

## Prevention of the expression of incubation behaviour using passive immunisation against prolactin in turkey hens (*Meleagris gallopavo*)

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**Summary** — The efficacy of injecting antibodies raised against turkey prolactin to prevent the expression of incubation behaviour has been investigated in turkey hens. Medium white turkey hens ( $n = 15 \times 2$ ) were injected three times weekly for 4 consecutive weeks starting on week 5 of egg production. The hens were injected im with a volume of 1 mL per injection for the 1st week and 0.5 mL thereafter, of normal rabbit serum or serum containing antibodies raised against turkey prolactin (Guémené et al, 1994a). None of the 15 passively immunised hens expressed incubation behaviour, whereas, more than half (53%) of the control hens did express it. Plasma prolactin concentrations observed in the two groups presented comparable profiles until week 9 and from week 19 of egg production onward. Differences were, therefore, observed from week 10 until week 17 with the non immunised hens showing higher plasma prolactin concentrations than the immunised ones. This difference was related to the presence of incubating hens in the control group. A higher percentage of non immunised hens disrupted egg production during the course of the study and consequently immunised hens laid more eggs than the control ones. No change in plasma LH and oestradiol concentrations can be related to the immunisation procedure. We conclude that prevention of incubation behaviour can be achieved using passive immunisation against prolactin, prevention which resulted in more egg production under our experimental protocol.

**passive immunisation / prolactin / incubation behaviour / turkey hens**

**Résumé** — Prévention de l'expression du comportement de couvain chez la dinde (*Meleagris gallopavo*) par immunisation passive dirigée contre la prolactine. L'efficacité de l'injection d'anticorps anti-prolactine pour prévenir l'expression du comportement de couvain a été testée

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chez la dinde. Des dindes de souche « Medium » ( $n = 15 \times 2$ ) ont été traitées à partir de la 5<sup>e</sup> semaine de production pendant 4 semaines, à raison de trois injections hebdomadaires. Les injections, de sérum normal de lapin pour le groupe contrôle et de sérum contenant des anticorps dirigés contre la prolactine de dinde (Guémené et al, 1994), sont réalisées par voie intramusculaire. Le volume administré est de 1 mL par injection pendant la 1<sup>re</sup> semaine et de 0,5 mL ultérieurement. Aucune des dindes passivement immunisées contre la prolactine n'a exprimé le comportement de couvaion alors que cela fut le cas de plus de la moitié des dindes contrôles. Les cinétiques pour les concentrations plasmatiques de prolactine des dindes des deux groupes expérimentaux sont comparables jusqu'en semaine 9 puis à nouveau à partir de la semaine 19 de production. Des différences sont donc observées depuis la semaine 10 jusqu'à la semaine 17 et les dindes témoins avaient alors des taux de prolactine plus élevés que les dindes traitées. Cette différence est imputable à la présence de dindes couveuses parmi les contrôles. Un pourcentage plus élevé des dindes témoins cessent de pondre au cours de l'étude et de ce fait l'intensité de ponte est plus faible pour ce groupe. Aucune des variations de concentrations de LH ou d'œstradiol ne peut être attribuée à la procédure d'immunisation. Nous concluons que l'expression du comportement de couvaion peut-être prévenue par immunisation passive avec des anticorps anti-prolactine ; prévention qui résulte en de meilleures performances de ponte dans nos conditions expérimentales.

### **immunisation passive / prolactine / couvaion / dinde**

## **INTRODUCTION**

Incubation behaviour remains the source of important economic losses to the turkey breeding industry, in terms of decreases in egg production and increases in labour cost. Indeed, expression of this behaviour generally leads to disruption of egg laying and furthermore not all the hens will resume egg production afterwards. Incubation behaviour is a physiological state that can be expressed by a high percentage, up to 70%, of the turkey hens starting after the peak of laying, which occurs around the 3rd or 4th week of the production period (Nestor et al, 1986; Guémené and Etches, 1989). Although, the use of management programmes limits the occurrence of incubation expression and, consequently, the number of eggs lost, farm managers spend more than 50% of their labour time in attempts to prevent and disrupt its expression (Guémené, 1992). Indeed, extra labour and special handling are necessary to recognize the first signs of incubation behaviour in order to submit the thus identified hens to physical curative treatments as early as possible.

On the other hand, it has been shown that disruption and prevention of incubation behaviour expression using physical means between the 5th and the 10th week of production will prevent, at least partially, its further development (Guémené and Williams, 1992). Nevertheless, prevention of incubation behaviour expression using adequate methods such as pharmacological treatments would lead to economic gains to the turkey industry.

