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Abstract

In the majority of vertebrates, survival of offspring to sexual maturation is important for increasing population size, and parental investment in the young is important for reproductive success. Consequently, parental care is critical for the survival of offspring in many species, and many vertebrates have adapted this behavior to their social and ecological environments. Parental care is defined as any behavior that is performed in association with one's offspring (Rosenblatt, Mayer, Siegel. Maternal behavior among nonprimate mammals. In: Adler, Pfaff, Goy, editors. Handbook of behavioral neurobiology. New York: Plenum; 1985. p. 229–98) and is well characterized in mammals and birds. In birds (class Aves), this is due to the high level of diversity across species. Parental behavior in birds protects the young from intruders, and generally involves nest building, incubation, and broody behavior which protect their young from an intruder, and the offspring are reared to independence. Broodiness is complexly regulated by the central nervous system and is associated with multiple hormones and neurotransmitters produced by the hypothalamus and pituitary gland. The mechanism of this behavior has been extensively characterized in domestic chicken (*Gallus domesticus*), turkey (*Meleagris gallopavo*), and pigeons and doves (family Columbidae). This chapter summarizes broodiness in birds from a physiology, genetics, and molecular biology perspective.

Keywords

Broodiness • Chicken • Incubation behavior • Pituitary gland • Prolactin (PRL) • PRL receptor • Signal transduction • Transcription • Vasoactive intestinal peptide (VIP)

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10.1 Broodiness

Parental care is defined as any behavior that is performed in association with one's offspring in vertebrates (Rosenblatt et al. 1985). The parental behavior is highly diversified in birds. At one end of the spectrum, species such as cuckoos (family Cuculidae) and cowbirds (*Molothrus* spp.), deposit their eggs in the nests of other birds and never engage in parental behavior. The megapodes (family Megapodiidae) also do not incubate their eggs themselves, but rather bury their eggs in a nest mound made of compost to keep them warm until hatching. In some species, such as chicken and duck, the female displays the parental behavior, while male parental care, such as is provided by the emperor penguin (*Aptenodytes forsteri*) in Antarctica for more than 2 months to protect and warm the egg, is much rarer. However, the most common pattern of parental behavior in birds is biparental care, where both males and females provide equivalent levels of parental care toward their eggs and offspring. Approximately 90% of avian species are believed to be monogamous, and both sexes exhibit this behavior to enhance their reproductive success (Nelson 1995).

The extent of broodiness depends on the developmental state of the young at hatching. In precocial birds such as chicken, turkey, and duck, the chicks are able to independently search for and eat food immediately after hatching. Therefore, maternal care in chickens involves nest building, incubation, and broody behavior, all of which protect their young from intruders. By contrast, in altricial birds, the newly hatched nestlings are usually helpless and so the parents take care of their chicks until fledging. Consequently, in these species, the parents not only carry out nest building and incubation of the eggs before hatching but also feed and brood their young.

10.1.1 Characteristics of Incubation Behavior

In domestic chicken (*Gallus domesticus*) and turkey (*Meleagris gallopavo*), incubation behavior is triggered by the accumulation of a sufficient num-

ber of eggs to incubate; for example, bantam hens lay 10–20 eggs (Lea and Sharp 1982). The onset of incubation behavior is preceded by an increased frequency of nest visits (Haller and Chermers 1961). Furthermore, in laying bantams, the time spent at the nest is gradually increased closer to the onset of incubation behavior (Lea et al. 1981). Incubation behavior is preceded by an increase in the amplitude and duration of a nocturnal surge in plasma prolactin (PRL) levels (Sharp 1980) which, together with estrogen, stimulates brood patch formation (Hutchison et al. 1967; Hutchison 1975)—a defeathered area on the belly skin that is well supplied with blood vessels to effectively transfer body heat to the eggs (Jones 1971).

When incubation begins, chicken and turkey hens spend almost all of their time at the nest warming their eggs, occasionally turning and rearranging them (El Halawani et al. 1984). During this time, nutrient and water intake is drastically reduced, which results in a dramatic decrease in body weight (Sherry et al. 1980; Zadworny et al. 1988). When hens are incubating their eggs, they display aggressive and defensive behaviors, such as clucking (Romanov et al. 2002). Hens can be removed from their nest and transferred to a different environment to stop the expression of incubation behavior (El Halawani et al. 1980; Sharp et al. 1988), but it takes several days to disrupt this. Furthermore, in the case of bantams, approximately 90% of hens never reneest following 72 h of nest deprivation (Sharp et al. 1988), and turkeys never resume incubation behavior after 5 days of nest deprivation (El Halawani et al. 1980). These behavioral changes are also under the control of hormones, including PRL.

10.2 Prolactin and Broodiness

The mechanism that controls broodiness has interested avian biologists for a long time, and poultry scientists have also sought to clarify how incubation behavior is expressed in domestic birds, because broodiness causes the ovary and oviduct to regress, which results in the cessation of egg laying and thus economic losses for the poultry industry.

In 1930s, Oscar Riddle and his colleagues provided the first evidence that PRL induces broodiness in pigeons and chicken (Riddle et al. 1933, 1935), and it has since been confirmed that PRL is involved in brooding of the chicks (Yamashina 1952) and incubation behavior (Saeki and Tanabe 1955). Following the development of a radioimmunoassay for avian PRL, it has been shown that the onset of incubation behavior in chicken is correlated with an increase of PRL in the plasma (Sharp et al. 1979, 1988), and that the blood PRL level remains high throughout the incubation period in chicken and turkey (Sharp et al. 1979, 1988; Zadworny et al. 1985, 1988; Kuwayama et al. 1992). Furthermore, active immunization against recombinant-derived PRL reduces the incidence, delays the development, or prevents the occurrence of incubation behavior in birds (March et al. 1994); while administration of ovine PRL induces incubation behavior in turkey (El Halawani et al. 1986; Youngren et al. 1991). Therefore, it is well established that PRL is

involved in the initiation and maintenance of incubation behavior in these strains (Fig. 10.1).

This characteristic increase in blood PRL levels during broodiness is common to both domestic and wild birds. Indeed, plasma PRL levels even increase during the breeding season in a brood parasite, the brown-headed cowbird (*Molothrus ater*) (Dufty et al. 1987), and in male emperor penguins (*Aptenodytes forsteri*) during egg incubation (Lormée et al. 1999). However, the plasma PRL levels differ between precocial and altricial birds: in precocial birds belonging to the Galliformes and Anseriformes, PRL levels rapidly decline following the hatching of chicks and can be modified by manipulating the young chicks (Kuwayama et al. 1992), while in altricial birds, plasma PRL levels decrease more slowly after hatching, presumably because brooding by the parent(s) is imperative for chicks to survive to fledging (Goldsmith 1983).

Since PRL has a specific role in controlling incubation behavior, it is expected that the

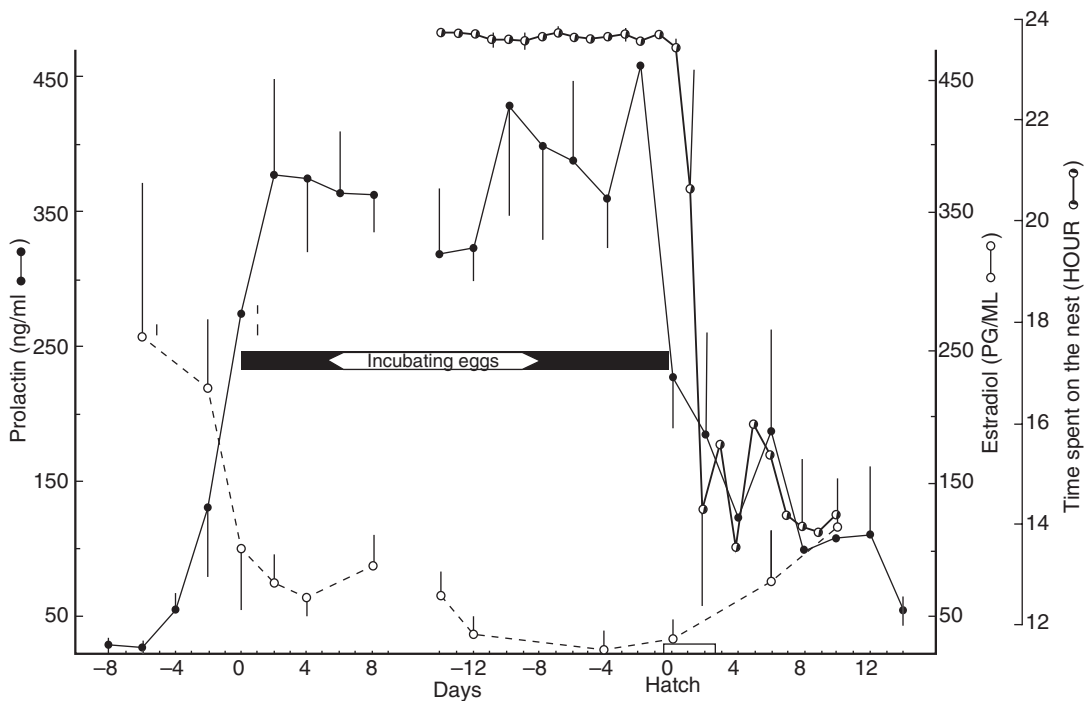


Fig. 10.1 Concentration of plasma PRL increased throughout the incubation period, and concentration of estradiol decreased at onset of incubation. In addition,

incubating hens spent over 95% of the day on their nest (adapted from Zadworny et al. 1988)

hypothalamus is the regulatory center for exhibiting broodiness. This hypothesis is supported by the observation that ^{125}I -labeled PRL specifically binds with the hypothalamic membrane in ring dove (*Streptopelia risoria*) (Buntin and Ruzycski 1987), with the binding sites localized in the preoptic area, tuberal hypothalamus, and bed nucleus of the pallial commissure (Buntin et al. 1993). It has been predicted that the PRL receptor (PRLR) in the preoptic hypothalamus mediates the expression of parental behavior, as lesions in this area block the development of incubation behavior in turkey (Youngren et al. 1989) and disrupt PRL-induced parental feeding behavior in ring dove (Slawski and Buntin 1995); and injections of PRL into the preoptic hypothalamus induce incubation behavior in turkey (Opel 1971). Furthermore, the development of incubation behavior in ring dove is associated with increased *c-fos* expression and the uptake of 2-[^{14}C] deoxyglucose in the preoptic hypothalamus (Georgiou et al. 1995; Sharp et al. 1996). The PRLR receptor in either the preoptic hypothalamus or the basal hypothalamus may also mediate the suppression of gonadotropin secretion (Buntin et al. 1988), and an injection of PRL into the ventromedial hypothalamus induces hyperphagia in ring dove (Hnasko and Buntin 1993).

High amounts of PRLR mRNA expression have been found in the basal and preoptic hypothalamus (Ohkubo et al. 1998a), suggesting the presence of indispensable, but perhaps different functions of PRL in these two areas. PRLRs in the basal hypothalamus occur in the same area as vasoactive intestinal polypeptide (VIP) neurons, which have a stimulatory effect on PRL synthesis and release (Sharp et al. 1989; Talbot et al. 1991, 1995). Thus, these neurons may be the targets for PRL action, allowing circulating levels of the hormone to exert a negative feedback control over its secretion. This is supported by the observation that intracerebroventricular injection of PRL in incubating ring doves suppresses the activity of basal hypothalamic VIP neurons, as assessed by quantitative immunocytochemistry (Saldanha and Silver 1995).

