Influence of 2–bromo-α–ergocryptine on Plasma Prolactin, Oestradiol-17β and Progesterone Levels in Domestic Hen

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ABSTRACT : This study investigated the effect of 2-bromo-α-ergocryptine (anti prolactin agent) on plasma levels of prolactin, oestradiol-17β and progesterone in domestic hen during the active period of lay. Fifty healthy female White Leghorn birds were administered with anti prolactin agent (2-bromo-α-ergocryptine, Sigma-USA., methane sulphonate salt, $C_{32}H_{40}BrN_5O_5.CH_4SO_3$) subcutaneously @100 μg/kg body weight at weekly intervals from 17th to 36th week of age. Another group of fifty birds as controls were given placebo in place of bromocriptine. The level of prolactin remained lower in treated birds than in the control birds from 19 to 36 weeks of age. Level of prolactin even in the control group was found to decrease during the peak production period. Oestradiol-17β and progesterone concentration in treated birds were significantly ($p<0.01$) higher than the controls during the treatment. Egg production, is positively correlated with oestradiol-17β ($r=0.02$; $r=0.67$) and progesterone ($r=0.49$; $r=0.90$) in control and treated groups respectively where as prolactin level is positively correlated with egg production in the control birds ($r=0.07$). Prolactin levels were negatively correlated with egg production ($r=-0.55$) in treated birds; and oestradiol-17β ($r=-0.71$; $r=-0.53$) and progesterone ($r=-0.22$; $r=-0.27$) respectively in control and treated groups. The total number of pause days during the treatment period decreased significantly ($p<0.01$) in the treated group compared to the control group. The reduction in pause days in treated group resulted in 1.76% increase in egg production over that in control group. The increase in egg laying days and the total egg production were found to be significant ($p<0.01$). These results indicate that a lower level of prolactin in circulatory blood enhances egg production in the domestic hen. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 8 : 1103-1109)***

Key Words : Sequence Length**,** Prolactin, Oestradiol, Progesterone, Domestic Hen

INTRODUCTION

Generally, hens lay eggs for a continues period of time and stop laying thereafter for one or few days (pause days). The exact physiological mechanism involved in pauses between the sequences is not fully known. Of late it was assumed that the higher levels of prolactin play a role in broodiness and cessation of egg lay during the active period of lay (Sharp et al., 1998). Prolactin inhibits reproductive function and probably acts at all levels of the hypothalamo-hypophysial-gonadal axis. For example, in turkeys systemic administration of prolactin decreases hypothalamic GnRH-I and II and plasma luteinizing hormone (LH) (Rozenboim et al., 1993), while incubation of anterior pituitary cells with prolactin inhibits LH β subunit gene expression (You et al., 1995). There is also evidence in the turkey that prolactin inhibits the steroidogenic activity of LH at the gonadal levels (Camper et al., 1977). It has been suggested that the increase in concentration of plasma prolactin during the incubation period may play a role in depressing LH secretion (El Halawani et al., 1993; Sharp et al., 1998; Nicholas et al., 1988) and inducing gonadal regression. Dopamine plays a role in the control of prolactin release at the level of anterior pituitary gland by inhibiting the stimulatory action of vasoactive intestinal peptide (VIP) through the D_2 receptor (Youngren et al., 1998). Further, elevated levels of serum prolactin, which has a negative effect on reproductive performance (Sharp et al., 1998) decreases egg production in poultry during the period of lay. Much of the work has been reported in turkeys (Burke et al., 1980; Burke et al., 1981; El Halawani et al., 1980; Zadworny et al., 1985; Sharp et al., 1989; El Halawani et al., 1988) with meager information on the domestic hen.

New approaches using immunological/neutralizing strategies to alter prolactin secretion for higher egg production is of economical interest and has been reported to be satisfactory at the experimental level (Crisostomo et al., 1998). However, until recently none were found appropriate for commercial use. Hence, this study was carried out to examine the influence of neutralization against prolactin, using bromoergocryptine to extend the clutch length by decreasing inter sequence pause days, thereby enhancing laying performance and egg production in domestic hen. This approach is of great practical interest, although their use needs to be carefully evaluated under commercial conditions. Even a slight increase in clutch length is achieved by manipulating the endocrine system; it may result in enormous increase in egg production with the available resources and under similar managemental practices.