Incubation behaviour is characterized by morphological, behavioural and physiological changes (Harvey and Bedrak, 1984). Thus a decrease in the circulating LH and steroid levels in plasma is concomitant with a rise in prolactin concentration while the hens express this behaviour (Cogger et al, 1979; Burke and Dennison, 1980; El Halawani et al, 1980; Lea et al, 1981; Zadworny et al, 1985). Much earlier studies also reported that higher prolactin concentrations were present in the pituitary gland at this specific physiological stage (Riddle et al, 1935; Saeki and Tanabe, 1955). Recent studies also demonstrated that prolactin pituitary contents or prolactin gene expression in

the pituitary were higher during incubation (Shimada et al, 1991; Talbot et al, 1991; Wong et al, 1991; Kansaku et al, 1994; Karatzas et al, 1997). Consequently, a causal involvement of prolactin in incubation behaviour expression in aves has long been hypothesized, although evidence of such a relationship has only been reported recently. Indeed, injections (Sharp et al, 1988; El Halawani et al, 1986) or intracranial perfusion (Youngren et al, 1991) of ovine prolactin induced incubation behaviour in bantam and turkey hens. In addition, placing incubating hens in individual cages without a nest induces a fall in plasma prolactin and disrupts well-established incubation behaviour (Burke et al, 1980; El Halawani et al, 1980; Proudman and Opel, 1981; Zadworny et al, 1985) whereas its disruption using pharmacological treatments (El Halawani et al, 1983; Guémené and Etches, 1989) or presence of poults or chicks (Opel and Proudman, 1988; Leboucher et al, 1993) is associated with a decrease in prolactin. More recently, it has been reported that active immunisations against prolactin (Guémené et al, 1994b, 1995; March et al, 1994; Crisóstomo-Pinto et al, 1995) or against its releasing factor, vasoactive intestinal peptide (VIP) (El Halawani et al, 1995; Sharp, 1996) were effective in preventing or delaying its occurrence. It has also been shown, both in mammals (Neri et al, 1964; Ferin et al, 1968; Goldman et al, 1972) and birds (Lea et al, 1981; Sharp et al, 1989; Lea et al, 1991) that when injected, heterologous antibodies possess antihormonal biological activity. Thus, a single injection of prolactin antiserum into incubating bantam hens results in an increase in LH (Lea et al, 1981). Otherwise, passive immunisation by injecting antibodies raised against VIP suppresses plasma prolactin and crop sac development in incubating ring doves (Lea et al, 1991) and induces disruption of incubation behaviour expression in bantam hens, an effect which can be prevented by prolactin injection (Sharp et al,

1989). These results also suggest that high levels of biologically active prolactin are a necessary aspect of incubation behaviour.

The present experiment was designed to investigate further the underlying mechanism that leads to incubation expression and to possibly find a complementary practical preventive procedure. Thus, we tested the hypothesis that the injection of serum containing antibodies raised against turkey prolactin will be effective in preventing the expression of incubation behaviour in turkey hens. In order to assess the effects, the rates of expression of incubation behaviour and egg laying as well as the changes in prolactin, LH and oestradiol concentrations were measured in passively immunised turkey hens.

## MATERIALS AND METHODS

### Animals

Medium white turkey hens ( $n = 30$ ) were transferred at 20 weeks of age and randomly allocated into two floor pens ( $1.33 \text{ hens/m}^2$ ). These pens were located in the same room, had wood shaving litter and were equipped with five nest boxes ( $0.57 \text{ m} \times 0.51 \text{ m} \times 0.51 \text{ m}$ ) per pen. Four mock eggs were placed in each nest. Hens were exposed to a lighting regiment of 6L-18D during the 8 weeks following their arrival and 14L-10D thereafter. The minimum light intensity at the level of the hens was 50 lux. Feed of commercial diet type and water were available ad libitum.

### Experimental approach

Passive immunisation was initiated on the 5th week of the production period while the hens were aged 35 weeks. The hens were injected intramuscularly with either rabbit immune serum containing antibodies raised against a recombinant turkey prolactin (Karatzas et al, 1993) (immune rabbit serum group, immunised,  $n = 15$ ) (Guémené et al, 1994a), or normal rabbit serum (normal rabbit serum group, control,  $n = 15$ ). A volume of 1 mL/hen/injection during the first week of treatment (5th week of the produc-

tion period) and 0.5 mL during the following 3 weeks (6th, 7th and 8th) were administered three times weekly (Monday, Wednesday and Friday).