Rearing behavior is essential for reproductive success. In altricial birds especially, where post-

hatch care is important for the hatchlings, the parents put a lot of effort into rearing their young, including feeding, protecting, and warming them. Plasma PRL levels significantly increase during late incubation and the post-hatch stage (Buntin 1996; Sockman et al. 2006; Angelier et al. 2016). During the rearing period, the presence of chicks is transmitted to the parents as various stimuli, including tactile, visual, auditory, and olfactory, which modify the neuroendocrine mechanisms (Numan and Woodside 2010). This change in the internal environment alters the behavior of the parents, which has a significant impact on fledging success. There is evidence that plasma PRL levels are positively correlated with the frequency of feeding and protecting the offspring (Vleck et al. 1991; Khan et al. 2001; Duckworth et al. 2003; Smiley and Adkins-Regan 2016), which, in turn, increases the number of chicks that successfully fledge (Smiley and Adkins-Regan 2016). Furthermore, it has been shown that the administration of PRL to non-breeding ring doves promotes feeding and care toward the nestlings (Wang and Buntin 1999).

By contrast, avian species that hatch precocial offspring exhibit crouching behavior immediately after their chicks hatch, and this behavior can be triggered in Galliformes by introducing newly hatched chicks to them (Richard-Yris and Leboucher 1987; Opel and Proudman 1989). In precocial species, the chicks are able to walk and search for food immediately after hatching. However, hens must protect them from lower temperatures during the first 2 weeks as their thermoregulatory mechanisms are not yet mature (Mills et al. 1997). These birds also display typical maternal behaviors such as clucking and food calling, where clucking (or the “follow-me call”; Field et al. 2007) is emitted to initiate following behavior in the chicks (Collias and Joos 1953; Collias 1987; Field et al. 2007), and food-calling is emitted to attract their young to food that they have found (Wauters and Richard-Yris 2002). These rearing behaviors from incubation onward are induced by tactile stimulation from the newly hatched chicks, occasionally in combination with visual and/or auditory stimuli, which causes a decline in PRL in the plasma (Richard-Yris et al. 1998).

10.2.1 Neuroendocrine Control of Pituitary Function in Association with Incubation Behavior

Reproduction is triggered by external stimuli that act on the central nervous system. These stimuli include photoperiod, ambient temperature, and the presence of eggs and chicks, and are transmitted to the brain via the sensory organs. These signals are then transmitted to the hypothalamus, which regulates the hypothalamus–pituitary–gonadal axis through the action of a variety of neuropeptides and neurotransmitters. The major hypothalamic neuropeptides that are involved in the regulation of reproduction and incubation behavior are gonadotropin-releasing hormone (GnRH) and VIP.

Two structurally different forms of GnRH have been identified in birds: GnRH-I and GnRH-II. Both of these forms stimulate luteinizing hormone (LH) in vivo and in vitro (Sharp et al. 1987; Guémené and Williams 1992); however, the physiological significance of GnRH-II is not yet fully understood. The release of GnRH-I (but not GnRH-II) into the hypophyseal portal system leads to regression of the gonad in domestic chicken (Sharp et al. 1990). The secretion of GHRH-I is stimulated by epinephrine and norepinephrine and inhibited by dopamine (DA) and enkephalins. GnRH-I particularly stimulates the secretion of LH and follicle-stimulating hormone (FSH) from the pituitary gland, which control follicle development and the sex steroid hormones in the ovary.

10.2.1.1 The Role of VIP with Dopamine on PRL Release

VIP is a PRL-releasing hormone that acts directly on the lactotrophs of the pituitary gland in birds to increase the release of PRL (Macnamee et al. 1986; Opel and Proudman 1988; El Halawani et al. 1990), and also induces PRL gene expression in the pituitary gland (El Halawani et al. 1997). Avian VIP is a 28 amino acid peptide that was originally isolated from the intestine of chicken (Nilsson 1975).

Alternative splicing generates VIP transcripts, the most abundant and shortest of which encode VIP (Talbot et al. 1995; You et al. 1995a). Active immunization against VIP prevents photoinduced PRL release (El Halawani et al. 1996) and PRL release in incubating chickens (Sharp et al. 1989), while passive immunization inhibits PRL and causes the termination of incubation behavior in chicken (Sharp et al. 1989). It has also been shown that VIP secretion is controlled by DA, and so DA also plays an important role in controlling incubation behavior. The VIP neuron is widely localized in the basal hypothalamus, projecting to the median eminence and having axons that terminate in the external layer. It has previously been found that VIP concentrations in the hypophyseal portal vein are correlated with blood PRL levels in turkey (You et al. 1995a); and the number of VIP neurons in the basal hypothalamus also increases during incubation in hens (Sharp et al. 1989; Cloues et al. 1990; Mauro et al. 1992). Furthermore, both PRL gene expression in the pituitary gland and VIP mRNA in the hypothalamus increase in response to tactile stimuli in incubating hens (Silver et al. 1988; Buntin et al. 1991; Massaro et al. 2007).

The mechanism of DAergic regulation of VIP in the hypothalamus has been extensively analyzed in domestic turkey. Although DA inhibits PRL release in mammals (Ben-Jonathan and Hnasko 2001), it stimulates VIP release in birds. Furthermore, DA has both a stimulatory and inhibitory effect on PRL release in birds, depending on the subtype of DA receptor (Youngren et al. 1995, 1996). The distribution of DA neurons in the hypothalamus has been established in a number of species, such as chicken (Knigge and Piekut 1985; Moons et al. 1995), turkey (Al-Zailaie and El Halawani 2000), Japanese quail (*Coturnix japonica*; Absil et al. 2001), and pigeon (Kiss and Péczely 1987; Durstewitz et al. 1998). DA neurons are distributed extensively through the hypothalamic nucleus, as well as in the infundibular nuclear complex (INF) where VIP neurons occur and in the preoptic area (POA) where GnRH neurons are localized.

DA activity in the hypothalamus is higher in incubating bantam hens than in laying or photorefractory hens (Macnamee and Sharp 1989). In ring dove, DA activity also increases in the periventricular region during the incubation period (Lea et al. 2001). To mediate the DA stimuli, VIP neurons express both D1 and D2 DA receptors, with the relative expression of each being modulated by physiological changes. Expression of D1 DA receptor is increased during incubation stage in the hypothalamus in turkey hens. In contrast, whereas D1 DA receptor and D2 DA receptor expresses in the anterior pituitary gland, D1 DA receptor is not altered, but D2 DA receptor is much reduced during incubation in turkey hens. There is also evidence that expression of the D2 DA receptor is increased in the pituitary gland of photorefractory hens, which characteristically exhibit hypoprolactinemia (Chaiseha et al. 2003).

10.2.1.2 Regulation of Gonadal Function During Broodiness

The plasma concentrations of LH and steroid hormones decrease during broodiness, resulting in regression of the ovary. This reduction in ovarian function may be associated with an increase in PRL (Opel and Proudman 1980; Zadworny et al. 1989). The PRL level increases at the end of the incubation period in all birds, and subsequently declines once the young have hatched. The hatching or introduction of chicks reduces the levels of circulating PRL in the parents, while the concentrations of LH and ovarian steroid hormones gradually increase (Opel and Proudman 1989; Lea et al. 1996; Richard-Yris 1998). The presence of altricial chicks stimulates PRL release in the parent (Silverin and Goldsmith 1984; Ketterson et al. 1990; Lea and Sharp 1991), while the removal of chicks immediately induces the release of LH and steroid hormones in the rearing chickens (Kuwayama et al. 1992). There is also evidence that non-rearing Thai native chicken hens recover laying faster than rearing hens. (Fig. 10.2)

Contact stimulation from chicks delays the decline in plasma PRL levels in the parent, and

inhibits the release of gonadotropin and gonadal steroid hormones (Chaiyachet et al. 2013b). A rise in plasma gonadotropin is correlated with an increase in GnRH-1 mRNA in the hypothalamus in chicken (Dunn et al. 1996; Dunn and Sharp 1999). The decline in gonadal function during broodiness is presumably an inhibitory effect caused by PRL. Systemic administration of PRL reduces GnRH-I and GnRH-II mRNA expression in the hypothalamus, as well as plasma LH in turkey hens (Rozenboim et al. 1993). There is also evidence that PRL directly suppresses LH β subunit mRNA expression in the anterior pituitary gland of turkey in culture (You et al. 1995b).

10.3 Molecular Biology of PRL

In the late 1980s, three independent groups cloned PRL cDNA from chicken (Hanks et al. 1989a; Watahiki et al. 1989), which marked the start of molecular-based analyses of the PRL system in birds. Avian PRL cDNA encodes a mature hormone of 199 amino acids and a 30 amino acid signal peptide, and the overall structure of avian PRL is highly conserved among vertebrate classes and species (Hanks et al. 1989a; Yasuda et al. 1991; Noso et al. 1992). cDNA cloning made it possible to produce recombinant protein in other organisms, and so recombinant-derived chicken PRL was expressed in *Escherichia coli*, allowing the immunological and biological activity of PRL to be characterized (Hanks et al. 1989b; Ohkubo et al. 1993). The estimated molecular mass of recombinant chicken PRL expressed in *E. coli* is 23 kDa, which is in close agreement with the predicted molecular mass based on chicken PRL cDNA (Watahiki et al. 1989) and turkey PRL cDNA (Karatzas et al. 1990). Recombinant chicken PRL specifically cross-reacted with anti-chicken PRL antibody (Hanks et al. 1989b) and anti-turkey PRL antibody (Ohkubo et al. 1993), and also stimulated proliferation of the pigeon crop sac mucosa with the same potency as authentic turkey PRL

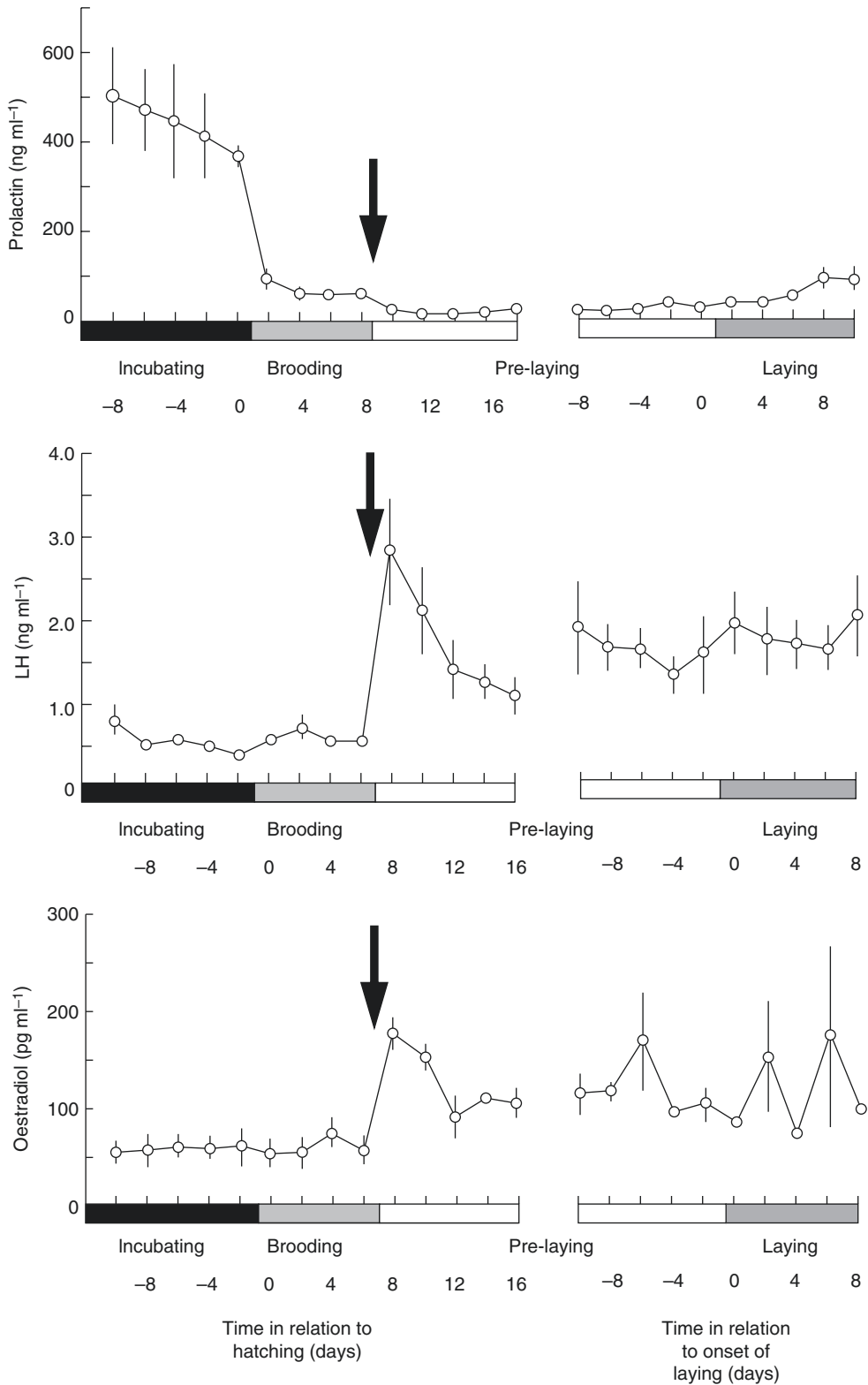
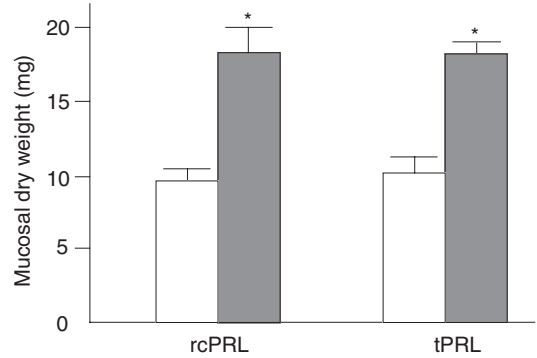


