Expression of transforming growth factor-β isoforms and their receptors in utero-vaginal junction of hen oviduct in presence or absence of resident sperm with reference to sperm storage

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Abstract

Our goal was to determine whether transforming growth factor β (TGFβ) isoforms were involved in the process of sperm survivability in the sperm-storage tubules (SST) in the utero-vaginal junction (UJ) of hen oviduct. The birds were artificially inseminated. The mRNA expressions of three types of TGFβ isoforms (TGFβ2, TGFβ3, and TGFβ4) and three types of receptors (TβR1, TβR2, and TβR3) were examined in the presence or in the absence of resident sperm in SST by semi-quantitative reverse transcriptase-PCR. The mRNA expression of TGFβs and TβRs in sperm was also examined. Immunocytochemistry and western blot were performed for TβR2 to confirm its localization in UJ. The sperm were observed at least 10 days after insemination by histology. The mRNA expressions of TGFβs and TβRs were significantly increased in UJ in the presence of resident sperm in SST. The mRNA expressions of TGFβs and TβRs were also observed in sperm. Immunohistochemistry revealed that TβR2 were located in lymphocytes in UJ and SST cells. The presence of TβR2 in UJ was also confirmed by western blot. These results suggest that enhanced expressions of TGFβs and TβRs in UJ may protect sperm in SST, probably by suppressing anti-sperm immunoreactions.


Introduction

In hen oviduct, sperm are stored for a prolonged period in the sperm-storage tubules (SST) in the utero-vaginal junction (UJ) and infundibulum with the UJ as the primary site for sperm storage (Fujii & Tamura 1963, Bobr et al. 1964, Bakst et al. 1994). The SST are formed by the invaginations of mucosal surface, where sperm are found to be close contact with the epithelium (Ashizawa & Nishiyama 1983). A number of studies have examined the features of sperm survivability in SST, and suggested that quiescence of spermatozoa during the storage (Holm et al. 2000), interaction of SST fluid outflow from SST cells and sperm mobility (Froman 2003, Zaniboni & Bakst 2004), and protection of sperm from immunoreaction (Das et al. 2005a, 2006) may be important events in this process. However, the precise mechanism of sperm survivability in the SST has not been established. There are reports that the leukocyte population was increased in UJ after artificial insemination (AI) in infertile hens and the local immunity of vagina and UJ may affect the sperm survivability (Higaki et al. 1995, Zheng et al. 2001, Das et al. 2005b). Thus, one of the requirements for prolonged sperm survivability in hen oviduct may be to suppress the immune response to sperm stored in SST. Transforming growth factor β (TGFβ) is one of the possible factors to suppress immunoresopne (Wahl et al. 1988, Mouri et al. 2002). Apart from the basic functions affecting growth, differentiation, and morphogenesis of cells (Sporn et al. 1986, 1987), TGFβ isoforms mediate immunosuppression by suppressing the proliferation of T- and B-lymphocytes in mammals and birds (Kehrl et al. 1986a, 1986b, Quere & Thorbecke 1990, Huang & Huang 2005). TGFβ in human seminal plasma may protect sperm from immune response in female reproductive tract (Nocera & Chu 1995, Srivastava et al. 1996). Thus, TGFβs may play an important role in the storage of sperm in SST by suppressing anti-sperm immune response. Chicken TGFβ superfamily consists of TGFβ2, TGFβ3, and TGFβ4 (Chowdhury et al. 2003). Three types of TGFβ receptors, namely types 1, 2, and 3 (TβR1, TβR2, and TβR3) were also identified in chicken (Chowdhury et al. 2004), and TβR2 binds with TGFβs directly and the complex is recognized by TβR1 (Massague 1996). However, the TβR3 does not participate in signal transduction but increases the receptor-binding affinity and cell responsiveness (Lopez et al. 1993).
Published information on the expression of TGFβ isoforms and their receptors in UVJ tissues or infundibulum are not available. If TGFβs are involved in the protection of sperm from immunoreaction, the possibility of their synthesis by the sperm should also be examined. However, this possibility has also not been reported yet. Therefore, the goal of this study was to determine whether the cells in UVJ, and potentially the sperm themselves, express mRNAs of TGFβ isoforms and their receptors (TβRs) and whether their expressions are changed during the storage of sperm in SST. In Experiment 1, histological observations of UVJ tissue with or without AI were performed to confirm the changes in the population of SST-containing sperm and that of the lymphocytes after AI. In Experiment 2, mRNA expressions of TGFβs and TβRs were investigated by reverse transcriptase (RT)-PCR. The expressions of TGFβs and TβRs by chicken sperm were also examined in Experiment 3. Finally, immunocytochemistry and western blot for TβR2 were performed to localize it in UVJ in Experiment 4.

