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APPLICATION OF MOLECULAR REPRODUCTIVE ENDOCRINOLOGY INTO POULTRY INDUSTRY

Seungkwon You and Yun Jaie Choi
School of Agricultural Biotechnology, Seoul National University,
Suweon 441-744, Korea

INTRODUCTION

Reproduction efficiency of broiler chickens and turkeys is low in comparison with egg type chickens. One component of this low efficiency is relatively poor egg production, which is related to variable propensity toward early cessation of egg laying and associated incubation behavior which have commonly been referred to as broodiness.

The disparity in egg production is due among other things to the general management of the breeder flocks and the inability of farm managers to identity and treat incubating hens before follicular atresia occurs(1).

Incubation behavioral patterns are a heritable trait and the incidence of the expression of incubation behavior can be reduced by selecting for increased egg production or low expression of incubation behavior can be reduced by selecting for increased egg production or low expression of incubation activity. As meat production is the single consumer and product of domestic broiler chicken and turkey production, broiler chicken and turkey breeders have not emphasized selection for reproductive parameters. In addition, it becomes increasingly difficult to be able to identify and treat potential incubating hens due to the increase in the number of hens per flock. Thus, relatively poor reproductive performance, partially attributed to induction of incubation behavior, remains a costly problem to broiler chicken and trukey breeders as well as producers of hatching eggs.

ENDOCRINOLOGY OF INCUBATION BEHAVIOR

The endocrinology and behavior associated with incubation are complex. Onset of incubation activity is correlated with declining levels of luteinizing hormone (LH), estradiol and progesterone and dramatically increasing circulating prolactin (PRL) levels. Incubation activity is also associated with aggressive nest-protective behavior, cessation of ovulation and ovarian regression(2, 3).

Numerous studies produced evidence implicating increased PRL secretion as a cause of the reduced circulating gonadotropins and ovarian regression that results with birds shift from egg laying to incubation. A six-to ten fold increase in circulating PRL level is associated with the initiation of incubation activity, while gonadotropin and gonadol steroid levels decline to ver low levels. A significant rise in circulating PRL level has been reported to occur either prior to or following increased nesting activity. The increase in circulating PRL level prior to increased nesting activity may reflect increased tactile sensitivity of the breast, so stimulation from the nest during egg laying promotes PRL secretion. Pituitary and circulation PRL content is maintained at high levels as long as nesting persits.

Depriving incubating hens of a nest by placing them in wire cages lowers circulating PRL levels to those characteristic of laying hens within 48 hours.

Changes in PRL gene expression (PRL gene transcription, PRL mRNA stability and steady-state PRL mRNA levels) and pituitary and plasma PRL levels were found to be related during the different stages of reproductive cycle. PRL mRNA and pituitary and plasma PRL all rese during photostimulation and the laying phase, peak during the incubation phase, and decline dramatically during the onset of photorefractoriness. These results together with the in vitro findings that spontaneous and stimulated PRL secretion was highest by lactotrophs from incubating hens, suggest interactive regulatory mechanisms between PRL gene expression and PRL secretory activity. Thus, under conditions of incubation activity, PRL is expressed and secreted at a high rate and shows an enhanced secretary responsiveness.

A suppressive effect of PRL on gonadotropin secretion is suggested by the association between high PRL levels and low LH levels in incubating hens. This is supported by the findings that the administration of PRL to incubating

hens results in an increase induces gonadal regression. Previous findings indicated that the LH-releasing mechanism is not impaired under the conditions of hyperprolartinemia in birds, since the high PRL levels in incubating hens do not depress LHRH-stimulated LH secretion. The amount of LH-subunit transcript is lowest in hyperprolactinemic, incubating hens, implaying that PRL may have a supprissive effect on the steady-state LH-mRNA level. Exposure of pituitary cells to high dose of PRL in vitro (to mimic a hyperprolactinemic state) decreased steady-state, as well as LHRH-stimulated LH-mRNA levels along with the secreted levels of LH. Thus, reduced amounts of LH-mRNA in the anterior piruitary may be partially responsible for the reduced concentrations of plasma LH in incubating hens. This is substantiated by findings that cultures of anterior pituitary cells from laying or incubating hens release LH in proprotion to the circulating concentrations of plasma LH. In addition, it appears that in the incubating turkey, PRL acts centrally to inhibit LHRH release, resulting in low concentration of plasma LH.

