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Gene Expression Profiles of LH, Prolactin and Their Receptors in Female Zi Geese (*Anser cygnoides*) during Development

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The objective of this work was to elucidate the gene expression profiles of luteinizing hormone (LH), prolactin (PRL) and their receptors during the developmental and egg laying stage. The expression of genes encoding pituitary LH and PRL, as well as those for the ovarian LH receptor (LHR) and PRL receptor (PRLR), was determined by quantitative real-time PCR in Zi geese on day 1 and at 1, 2, 3, 4, 5, 6, 7 and 8 months of age, respectively. The expression of LH and LHR fluctuated and increased as the geese aged. The expression of LH was significantly higher at 5 to 8 months of age than in 1 day old geese ($P<0.05$). The expression of LHR was higher at 8 months than at 1 day, at 1 to 4 months and at 6 months ($P<0.05$). The expression of PRL decreased from day 1, followed by an increase from 3 months, and reached the highest values at 8 months of age in the study. The difference in PRL expression between 7 and 8 months of age was significant ($P<0.05$). The expression of PRLR decreased initially and this was followed by a fluctuating increase from 5 months until 8 months of age. The expression of PRLR in 1 to 8 month old geese was significantly lower than at day 1 ($P<0.05$). These results suggest that LH and PRLR may play an important role in ovarian development and the egg-laying process in Zi geese.

Key words: Zi goose, luteinizing hormone, prolactin, receptor, quantitative real-time PCR.

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Luteinizing hormone (LH) induces many enzymes that are involved in steroid biosynthesis. Mice deficient in LH or in LH receptor (LHR) genes are infertile, with defects in steroidogenesis (LEI *et al.* 2001; ZHANG *et al.* 2001; MA *et al.* 2004). LH, as well as follicle-stimulating hormone (FSH), is essential for sexual maturation and egg production. In birds, ovulation and oviposition are processes controlled by LH and sex steroids, including progesterone (SANDHU *et al.* 2008). The expression profiles of LH in the pituitary and LHR in the ovary of geese during embryonic development and the egg-laying period have been studied extensively in avian species (BRUGGEMAN *et al.* 2002; RANGEL *et al.* 2006; NI *et al.* 2007; GRZEGORZEWSKA *et al.* 2009). However, whether LH can regulate ovarian development and/or folliculogenesis remains to be determined by further experimentation. Prolactin (PRL), produced in the anterior pituitary, is postulated to play a critical role in the onset and maintenance of incubation behavior in birds (SHARP *et al.* 1988; MARCH *et al.* 1994; JIANG *et al.* 2005; GUO *et al.* 2008; LIU *et al.* 2008). LH and PRL play important roles in the synchronization and regulation of reproductive seasonality in both long- and short-day breeding birds (SHARP & BLACHE 2003).

Zi geese (*Anser cygnoides*) are a native breed of the temperate zone of Heilongjiang Province in China. The number of eggs laid by one Zi goose in

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the annual cycle ranges only from about 80 to 100. Studies on improving the performance of Zi geese have become more common in recent years (KANG *et al.* 2009). Our previous studies demonstrated that the FSH receptor (FSHR) should play a pivotal role in mediating the responsiveness of the goose ovary to FSH during the developmental and egg-laying stages (KANG *et al.* 2009; KANG *et al.* 2010). However, changes in the expression of LH and PRL during the developmental and egg-laying stages remain to be determined. Furthermore, to our knowledge, although there have been numerous studies published concerning the expression of LH, LHR, PRL and PRLR, there are no data regarding their simultaneous measurement and analysis during the developmental and egg-laying stages in avian species. Therefore, the purpose of the present study was to investigate the gene expression profiles of LH and PRL in the pituitary and their receptors in the ovaries in 1 day old Zi geese and at the ages of 1 to 8 months.

