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Sensitivity of the oviduct to oestrogens in broiler and layer chickens: differential response in the induction of ovalbumin gene expression

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Summary. There are large differences between broiler and layer chickens in reproductive performance. These differences are correlated with differences in the tissue sensitivity of the oviductal magnum to oestradiol stimulation and demonstrated by alterations in relative ovalbumin synthesis. This biochemical correlate of selection for fertility also differs between 'male-parent' and 'female-parent' lines of broilers. Ovalbumin synthesis in female-parent lines, selected for fertility as well as growth, shows a greater response to saturating doses of oestradiol than male-parent lines selected for growth alone. Sub-saturating doses of oestradiol produced an even greater difference between the strains. Because of the substantial amount of information available on the molecular biology of steroid induction of egg white proteins, it should now be possible to identify the level at which these differences in gene expression occur.

Key words: Broiler chickens – Layer chickens – Oviduct sensitivity – Ovalbumin expression – Selection

Introduction

Modern broiler chickens result from the successful application of quantitative genetic techniques (for review see Robertson 1980; Chambers et al. 1981). They also illustrate some of the problems that can arise after intense selection. Current lines of broilers have been selected for weight-for-age and are 3,5–4 fold the weight of layer strains at 6,5 weeks of age (Chambers et al. 1981; Hocking et al. 1985; Bulfield and Middleton 1987); these are usually referred to as 'male-parent' lines. They suffer, however, from unwanted correlated problems such as poor fertility and leg weakness. We have attempted to analyse the physiological bases of both muscle growth (Bulfield and Middleton 1987) and deleterious associated characteristics. The eventual aim of this analysis is to uncover some of the genes and products involved to aid conventional selection (Bulfield 1980) and the application of genetic manipulation techniques (Bulfield 1985).

It is well known that male-parent lines of commercial broilers have poor fertility; they are overweight at the onset of puberty and commercial breeders subject them, at the start of their breeding life, to several days of severe dietary restriction to reduce their weight (Watson 1975; Pearson and Shannon 1979).

For example, it has been shown that broiler breeders have defective ovarian function with multiple ovulation and atretic follicles; the number of follicles can be reduced and rate of lay improved by restricted feeding (Hocking et al. 1987). Breeding companies have also attempted to improve the reproductive performance by selecting some foundations stocks – usually referred to as 'female-parent' lines – for fertility as well as weight-for-age. Differences in fertility between female-parent and male-parent lines of broilers and layer strain chickens could be due to genetic changes in several areas of reproductive physiology, including circulating levels of sex hormones or target tissue sensitivity.

In an attempt to estimate target tissue sensitivity to sex hormones we analysed the response of oviductal ovalbumin synthesis to induction by injecting varying levels of 17 β oestradiol in newly hatched chickens (Brant and Nalbandov 1956; Palmiter 1972). There are significant strain differences in the induction of the ovalbumin gene by oestradiol both between female-parent and male-parent broiler lines and layer chick-

ens; in general, increased sensitivity to oestradiol paralleled increased fertility.

Materials and methods

Animals

One-day-older broiler and layer chickens were obtained from commercial breeders or from our own flocks at IAPGR, Edinburgh Research Station (formerly the AFRC Poultry Research Centre).

Hormone treatment

The oviduct was stimulated to differentiate by 17 β oestradiol treatment of animals at 1 week of age for a 10-day period. Treatment was withdrawn for 10 days; secondary hormone stimulation followed. With preliminary experiments, within strain variation in ovalbumin concentration was reduced during the period of secondary stimulation; this also has the advantage of separating oviduct differentiation from specific ovalbumin synthesis. Two protocols of hormone administration were used: one using hormone implants, the other using subcutaneous injections of hormone in olive oil.

The implantation protocol used a 30 mg pellet of 17 β oestradiol (Sigma, UK) compacted with 5 mg of cellulose and implanted subcutaneously in the neck. The first implant was inserted at 8 days after hatch and withdrawn 9 days later. Ten days after withdrawal of the first implant, a second implant was inserted and the animals were killed either 2, 4 or 7 days later.

