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PATTERNS OF PROLACTIN SECRETION IN RELATION TO INCUBATION FAILURE IN A TROPICAL SEABIRD, THE RED-FOOTED BOOBY

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Abstract. Prolactin levels rapidly drop after breeding failure in several terrestrial bird species, but in penguins prolactin secretion can be maintained well after failure. We measured prolactin secretion in relation to reproductive failure in a tropical seabird, the Red-footed Booby *(Sula sula)*. Incubation failure was recorded in 7 nests (2 accidental losses, 5 desertions). Prolactin titers significantly decreased after incubation failure. In birds that accidentally lost their egg, prolactin titers declined but measurements 12 to 24 hr after failure were still above basal levels. Birds naturally deserting their egg exhibited prolactin titers typical of basal levels 6-24 hr after abandonment. Two birds showed lower prolactin concentration as early as 4-8 days before nest desertion, suggesting that a decline in prolactin levels may precede egg desertion. This study shows that in this tropical seabird, incubation failure results in a rapid cessation of prolactin secretion, as it does for terrestrial birds.

Key words: incubation failure, prolactin, Red-footed Booby, seabirds, spontaneous egg desertion, Sula sula.

Relación entre los Niveles de Prolactina y el Fracaso Reproductivo en un Ave Marina Tropical, *Sula sula*

Resumen. Los niveles de prolactina de varias especies de aves terrestres disminuyen rápidamente después del fracaso reproductivo. Sin embargo, en los pingüinos la secreción de prolactina tiende a mantenerse inalterada después del fracaso reproductivo. Medimos los niveles de prolactina en relación al fracaso reproductivo en un ave marina tropical, *Sula sula*. Se observaron 7 eventos de fracaso reproductivo (2 pérdidas accidentales de huevos y 5 deserciones de nidos). En general, los niveles de prolactina disminuyeron significativamente después del fracaso reproductivo. En las aves que perdieron accidentalmente sus huevos, los niveles de prolactina disminuyeron, pero las medidas registradas 12 a 24 horas después del fracaso fueron superiores a los niveles básales. Los niveles de prolactina de individuos que espontáneamente abandonaron sus nidos, medidos 6 a 24 horas después del abandono, fueron similares a los niveles básales. Dos individuos presentaron concentraciones de prolactina bajas 4 a 8 días antes del abandono de sus nidos, sugiriendo que una disminución del nivel de prolactina podría preceder el abandono de los nidos. Este estudio demuestra que el fracaso de incubación de esta especie de ave marina tropical conlleva a una rápida cesación de la secreción de prolactina, tal como ha sido observado en las aves terrestres.

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Several studies conducted on domestic bird species have shown that tactile and visual stimuli from nests or eggs trigger prolactin secretion, and that the removal of these stimuli results in a decrease in plasma prolactin and the termination of incubation behavior (review in Buntin 1996). For example, in female Canaries *(Serinus canaria),* prolactin titers decreased significantly 3 hr after removal of the nest and returned to basal values after 24 hr (Goldsmith et al. 1984). In bantam hens *(Gallus gallus),* plasma prolactin de-creased to 50% above the basal values 18-24 hr after nest deprivation, and were similar to basal values after 48 hr (Sharp et al. 1988). Similarly, in a domestic turkey hen *(Meleagris gallopavo),* plasma levels of circulating prolactin returned to basal levels 24 hr after nest deprivation (El Halawani et al. 1980).

However, such a pattern does not appear as a general rule among birds. In male Adélie Penguins *(Pygoscelis adeliae),* there was a modest 20% decrease in prolactin levels 2 weeks after breeding failure, whereas no drop in prolactin concentration was observed in females (Vleck et al. 2000). Similarly, prolactin titers of King Penguins *(Aptenodytes patagonicus)* were still 10 times above basal levels more than one month after failure (Jouventin and Mauget 1996). Plasma prolactin levels remained nearly unchanged 2 months after breeding failure in the Emperor Penguin *(Aptenodytes forsteri,* Lormée et al. 1999). Thus, reproductive failure does not seem to result in an abrupt cessation of prolactin secretion in penguins as opposed to other terrestrial species.

This pattern is not found in all seabird species: prolactin levels fall to basal levels 24 hr after incubation failure in the Cape Gannet *(Sula capensis,* Hall 1986). Thus, further studies investigating the relationship between prolactin secretion and breeding failure in sea-birds are needed to identify general patterns.