Egg production and incubation behaviour were recorded daily during a 25 week production period, which started with the laying of the first egg by the flock. Identification of laying or out-of-lay hens was performed five times throughout the study, by oviductal palpation for 5 consecutive days on the 4th, 11th, 17th, 21th and 25th week of the production period. The presence or absence of an egg in the oviduct confirmed if the hen was actively laying or out-of-lay, respectively. Nest-boxes and pens were checked four times daily (0900, 1100, 1400, and 1600 hours). Hens were considered to be incubating eggs when recorded in the nest boxes or lying in the same place a minimum of three out of four checks on 3 consecutive days. Live body weights were recorded at the time of arrival (-9 [22 weeks of age]), 1 (-2) week before photostimulation and on weeks 9, 12, 17, 21 and 25 of the production period.

Blood samples (5 mL) were obtained by puncture from the wing vein into heparinized syringes. Samples were first collected 1 week before photostimulation, in week 1, weekly from week 5 until week 13, and in weeks 17, 21 and 25 of the production period. Moreover, three samples were collected in week 5 (Monday, Wednesday and Friday), and two in weeks 6 and 7 (Monday and Friday). Plasma was separated by centrifugation of the blood samples for 10 min at 2 000 *g* at 4 °C and stored at -20 °C until required for hormonal level measurements.

### Radioimmunoassay

Prolactin and LH concentrations (ng/mL plasma) were determined in triplicate by radioimmunoassay using the methods of Guémené et al (1994a) and Sharp et al (1987), respectively. In order to alleviate interassay variation, all samples were assayed in a single assay for both prolactin and LH. The within-assay coefficient of variation was 7.3% and 8.0% for prolactin and LH, respectively.

Oestradiol levels (pg/mL) were measured in duplicate by using the method of Etches et al (1984). The intraassay and interassay coefficient of variation for the six assays needed for this study were 3.6 and 12.6%, respectively.

### Statistical analysis

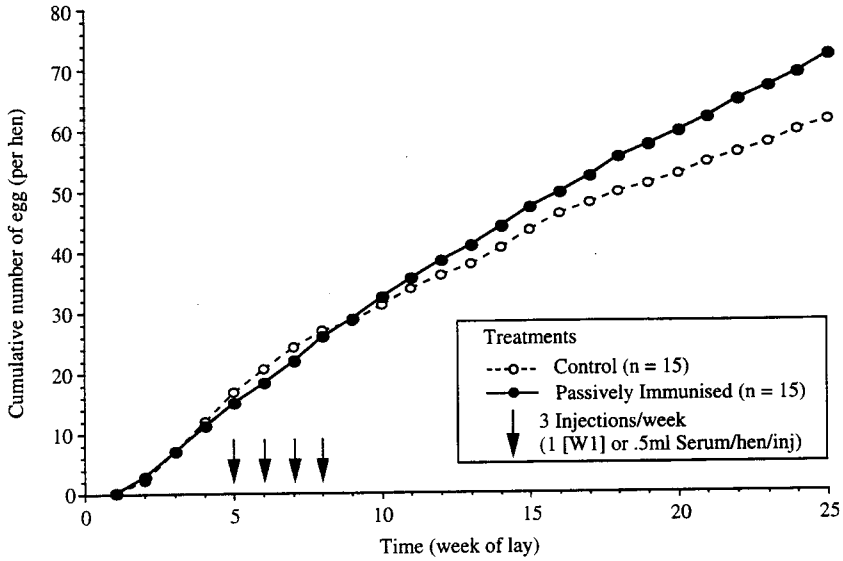
Hormonal and body weight data were compared using an analysis of variance (ANOVA) and Fisher protected least significant difference (PLSD) post hoc test if appropriate ( $P < 0.05$ , ANOVA). The comparisons of the rates of expression of incubation behaviour and of disruption of egg laying were performed using a Chi-square test analysis. The statistical analyses were done using the Statview™ II program (Abacus Concept Inc, Berkeley, CV, USA) for the Apple Mac-Intosh computer. The standard error of the mean was used to express data variability.