Fig. 10.2 In response to removal of chicks there was an immediate and rapid increase in concentration of LH and estradiol in the plasma of hens (adapted from Kuwayama et al. 1992)

Fig. 10.3 Recombinant-derived chicken (White Leghorn) PRL increased the weight of pigeon crop sac mucosa to a degree comparable to that induced by turkey PRL purified from pituitary gland (adapted from Ohkubo et al. 1993)



purified from the anterior pituitary gland (Ohkubo et al. 1993) (Fig. 10.3).

The occurrence of multiple isoforms of PRL in the anterior pituitary gland has been established in turkey (Proudman and Corcoran 1981; Corcoran and Proudman 1991; Arámburo et al. 1992; Karatzas et al. 1993) and chicken (Arámburo et al. 1992), and is known to result from posttranslational modification. Some of these isoforms are known to be glycosylated (Proudman and Corcoran 1981; Corcoran and Proudman 1991; Karatzas et al. 1993). Glycosylated PRL has also been observed in the pituitary gland of mammals (Lewis et al. 1989; Young et al. 1990; Bollengier et al. 1993) and reptiles (Noso et al. 1992). In mammals, glycosylated PRL exhibits less biological activity in stimulating mitogenesis in the pigeon crop sac (Lewis et al. 1989) and the Nb2 cells (Markoff et al. 1988; Sinha et al. 1991) than non-glycosylated PRL, but exhibits greater lactogenic activity (Young et al. 1990). The proportion of glycosylated PRL in the pituitary gland of turkey embryos increases alongside increases in the PRL content (Bédécarrats et al. 1999a). It should also be noted that, in turkey, there is a larger amount of glycosylated PRL in the pituitary gland during incubation than in any other physiological condition (Bédécarrats et al. 1999b). Thus, the occurrence of glycosylated PRL may modify physiological properties, such as incubation behavior.

The PRL gene has been cloned and characterized in a number of birds, including turkey (Kurima et al. 1995), chicken (Ohkubo et al. 2000), and duck (Kansaku et al. 2005). The avian

PRL gene possesses common exon/intron arrangements and exhibits a length of approximately 6 kb from the first exon to the last exon in these species. The chicken PRL gene has been mapped to chromosome 2 in the chicken genome (Miao et al. 1999), while the turkey PRL gene is localized on chromosome 3.

10.3.1 Transcriptional Regulation of the PRL Gene

Lactotrophs and somatotrophs are mainly localized in the cephalic and caudal lobes, respectively, of the anterior pituitary gland in birds (Hansen and Hansen 1977; Józsa et al. 1979; Ramesh et al. 1996). PRL mRNA expression is markedly higher in incubating hens than in laying hens (Talbot et al. 1991). During incubation, the lactotrophs increase in size in the pituitary of turkey hens. In addition, localization of the lactotrophs and somatotrophs is altered, with PRL-producing cells becoming spread across half of the ventral region of the caudal lobe of the anterior pituitary gland in turkey (Ramesh et al. 1996). It is known that mammosomatotrophs that can differentiate into both lactotrophs and somatotrophs exist in the adenohypophysis in mammals (Porter et al. 1990), and the same characteristic cells are also observed in the pituitary gland of turkey, where they act as PRL-producing cells during incubation (Ramesh et al. 1998). The termination of incubation behavior causes a decline in the blood PRL level, the induction of apoptosis of

the lactotrophs, and the disappearance of the mammosomatotrophs in turkey hens (Ramesh et al. 2001).

The regulation of PRL gene expression in association with broodiness has also been thoroughly investigated. During the incubation period, PRL mRNA in the pituitary gland is upregulated in chicken (Talbot et al. 1991; Shimada et al. 1991) and turkey (Wong et al. 1991; Ramesh et al. 1996). VIP is a major PRL-releasing factor in birds, which contributes to the maintenance of incubation behavior in chicken (Sharp et al. 1989). VIP also plays an important role in controlling PRL gene expression in the anterior pituitary gland in chicken (Sharp et al. 1989; Talbot et al. 1991, 1995; Kansaku et al. 1995) and turkey (Youngren et al. 1994; El Halawani et al. 1995). It has been shown that thyrotrophin-releasing hormone (TRH) stimulates PRL gene expression in the chicken pituitary gland (Shimada et al. 1991), as previously observed in mammals (Murdoch et al. 1985; Laverrière et al. 1988).

The transactivation of genes is complexly regulated by transcription factors and other regulatory molecules that are directly or indirectly associated with the target gene. Transcription of

the chicken PRL gene has been extensively investigated (Ohkubo et al. 2000). Pituitary-specific transcription factor (Pit-1) is involved in regulating transcription of the PRL gene, as well as the growth hormone and thyroid-stimulating hormone- β (TSH β) genes (Steinfeldt et al. 1992). The PRL gene in mammals possesses several putative Pit-1 binding sites in the *PRL* promoter. The proximal promoter region of the PRL gene is highly conserved between chicken and mammals, with two putative Pit-1 binding sites corresponding to the P2 and P3 regions of the rat *PRL* promoter (Mangalam et al. 1989) being conserved in the chicken and turkey *PRL* promoter (Ohkubo et al. 2000). Pit-1 dependent gene expression is enhanced by cyclic adenosine monophosphate (cAMP) (Peers et al. 1991; Liang et al. 1992; Gaiddon et al. 1995) and other factors (Howard and Maurer 1995) interacting with the proximal promoter region. In an analysis that used the gel mobility shift assay and luciferase assay, it was shown that a putative Pit-1 binding element between -76 and -133 in chicken *PRL* binds with Pit-1 to activate its promoter, which is stimulated by an increase in intracellular cAMP (Ohkubo et al. 2000) (Fig. 10.4).

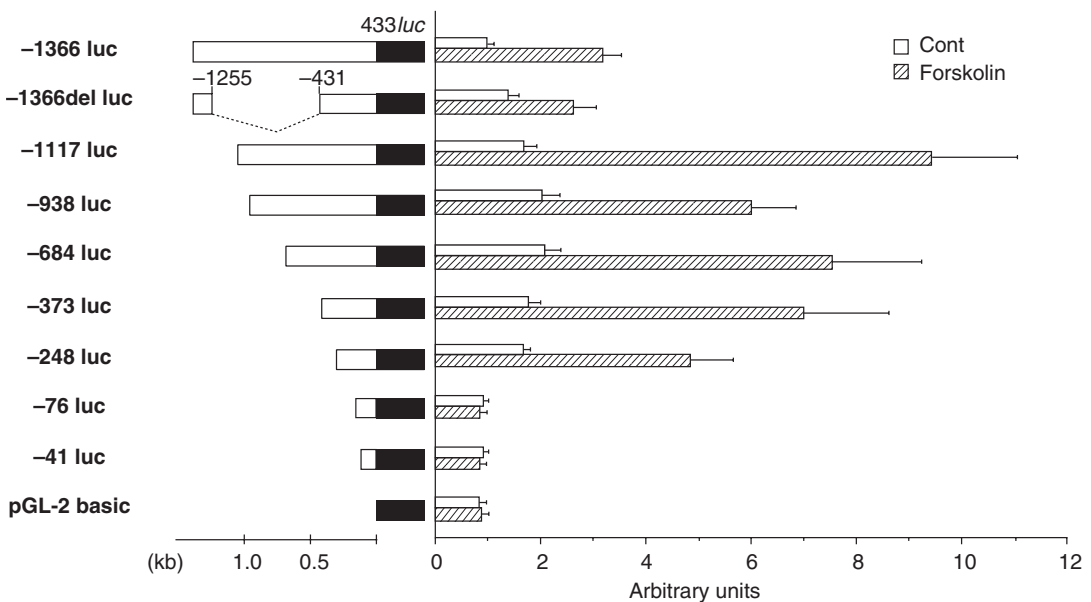


Fig. 10.4 Forskolin activated the *PRL* gene promoter in GH3 cells, which indicates that cAMP is involved in transactivating PRL gene (adapted from Ohkubo et al. 2000)

There is evidence that cyclic AMP responsive element binding protein (CBP) (Chrivia et al. 1993) and p/CAF (Yang et al. 1996) form a coactivator complex, which interacts with nuclear coactivator to mediate the nuclear receptor function (Torchia et al. 1997). This mechanism is involved in the Pit-1 dependent activation of the PRL gene. CBP stimulated by cAMP directly binds to the POU-specific domain of Pit-1 and induces *PRL* promoter activity (Tolón et al. 1998; Xu et al. 1998). It has been reported that VIP, a PRL-releasing factor in birds, increases the cellular cAMP concentration in the perfused chicken anterior pituitary gland (Kansaku et al. 1998), which induces PRL gene expression in the chicken anterior pituitary gland (Kansaku et al. 1995). Thus, the cAMP/protein kinase A (PKA) pathway may involve VIP-dependent PRL gene expression in the chicken anterior pituitary. In addition, the VIP receptor expresses predominantly in the cephalic lobe of the anterior pituitary gland, where PRL-producing cells are localized in chicken (Kansaku et al. 1998). Therefore, PRL gene expression in chicken may be cooperatively promoted by Pit-1 and cAMP-induced CBP, which probably form a complex, by binding to the proximal Pit-1 binding site.

10.3.2 PRL Receptor (PRLR)

The cDNA of avian PRLR was first isolated from chicken in 1992 (Tanaka et al. 1992), followed by pigeon (Chen and Horseman 1994), turkey (Zhou et al. 1996), and various other birds such as duck (*Anas platyrhynchos*) and great tit (*Parus major*). The PRLR gene has been mapped to the Z chromosome in chicken (Dunn et al. 1998). Avian PRLR possesses two repeated ligand-binding domains, as is found in reptilian PRLR (Kato et al. 2005). This double extracellular domain is the result of the duplication of exons 3–7 of mammalian PRLR, and so 5'-rapid amplification of cDNA ends (5'-RACE) suggested that at least ten non-coding exons are located upstream of the initiation codon in chicken PRLR (Bu et al.

