Brief Communication

Oocyte cumulus complex quality and oviduct transportation velocity in systemic autoimmune disease model mice

Marina Hosotani[1], Osamu Ichii[2,3], Takafumi Watanabe[1] and Yasuhiro Kon[2]

[1]Laboratory of Veterinary Anatomy, Department of Veterinary Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu 069-8501, Japan; [2]Laboratory of Anatomy, Department of Basic Veterinary Science, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan; [3]Laboratory of Agrobiomedical Science, Faculty of Agriculture, Hokkaido University, Sapporo 060-0818, Japan
Corresponding author: Marina Hosotani. Email: m-hosotani@rakuno.ac.jp

Impact Statement

Oocyte transportation consists of two steps: adhesion of cumulus cells to the ciliary tip of ciliated epithelial cells and transportation of cumulus–oocyte complexes (COCs) by ciliary beating. In the former step, the pathological factors that alter the interaction between cumulus cells and cilia are not currently understood. In this study, by using autoimmune disease-prone MRL/MpJ-*Fas*$^{lpr/lpr}$ mice that exhibited the oocyte transportation disorder by abnormal morphofunction of oviductal ciliated epithelium, we revealed that COC transportation property was determined by both the ciliary function in the infundibulum and the properties of COCs. Furthermore, we showed that the transportation velocity of COCs (TVCs) was recovered by the properties of cumulus cells and the healthy morphofunction of oviductal ciliated epithelium. These findings contribute to further investigations on novel immunological factors in COCs that can achieve efficient oocyte transportation and related processes, which provide the potential for understanding the pathogenesis of tubal infertility.

Abstract

Oocyte transportation by the oviduct involves the interaction between ciliated epithelial cells and cumulus cells. To determine whether the quality of cumulus–oocyte complexes (COCs) changes the transportation property of COCs, we compared the transportation velocity of COCs (TVC) by the infundibulum *ex vivo* with various combinations of infundibula and COCs collected from different mice. We used young and aged C57BL/6N and MRL/MpJ, and MRL/MpJ-*Fas*$^{lpr/lpr}$ mice as the strains with intact female reproductive function and the systemic autoimmune disease model exhibiting oocyte pick-up dysfunction owing to the morphofunctional abnormality of ciliated epithelium, respectively. The TVC of aged MRL strains was less than that of aged C57BL/6N mice, suggesting that aging affects the transportation of COCs in MRL strains. The TVC of aged MRL/MpJ-*Fas*$^{lpr/lpr}$ mice was the least among all examined combinations, whereas the TVC accelerated when the infundibulum or COCs were collected from other strains. These results indicate that the transportation property of COCs is determined not only by the ciliary function in the infundibulum but also by the properties of COCs.

Keywords: Autoimmune disease, cumulus cell, cilia, *ex vivo* experiment, oocyte transportation, oviduct

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Introduction

The mechanism of oocyte pick-up and transportation by the oviductal infundibulum has not been fully understood in mammalian species. Among the oviductal epithelium of the infundibulum, ampulla, and isthmus, the infundibulum and ampulla have the highest percentage of ciliated epithelial cells.[1] This histology indicates that ciliary beating is the primary factor facilitating oocyte transportation and pick-up by the infundibulum, whereas muscular peristalsis mainly maintains oocyte transportation in the isthmus.[2,3] In advance of the transportation of COCs by the ciliary beating of ciliated epithelial cells, the adhesion between cumulus cells and the ciliary tip appears to be a pivotal process in the oocyte transportation in the infundibulum. Therefore, we hypothesized that not only the ciliary function but also the physical or physiological quality of COCs influences oocyte transportation and the following success of oocyte pick-up in the infundibulum.

