Expression of Prolactin Receptor on the Surface of Quail Spermatozoa

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Prolactin receptor (PRLR) is expressed in a wide variety of tissues and mediates diverse biological actions of prolactin (PRL). In mammals, PRL signaling is thought to be involved not only in the process of spermatogenesis and steroidogenesis in the testis, but also in the survival of ejaculated sperm. In avian species, although the expression of PRLR with several variants in the testis was reported, the role of PRL in testicular function is still unclear. The aim of this study was to examine the expression of PRLR in the testis and mature sperm in quail. It is revealed that PRLR was mainly localized in the round- and elongated-spermatid by immunohistochemical analysis on the testis suggesting that PRL signaling may participate in the spermatogenesis. Western blot analysis confirmed the presence of PRLR in the plasma membrane of the ejaculated sperm (SPML), whereas the size of PRLR in the sperm was smaller than that in the hypothalamus. Moreover, PRLR was detected on the surface of the midpiece and flagellum of sperm by immunostaining. To evaluate the functionality of the sperm PRLR, the dot blot assay was performed to test the binding of pituitary PRL to PRLR in the SPML, and resulted in the detection of specific binding of PRL to the component of SPML, most likely to sperm PRLR. Furthermore, when the ejaculates were incubated with pituitary PRL to investigate the role of PRL on the sperm, the occurrence of spontaneous acrosome reaction was significantly decreased. In addition, the expression of PRL on the surface of utero-vaginal junction of oviduct was detected by immunohistochemistry. These results may suggest a novel system that the interaction between oviductal PRL and sperm PRLR is involved in the maintenance of the fertilizability of the spermatozoa through the prevention of the spontaneous acrosome reaction in Japanese quail.

Key words: acrosome reaction, prolactin receptor, quail, sperm, testis, utero-vaginal junction

Introduction

Prolactin (PRL) is mainly produced by lactotroph cells in anterior pituitary gland, and exerts diverse biological actions. PRL exerts its function through binding to its membrane-bound receptor (PRLR), which is expressed in a wide variety of target tissues (Bole-Feysot *et al*., 1998; Marano and Ben-Jonathan, 2014). The PRLR gene in mammalian species is comprised of 11 exons and produces the receptor with extracellular, transmembrane and cytoplasmic domains. There are two major isoforms referred to as long and short form with intact- or truncated-cytoplasmic domain of carboxy terminus, respectively (Boutin *et al*., 1988; Shirota *et al*., 1990). Although the functions of the isoforms are not well

established, the existence of several splice variants of the receptor had been reported (Freeman *et al*., 2000; Trott *et al*., 2003). In addition, the presence of many types of PRL-like peptide (Duckworth *et al*., 1986; Toft and Lnzer, 1999; Dai *et al*., 2000; Soares, 2004) as well as extra-pituitary PRL had been reported (Ben-Jonathan *et al*., 1996; Bole-Feysot *et al*., 1998; Harvey *et al*., 2014), thus the function of PRL might be more complex than our classical understanding.

In avian species, multiple actions of PRL have also been reported including the modulation of immune responsiveness (Skwarlo-Sonta *et al*., 1986), crop-sac development (Nicoll 1967), the induction of broody behavior (Riddle *et al*., 1935) and control of follicular development and egg production (Camper and Burke 1977, Sharp *et al*., 1989, Zadworny *et al*., 1989, Li *et al*., 2011). In birds, such as chicken (Tanaka *et al*., 1992), turkey (Zhou *et al*., 1996) and pigeon (Chen and Horseman, 1994), PRLR had been identified by cDNA cloning, and the tissue distribution of the receptor gene is as ubiquitous as in mammalian species (Zhou *et al*., 1996).

Although a wide spread expression of the receptor may indicate the diverse physiological functions of PRL signaling via PRLR, an elucidation of authentic functions of avian PRL based on experimental evidence is not well investigated.

In mammalian species including human, mice and rats, it is reported that both PRL and its receptor are expressed in the testis (Ouhtit *et al*., 1993; Hondo *et al*., 1995; Untergasser *et al*., 1997; Hair *et al*., 2002; Parthasarathy and Balasubramanian, 2008; Ishida *et al*., 2010). Because the receptor had been expressed in either spermatogenic or somatic cells (i. e. Leydig and Sertoli cells), PRL is thought to be involved in the process of both spermatogenesis and steroidogenesis in the testis. In contrast, Binart *et al*. (2003) demonstrated that male mice with a targeted disruption of the PRLR appeared normal in their reproductive parameters (e. g. circulating gonadotropins level, prostate weight, testis morphology and total number of sperm) and were fertile with natural mating.

In birds, the expression of several kinds of receptor variant in the testis was reported (Mao *et al*., 1999; Tanaka *et al*., 2000), however, the role of PRL in testicular function such as sperm production as well as fertilization is poorly understood. In this study, we aimed to study the expression of PRLR in the testis and mature sperm to advance our understanding of the role of PRL signaling in fertilization in birds. In addition, we reported here that the PRLR expressed on the surface of quail spermatozoa might contribute to the preservation of the sperm fertilizability by inhibiting spontaneous acrosome reaction.