Material and Methods

Animals and tissue collection

The experiment was carried out on 45 female Zi geese (*Anser cygnoides*) according to the regulations of the Animal Ethics Committee of the College of Animal Husbandry and Veterinary Medicine of Jilin University (No.58/2008 of the May 8th, 2008).

The experiment was carried out in Datong district (124.83°E, 46.04°N), Heilongjiang Province. On a local breeding farm about 120 eggs from different parent animals (3 males and 18 females) were hatched, a further 100 geese were raised according to the standard program used at the farm. For the experiment, 45 female geese were selected from this flock (about 2-4 experimental animals were from the same parents). Geese were fed *ad libitum* with rice grain and were supplemented with green grass or water plants whenever possible during the experiment.

Zi geese were exposed to a natural photoperiod throughout the experimental period. The experiment began on October 15th, 2008 and ended on the June 15th, 2009. During the breeding season, from April to November, Zi geese at approximately 6 months of age began to lay eggs. Eggs were collected daily from 6:00 to 14:00, and the date of egg-laying was recorded.

Five geese were killed at the age of 1 day and at 1, 2, 3, 4, 5, 6, 7 and 8 months of age, respectively. Only non-broody or animals not showing signs of broodiness were selected. In general, Zi geese brood after laying 10-20 eggs. The laying performance of the experimental animals was low, thus they were considered as non-broody.

To obtain samples, the animals were killed by exsanguination at 10:00-11:00 a.m. (Zi geese ovulate eggs between 7:00 - 9:00 a.m., thus the egg-laying geese were killed approximately 2-3 hours after oviposition). Body weight, ovarian and oviduct weight were recorded, and their pituitaries and ovarian stromas were collected. These tissues were frozen in liquid nitrogen immediately after removal.

Total RNA isolation and reverse transcription PCR

Total RNA was prepared from the pituitary glands and with Trizol reagent (Invitrogen Corporation, Carlsbad, California, USA), and stored at -70°C until analysis. The cDNA was synthesized using SuperScript III Reverse Transcriptase (Invitrogen). Reverse transcriptase negative control reactions were performed to ensure the absence of genomic DNA contamination. Gene-specific primers were designed by Primer Premier 5.00 and synthesized by Shanghai Sangon (Shanghai, China). The sequences of the primers are listed in Table 1. The 50 μl reaction consisted of 2 μl of cDNA, 8 μl of 2.5 mmol/l deoxynucleoside triphosphate (dNTP) mix, 1 μl of 20 μmol/l PCR forward primer and PCR reverse primer, 5 μl of 10×LA PCR Buffer, 0.5 μl of 5U/μl LA Taq™ (Takara Bio Inc., Dalian, China), and 32.5 μl of sterile MilliQ water. Thermal cycling was performed with an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 40 s at 94°C, and 52-56°C for 30 s, 72°C for 40 s, and then a final extension at 72°C for 10 min.

Construction of cDNA plasmid

The recombinant plasmids containing LH, LHR, PRL, PRLR and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA were termed pLH, pLHR, pPRL, pPRLR and pGAPDH, respectively. The sequences of the above genes were obtained from GenBank. These constructs were prepared from the total RNA of the ovaries of Zi geese at 8 months of age, and the complementary double-stranded cDNA fragments were subcloned into the pGEM-T Easy Vector System (Promega, Madison, Wisconsin, USA) as described previously (KANG *et al.* 2010), then sequenced by Shanghai Sangon (Shanghai, China).

Quantitative real-time PCR

The quantitative real-time PCR (qRT-PCR) was performed on first strand cDNA using the Line-Gene K Real-time PCR Detection System and software (Bioer Technology, Hangzhou, China) with SYBR® Premix Ex Taq™ (Takara). Briefly, the 25 μl reaction consisted of 0.5 μl of cDNA,