MRL/MpJ-*Fas*$^{lpr/lpr}$ (lpr) mice are models of severe systemic autoimmune disease and show abnormalities in female reproductive function due to local inflammation in the ovary

and oviduct, including premature ovarian failure, decreased number of ovulations, oocyte pick-up dysfunction, and abnormal morphofunction of ciliated epithelial cells.[4–6] In the infundibulum of lpr mice, a decreased number of ciliated epithelial cells, elongation of cilia, and slow and randomized oriented ciliary beating are involved in oocyte pick-up dysfunction.[5,6] However, whether the quality of cumulus cells affects the properties of oocyte transportation in lpr mice has not been investigated. In this study, we compared the TVCs by the infundibulum under *ex vivo* conditions with different combinations of infundibula and COCs collected from C57BL/6N (B6), MRL/MpJ (MpJ), and lpr mice; the former two strains were used as control strains with intact functioning of oocyte transportation and pick-up. This study revealed that both the abnormalities of ciliated epithelium and the quality of cumulus cells were involved in healthy oocyte transportation by the infundibulum.

Materials and methods

Animal experiments were approved by the School of Veterinary Medicine, Rakuno Gakuen University, Japan (approval no. VH19A6). The animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals, Rakuno Gakuen University. Female B6, MpJ, and lpr mice of three and six months of age were obtained from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). Previous studies have reported that autoimmune disease is more severely exacerbated in female lpr mice at six months of age compared with those at three months of age.[5,6] The mice were housed in groups in plastic cages at 18°C–26°C under a 12-h light/dark cycle with free access to a commercial diet and water. Pregnant mare serum gonadotropin (PMSG, ASKA Animal Health Co., Ltd., Tokyo, Japan) was injected intraperitoneally into the mice (200 µL of 37.5 IU/mL gonadotropin per mouse). Forty-eight hours after the PMSG injection, the mice were injected intraperitoneally with the same dose of human chorionic gonadotropin (hCG, ASKA Animal Health Co., Ltd.). The superovulation treatment procedure was based on that of a previous report.[7] Twenty-four hours after the hCG injection, all mice were euthanized by cervical dislocation under deep anesthesia using a mixture of medetomidine (0.3 mg/kg), midazolam (4 mg/kg), and butorphanol (5 mg/kg). The oviducts including the infundibulum and ampulla were removed and immediately placed in D-MEM (high glucose) with L-glutamine and phenol red (D-MEM) (FUJIFILM Wako Pure Chemical Co., Ltd.) at 37°C without any fixation. Under a stereomicroscope, the infundibulum was detached from the oviduct. The COCs were obtained from the ampulla using micro-dissecting scissors (Figure 1(A)). The infundibulum was placed on a glass chamber slide with a drop of D-MEM, and COCs were then added to the infundibulum epithelium using a glass cannula (Figure 1(B)). The glass slide was incubated at 37°C on a thermoplate (TPi-SX, Tokai Hit Co., Ltd., Shizuoka, Japan), observed under a phase contrast microscope (Nikon ECLIPSE E200LED, Nikon Co., Ltd., Tokyo, Japan), and recorded using a HAS-U1 high-speed camera (Ditect Co., Ltd., Tokyo, Japan) at 200 frames per second and a shutter speed of 1/100. The videos were cropped into 3 s fragments. The representative video of the COCs transportation in the combination of (6m B6-6m B6) was shown in **Movie 1**. The trajectory distance of three cumulus cells in focus (µm) was calculated using the Particle tracker plugin of the Image J software (National Institutes of Health, Bethesda, MD, USA) and averaged (Figure 1(C)). The transportation velocity of the COC (TVC) was calculated as follows: TVC (µm/s) = the average distance of the trajectory of cumulus cells in focus (µm)/3 (s). The combinations of infundibula and COCs collected from the mice are summarized in Table 1. In this experiment, no muscular peristalsis was observed. Results are expressed as mean ± standard error of the mean (SEM) and statistically analyzed in a non-parametric manner. Data from three or more groups were compared using Tukey's test ($P < 0.05$). Dunnett's test was used to compare multiple groups with the control ($P < 0.05$).