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MATERIALS AND METHODS

Animals

One hundred White Leghorn birds (Bob Cob strain), housed in individual cages (one bird per cage, 12×12×12 inches) under two tier battery system birds were randomly allocated to both tires and divided into two groups of 50 birds each, at the age of 12 weeks. The birds were housed under normal husbandry conditions with ventilation and light schedule of 16L:8D. Feed was provided (Energy 50%, CP 30%, bran 15%, lime 5%)as recommended by Ranjhan (1980). The same amount of feed was offered in both control and treated groups and feed intakes were not affected by the treatment. The feed efficiency was 2.58 kgs in control comapared to 2.48 kgs in the treated birds. Clean water was made available round the clock through out the experimental period.

Modulation of prolactin using 2-bromo-α-ergocryptine

At 17th week of age the birds in treatment group were given 2-bromo-α-ergocryptine @100 µg/kg body weight subcutaneously at weekly interval (Reddy et al., 2001). Controls were administered with placebo (only vehicle without bromoergocryptine). Egg production for individual birds were recorded daily from the start of egg laying, for calculating sequence length, inter-sequence pause days, total egg production in treated and control groups. The total number of pause days during a 11 week period was recorded.

Blood samples were collected between 11:00 to 12:00 hours from each bird commencing from 13 weeks of age and at weekly intervals and continued until the end of experimental period. Plasma was separated and stored at -20°C for hormone assay. Chicken prolactin anti serum, chicken prolactin iodination grade and pure chicken prolactin were obtained from Dr. A. F. Parlow, NIADDK, California, USA. Plasma Prolactin levels were estimated by radioimmunoassay (RIA) using the method described by Kaprowski and Tucker (1971) using highly specific antiserum to chicken prolactin (Rabbit) AFP-151040789. Intra and inter assay coefficient of variation for prolactin were 7.22% and 9.50%, respectively and the sensitivity of the method was 5 ng/tube. The antiserum had a specificity of 100% for chicken prolactin and less than 1% for chicken growth hormone. Highly purified chicken prolactin AFP-44448B:1 ampoule, approximately 100 micrograms was provided and stored in 20-30 micrograms of aliquots. Oestradiol 17β and progesterone and chemicals used for RIA of hormones and 2-bromo-α-ergocryptine (methane sulphonate salt, $C_{32}H_{40}BrN_5O_5.CH_4SO_3$) were purchased from Sigma Chemicals Co. (St.Louis, MO, USA). Oestradiol antisera (#244Anti-estradiol-6-BSA Serum) and progesterone antisera (#337anti-progesterone-11-BSA serum) lyophilized were procured from Prof. G. D. Niswender, Colorado, USA. Radiochemicals viz. (2,4,6,7-^{3}H) Oestradiol, 85.0 Ci/mmol and (1,2,6,7-^{3}H) Progesterone, 93.0 Ci/mmol were purchased from Amersham Life Science, Nycomed Amersham plc, England, UK. Plasma progesterone and oestradiol-17β were estimated using RIA following the method described by Hall and Sufi (1981). Intra and inter assay coefficient of variation for oestradiol-17β were 4.76% and 6.22%, respectively and 6.5% and 9.63% respectively for progesterone and the sensitivity of the method was 2 pg/tube for oestradiol-17β and 25 pg/tube for progesterone.

Statistics

Completely Randomized Design was used in this study. The data on prolactin, oestradiol-17β and progesterone were subjected to one-way analysis of variance to test the statistical differences in hormone measurements between the treated and control birds. The data on egg production and prolactin, oestradiol-17β and progesterone were subjected to Pearson's correlation coefficient analysis to study the influence of these hormones on egg production. The statistical analyses were carried out following the methods described by Snedecor and Cochran (1994).