At the onset of incubation, the ovary, which contains a full complement of large preovulatory follicles, suddenly undergoes follicular atresia. Follicular atresia begins with the largest preovulatary follicles and proceeds down the follicular hierachy until all the preovulatory follicles have become atresic. During ovarian regression associated with the onset of incubation, follicular atresia is extensive five days after the last egg is laid. Circulating concentrations of LH decrease concurrently with the increase in nesting behavior which precedes the full expression of incubation behavior and prior to any visible evidence of follicular atresia.

The mechanism underlying the decrease in concentrations of plasma ovarian steroids and subsequent ovarian regression at the onset of incubation are not known, but may involve the associated decline in plasma gonadotropins and the increase the plasma PRL. PRL is a pricandidate because it induces ovarian regression and diminishes thecal aromatase activity when administered exogenously, and inhibits the ability of injected LH to stimulate testosterone and estrogen secretion. In addition, stimulation of steroidogenesis in ovorian follicular theca cells by LH in vitro is suppressed by prior treatment of hens with ovine PRL. However, LH-induced

progesterone and testosterone secretion by granulosa and theca cells obtained from preovulatory follicles from hens at the onset of incubation is greater than that by theca cells from follicles of laying hens. Therefore, it appears that granulasa and theca cells remain responsive to LH until well after the initiation of ovarian regression associated with incubation behavior. Thus, ovarian collapse may result from decreased LH secretion.

FOLLICULAR DEVELOPMENT AND GONADOTROPIN RECEPTORS GENE EXPRESSION

The gonadotropins, FSH and LH, are heterodimeric glycoproteins produced with the anterior pituitary that, in the female, act primarily at the level of the ovarian follicle. While LH is generally thought to be the more active gonadotropin in promoting progesterone production from preovulatory follicle granulosa cells, FSH has been reported to demonstrate limited to biological activity when steroid production is evaluated as a physiological endpoint. By contrast, results from in vitro studies indicate that recombinant human (rh)FSH acts within prehierarchical (6 to 8 mm diameter) follicle granulosa cells to increase levels of cytochrome p450 cholesterol side-chain cleavage (p450scc) and p450 17-hydroxylase (p45017 OH) mRNA, initiate p450ssc and p45017 OH enzyme activity, and promote progesterone and androgen synthesis, and promote progesterone and androgen synthesis. There is also evidence that FSH can induce modest, but significant, progesterone, androgen, and estrogen production from the prehierachical follicle theca layer in vivo and prevent granulosa cells from undergoing aopoptosis in vitro. Moreover, relatively low levels of FSH binding have been detected within ovarian stroma, the theca layer, and granulosa tissue, and such binding generally decreases follicle develpedment. In consistent, as the follicle developed from the prehierarchical (6 to 8 mm diameter) to the largest preovalatory (F1 follicle) stage, FSH-R mRNA levels progressively declined within both the granulosa and theca layers. Moreover, FSH-R mRNA levels were lower in whole atretic than in morphologically normal 3- to 5-mm follicles. The pattern of FSH-R mRNA expression within the granulosa layer during follicle development was notably different from that of LH-R mRNA expressionin that LH-R mRNA

levels increase to become readily detecable coincident with dramatically increased steroidogenic capacity during the last few days before oculation of the follicle. On the other hand, highest levels of FSH-R mRNA in 6- to 8-mm (prehierarchical)follicles were consistent with a role for the FSH-R in maintaining the viability of prehierarchical follicles and in initiating granulosa cell differentiation at the time when follicles are selected into the preovulatory hierarchy. This shift from an FSH-dominant to an LH-dominant environment in granulosa cells during follicle development is not unlike that which occurs in mammalian granulosa cells during the transition from the prenatal and antral stages to the preovulatory stage of differentitation.

REGULATION OF PRL BY VASOACTIVE INTESTINAL PEPTIDE

The medial basal hypothalamus contains the final neural elements that regulate anterior pituitary secretion of PRL in the birds. Acute electircal stimulation (ES) throughouta large region of the hypothalamus elicits a rapid rise in circulating PRL. This region extends from the media preoptic area, caudally through the ventromedial nucleus and infundibular nuclear complex, then ventrally to the median eminence. The amount of PRL released in response to ES of the hypothalamus differs among birds in different reproductive stages. Electircally stimulated PRL release is greatest in incubating hens and occurs to a lesser extent in laying, photorefractory, and short-day birds. The differencein the PRL response to ES may be related to changes in hypothalamic vasoactive intestinal peptide (VIP). VIP concentrations in portal blood plasma were lowest in non-photostimulated, reproductively inactive hens and highest in incubating hens, with laying and photorefractory hens having intermediate levels. These differences in portal blood VIP concentrations mirrored those of PRL in the general circulation.

