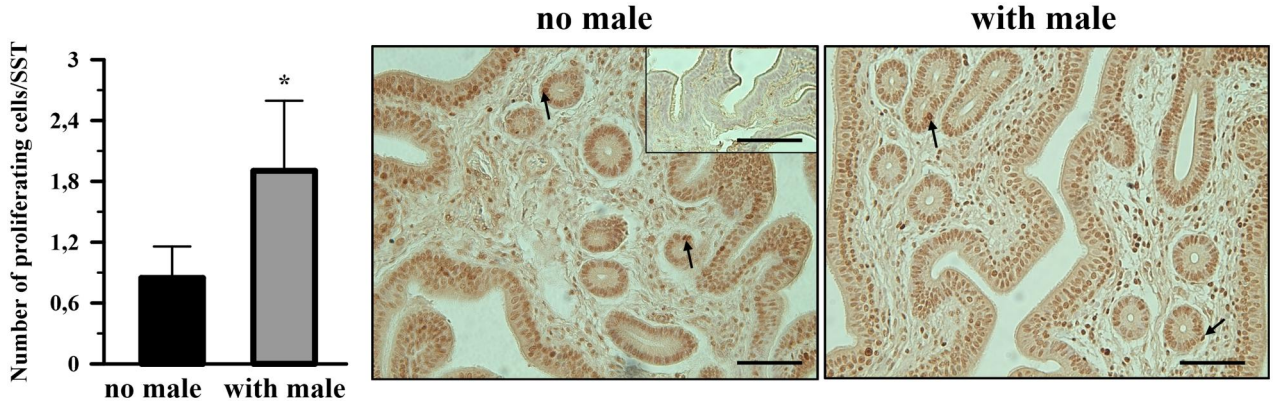

Figure 2. Immunohistochemical localisation of PCNA-positive cells in the sperm storage tubules (SST) of quails. Arrows depict proliferating cells (deep brown). Insert in micrograph indicates negative control. Bar = 20 μm. Chart represents the proliferating index of the cells in SSTs. Asterisk (*) indicates statistically significant differences between groups ($p < 0.05$).