Table 1

List of primer sequences used for quantitative real-time PCR

Target gene	Sequence of primer (5'-3')	Accession Number	Species	PCR products (bp)	Annealing temperature
LH	Forward: GTGACAGTGGCGGTGGAGAA Reverse: CCCAAAGGGCTGCGGTA	GenBank: L35519	*Meleagris gallopavo*	110	56°C
LHR	Forward: GTAACACTGGAATAAGGGAAT Reverse: GAAGGCTTGACTGTGGATA	GenBank: EU049613	*Anas platyrhynchos*	191	52°C
PRL	Forward: CCTGAAGACAAGGAGCAAGC Reverse: AGAATGAACCCGCCCAAC	GenBank: DQ062571	*Anser anser*	222	56°C
PRLR	Forward: GATCCTCGCTGTCCTCTACCTCT Reverse: GCCTTTATCCTACCACCAGTTCC	GenBank: EU078175	*Anser anser*	175	55°C
GAPDH	Forward: GTGGTGCAAGAGGCATTGCTGAC Reverse: GCTGATGCTCCCATGTTCGTGAT	GenBank: AY436595	*Anas platyrhynchos*	86	52°C ~56°C

12.5 μl of SYBR® Premix Ex Taq™ (2 × concentration), 0.5 μl of 20 μmol/l of PCR forward primer and PCR reverse primer, and 11 μl of nuclease-free water. Thermal cycling was performed as mentioned. For the generation of standard curves, the pLH, pLHR, pPRL, pPRLR or pGAPDH standards were also run. The level of expression of the LH, LHR, PRL and PRLR genes was calculated relative to GAPDH (the internal normalizer) using the double-standard curve method (KANG *et al.* 2009).

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SAS 9.0 statistical software for Windows (SAS Institute Inc., Cary, NC, USA). Values were expressed as means ± SD and were considered significantly different at $P<0.05$.

Results

Changes in growth and reproductive parameters

Body weight and food intake showed a fluctuating increase from the age of 1 day (Fig. 1). Body weight among 1 day old geese and those 1 to 5 months old was significantly different ($P<0.05$), however values for geese 6 to 8 months of age were not significantly different ($P>0.05$). Food intake among 1 day old geese and 1 to 4 month old birds was significantly different ($P<0.05$), but not for individuals 5 to 8 months of age ($P>0.05$).

The geese began to lay eggs on 12 April, and on average 5, 12 and 15 eggs were laid by a goose during one month at the age of 6, 7 and 8 months, respectively. Ovarian and oviduct weight of Zi geese at the age of 1 day and 1 to 4 months did not significantly increase (Fig. 2). However, ovarian and oviduct weight began to increase rapidly from February 2009 (approximately at the age of 4 months). Ovarian and oviduct weight among geese 5 to 8 months of age was significantly different ($P<0.05$).

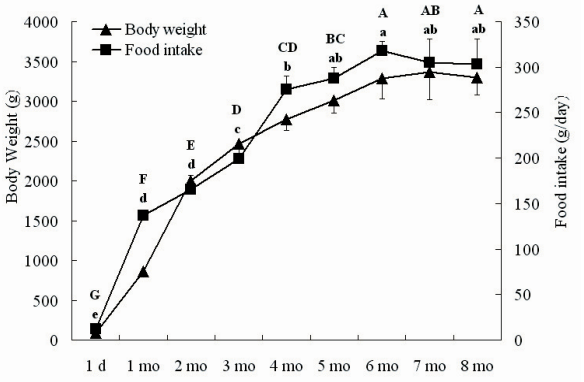

Fig. 1. Changes of body weight and food intake in Zi geese (n=5) at the age of 1 day and 1 to 8 months. Values are means ± SD. The significance of differences in body weight and food intake was determined by ANOVA. Common letters indicate a lack of significant differences between groups (capital letters: body weight; lowercase letters: food intake).