The injection protocol used 17 β oestradiol finely ground in a pestle and mortar and dispersed in olive oil at 45 °C by alternatively sonicating and vortexing; this produced an emulsion fine enough to inject. Primary injections of 2 mg in 0.2 ml of olive oil were made at 5 days after hatch and continued daily for 10 days. Treatment was withdrawn for 10 days and then varying concentrations of hormone (0.22 mg–6 mg per day) were injected daily for 2 days and the animals killed.

Preparation of ovalbumin antiserum

Antiserum to purified ovalbumin (5× recrystallised, Miles, UK) was raised in rabbits by multisite subcutaneous injections of 1 mg (in total) in Freunds complete adjuvent (Difco, UK) at monthly intervals. The titres of batches of antiserum obtained from the rabbits at various intervals were determined by the precipitation of purified unlabelled ovalbumin and ^{14}C methylated ovalbumin (New England Nuclear, Dupont, UK) and each was checked for its ability to precipitate ^{14}C ovalbumin completely from a known experimental homogenate (see below).

Incubation of oviduct samples

The magnum of the oviduct was removed aseptically, weighed and placed in 10 ml of sterile Hanks medium pH 7.4 (Miles, UK). It was then cut into small pieces of about 10 mg and 30 pieces were placed into 1 ml of sterile Hanks medium containing 1.5 µCi of high specific activity ^{14}C amino acids (Amersham International, UK) in a sterile disposable 30 ml Universal tube (Sterilin, UK), gassed with 95% O_2/5% CO_2 (British Oxygen, UK) for 30 s and incubated in a vigorously shaking waterbath at 41 °C. After 1 h the tubes were plunged into ice to stop the reaction, the pieces of magnum removed, blotted, and homogenised 5 times of 0.25 ml of a buffer containing 25 m*M* Tris pH 7.4, 5 m*M* $MgCl_2$, 25 m*M* NaCl 1% Triton X-100, using a Tri-R homogeniser (Camlab, UK) at full speed. One milliliter of buffer, without Triton X-100, was added, re-homogenised and centrifuged for 20 min at 3,000 g_{av} at 5 °C. The supernatant could be used immediately for the biochemical determination or frozen at −70 °C for up to 6 months before use without affecting the results.

Biochemical determination of relative ovalbumin concentration

Ovalbumin synthesis in the oviduct was determined according to the method of Palmiter (1972). The amount of ^{14}C amino acids incorporated into ovalbumin and into total protein in 1 h of incubation of magnum tissue was estimated by precipitation with ovalbumin specific antiserum and TCA, respectively. For estimation of incorporation into ovalbumin, 10 µl of the supernatant from the tissue homogenates was incubated with 100 µl of the appropriate concentration of anti-ovalbumin antiserum (diluted in sterile 0.9% NaCl), 20 µl of 1% Triton X-100 and 200 µl of the homogenisation buffer (without Triton X-100). These were incubated at room temperature for 30 min and centrifuged for 5 min at 3,000 g_{av}; the supernatant was discarded and the precipitate washed twice and broken up gently with a glass rod in 350 µl of buffer. After the second wash, the precipitate was incubated with 100 µl of NCS (Nuclear Chicago) overnight with intermittent vortexing; when the precipitate was dissolved, 3 ml of Fisofluor I (Fisons, UK) was added and the mixture was incubated in mini-scintillation vials at 5 °C overnight (to remove the chemiluminescence) and counted in an LKB Wallac 1218 Rackbeta liquid scintillation counter. Background controls were prepared by substituting 100 µl of sterile 0.9% NaCl for the anti-ovalbumin antiserum in the incubation.

The incorporation of ^{14}C amino acids into total protein was determined by adding 10 µl of the supernatant from the tissue homogenate to 300 µl of 5% cold TCA at 5 °C for 15 min, centrifuging at 3,000 g_{av} for 15 min and washing the precipitate twice (and broken up with a glass rod) in 350 µl of 5% TCA. The final precipitate was incubated in 100 µl NCS and counted as described above.

From the estimate of ^{14}C cpm in the anti-ovalbumin antiserum precipitated protein and the TCA precipitated protein, the ovalbumin synthesis as a percentage of total protein synthesis in the magnum could be calculated (Palmiter 1972).

