Available online at www.sciencedirect.com

ScienceDirect

General and Comparative Endocrinology 151 (2007) 220–229

GENERAL AND COMPARATIVE ENDOCRINOLOGY

www.elsevier.com/locate/ygcen

Effects of artificial eggs on prolactin secretion, steroid levels, brood patch development, incubation onset and clutch size in the yellow-eyed penguin (*Megadyptes antipodes*)

Melanie Massaro *, Alvin N. Setiawan, Lloyd S. Davis

Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

Received 24 May 2006; revised 22 January 2007; accepted 25 January 2007
Available online 9 February 2007

Abstract

Several studies have shown that the transition from egg laying to incubation behavior in birds is associated with changes in plasma levels of prolactin and steroid hormones. However, any effect of the tactile and visual input provided by eggs at initiating these hormonal changes has not been fully investigated in wild birds. A few days before yellow-eyed penguins, *Megadyptes antipodes*, started egg laying, we placed an artificial egg into their nests or under cages next to their nest. We then investigated the effect of the tactile and/or visual stimulus of such an artificial egg on prolactin secretion, steroid hormone levels (total androgen, estradiol and progesterone), brood patch development, incubation onset and clutch size in these penguins. Prolactin levels rose in females in response to having an artificial egg in the nest, while they declined considerably in males. Total androgen concentrations in males were less than 7% of those of control males and the levels prior to egg placement. Brood patch width increased in both males and females. Additionally, an egg in the nest caused yellow-eyed penguin pairs to attend and sit prone on their nest more frequently. Females that initiated egg laying 1 or 2 days after placement of the artificial egg in the nest, laid a full clutch of two eggs, while most other females that were exposed to an artificial egg in their nest, laid only a single egg. In contrast, the visual stimulus of an artificial egg alone (that was placed under a cage) did not influence hormone levels, brood patch development, incubation behavior or clutch size. The stimulation of an egg in the nest influences prolactin and total androgen levels in yellow-eyed penguins, particularly in males. While brood patch development and incubation behavior were initiated and egg laying was terminated in response to an artificial egg in the nest, the exact endocrine mechanisms underlying these physiological and behavioral changes remain poorly understood. We encourage further studies on other bird species taking an experimental approach to investigate the direct influence of hormones in initiating brood patch development and incubation behavior. Moreover, such experimental studies will widen our understanding of the endocrine mechanisms that regulate clutch size.

Keywords: Brood patch development; Clutch size; Egg laying; Hormones; Incubation onset; *Megadyptes antipodes*; Parental behavior; Yellow-eyed penguin

1. Introduction

There is considerable evidence that incubation behavior in birds is affected by plasma levels of prolactin (e.g., Goldsmith, 1991; Buntin, 1996; Lormée et al., 1999). Prolactin secretion is known to increase steadily in incubating birds during egg laying and incubation, when it usually reaches its highest levels, before declining immediately after hatching in precocial species or at the end of the brooding period in altricial species (Goldsmith, 1991; Buntin, 1996; Vleck, 2002). In contrast, plasma levels of gonadal steroids are generally at their highest during the courtship and egg laying period and decline as incubation is commenced (e.g., Groscolas et al., 1986; Hector et al., 1986; Ball, 1991). Of particular interest is the endocrine control of the transition from courtship to incubation behavior as the exact timing of incubation onset in relation to egg laying may influence

* Corresponding author. Present address: School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand. Fax: +64 3 364 2590.

E-mail address: melanie.massaro@canterbury.ac.nz (M. Massaro).

0016-6480/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2007.01.034

clutch size (Meijer, 1995) and the degree of hatching asynchrony in altricial birds (Magrath, 1990; Ricklefs, 1993; Stoleson and Beissinger, 1995). Embryonic development in birds does not usually start until a parent incubates (Dent, 1975), so that if parents delay incubation onset until the clutch is complete, their eggs usually hatch synchronously. However, if parents start incubation before egg laying has been completed, their eggs typically hatch over a period of days (Ricklefs, 1993). Such hatching asynchrony can create competitive hierarchies among offspring which often lead to starvation or siblicide of the smallest sibling (Ricklefs, 1993; Viñuela, 1997). In addition, the onset of incubation can also be associated with the termination of egg laying as both mechanisms might be controlled by the same hormonal changes (Eisner, 1960; Meijer et al., 1990; Meijer, 1995).