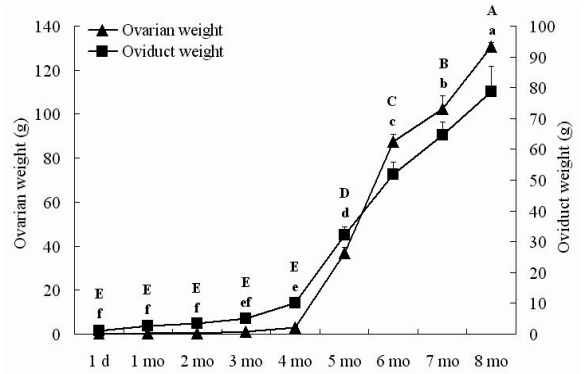

Fig. 2. Changes of ovarian and oviduct weight in Zi geese (n=5) at the age of 1 day and 1 to 8 months. Values are means ± SD. The significance of differences in ovarian and oviduct weight was determined by ANOVA. Common letters indicate non-significant differences between groups (capital letters: ovarian weight; lowercase letters: oviduct weight).

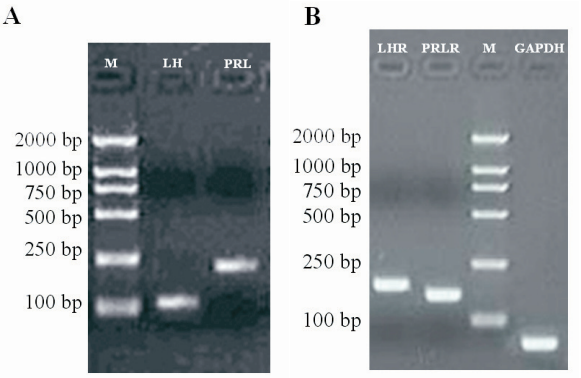

Fig. 3. Amplification of pituitary LH and PRL, ovarian LHR, PRLR and GAPDH. The amplicons of the 110-bp luteinizing hormone gene (LH) and 224-bp prolactin gene (PRL) were separated in agarose gels (A). The amplicons of the 191-bp luteinizing hormone receptor gene (LHR), 175-bp prolactin receptor gene (PRLR) and 86-bp glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) were separated (B). A 2000-bp molecular weight marker (M) was used.

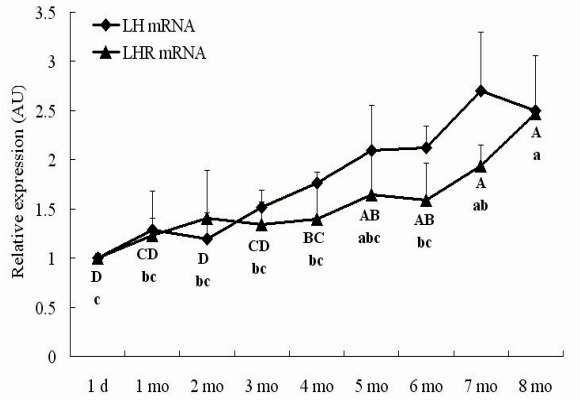

Fig. 4. Quantification of LH and LHR in Zi geese (n=5) at the age of 1 day and 1 to 8 months. The level of LH and LHR expression was normalized to glyceraldehyde-3-phosphate dehydrogenase. The level of expression is presented in arbitrary units (AU). Values are means ± SD. The significance of differences in the levels of expression of LH and LHR mRNA was determined by ANOVA. Common letters indicate a lack of significant differences between groups (capital letters: the amount of pituitary LH mRNA; lowercase letters: the amount of ovarian LHR mRNA).