Results

Response of the ovalbumin gene to oestradiol implants

Initial experiments showed that induction of ovalbumin synthesis within a strain was less variable if a period of secondary stimulation by oestradiol was used after a period of withdrawal from the primary stimulation ("Materials and methods"; Palmiter 1972). Use of 30 mg implants with mice has shown that they produce large and saturating doses of hormone (Middleton and Bulfield 1987). The first series of experiments assessed the effects of large doses of oestradiol on the expression of the ovalbumin gene in a number of genetically different strains of chickens, some selected and some unselected for fertility.

Table 1. Response in oviductal ovalbumin synthesis to secondary induction by implants of 17β oestradiol for various times

Strains of chickens	Oviduct ovalbumin concentration[a] days after oestradiol induction[b]		
	2 days (n)	4 days (n)	7 days (n)
Commercial layers:			
Line 1	36.4 ±37.9 (7)	49.5±4.03 (5)	61.4 ±3.77 (5)
Line 2	52.5 ± 1.97 (5)	54.5±3.38 (5)	71.1 ±4.13 (5)
Average	44.5 ± 8.04	52.0±2.50	66.3 ±4.85
Unimproved layers:			
J line	20.0 ±1.49 (7)	36.1±2.17 (8)	–
T line	19.1 ±0.84 (5)	31.8±1.42 (6)	–
S line	–	29.8±1.36 (8)	40.3 ±2.25 (7)
Average	19.6 ±0.45	32.6±1.86	–
Female-parent broilers:			
Breeder A line 3	16.7 ±1.98 (6)	12.3±2.15 (6)	14.6 ±0.98 (4)
Breeder A line 4	13.2 ±1.97 (6)	18.2±1.88 (7)	23.8 ±4.71 (5)
Breeder A line 5	26.1 ±3.86 (6)	20.9±5.38 (6)	13.4 ±4.24 (5)
Average breeder A	18.7 ±3.85	17.1±2.54	17.3 ±3.28
Breeder B line 6	30.4 ±5.94 (5)	25.2±3.50 (5)	17.0 ±2.71 (6)
Male-parent broilers:			
Breeder A line 7	10.2 ±1.27 (8)	10.5±0.71 (5)	6.15±2.03 (4)
Breeder A line 8	9.67±2.65 (6)	8.70±1.55 (8)	13.7 ±4.32 (5)
Breeder A line 9	6.07±2.09 (7)	15.7±5.89 (6)	6.30±3.66 (3)
Average breeder A	8.65±1.30	11.6±2.10	8.72±2.49
Breeder B line 10	17.3 ±3.27 (4)	21.0±5.00 (5)	14.2 ±3.52 (7)

[a] Ovalbumin concentration expressed as a percentage of total protein concentration (see "Materials and methods")
[b] 30 mg β oestradiol implants for 2, 4 or 7 days after an initial implant of 9 days followed by a 10 day withdrawal period (see "Materials and methods"); birds were killed at 29, 31 or 34 days after hatch

Animals from four strains were used: (a) two improved egg layer strains from a commercial breeder, (b) three unimproved layer strains from our own stocks (unselected for at least 25 years), (c) four current commercial 'female-parent' broiler lines selected for both weight-for-age and fertility, and (d) four current commercial 'male-parent' broiler lines selected solely for weight-for-age. Animals from these strains were killed 2, 4 and 7 days after the second implant had been inserted and the percentage of ovalbumin synthesis determined (see "Materials and methods"; Table 1).

Among layer strains, animals from the current selection lines of the commercial breeder responded best to oestradiol treatment and produced the highest concentration of ovalbumin in the oviduct at all three times points (Table 1). The response of all the layer strains was generally higher than all the broiler strains, and among the broiler strains the female-parent lines generally had a higher response than the male-parent lines.

The differences between the female-parent and male-parent lines of broilers are particularly interesting as both types of strains are derived from the same base population. This has been examined in more detail using oestradiol injections instead of implants to enable the dose of oestradiol during the second stimulatory period to be varied.

Response of the ovalbumin gene to injections of varying concentrations of oestradiol

Animals from a female-parent and a male-parent line of broilers were injected with oestradiol (in olive oil) for a primary stimulatory period, followed by withdrawal and then stimulation for a second time with injections of varying concentrations of oestradiol from 0.22 mg–6 mg per day (a 27-fold range).