Materials and Methods

Birds and tissue collection

Healthy White Leghorn laying hens (aged approximately 50 weeks), regularly laying five or more eggs in a sequence were kept in individual cages under a regimen of 14 h light:10 h darkness and provided with commercial feed and water ad libitum. The hens were inseminated in Experiments 1, 2, and 4, and examined at two different terms, namely short- (trial 1) and long-term after AI (trial 2). In trial 1, the birds were divided into four groups: 0, 1, 12, and 24 h after AI (n=4 each). In trial 2, birds were also divided into four groups: 0, 1, 10, and 20 days after AI (n=4 each). Semen used for AI and RT-PCR analysis of TGFβs and TβRs expressions in sperm (Experiment 3) was collected from White Leghorn roosters (n=3) kept under the similar condition. For the insemination, 0.05 ml undiluted fresh semen containing approximately 2×10⁸ sperm were intravaginally introduced from the cloaca using a plastic syringe. When the oviductal tissues were collected, the birds were euthanatized by decapitation under anesthesia with Nembutal (Abbott Laboratories, IL, USA) after approximately 5 h oviposition. The handling of birds was performed in accordance with the regulation by Animal Experiment Committee of Hiroshima University.

Experiment 1. Histological observation

A part of UVJ tissues of all female birds (trials 1 and 2) were fixed with Bouin’s solution followed by embedding in paraffin in the usual manner. Paraffin sections (4 µm in thickness) were prepared and stained with hematoxylin and eosin. The ratio of the SST-containing sperm and the lymphocyte frequencies in the lamina propria were analyzed under a light microscope with a computer-assisted image analysis system (Image-Pro Plus; Media Cybernetics, Silver Spring, MD, USA) as described previously (Yoshimura et al. 2004). The lymphocytes were identified histologically with densely stained, small and round nuclei. Their frequencies in the lamina propria were analyzed by counting 15 different regions (approximately, 7–10×10⁴ µm² area in each count) randomly selected from three UVJ sections. Then, the number of lymphocytes in 5×10⁴ µm² areas was calculated.

Experiment 2. RT-PCR analysis for expression of TGFβs and TβRs in UVJ

Changes in the expressions for TGFβs mRNA (TGFβ2, TGFβ3, and TGFβ4) and TβRs mRNA (TβR1, TβR2, and TβR3) after AI were observed. In trial 1, four different oviductal segments, namely the infundibulum, uterus, UVJ, and vagina were collected. In trial 2, since significant changes in the expressions for TGFβs and TβRs were observed only in UVJ in trial 1, only the UVJ tissues were collected.

Extraction of total RNA

Total RNA was extracted from the mucosal tissues of oviductal segments using Sepasol RNA I Super (Nacalai Tesque, Inc., Kyoto, Japan) as described previously by Barua & Yoshimura (2004). The pellet of RNA was suspended in TE buffer, incubated with DNase I (Roche) at a concentration of 10 U/µl, and the RNA concentration was measured with Gene Quant Pro (Amersham Pharmacia Biotech), and stored at −80 °C until analysis.