## RESULTS

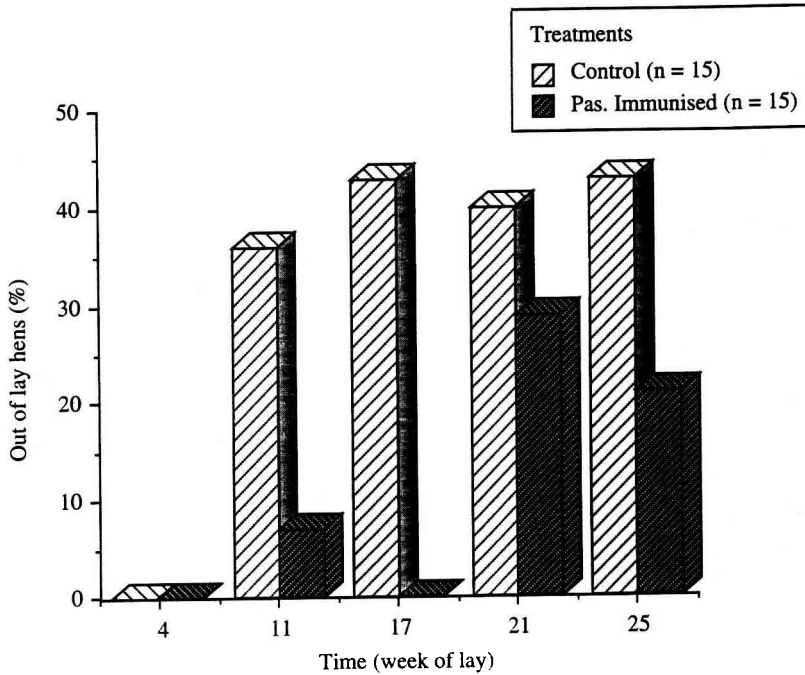
### Egg production

Egg production started 18 and 21 days after photostimulation in immunised and control groups, respectively. The highest rate of egg laying occurred between weeks 3 and 5 of the production period for both experimental groups, but although slightly lower peak production was observed for the immunised hens (data not shown), the overall average number of egg laid per hen during the 25-week experimental period was 61 and 72 eggs/hens for the control and immunised hens, respectively (fig 1).

All hens from both groups were actively laying on the 4th week of the production period. Afterwards, significantly higher ( $P < 0.05$ ) percentages of out-of-lay hens were observed in the control hens on the 11th, 17th and 25th week of the production period (fig 2). The results of the vaginal palpation indicated that 5-6 (36-43%) out of 14 of the control hens were out-of-lay from the 11th week of the production period, onward. Conversely, the number of hens recorded as out-of-lay from the immunised group of hens was 1 (7%), 0 (0%), 4 (29%) and 2 (14%), respectively during the last four palpation periods. Two out of eight and three out of five hens from the control and immunised groups respectively, identified as out-



**Fig 1.** Cumulative egg production in turkey hens injected with normal rabbit serum or serum containing antibodies raised against turkey prolactin.



**Fig 2.** Percentage of out-of-lay hens in turkeys injected with normal rabbit serum or serum containing antibodies raised against turkey prolactin.

of-lay during the course of the study did resume egg laying later on.

### Incubation behaviour

None of the 15 hens injected with prolactin antibodies (immunised hens) showed any sign of incubation behaviour, whereas 8 out of 15 hens (53%) from the control group did. Onset of incubation behaviour expression for this group took place between the 5th and 18th week of the production period and maximal incidence was during the 8th week of egg production (fig 3).

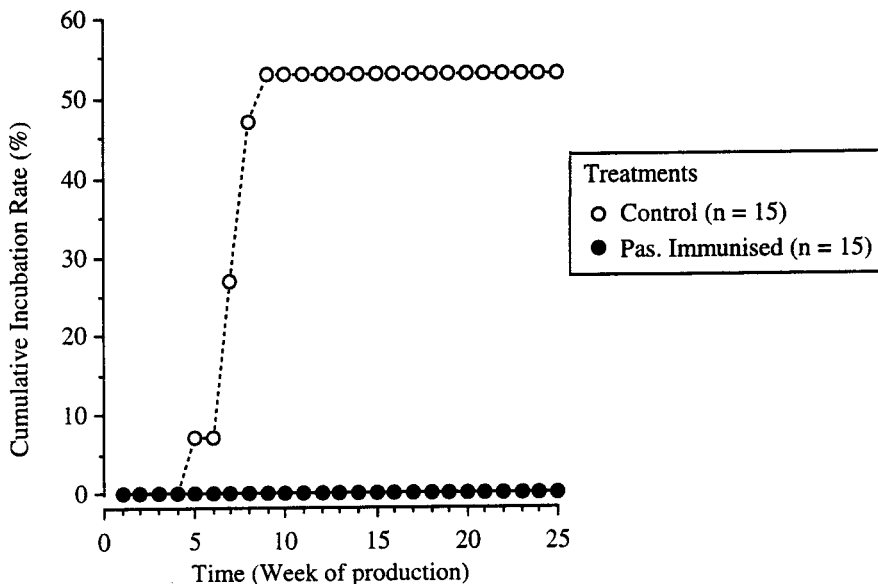
### Body weights

At the time of transfer, mean body weights were approximately 8 kg and comparable for both groups of hens (data not shown). Significant ( $P < 0.05$ ) increases occurred during the following 7 weeks and live body weights