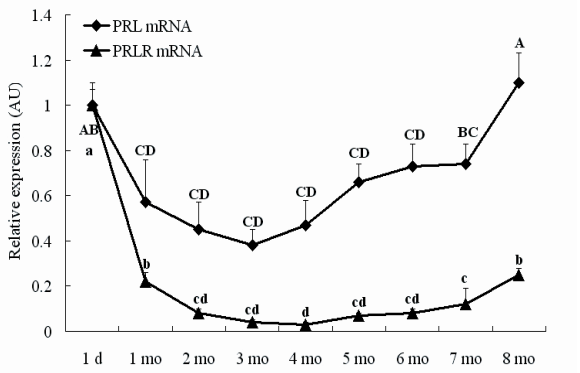

Fig. 5. Quantification of PRL and PRLR in Zi geese (n=5) at the age of 1 day and 1 to 8 months. The level of PRL and PRLR gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase. The level of expression is presented in arbitrary units (AU). Values are means ± SD. The significance of differences in the levels of expression of LH and LHR mRNA was determined by ANOVA. Common letters indicate non-significant differences between groups (capital letters: the amount of pituitary PRL mRNA; lowercase letters: the amount of ovarian PRLR mRNA).

Amplification of target gene

LH, PRL and GAPDH genes in pituitary glands and LHR, PRLR and GAPDH genes in ovaries were detected in Zi geese at the age of 5 months. The products corresponded to the approximate predicted size, and their specificity was appropriate for the qRT-PCR (Fig. 3).

Quantification of LH and LHR

With age, the levels of LH and LHR gene expression showed a slightly fluctuating increase (Fig. 4). The highest value of pituitary LH was determined at 7 months of age, and this was 2.7±0.6 fold higher than that at 1 day ($P<0.05$). The level of expression of LH among 1 day old geese and those 1 to 3 months of age was not significantly different ($P>0.05$), as well as that of geese 5 to 8 months of age. However, the expression of LH was much higher in 5 to 8 month old animals than in 1 day old geese ($P<0.05$). The expression of LHR in the ovaries fluctuated from 1 day, and reached the highest value at 8 months in the study. The highest value of ovarian LHR expression was 2.5±0.07 fold higher than that at 1 day ($P<0.05$). The expression of LHR was higher at 8 months than that at 1 day, at 1 to 4 months and at 6 months ($P<0.05$).

Quantification of PRL and PRLR

The change in regulation of PRL and PRLR expression was different from that of LH and LHR (Fig. 5). The level of PRL expression decreased from the post hatch period, then fluctuated from 3 months of age, and reached the highest value at 8 months of age in the study. The level of PRL expression between geese at 1 day and at 8 months of age was not significantly different ($P>0.05$). The differences among geese at 1 to 7 months old were not significant ($P>0.05$). The expression of PRL was significantly higher at 1 day and at 8 months old than at 1 to 7 months old ($P<0.05$). The level of PRLR in the ovaries decreased initially as the geese aged, then fluctuated from 5 months old until 8 months old. The level of PRLR expression in 1 to 8 month old geese was much lower than expression in 1 day old geese ($P<0.05$). The expression of PRLR was not significantly different in 8 month old birds from that at of geese aged 1 month ($P>0.05$). The differences among geese at 2, 3, 4, 5 and 6 months were also insignificant ($P>0.05$).

Discussion

Numerous studies have been conducted on the expression of pituitary LH and ovarian LHR genes during embryonic development and reproductive

stages in mammalian and avian speices (SAWITZKE & ODELL 1991; SHEMESH *et al.* 2001; CICCONE *et al.* 2005; BRONNEBERG *et al.* 2009). However, the changes in LH and LHR expression in avian species during the developmental and egg-laying stages remain to be determined. Therefore, the aim of this study was to determine the profiles of pituitary LH expression and ovarian LHR expression, and the changes of body weight, food intake, ovarian and oviduct weight in Zi geese during the developmental and egg-laying stages.

The change of body weight was coincident with that of food intake during the developmental and egg laying stages. Similar results were obtained by IZUMI et al., who demonstrated that a positive correlation was observed between food intake and body weight in geese (IZUMI *et al.* 1992). Zi geese usually begin to lay eggs at the age of 6 months. The amount of expressed follicle-stimulating hormone receptor in the ovary of Zi geese aged 4 months began to increase significantly, and the ovarian follicle developed rapidly (KANG *et al.* 2010). Therefore, it is not surprising that ovarian and oviduct weight began to increase rapidly during two months before the egg laying period.

