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Genetic selection on abdominal fat content alters the reproductive performance of broilers

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The effects of obesity on reproduction have been widely reported in humans and mice. The present study was designed to compare the reproductive performance of lean and fat chicken lines, divergently selected for abdominal fat content. The following parameters were determined and analyzed in the two lines: (1) reproductive traits, including age at first egg and total egg numbers from generations 14 to 18, absolute and relative testicular weights at 7, 14, 25, 30, 45 and 56 weeks of age, semen quality at 30, 45 and 56 weeks of age in generation 18, and fertility and hatchability from generations 14 to 18; (2) reproductive hormones at 7, 14, 25, 30, 45 and 56 weeks of age in generation 18; (3) and the relative mRNA abundance of genes involved in reproduction at 7, 14, 25, 30, 45 and 56 weeks of age in generation 18. In females, birds in the lean line laid more eggs from the first egg to 40 weeks of age than the birds in the fat line. In male broilers, the birds in the lean line had higher absolute and relative testicular weights at 7, 14 and 25 weeks of age, but lower absolute and relative testicular weights at 56 weeks of age than the birds in the fat line. Male birds in the lean line had greater sperm concentrations and larger numbers of motile and morphologically normal sperms at 30, 45 and 56 weeks of age than the birds in the fat line. Fertility and hatchability were also higher in the lean line than in the fat line. Significant differences in the plasma levels of reproductive hormones and the expression of reproduction-associated genes were also found at different ages in the lean and fat birds, in both males and females. These results suggest that reproductive performance is better in lean birds than in fat birds. In view of the unique divergent lines used in this study, these results imply that selecting for abdominal fat deposition negatively affects the reproductive performance of birds.

Keywords: selection, abdominal, fat, reproduction, broilers

Implications

In this study, we compared the reproductive performance between two chicken lines divergently selected for abdominal fat content, including reproductive traits, plasma concentrations of reproductive hormones and genes involved in the reproduction. We found lean birds have better reproductive performance compared with the fat ones. Our research is helpful for current broiler breeding programs and for improving the understanding of the relationship between obesity and reproduction in birds.

Introduction

Obesity has been associated with reproductive dysfunction in many species at different levels, including reproductive hormone levels, oocyte development, semen quality, etc. In humans, it is reported that obese men have lower sperm count and total testosterone (T) (Jensen *et al*., 2004), and obese women have poor conception and implantation rates (Brewer and Balen, 2010). In mice, obesity negatively affects the female reproductive capacity by reducing the number of oocytes and preantral follicles (Sagae *et al*., 2012). The adverse impact of obesity on reproduction has also been reported in domestic livestock. Arnett *et al*. (1971) found that obese cows lost more calves at parturition, and produced less milk than normal cows. In leptin-resistant obese sows, the corpora lutea are less functional during the estrous cycle and early pregnancy, which may contribute to their low reproductive efficiency (Astiz *et al*., 2013).

The effects of obesity on reproduction in chicken have been the subject of research interest for many years. It has been shown that diet-induced obesity has no significant effect on testes weight, semen volume or sperm density in

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male White Leghorn cocks (Parker and Arscott, 1972). In females, initial egg production under *ad libitum* feeding was lower in the fat line (Whitehead *et al*., 1990). Compared with *ad libitum* feeding, food restriction enhanced egg production in both lines (Hocking and Whitehead, 1990). The ovaries of fat line females fed *ad libitum* had a high proportion of atretic yellow follicles and there was more extensive atresia in small white follicles of fat line birds even when food was severely restricted (Hocking and Whitehead, 1990). Recently, it has also been demonstrated that diet-induced obesity compromises the ovarian function of hens by altering ovarian hormone production and exerting proapoptotic effects on granulosa cells (Walzem and Chen, 2014). Although the influences of obesity on reproduction in male fowl have been studied by feeding them high-energy rations, as far as we know, there is no report of the effects of obesity on genetically obese male broilers. The effects of genetic obesity on the reproduction of female broilers have not yet been studied comprehensively.

Therefore, in this study, the reproductive performance, including the reproductive traits, reproductive hormones and the transcription of genes associated with reproduction, were compared, between the genetically lean and fat lines established by divergent selection for abdominal fat content at Northeast Agricultural University (NEAUHLF).

Material and methods

Ethics statement

All animal work was conducted according to the guidelines for the care and use of experimental animals established by the Ministry of Science and Technology of the People's Republic of China (Approval no.: 2006-398) and approved by the Laboratory Animal Management Committee of the NEAUHLF.