Results and discussion

Track images of the trajectory of cumulus cells in all the examined combinations are summarized in Figure 1(D). The TVC in the combinations of both infundibula and COCs collected from B6 mice at three or six months of age was approximately 5–7 µm/s (Figure 1(E)). In contrast, the TVC in the combinations of both infundibula and COCs collected from MpJ or lpr mice at three or six months of age was approximately 1–4 µm/s (Figure 1(E)). In particular, the TVCs in the combination of (6m MpJ-6m MpJ) and (6m lpr-6m lpr) were significantly less than that in the combination of (6m B6-6m B6). The TVC in the combination of (6m lpr-6m lpr) was the least of all combinations. The TVC of B6 mice tended to be greater than that of MRL strains. It was estimated that the ciliary function of the infundibulum of B6 mice had a higher baseline of TVC than MRL strains. The dynamics of COCs governed by ciliary beating and muscular peristalsis, and the luminal secretory flow controls the accurate timing of oocyte transportation in the ampulla.[8] Thus, the TVC changing the timing of oocyte transportation is one of the indices that determines the transportation efficiency of oocytes and accurate female reproductive function. Our results indicate that the transportation efficiency of oocytes by the infundibulum deteriorates with aging in the MRL strains. The morphological differences in the COCs between B6 and MRL strain mice were not significant under the stereomicroscope, while the number of cumulus cells composing the COCs of B6 mice at six months of age seemed fewer than that of other strains (Supplementary Figure 1). Aging leads to a decrease in oocyte quality in both humans and mice because of factors such as altered mitochondrial function and the expression profile of transcriptions.[9,10] The difference in the genetic background between B6 and MRL strain mice appears to amplify the effect of aging on COC quality. The ovulation phenotype is different between B6 and MRL strains at three months of age; under the superovulation treatment, MRL strains produced poorer quality oocytes with a lower fertilization rate compared to B6 mice.[11] Despite the limitation that there are no reports investigating the effects of aging on oocyte quality and the quality of cumulus cells between B6 and MRL strains, it is suggested that the property of cumulus cells governed by the genetic background determines the transportation efficiency of COCs in mice and that aging changes the properties of COCs in MRL strains more sensitively than in B6 mice.