In the present study, we found that the levels of LH and LHR expression in Zi geese during the developmental period were relatively constant, and were lower than that during the egg-laying stage. Similar results were obtained by YANG *et al*. 2005, who demonstrated that no significant changes in plasma LH were observed during the development of female ducks. The levels increased from day 135 and reached the highest value on day 165 post-hatch (YANG *et al.* 2005). Furthermore, plasma LH concentrations in Magang ganders were low in the non-breeding season, from April to July, and increased to high levels in the normal breeding season, from August to March (SHI *et al.* 2007). The expression of LHR in the ovaries increased in pre-pubertal ducks from 1 to 90 days of age, reaching the highest value at 90 days (NI *et al.* 2007). In this study, similar results were detected in the ovaries of Zi geese during the developmental stage. Therefore, the lower level of LH expression in the pituitary glands of the pre-pubertal geese may have been a consequence of higher levels of estrogen and other steroids which suppress LH secretion (SHARP 1975). After the breeding seasons and at 6 months of age, usually from April to November, Zi geese begin to lay eggs. Therefore, it is not surprising that the expression of pituitary LH and ovarian LHR was maintained at relatively high levels during this period. Interestingly, the change in the expression of LHR was not more striking than that of FSHR (KANG *et al.* 2009). These findings indicate that LH may play an important role in the control of ovarian growth and in maintenance of the process of reproduction. However, LH could act as a more important modulator of processes associated with egg-laying when compared with ovarian growth. Ovarian development is regulated by gonadotropin, and is highly responsive to the regulation of FSH during the early stage; in contrast, the ovary is likely to be more responsive to LH in the period close to the onset of sexual maturation (NI *et al.* 2007).

PRL plays a pivotal role in broodiness behavior and its function is manifested through PRLR (TANAKA *et al.* 1992). The highest level of expression of PRLR in the ovaries of Eastern Zhejiang White Geese was found at the incubation stage; a lower level of expression occurred during the egg-laying stage, and the lowest level of expression was found in the out-of-lay stage (CHU *et al.* 2008). Furthermore, the levels of PRL and PRLR expression decreased gradually from day 1, followed by fluctuation, and then reached the highest value in 8 month old birds in the experiment. The changes in expression of pituitary PRL and ovarian PRLR were synchronized; both decreased continuously from the neonatal stage, and then increased until the egg-laying stage. The increase in PRLR expression lagged slightly behind that of PRL. When the level of expression of PRLR increases to a certain value, PRL combines with PRLR in the ovaries, and this initiates the biological actions of PRL, which lead to broodiness behavior in geese (SHI *et al.* 2007; DAWSON & SHARP 2010; XU *et al.* 2010). Therefore, the increase in expression of pituitary PRL and ovarian PRLR may be a signal for broodiness behavior in geese. However, the threshold values of PRL and/or PRLR required for the onset of broodiness need to be determined in further studies.

Interestingly, the changes in LH and LHR expression were opposite to those in PRL and PRLR expression. During the early development of Zi geese, the levels of LH and LHR expression increased gradually, however those of PRL and PRLR expression decreased at first, and then increased. In White Leghorn hens the plasma concentration of PRL significantly decreased ($P<0.05$) whereas plasma LH significantly increased ($P<0.05$) after administration of an anti-PRL agent (2-bromo-α-ergocriptine). The increase in LH might have resulted from the removal of the suppressive effect of PRL on LH (REDDY *et al.* 2007). Furthermore, in cockatiels, nest inspection and laying were characterized by high LH levels while high PRL levels occurred during incubation and feeding of nestlings in females (Sharp & Blache 2003), in agreement with the present study. However, it remains to be determined whether the progressive increase in expression of LH and LHR results from the reduction in expression of PRL

and PRLR during the developmental and egg-laying stages in Zi geese.