Semi-quantitative RT-PCR

The semi-quantitative RT-PCR was performed as described previously by Subedi & Yoshimura (2005). The RNA samples were reverse transcribed using ReverTra Ace (Toyobo Co. Ltd, Osaka, Japan) as described by the manufacturer’s instructions. The primers used for TGFβ2 (Burt & Paton 1991; Accession no. NM001031045), TGFβ3 (Jakowlew et al. 1992; Accession no. S46000), and TGFβ4 (Jakowlew et al. 1998) and their receptors TβR1 (Accession no. U38622), TβR2 (Barnett et al. 1994; Accession no. NM205428), and TβR3 (Barnett et al. 1994; Accession no. NM204339) and also for chicken β-actin (Kost et al. 1983; Accession no. X00182) are shown in Table 1. An aliquot of cDNA corresponding to 1 µg initial total RNA was used as a template in a volume of 25 µl reaction mixture for PCR. The mixture was denatured at 95 °C for 1 min followed by 30 cycles of 95 °C for 1 min, 58 °C for 1 min to anneal, 72 °C for 1 min for extension and a final extension was done at 72 °C for 10 min in a Programmable Thermal Controller PTC-100 (MJ Research, Inc., Waltham, MA,
USA). In the preliminary experiments, different numbers of cycles (25, 30, 35, and 40 cycles) for TGFβs and TβRs were tested in each sample to optimize the amplification and it was confirmed that 30 cycles were optimal for the detection of quantitative differences between the samples. The PCR products were electrophoresed in a 3% (w/v) agarose gel with 0.4% ethidium bromide. The samples were tested in each sample to optimize the amplification with reference to that of density of bands of TGFβ.

Table 1 List of the PCR primers of TGFβ isomers and their receptors.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences</th>
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<tbody>
<tr>
<td>TGFβ2</td>
<td>F: 5′-AGGAATGTCCAGGATAAT-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-ATTITGCGTTGTTGCCAAA-3′</td>
</tr>
<tr>
<td>TGFβ3</td>
<td>F: 5′-CAGATCCTGCGTCCCTACA-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-GAGGCCTGAGTACGTCGTA-3′</td>
</tr>
<tr>
<td>TGFβ4</td>
<td>F: 5′-CACCAGACTGCCTCCGGC-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-GGTGGGCTCAGATGTAC-3′</td>
</tr>
<tr>
<td>TβR1</td>
<td>F: 5′-GGTCCCAATGATCCAGT-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-TGCTGATGAGGATCTGACA-3′</td>
</tr>
<tr>
<td>TβR2</td>
<td>F: 5′-CTATGCAAATCTGAGCCG-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-ATACCTCCTTTCTCTCTC-3′</td>
</tr>
<tr>
<td>TβR3</td>
<td>F: 5′-TGCACTGCTCTACACAGA-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-GCTGACAAAGAGAAAAATC-3′</td>
</tr>
<tr>
<td>β-actin</td>
<td>F: 5′-TCCACGACCTTTCTTG-3′</td>
</tr>
<tr>
<td></td>
<td>R: 5′-CTCTCCTGCATCGTCC-3′</td>
</tr>
</tbody>
</table>

It was examined whether the addition of sperm to isolated UVJ tissue causes the changes in their expression. Sperm were added to the isolated UVJ tissue at the ratio of 0, 1.5×10^3, 3×10^3, and 7.5×10^3 sperm/mg UVJ tissue, which is equivalent to 0, 0.5, 1, and 2% of the inseminated sperm (2×10^6 sperm) that calculated based on the total UVJ mucosal tissue weight (600 mg) respectively. However, Bakst et al. (1994) described the number of sperm that enter SST was <1% of the inseminated sperm. Total mRNA was collected from the mixed samples and their expressions of TGFβs and TβRs were observed in the same manner as described previously.