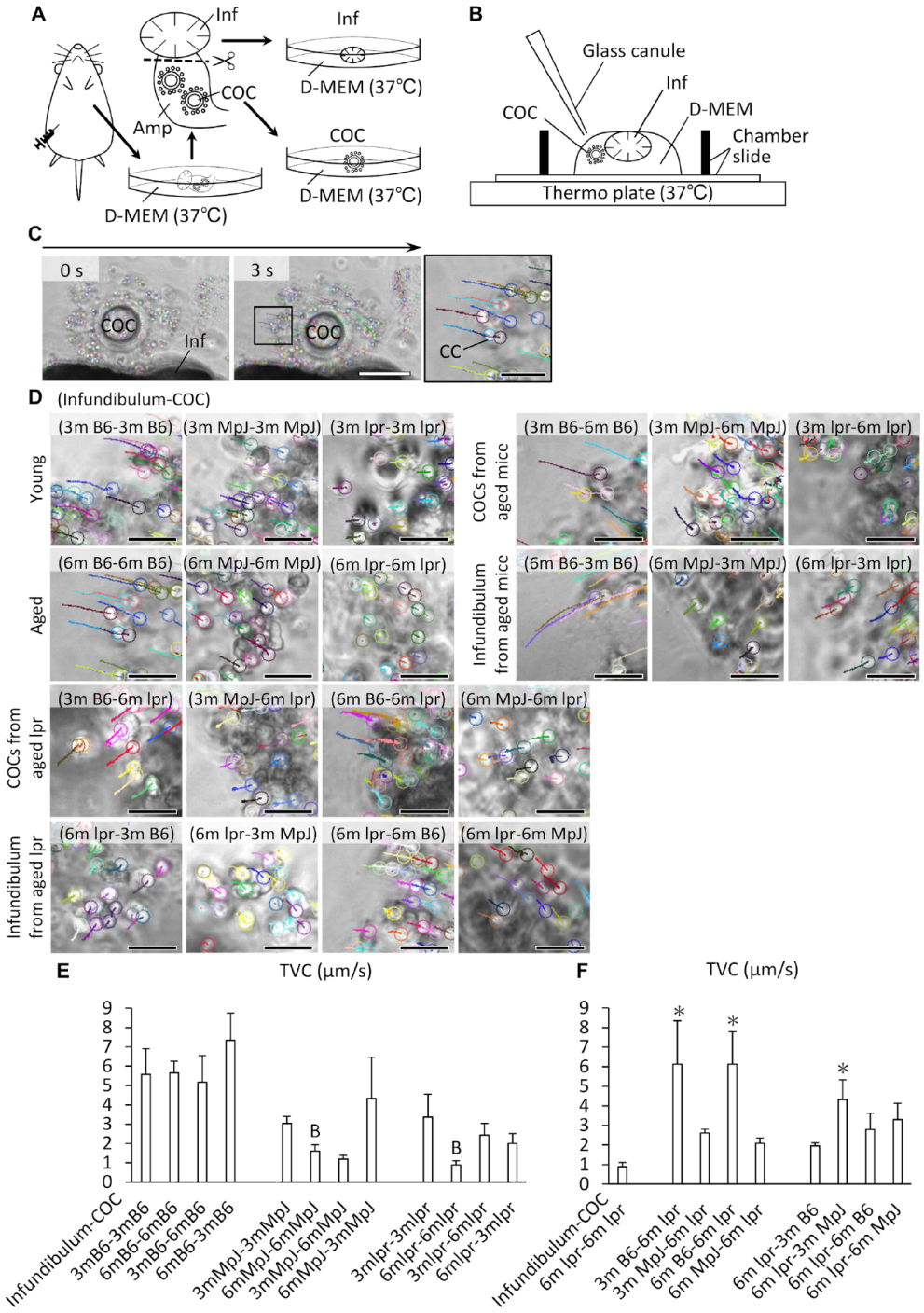

Figure 1. The *ex vivo* transportation of the cumulus–oocyte complex (COC) in various combinations of infundibula and COCs collected from C57BL/6N (B6), MRL/MpJ (MpJ), and MRL/MpJ-*Fas*$^{lpr/lpr}$(lpr) mice at three months of age (3m) and six months of age (6m). (A) Scheme of the sampling of infundibulum (Inf) and COCs from mice. D-MEM: D-MEM (high glucose) with L-glutamine and phenol red. (B) Scheme of the preparation for the observation of the *ex vivo* transportation of COCs by the infundibulum (Inf). (C) The representative cropped images of the trajectory of cumulus cells (CCs) are tracked by colored lines from 0 to 3 s. Black square is magnified in the right image. White bar = 100 μm. Black bar = 25 μm. (D) The trajectory of CCs in all combinations of infundibula and COCs. Black bars = 25 μm. (E) The transportation velocity of COCs (TVC) in the combinations of infundibula and COCs collected from strain-matched mice. B: Comparison with (6m B6-6m B6) (Tukey test, $P < 0.05$). $n = 4$ for all combinations. (F) The transportation velocity of COCs (TVC) in the combinations with either the infundibulum or COCs collected from lpr at six months of age. Amp: ampulla; Syringe: superovulation treatment; Scissor: cutting using micro-dissecting scissor; Inf: infundibulum.
*Comparison with (6m lpr-6m lpr) (Dunnett's test, $P < 0.05$). $n = 4$ for all combinations.