Animals

The NEAU broiler lines divergently selected for abdominal fat content (NEAUHLF) have been selected since 1996 using abdominal fat percentage (AFP) and plasma very low-density lipoprotein (VLDL) concentration as selection criteria (Guo *et al*., 2011). The 0 generation (G_0) of the two lines came from the same grandsire line originating from the Arbor Acres breed, which was then divided into two lines according plasma VLDL concentration at 7 weeks. From G_1 to G_{18}, plasma VLDL concentrations were measured for all male birds at 7 weeks, and AFP of the male birds in the first hatch was measured after slaughter at 7 weeks. Sibling birds from the families with lower (lean line) or higher (fat line) AFP than the average value of the population were selected as candidates for breeding, considering the BW at 7 weeks and plasma VLDL concentration of male birds in the second hatch and egg production of female birds in both hatches. A significant difference in AFP at 7 weeks of age has been found between the two lines since G_4. Selection was continued for 18 generations. Birds from G_{14} to G_{18} were used in the current study.

For males (females), the birds of two lines had access to feed and water *ad libitum* from hatching to 7 weeks (from hatching to 2 weeks), and were equally feed restricted from 8 weeks to 56 weeks of age (from 3 weeks to 56 weeks of age) according to the guidelines given in *Arbor Acres Plus Broiler Parent Stock Management Handbook* (2014). All birds were kept in the same environmental conditions.

Measurement of carcass traits

Birds from the second hatch of G_{18} were slaughtered at 7, 14, 25, 30, 45 or 56 weeks of age. BW and abdominal fat weight (AFW) were measured, and AFW was calculated as a percentage of BW (AFP). In all, 30 males (15 from the lean line and 15 from the fat line) and 30 females (15 from the lean line and 15 from the fat line) were used at each of the ages described above.

Reproductive trait determination in lean and fat lines

Ages at first egg and total egg numbers in lean and fat female birds. The lean and fat lines' ages at first egg (AFE) and total egg numbers (EN) from the first egg to 40 weeks of age in both hatches of every generation were calculated. To determine AFE and EN, a total of 2641 (1303 from the lean line and 1338 from the fat line) and 2117 (1108 from the lean line and 1009 from the fat line) female birds from five generations (G_{14} to G_{18}) were used, respectively.

Testicular weights and semen quality characteristics of lean and fat male birds. Testicular weight (TW) was measured and TW was calculated as a percentage of BW (TWP) after the chicken from the second hatch were slaughtered at 7, 14, 25, 30, 45 and 56 weeks of age in G_{18}. In all, 30 males (15 from the lean line and 15 from the fat line) were used at each age. The semen quality characteristics, including semen volume, semen pH, sperm concentration, percentage of motile spermatozoa and percentage of spermatozoa with an abnormal morphology, were determined at 30, 45 and 56 weeks of age in G_{18}. In all, 60 males (30 from the lean line and 30 from the fat line) were used and the ejaculate of each male was collected three times at each age. The procedures were performed as described in detail by Peters *et al*. (2008) and carried out by the same experienced person to avoid subjective assessments. In brief, the semen of each male was collected three times a week by the back massage method. The semen volume was measured by a graduated tube, and the semen pH was detected by the pH-indicator paper ranging from 6.4 to 8.0. Then, the semen was immediately diluted by 1 μl of semen with 199 μl of 0.75% NaCl, and submitted for examining the following sperm characteristics. The sperm concentration was detected under the microscope (40×10) by counting the spermatozoa using the hemocytometer. To determine the sperm motility, a drop of semen was placed onto a microscope slide by the micropipette, and then spread by a glass cover slip, to assess the percentage of progressing motile sperm under a microscope (40×10). The sperm with abnormal morphology was observed by staining the slides of sperm samples with 0.5% gentian violet solution

under the microscope (40×10), and a minimum of 500 sperms were counted on each slide to calculate the abnormal sperm per sample.

Fertility and hatchability of lean and fat lines. In every generation, eggs from the first hatch were collected for 15 to 18 days after two rounds of artificial insemination following family establishment (one sire: four dams), and eggs from the second hatch were collected for another 15 to 18 days after the first hatch. The lean and fat lines' egg fertility and hatchability in both two hatches of every generation were calculated. A total of 1240 individuals from five generations (G_{14} to G_{18}) were used (620 from the lean line and 620 from the fat line).

Blood sampling and measurement of reproductive hormones in plasma

All birds were fasted for 10 h, and venous blood samples were collected before slaughtering in 1.5 ml tubes containing EDTA-Na_2 at 7, 14, 25, 30, 45 and 56 weeks of age in G_{18}. In all, 60 females (30 from the lean line and 30 from the fat line) and 60 males (30 from the lean line and 30 from the fat line) from the second hatch were used at each of the age. After centrifugation, the plasma was separated and stored at −20°C for later analysis. The plasma concentrations of reproductive hormones, including FSH, LH and estrogen (E_2) in the lean and fat females, and FSH, LH and T in the lean and fat males, were measured by radioimmunoassay (RIA) using RIA kits (Beifang Biotechnology Institution, Beijing, China) with previously described methods (Goldsmith and Follett, 1983; Dorgan *et al*., 2002). Detailed information of the RIA kits used in the present study were provided in the Supplementary Table S1.