2013), and tissue-specific transcription of PRLR is regulated by alternative promoters on the PRLR gene. In addition, truncated PRLRs that lack extracellular and transmembrane domains have been cloned in the testis of sexually mature chickens (Mao et al. 1999; Tanaka et al. 2000), and an alternative promoter is also involved in the transcription of these testis-specific isoforms (Tanaka et al. 2000).

It has been suggested that the variety of actions of PRL is accompanied by complex mechanisms involved in the transcription of PRLR in the target tissues of chicken. PRLR is categorized as a class I cytokine receptor, which has several features in common with intracellular signal transduction, such as the WSXWS motif and Box 1 (Bazan 1989, 1990; Itoh et al. 1990; Lebrun et al. 1995). The overall structure of avian PRLR is similar to that of mammals, except the extracellular domain has two homologous repeated units. Each repeated region has all cysteine pairs and a WSXWS motif that is characteristic of the conserved ligand-binding domain in mammalian PRLRs (Kelly et al. 1991). An in-vitro binding assay showed that the membrane-proximal PRL-binding domain specifically binds with PRL in chicken and pigeon PRLR (Chen and Horseman 1994; Bu et al. 2013).

PRLR mRNA is expressed widely in the central and peripheral tissues of chicken (Tanaka et al. 1992; Ohkubo et al. 1998a, b) and turkey (Zhou et al. 1996), which reflects the expression of PRL with its many physiological functions. It has been shown that the hypothalamus, which is expected to be a regulatory center for broodiness, has a high level of PRLR mRNA expression in chicken (Ohkubo et al. 1998a, b) and turkey (Zhou et al. 1996), as does the anterior pituitary gland (Ohkubo et al. 1998a, b; Zhou et al. 1996). PRL suppresses mRNA expression of the LH β subunit in chicken (Kansaku et al. 1994) and turkey (You et al. 1995b), and the level of LH β mRNA expression in the cephalic and caudal lobes of the anterior pituitary gland are inversely correlated with the expression levels of PRL mRNA in bantam hens (Kansaku et al. 1994).

Therefore, PRL may directly regulate pituitary function. In the peripheral tissues, a high level of PRL mRNA expression is found in the kidney and skin in chicken (Ohkubo et al. 1998a), and in the kidney and adrenal gland in turkey (Pitts et al. 2000). Localization of PRLR in these tissues is consistent with PRL playing a role in osmoregulation (Harvey et al. 1984; Murphy et al. 1986), brood-patch formation (Hutchison et al. 1967), and molting (Juhn and Harris 1958). In mammals, there are multiple isoforms of PRLR as a result of alternative splicing, which differ in the size of the intracellular domain and modulate physiological actions by altering intra-cellular signaling (Kelly et al. 1991). However, different isoforms have not been identified in birds.

10.3.2.1 Signal Transduction of PRL

In mammals, it has been established that PRL coupled with PRLR phosphorylates Janus kinase 2 (JAK2), which, in turn, phosphorylates signal transducer and activator of transcription 5 (STAT5) resulting in signal transduction (Gouilleux et al. 1994; Miyoshi et al. 2001). Avian PRLR also activates the JAK2-STAT5 pathway via PRL in vitro. Despite avian PRLR having two repeated ligand-binding domains, PRL specifically binds with the second domain that is close to the transmembrane region to transduce its signal (Bu et al. 2013).

In ring dove, phosphorylated STAT5 (pSTAT5) becomes localized in the brain as a result of various physiological states. The pSTAT5-immunoreactive (pSTAT5-ir) cells are distributed in the POA, suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), ventromedial nucleus (VMN), lateral hypothalamic region (LHy), and tuberal hypothalamic region (TU) in the hypothalamus, and in the lateral septum (LS) and lateral bed nucleus of the stria terminalis (BSTL), which are also known to contain localized PRLR. The density of pSTAT5-ir cells is higher in all of these regions except TU during the late incubation stage, when the PRL level peaks, than during the pre-laying stages, such as courtship and nest building, when plasma PRL

concentrations are low (Buntin and Buntin 2014). Pigeon-administered PRL results in a similar distribution of pSTAT5-positive cells in the brain to that which occurs in pigeons during the breeding season, and also significantly increases the number of pSTAT5-ir cells in the POA, PVN, and TU. PRL binding sites are distributed in these areas, and c-fos immunoreactive cells are induced when the parent is introduced to its hatchling (Buntin et al. 2006). VIP-expressing neurons are localized in the TU (den Boer-Visser and Dubbeldam 2002) and mesotocin is expressed in the PVN (Goossens et al. 1977; Barth et al. 1997; Bons 1980; Thayananuphat et al. 2011). Mesotocin is also associated with parental behavior, with mesotocin neurons increasing during late incubation in turkey (Thayananuphat et al. 2011). Thus, multiple pathways are suggested in PRL signaling, with the JAK2-STAT5 pathway at least partly being involved in regulating parental behavior in birds.

10.4 The Genetics of Incubation Behavior

Broodiness is essential for the breeding success of birds. However, during domestication, several strains that never get broody have been established, such as White Leghorn. The red junglefowl (*Gallus gallus*), which is believed to be the ancestor of domestic chicken (Akishinomiya et al. 1994, 1996), and other strains that have not undergone large amounts of genetic selection still exhibit incubation behavior, though. Incubation behavior in chicken is influenced by environmental factors, such as high temperatures, photoperiod, and the existence of a nest and egg. However, different individuals from a particular strain within the same environment exhibit variation in the occurrence and frequency of incubation behavior, indicating that there is a genetic basis to incubation behavior in chicken.

Spontaneously occurring mutations are accumulated in specific populations during the domestication process, allowing strains that do

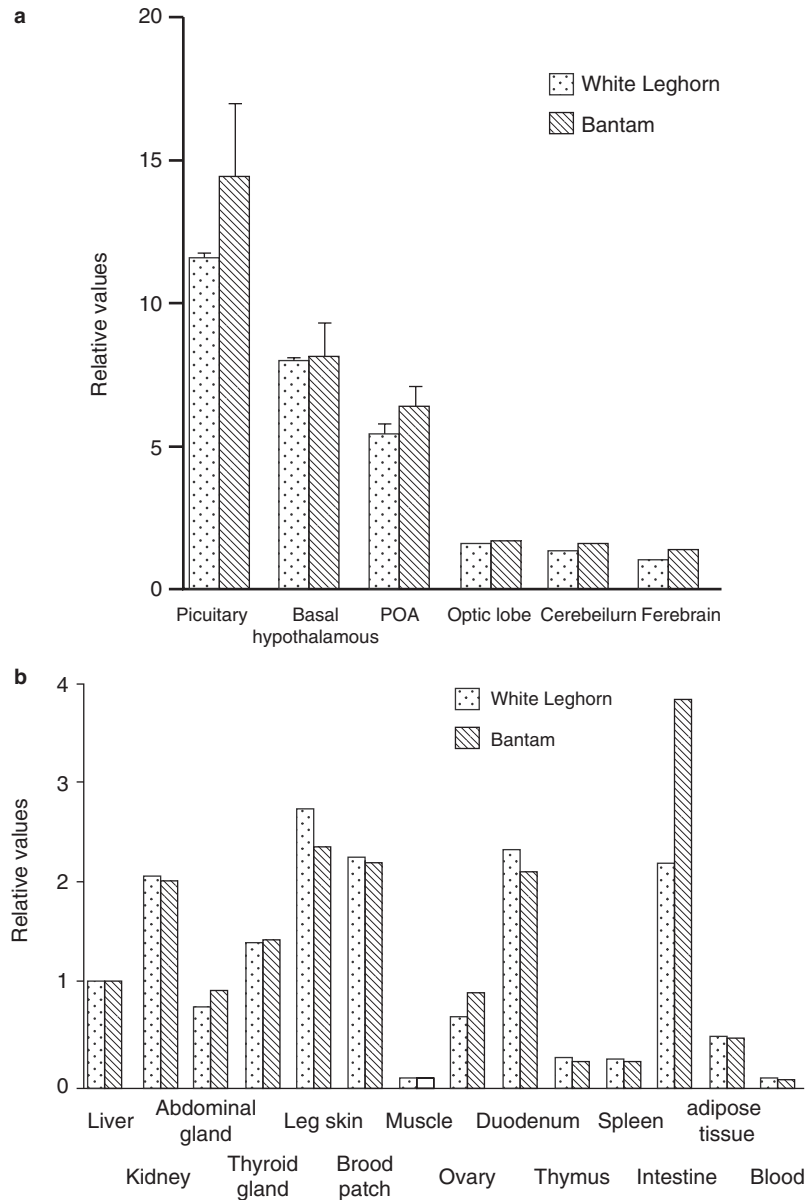
not exhibit incubation behavior to be established. Research of relationship between incubation behavior and heredity has been demonstrated for a long time. Punnett and Bailey (1920) pointed out that more than one independent autosomal gene is involved in the regulation of incubation behavior. Goodale et al. (1920) hypothesized that non-broody hens lack an allele that is involved in incubation behavior or possess an inhibitory gene(s) for the behavior—and this hypothesis is supported by the finding that the offspring that result from mating two different strains exhibit a higher incidence of broodiness (Hutt 1949). In addition, several researchers have reported that genes on the sex chromosome may be involved in broodiness (Warren 1930; Roberts and Card 1933; Saeki 1957; Saeki and Inoue 1979). However, Hays (1940) found no evidence for Z-linked genes being associated with broodiness in a study on a population of Rhode Island Red chickens. A recent analysis using an F1 generation cross of males from a non-broody breed (White Leghorn) and females from a broody breed (bantam) also showed that the major genes that affect incubation behavior may not be localized on the Z chromosome (Romanov et al. 1999)—and this finding is consistent with the reanalysis by Mead and Curnow (1983) of the data obtained by Saeki (1957) and Saeki and Inoue (1979).

PRL plays a fundamental role in the initiation and maintenance of incubation behavior in domestic chicken (El Halawani et al. 1986; Youngren et al. 1991; March et al. 1994). Hormonal action occurs via a specific receptor that is expressed in the target tissues, which also involve a specific signal transduction pathway. With regard to PRL signaling, the major pathway involves PRLR activating the JAK2-STAT5 cascade, and molecular analyses have revealed that the PRLR and JAK2 genes are localized on the Z chromosome (Dunn et al. 1998; Wong et al. 2004). Therefore, it is likely that genes on the Z chromosome are involved in incubation behavior. However, it is unlikely

that it is a loss of function of PRLR and JAK2 that accounts for the difference in the manifestation of incubation behavior between non-broody and broody breeds. Northern blot analyses have shown that the PRLR transcripts expressed in the tissues of White Leghorn and bantam hens are the same size, and that the localization and amount of PRLR mRNA also does not differ between the two breeds (Ohkubo et al. 1998a). Furthermore, White Leghorn PRLR expressed in chicken hepatoma cells phosphorylates STAT5 and activates the STAT5 inducible gene after PRL stimulation (Fig. 10.5); and as with other genes related to the PRL system, both recombinant-derived White Leghorn and bantam PRL proliferate pigeon crop sac mucosa (Hanks et al. 1989b; Ohkubo et al. 1993), indicating that PRL is functional regardless of the behavioral difference. There is also evidence that VIP is able to induce PRL release from the anterior pituitary gland in White Leghorn (Ohkubo et al. 1998), strongly suggesting that the absence of incubation behavior in this breed cannot be explained by an inability of VIP to stimulate PRL release. Therefore, together these findings show that PRL and PRLR are not causative genes for the lack of broodiness in White Leghorn (Fig. 10.6).