The direct effects of hormones on incubation (sitting) behavior have been studied extensively in chickens, *Gallus gallus*, turkeys, *Meleagris gallopavo*, and ring doves, *Streptopelia risoria*, but they are virtually unknown in most other species of birds (but see Sockman et al., 2000). In galliform birds, where circulating prolactin levels increase abruptly around the time of egg laying, the synergistic interactions of prolactin and gonadal steroids induce sitting behavior (El Halawani et al., 1986; Lea, 1987; Buntin, 1996). In American kestrels, *Falco sparverius*, experimental administration of ovine prolactin enhanced incubation behavior (Sockman et al., 2000). Conversely, in ring doves, the synergistic interactions of estradiol and progesterone are sufficient to induce incubation (Cheng and Silver, 1975). Correlational studies in wandering albatross, *Diomedea exulans*, and macaroni penguins, *Eudyptes chrysolophus*, suggested similarly that estradiol and progesterone play a more important role than prolactin in the induction of incubation (Hector and Goldsmith, 1985; Williams and Sharp, 1993). These species are characterized by a delay in the increase in plasma prolactin until incubation is already well established. Overall, there appears to be considerable interspecies variation in which hormones facilitate incubation onset. Moreover, evidence remains inconclusive about what mechanisms ultimately underlie these changes in hormone levels that lead to incubation onset and may terminate egg laying. For example, in European starlings, *Sturnus vulgaris*, and great tits, *Parus major*, prolactin secretion is induced by changes in the photoperiod (Dawson and Goldsmith, 1985; Silverin and Goldsmith, 1997), while in several seabirds prolactin secretion appears to be triggered by an endogenous mechanism (Hector and Goldsmith, 1985; Garcia et al., 1996; Lormée et al., 1999). Alternatively, tactile stimulation of the brood patch by eggs has been shown to induce prolactin secretion in mallards, *Anas platyrhynchos* (Hall and Goldsmith, 1983; Hall, 1987), but not necessarily in other birds (El Halawani et al., 1980; Bedrak et al., 1981). However, since egg or nest removals cause plasma prolactin levels to drop not only in mallards, but also in ruffed grouse, *Bonasa umbellus*, ring doves and canaries, *Serinus canarius*, tactile contact is certainly important in maintaining high prolactin levels once incubation is advanced (Etches et al., 1979; Goldsmith, 1983; Goldsmith et al., 1984; Ramsey et al., 1985). Brooding eggs and prolactin secretion seem to reinforce each other during the incubation period, whereby prolactin sustains sitting behavior, and sitting activity in turn promotes further prolactin secretion (Buntin, 1996). Within this mechanism, the brood patch plays a central role in mediating this positive feed-back between hormonal secretion and incubation behavior, and its development is in itself advanced through increased hormone release (Jones, 1971). In addition, the decline in gonadal steroid levels may also be related to the presence of eggs in nests since steroid levels usually decline considerably after egg laying (Hector et al., 1986; Williams, 1992; Cherel et al., 1994) and failed breeders have higher steroid levels than successful breeders (Fowler et al., 1994). While both brood patch development and sitting behavior can be artificially induced by hormonal treatment in several species of non-breeding birds (Jones, 1971; Buntin, 1996), the influence of the eggs in initiating this positive feed-back between hormone secretion, brood patch development and incubation onset has never been investigated simultaneously.

If the visual or tactile stimulus of eggs in nests can change prolactin and steroid hormone levels, then these changes might also cause the cessation of egg laying. It has been shown that administration of prolactin to laying females results in the cessation of laying in chickens and turkeys (Riddle et al., 1935; Opel and Proudman, 1980), but not in American kestrels (Sockman et al., 2000). Prolactin directly inhibits the growth of small follicles in the ovary (Zadworny et al., 1989), while it also limits the gonadal activity indirectly by reducing the gonadotropin secretion (Buntin et al., 1999). Alternatively, contact with eggs could potentially inhibit the production of gonadotropin releasing hormone (GnRH-I), which controls the secretion of the follicle stimulating (FSH) and luteinizing hormones (LH) (Haywood, 1993). As the growth of the ovarian follicles depends on FSH and LH, tactile contact with eggs could theoretically cause termination of laying or change egg laying patterns (Haywood, 1993).

In this study we conducted two experiments to examine the effect of visual and tactile stimulation by an egg on hormone secretion, brood patch development, egg laying and incubation onset in the yellow-eyed penguin, *Megadyptes antipodes*. Yellow-eyed penguins generally lay two similar-sized eggs in Sept or Oct, and both sexes contribute equally to incubation and chick rearing (Richdale, 1957). Although females lay their two eggs 3–5 days apart, both eggs usually hatch on the same day (Lamey, 1990). Throughout the incubation period, males and females relief each other from incubation duties every 1–2 days (Seddon, 1989; Darby and Seddon, 1990). The length of incubation varies considerably among pairs in this species from 35 to 54 days (Massaro et al., 2004) as some birds seem to delay incubation onset and have a slower brood patch development than others (Seddon, 1989). For the first experiment, we placed an artificial egg into several nests prior to egg laying and