Two of the best characterized physiological effects of PRL are : i) the induction and maintenance of incubation behavior in birds and their associated ovarian regression, and ii) the proliferation of crop sac tissues during the latter stages of incubation in members of the pigeon family. The behavioral and hormonal characteristics of incubating hens were absent in VIP immunized

hens. Termination of incubation behavior and reduction of circulating PRL were induced by passive immunization of incubating hens with VIP antibodies. In incubating ring doves, passive immunization by treatment with anti-VIP serum depressed plasma PRL levels and prevented the proliferation of crop sac tissue. Similarly, neutralizing VIP availability by active immunization against VIP completely eliminated the PRL release induced by electrical stimulation of the turkey hypothalamus. These finding taken together indicate that no other PRL-releasing factor needs to be invoked to explain the incubation associated hyperprolactinemia in birds.

MANAGEMENT FOR THE DISRUPTION OF INCUBATION ACTIVITY

If the incubating hen is not treated in a manner to interrupt incubation activity, many hens will continue incubating indefinitely. The longer incubation activity is allowed to persist, the more complete ovarian regression will be associated. For this reason, early detection and interrruption of incubation activity is desirable. Alternatively, if incubation activity is allowed to persist for several days prior to interruption attempts, incubation-associated ovarian regression will prevent the economically important return to egg production.

A variety of techniques have been used with some success in blocking incubation behavior. The technique most widely used involves moving the potential incubating hens from their familiar pens to a strange and less comfortable one without nests (standard broody coop, wire floored pen, cages). These techniques require expertise in order to diagnose the onset of incubation before ovarian regression begins. Identification of incubating hen is dependent on detection of increased nesting activity Recently, it has been clearly shown that immunization of turkey hens against VIP prevents the rise in circulating PRL levels that normally occurs during the reproductive cycle. Since VIP is the brain hormone which is necessary for stimulating the secretion of PRL, suppression of VIP through an effective vaccination procedures prevents both the increase in PRL that normally occur in laying hens, and the very high circulating PRL levels that are needed to cause incubation and regression of the ovary. Despite the relatively low circulating PRL levels, laying hens

immunized to suppress VIP use the nests at a normal frequency. Egg production in immunized hen is maintained at the same level as in non-immunized hens that do not exhibit incubation during the reproductive cycle. These results show that vaccination against VIP can be an effective treatment in the prevention of incubation.

REFERENCES

El Halawani, M. E., W. H. Burke and P. T. Dennison. 1980a. Effect of nest-deprivation on serum prolactin level in nesting female turkeys. Biol. Reprod. 23: 118.

El Halawani, M. E., W. H. Burke and P. T. Dennison. 1980b. Effect of -chloro-phenylalanine on the rise in serum prolactin associated with nesting in broody turkey. Biol. Reprod. 23: 815.

El Halawani, M. E., S. Gehrer, B. Hargis and T. porter. 1988. Incubation behavior in the domestic turkey: physiological correlates. CRC Crit. Rev. Poult. Biol. (4): 285.

El halawani, M. E., J. L. Silsby, O. M. Youngren and R. E. Phillips. 1991. Enhanced luteinizing hormone release by luteinizing hormone-releasing hormone in incubating female turkeys *(Meleagris gallopavo)*. Biol. Reprod. 36: 884.

El Halawani, M. E., J. L. Silsby and S. C. Fehner. 1988. Basal and hypothalamic extract-induced luteinizing hormone and prolactin secretion by cultured anterior pituitary cells from female turkeys in various stages of the reproductive cycle. Gen. Comp. Endocrinol. 71: 45.

El Halawani, M. E., J. L. Silsby and L.J. Mauro. 1990. Enhanced vasoactive intestinal peptide-induced prolactin secretion from anterior pituitary cells of incubating turkey *(Meleagris gallopavo)*. Gen.Comp. Endocrinol . 80:138.