**Experiment 4. Immunohistochemistry and western-blot analysis for TβR2**

Immunostaining for TβR2 in the tissue of UVJ was performed using paraffin sections prepared in Experiment 1. After deparaffinization, sections were washed with PBS for 15 min (5 min×3 times), and autoclaved for 1 min in 2 mM citric acid (pH 6.0) to enhance antigenicity. The sections were incubated overnight at 4°C with sheep anti-chicken TβR2 polyclonal antibody (Abcam Ltd, Cambridge, UK) diluted to 1:100 in PBS containing 0.05% BSA (Nacalai Tesque, Inc., Kyoto, Japan). After washing with PBS (5 min×3 times), the sections were incubated with biotinylated anti-sheep IgG (Abcam Ltd) for 1 h and with avidin–peroxidase complex (Nichirei Corporation, Tokyo, Japan) for 30 min. The sections were washed in PBS (5 min×3 times) and immunoprecipitates were visualized by incubating with 0.02% (w/v) 3’, 3’-diaminobenzidine tetrahydrochloride (Nacalai Tesque, Inc., Kyoto, Japan) and 0.001% (v/v) H2O2 in 0.05 M Tris–HCl buffer (pH 7.6). The slides were counterstained with hematoxylin, dehydrated, and covered.

For western-blot analysis, the UVJ tissue of non-inseminated birds and fresh sperm were homogenized in five times the volume of homogenization buffer consisting of 10 mM Tris–HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid, and 1 mM phenylmethylsulfonyl fluoride with a Polytron homogenizer (Kinematica AG, Littau, Lucerne, Switzerland). The samples were centrifuged at 12 000 g for 10 min, the supernatant was again centrifuged at 45 000 g for 1 h, and the supernatant was collected. The samples were separated by SDS-PAGE, 10% separating gel and 4% stacking gel, as described by Yoshimura et al. (1997). Briefly, the protein concentrations were measured using protein- assay reagent (Bio-Rad Lab.) as described by the manufacturer. Each sample of 62.5 μg in 50 μl was mixed with 20 μl sample buffer (35% (v/v) glycerol, 12% (v/v) mercaptoethanol, 7.2% (w/v) SDS, 0.15 M Tris–HCl (pH 6.8), 0.06% (v/v) bromophenol blue) and boiled for 2 min. The sample, 10 μl, was loaded onto gels and run at 80 V.
in the stacking gel and at 120 V in the separating gel. After SDS-PAGE, the samples were electrophoretically transferred onto the nitrocellulose membrane (Hybond-C, Amersham Int.). The membrane was washed with western buffer (0.02 M Tris–HCl (pH 7.4), 0.15 M NaCl, 0.5% Tween-20, and 0.05% (w/v) BSA) for 30 min (10 min × 3 times) and incubated with 10% Block Ace (Dainihon Pharmaceutical Co., Osaka, Japan) in western buffer for 30 min. The membrane was then incubated with sheep anti-chicken TβR2 polyclonal antibody (Abcam Ltd) diluted to 1:1000 with western buffer for overnight. Following washing with western buffer for 45 min (15 min × 3 times), they were incubated with biotinylated anti-sheep IgG (Abcam Ltd) diluted to 1:10 000 for 2 h and with avidin–peroxidase complex (Nichirei Co., Tokyo, Japan) for 1 h. The membrane was washed with western buffer for 30 min (10 min × 3 times) and the immunoprecipitates on the membrane were visualized by incubating in a reaction mixture of 0.02% (w/v) 3,3’-diaminobenzidine tetrahydrochloride (Nacalai Tesque, Inc.) and 0.001% (v/v) H2O2 in 0.05 M Tris–HCl buffer (pH 7.6).

Statistical analysis

The significance of differences in TGFβs and TβRs expressions (the ratio of TGFβs or TβRs/β-actin mRNAs) within each oviductal segment (infundibulum, uterus, UVJ, or vagina) was examined among different hours after AI (trial 1) or different days (trial 2) by one-way ANOVA, followed by Duncan’s (1955) multiple range test. Differences were considered significant when P value was <0.05.

Results

Experiment 1. Histological observation

In the UVJ, the SST were distributed in the lamina propria of mucosal folds. The SST consisted of single layer of non-ciliated epithelial cells. No sperm was observed in the lumen of SST in non-inseminated birds (Fig. 1A), whereas there were SST filled with sperm in the inseminated birds of trials 1 and 2 (Fig. 1B and C) except for 20 days after AI (Fig. 1D). The ratio of SST structure containing sperm was approximately 35–50% at 1, 12, and 24 h after AI (trial 1; Fig. 1D), and showed a gradual decrease on 10 days (trial 2; Fig. 2B). The number of lymphocytes in the stroma surrounding the SST was not changed significantly during experimental periods in both trials 1 and 2 (Fig. 2C and D).