Materials and Methods

Animals and Tissue Preparation

Male and female Japanese quail, *Coturnix japonica*, 8−20 weeks of age (Motoki Corporation, Tokorozawa, Japan), were maintained individually under a photoperiod of 14L: 10D (with the light on at 05:00) and provided with water and a commercial diet (Motoki Corporation) *ad libitum*. Females were decapitated and the utero-vaginal junction (UVJ) of the oviduct was dissected according to the method of Ito *et al.* (2011). The testis was isolated from mature male after decapitation. All experimental procedures for the care and use of animals in the present study were approved by the Animal Care Committee of Shizuoka University (Approval number: 26-12).

Preparation of Antisera

Rabbit polyclonal anti-quail PRLR antiserum (anti-PRLR) was raised against bacterially expressed His-tagged PRLR protein. We produced two types of antisera raised against the extracellular (from aa^1-aa^{413}) and intracellular domain (from aa^{471} to aa^{830}) of the quail receptor (GenBank accession number: LC074729) because full length of the receptor is found to be difficult to express in *Escherichia coli*. The sense and antisense primers used were 5′- TCGCTCGAG-GTGGAAATCATGAAACAG-3′ and 5′- TGGAAGCTTT-GGTTTGCAGTGAATCTGTA-3′ for extracellular domain and 5′- GTACTCGAGATCCTACCACCAGTTCCAGGAC-3′ and 5′- CTAAAGCTTCCATTCTGTAGCATTACCTGAT-3′ for intracellular domain respectively. Each PCR product

was digested with *Xho*I and *Hind*III, and ligated into pCold TF DNA vector (Takara Biomedical) treated with the same restriction enzymes. The resulting construct was transformed into competent *Escherichia coli*, strain *BL21* (Takara Biomedical), and an ampicillin-resistant clone was selected after the nucleotide sequence analysis was performed. Recombinant PRLR was expressed in the presence of 1 mM isopropyl-β-thiogalactopyranoside at 15℃ for 24 h, and the protein was purified from the cell lysate using nickel resin (Novagen, Madison, WI, USA) according to the manufacturer's instructions. The purity of the recombinant protein was verified by SDS-PAGE followed by CBB staining.

Female New Zealand White rabbits (SLC, Hamamatsu, Japan) were immunized with the recombinant PRLR (300 μg of protein) as described previously (Kuroki and Mori, 1995) and resulting antisera raised against extracellular or intracellular domain of the receptor were mixed at 1:1 ratio. To verify the specificity of anti-PRLR antiserum, we preincubated the antiserum with the antigen protein (1 mg/ml) at 4℃ overnight before use.

The antiserum against chicken PRL (anti-PRL) used in the present study was raised in rabbit, and was characterized as described previously (Hiyama *et al*., 2009). The amino acid sequence similarity of chicken and quail PRL shows more than 90%.

Semen Collection and Preparation

Ejaculated semen was obtained from male quail during mating prior to ejaculation according to the procedure of Kuroki and Mori (1997). Semen obtained from two to three males was suspended in Hanks balanced salt solution containing 1.25 mM CaCl$_2$ and 1.8 mM MgSO$_4$. The concentrations of sperm were measured with a hemocytometer and sperm viability was assessed using a LIVE/DEAD sperm viability kit according to the manufacturer's instructions (Molecular Probes, Eugene, OR, USA). Sperm were incubated in the presence or absence of chicken PRL (generous gift from NIDDK) at 39℃. The acrosome status of the spermatozoa was observed based on the presence (acrosome-intact sperm) or absence (acrosome-reacted sperm) of the acrosome, which is observed as a propidium iodide-negative structure located on the tip of the sperm nucleus as previously described (Sasanami *et al*., 2007).

To obtain the sperm membrane lysates (SPML), the ejaculates were washed three times with ice-cold PBS with repeated centrifugation at 800$\times$g for 3 min at 4℃, and the final pellet was suspended in cavitation buffer containing 150 mM NaCl, 20 mM HEPES (pH 7.4). The suspension was cavitated with a cell disruptor (Parr Instrument Company, Moline, IL, USA) at 400 pound per square inch gauge (psig), and the cellular debris was removed by centrifugation at 10,000$\times$g for 10 min. The supernatants were further centrifuged at 158,000$\times$g for 30 min, and the precipitates were suspended in cavitation buffer containing 0.1% Triton X-100, 0.1 mM PMSF, 0.5 μg/ml leupeptin and 10 μg/ml soybean trypsin inhibitor. The SPML samples were stored at −80℃ until use.

Western Blot Analysis

Testis, hypothalamus or pituitary was homogenized in PBS containing 0.1 mM PMSF, 0.5 μg/m*l* leupeptin and 10 μg/m*l* soybean trypsin inhibitor and the supernatant was obtained by centrifugation at 20,000$\times g$ for 10 min. Protein concentration of each lysate was determined using a BCA protein assay kit (Pierce, Rockford, IL). Aliquots (10 μg) of the solubilized materials were subjected to one-dimensional SDS-PAGE with 12% separating gel as described previously (Laemmli, 1970). The protein was transferred onto polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore, Bedford, MA). PVDF membrane was blocked with 5% non-fat skim milk in saline buffered at pH 7.4 with 10 mM Tris-HCl containing 0.1% Tween 20 (TBS-T) and incubated for 1 h with anti-PRLR (1:2,000) or anti-PRL (1:5,000). After reacting, bands were visualized by a chemiluminescent technique (LAS500, GE Healthcare) using horseradish peroxidase-conjugated anti-rabbit IgG (Cappel, Durham, NC) as secondary antibody.