In this paper, we report data on prolactin secretion in relation to incubation failure in the Red-footed Booby *(Sula sula)* a long-lived tropical seabird. In this species, high levels of prolactin are found during incubation and brooding periods (Lormée et al. 2000). Afterward, prolactin titers return to basal values during the rearing of the thermally independent chick (Lormée et al. 2000). Prolactin data reported here were

obtained from birds that accidentally lost their egg and from birds that naturally deserted their egg. To our knowledge this is the first report on prolactin changes in relation to egg desertion.

METHODS

The study was carried out in September 1998 at Europa Island, Mozambique Channel, Western Indian Ocean (22°20'S, 40°22'E), in large colonies of Red-footed Boobies (3000 pairs; Le Corre and Jouventin 1997). At Europa, Red-footed Boobies breed from July to December in trees, and the parental phase (incubation and chick rearing) lasts for 145 days (Lormée et al. 2000). Males and females incubate the single egg during 45 days and take roughly equal share of incubation (average duration of incubation shifts: ca. 30 hr). Foraging birds return to the nest every day and roost close to the nest when not incubating. Males and females incubate during night and day (Lormée and Chastel, unpubl. data).

During a preliminary survey of prolactin changes in relation to breeding stages (Lormée et al. 2000), we collected blood samples during the incubation period. Incubating birds were captured with a landing net, marked with a metal band and weighed to the nearest 5 g using a Pesola balance. Blood samples (300 µL to 1 mL) were collected from the tibial vein into heparinized tubes, placed into ice and centrifuged as soon as possible. Plasma (for prolactin assay) and red cells (for sexing) were then stored at -20°C. Boobies were sexed using voice dimorphism and a molecular method (Lormée et al. 2000). Nests were checked daily to determine hatching success. To reduce possible disturbance during daily checks, one of the adults was dyed with picric acid on crown and belly, to allow individual identification from a distance.

Incubation failure was recorded in 7 nests: in nests A and B the egg accidentally fell from the nest, and in nests C, D, E, F, and G, birds spontaneously deserted their egg. When incubation failure was observed, birds were recaptured and weighed as soon as possible while roosting near the nest, and a second blood sample was taken. Birds that hatched their egg were sampled only once (Lormée et al. 2000). Blood samples were taken from 4 to 28 September, between 07:30 and 17:30. We measured plasma prolactin using radioimmunoassays at the Centre d'Étude Biologiques de Chizé. Our prolactin assay has been previously validated for Red-footed Boobies (Lormée et al. 2000). All samples were assayed in the same assay, and the intra-assay coefficient of variation was 7.8%.

STATISTICAL ANALYSES

Prolactin levels and body masses of birds whose nests failed were compared to birds with successful clutches using Student's *t*-tests. Wilcoxon signed-ranks tests were used to compare prolactin levels and body masses before and after incubation failure. We evaluated the relationship between date or time of day and prolactin levels using Spearman rank-order correlations. All values are expressed as means ± SE. All computations were performed using SYSTAT (Wilkinson 1997).

TABLE 1. Body mass and plasma levels of prolactin in male and female Red-footed Boobies before incubation failure compared to those of birds incubating an egg that later hatched. Values are means ± SE, sample size in parentheses.

	Prolactin (ng mL^{-1})	Body mass (g)
Males		
Failed	15.4 ± 2.2 (4)	869.2 ± 29.5 (4)
Successful	17.1 ± 0.5 (11)	878.0 ± 16.0 (11)
Females		
Failed	20.2 ± 1.6 (5)	987.6 ± 20.2 (5)
Successful	23.5 ± 1.8 (13)	993.0 ± 17.0 (13)

RESULTS

Prolactin levels and body masses from 9 birds (4 males and 5 females) were obtained from 16 to 4 days before incubation failure and from 6 hr to 6 days after incubation failure. Each bird was bled and weighed only once before, and only once after, incubation failure. There was no effect of date or hour on prolactin levels measured before or after incubation failure (Spearman rank-order correlations, all $r_s <$ 0.30, all $P > 0.4$, $n = 9$ for all tests). Plasma levels of prolactin and body masses measured before incubation failure were similar to those found in birds incubating an egg (Table 1, all $t < 1.1$, all $P > 0.29$; df = 13 for males and 16 for females).

Prolactin titers decreased after incubation failure (males: Z = 1.85, $P = 0.06$; females, Z = 2.02, $P =$ 0.04, Fig. 1). The decrease in prolactin levels was particularly marked in nests C, D, E, F, and G, where birds naturally deserted their egg and exhibited very low prolactin titers typical of basal levels, even 6-12 hr after the egg was recorded alone in the nest for the first time. Compared to other birds, the female from nest E and the male from nest D showed lower prolactin concentrations 8 and 4 days before nest desertion, respectively. Body mass did not significantly change after incubation failure (males: Z = 1.46, $P =$ 0.15; females, Z = 0.94, $P = 0.35$, Fig. 2).