The infundibulum of lpr mice at six months of age shows oocyte pick-up dysfunction owing to the abnormal morpho-function of the ciliated epithelium.[6] To determine the detailed transportation property of the COCs by the infundibulum of lpr mice at six months of age, we examined the TVC in the infundibula collected from these mice and transported the COCs collected from other strains. The TVC in the combinations of (6m lpr-3m B6), (6m lpr-3m MpJ), (6m lpr-6m B6), and (6m lpr-6m MpJ) tended to be faster than that of (6m lpr-6m lpr) (**Movie 2**), especially for COCs collected from MpJ

Table 1. Combination of infundibulum and cumulus–oocyte complexes collected from C57BL/6N, MRL/MpJ, and MRL/MpJ-*Fas*$^{lpr/lpr}$ at three and six months of age.

Group	Combination (infundibulum-COCs)
Young	(3m B6-3m B6), (3m MpJ-3m MpJ), (3m lpr-3m lpr)
Aged	(6m B6-6m B6), (6m MpJ-6m MpJ), (6m lpr-6m lpr)
COCs collected from aged mice	(3m B6-6m B6), (3m MpJ-6m MpJ), (3m lpr-6m lpr)
Infundibulum collected from aged mice	(6m B6-3m B6), (6m MpJ-3m MpJ), (6m lpr-3m lpr)
COCs collected from aged MRL/lpr	(3m B6-6m lpr), (3m MpJ-6m lpr), (6m B6-6m lpr), (6m MpJ-6m lpr)
Infundibulum collected from aged MRL/lpr	(6m lpr-3m B6), (6m lpr-3m MpJ), (6m lpr-6m B6), (6m lpr-6m MpJ)

COC: cumulus–oocyte complex; B6: C57BL/6N; MpJ: MRL/MpJ; lpr: MRL/MpJ-*Fas*$^{lpr/lpr}$; 3m: three months of age; 6m: six months of age.

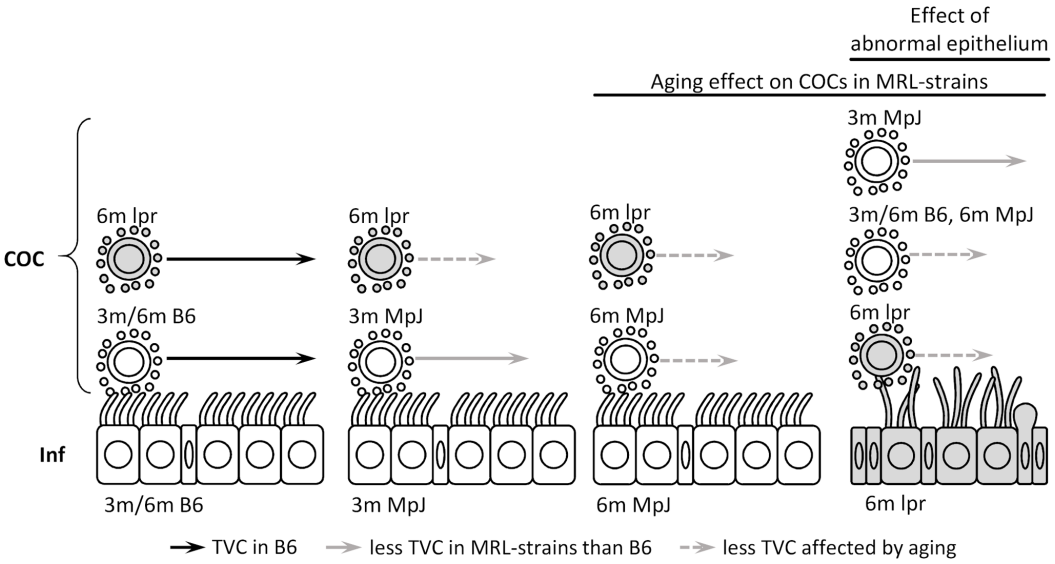

Figure 2. Summary of transportation velocity of cumulus–oocyte complexes (TVC). The infundibula of C57BL/6N (B6) mice at three months of age (3m) and six months of age (6m) transported COCs collected from MRL/MpJ-*Fas*$^{lpr/lpr}$(lpr) mice of six months of age at a healthy speed. The TVC of the infundibulum of MRL/MpJ (MpJ) and lpr at six months lost speed with aging, while the infundibulum of lpr at six months transported COCs collected from MpJ at three months at a healthy speed.
Inf: infundibulum.