Dot Blot Analysis

The dot blot assay was performed according to Kubo *et al.* (2010). A PVDF sheet (Immobilon-P; Millipore) wetted with methanol and then with PBS was set in a dot blotter (Bio-Rad Laboratories), and the sperm plasma membrane extracts was added to each well. Twenty minutes later, the solution was removed by suction, and the wells were washed by suction with PBS three times. Thereafter, the solution in the well was removed. The wells were blocked for 30 min with blocking reagent N101 (NOF Corporation, Japan). After blocking, the wells were rinsed once with PBS and quail pituitary extracts was added to each well, and the wells were incubated for 30 min. After the incubation, the wells were washed twice with PBS and were again blocked with the blocker in the same manner. After wells were washed with PBS, anti-PRL (1:2,500) was added to each well and the wells were incubated for 30 min. The wells were washed three times with PBS containing 0.1% (w/v) Tween 20, and the bound antibodies were labeled for 30 min with peroxidase-conjugated anti-rabbit IgG (GE Healthcare) 5,000-fold diluted with 5% (w/v) skim milk in PBS. The wells were washed three times with 0.1% (w/v) Tween 20 in PBS, and the PVDF sheet removed from the blotter was washed again with vigorous shaking three times and then subjected to a chemiluminescent detection system as described above.

Immunohistochemistry

To observe the localization of the PRLR in the testis, the isolated testis were fixed in Bouin's fixative solution and embedded in Paraplast (Oxford Labware, St. Louis, MO). The isolated UVJ mucosa was also processed for immunohistochemistry to observe the expression of PRL protein. Immunohistochemical techniques were as described previously (Sasanami *et al*., 2002) using anti-PRLR (1:200) or anti-PRL (1:200) for the sections of testis or UVJ, respectively. The immunolabeled sections were examined under an interference–contrast photomicroscope (BX 50, Olympus Optics, Tokyo, Japan).

Ejaculated sperm were suspended in sperm extender and washed three times with repeated centrifugation at 800$\times g$ for 3 min. After washing, sperm were fixed in formaldehyde (final concentration, 3.7% (v/v)). The fixed sperm were then smeared on a microscope glass slide, and the PRLR on the surface of the spermatozoa was detected immunologically as described previously (Sasanami *et al*., 2011).

Data Analysis

Data were expressed as means±SE. An arcsine square-root transformation of the percentage data was performed and the transformed data was compared by Student's t-test. Differences were considered statistically significant when $P < 0.05$.

Results and Discussion

To investigate the expression of PRLR, we produced antisera against bacterially expressed PRLR. Western blot analysis demonstrated that the SPML contain immunoreactive band migrating around 70 kDa (Fig 1, left panel, lane 1). This is not due to the contamination of seminal plasma protein since we failed to detect 70 kDa band in the sample of seminal plasma (Fig 1, left panel, lane 2). Importantly, the size of this molecule is much smaller than that of the immunoreactive band in the lysate of hypothalamus (approximately 97 kDa, Fig 1, lane 3), indicating the presence of
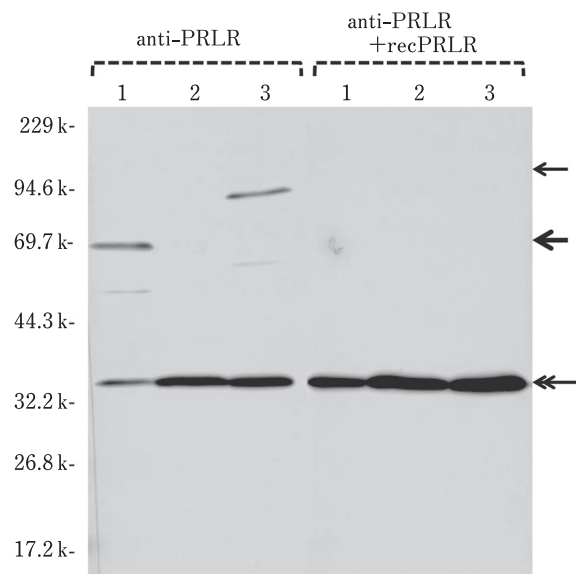

Fig. 1. **Production of anti-prolactin receptor antiserum (anti-PRLR).** The plasma membrane of the ejaculated sperm was isolated and 10 μg aliquots of the SDS-solubilized protein were detected by Western blot using anti-PRLR (lane 1, left panel). Same sample was detected with anti-PRLR preincubated with antigen protein (lane 1, right panel). Seminal plasma (10 μg, lane 2) or homogenates of hypothalamus (10 μg, lane 3) was also detected by same procedure. The representative blots of the repeated experiments were shown. Thin arrow, 97 kDa protein in the homogenates of hypothalamus; bold arrow, 70 kDa protein in the sperm plasma membrane; double arrow, 33 kDa protein that non-specifically reacted with anti-PRLR.

structural differences in the receptor on the sperm with that of hypothalamus. Although the nature of this difference in size is not known, Mao *et al*. (1999) reported that several kind of mRNAs derived from alternative splicing of PRLR gene are present in the testis of chicken. In addition, differential *N* oligosaccharides modifications of the protein might contribute to the structural difference between them since there are totally 13 sites for a potential *N* glycosylation in the amino acid sequence of quail PRLR (GenBank accession number: LC074729). Further studies are required to uncover the structural properties of sperm PRLR. Because the bands detected around 33 kDa that reacted with anti-PRLR did not fade away by antigen absorption (right panel, lanes 1−3), this protein appears to react non-specifically with the antibody.