DISCUSSION

In the Red-footed Booby, prolactin levels declined after incubation failure. Such a pattern has also been observed in some terrestrial, domestic species (Buntin 1996) and in another seabird, the Cape Gannet, where basal prolactin titers were found 24 hr after experimental egg removal (Hall 1986).

During this study, nest desertion was observed at five nests allowing us to observe changes in prolactin secretion. Plasma levels of prolactin from birds sampled 6, 12, and 24 hr after egg desertion were very low (<5 ng mL^{-1}) and close to levels found in prelaying birds (3-6 ng mL^{-1}, Lormée et al. 2000). The de-crease in prolactin levels seemed to be more rapid in birds that deserted compared to birds that accidentally lost their egg. This suggests that different processes (egg loss and desertion) may cause variation in prolactin levels.

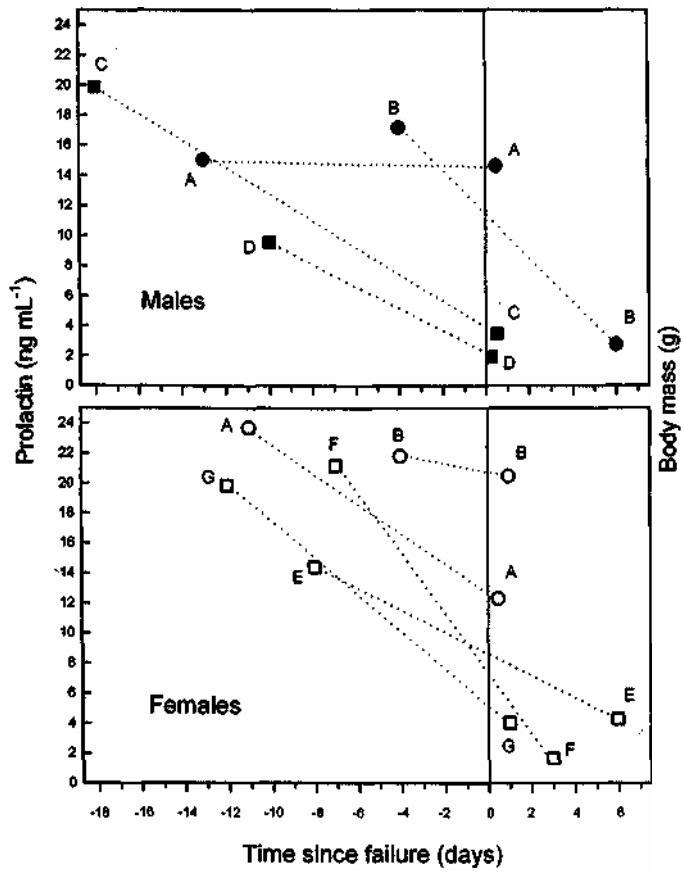

FIGURE 1. Changes in plasma levels of prolactin in relation to incubation failure in male and female Red-footed Boobies. In nests A and B the egg accidentally fell from the nest (circles) and in nests C, D, E, F, and G, birds spontaneously deserted (squares).