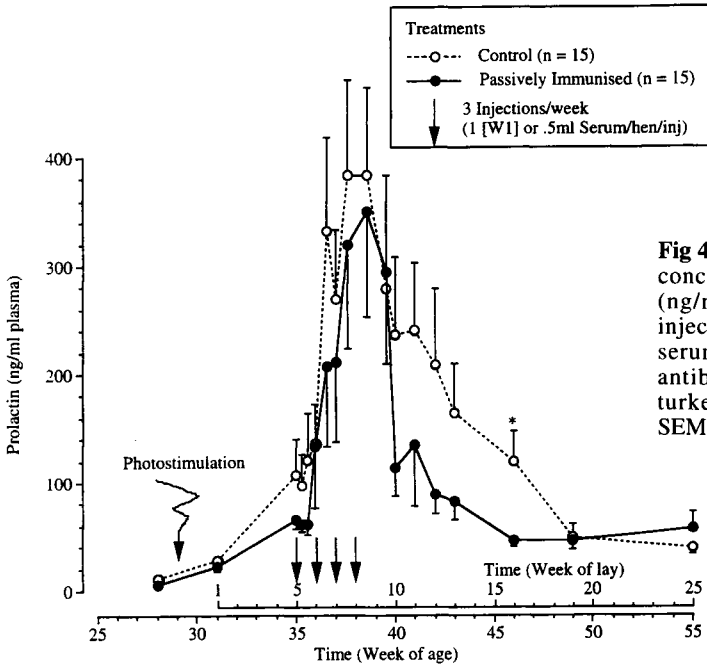
were  $9.5 \pm 0.3$  kg for both groups. After photostimulation, mean body weights for both groups did not differ significantly ( $P > 0.05$ ).

### Prolactin

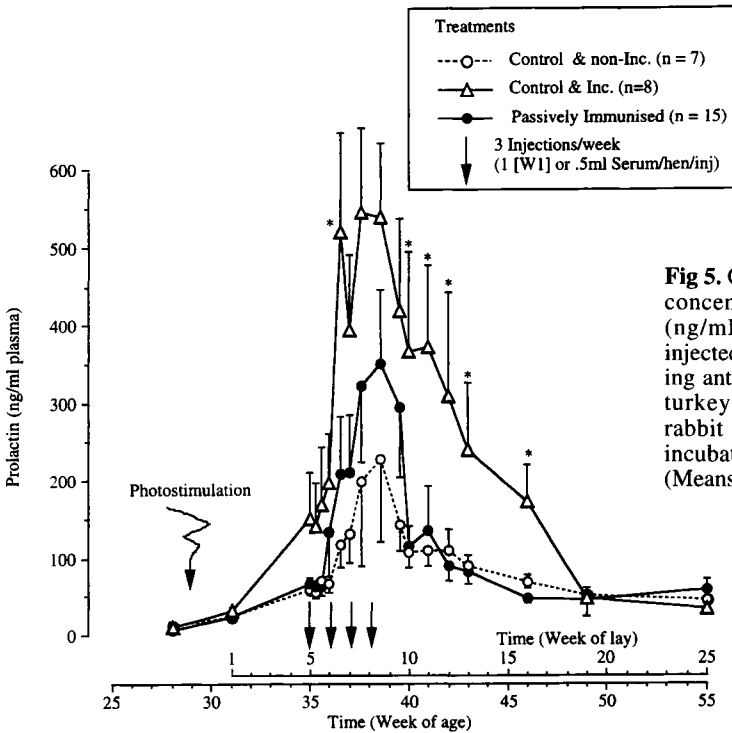
Plasma concentrations of prolactin were low in sexually immature hens, reached maximum levels around the 8th week of the production period, then gradually declined with time. The overall changes of level of prolactin in the plasma occurred concomitantly for both groups, however the amplitude of the variations differed (fig 4). Higher levels of prolactin were measured from week 10 until week 17 of the production period for the control group and the difference reached significance level ( $P < 0.05$ ) on the 17th week. During this specific period, the hens that did express incubation behaviour had significantly higher prolactin concentrations ( $P < 0.05$ ) than those that did not (fig 5). Although no significant difference was



**Fig 3.** Cumulative rate of expression of incubation behaviour (%) in turkey hens injected with normal rabbit serum or serum containing antibodies raised against turkey prolactin.