RESULTS

Effect of 2-bromo-α-ergocryptine on egg production

The egg laying in treatment group commenced in the 19th week of age compared to 20th week in the control group. Egg laying in the treated and control group increased slowly from 19th to 23rd week from 0.85% to 68.57% and from 0% to 66.57% in the 23rd week respectively. All the birds started to lay eggs from the 24th week of age. From the age of 24th to 34th weeks the average number of eggs laid per bird were 73.22±0.66 in the treatment group as against 71.44±0.04 in the control group. The difference in egg production per week per bird was significant between two groups during all weeks except during the 29th week. In all weeks where differences are significant except at 24th week of age, the mean values in the treatment group were significantly higher in comparison to values in control group (figure 1) at 24th week the values in control group were significantly higher than that in the treatment group. The total number of pause days per bird during the 11 week period (77 days) were 3.42±0.40 in the treatment group compared to 5.26±0.40 in control group. Egg production in treatment group between 24 and 34 weeks of age is 1.76 per cent higher than the control group.

Effect of 2-bromo-α-ergocryptine on prolactin levels

The plasma prolactin level in the control group varied

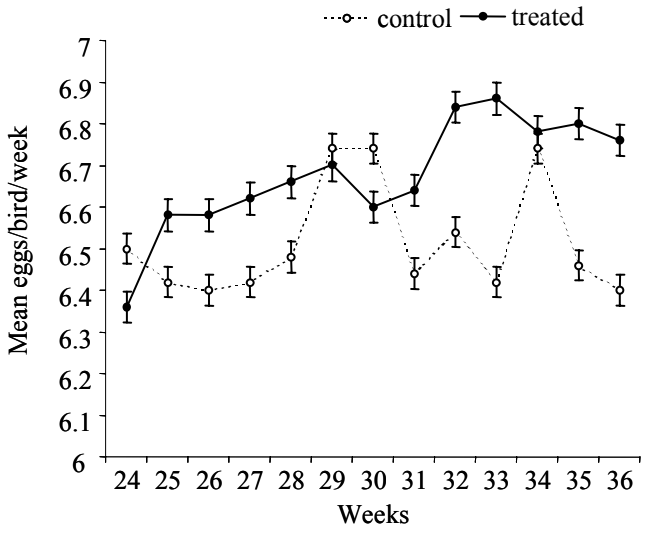

Figure 1. Weekly mean egg production in control and treated birds

between 428.76±37.28 ng/ml to 649.89±29.47 ng/ml during 17th to 23rd week of age (figure 2). In the treatment group the level of plasma prolactin decreased from 635.90±43.02 ng/ml to 118.39±8.83 ng/ml during 17th to 23rd week of age. In the control group a concomitant decrease in the level of prolactin to 247.99±30.15 ng/ml was observed during 25th week of age. The changes in the level of prolactin in birds of control group indicated that as the birds attained the peak level of egg production the prolactin level decreased even in control group but in the treatment group the decrease was of greater magnitude due to the administration of bromoergocryptine for five weeks. The level of prolactin in the treatment group from 23 to 34 weeks of age fluctuated between 118.39±8.83 ng/ml and 215.92±20.44 ng/ml whereas in the control group it showed a trend of progressive increase from 247.99±30.15 ng/ml at 25 weeks of age to 512.45±42.17 ng/ml and 511.50±49.43 ng/ml on 33 and 34 weeks of age respectively.

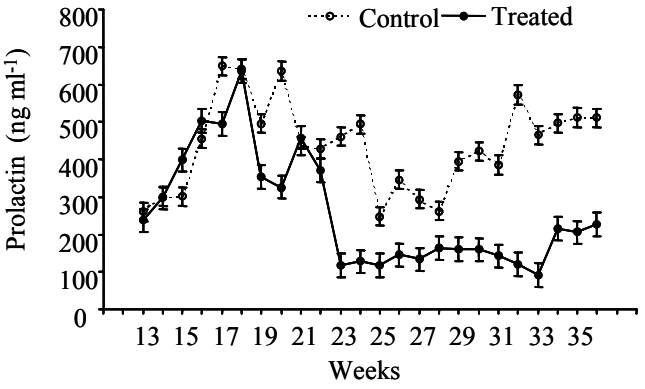

Figure 2. Plasma prolactin levels in control and treated birds during different weeks of lay

Effect of 2-bromo-α-ergocryptine on oestradiol 17-β and progesterone levels

The plasma oestradiol-17β level in birds of control group varied between 72±0.90 pg/ml to 239.14±2.56 pg/ml during 13th to 36th week of age (figure 3). In birds of treatment group the level of plasma oestradiol-17β increased from 81.32±2.1 pg/ml during 13th week of age to 248.07±1.50 pg/ml during 36th week of age. The progesterone secretion in the two groups also followed a similar pattern as oestradiol-17β and is presented in (figure 4). However intermittent hormonal fluctuations were observed in both control and treated groups.