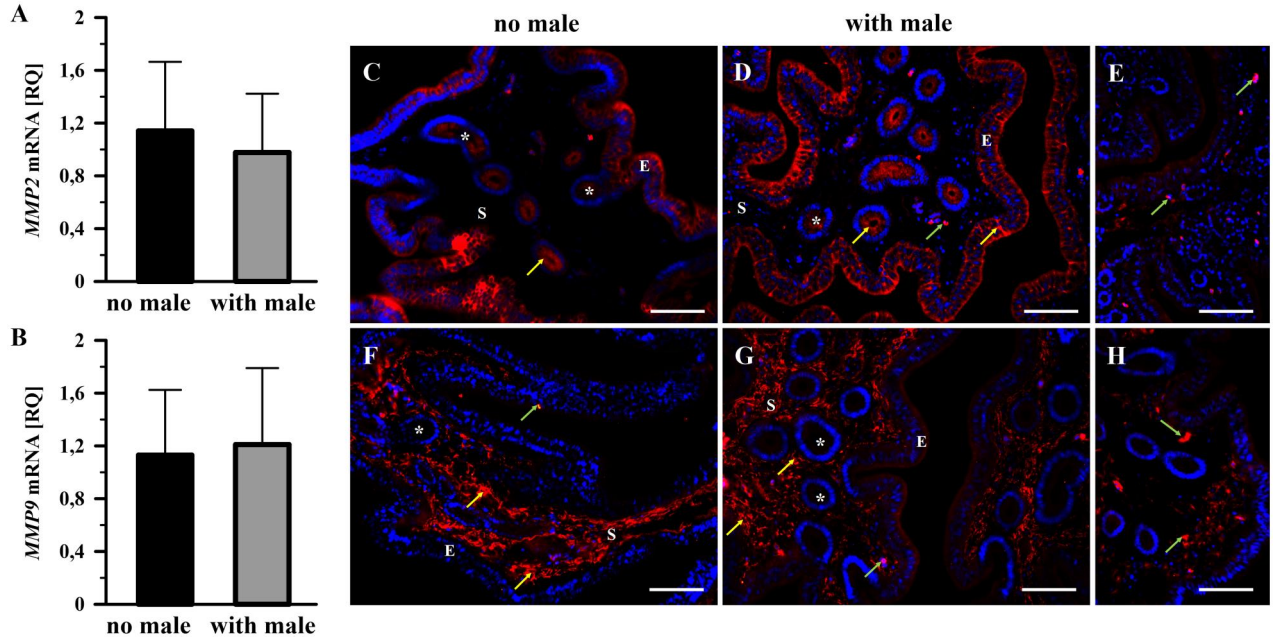

Figure 3. Relative mRNA expression and immunofluorescent localisation of the MMP-2 (A, C, D) and MMP-9 (B, F, G) protein in the sperm storage tubules of quails. (E and H) show negative controls. Yellow arrows indicate immunopositive reaction. Green arrows indicate non-specific reaction. S: stroma (muscles + connective tissue); E: luminal epithelium; asterisks (*), sperm storage tubule. Scale bars = 20 μm.

did not exhibit positive staining, excluding red blood cells which always show non-specific fluorescence (Figure 3E,H).

Discussion

We hypothesised that physical contact and mating stimulate early SST development by enhancing epithelial proliferation and tissue remodelling in the quail uterovaginal junction UVJ. To test this theory, the UVJ morphometric characteristics, cell proliferation, and expression of MMP2 and MMP9 mRNA transcripts were evaluated in females exposed to males and in females isolated from males at the beginning of the laying cycle.

The most significant impact of mating and the presence of males was observed in morphometric analyses, where males stimulated the development and increased the number of SSTs. These results can be associated with the preparation of the female reproductive tract for the production period. For practical purposes in parent stock, it is important to know whether these changes are transient adaptations or stable anatomical changes. It can be assumed, that in parent stock, both sexes are in constant contact, meaning that this physical stimulus is continuous, so it

can be assumed that the effect should be long-term. The development of SST is important from the perspective of the proven significance of SST in the reproduction of the parent flock. SSTs are important for fertility, as hens from the high-fertility line were found to have more SSTs, than hens from the low-fertility line (Brillard et al. 1998; Yang et al. 2021). Therefore, factors affecting the early development of SSTs should be considered to ensure a high fertility rate in the laying cycle (Sasanami et al. 2013). Other factors have been described as influencing the development of the uterovaginal junction in poultry. Photostimulation is the main breeding measure for developing the reproductive system. Bakst et al. (2010) demonstrated that hens experienced a gradual increase in the number and length of folds in the uterovaginal junction after the increase of daylight length to 16h. However, no significant differences in the number of SSTs were observed after photostimulation. The impact of mating has only been shown to quickly upregulate immune-modulatory and pH-regulatory genes within 24 h after mating, supporting sperm survival in the reproductive tract (Atikuzzaman et al. 2015). Other studies were dedicated to study impacts of insemination. For instance, Kosonsiriluk et al. (2023) observed that insemination whether *via* sperm or sham did not affect the expression of development-related genes in turkeys until two days post-treatment. The effects of insemination on the number and size of SSTs were described in studies where repeated insemination was shown to decrease the number of SSTs and accelerate ageing at the uterovaginal junction (Das et al. 2005; Kosonsiriluk et al. 2023). In the current study, 14 days of constant exposure to a male had a significant impact on the development of the uterovaginal junction and SSTs of quails at the start of laying. For this reason, earlier exposure to males may be beneficial at the onset of the laying cycle; however, further experiments would be necessary to evaluate the long-term effects of physical contact with male on SST development. It can be speculated that the presence of sperm in the uterovaginal junction does not only play a role in SST development, but that physical contact with the male is an additional factor which further supports SST development. Nevertheless, there are no articles that support this speculation. Additionally, the distinction between the effects of mating and the presence of males is unclear due to lack of experimental separation.

To better understand the process of SST development, subsequently in the study, the proliferating cells were identified in SSTs by PCNA immunostaining. It was found that hens exposed to the contact with males had a significantly higher number of proliferating cells in SSTs, than those kept without males. This finding lends further support to the hypothesis that the presence of males may promote cellular proliferation within SSTs, leading to SST enlargement and an increase in their functional capacity for sperm storage. It is also possible that this may contribute to enhancing the ability of SSTs to support sperm maintenance, viability, and regulated release. Similar effects of male stimulation on oviductal proliferation have previously been reported in quails, where mating has been shown to increase epithelial cell turnover and SST development (Ito et al. 2011). Enhanced proliferative activity could, therefore, be related to the physiological preparation of the female reproductive tract for sperm reception and storage over a prolonged period.