**Fig 4.** Changes in the plasma concentrations of prolactin (ng/mL) in turkey hens injected with normal rabbit serum or serum containing antibodies raised against turkey prolactin. (Means  $\pm$  SEM, \*:  $P < 0.05$ ).



**Fig 5.** Changes in the plasma concentrations of prolactin (ng/mL) in turkey hens injected with serum containing antibodies raised against turkey prolactin or normal rabbit serum and expressed incubation behaviour or not (Means  $\pm$  SEM, \*:  $P < 0.05$ ).

found with the prolactin concentrations measured for the non-incubating control hens, prolactin levels of the treated hens appeared to be somehow intermediate (fig 5).

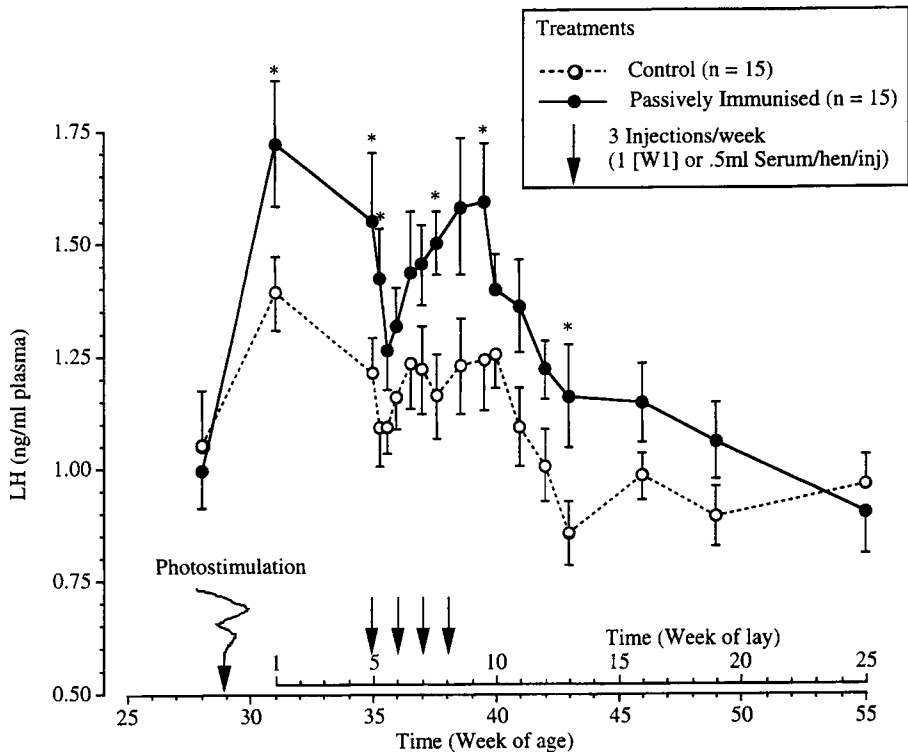
Subsequently, LH tended to be maintained at higher levels in the immunised hens than in the control hens.

## LH

The changes in plasma concentration of LH are shown in figure 6. Before photostimulation, LH levels were comparable for both groups ( $1.06 \pm 0.12$  [control] versus  $0.99 \pm 0.08$  ng/mL [immunised]). Following photostimulation, the increases occurring in plasma LH were of lower amplitude for the control hens (control) ( $1.39 \pm 0.08$  versus  $1.72 \pm 0.14$  ng/mL [immunised];  $P < 0.05$ ).

## Oestradiol

Changes in plasma oestradiol levels occurring throughout the experimental period are illustrated in figure 7. Oestradiol concentrations rose to a peak after photostimulation and decreased thereafter. Afterwards, although no significant difference was found ( $P > 0.05$ ), more fluctuations in oestradiol levels between subsequent sampling were observed for the control hens.



