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Role of the serotonergic axis in the reproductive failure associated with aging broiler breeder roosters

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ABSTRACT

Reproductive failure associated with aging is a well-known phenomenon. However, the mechanism by which this failure occurs in broiler breeder roosters is still unclear. A previous study conducted in our laboratory, comparing young and aging broiler breeder roosters, demonstrated an elevation in hypothalamic vasoactive intestinal peptide (VIP) and pituitary prolactin (PRL) gene expression accompanied by a deterioration of gonadal axis function. This resulted in a decrease in semen-quality variables as roosters aged. The objective of this study was to examine the involvement of the serotonergic axis in the age-associated reproductive failure in broiler breeder roosters. Cobb roosters aged 64 wk were divided into 3 groups (n = 20 each): parachlorophenylalanine (PCPA) administration, active immunization against chicken VIP, and controls. At 69 wk of age, each group was divided into 2 equal subgroups: 1 received ovine PRL and the other served as controls. Weekly semen volume, concentration and motility, and plasma testosterone, estradiol, and PRL concentrations were examined. At the end of the experiment, roosters were euthanized, testes were weighed, and hypothalamus and pituitary were removed to assay the expression of genes encoding hypothalamic GnRH-I, pituitary FSH, pituitary LH, hypothalamic VIP, and pituitary PRL. Both PCPA administration and active immunization against chicken VIP significantly increased testis weight, semen volume, sperm concentration, ejaculation grade, plasma testosterone level, and GnRH-I, FSH and LH gene expression compared with controls ($P \leq 0.05$). In addition, a decrease in plasma estradiol and PRL concentrations and VIP and PRL gene expression was observed in PCPA- and VIP-immunized birds compared with controls ($P \leq 0.05$). Administration of PRL in all groups decreased gonadal axis function and semen-quality variables ($P \leq 0.05$). Collectively, these results suggest that the increasing expression levels of the serotonergic axis in aging broiler breeder roosters inhibit proper gonadal function and reproductive performance. This article establishes for the first time the inhibitory role of serotonin on reproduction in aging roosters.

1. Introduction

Fertility of domestic roosters begins to decline at the age of 45 wk, as manifested by a significant reduction in gonadal axis activity [1–5]. Studies on aging male quails have shown a significant reduction in the secretion and concentration of

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hypothalamic GnRH-I [2], resulting in reduced synthesis and release of pituitary LH and pituitary FSH [1,5].

Another axis which has been shown to be involved in reproduction is the serotonergic axis (serotonin–vasoactive intestinal peptide [VIP]–prolactin [PRL]). There are numerous studies on the proximity localization of serotonin and VIP in the lung [6], bone, [7] and gastrointestinal tract [8] of mammals with no evidence on their influence on one another. However, the role of the serotonergic axis has been studied a lot in reproduction. High levels of serotonin, which is synthesized in the hypothalamus [9], have been reported to inhibit avian reproduction [10–12]. Elevation of serotonin levels directly inhibits GnRH-I synthesis [13] and LH secretion [10,12]. Conversely, blockade of the serotonergic system generally stimulates gonadotropin secretion and enhances gonadal development [10].

Serotonin also inhibits reproduction indirectly through VIP [13–16], which is known as the major avian PRL-releasing factor [17,18]. In early experiments with male chickens, high PRL levels were found to induce testicular regression [19,20]. In addition, PRL is well documented to inhibit GnRH-I and LH secretion and to dampen gonadal activity [21,22].

Previous studies in our laboratory on White Leghorn (WL) roosters actively immunized against chicken VIP (cVIP) showed that expression of hypothalamic VIP and pituitary PRL messenger RNA (mRNA) genes is lower in aging vs young roosters [23,24]. Active immunization of aging WL roosters against cVIP decreased pituitary PRL, hypothalamic GnRH-I, pituitary LH, and pituitary FSH mRNA gene expression, and decreased the examined reproductive parameters. Administration of ovine PRL (oPRL) to the immunized WL roosters increased their reproductive parameters, indicating the requirement for VIP and PRL in aging roosters [24]. However, a comparison of young and aging broiler breeder rooster reproduction showed a decrease in gonadal axis function and an increase in VIP–PRL expression in aging birds [24,25].