A quantitative trait loci (QTL) analysis based on an F2 generation established from White Leghorn and Silkie, a broody breed, indicated that particular QTL that are associated with incubation behavior are not located on the Z chromosome or chromosome 2, where the PRLR and PRL genes respectively are localized (Basheer et al. 2015). By contrast, significant QTL that affect incubation status and the early onset of incubation behavior have been found on chromosomes 1, 5, 8, 13, 18, 19, and 26, and linkage group E22C19W28. The peak position of these loci on chromosome 5 matches the largest selective sweep between domesticated chicken breeds and red junglefowl (Rubin et al. 2010), and there are several genes associated with photoperiodism and reproduction, such as

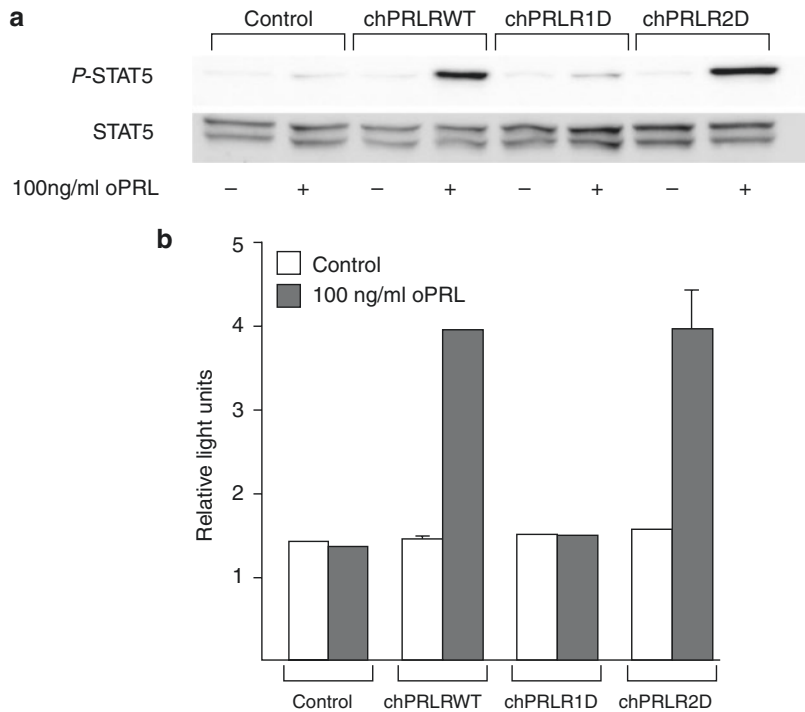
Fig. 10.5 PRLR mRNA is expressed widely in central (a) and peripheral (b) tissues. The largest amount of expression is found in the pituitary gland and hypothalamus in brain, which did not differ significantly between a non-broody breed, White Leghorns, and a broody breed, bantams, at laying period (adapted from Ohkubo et al. 1998a)



thyroid-stimulating hormone receptor (TSHR) and thyroid hormone deiodinase 2 (DIO2), in chicken (Nakao et al. 2008). A single nucleotide polymorphism (SNP) analysis showed that the peak position of the QTL is close to TSHR (Basheer et al. 2015), which is a characteristic locus for the selective sweep of the Silkie breed

(Fan et al. 2013). As for DIO2, TSHR is a key trigger for the onset of seasonal breeding and increases the synthesis of triiodothyronine. Therefore, these findings suggest that the thyroid hormone axis is involved not only in seasonal reproduction, but also in the expression of incubation behavior in birds.

Fig. 10.6 PRL phosphorylates STAT5 (a) and activates STAT inducible reporter gene in Leghorn male hepatoma (LMH) cells. (b) PRL may specifically bind with the 2nd ligand domain (2D), but not with 1st domain (1D) to phosphorylate STAT5, and initiate following signal transduction



References

- Abzil P, Foidart A, Hemmings HC Jr, Steinbusch HW, Ball GF, Balthazart J. Distribution of DARPP-32 immunoreactive structures in the quail brain: anatomical relationship with dopamine and aromatase. *J Chem Neuroanat.* 2001;21:23–39.
- Akishinomiya F, Miyake T, Sumi S, Takada M, Ohno S, Kondo N. One subspecies of the red junglefowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. *Proc Natl Acad Sci U S A.* 1994;91:12505–9.
- Akishinomiya F, Miyake T, Takada M, Shingu R, Endo T, Gojobori T, Kondo N, Ohno S. Monophyletic origin and unique dispersal patterns of domestic fowls. *Proc Natl Acad Sci U S A.* 1996;93:6792–5.
- Al-Zailaie K, El Halawani M. Neuroanatomical relationship between immunoreactive dopamine and vasoactive intestinal peptide neurons in the turkey hypothalamus. *Poult Sci.* 2000;79(Suppl 1):50.
- Angelier F, Wingfield JC, Tartu S, Chastel O. Does prolactin mediate parental and life-history decisions in response to environmental conditions in birds? A review. *Horm Behav.* 2016;77:18–29.
- Arámburo C, Montiel JL, Proudman JA, Berghman LR, Scanes CG. Phosphorylation of prolactin and growth hormone. *J Mol Endocrinol.* 1992;8:183–91.
- Barth SW, Bathgate RA, Mess A, Pary LJ, Ivell R, Grossmann R. Mesotocin gene expression in the diencephalon of domestic fowl: cloning and sequencing of the MT cDNA and distribution of MT gene expressing neurons in the chicken hypothalamus. *J Neuroendocrinol.* 1997;9:777–87.
- Basheer A, Haley CS, Law A, Windsor D, Morrice D, Talbot R, Wilson PW, Sharp PJ, Dunn IC. Genetic loci inherited from hens lacking maternal behaviour both inhibit and paradoxically promote this behaviour. *Genet Sel Evol.* 2015;47:100. doi:10.1186/s12711-015-0180-y.
- Bazan JF. A novel family of growth factor receptors: a common binding domain in the growth hormone, prolactin, erythropoietin and IL-6 receptors, and the p75 IL-2 receptor beta-chain. *Biochem Biophys Res Commun.* 1989;164:788–95.
- Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. *Proc Natl Acad Sci U S A.* 1990;87:6934–8.
- Bédécarrats G, Guémené D, Morvan C, Kühnlein U, Zadworny D. Quantification of prolactin messenger ribonucleic acid, pituitary content and plasma levels of prolactin, and detection of immunoreactive isoforms of prolactin in pituitaries from turkey embryos during ontogeny. *Biol Reprod.* 1999a;61:757–63.
- Bédécarrats G, Guémené D, Morvan C, Crisóstomo-Pinto S, Kühnlein U, Zadworny D. In vitro release of isoforms of prolactin from pituitary glands of turkey hens at different physiological stages. *Gen Comp Endocrinol.* 1999b;113:105–11.
- Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. *Endocr Rev.* 2001;22:724–63.
- Bollengier F, Geerts A, Matton A, Mahler A, Velkeniers B, Hooghe-Peters E, Vanhaeist L. Identification and

- localization of 23,000 and glycosylated rat prolactin in subcellular fractions of rat anterior pituitary and purified secretory granules. *J Neuroendocrinol.* 1993;5:669–76.
- Bons N. The topography of mesotocin and vasotocin systems in the brain of the domestic mallard and Japanese quail: immunocytochemical identification. *Cell Tissue Res.* 1980;213:37–51.
- Bu G, Ying Wang C, Cai G, Leung FC, Xu M, Wang H, Huang G, Li J, Wang Y. Molecular characterization of prolactin receptor (cPRLR) gene in chickens: gene structure, tissue expression, promoter analysis, and its interaction with chicken prolactin (cPRL) and prolactin-like protein (cPRL-L). *Mol Cell Endocrinol.* 2013;370:149–62.
- Buntin JD. Neural and hormonal control of parental behavior in birds. *Adv Stud Behav.* 1996;25:161–213.
- Buntin JD, Buntin L. Increased STAT5 signaling in the ring dove brain in response to prolactin administration and spontaneous elevations in prolactin during the breeding cycle. *Gen Comp Endocrinol.* 2014;200:1–9.
- Buntin JD, Ruzycski E. Characteristics of prolactin binding sites in the brain of the ring dove (*Streptopelia risoria*). *Gen Comp Endocrinol.* 1987;65:243–2553.
- Buntin JD, Lea RW, Figge GR. Reductions in plasma LH concentration and testicular weight in ring doves following intracranial injection of prolactin and growth hormone. *J Endocrinol.* 1988;118:33–40.
- Buntin JD, Becker GM, Ruzycski E. Facilitation of parental behavior in ring doves by systemic or intracranial injections of prolactin. *Horm Behav.* 1991;25:424–44.
- Buntin JD, Ruzycski E, Witebsky J. Prolactin receptors in dove brain: autoradiographic analysis of binding characteristic in discrete brain regions and accessibility to blood-borne prolactin. *Neuroendocrinology.* 1993;57:738–50.
- Buntin L, Berghman LR, Buntin JD. Patterns of fos-like immunoreactivity in the brains of parent ring doves (*Streptopelia risoria*) given tactile and non-tactile exposure to their young. *Behav Neurosci.* 2006;120:651–64.
- Chaiseha Y, Youngren O, Al-Zailaie K, El Halawani M. Expression of D1 and D2 dopamine receptors in the hypothalamus and pituitary during the turkey reproductive cycle: colocalization with vasoactive intestinal peptide. *Neuroendocrinology.* 2003;77:105–18.
- Chaiyachet OA, Chokchaloemwong D, Prakobsaeng N, Sartsoongnoen N, Kosonsiriluk S, Rozenboim I, El Halawani ME, Porter TE, Chaiseha Y. Neuroendocrine regulation of rearing behavior in the native Thai hen. *Acta Histochem.* 2013b;115:209–18.
- Chen XJ, Horseman ND. Cloning, expression and mutational analysis of the pigeon prolactin receptor. *Endocrinology.* 1994;135:269–76.
- Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH. Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature.* 1993;365:855–9.
- Cloues R, Ramos C, Silver R. Vasoactive intestinal polypeptide-like immunoreactivity during reproduction in doves: influence of experience and number of offspring. *Horm Behav.* 1990;24:215–31.
- Collias NE. The vocal repertoire of the red junglefowl—a spectrographic classification and the code of communication. *Condor.* 1987;89:510–24.
- Collias N, Joos M. The spectrographic analysis of sound signals of the domestic fowl. *Behaviour.* 1953;5:175–88.
- Corcoran DH, Proudman JA. Isoforms of turkey prolactin: evidence for differences in glycosylation and in tryptic peptide mapping. *Comp Biochem Physiol.* 1991;99B:563–70.
- den Boer-Visser AM, Dubbeldam JL. The distribution of dopamine, substance P, vasoactive intestinal polypeptide and neuropeptide Y immunoreactivity in the brain of the collared dove, *Streptopelia decaocto*. *J Chem Neuroanat.* 2002;23:1–27.
- Duckworth RA, Badyaev AV, Parlow AF. Elaborately ornamented males avoid costly parental care in the house finch (*Carpodacus mexicanus*): a proximate perspective. *Behav Ecol Sociobiol.* 2003;55:176–83.
- Duffy AM Jr, Goldsmith AR, Wingfield JC. Prolactin secretion in a brood parasite, the brown-headed cowbird, *Molothrus ater*. *J Zool.* 1987;212:669–75.
- Dunn IC, Sharp PJ. Photo-induction of hypothalamic gonadotrophin-releasing hormone-I mRNA in the domestic chicken: a role for oestrogen? *J Neuroendocrinol.* 1999;11:371–5.
- Dunn IC, Beattie KK, Maney D, Sang HM, Talbot RT, Wilson PW, Sharp PJ. Regulation of chicken gonadotropin-releasing hormone-I mRNA in incubating, nest-deprived and laying bantam hens. *Neuroendocrinology.* 1996;63:504–13.
- Dunn IC, McEwan G, Okhubo T, Sharp PJ, Paton IR, Burt DW. Genetic mapping of the chicken prolactin receptor gene: a candidate gene for the control of broodiness. *Br Poult Sci.* 1998;39(Suppl):S23–4.
- Durstewitz D, Kröner S, Hemmings HC Jr, Güntürkün O. The dopaminergic innervation of the pigeon telencephalon: distribution of DARPP-32 and co-occurrence with glutamate decarboxylase and tyrosine hydroxylase. *Neuroscience.* 1998;83:763–79.
- El Halawani ME, Burke WH, Dennison PT. Effect of nest-deprivation on serum prolactin level in nesting female turkeys. *Biol Reprod.* 1980;23:118–23.
- El Halawani ME, Burke WH, Millam JR, Fehrer SC, Hargis BM. Regulation of prolactin and its role in gallinaceous bird reproduction. *J Exp Zool.* 1984;232:521–9.
- El Halawani ME, Silsby JL, Behnke EJ, Fehrer SC. Hormonal induction of incubation in ovariectomized female turkey (*Meleagris gallopavo*). *Biol Reprod.* 1986;35:59–67.
- El Halawani ME, Silsby JL, Mauro LJ. Vasoactive intestinal peptide is a hypothalamic prolactin-releasing neuropeptide in the turkey (*Meleagris gallopavo*). *Gen Comp Endocrinol.* 1990;78:66–73.
- el Halawani ME, Youngren OM, Rozenboim I, Pitts GR, Silsby JL, Phillips RE. Serotonergic stimulation of prolactin secretion is inhibited by vasoactive intestinal