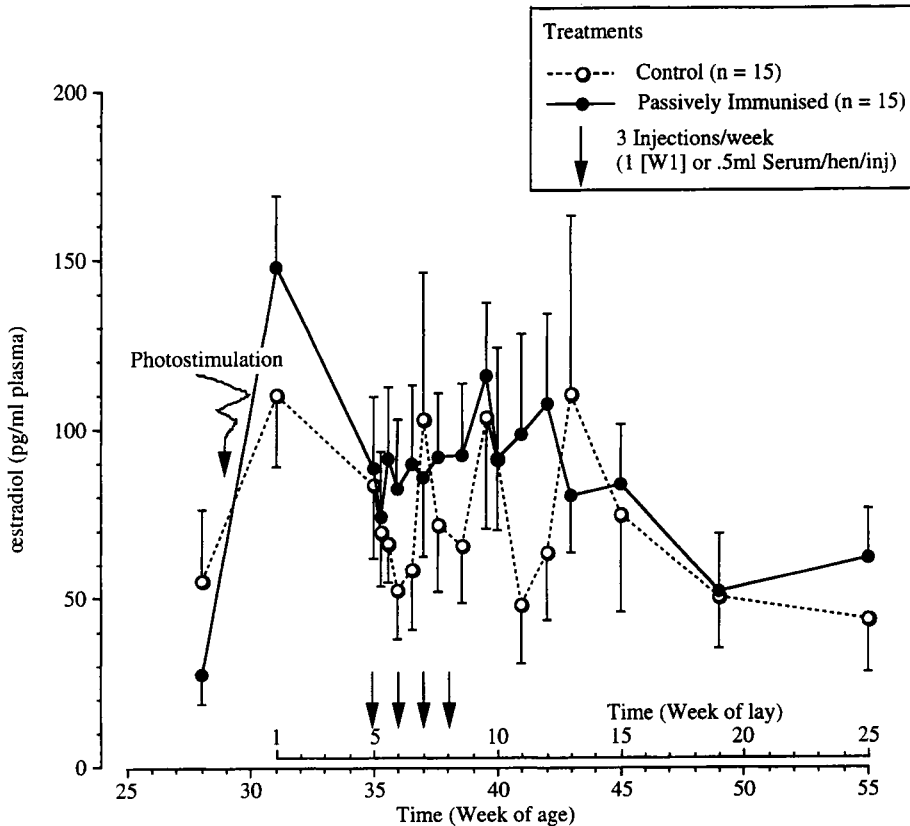
**Fig 6.** Changes in the plasma concentrations of LH (ng/mL) in turkey hens injected with serum containing antibodies raised against turkey prolactin or normal rabbit serum. (Means  $\pm$  SEM, \*;  $P < 0.05$ ).



## DISCUSSION

The results of the present study show that the prevention of the expression of incubation behaviour can be effectively achieved by means of passive immunisation using rabbit serum containing antiturkey prolactin antibodies. Indeed, whereas 8 out of 15 from the control group did express this behaviour, none of the treated hens did so despite the presence of environmental stimuli such as the availability of nest sites or presence of mock eggs in the nest boxes, factors which are well known to exert a stimulatory influ-

ence upon rate of expression of incubation behaviour (Guémené, 1992). Elevated blood levels of prolactin have been clearly demonstrated as a key component of incubation in galliforms (Burke and Dennison, 1980; Burke et al, 1981; El Halawani et al, 1980; Proudman and Opel, 1981) and termination of the behaviour by either natural or artificial (El Halawani et al, 1980; Richard-Yris et al, 1987; Opel and Proudman, 1988; Zadworny et al, 1988; Guémené and Etches, 1990; Leboucher et al, 1990; Kuwayuma et al, 1992) mechanisms results in a precipitous drop in concentration. In addition the



**Fig 7.** Changes in the plasma concentrations of oestradiol (pg/mL) in turkey hens injected with normal rabbit serum or serum containing antibodies raised against turkey prolactin. (Means  $\pm$  SEM, \*;  $P < 0.05$ ).

injection of ovine prolactin has been clearly shown to induce incubation behaviour (Hargis et al, 1987; El Halawani et al, 1991) although these latter results are somewhat equivocal (Opel and Proudman, 1980). In the current study, we hypothesize that an increase in concentration of bioactive prolactin was at least partially blocked via immunoneutralisation and thus a critical threshold concentration (Zadworny et al, 1986) for the expression of incubation behaviour was not achieved. This effect is similar to that observed when endogenous prolactin levels are manipulated via active immunisation protocols against either prolactin (Guémené et al, 1994b; March et al, 1994; Crisóstomo-Pinto et al, 1995) or a major prolactin releasing factor, VIP (El Halawani et al, 1995; Sharp, 1996), in galliforms. The results of the present study taken together with the results of these other authors clearly confirm the hypothesis that prolactin plays a key role in the process of expression of incubation behaviour. It is also clear from the present results that incubation behaviour expression will occur after the peak of lay at a period of time characterized by a peak in prolactin concentrations and that expression of this behaviour will trigger even higher circulatory prolactin levels. Disruption of incubation by deprivation of nest will induce a fall in prolactin concentrations (El Halawani et al, 1980; Proudman and Opel, 1981, Zadworny et al, 1985) whereas renesting will boost its concentration (El Halawani et al, 1980) as well as placement of a laying hen in the nest (Burke et al, 1981). It is therefore clear that nesting is a key factor in maintaining and even promoting a further increase in prolactin concentrations during incubation as observed in the present study. The present results also confirm the previously reported observations (Etches and Cheng, 1982; Guémené and Williams, 1994) showing that initial increases in prolactin concentration occur before the onset of incubation behaviour and independently of it.

