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

Short running title: Cumulus cell property changes oviduct transportation velocity

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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

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 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.

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 cumulus-oocyte complexes (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 transportation

velocity of COCs 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 to those at three months of age.[5,6] The mice were housed in groups in plastic cages at 18–26 °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 immidiately 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 1A)**. 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 1B**). 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 1C**). 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 1D**. 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 1E**). 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 1E**). 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.

119 The morphological differences in the COCs between B6 and MRL-strain mice were not significant under
120 the stereomicroscope, while the number of cumulus cells composing the COCs of B6 mice at six months
121 of age seemed fewer than that of other strains (Supplementary Figure 1). Aging leads to a decrease in oocyte
122 quality in both humans and mice because of such factors as altered mitochondrial function and the
123 expression profile of transcriptions.[9,10] The difference in the genetic background between B6 and MRL-
124 strain mice appears to amplify the effect of aging on COC quality. The ovulation phenotype is different
125 between B6 and MRL strains at three months of age; under the superovulation treatment, MRL strains
126 produced poorer quality oocytes with a lower fertilization rate compared to B6 mice.[11] Despite the limitation
127 that there are no reports investigating the effects of aging on oocyte quality and the quality of cumulus cells
128 between B6 and MRL-strains, it is suggested that the property of cumulus cells governed by the genetic
129 background determines the transportation efficiency of COCs in mice, and that aging changes the properties
130 of COCs in MRL-strains more sensitively than in B6 mice.

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

137 age **(Movie 3)**, which had a significantly increased TVC **(Figure 1F)**. These results indicate that even the
138 infundibulum with abnormal ciliary morphofunction has the potential to efficiently transport COCs
139 collected from healthy mice. In addition, the significant recovery in the TVC in the combination of (6m lpr-
140 3m MpJ) but the insignificant recovery in the combination of (6m lpr-3m B6) and (6m lpr-6m B6) implies
141 a strain-dependent interaction between the COCs and the ciliated epithelium of infundibulum.

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

150 The transportation of COCs primarily consists of two processes: adhesion of cumulus cells to the ciliary
151 tip of ciliated epithelial cells and transportation of COCs by ciliary beating.[12] In the former process,
152 adhesive bonds formed by electrostatic or physical interactions between cilia on the infundibulum and
153 cumulus cells have been reported to facilitate oocyte transportation.[13,14] MRL strains are used as models of
154 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: M.H. and O.I.; Methodology: M.H. and T.W.; Validation: M.H., O.I., and T.W.; Formal analysis: M.H.; Investigation: M.H.; Resources: M.H.; Data curation: M.H.; Writing - original draft: M.H.; Writing - review & editing: M.H., O.I., T.W., and Y.K.; Visualization: M.H.; Supervision: O.I. and Y.K.; Project administration: O.I. and Y.K.; Funding acquisition: M.H.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article .

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Figure Legends

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. Amp: Ampulla. Syringe: Superovulation treatment. Scissor: Cutting using micro-dissecting scissor.

(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 sec. Black square is magnified in the right image. Inf: Infundibulum. 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. *: comparison with (6m lpr-6m lpr) (Dunnett's test, $P < 0.05$). n = 4 for all combinations.

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.