The immunohistochemical analysis on the sections of the testis shown in Fig. 2A revealed that immunoreactive PRLR proteins were mainly localized in the round spermatids and elongated spermatids. Although a weak signal nearly equivalent to background level was observed, such strong signals were not seen in the spermatocytes, spermatogonia, Sertoli cells as well as Leydig cells. Importantly, no such intense signals were observed when the specimens were incubated with anti-PRLR pre-incubated with antigen proteins (Fig. 2B), indicating that the 33 kDa proteins on the blot are not reactive to our antibody on the paraffin sections. In mammalian species, it is reported that PRLR protein are detected in Leydig cells in addition to spermatogenic cells, indicating the involvement of PRL signaling in the process of steroidogenesis (Hair *et al*., 2002). Although we can not explain this discrepancy, mRNA of PRLR was detected in the isolated Leydig cells by conventional RT-PCR (data not shown). Additional experiments elucidating the role of PRL signaling on the steroidogenesis in the testis of quail will remain in the future studies.

To localize the PRLR proteins in the spermatozoa, we next performed immunostaining of the ejaculated sperm with anti-PRLR. As a result, immunoreactive signals were clearly observed on the surface of the midpiece and flagellum of the sperm (Fig. 3, panels A and C). On the other hand, sperm head was not stained by the antibody (arrows in panels A and C), indicating PRLR proteins mainly localized on the surface of the midpiece and flagellum of the sperm. These signals were considered specific as no such signal was observed when the same preparations were incubated with anti-PRLR antiserum pre-incubated with antigen proteins (panels B and D). This staining pattern is coincident with that of human sperm in that immunoreactive PRLR are mainly localized in the midpiece and flagellum (Pujianto *et al*., 2010). To evaluate the binding of the sperm PRLR to PRL, we tested whether the PRLR in SPML interacts with pituitary PRL based on the dot blot assay (Kubo *et al.,* 2010). Our anti-PRL is confirmed to recognize pituitary PRL (Fig. 4A) in accordance with the results of our previous study (Hiyama *et al*., 2009). As shown in Figure 4B, anti-PRL recognized immunoreactive materials that interact with the SPML component immobilized on a PVDF membrane. When the pituitary ex-

tracts and /or SPML were omitted from the assay, the immunoreactive signal was diminished to a background level or lower, indicating the specificity and the reliability of the assay performed here. These results indicated that pituitary PRL interact with SPML component, most probably via sperm PRLR. Taken together with the results of Western blot analysis, the 70 kDa PRLR on the surface of the ejaculated spermatozoa is functional receptor and might play a role for sperm function.