The changes in plasma prolactin occurring after the peak of egg production were not of a similar magnitude in both groups. This difference was related to the occurrence of incubation behaviour in the control group, and hens expressing this behaviour had significantly higher levels of prolactin than those which did not. However, we could have also expected an increase in immunoreactive prolactin owing to a decrease in bioactive prolactin resulting from antibody injections. Indeed such an hyperprolactinemia was previously reported to occur in laying turkey hens following active immunisation against prolactin (Crisóstomo-Pinto et al, 1995). In the present study, although prolactin levels measured for the treated hens were somehow higher than those of the non incubating control hens, they did not differ significantly. We can therefore hypothesize that lower levels of bioactive prolactin were probably present in the immunised hens. It has also been shown that a single injection of antiprolactin antibodies will induce an increase in LH levels following a delay of 24 h in incubating bantam hens (Lea et al, 1981). Such an effect was not observed in incubating turkey hens (Crisóstomo-Pinto et al, unpublished data) or in the present experiment with laying hens since the treatment cannot account for the overall observed changes in LH levels. Although an inverse relationship between circulating levels of prolactin and LH has been shown in ovariectomised incubating turkey hens (Zadworny and Etches, 1987), it appears from different studies that LH changes are at least in part independent of plasma prolactin. First, incubation was not associated with any fall in LH circulating levels in hens from a similar genetic origin (Guémené and Williams, 1994) and LH levels were not affected by injection of anti-VIP antibodies in incubating bantam hens, whereas, a fall in prolactin concentrations was observed (Sharp et al, 1989). Likewise, intracranial perfusion of prolactin had no

effect upon LH levels (Youngren et al, 1991).

The optimal treatment protocol to prevent incubation behaviour in turkeys has not been addressed in the current study. This protocol was based on our observations of the effects of carefully managed physical treatment protocols to prevent the behaviour (Guémené and Williams, 1992); however, it is more than likely that a variety of factors, such as the physiological stages at which treatment is initiated, the number and dose level of injection, and the strain of hen used, may affect the success of the immunoneutralisation protocol. For example, in laying bantam hens active immunisation against prolactin using a treatment regime of four injections, separated by 6 weeks, did not significantly reduce the incidence of incubation behaviour, whereas it delayed its expression when application was started before sexual maturity (March et al, 1994). Similarly, active immunisation against turkey prolactin in turkey hens, when started before photostimulation, fully prevented incubation behaviour (Guémené et al, 1994; Crisóstomo-Pinto et al, 1995). Conversely, a single injection of prolactin antiserum into incubating bantam hens is ineffective in terms of incubation disruption (Lea et al, 1981). The failure of the latter treatment is likely to be related to the observation that an acute injection may have no incidence on a hormone's biological effect, whereas multiple injections will promote anti-hormonal effects (Neri et al, 1964). Although the current treatment regime completely blocked the expression of incubation behaviour, it is possible that fewer injections may have achieved the same effect. Further studies using different treatment regimes and/or strains are required to determine the optimal protocol for commercial applications.

It appears, at least in part, that the cessation of egg laying was due to the expression of incubation behaviour. The fact that the treatment lowered the percentage of out-

of-lay hens thus contributed to a greater number of eggs laid per hen in the immunised group during the experimental period. This result supports the hypothesis that high levels of prolactin are able to interfere with the mechanisms of steroidogenesis resulting in ovarian regression. Prolactin has been reported to exert antigonadal activity by inhibiting oestradiol secretion from small follicles (Zadworny et al, 1989). However, the mechanisms by which prolactin would have exerted such an effect in the present study remain obscure since we did not observe any consistent effect upon circulatory LH or oestradiol concentrations. Inappropriate timing of blood sampling may have masked some significant changes in oestradiol, since greater variability in basal levels were observed for the control groups.

In conclusion, the results reported here support the idea that prolactin plays a key role in the onset of incubation behaviour and that specific antibodies can act as anti-hormones to prevent its expression. As a consequence, such a treatment will at least partly prevent stoppages in egg laying and lead to a higher egg-laying rate. Further studies will be necessary, however, to clarify the most efficient timing and doses necessary for various commercial strains before such a treatment can be applied on a commercial basis.

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