- peptide immunoneutralization in the turkey. *Gen Comp Endocrinol.* 1995;99:69–74.
- el Halawani ME, Pitts GR, Sun S, Silsby JL, Sivanandan V. Active immunization against vasoactive intestinal peptide prevents photo-induced prolactin secretion in turkeys. *Gen Comp Endocrinol.* 1996;104:76–83.
- el Halawani ME, Youngren OM, Pitts GR. Vasoactive intestinal peptide as the avian prolactin releasing factor. In: Harvey S, Etches R, editors. *Prospectives in Avian Endocrinology*, Bristol: The Society of Endocrinology. 1997. pp 403–416.
- Fan WL, Ng CS, Chen CF, Lu MY, Chen YH, Liu CJ, Wu SM, Chen CK, Chen JJ, Mao CT, Lai YT, Lo WS, Chang WH, Li WH. Genome-wide patterns of genetic variation in two domestic chickens. *Genome Biol Evol.* 2013;5:1376–92.
- Field SE, Rickard NS, Toukhsati SR, Gibbs ME. Maternal hen calls modulate memory formation in the day-old chick: the role of noradrenaline. *Neurobiol Learn Mem.* 2007;88:321–30.
- Gaiddon C, Mercken L, Bancroft C, Loeffler JP. Transcriptional effects in GH3 cells of Gs alpha mutants associated with human pituitary tumors: stimulation of adenosine 3',5'-monophosphate response element-binding protein-mediated transcription and of prolactin and growth hormone promoter activity via protein kinase A. *Endocrinology.* 1995;136:4331–8.
- Georgiou GG, Sharp PJ, Rea RW. [¹⁴C]-2 deoxyglucose uptake in the brain of ring dove (*Streptopelia risoria*). II. Differential uptake at the onset of incubation. *Brain Res.* 1995;700:137–41.
- Goldsmith AR. Prolactin in avian reproductive cycles. In: Balthazart J, Prove JE, Gilles R, editors. *Hormones and behaviour in higher vertebrates*. Berlin: Springer-Verlag; 1983. p. 375–87.
- Goodale HD, Sanborn R, White D. Broodiness in domestic fowl: data concerning its inheritance in the Rhode Island Red breed. *Massachusetts Agr Exp Sta Bull.* 1920;199:94–116.
- Goossens N, Bläher S, Oksche A, Vandesande F, Dierickx K. Immunocytochemical investigation of the hypothalamo-neurohypophysial system in birds. *Cell Tissue Res.* 1977;184:1–13.
- Guilleux F, Wakao H, Mundt M, Groner B. Prolactin induces phosphorylation of Tyr694 of Stat5 (MGF), a prerequisite for DNA binding and induction of transcription. *EMBO J.* 1994;13:4361–9.
- Guémené D, Williams JB. Comparison of the basal and luteinising hormone-releasing hormone induced luteinising hormone release by perfused hypophyses from turkey hens (*Meleagris gallopavo*) at different physiological stages. *Br Poult Sci.* 1992;33:153–63.
- Haller RW, Chermis FL Jr. A comparison of several treatments on terminating broodiness in broad breasted bronze turkeys. *Poult Sci.* 1961;40:155–63.
- Hanks MC, Alonzi JA, Sharp PJ, Sang HM. Molecular cloning and sequence analysis of putative chicken prolactin cDNA. *J Mol Endocrinol.* 1989a;2:21–30.
- Hanks MC, Talbot RT, Sang HM. Expression of biologically active recombinant-derived chicken prolactin in *Escherichia coli*. *J Mol Endocrinol.* 1989b;3(1):15–21.
- Hansen GN, Hansen BL. Light and electron microscopic identification of pituitary cells containing growth hormone and prolactin in the pigeon (*Columba livia*), using the immunoglobulin-enzyme bridge technique. *Gen Comp Endocrinol.* 1977;32:99–107.
- Harvey S, Hall TR, Chadwick A. Growth hormone and prolactin secretion in water-deprived chickens. *Gen Comp Endocrinol.* 1984;54:46–50.
- Hays FA. Inheritance of broodiness in Rhode Island Reds. *Massachusetts Agr Exp Sta Bull.* 1940;377:1–11.
- Hnasko RM, Buntin JD. Functional mapping of neural sites mediating prolactin-induced hyperphage in doves. *Brain Res.* 1993;623:257–66.
- Howard PW, Maurer RA. A composite Ets/Pit-1 binding site in the prolactin gene can mediate transcriptional responses to multiple signal transduction pathway. *J Biol Chem.* 1995;270:20930–6.
- Hutchison RE. Effect of ovarian steroids and prolactin on the sequential development of nesting behavior in female budgerigars. *J Endocrinol.* 1975;67:29.
- Hutchison RE, Hinde RA, Steel E. The effect of oestrogen, progesterone and prolactin on brood patch formation in ovariectomized canaries. *J Endocrinol.* 1967;39:379–85.
- Hutt FB. *Genetics of the fowl*. New York, NY: McGraw-Hill Book Company Inc.; 1949.
- Itoh N, Yonehara S, Schreurs J, Gorman DM, Maruyama K, Ishii A, Yahara I, Arai K, Miyajima A. Cloning of an interleukin-3 receptor gene: a member of a distinct receptor gene family. *Science.* 1990;247:324–7.
- Jones RE. The incubation patch of birds. *Biol Rev.* 1971;46:315–39.
- Józsa R, Scanes CG, Vigh S, Mess B. Functional differentiation of the embryonic chicken pituitary gland studied by immunohistological approach. *Gen Comp Endocrinol.* 1979; 39(2):158–163.
- Juhn M, Harris PC. Molt of capon feathering with prolactin. *Proc Soc Exp Biol Med.* 1958;98:669–72.
- Kansaku N, Shimada K, Terada O, Saito N. Prolactin, growth hormone, and luteinizing hormone-beta subunit gene expression in the cephalic and caudal lobes of the anterior pituitary gland during embryogenesis and different reproductive stages in the chicken. *Gen Comp Endocrinol.* 1994;96:197–205.
- Kansaku N, Shimada K, Saito N. Regionalized gene expression of prolactin and growth hormone in the chicken anterior pituitary gland. *Gen Comp Endocrinol.* 1995;99:60–8.
- Kansaku N, Shimada K, Saito N, Hidaka H. Effects of protein kinase A inhibitor (H-89) on VIP- and GRF-induced release and mRNA expression of prolactin and growth hormone in the chicken pituitary gland. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol.* 1998;119:89–95.
- Kansaku N, Ohkubo T, Okabayashi H, Guémené D, Kuhnlein U, Zadworny D, Shimada K. Cloning of

- duck PRL cDNA and genomic DNA. *Gen Comp Endocrinol.* 2005;141:39–47.
- Karatzas CN, Zadworny D, Kuhnlein U. Nucleotides sequence of turkey prolactin. *Nucleic Acids Res.* 1990;18:3071.
- Karatzas CN, Guémené D, Zadworny D, Kuhnlein U. Production and characterization of recombinant turkey prolactin. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 1993;106(2):273–280.
- Kato K, Ikemoto T, Park MK. Identification of the reptilian prolactin and its receptor cDNAs in the leopard gecko, *Eublepharis macularius*. *Gene.* 2005;346:267–76.
- Kelly PA, Djiane J, Postel-Vinay MC, Ederly M. The prolactin/growth hormone receptor family. *Endocr Rev.* 1991;12:235–51.
- Ketterson ED, Nolan V Jr, Wolf L, Goldsmith AR. Effect of sex, stage of reproduction, season, and mate removal on prolactin in dark-eyed juncos. *Condor.* 1990;92:922–30.
- Khan MZ, McNabb FM, Walters JR, Sharp PJ. Patterns of testosterone and prolactin concentrations and reproductive behavior of helpers and breeders in the cooperatively breeding red-cockaded woodpecker (*Picoides borealis*). *Horm Behav.* 2001;40:1–13.
- Kiss JZ, Péczely P. Distribution of tyrosine-hydroxylase (TH)-immunoreactive neurons in the diencephalon of the pigeon (*Columba livia domestica*). *J Comp Neurol.* 1987;257:333–46.
- Knigge KM, Piekut DT. Distribution of CRF- and tyrosine hydroxylase-immunoreactive neurons in the brainstem of the domestic fowl (*Gallus domesticus*). *Peptides.* 1985;6:97–101.
- Kurima K, Proudman JA, El Halawani ME, Wong EA. The turkey prolactin-encoding gene and its regulatory region. *Gene.* 1995;156:309–10.
- Kuwayama T, Shimada K, Saito N, Ohkubo T, Sato K, Wada M, Ichinoe K. Effects of removal of chicks from hens on concentrations of prolactin, luteinizing hormone and oestradiol in plasma of brooding Gifujidori hens. *J Reprod Fertil.* 1992;95:617–22.
- Laverrière JN, Tixier-Vidal A, Buisson N, Morin A, Martial JA, Gourdji D. Preferential role of calcium in the regulation of prolactin gene transcription by thyrotropin-releasing hormone in GH3 pituitary cells. *Endocrinology.* 1988;122:333–40.
- Lea RW, Sharp PJ. Plasma prolactin concentrations in broody turkeys: lack of agreement between homologous chicken and turkey prolactin radioimmunoassay. *Br Poult Sci.* 1982;23:451–9.
- Lea RW, Sharp PJ. Effects of presence of squabs upon plasma concentrations of prolactin and LH and length of time of incubation in ringdoves on “extended” incubatory patterns. *Horm Behav.* 1991;25:275–82.
- Lea RW, Dods AS, Sharp PJ, Chadwick A. The possible role of prolactin in the regulation of nesting behaviour and the secretion of luteinizing hormone in broody bantams. *J Endocrinol.* 1981;91:89–97.
- Lea RW, Richard-Yris MA, Sharp PJ. The effect of ovariectomy on concentrations of plasma prolactin and LH and parental behavior in the domestic fowl. *Gen Comp Endocrinol.* 1996;101:115–21.
- Lea RW, Clark JA, Tsutsui K. Changes in central steroid receptor expression, steroid synthesis, and dopaminergic activity related to the reproductive cycle of the ring dove. *Microsc Res Tech.* 2001;55:12–26.
- Lebrun JJ, Ali S, Goffin V, Ullrich A, Kelly PA. A single phosphotyrosine residue of the prolactin receptor is responsible for activation of gene transcription. *Proc Natl Acad Sci U S A.* 1995;92:4031–5.
- Lewis UJ, Singh RN, Lewis LJ. Two forms of glycosylated human prolactin have different pigeon crop sac-stimulating activities. *Endocrinology.* 1989;124:1558–63.
- Liang J, Kim KE, Schoderbek WE, Maurer RA. Characterization of a nontissue-specific, 3', 5'-cyclic adenosine monophosphate-responsive element in the proximal region of the rat prolactin gene. *Mol Endocrinol.* 1992;6:885–92.
- Lormée H, Jouventin P, Chastel O, Mauget R. Endocrine correlates of parental care in an Antarctic winter breeding seabird, the emperor penguin, *Aptenodytes forsteri*. *Horm Behav.* 1999;35:9–17.
- Macnamee MC, Sharp PJ. The functional activity of hypothalamic dopamine in broody bantam hens. *J Endocrinol.* 1989;121:67–74.
- Macnamee MC, Sharp PJ, Lea RW, Sterling RJ, Harvey S. Evidence that vasoactive intestinal polypeptide is a physiological prolactin-releasing factor in the bantam hen. *Gen Comp Endocrinol.* 1986;62:470–8.
- Mangalam HJ, Albert VR, Ingraham HA, Kapiloff M, Wilson L, Nelson C, Elsholtz H, Rosenfeld MG. A pituitary POU domain protein, Pit-1, activates both growth hormone and prolactin promoters transcriptionally. *Genes Dev.* 1989;3:946–58.
- Mao JN, Burnside J, Li L, Tang J, Davolos C, Cogburn LA. Characterization of unique truncated prolactin receptor transcripts, corresponding to the intracellular domain, in the testis of the sexually mature chicken. *Endocrinology.* 1999;140:1165–74.
- March JB, Sharp PJ, Wilson PW, Sang HM. 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.* 1994;101:227–33.
- Markoff E, Sigel MB, Lacour N, Seavey BK, Friesen HG, Lewis UJ. Glycosylation selectively alters the biological activity of prolactin. *Endocrinology.* 1988;123:1303–6.
- Massaro M, Setiawan AN, Davis LS. Effects of artificial eggs on prolactin secretion, steroid levels, brood patch development, incubation onset and clutch size in the yellow-eyed penguin (*Megadyptes antipodes*). *Gen Comp Endocrinol.* 2007;151:220–9.
- Mauro LJ, Youngren OM, Proudman JA, Phillips RE, el Halawani ME. Effects of reproductive status, ovariectomy, and photoperiod on vasoactive intestinal peptide in the female turkey hypothalamus. *Gen Comp Endocrinol.* 1992;87:481–93.
- Mead R, Curnow RN. *Statistical methods in agriculture and experimental biology.* London, UK: Chapman and Hall; 1983.