These results prompt us to investigate the role of PRL on the sperm functions. When we incubated the ejaculates with purified chicken PRL, the occurrence of spontaneous acrosome reaction was decreased in a dose related manner (Fig. 5A). The percentage of acrosome reacted sperm during incubation without PRL increased markedly over time, however, the change was minimal when PRL was added in the incubation mixture (Fig. 5B). This PRL effects is not thought to be a nonspecific action due to the presence of a protein because our previous results indicated the purified ZP3 protein does not affect the occurrence of the spontaneous acrosome reaction (Sasanami *et al*., 2007). In contrast, neither sperm motility nor intracellular ATP level was affected in the presence of the PRL during incubation (data not shown). These results indicated that PRL might inhibit spontaneous acrosome reaction that will impede the fertilizability of the sperm, thus this hormone might maintain the sperm fertilizability until the time of fertilization in birds. The acrosomal contents were required to penetrate the perivitelline membrane, an investment of avian ovum homologous to mammalian zona pellucida at the time of fertilization. Thus, acrosomal components such as sperm acrosin (Sasanami *et al*., 2011) or proteasome (Sasanami *et al*., 2012) that are required for fertilization are not prematurely released until the time of encounter of the sperm and eggs. Recent findings in human indicated that PRLR is expressed on the surface of the spermatozoa and PRL may act as a prosurvival factor for sperm that is responsible for preventing caspase activation thereby inhibiting capacitation (Pujianto *et al*., 2010). They concluded that PRL in the seminal plasma that is transported to the female genital tract along with sperm may exert prosurvival effects for successful fertilization. In contrast, when we examined the quail seminal plasma by Western blot using anti-PRL, a positive signal was not detected (data not shown), instead, the immunohistochemical detection clearly demonstrated that PRL is expressed in the utero-vaginal junction of the oviduct (Fig. 6). It is now accepted that the female reproductive tract actively eliminates the spermatozoa as foreign body. Indeed, Kawano *et al*. (2007, 2014) discovered a sperm protection system in mice in that seminal vesicle secretion 2 (SVS2) protein coats sperm surface and protects the sperm from the cytotoxic factor derived from the uterus. In birds, the oviductal immune system may also affect the survivability of the sperm in the oviduct (Das *et al*., 2008). Very recently we also found that cloacal gland secretions, the major seminal plasma in quail, contains prostaglandin F$_{2\alpha}$, which act to open the entrance of sperm storage tubules that serve as "sperm shelter" for sperm in the oviduct (Sasanami
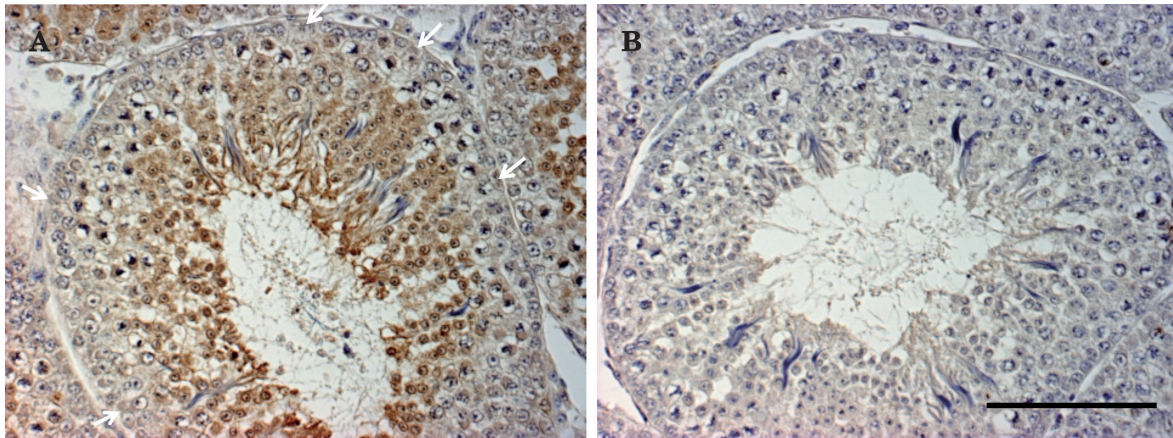
Fig. 2. **Immunohistochemical detection of prolactin receptor in testis.** Sections of testis of mature male were processed for immunohistochemical observation using anti-PRLR (A) or anti-PRLR preincubated with antigen protein (B). The sections were lightly stained by hematoxylin. Arrows shown in panel A represent Sertoli cell. Shown are representative of repeated experiments. Bar＝100 μm.
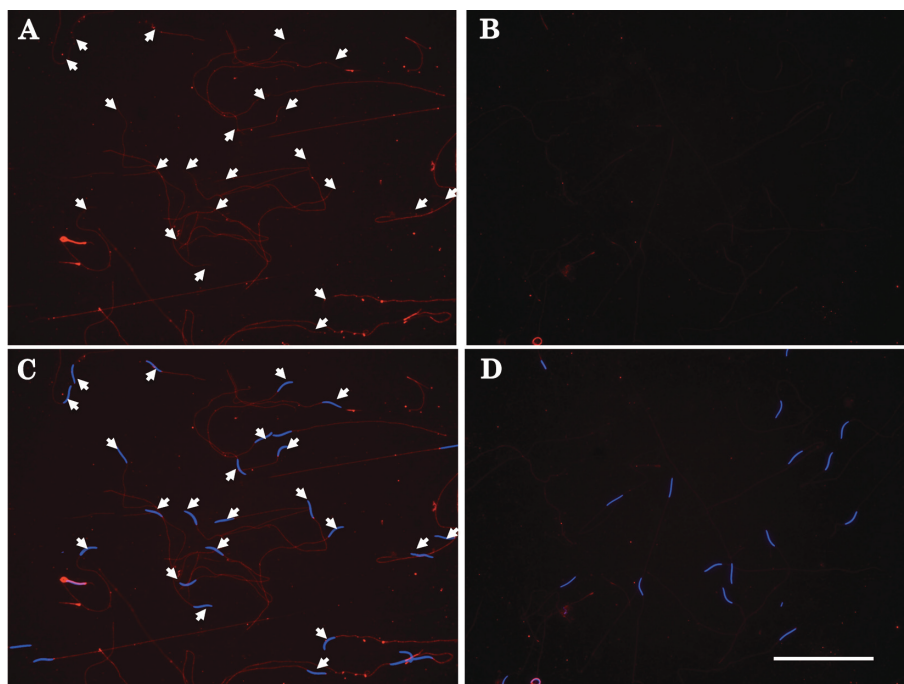

Fig. 3. **Immunohistochemical localization of prolactin receptor in ejaculated sperm.** The smeared specimens of the ejaculated sperm were detected with anti-PRLR. The immunoreactive materials with anti-PRLR (A) can be seen in the midpiece and flagellum but not detected in the head of the sperm. (B) These signals were not observed when the specimen was detected by normal rabbit serum. The sperm nuclei in (A) were stained with DAPI (blue) and the image was merged (C). The control specimen was also stained with DAPI and merged with immunostaining (D). Arrows shown in panels (A) and (C) represent sperm head. Shown are representative of repeated experiments. Bar＝100 μm.
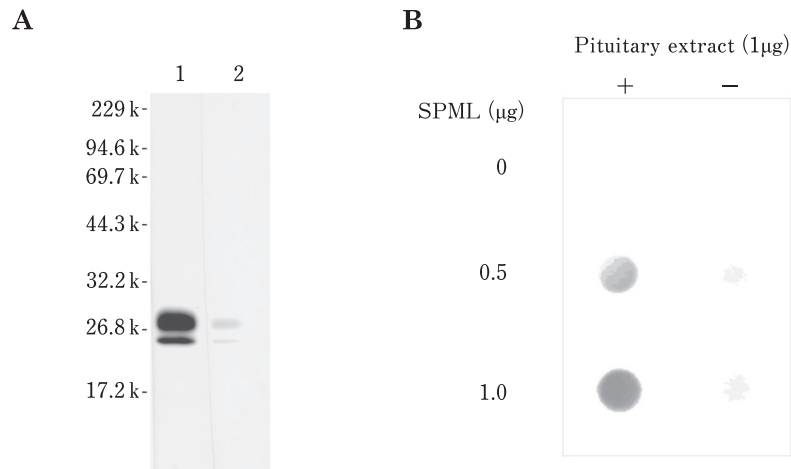
A   **B**