Experiment 2. RT-PCR analysis for expressions of TGFβs and TβRs in UVJ

Trial 1

The expressions of TGFβ2, TGFβ3, and TGFβ4 were observed in the infundibulum, uterus, UVJ, and vagina. Figures 3–5 show the changes in the expressions of TGFβs in the oviduct until 24 h after AI. Differences in the expressions for TGFβ2 were not significant among 0, 1, 12, and 24 h after AI in the infundibulum, uterus, and vagina (Fig. 3A, B and D), however, that in the UVJ was significantly increased at 1 h after insemination and showed a gradual decreasing tendency (Fig. 3 C). The expressions for TGFβ3 and TGFβ4 also did not show any significant changes within 24 h after AI in the infundibulum, uterus, and vagina (Figs 4A, B and D and 5A, B)

Figure 1 Sections of sperm-storage tubules (SST) in utero-vaginal junction of hens with or without insemination. (A) Before insemination. (B) 1 day after insemination. (C) 10 days after insemination. (D) 20 days after insemination. Note that SST are filled with sperm 1 and 10 days after insemination (arrow heads; (B) and (C)), whereas no sperm is located in SST before and 20 days after insemination (arrows; (A) and (D)). (E) Surface epithelium; Lp, lamina propria; scale bars = 50 μm. HE staining. The inserts of (B) and (C) (scale bar, 20 μm) show the higher magnification of single SST with sperm in lumen (arrows).
and D). However, in UVJ, the expression of TGFβ3 was significantly increased during 1–12 h after AI and then decreased at 24 h after AI (Fig. 4C). The expression of TGFβ4 in UVJ was significantly greater during 1–24 h compared with the 0-h group (Fig. 5C).

The expressions of TβR1, TβR2, and TβR3 were observed in the infundibulum, uterus, UVJ, and vagina.

Differences in the expressions for TβR1 and TβR2 were insignificant among 0, 1, 12, and 24 h after AI within the infundibulum, uterus, or vagina (Figs 6A, B and D and 7A, B and D). In contrast, expressions of TβR1 and TβR2 in the UVJ were significantly increased at 1 h after AI and kept higher up to 24 h (Figs 6C and 7C). However, the expressions for TβR3 did not show significant change within 24 h after AI in any of the oviductal segments observed in the present study (Fig. 8).

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Figure 6 Changes in the expression of TβR1 in the tissues of (A) infundibulum, (B) uterus, (C) UVJ, and (D) vagina within 24 h after insemination. Values show the mean ± S.E.M. (n = 4 for each value) of the ratio of TβR1 mRNA to β-actin mRNA. Values with different letters are significantly different (P < 0.05).

Figure 7 Changes in the expression of TβR2 in the tissues of (A) infundibulum, (B) uterus, (C) UVJ, and (D) vagina within 24 h after insemination. Values show the mean ± S.E.M. (n = 4 for each value) of the ratio of TβR2 mRNA to β-actin mRNA. Values with different letters are significantly different (P < 0.05).

Figure 8 Changes in the expression of TβR3 in the tissues of (A) infundibulum, (B) uterus, (C) UVJ, and (D) vagina within 24 h after insemination. Values show the mean ± S.E.M. (n = 4 for each value) of the ratio of TβR3 mRNA to β-actin mRNA.

Figure 9 Changes in the expression of TGFβ isoforms and their receptors in the tissue of uterus-vaginal junction within 20 days after insemination. (A) TGFβ2, (B) TGFβ3, (C) TGFβ4, (D) TβR1, (E) TβR2, and (F) TβR3. Values show the mean ± S.E.M. (n = 4 for each value) of the ratio of TGFβs or TβRmRNA to β-actin mRNA. Values with different letters are significantly different (P < 0.05).
The expressions of TβR1 and TβR2 in UVJ were significantly increased on 1 day after AI and kept higher until 10 days followed by decreasing on 20 days that showed a same level as 0 day (Fig. 9D and E). However, expression of TβR3 in the tissue of UVJ did not show any significant differences during 0–20 days (Fig. 9F).