In this study, the expression of LH, LHR, PRL and PRLR was determined in Zi geese during the development and egg-laying stages. The expression of LH and LHR fluctuated as the geese aged. Pituitary expression of PRL decreased during the early developmental stage, and then fluctuated and reached the highest value at the egg-laying stage in the experiment. These results suggest that LH and PRL may play an important role in ovarian development and the egg-laying process in Zi geese.

References

BRONNEBERG R. G., VERNOOIJ J. C., STEGEMAN J. A., TAVERNE M. A. 2009. Follicle dynamics and its relation with plasma concentrations of progesterone, luteinizing hormone and estradiol during the egg-laying cycle in ostriches. Reprod. Domest. Anim. **44**: 705-713.

BRUGGEMAN V., VAN AS P., DECUYPERE E. 2002. Developmental endocrinology of the reproductive axis in the chicken embryo. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. **131**: 839-846.

CHU X. H., HU J. P., LU L. Z., WANG Y. Q., CHEN W. H., XU N. Y. 2008. Cloning of the prolactin receptor gene and studying on its expression characteristics in eastern zhejiang white geese. Acta Vet. Zootech. Sinica **39**: 823-826.

CICCONE N. A., SHARP P. J., WILSON P. W., DUNN I. C. 2005. Changes in reproductive neuroendocrine mRNAs with decreasing ovarian function in ageing hens. Gen. Comp. Endocrinol. **144**: 20-27.

DAWSON A., SHARP P. J. 2010. Seasonal changes in concentrations of plasma LH and prolactin associated with the advance in the development of photorefractoriness and molt by high temperature in the starling. Gen. Comp. Endocrinol. **167**: 122-127.

GRZEGORZEWSKA A. K., SECHMAN A., PACZOSKA-ELIASIEWICZ H. E., RZĄSA J. 2009. The expression of pituitary FSHbeta and LHbeta mRNA and gonadal FSH and LH receptor mRNA in the chicken embryo. Reprod. Biol. **9**: 253-269.

GUO L., YANG H. M., LI P., KANG B. 2008. Cloning and optimizing prokaryotic induced expression conditions of prolactin in White Goose. Yi Chuan **30**: 1433-1438.

IZUMI T., SHIMADA K., SAITO N., ISHIDA H., SATO K., UCHIDE K., TOMITA Y., SAKAKIDA S., NISHIDA E. 1992. Changes in body weight, egg production, hackle growth, and plasma sex steroid hormones and prolactin during the annual reproductive cycle in domestic geese. Japanese Poult. Sci. **29**: 378-388.

JIANG R. S., XU G. Y., ZHANG X. Q., YANG N. 2005. Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. Poult. Sci. **84**: 839-845.

KANG B., JIANG D. M., ZHOU R. J., YANG H. M. 2010. Expression of follicle-stimulating hormone receptor (FSHR) mRNA in the ovary of Zi geese during developmental and egg laying stages. Folia Biol. (Kraków) **58**: 61-66.

KANG B., GUO J. R., YANG H. M., ZHOU R. J., LIU J. X., LI S. Z., DONG C. Y. 2009. Differential expression profiling of ovarian genes in prelaying and laying geese. Poult. Sci. **88**: 1975-1983.

LEI Z. M., MISHRA S., ZOU W., XU B., FOLTZ M., LI X., RAO C. V. 2001. Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. Mol. Endocrinol. **15**: 184-200.

LIU Z., SHI Z. D., LIU Y., LI M. Y., HUANG Y. M., YAO B. H. 2008. Molecular cloning and characterisation of the Magang goose prolactin gene. Gen. Comp. Endocrinol. **155**: 208-216.

MA X., DONG Y., MATZUK M. M., KUMAR T. R. 2004. Targeted disruption of luteinizing hormone beta-subunit leads to hypogonadism, defects in gonadal steroidogenesis, and infertility. Proc. Natl. Acad. Sci. U S A **101**: 17294-17299.