Table 2. Response in oviductal ovalbumin syntheses to secondary induction by injections of various amounts of 17β oestradiol for 2 days

Strains of chickens	Oviduct ovalbumin concentration[a] after daily injection[b] of varying amounts of oestradiol			
	6 mg (n)	2 mg (n)	0.67 mg (n)	0.22 mg (n)
Female-parent line	68.5 ± 13.5 (7)	76.6 ± 11.4 (5)	77.3 ± 13.1 (5)	61.7 ± 6.57 (5)
Male-parent line	43.8 ± 1.92 (5)	32.4 ± 2.30 (5)	33.4 ± 3.27 (5)	16.6 ± 3.76 (4)
Ratio: female-line/male-line	1.56	2.36	2.31	3.72

[a] Ovalbumin concentration expressed as a percentage of total protein concentration (see "Materials and methods")
[b] Injection of varying amounts of oestradiol for 2 days after initial injections for 10 days followed by a 10 day withdrawal period (see "Materials and methods"); birds were killed at 27 days after hatch

Using this injection protocol a clearer picture of the differences between the female-parent and male-parent broiler strains emerged (Table 2). At all concentrations of oestradiol the oviducts from the female-line had higher concentrations of ovalbumin, varying from 56% higher than the male-parent line at 6 mg/day to 3.72 fold higher at 0.22 mg/day; the magnum of the oviduct was almost the same size after secondary stimulation in the two strains (at 2 mg/day 1.009 ± 0.097 g for the female-parent strain compared with 0.995 ± 0.032 g for the male-parent strain).

The highest concentrations of ovalbumin presented in Table 2 are higher than those for the comparable strains from the experiment with implants in Table 1, demonstrating that the implants probably do not produce a saturating dose of the hormone. All injection protocols produce magnum ovalbumin concentrations of 70%–80% of total protein synthesis in a commercial layer strain (results not shown).

Discussion

The physiological and genetical causes of the poor fertility of broiler breeders are unknown. The fat content of the animals does not appear to be related to reproductive performance (Chaney and Fuller 1975; Hocking et al. 1985) and the role of circulating levels of reproductive hormones and sensitivity of the target tissues are unknown. It is therefore significant that there is a wide variation in response in oviduct ovalbumin synthesis among chicken strains to a large implanted dose of oestradiol that parallels the fertility of the strains (Table 1). It is particularly interesting that female-parent lines of broilers are more sensitive to oestradiol than the male-parent lines of broilers, as both sets are from the same base population. There is also a difference between lines obtained from two different commercial breeders, although in each case the female-parent line(s) responds better than the male-parent line(s).

The differences between male-parent and female-parent lines of broiler were investigated in more detail by injections of varying amounts of oestradiol. The response of oviductal ovalbumin synthesis to oestradiol declined throughout the dose range from 6 mg/day to 0.22 mg/day in the male-parent line, but in the female-parent line a significant decline in response probably only began at 0.22 mg per day (Table 2). These results demonstrate that not only does oviductal ovalbumin synthesis in female-line broilers show a greater response to saturating levels of oestradiol than that in male-line animals, but they also show an even greater difference in response to sub-saturating doses of oestradiol (there is a 1.56 fold difference between the strains at 6 mg/day compared with a 3.72 fold difference at 0.22 mg/day; Table 2).

These results indicate that selection for fertility in both layer and broiler chickens results in a greater response of oviductal ovalbumin synthesis to oestradiol. Two questions remain: first, whether the differences in ovalbumin synthesis represent a greater overall tissue sensitivity to oestradiol; and second, at what stage in the molecular events between the circulating levels of oestradiol and the increase in ovalbumin synthesis is control exercised.

Fortunately, much is known about the molecular control of egg-white protein induction by steroid hormones (for review see O'Malley et al. 1979, 1983); the ovalbumin (McReynolds et al. 1977; Dugaiczyk et al. 1979; Royal et al. 1979), conalbumin (Cochet et al. 1979), ovomucoid (Catterall et al. 1979) and lysozyme (Jung et al. 1980) genes have all been cloned by recombinant DNA techniques, as well as the genes for the chicken oestrogen receptor (Krust et al. 1986) and progesterone receptor (Jeltsch et al. 1986). Therefore the expression of these genes in oviducts from the different strains can be quantified and the point(s) of control of the regulation of ovalbumin gene expression identified.

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