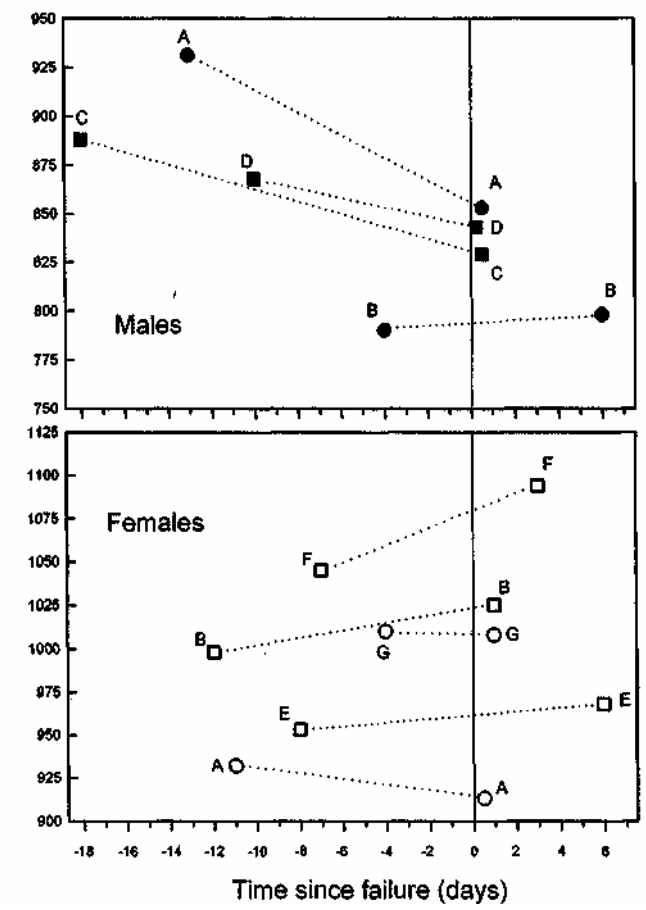

FIGURE 2. Changes in body mass in relation to incubation failure in male and female Red-footed Boobies. In nests A and B the egg accidentally fell from the nest (circles) and in nests C, D, E, F, and G, birds spontaneously deserted (squares).

Interestingly, the male from nest D and the female from nest E had lower prolactin levels as early as 4 and 8 days before egg desertion, suggesting that in the Red-footed Booby, a decline in prolactin levels precedes egg desertion. This decline in prolactin levels was associated with a progressive disruption of incubation, this process taking 4-7 days (male from nest D and female from nest E, respectively).

Egg desertion was not associated with low body mass in our study. On the other hand, low levels of prolactin have been measured in King Penguins reaching a critical threshold in body reserves (phase III; Cherel et al. 1994). A decrease in plasma prolactin in relation to a progressive increase in protein loss could thus be a main component in the control of spontaneous desertion after long incubation periods in fasting penguins (Groscolas 1990). Contrary to King or Emperor Penguins which fast ashore for prolonged periods (>1 month), Red-footed Boobies fast for 20-39 hr *(n* = 34) per incubation shift and show little or no mass change during breeding (Lormée et al., in press). As incubating Red-footed Boobies do not rely on large fat reserves for successful incubation, a decrease in prolactin levels associated with a depletion of body reserves is unlikely to occur.

The ultimate cause of spontaneous egg desertion in these Red-footed Boobies was unknown. We could not check whether eggs from deserted nests were fertile or addled because unattended eggs were rapidly eaten by Pied Crows *(Corvus albus).* Interestingly, the male from nest D was probably a first-year breeder because some of the plumage and the bill were typical of 2-3-year-old immatures. This male showed a low prolactin level 4 days before egg desertion and indeed, incubated inadequately, often leaving the nest for a few minutes, and even trying to copulate with the female while she was incubating.

In seabirds, the pattern of prolactin secretion after breeding failure seems to differ among species: in sulids (Hall 1986, this study), prolactin declines in the few days following incubation failure. On the other hand, elevated prolactin can be maintained well after breeding failure in penguins (Jouventin and Mauget 1996, Lormée et al. 1999, Vleck et al. 2000). Could this be explained by the fact that Red-footed Boobies and Cape Gannets are able to lay a replacement clutch, contrary to most penguins (Hall 1986)? During our study, females from nests A, B, F, and G laid replacement clutches within 3 weeks. High prolactin levels are known to depress gonadotropin secretion and a decrease in prolactin secretion is needed to remove this depressive effect and begin a new reproductive cycle (Sharp et al. 1998). Such an interpretation is, however, impaired by data obtained on Gentoo Penguins *(Pygoscelis papua;* Mauget et al. 1995). At the Crozet Islands,

birds lay replacement clutches and elevated prolactin levels are found in birds courting after a breeding failure (Mauget et al. 1995), a pattern also observed in a multiple-brooding passerine, the Song Sparrow *(Melospiza melodia;* Wingfield and Goldsmith 1990). In King and Emperor Penguins, the long-term maintenance of prolactin levels after breeding failure has been thought to represent the byproduct of an endogenous regulation of prolactin secretion independent of external stimuli (egg or chick; Garcia et al. 1996, Lormée et al. 1999). Such a pattern of prolactin secretion may have evolved to maintain parental care despite long absences (up to several months) at sea from the breeding colony (Garcia et al. 1996; Lormée et al. 1999). At Europa, Red-footed Boobies foraged for a maximum of 13 hr per day during incubation (mean: 11.1 ± 0.4 hr, n = 34 foraging trips). In coastal foragers like Red-footed Boobies, such short absences at sea may not be sufficient to promote a regulation of prolactin secretion independent of external stimuli, as found in offshore-foraging penguins.

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