- Miao Y-W, Burt DW, Paton IR, Sharp PJ, Dunn IC. Mapping of the chicken prolactin gene to chromosome 2. *Anim Genet.* 1999;30:462–78.
- Mills AD, Crawford LL, Domjan M, Faure JM. The behavior of the Japanese or domestic quail *Coturnix japonica*. *Neurosci Biobehav Rev.* 1997;21:261–81.
- Miyoshi K, Shillingford JM, Smith GH, Grimm SL, Wagner KU, Oka T, Rosen JM, Robinson GW, Hennighausen L. Signal transducer and activator of transcription (Stat) 5 controls the proliferation and differentiation of mammary alveolar epithelium. *J Cell Biol.* 2001;155:531–42.
- Moons L, D'Hondt E, Pijcke K, Vandesande F. Noradrenergic system in the chicken brain: immunocytochemical study with antibodies to noradrenaline and dopamine-beta-hydroxylase. *J Comp Neurol.* 1995;360:331–48.
- Murdoch GH, Waterman M, Evans RM, Rosenfeld MG. Molecular mechanisms of phorbol ester, thyrotropin-releasing hormone and growth factor stimulation of prolactin gene expression. *J Biol Chem.* 1985;260:11852–8.
- Murphy MJ, Brown PS, Brown SC. Osmoregulatory effects of prolactin and growth hormone in embryonic chicks. *Gen Comp Endocrinol.* 1986;62:485–92.
- Nakao N, Ono H, Yamamura T, Anraku T, Takagi T, Higashi K, Yasuo S, Katou Y, Kageyama S, Uno Y, Kasukawa T, Iigo M, Sharp PJ, Iwasawa A, Suzuki Y, Sugano S, Niimi T, Mizutani M, Namikawa T, Ebihara S, Ueda HR, Yoshimura T. Thyrotrophin in the pars tuberalis triggers photoperiodic response. *Nature.* 2008;452:317–22.
- Nelson RJ. An introduction to behavioral endocrinology. Sunderland, MA, USA: Sinauer Associates; 1995.
- Nilsson Ann. Structure of the vasoactive intestinal octacosapeptide from chicken intestine. The amino acid sequence. *FEBS Letters.* 1975;60(2):322–326.
- Noso T, Swanson P, Lance VA, Kawachi H. Isolation and characterization of glycosylated and non-glycosylated prolactins from alligator and crocodile. *Int J Pept Protein Res.* 1992;39:250–7.
- Numan M, Woodside B. Maternity: neural mechanisms, motivational processes, and physiological adaptations. *Behav Neurosci.* 2010;124:715–41.
- Ohkubo T, Tanaka M, Nakashima K, Shimada K, Saito N, Sato K. High-level expression of biologically active chicken prolactin in *E. coli*. *Comp Biochem Physiol Comp Physiol.* 1993;105:123–8.
- Ohkubo T, Tanaka M, Nakashima K, Talbot RT, Sharp PJ. Prolactin receptor gene expression in the brain and peripheral tissues in broody and nonbroody breeds of domestic hen. *Gen Comp Endocrinol.* 1998a;109:60–8.
- Ohkubo T, Tanaka M, Nakashima K, Sharp PJ. Relationship between prolactin receptor mRNA in the anterior pituitary gland and hypothalamus and reproductive state in male and female bantams (*Gallus domesticus*). *Gen Comp Endocrinol.* 1998b;111:167–76.
- Ohkubo T, Tanaka M, Nakashima K. Molecular cloning of the chicken prolactin gene and activation by Pit-1 and cAMP-induced factor in GH3 cells. *Gen Comp Endocrinol.* 2000;119:208–16.
- Opel H. Induction of incubation behavior in the hen by brain implants of prolactin. *Poult Sci.* 1971;50:1613.
- Opel H, Proudman JA. Failure of mammalian prolactin to induce incubation behavior in chickens and turkeys. *Poult Sci.* 1980;59:2550–8.
- Opel H, Proudman JA. Stimulation of prolactin release in turkeys by vasoactive intestinal peptide. *Proc Soc Exp Biol Med.* 1988;187:455–60.
- Opel H, Proudman JA. Plasma prolactin levels in incubating turkey hens during pipping of the eggs and after introduction of poults into the nest. *Biol Reprod.* 1989;40:981–7.
- Peers B, Monget P, Nalda MA, Voz ML, Berwaer M, Belayew A, Martial JA. Transcriptional induction of the human prolactin gene by cAMP requires two cis-acting elements and at least the pituitary-specific factor Pit-1. *J Biol Chem.* 1991;266:18127–34.
- Pitts GR, You S, Foster DN, El-Halawani ME. Evidence for multiple prolactin receptor transcripts in the turkey. *Poult Sci.* 2000;79:355–62.
- Porter TE, Hill JB, Wiles CD, Frawley LS. Is the mammosomatotrope a transitional cell for the functional interconversion of growth hormone- and prolactin-secreting cells? Suggestive evidence from virgin, gestating, and lactating rats. *Endocrinology.* 1990;127:2789–94.
- Proudman JA, Corcoran DH. Turkey prolactin: purification by isotachopheresis and partial characterization. *Biol Reprod.* 1981;25:375–84.
- Punnett RC, Bailey PG. Genetic studies in poultry: II. Inheritance of egg-colour and broodiness. *J Genet.* 1920;10:277–92.
- Ramesh R, Proudman JA, Kuenzel WJ. Changes in pituitary somatotroph and lactotroph distribution in laying and incubating turkey hens. *Gen Comp Endocrinol.* 1996;104:67–75.
- Ramesh R, Solow R, Proudman JA, Kuenzel WJ. Identification of mammosomatotrophs in the turkey hen pituitary: increased abundance during hyperprolactinemia. *Endocrinology.* 1998;139:781–6.
- Ramesh R, Kuenzel WJ, Proudman JA. Increased proliferative activity and programmed cellular death in the turkey hen pituitary gland following interruption of incubation behavior. *Biol Reprod.* 2001;64:611–8.
- Richard-Yris MA, Leboucher G. Responses to successive test of induction of maternal behaviour in hens. *Behav Processes.* 1987;15:17–26.
- Richard-Yris MA, Sharp PJ, Wauters AM, Guémené D, Richard JP, Forasté M. Influence of stimuli from chicks on behavior and concentrations of plasma prolactin and luteinizing hormone in incubating hens. *Horm Behav.* 1998;33:139–48.
- Riddle O, Bates RW, Dykshorn SW. The preparation, identification and assay of prolactin—a hormone of the anterior pituitary. *Am J Physiol.* 1933;105:191–216.
- Riddle O, Bates RW, Lahr EL. Prolactin induces broodiness in fowl. *Am J Physiol.* 1935;111:352–60.