at three months of age (**Movie 3**), which had a significantly increased TVC (Figure 1(F)). These results indicate that even the infundibulum with abnormal ciliary morphofunction has the potential to efficiently transport COCs collected from healthy mice. In addition, the significant recovery in the TVC in the combination of (6m lpr-3m MpJ) but the insignificant recovery in the combination of (6m lpr-3m B6) and (6m lpr-6m B6) implies a strain-dependent interaction between the COCs and the ciliated epithelium of infundibulum.

To determine whether the properties of the COCs produced by lpr mice at six months of age affect the transportation property of COCs, we examined the TVC of COCs collected from these mice that were transported by infundibula collected from other strains. The results showed that the TVC in the combinations of (3m B6-6m lpr), (3m MpJ-6m lpr), (6m B6-6m lpr), and (6m MpJ-6m lpr) tended to be faster than that of (6m lpr-6m lpr), and the TVC of the infundibulum collected from B6 was significantly increased (Figure 1(F)). These results indicate that the transportation efficiency of COCs is determined not only by the ciliary morphofunction in the infundibulum but also by the properties of COCs. In addition, we suggest that the primary role of the cilia is in oocyte transportation than the role of the quality of COCs.

The transportation of COCs primarily consists of two processes: adhesion of cumulus cells to the ciliary tip of ciliated epithelial cells and transportation of COCs by ciliary beating.[12] In the former process, adhesive bonds formed by electrostatic or physical interactions between cilia on the infundibulum and cumulus cells have been reported to facilitate oocyte transportation.[13,14] MRL strains are used as models of systemic autoimmune disease, and the lymphoproliferation mutation (lpr) of the Fas cell surface death receptor gene in lpr mice exacerbates autoimmune abnormality,[15] resulting in severe inflammation in the ovaries and oviducts.[4,6] Although the pathological factors that alter the interaction between cumulus cells and cilia *in vivo* is not currently understood, further studies comparing ultrastructure, cell polarity, electrostatic properties, biochemical properties, and transcriptional variety of cumulus cells in B6, MpJ, and lpr mice will contribute to revealing the novel immunological factors in COCs necessary to achieve healthy oocyte transportation.

As summarized in Figure 2, the property of cumulus cells related to the transportation efficiency of oocytes changes with aging in MRL strains, but the efficiency is recovered by the property of COCs and the healthy morphofunction of ciliated epithelium of the infundibulum. Although the

less TVC compared to the healthy conditions does not necessarily result in the failure of oocyte pick-up, the disturbance of the accurate timing of oocyte transportation by the infundibulum potentially impairs oocyte pick-up combined with the abnormality of oviductal morphofunction. In addition to the abnormality of ciliated epithelium in the infundibulum in lpr mice at six months of age,[6] the altered properties of cumulus cells are suggested to be one of the factors altering oocyte pick-up and transportation in mice. These findings contribute to further understanding of the oocyte transport process by the infundibulum and female reproductive failure in patients with immune abnormalities, such as autoimmune disease, thus improving assisted reproductive technology.

AUTHORS' CONTRIBUTIONS

Conceptualization: MH and OI; Methodology: MH and TW; Validation: MH, OI, and TW; Formal analysis: MH; Investigation: MH; Resources: MH; Data curation: MH; Writing – original draft: MH; Writing – review & editing: MH, OI, TW, and YK; Visualization: MH; Supervision: OI and YK; Project administration: OI and YK; Funding acquisition: MH.