Correlation between egg production, prolactin and steroid hormone levels

Egg production is positively correlated with oestradiol-17β (r=0.02; r=0.67) and progesterone (r=0.49; r=0.90) in control and treated groups respectively, where as prolactin level is positively correlated with egg production in the control birds (r=0.07). Prolactin levels were negatively correlated with egg production (r=-0.55) in treated birds and oestradiol-17β (r=-0.71; r=-0.53) and progesterone (r= -0.22; r=- 0.27) respectively in control and treated groups.

DISCUSSION

The regulation and interplay of various hormones like progesterone, oestradiol, testosterone, LH and prolactin in the ovulatory cycle are still not very clear but some related concepts have been reported. Prolactin is reported to have a

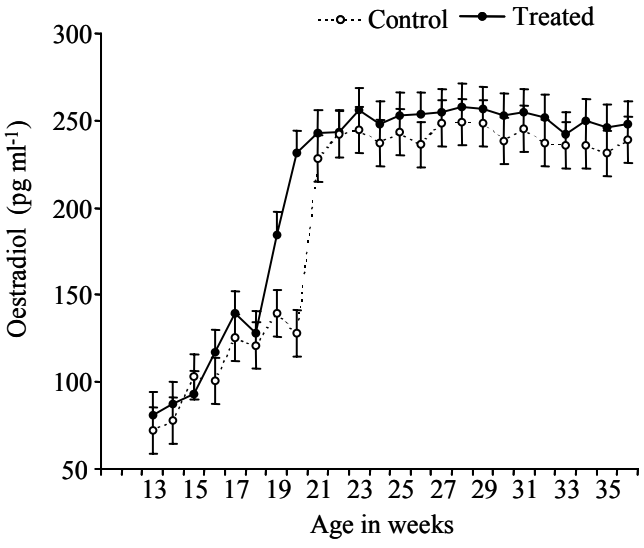

Figure 3. Plasma oestradiol 17 beta concentration in control and treated birds

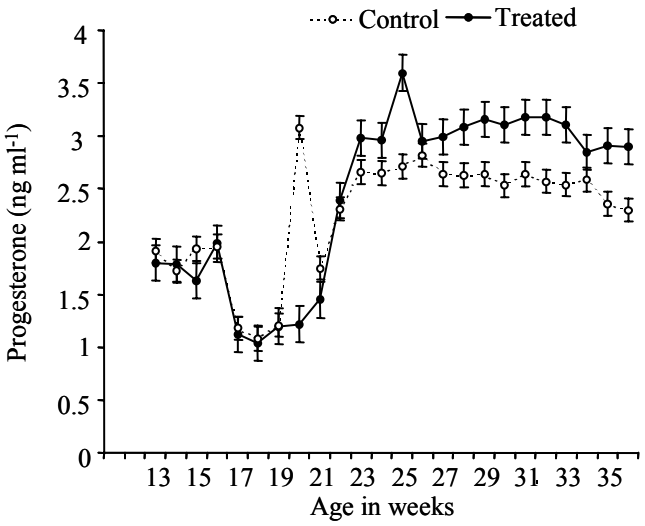

Figure 4. Plasma progesterone level in control and treated birds

negative effect on gonadotrophins particularly on the preovulatory surge of LH. Low levels of prolactin in treated group as a result of administration of bromocriptine might have abolished the negative effect of prolactin on gonadotrophins particularly preovulatory surge of LH (Guemene and Williams, 1994) and enhanced steroidogenesis in the gonads (Porter et al., 1991), increased the levels of oestradiol, progesterone and testosterone (Porter et al., 1991; Dajee et al., 1998) in turn influencing the feed back on the anterior pituitary for eliciting LH surge for ovulation (Emmerson et al., 1991). This may be attributed to decreasing the interval of ovulatory cycle to 24 h or even less (Robinson et al., 1990) resulting in an increase in the sequence length and thus reducing the pause days with concomitant increase in egg production as observed in this study. Further, the changes in the level of prolactin in the control group indicate that, as the birds attained the peak level of egg production the prolactin level decreased. But in the treatment group the decrease in prolactin level was of greater magnitude due to the administration of bromoergocryptine for five weeks leading to enhanced steroidogenesis, oviposition and persistency of egg laying. Prolactin neutralization has been shown to elicit longer ovulatory sequences, lesser inter-sequence pause days and an increase in egg production.

The studies on role of steroid hormones in the initiation have been shown that while progesterone (Youngren et al., 1993) is most important in inducing the preovulatory surge of LH and ovulation, oestrogen is important in priming of the hypothalamo- pituitary system (Guemene and Williams, 1994) and formation of the egg. The rise in oestrogen levels after inhibiting the high levels of prolactin suggests that prolactin levels might be interfering with the oestrogen synthesis. Similar observations have been reported in turkey (Wong et al., 1991) where oestradiol secretion was suppressed in TRH (prolactin stimulant) treated ewes compared to controls. In the rat, the effect of prolactin on oestradiol production indicated that hyperprolactinemia reduced oestrogen synthesis (Fortune et al., 1986). From the rat model, oestradiol production was believed to require the interaction of the two types of follicular cells, theca cells producing androgen for aromatization and granulosa cells converting them to oestradiol. Granulosa cell