El Halawani, M. E., J. L. Silsby and I. Rozenboim. 1995. Increased egg production by active immunization against vasoactive intestinal peptide in the turkey *(Meleagris gallopavo)*. Biol. Reprod. 52:179.

El Halawani, M. E., J. L. Silsby., O.M. Youngren and R.E.Phillips. 1987. Exogenous prolactin delays photo-induced sexual maturity and suppress ovatiectomy-induced luteinizing hormone secretion in the turkey

(Meleagris gallopavo). Biol. Reprod. 44:420.

El Halawani, M. E., O.M. Youngren., J. L. Silsby and R.E.Phillips. 1987. Prolactin release following electrical stimulation of the brain in female turkeys *(Meleagris gallopavo)*. Gen. Comp. Endocrinol. 65:1.

Hall, T. R. and A. R. Goldsmith. 1983. Factors affecting prolactin secretion during breeding and incubation in the domestic duck *(Anas platyrhynchos)*. Gen. Comp. Endocrinol. 49: 270.

Hargis, B. M. 1987. Anti-ovarian activity of prolactin in the domestic turkey hen. Ph. D. Thesis, University of Minnesota.

Johnson A.L,. 1993. Regulation of follicle differentiation by gonadotrophins and growth factors. Poult Sci. 72:867-873.

Johnson. A. L. J. T. Bridgham., J. P. Witty., J. L. Tilly. Susceptibility of avian granulosa cells to apoptosis is dependent upon stage of follicle development and is related to endogenous levels of bcl-xlong gene expression. Endocrinology. 137:2059-2066.

Krishnan, K. A., J. A. Proudman and J. M. Bahr. 1992. Purification and characterization of chicken follicle-stimulating hormone. Comp. Biochem. Physiol. 102: 67-75.

Lea, R. W., A. S. M. Dods, P. J. Sharp and A. Chadwick. 1981. The possible role of prolactin in the regulation of nesting behavior and the secretion of luteinizing hormone in broody bantams. J. Endocrinol. 91: 89.

Lea, R. W., R. T. Talbot and P. J. Sharp. 1991. Passive immunization against chicken vasoactive intestinal polypeptide suppresses plasma prolactin and crop sac development in incubating ring doves. Horm. Behav. 25: 283.

Li. Z., A. L. Johnson. 1993. Regulation of P450 cholesterol side-chain cleavage mRNA expression and progesterone production in hen granulosa cells. Biol Reprod. 49:463-469.

Li. Z., A. L. Johnson. 1993. Expression and regulation of cytochrome P450 17a-hydroxylase mRNA levels and androstenedione production in hen granulosa cells. Biol Reprod. 49:1293-1302.

Lien, R. J., A. Pusztai and M. E. El Halawani. 1989. Influence of the administration of ovine prolactin to laying turkeys on subsequent ovarian steroidogenesis in vitro. Poultry Sci. 68: 86.

Morrison, T. O., R. J. Lien and M. E. El Halawani. 1990. Influence of ovine prolactin administration on in vivo steroidogenic responses of laying

turkeys to ovine LH (oLH). Poultry Sci. 69: 96.

Nestor, K. E., W. L. Bacon, and P. A. Renner. 1986. The influence of time of application of broody hen treatments on egg production of turkeys. Poultry sci. 65: 1405.

Porter, T. E., J. L. Silsby, E. J. Behnke, T. R. Knapp and M. E. El Halawani. 1991. Ovarian steroid production in vitro during gonadal regression in the turkey. I. Changes associated with incubation behavior. Biol. Reprod. 45: 581.

Proudman, J. A. and H. Opel. 1980. Plasma prolactin levels in broody turkeys. Poultry Sci. 59: 1653.

Robinson, F. E. R. J. Etches, C. E. Anderson-Langmuir, W. H. Burke, F. J. Cunningham, S. Ishii, P.J. Rozenboim, I., C. H. Tabibzadeh, J. L. Silsby and M. E. El Halawani. 1993. The effect of ovine prolactin (oPRL) administration on hypothalemic vasoactive intestinal peptide (VIP), gonadotropin-releasing hormones I and II (GnRH-I, II) content, and anterior pituitary VIP recpetors in laying turkey hens. Biol Reprod. 48: 1248.