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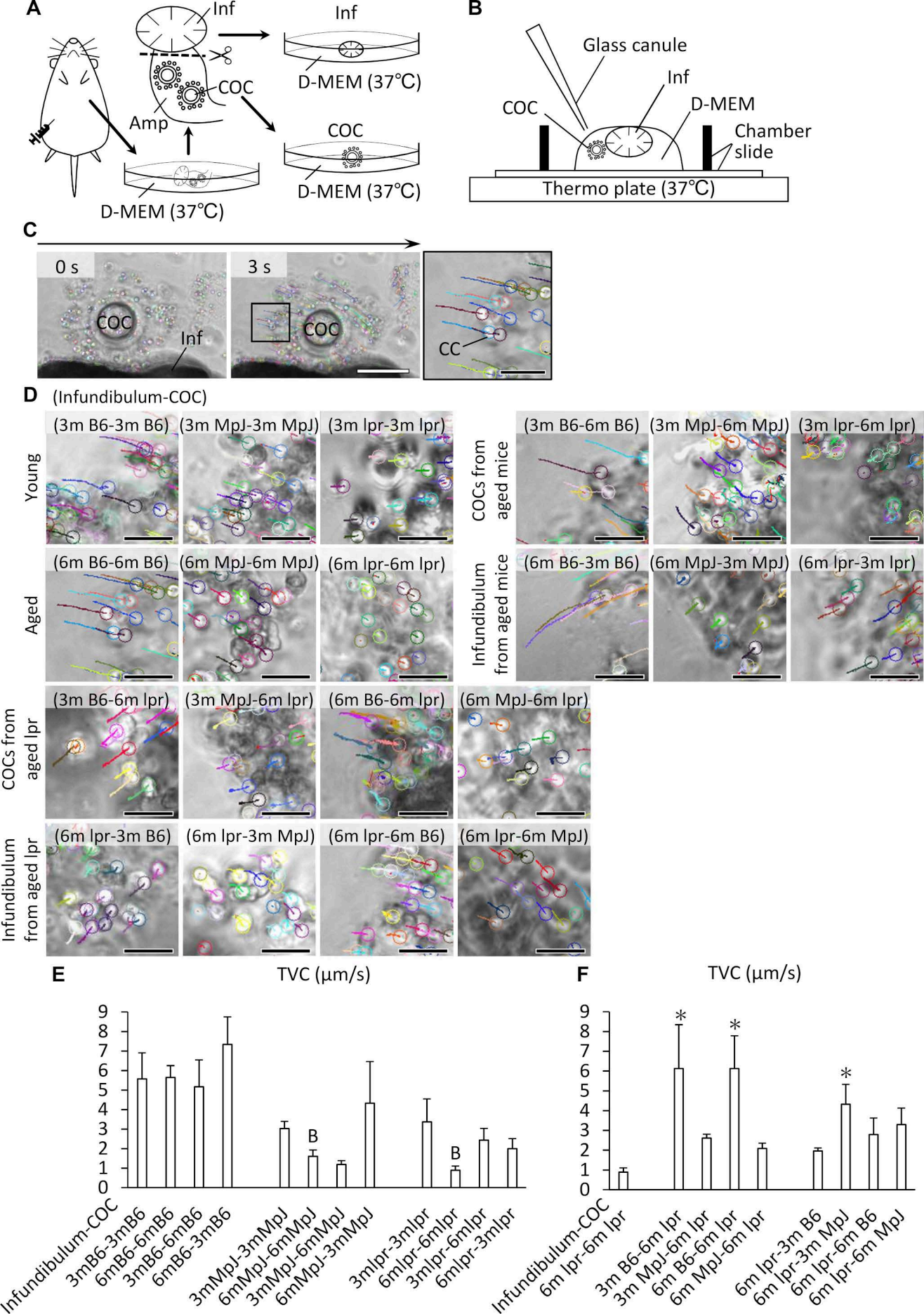
A
Inf
Inf
D-MEM (37℃)
COC
Amp
COC
D-MEM (37℃)
D-MEM (37℃)
B
Glass canule
Inf
COC
D-MEM
Chamber slide
Thermo plate (37℃)
C
0 s
3 s
COC
Inf
COC
CC
D
(Infundibulum-COC)
(3m B6-3m B6)
(3m MpJ-3m MpJ)
(3m lpr-3m lpr)
Young
COCs from aged mice
(3m B6-6m B6)
(3m MpJ-6m MpJ)
(3m lpr-6m lpr)
(6m B6-6m B6)
(6m MpJ-6m MpJ)
(6m lpr-6m lpr)
Aged
Infundibulum from aged mice
(6m B6-3m B6)
(6m MpJ-3m MpJ)
(6m lpr-3m lpr)
(3m B6-6m lpr)
(3m MpJ-6m lpr)
(6m B6-6m lpr)
(6m MpJ-6m lpr)
COCs from aged lpr
(6m lpr-3m B6)
(6m lpr-3m MpJ)
(6m lpr-6m B6)
(6m lpr-6m MpJ)
Infundibulum from aged lpr