is believed to be the principle site of follicular androgen aromatization with oestradiol-17β being the major product. Granulosa cells have been reported to have receptors for prolactin (Dunaif et al., 1982) while theca cells did not appear to bind prolactin (Dunaif et al., 1982). Thus, the high prolactin levels may either interfere with the synthesis of androgens (or) aromatization of androgens to oestrogens (Henderson et al., 1982). Thus, FSH stimulates granulosa cell aromatase activity, leading to oestradiol-17β formation (Hillier, 1981; Guemene et al., 1994). The high levels of prolactin have been reported to reduce to the FSH induced aromatase activity (Fortune et al., 1986; Robinson et al., 1990) The infusion of prolactin locally into the ovarian arterial circulation reduced the steroid secretion (McNeilly et al., 1982). In the current study administration of anti prolactin agent as observed in this study has increased the estrogen levels in treated group. Since androstenedione is thecal in origin and its release is stimulated by LH, it seems probable that the high level of prolactin may inhibit ovarian follicular steroidogenesis not only by interfering with aromatize but also by reducing the production by the theca cells of the androgen precursor necessary for estrogen production (McNeilly et al., 1982). Among the treated birds in the present study it could be presumed that the increase in estrogen could have been due to decreased prolactin concentration. The oestrogen elevation, logically, is thus the effect of the treatment of bromoergocryptine administration and prolactin inhibition. Hence the elevated levels of prolactin found in control group lead to reduced performance of egg production with increase in inter sequence pause days during the period of lay. Therefore the reproductive activity and poor efficiency of the control group associated with of presence of long inter sequence pause days may be due to impairment of estrogen synthesis by high prolactin levels and keeping prolactin under check can overcome the problem of longer inter sequence pause days and broodiness in birds for higher egg production.

After administration of bromoergocryptine, the progesterone levels were significantly higher than the control group (figure 4). In earlier studies in farm animals (Niswender, 1974) and humans, it was suggested that the low concentration of prolactin is required for the production of progesterone by human (MacNatty et al., 1974) turkeys (Porter et al., 1991) and mouse granulose cells *in vitro*

(MacNatty et al., 1976); high concentrations of prolactin are inhibitory (MacNatty et al., 1974; Dajee et al., 1998) to progesterone synthesis. High prolactin levels are reported to inhibit progesterone synthesis in porcine granulose cells in vitro (Veldhius et al., 1981) and affect the metabolism of progesterone (Wang et al., 1979). In context to the above Marrone et al. (1985) observed that steroid metabolites produced by theca cells from the adult domestic hen will be mainly metabolized to 5β or 5α but not to 20β or 20α metabolites. However, a critical study both in vivo and in vitro needs to be done on ovarian tissues to confirm such a situation among avian species. The data of present study suggests that blocking of prolactin is bringing about a change in circulatory blood. But this does not eliminate the possibility of the involvement of prolactin in enzyme activation at ovarian tissue cells.