Scanes, C. G.and J. H. Fagioli. 1980. Effects of mammalian and avian gonadotrophins on in vitro progesterone production by avian ovarian granulosa cells. Gen. Comp. Endocrinol. 41: 1-7

Sharp, R. T. Talbot. Steroidogenic relationships of gonadotrophin hormones in the ovary of the hen (Gallus domesticus). Gen. Comp. Endocrinol. 69:455-466

Sharp, P. J., M-C. Macmamee, R. J. Sterling R. W. Lea and H-C. Pedersen. 1988. Relationships between prolactin, LH and broody behavior in bantam hands. J. Endocrinology. 188: 279.

Sharp, P. J.,G. J. Sterling, R. T. Talbot and N. S. Huskisson. 1989. The role of hypothalamic vasoactive intestinal polypeptide in the maintenance of prolactin secretion in incubating bantam hens: Observations using passive immunization, radioimmunoassay and immunohistochemistry. J. Endocrinol. 122: 5

Tong, Z., G. R. Pitts, D. N. Foster and M. E. EL Halawani. 1997. Transcriptional and post-transcriptional regulation of prolactin during the turkey reproductive cycle. J. Mol. Endocrinol. 18: 223.

Wells, J. W., M. A. Walker, J. Culbert and A. B. Gilbert. 1985. Comparison of the response in vivo to luteinizing hormone and follicle stimulating hormone of the six follicles from the ovarian hierarchy in the chicken (Gallus domesticus). Gen. Comp. Endocrinol. 59: 369-374.

Wong, E. A., N. Ferrin, J. L. Silsby and M. E. EL Halawani. 1991. Cloning of a turkey prolactin cDNA: expression of prolactin mRNA throughout the reproductive cycle of the domestic turkey *(Meleagris gallopavo)*. Gen. Comp. Endocrinol. 83: 18.

Wong, E. A., J. L. Silsby, s. Ishii and M. E. EL Halawani. 1992. Pituitary luteinizing hormone and prolactin mRNA levels are inversely related in laying and incubating turkey hens. Biol. Reprod. 47: 598.

You, S., L. K. Foster, J. L. Silsby, M. E. EL Halawani and D. N. Foster. 1995a. Sequence analysis of the turkey LH -subunit and its regulation by gonadotropin-releasing hormone and prolactin in cultured pituitary cells. J. Mol. Endocrinol. 14: 117.

You, S., J. L. Silsby, J. Farris, D. N. Foster and M. E. EL Halawani. 1995b. Tissue-specific alternative splicing of turkey preprovasoactive intestinal peptide messenger ribonucleic acid, its regulation, and correlation with prolactin secretion. Endocrinology, 136: 2602.

You. S., J. T. Bridgham., D. N. Foster., A. L. Johnson. 1996. Characterization of the chicken follicle-stimulating hormone receptor (cFSH-R) complementary deoxyribonucleic acid, and expression of cFSH-R messenger ribonucleic acid in the ovary. Biol Reprod. 55:1055-1062.

You. S., H. Kim., C-C.Hsu., M. E. El Halawani., D. N. Foster. 2000. Three different turkey luteinizing hormone receptor (tLH-R) isoforms I : Characterization of alternatively spliced tLH-R isoforms and their regulated expression in diverse tissues. Biol Reprod. 62:108-116.

You. S., H. Kim., M. E. El Halawani., D. N. Foster. 2000. Three different turkey luteinizing hormone receptor (tLH-R) isoforms II : Characterization of differentially regulated tLH-R messenger ribonucleic acid isoforms in the ovary. Biol Reprod. 62:117-124.

Youngren, O. M., Y. Chaiseha, R. Phillips and M. E. EL Halawani. 1996. Vasoactive intestinal peptide concentrations in turkey hypophysial portal blood differ across the reproductive cycle. Gen. Comp. Endocrinol. 103: 323.

Youngren, O. M., M. E. EL Halawani, J. L. Silsby and R. E. Phillips. 1992. Effect of reproductive condition on luteinizing hormone and prolactin release induced by electrical stimulation of the turkey hypothalamus. Gen. Comp. Endocrinol. 89: 220.

Youngren, O. M., J. L. Silsby, I. Rozenboim. R. E. Phillips and M. E. EL Halawani. 1994. Vasoactive intestinal peptide mediates prolactin secretion induced by electrical stimulation of the turkey hypothalamus. Gen. Comp. Endocrinol. 95: 330.