E
TVC (μm/s)
B
B
Infundibulum-COC
3mB6-3mB6
6mB6-6mB6
3mB6-6mB6
6mB6-3mB6
3mMpJ-3mMpJ
6mMpJ-6mMpJ
3mMpJ-6mMpJ
6mMpJ-3mMpJ
3mlpr-3mlpr
6mlpr-6mlpr
3mlpr-6mlpr
6mlpr-3mlpr
F
TVC (μm/s)
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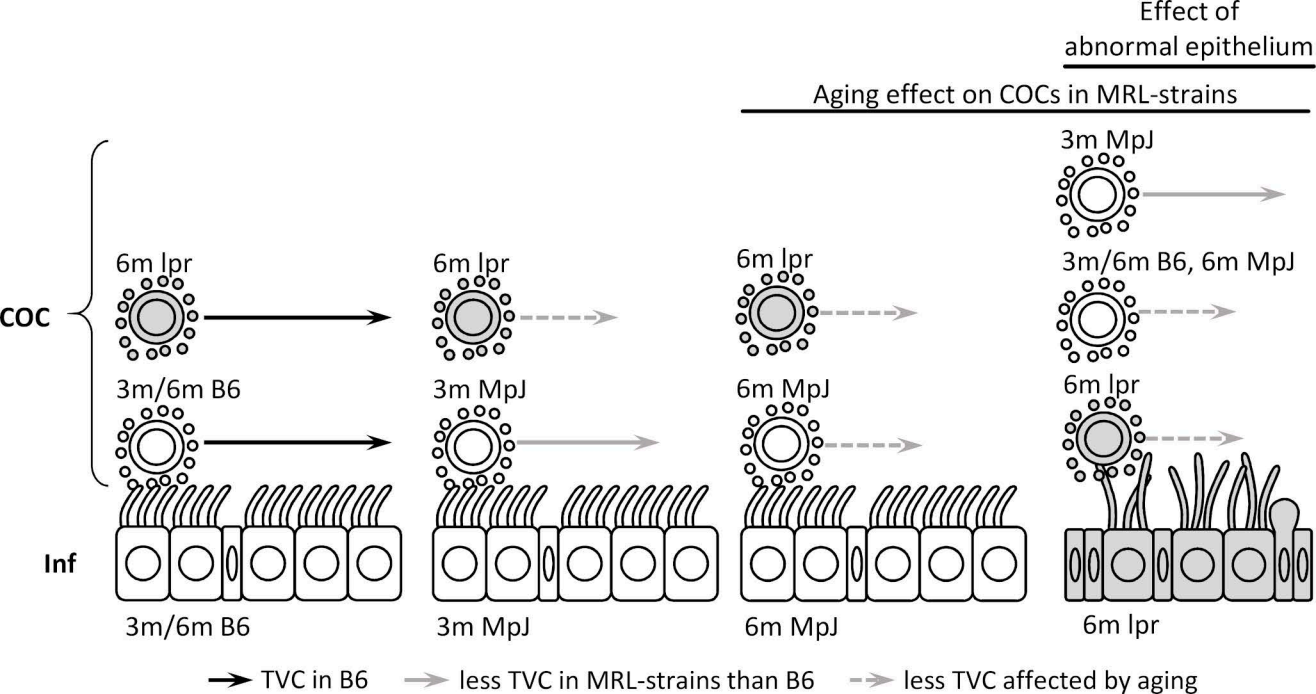
Infundibulum-COC
6m lpr-6m lpr
3m B6-6m lpr
3m MpJ-6m lpr
6m B6-6m lpr
6m MpJ-6m lpr
6m lpr-3m B6
6m lpr-3m MpJ
6m lpr-6m B6
6m lpr-6m MpJ


Effect of
abnormal epithelium
Aging effect on COCs in MRL-strains
3m MpJ
COC
6m lpr
6m lpr
6m lpr
3m/6m B6, 6m MpJ
3m/6m B6
3m MpJ
6m MpJ
6m lpr
Inf
3m/6m B6
3m MpJ
6m MpJ
6m lpr
TVC in B6
less TVC in MRL-strains than B6
less TVC affected by aging