Bromoergocryptine may bring about an increase in egg production acting on the hypothalamo hypophysial gonadal axis by inhibiting the release of prolactin. There is a fundamental difference in prolactin secretion between the mammals and the birds. In mammals bromoergocryptine is a stimulant of dopaminergic receptors, inhibitor of Prolactin secretion at hypothalamus region whereas in birds it acts at the anterior pituitary gland level (Youngren et al., 1998) rather than at the hypothalamic region. On the other hand in birds prolactin secretion is also directly under the control of vasoactive intestinal peptide (VIP). Therefore, bromocriptine as a dopamine agonist might have acted at the anterior pituitary by inhibiting the stimulatory effects of VIP on prolactin release, with concomitant decrease in prolactin secretion (Youngren et al., 1998). There is no indication whether circulating prolactin levels affects the sequence length or inter sequence pause days in domestic hen directly or indirectly by the gonadotropic hormones and their ovarian receptors. The rat models showed that high levels of prolactin act directly on granulosa cells to suppress progesterone and oestradiol secretion by inhibiting steroidogenic enzymes (Dorrington and Gore-Langton, 1981 and Gitany Goren et al., 1989). The inhibitory effect of prolactin on steroidogenesis might be attributable to reduced number of ovarian LH receptors (Gitany-Goren et al., 1989). It is therefore, likely that, low levels of prolactin could modulate LH receptors for ovulation (Chen et al., 1988). Thus antisteroidogenic effect of prolactin and modulation of the ovarian LH receptors leads to alterations in ovulation. The extent to which depressed steroidogenic activities due to alterations in the circulatory prolactin levels are not readily discernible.

Results of the present study showed that progressive increase in egg production occurred concurrently with a reduction in circulating prolactin levels (figure 2), increase in oestradiol-17β (figure 3) and progesterone (figure 4) After the administration of bromoergocryptine, it is possible that decreased prolactin levels are attributed to increase in oestradiol and progesterone levels which may act as a positive feedback on secretion of LH and subsequent ovulation (El Halawani et al., 1993).

The mechanisms responsible for ovulation and for its failure, which lead to skipped days, have been much studied but little clarified. Many studies have shown that LH of mammalian origin will induce ovulation in hens. The present experiment showed that the cessation of egg lay between the ovulatory sequences of chicken is usually caused by increased prolactin secretion from the anterior pituitary gland. It is further stated that prolactin is antigonadotrophic, presumably either blocking the secretion of pituitary gonadotrophin or blocking their action on the gonad. Prolactin neutralization has been shown to elicit longer ovulatory sequences, lesser intersequence pause days and an increase in egg production.

We conclude that, it is proved that the higher level of prolactin decreases the egg production. Immunization of birds against prolactin using molecular biological approaches has a tremendous impact on egg production. Under our experimental conditions, bromoergocryptine was effective in suppressing the prolactin levels for eliciting longer sequences during the period of study. On the other hand, in control birds, higher levels of prolactin led to decreased egg production than the treated birds not over full extent of the experiment. This indicates that prolactin is having a negative effect on sequence length and egg production. Although, further studies regarding optimization of the anti prolactin agent's dosage, schedule that is required for eliciting higher egg production and possible secondary consequences of the presence of the anti prolactin agent in the egg yolk upon embryonic and poult development are needed, the use of such an approach commercially to elicit higher egg production may be effective in the near future if not by using this drug, but it is possible to extend the sequence length by recombinant DNA technology by immunizing the birds against prolactin. Furthermore, such an approach and procedure could be extended to other species of economic importance such as ducks, geese and quail.

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