Fig. 4.   **Detection of prolactin binding to the sperm plasma membrane.** (A) The extracts of pituitary (1 μg proteins) was separated on SDS-PAGE and was detected by Western blotting using anti-prolactin antiserum (lane 1) or anti-PRL antiserum preincubated with antigen protein (lane 2). SDS-solubilized sperm plasma membrane (0, 0.5 or 1 μg proteins) was dot blotted onto a PVDF and then was incubated with the extracts of pituitary (1 μg/ml). The dots were incubated with anti-PRL antiserum and the bound prolactin was immunologically detected. Shown are results representative of three repeated experiments.
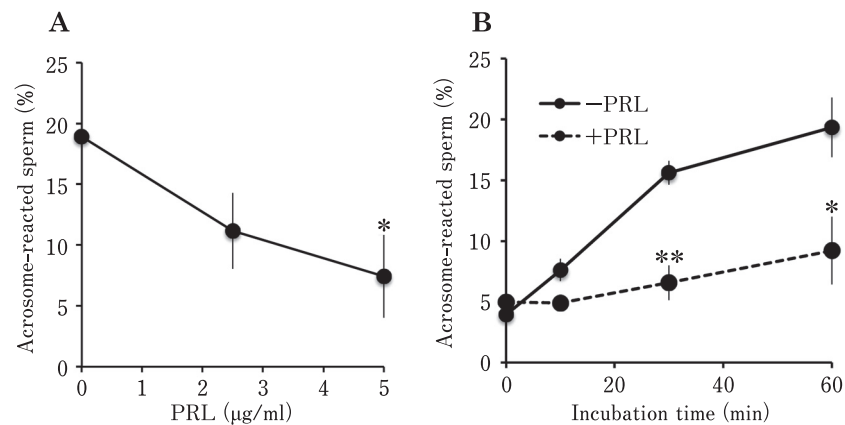
A   **B**

Fig. 5.   **Effects of chicken prolactin on the acrosome reaction of quail sperm.** (A) The ejaculated sperm were suspended in the medium containing 0, 2.5 or 5 μg/ml chicken PRL and were incubated at 39℃ for 30 min. Data shown are the mean±SE of 3 experiments. Value with * is significantly different from 0 μg/ml control ($P<0.05$). (B) The ejaculated sperm were incubated in the presence (+PRL) or absence (−PRL) of chicken PRL (5 μg/ml) at 39℃ for 0, 10, 30 or 60 min. The acrosome status of the sperm after the incubation was observed under fluorescent microscope. Data shown are the mean±SE of 3 experiments. Value with * or ** is significantly different from "−PRL" at respective incubation time (* or ** denotes $P<0.05$ or $P<0.01$, respectively).

et al., 2015). The results obtained in this study suggest a novel system that a female factor, oviductal PRL prevents spontaneous acrosome reaction that might directly link to the maintenance of the fertilizability of the spermatozoa in Japanese quail. The question of whether this unique mechanism works in the quail reproductive system is also functional in other birds remains to be solved in the future studies.
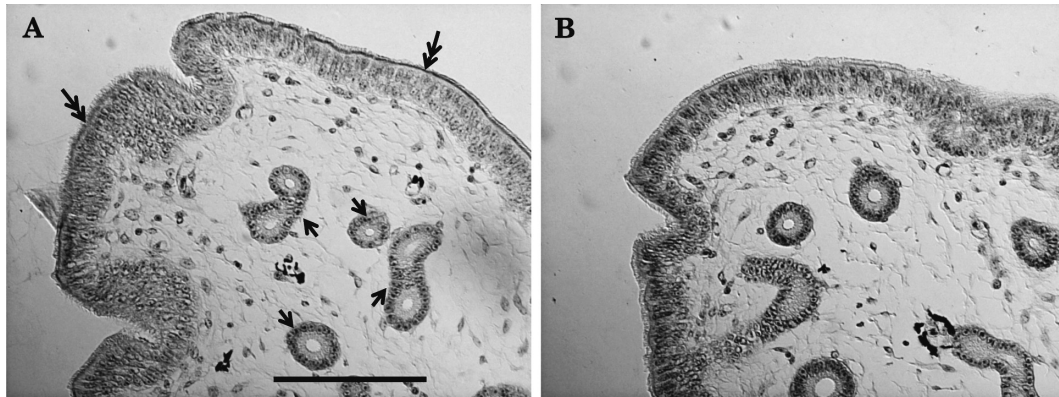
Fig. 6. **Expression of prolactin in the utero-vaginal junction.** Sections of utero-vaginal junction of mature female were processed for immunohistochemical observation using anti-PRL (A) or anti-PRL preincubated with antigen protein (B). Positive signal was detected on the surface epithelium (Double allows) but not in the sperm storage tubules (Allows) of utero-vaginal junction. The sections were lightly stained by hematoxylin. Shown are representative of repeated experiments. Bar＝100 μm.