**Experiment 3. Expression of TGFβs and TβRs by sperm**

Clear PCR products showing the expression of the TGFβ2, TGFβ3, and TGFβ4 and TβR1, TβR2, and TβR3 were observed in sperm samples (Fig. 10A and B). The expressions of TGFβs and TβRs were insignificant among the tissues of UVJ added with 0, 1.5×10^3, 3×10^3, and 7.5×10^3 sperm/mg UVJ tissue (Fig. 11).

**Experiment 4. Immunocytochemistry and western-blot analysis for TβR2**

Immunoreactive TβR2 were observed in the lymphocytes in the lamina propria (Fig. 12A). The cells in SST were also stained positive with a strong intensity as compared to those of the surface epithelium. Western-blot analysis confirmed the presence of TβR2 in UVJ tissue and in sperm, which was observed approximately at 75 kDa (Fig. 12B).

**Discussion**

We are reporting that the TGFβs (TGFβ2, TGFβ3, and TGFβ4) and TβRs (TβR1, TβR2, and TβR3) are expressed in UVJ of hen oviduct and changed in association with the presence of sperm in the SST after AI. The significant findings of this study are: (i) among the four oviductal segments (infundibulum, uterus, UVJ, and vagina), the expressions for TGFβs and TβRs were increased only in the tissue of UVJ during the storage of sperm in SST; (ii) the sperm also expressed TGFβs and TβRs; and (iii) lymphocytes and SST cells were immunopositive for TβR2.

The expressions of TGFβ2, TGFβ3, and TGFβ4 in UVJ were significantly increased within 1 h after AI and that of TGFβ4 was kept higher even during 24 h–10 days after AI. In case of their receptors, expressions of TβR1 and TβR2 but not TβR3 were increased at 1 h after AI and kept higher until 10 days. The UVJ is the primary site for sperm storage, where abundant SST distribute (Fujii & Tamura 1963, Bakst et al. 1994). The ratio of SST-containing sperm was high on 1 day after AI and gradually decreased by 10 days, followed by decreasing to a negligible level on 20 days. Thus, there seems a close association between sperm storage and expressions levels of TGFβs and their receptors. Among the three types of TGFβs, the TGFβ4 isoform may be the most noticeable molecule for this event as it maintained higher expression level for the longest term, which was similar to the term of sperm residence in SST. In contrast, the other three oviductal segments, namely infundibulum, uterus, and vagina did not show increase in expressions for any of the ligands or their receptors. We suggest that increased expressions of TGFβs and their receptors occur specifically in UVJ after insemination. The reason why the infundibulum, which is known as secondary sperm storage site, failed to show increase in expressions for any of the ligands or receptors may be due to that the amount of sperm storage in their SST is small.
We report the novel expressions of TGFβ2, TGFβ3, and TGFβ4, and TβR1, TβR2, and TβR3 by chicken sperm observed in the present study. In the previous reports, TGFβ1 was detected in human sperm by immunostaining (Chu et al. 1996) and the presence of TGFβ1, TGFβ2, and TGFβ3 were identified in human seminal plasma (Nocera & Chu 1995, Srivastava et al. 1996). It has also been reported that the majority of TGFβ in seminal fluids is in a latent form, which becomes activated in female reproductive tract after insemination (Robertson 2005). These results suggest that sperm likely produce TGFβs in different species, including birds and mammals. However, the addition of 7.5 × 10³ sperm to per milligram isolated mucosal UVJ tissue did not cause significant differences in the expression of TGFβs and TβRs. Bakst et al. (1994) reported that the number of sperm that enter the SST is <1%, and approximately similar number of sperm were added to the UVJ tissue in this study. Therefore, the expressions of TGFβs and TβRs in UVJ after AI might be increased by the interaction of SST cells and sperm, rather than the simple addition of sperm expression to the UVJ tissue expression. Sperm may influence some gene expressions and secretory proteomic profiles in the reproductive tract of mammals that may be related to sperm transport and selection (Fazeli et al. 2004, Georgiou et al. 2005). Long et al. (2003) reported the increase of gene expression for avidin in UVJ of turkey hens in response to insemination, and suggested that it might provide the nutrient sources of biotin or related vitamins for the resident sperm. These reports suggest that sperm may influence the gene expressions of some molecules in the oviduct of both mammals and birds. Thus, sperm could also induce the gene expression of TGFβs and their receptors in the UVJ. The TGFβs could be synthesized by sperm until ejaculation, however, it is not confirmed whether they are still synthesized even during the traveling in the oviduct. If sperm synthesize TGFβs even while traveling the oviduct, it may play roles in protecting themselves.