DECLARATION OF CONFLICTING INTERESTS

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ORCID IDS

Marina Hosotani https://orcid.org/0000-0003-0360-3360
Osamu Ichii https://orcid.org/0000-0002-6643-1554

SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

REFERENCES

1. Koyama H, Shi D, Fujimori T. Biophysics in oviduct: planar cell polarity, cilia, epithelial fold and tube morphogenesis, egg dynamics. *Biophys Physicobiol* 2019;**16**:89–107
2. Yuan S, Wang Z, Peng H, Ward SM, Hennig GW, Zheng H, Yan W. Oviductal motile cilia are essential for oocyte pickup but dispensable for sperm and embryo transport. *Proc Natl Acad Sci U S A* 2021;**118**: e2102940118
3. Faussone-Pellegrini MS, Bani G. The muscle coat morphology of the mouse oviduct during the estrous cycle. *Arch Histol Cytol* 1990;**53**:167–78
4. Otani Y, Ichii O, Otsuka-Kanazawa S, Chihara M, Nakamura T, Kon Y. MRL/MpJ- Fas lpr mice show abnormalities in ovarian function and morphology with the progression of autoimmune disease. *Autoimmunity* 2015;**48**:402–11
5. Hosotani M, Ichii O, Nakamura T, Kanazawa SO, Elewa YHA, Kon Y. Autoimmune abnormality affects ovulation and oocyte-pick-up in MRL/MpJ-Fas lpr/lpr mice. *Lupus* 2018;**27**:82–94
6. Hosotani M, Ichii O, Nakamura T, Masum MA, Otani Y, Elewa YHA, Kon Y. Altered ciliary morphofunction in the oviductal infundibulum of systemic autoimmune disease-prone MRL/MpJ-Fas lpr/lpr mice. *Cell Tissue Res* 2020;**380**:627–41
7. Takeo T, Hoshii T, Kondo Y, Toyodome H, Arima H, Yamamura K, Irie T, Nakagata N. Methyl-beta-cyclodextrin improves fertilizing ability of C57BL/6 mouse sperm after freezing and thawing by facilitating cholesterol efflux from the cells. *Biol Reprod* 2008;**78**:546–51
8. Wang S, Larina IV. In vivo dynamic 3D imaging of oocytes and embryos in the mouse oviduct. *Cell Rep* 2021;**36**:109382
9. Babayev E, Wang T, Szigeti-Buck K, Lowther K, Taylor HS, Horvath T, Seli E. Reproductive aging is associated with changes in oocyte mitochondrial dynamics, function, and mtDNA quantity. *Maturitas* 2016;**93**: 121–30
10. Ge ZJ, Schatten H, Zhang CL, Sun QY. Oocyte ageing and epigenetics. *Reproduction* 2015;**149**:R103–14
11. Hosotani M, Ichii O, Nakamura T, Masum MA, Otani Y, Otsuka-Kanazawa S, Elewa YHA, Kon Y. MRL/MpJ mice produce more oocytes and exhibit impaired fertilisation and accelerated luteinisation after superovulation treatment. *Reprod Fertil Dev* 2019;**31**:760–73
12. Kölle S, Dubielzig S, Reese S, Wehrend A, König P, Kummer W. Ciliary transport, gamete interaction, and effects of the early embryo in the oviduct: ex vivo analyses using a new digital videomicroscopic system in the cow. *Biol Reprod* 2009;**81**:267–74
13. Norwood JT, Hein CE, Halbert SA, Anderson RG. Polycationic macromolecules inhibit cilia-mediated ovum transport in the rabbit oviduct. *Proc Natl Acad Sci U S A* 1978;**75**:4413–6
14. Lam X, Gieseke C, Knoll M, Talbot P. Assay and importance of adhesive interaction between hamster (*Mesocricetus auratus*) oocyte-cumulus complexes and the oviductal epithelium. *Biol Reprod* 2000;**62**:579–88
15. Santiago-Raber ML, Laporte C, Reininger L, Izui S. Genetic basis of murine lupus. *Autoimmun Rev* 2004;**3**:33–9

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