References

Ben-Jonathan N, Mershon JL, Allen DL and Steinmetz RW. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. Endocrine Reviews, 17: 639−669. 1996.

Binart N, Melaine N, Pineau C, Kercret H, Touzalin AM, Imbert-Bolloré P, Kelly PA and Jégou B. Male reproductive function is not affected in prolactin receptor-deficient mice. Endocrinology, 144: 3779−3782. 2003.

Bole-Feysot C, Goffin V, Edery M, Binart N and Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocrine Reviews, 19: 225−268. 1998.

Boutin JM, Jolicoeur C, Okamura H, Gagnon J, Edery M, Shirota M, Banville D, Dusanter-Fourt I, Djiane J and Kelly PA. Cloning and expression of the rat prolactin receptor, a member of the growth hormone/prolactin receptor gene family. Cell, 53: 69−77. 1988.

Camper PM and WH Burke. Serum oestradiol and progesterone levels of laying turkey hens following acute treatment with mammalian luteinizing hormone or follicle stimulating hormone. General and Comparative Endocrinology, 31: 224−232. 1977.

Chen X and Horseman ND. Cloning, expression, and mutational analysis of the pigeon prolactin receptor. Endocrinology, 135: 269−276. 1994.

Dai G, Wang D, Liu B, Kasik JW, Müller H, White RA, Hummel GS and Soares MJ. Three novel paralogs of the rodent prolactin gene family. Journal of Endocrinology, 166: 63−75. 2000.

Das SC, Isobe N and Yoshimura Y. Mechanism of prolonged sperm storage and sperm survivability in hen oviduct: a review. American Journal of Reproductive Immunology, 60: 477−481. 2008.

Duckworth ML, Peden LM and Friesen HG. Isolation of a novel prolactin-like cDNA clone from developing rat placenta. Journal of Biological Chemistry, 261: 10879−10884. 1986.

Freeman ME, Kanyicska B, Lerant A and Nagy G. Prolactin: structure, function, and regulation of secretion. Physiological Reviews, 80: 1523−1631. 2000.

Hair WM, Gubbay O, Jabbour HN and Lincoln GA. Prolactin receptor expression in human testis and accessory tissues: localization and function. Molecular Human Reproduction, 8: 606−611. 2002.

Harvey S, Martínez-Moreno CG, Luna M and Arámburo C. Autocrine/paracrine roles of extrapituitary growth hormone and prolactin in health and disease: An overview. General and Comparative Endocrinology, 220: 103−111. 2015.

Hiyama G, Kansaku N, Kinoshita M, Sasanami T, Nakamura A, Noda K, Tsukada A, Shimada K and Zadworny D. Changes in post-translational modifications of prolactin during development and reproductive cycles in the chicken. General and Comparative Endocrinology, 161: 238−245. 2009.

Hondo E, Kurohmaru M, Sakai S, Ogawa K and Hayashi Y. Prolactin receptor expression in rat spermatogenic cells. Biology of Reproduction, 52: 1284−1290. 1995.

Ishida M, Yoshida M, Fukuta S, Uemura K, Iijima M, Horiguchi K and Harigaya T. Analysis of prolactin gene expression and cleaved prolactin variants in the mouse testis and spermatozoa. Journal of Reproduction and Development, 56: 567−574. 2010.

Ito T, Yoshizaki N, Tokumoto T, Ono H, Yoshimura T, Tsukada A, Kansaku N and Sasanami T. Progesterone is a sperm-releasing factor from the sperm-storage tubules in birds. Endocrinology, 152: 3952−3962. 2011.

Kawano N and Yoshida M. Semen-coagulating protein, SVS2, in mouse seminal plasma controls sperm fertility. Biology of Reproduction, 76: 353−361. 2007.

Kawano N, Araki N, Yoshida K, Hibino T, Ohnami N, Makino M, Kanai S, Hasuwa H, Yoshida M, Miyado K and Umezawa A. Seminal vesicle protein SVS2 is required for sperm survival in the uterus. Proceedings of the National Academy of Sciences of the United States of America, 111: 4145‒4150. 2014.

Kubo H, Shiga K, Harada Y and Iwao Y. Analysis of a sperm surface molecule that binds to a vitelline envelope component of Xenopus laevis eggs. Molecular Reproduction and Development, 77: 728‒735. 2010.

Kuroki M and Mori M. Origin of 33 kDa protein of vitelline membrane of quail egg: immunological studies. Development Growth and Differentiation, 37: 545‒550. 1995.

Kuroki M and Mori M. Binding of spermatozoa to the perivitelline layer in the presence of a protease inhibitor. Poultry Science, 76: 748‒752. 1997.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680‒685. 1970.

Li WL, Liu Y, Yu YC, Huang YM, Liang SD and Shi ZD. Prolactin plays a stimulatory role in ovarian follicular development and egg laying in chicken hens. Domestic Animal Endocrinology, 41: 57‒66. 2011.

Mao JN, Burnside J, Li L, Tang J, Davolos C and Cogburn LA. Characterization of unique truncated prolactin receptor transcripts, corresponding to the intracellular domain, in the testis of the sexually mature chicken. Endocrinology, 140: 1165‒1174. 1999.

Marano RJ and Ben-Jonathan N. Minireview: Extrapituitary prolactin: an update on the distribution, regulation, and functions. Molecular Endocrinology, 28: 622‒633. 2014.