In view of our results showing the inverse relations of the gonadal axis and VIP–PRL in aging broiler breeder roosters, the objective of the present study was to examine the involvement of serotonin, VIP, and PRL in the reproductive failure associated with aging broiler breeder roosters.

2. Materials and methods

2.1. Experimental animals

All procedures were approved by the Animal Care and Welfare Committee of The Hebrew University of Jerusalem. Breeder roosters aged 60 wk were brought from the same mixed sex flock (Cobb; n = 60; body weight [BW], 4.92 ± 0.2 kg). Roosters were housed in individual cages for 4 wk before the experiment to prevent different parameters that would impact reproductive capability. Roosters were kept under photostimulatory conditions (16 light:8 dark) with a light intensity of 0.1 W/m^2 achieved by white fluorescent lamps. Birds were subjected to a commercial restricted feeding program with daily administration of feed, as recommended by the Cobb 500 Breeder Management Guide with modifications for birds reared in cages. The roosters' diet contained 14.5% crude protein and 2,700 kcal/kg.

Roosters aged 64 wk were divided into 3 groups (n = 20). The first group was actively immunized against cVIP, the second was treated with parachlorophenylalanine (PCPA; Sigma, St. Louis, MO, USA) and the third group, serving as a control, received adjuvant and keyhole limpet hemocyanin (KLH).

Active immunization against cVIP was conducted as described previously [13,25]. Briefly, the primary subcutaneous injection contained 125 μg cVIP conjugated to KLH in 1-mL Freund's complete adjuvant adjusted to 2 mL with distilled water and was given at 64 wk of age. A booster shot of 25 μg cVIP conjugated to KLH in 1-mL Freund's incomplete adjuvant adjusted to 2 mL with distilled water was given subcutaneously at 68 wk of age. Treatment with PCPA was given orally in gelatin capsules (50 mg/kg BW per d for 3 consecutive d) at 64 wk of age and was repeated every other week using the same procedure, ie, at 66, 68, and 70 wk of age.

At 69 wk of age, each group was divided into 2 subgroups (n = 10). The first subgroup was injected intramuscularly with 1.5 mg of oPRL per bird daily for 10 d. The oPRL was dissolved in a saturated solution of polyvinylpyrrolidone saline [26]. The second subgroup, serving as a control, received only polyvinylpyrrolidone saline. The experiment was terminated at 71 wk of age.

2.2. Blood sampling

Weekly heparinized blood samples were drawn from the brachial vein for determination of plasma steroid and PRL concentrations. Plasma samples were stored at −20°C until assay.

2.3. Hormone analysis

Plasma estradiol and testosterone were measured in a single enzyme-linked immunosorbent assay (ELISA) according to a previously described protocol [27] using primary antibody and tracer dilutions as described previously [23]. Plasma PRL was assayed by competitive ELISA using biotinylated anti-chicken PRL tracers that shows broad phylogenetic recognition of avian PRL with no cross-reactivity to oPRL as described previously [28].

2.4. Semen-quality variables

Semen was sampled once a week using the "abdominal massage method" [29]. Semen volume, sperm concentration, and ejaculation grade [30] were determined.

Ejaculation was graded as the response to the massage procedure as follows: 0, no erection of the phallus; l, erection with no fluid; 2, erection with secretion of fluid only; 3 to 8, secretion of semen with increasing amounts of spermatozoa.

2.5. Tissue sampling

Birds were killed by euthanasia at 71 wk of age (injection of pentobarbitone sodium at 1 mL/1.5 kg BW; Chemical and Technical Supplies, Chemical Industries Ltd, Kiryat Malachi, Israel). Immediately after the injection, a block of