MARCH J. B., SHARP P. J., WILSON P. W., SANG H. M. 1994. Effect of active immunization against recombinant-derived chicken prolactin fusion protein on the onset of broodiness and photoinduced egg laying in bantam hens. J. Reprod. Fertil. **101**: 227-233.

NI Y., ZHOU Y., LU L., GROSSMANN R., ZHAO R. 2007. Developmental changes of FSH-R, LH-R, ER-beta and GnRH-I expression in the ovary of prepubertal ducks (*Anas platyrhynchos*). Anim. Reprod. Sci. **100**: 318-328.

RANGEL P. L., SHARP P. J., GUTIERREZ C. G. 2006. Testosterone antagonist (flutamide) blocks ovulation and preovulatory surges of progesterone, luteinizing hormone and oestradiol in laying hens. Reproduction **131**: 1109-1114.

REDDY I. J., DAVID C. G., RAJU S. S. 2007. Effect of suppression of plasma prolactin on luteinizing hormone concentration, intersequence pause days and egg production in domestic hen. Domest. Anim. Endocrinol. **33**: 167-175.

SANDHU M. A., RAHMAN Z. U., HASAN I. J., RAHMAN S. U., YOUSAF M. S. 2008. Changes in luteinizing hormone-containing gonadotrophs after moulting induced by fasting and zinc in laying hens (*Gallus domesticus*). J. Anim. Physiol. Anim. Nutr. (Berl.) 92: 668-676.

SAWITZKE A. L., ODELL W. D. 1991. Uterine binding sites for LH/hCG can be modulated by hormonal status in rabbits and rats. Acta Endocrinol. (Copenh.) **124**: 322-330.

SHARP P. J. 1975. A comparison of variations in plasma luteinizing hormone concentrations in male and female domestic chickens (*Gallus domesticus*) from hatch to sexual maturity. J. Endocrinol. **67**: 211-223.

SHARP P. J., BLACHE D. 2003. A neuroendocrine model for prolactin as the key mediator of seasonal breeding in birds under long- and short-day photoperiods. Can. J. Physiol. Pharmacol. **81**: 350-358.

SHARP P. J., MACNAMEE M. C., STERLING R. J., LEA R. W., PEDERSEN H. C. 1988. Relationships between prolactin, LH and broody behaviour in bantam hens. J. Endocrinol. **118**: 279-286.

SHEMESH M., MIZRACHI D., GUREVICH M., SHORE L. S., REED J., CHANG S. M., THATCHER W. W., FIELDS M. J. 2001. Expression of functional luteinizing hormone (LH) receptor and its messenger ribonucleic acid in bovine endometrium: LH augmentation of cAMP and inositol phosphate *in vitro* and human chorionic gonadotropin (hCG) augmentation of peripheral prostaglandin *in vivo*. Reprod. Biol. **1**: 13-32.

SHI Z. D., HUANG Y. M., LIU Z., LIU Y., LI X. W., PROUDMAN J. A., YU R. C. 2007. Seasonal and photoperiodic regulation of secretion of hormones associated with reproduction in Magang goose ganders. Domest. Anim. Endocrinol. **32**: 190-200.

TANAKA M., MAEDA K., OKUBO T., NAKASHIMA K. 1992. Double antenna structure of chicken prolactin receptor deduced from the cDNA sequence. Biochem. Biophys. Res. Commun. **188**: 490-496.

XU H., SHEN X., ZHOU M., FANG M., ZENG H., NIE Q., ZHANG X. 2010. The genetic effects of the dopamine D1 receptor gene on chicken egg production and broodiness traits. BMC Genet. **11**: 17.

YANG P., MEDAN M. S., WATANABE G., TAYA K. 2005. Developmental changes of plasma inhibin, gonadotropins, steroid hormones, and thyroid hormones in male and female Shao ducks. Gen. Comp. Endocrinol. **143**: 161-167.

ZHANG F. P., POUTANEN M., WILBERTZ J., HUHTANIEMI I. 2001. Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. Mol. Endocrinol. **15**: 172-183.