Besides cell division, critical to tissue development are changes in ECM architecture and composition, realised in large part by MMPs, the key players in the molecular communication between cells and the ECM. Thus, next in the study the patterns of gene expression and protein localisation of the most widespread MMPs were evaluated in uterovaginal tissue for the first time. By contrast to the number of PCNA+ cells, no significant differences were observed in *MMP2* and *MMP9* mRNA expression between the two groups of quails. Matrix metalloproteinases are key regulators of ECM turnover and have been implicated in tissue reorganisation during oviduct development, regression, and rejuvenation in chickens (Leśniak-Walentyn and Hrabia 2016a, 2016b). The lack of changes in *MMP* transcript abundance in the present study may suggest that the remodelling of oviductal SSTs associated with changes in SST proportions and activity may not depend on the transcriptional regulation of these enzymes, but rather on post-translational modulations, the local activation of latent proteins, and/or inhibition of active proteins. Alternatively, MMPs localised around SSTs (mainly MMP9), as well as in the cells (MMP2) may play a more prominent role in the formation or regression of SSTs than in the short-term physiological adaptation to the presence of a male. It is apparent that the immunofluorescent method is limited in its capacity to estimate protein quantity in tissue; its efficacy is primarily confined to the estimation of cellular localisation. Consequently, there is a necessity for further studies to be conducted in order to examine MMP protein levels by means of Western blotting, in addition to the measurement of MMP activity. The information obtained has the potential to

facilitate a more profound comprehension of the distinct function of MMPs in avian SSTs.

Based on the results of the current study, exposure to males during the final stage of rearing period could improve SST development. There is little information about the impact of mixed-sex rearing on the development of the reproductive tract. However, Broadus et al. (2022) conducted an experiment with ducks in which they gave drakes physical access to females during the breeding period. A second group of drakes only had visual contact with females. During the production period, the researchers evaluated egg fertilisation and testosterone levels in the drakes. Physical contact with females in rearing had no statistically significant effect on fertilisation rates or testosterone levels in males in production period. However, this study was limited by the fact that there was no group of drakes that had no contact with females, either physical or visual. This opens up scope for further research in connection with the results of the current study, which compared quails with physical contact only and quails with no physical and visual contact.

In summary, it is necessary to mention several limitations of the study. SSTs are a phenomenon specific to birds; however, in order to apply the results of this study to other poultry species, additional experiments should be performed. For the practical applicability of the results, it is necessary to conduct the experiment with a higher number of repetitions. A longer-term experiment is also necessary to study the long-term effect on SST and UVJ development after a single exposure to males. At the same time, the experimental procedure used in this study does not allow for the discrimination between the effects of physical contact, mating behaviour, sperm presence, or male-derived cues, so these influences act simultaneously and their individual effects have not been isolated.

Conclusion

This study demonstrates that the maturation of the uterovaginal junction and the development of SST in Japanese quail are modulated by social and sexual stimuli, suggesting a plastic reproductive system that responds to the presence of a male. By showing that male presence and mating behaviour significantly catalyse SST expansion and PCNA+ cellular activity, these findings suggest a practical management strategy: introducing males early at the onset of egg-laying may improve the female's sperm storage capacity to maximise early-stage fertilisation. However, because the current procedure does not decouple the individual effects of physical contact, pheromonal cues, and sperm presence, future research must utilise experimental setups to identify the specific drivers of this maturation. Furthermore, while these results provide a foundation for parent flock management, longitudinal studies across diverse poultry species are necessary to determine the long-term persistence of this effect and its broader applicability to avian reproductive science.

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Ethical approval

The use of animals in this study was approved by the Institutional Ethics Committee for Animal Studies at the Faculty of AgriSciences, Mendel University in Brno (protocol code: 57199/2020–MZE-18134).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data that support the findings of this study are available from the corresponding author, [VZ], upon reasonable request.

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