- Roberts E, Card LE. Inheritance of broodiness in the domestic fowl. Proceedings of the 5th World's Poultry Congress; Vol 2; 1933. p. 353–8.
- Romanov MN, Talbot RT, Wilson PW, Sharp PJ. Inheritance of broodiness in the domestic fowl. *Br Poult Sci.* 1999;40(Suppl):S20–1.
- Romanov MN, Talbot RT, Wilson PW, Sharp PJ. Genetic control of incubation behavior in the domestic chicken. *Poult Sci.* 2002;81:928–31.
- Rosenblatt JS, Mayer AD, Siegel HI. Maternal behavior among nonprimate mammals. In: Adler NT, Pfaff D, Goy RW, editors. *Handbook of behavioral neurobiology.* New York: Plenum; 1985. p. 229–98.
- Rozenboim I, Silsby JL, Tabibzadeh C, Pitts GR, Youngren OM, el Halawani ME. Hypothalamic and posterior pituitary content of vasoactive intestinal peptide and gonadotropin-releasing hormones I and II in the turkey hen. *Biol Reprod.* 1993;49:622–6.
- Rubin CJ, Zody MC, Eriksson J, Meadows JR, Sherwood E, Webster MT, Jiang L, Ingman M, Sharpe T, Ka S, Hallböök F, Besnier F, Carlborg O, Bed'hom B, Tixier-Boichard M, Jensen P, Siegel P, Lindblad-Toh K, Andersson L. Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature.* 2010;464:587–91.
- Saeki Y. Inheritance of broodiness in Japanese Nagoya fowl, with special reference to sex-linkage and notice in breeding practice. *Poult Sci.* 1957;36:378–83.
- Saeki Y, Inoue Y. Body growth, egg production, broodiness, age at first age and egg size in red jungle fowls, and attempt at their genetic analyses by reciprocal crossing with White Leghorns. *Jpn Poult Sci.* 1979;16:121–5.
- Saeki Y, Tanabe Y. Changes in prolactin content of fowl pituitary during broody periods and some experiments on the induction of broodiness. *Poult Sci.* 1955;34:909–19.
- Saldanha CJ, Silver R. Intraventricular prolactin inhibits hypothalamic vasoactive intestinal polypeptide expression in doves. *J Neuroendocrinol.* 1995;11:881–7.
- Sharp PJ. Female reproduction. In: Epplé A, Stetson MH, editors. *Avian endocrinology.* London and New York: Academic Press; 1980. p. 435–54.
- Sharp PJ, Scanes CG, Williams JB, Harvey S, Chadwick A. Variations in concentrations of prolactin, luteinizing hormone, growth hormone and progesterone in the plasma of broody bantams (*Gallus domesticus*). *J Endocrinol.* 1979;80:51–7.
- Sharp PJ, Dunn IC, Talbot RT. Sex differences in the LH responses to chicken LHRH-I and -II in the domestic fowl. *J Endocrinol.* 1987;115:323–31.
- Sharp PJ, Macnamee MC, Sterling RJ, Lea RW, Pedersen HC. Relationships between prolactin, LH and broody behaviour in bantam hens. *J Endocrinol.* 1988;118:279–86.
- Sharp PJ, Sterling RJ, Talbot RT, 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. *J Endocrinol.* 1989;122:5–13.
- Sharp PJ, Talbot RT, Main GM, Dunn IC, Fraser HM, Huskisson NS. Physiological roles of chicken LHRH-I and -II in the control of gonadotrophin release in the domestic chicken. *J Endocrinol.* 1990;124:291–9.
- Sharp PJ, Li Q, Georgiou G, Lea RW. Expression of *fos*-like immunoreactivity in the hypothalamus of the ring dove (*Streptopelia risoria*) at the onset of incubation. *J Neuroendocrinol.* 1996;8:291–8.
- Sherry DF, Mrosovsky N, Hogan J. Weight loss and anorexia during incubation in birds. *J Comp Physiol Psychobiol.* 1980;94:89–98.
- Shimada K, Ishida H, Sato K, Seo H, Matsui N. Expression of prolactin gene in incubating hens. *J Reprod Fertil.* 1991;91:147–54.
- Silver R, Witkovsky P, Horvath P, Alones V, Barnstable CJ, Lehman MN. Coexpression of opsin- and VIP-like-immunoreactivity in CSF-contacting neurons of the avian brain. *Cell Tissue Res.* 1988;253:189–98.
- Silverin B, Goldsmith A. The effects of modifying incubation on prolactin secretion in free-living pied flycatchers. *Gen Comp Endocrinol.* 1984;55:239–44.
- Sinha YN, DePaolo LV, Haro LS, Singh RN, Jacobsen BP, Scott KE, Lewis UJ. Isolation and biochemical properties of four forms of glycosylated porcine prolactin. *Mol Cell Endocrinol.* 1991;80:203–13.
- Slawski BA, Buntin JD. Preoptic area lesions disrupt prolactin-induced parental feeding behavior in ring doves. *Horm Behav.* 1995;29:248–66.
- Smiley KO, Adkins-Regan E. Prolactin is related to individual differences in parental behavior and reproductive success in a biparental passerine, the zebra finch (*Taeniopygia guttata*). *Gen Comp Endocrinol.* doi:10.1016/j.ygcen.2016.03.006. [Epub ahead of print].
- Sockman KW, Sharp PJ, Schwabl H. Orchestration of avian reproductive effort: an integration of the ultimate and proximate bases for flexibility in clutch size, incubation behaviour, and yolk androgen deposition. *Biol Rev Camb Philos Soc.* 2006;81:629–66.
- Steinfelder HJ, Radovick S, Mroczynski MA, Hauser P, McClaskey JH, Weintraub BD, Wondisford FE. Role of a pituitary-specific transcription factor (Pit-1/GHF-1) or a closely related protein in cAMP regulation of human thyrotropin-beta subunit gene expression. *J Clin Invest.* 1992;89:409–19.
- Talbot RT, Hanks MC, Sterling RJ, Sang HM, Sharp PJ. Pituitary prolactin messenger ribonucleic acid levels in incubating and laying hens: effects of manipulating plasma levels of vasoactive intestinal polypeptide. *Endocrinology.* 1991;129:496–502.
- Talbot RT, Dunn IC, Wilson PW, Sang HM, Sharp PJ. Evidence for alternative splicing of the chicken vasoactive intestinal polypeptide gene transcript. *J Endocrinol.* 1995;15:81–91.
- Tanaka M, Maeda K, Okubo T, Nakashima K. Double antenna structure of chicken prolactin receptor deduced from the cDNA sequence. *Biochem Biophys Res Commun.* 1992;188:490–6.
- Tanaka M, Yamamoto I, Hayashida Y, Nakao N, Ohkubo T, Wakita M, 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. *Biochim Biophys Acta*. 2000;1491:279-84.
- Thayananuphat A, Youngren OM, Kang SW, Bakken T, Kosonsiriluk S, Chaiseha Y, El Halawani ME. Dopamine and mesotocin neurotransmission during the transition from incubation to brooding in the turkey. *Horm Behav*. 2011;60:327-35.
- Tolón RM, Castillo AI, Aranda A. Activation of the prolactin gene by peroxisome proliferator-activated receptor-alpha appears to be DNA binding-independent. *J Biol Chem*. 1998;273:26652-61.
- Torchia J, Rose DW, Inostroza J, Kamel Y, Westin S, Glass CK, Rosenfeld MG. The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. *Nature*. 1997;387:677-84.
- Vleck CM, Mays NA, Dawson JW, Goldsmith AR. Hormonal correlates of parental and helping behavior in cooperatively breeding Harris' hawks (*Parabuteo unicinctus*). *Auk*. 1991;108:638-48.
- Wang Q, Buntin JD. The roles of stimuli from young, previous breeding experience, and prolactin in regulating parental behavior in ring doves (*Streptopelia risoria*). *Horm Behav*. 1999;35:241-53.
- Warren DC. Cross bred poultry, Kansas Agr Exp Sta Bull. 1930;252:5-54.
- Watahiki M, Tanaka M, Masuda N, Sugisaki K, Yamamoto M, Yamakawa M, Nagai J, Nakashima K. Primary structure of chicken pituitary prolactin deduced from the cDNA sequence. Conserved and specific amino acid residues in the domains of the prolactins. *J Biol Chem*. 1989;264:5535-9.
- Wauters AM, Richard-Yris MA. Mutual influence of the maternal hen's food calling and feeding behavior on the behavior of her chicks. *Dev Psychobiol*. 2002;41:25-36.
- Wong EA, Ferrin NH, Silsby JL, el Halawani ME. Cloning of a turkey prolactin cDNA: expression of prolactin mRNA throughout the reproductive cycle of the domestic turkey (*Meleagris gallopavo*). *Gen Comp Endocrinol*. 1991;83:18-26.
- Wong GK, Liu B, Wang J, Zhang Y, Yang X, Zhang Z, Meng Q, Zhou J, Li D, Zhang J, Ni P, Li S, Ran L, Li H, Zhang J, Li R, Li S, Zheng H, Lin W, Li G, Wang X, Zhao W, Li J, Ye C, Dai M, Ruan J, Zhou Y, Li Y, He X, Zhang Y, Wang J, Huang X, Tong W, Chen J, Ye J, Chen C, Wei N, Li G, Dong L, Lan F, Sun Y, Zhang Z, Yang Z, Yu Y, Huang Y, He D, Xi Y, Wei D, Qi Q, Li W, Shi J, Wang M, Xie F, Wang J, Zhang X, Wang P, Zhao Y, Li N, Yang N, Dong W, Hu S, Zeng C, Zheng W, Hao B, Hillier LW, Yang SP, Warren WC, Wilson RK, Brandström M, Ellegren H, Crooijmans RP, van der Poel JJ, Bovenhuis H, Groenen MA, Ovcharenko I, Gordon L, Stubbs L, Lucas S, Glavina T, Aerts A, Kaiser P, Rothwell L, Young JR, Rogers S, Walker BA, van Hateren A, Kaufman J, Bumstead N, Lamont SJ, Zhou H, Hocking PM, Morrice D, de Koning DJ, Law A, Bartley N, Burt DW, Hunt H, Cheng HH, Gunnarsson U, Wahlberg P, Andersson L, Kindlund E, Tammi MT, Andersson B, Webber C, Ponting CP, Overton IM, Boardman PE, Tang H, Hubbard SJ, Wilson SA, Yu J, Wang J, Yang H, International Chicken Polymorphism Map Consortium. A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. *Nature*. 2004;432:717-22.
- Xu L, Lavinsky RM, Dasen JS, Flynn SE, McInerney EM, Mullen T-M, Heinzel T, Szeto D, Korzus E, Kurokawa R, Aggarwal AK, Rose DW, Glass CK, Rosenfeld MG. Signal-specific co-activator domain requirements for Pit-1 activation. *Nature*. 1998;395:301-6.
- Yamashina Y. Notes on experimental brooding induced by prolactin injections in the domestic cock. *Annot Zool Japon*. 1952;25:135-42.
- Yang X-Y, Ogryzko VV, Nishikawa J, Howard BH, Nakatani Y. p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature*. 1996;382:319-24.
- Yasuda A, Yamaguchi K, Kobayashi T, Yamamoto K, Kikuyama S, Kawachi H. The complete amino acid sequence of prolactin from bullfrog (*Rana catesbeiana*). *Gen Comp Endocrinol*. 1991;83:218-26.
- You S, Silsby JL, Farris J, Foster DN, el Halawani ME. Tissue-specific alternative splicing of turkey preprovasoactive intestinal peptide messenger ribonucleic acid, its regulation, and correlation with prolactin secretion. *Endocrinology*. 1995a;136:2602-10.
- You S, Foster LK, Silsby JL, el Halawani ME, Foster DN. Sequence analysis of the turkey LH beta subunit and its regulation by gonadotrophin-releasing hormone and prolactin in cultured pituitary cells. *J Mol Endocrinol*. 1995b;14:117-29.
- Young KH, Buhi WC, Horseman N, Davis J, Kraeling R, Linzer D, Bazer FW. Biological activities of glycosylated and nonglycosylated porcine prolactin. *Mol Cell Endocrinol*. 1990;71:155-62.
- Youngren OM, El Halawani ME, Phillips RE, Silsby JL. Effects of preoptic and hypothalamic lesions in female turkeys during a photoinduced reproductive cycle. *Biol Reprod*. 1989;41:610-7.
- Youngren OM, El Halawani ME, Silsby JL, Phillips RE. Intracranial prolactin perfusion induces incubation behavior in turkey hens. *Biol Reprod*. 1991;44:425-43.
- Youngren OM, Silsby JL, Rozenboim I, Phillips RE, el Halawani ME. Active immunization with vasoactive intestinal peptide prevents the secretion of prolactin induced by electrical stimulation of the turkey hypothalamus. *Gen Comp Endocrinol*. 1994;95:330-6.
- Youngren OM, Pitts GR, Phillips RE, el Halawani ME. The stimulatory and inhibitory effects of dopamine on prolactin secretion in the turkey. *Gen Comp Endocrinol*. 1995;98:111-7.
- Youngren OM, Pitts GR, Phillips RE, el Halawani ME. Dopaminergic control of prolactin secretion in the turkey. *Gen Comp Endocrinol*. 1996;104:225-30.
- Zadworny D, Walton JS, Etches RJ. The relationship between plasma concentrations of prolactin and con-

- sumption of feed and water during the reproductive cycle of the domestic turkey. *Poult Sci.* 1985;64:401–10.
- Zadoworny D, Shimada K, Ishida H, Sumi C, Sato K. Changes in plasma levels of prolactin and estradiol, nutrient intake, and time spent nesting during the incubation phase of broodiness in the Chabo hen (Japanese bantam). *Gen Comp Endocrinol.* 1988;71:406–12.
- Zadoworny D, Shimada K, Ishida H, Sato K. Gonadotropin-stimulated estradiol production in small ovarian follicles of the hen is suppressed by physiological concentrations of prolactin in vitro. *Gen Comp Endocrinol.* 1989;74:468–73.
- Zhou JF, Zadoworny D, Guémené D, Kuhnlein U. Molecular cloning, tissue distribution, and expression of the prolactin receptor during various reproductive states in *Meleagris gallopavo*. *Biol Reprod.* 1996;55:1081–90.