Immunohistochemical examination confirmed the presence of TβR2 in lymphocytes and SST cells, suggesting a possibility of interaction between TGFβs and these cells. The specificity of the immunoreaction was confirmed by western-blot analysis. The TGFβs and its receptors exert a potent inhibitory effect on B-cell proliferation and differentiation (Kehrl et al. 1986a). The TGFβ1 in mammalian species, which is thought to be an ortholog of TGFβ4 in avian species (Pan & Halper 2003, Halper et al. 2004), suppressed immune response by maintaining development of suppressor T-cells in addition to the direct suppressive effect on the proliferation of B- and T-cells in chicken (Quere & Thorbecke 1990). In a recent study, Huang & Huang (2005) explained the involvement of TβR-V-signaling cascade along with TβR1/TβR2 for mediating the inhibitory function of TGFβs on various type of cell proliferation. Thus, these reports suggest that TGFβs exert suppressive effect on T- and B-cell proliferation or differentiation. Previous report described that lymphocyte population was increased in UVJ and sperm were not stored in SST in infertile hens after AI (Das et al. 2005b). Plasma cells were also shown in the UVJ of infertile hens (Van Krey et al. 1987). These results suggested that immune response to sperm may occur in infertile hens, resulting in the decline of sperm number stored in SST. Decline of fertility caused by immunoreponse to sperm has also been suggested in mammals (Mettler 1978). Our results showing the elevated levels of TGFβs and the presence of TβR2 in lymphocytes in UVJ suggest that TGFβs produced by UVJ tissue and sperm may suppress immune response to sperm to maintain the survivability of them in the SST. In the present study with healthy hens, the population of stromal lymphocytes was not significantly different between inseminated and non-inseminated birds. We assume that this is the normal dynamics of lymphocytes in UVJ stroma in healthy birds and TGFβ might be involved in the suppression of increased lymphocyte population.

The SST cells showed the stronger immunoreactivity for TβR2 than the surface epithelium of UVJ. Sperm expressed TβR1, TβR2, and TβR3 with negligible immunoreactivity in SST on the immunostained sections.
which might be due to the fewer amount of receptor molecules. The interaction of TGFβs with the receptors in the SST cells and sperm may also be responsible for the survivability of sperm in SST.

In conclusion, we have provided evidence that the mRNA for TGFβ2, TGFβ3, and TGFβ4 and TβR1, TβR2, and TβR3 are expressed in the hen oviduct and in sperm, and their expressions in UVJ are increased with AI. The increase of expressions in UVJ might be caused by the stimuli of sperm stored in SST. The enhanced expressions of TGFβs and TβRs in UVJ may be a mechanism responsible for the survivable of sperm during their storage in SST, probably via suppression of anti-sperm immunoreactions.

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References


Duncan B 1955 Multiple range and F test. Biometrics 11 1–42.


Fuji S & Tamura T 1963 Location of sperms in the oviduct of the domestic fowl with special reference to storage of sperms in the vaginal gland. Journal of Faculty of Fisheries and Animal Husbandry, Hiroshima University 5 145–163.


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