Nicoll CS. Bio-assay of prolactin. Analysis of the pigeon crop-sac response to local prolactin injection by an objective and quantitative method. Endocrinology, 80: 641‒655. 1967.

Ouhtit A, Morel G and Kelly PA. Visualization of gene expression of short and long forms of prolactin receptor in rat reproductive tissues. Biology of Reproduction, 49: 528‒536. 1993.

Parthasarathy C and Balasubramanian K. Differential effect of corticosterone deficiency on the expression of LH, prolactin and insulin receptors on rat Leydig cells. Molecular and Cellular Endocrinology, 285: 34‒42. 2008.

Pujianto DA, Curry BJ and Aitken RJ. Prolactin exerts a prosurvival effect on human spermatozoa via mechanisms that involve the stimulation of Akt phosphorylation and suppression of caspase activation and capacitation. Endocrinology, 151: 1269‒1279. 2010.

Riddle O, Bates RW and Lahr EL. Prolactin induces broodiness in fowl. American Journal of Physiology, 111: 352‒360. 1935.

Sasanami T, Pan J, Doi Y, Hisada M, Kohsaka T and Toriyama M. Secretion of egg envelope protein ZPC after C-terminal proteolytic processing in quail granulosa cells. European Journal of Biochemistry, 269: 2223‒2231. 2002.

Sasanami T, Murata T, Ohtsuki M, Matsushima K, Hiyama G, Kansaku N and Mori M. Induction of sperm acrosome reaction by perivitelline membrane glycoprotein ZP1 in Japanese quail (Coturnix japonica). Reproduction, 133: 41‒49. 2007.

Sasanami T, Yoshizaki N, Dohra H and Kubo H. Sperm acrosin is responsible for the sperm binding to the egg envelope during fertilization in Japanese quail (Coturnix japonica). Reproduction, 142: 267‒276. 2011.

Sasanami T, Sugiura K, Tokumoto T, Yoshizaki N, Dohra H, Nishio S, Mizushima S, Hiyama G and Matsuda T. Sperm proteasome degrades egg envelope glycoprotein ZP1 during fertilization of Japanese quail (Coturnix japonica). Reproduction, 144: 423‒431. 2012.

Sasanami T, Izumi S, Sakurai N, Hirata T, Mizushima S, Matsuzaki M, Hiyama G, Yorinaga E, Yoshimura T, Ukena K and Tsutsui K. A unique mechanism of successful fertilization in a domestic bird. Scientific Reports, 5: 7700. 2015.

Sharp PJ, Sterling RJ, Talbot RT and Huskisson NS. The role of hypothalamic vasoactive intestinal polypeptide in the maintenance of prolactin secretion in incubating bantam hens, observations using passive immunization, radioimmunoassay and immunohistochemistry. Journal of Endocrinology, 122: 5‒13. 1989.

Shirota M, Banville D, Ali S, Jolicoeur C, Boutin JM, Edery M, Djiane J and Kelly PA. Expression of two forms of prolactin receptor in rat ovary and liver. Molecular Endocrinology, 4: 1136‒1143. 1990

Skwarło-Sońta K, Rosołowska-Huszcz D, Sotowska-Brochocka J and Gajewska A. Daily variations in response of certain immunity indices to prolactin in White Leghorn chickens. Experimental and Clinical Endocrinology, 87: 195‒200. 1986.

Soares MJ. The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal-fetal interface. Reproductive Biology and Endocrinology, 2: 51. 2004.

Tanaka M, Maeda K, Okubo T and Nakashima K. Double antenna structure of chicken prolactin receptor deduced from the cDNA sequence. Biochemical and Biophysical Research Communications, 188: 490‒496. 1992.

Tanaka M, Yamamoto I, Hayashida Y, Nakao N, Ohkubo T, Wakita M and Nakashima K. Two novel first exons in the prolactin receptor gene are transcribed in a tissue-specific and sexual maturation-dependent manner to encode multiple 5′-truncated transcripts in the testis of the chicken. Biochimica et Biophysica Acta, 1491: 279‒284. 2000.

Toft DJ and Linzer DI. Prolactin (PRL)-like protein J, a novel member of the PRL/growth hormone family, is exclusively expressed in maternal decidua. Endocrinology, 140: 5095‒5101. 1999.

Trott JF, Hovey RC, Koduri S and Vonderhaar BK. Alternative splicing to exon 11 of human prolactin receptor gene results in multiple isoforms including a secreted prolactin-binding protein. Journal of Molecular Endocrinology, 30: 31‒47. 2003.

Untergasser G, Kranewitter W, Schwärzler P, Madersbacher S, Dirnhofer S and Berger P. Organ-specific expression pattern of the human growth hormone/placental lactogen gene-cluster in the testis. Molecular and Cellular Endocrinology, 130: 53‒60. 1997.

Zadworny D, Shimada K, Ishida H and Sato K. Gonadotropin-stimulated estradiol production in small ovarian follicles of the hen is suppressed by physiological concentrations of prolactin in vitro. General and Comparative Endocrinology, 74: 468‒473. 1989.

Zhou JF, Zadworny D, Guémené D and Kuhnlein U. Molecular cloning, tissue distribution, and expression of the prolactin receptor during various reproductive states in Meleagris gallopavo. Biology of Reproduction, 55: 1081‒1090. 1996.