Tissue collection and RNA extraction

The hypothalamus, pituitary and ovary were collected from each female and the hypothalamus, pituitary and right testis of each male in G_{18} immediately after slaughtering at 7, 14, 25, 30, 45 or 56 weeks of age. In all, 30 females (15 from the lean line and 15 from the fat line) and 30 males (15 from the lean line and 15 from the fat line) were used at each of the ages tested. All the tissues were washed with 0.75% NaCl solution and then snap frozen in liquid nitrogen and stored at −80°C until RNA extraction. Total RNA was extracted from the frozen tissues using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol, and diluted in nuclease-free water. RNA quality was assessed by visualization of the 18S and 28S ribosomal RNA bands on a denaturing formaldehyde agarose gel. Only RNA with a 28S:18S ratio between 1.8 and 2.1 was used for reverse transcription. Then, the RNA was stored at −80°C for further analysis.

First-strand complementary synthesis and quantitative real-time PCR

Complementary DNA (cDNA) was synthesized from 1 μg of total RNA using the ImProm-II™ Reverse Transcription System (Promega, Madison, WI, USA), according to the manufacturer's instructions. Gene expression was detected with quantitative real-time PCR (qPCR) on a 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Each qPCR reaction contained 3.6 μl of FastStart Universal SYBR Green Master Mix (Roche, Indianapolis, IN, USA), 0.2 μl (10 mM) of each primer, 1 μl of cDNA and H_2O to a final volume of 10 μl. The thermal cycling conditions included an initial hold at 95°C for 10 min and 40 cycles at 95°C for 15 s and 60°C for 1 min. All the samples were assayed in triplicate. The target genes were gonadotropin-releasing hormone 1(*GNRH1*) in the hypothalamus, FSHβ polypeptide (*FSHB*) and LHβ polypeptide (*LHB*) in the pituitary, and *FSHB*, *LHB*, estrogen receptor 1 (*ESR1*) and insulin receptor (*INSR*) in the ovary or testis. The PCR efficiency of *GNRH1, FSHB*, *LHB*, *ESR1*, *INSR* and non-POU domain containing, octamer-binding (*NONO*) were 98.96%, 96.30%, 103.18%, 96.45%, 102.65% and 98.68%, respectively. The expression of each target gene was normalized to the expression of the reference gene, *NONO*. The relative fold differences in expression were calculated using the $2^{-\Delta C_T}$ method (Schmittgen and Livak, 2008), in which $\Delta C_T = C_T$ (target gene) $- C_T$ (NONO). The primer sequences are given in the Supplementary Table S2.

Statistical analysis

All the data were tested for normality. The specific statistical methods used were as follows. (1) Data for BW, AFW, AFP, TW, TWP and gene expression levels, which were normally or approximately normally distributed and had small sample numbers ($n = 15$), were analyzed with a *t* test. The results of the analysis of those data are presented as means and SEM. (2) Data for AFE, EN, semen quality characteristics, fertility, hatchability and plasma reproductive hormonal levels, which were normally or approximately normally distributed and had larger sample numbers ($n \geqslant 30$), were assessed with a GLM procedure. Model (1) was used for the analysis of AFE, EN, fertility and hatchability, which fitted line (*L*), generation (*G*) and hatch (*H*) as fixed effects. Model (2) was used for the analysis of the semen quality characteristics and plasma reproductive hormonal levels, which fitted with the line (*L*) as fixed effects, as follows:

$$Y = \mu + L + G + H + F(L) + D(F, L) + e \quad (1)$$

$$Y = \mu + L + F(L) + D(F, L) + e \quad (2)$$

In both model (1) and model (2), Y was the dependent variable, μ was the population mean, F (L) was a random effect of the family nested within the line, D (F, L) was a random effect of the dam nested within the line and the family and e was the random error. The results of the analysis of these data are presented as least squares means and SEM. All the statistical analyses were performed with the JMP 11.0 software (SAS Institute Inc., Cary, NC, USA), with a statistical significance level of $P < 0.05$.

Results

Carcass traits of lean and fat birds

To clarify the background of the lean and fat females and males used in this study, BW and AFW of each female and male from G_{18} were measured and AFP was calculated at 7, 14, 25, 30, 45 and 56 weeks of age. As shown in the Supplementary Tables S3 and S4, AFW and AFP were significantly higher in the fat females and males than in the lean ones ($P < 0.05$ or $P < 0.01$). However, there was no significant difference in BW between the lean and fat females or males at any time points.

Reproductive traits in lean and fat lines

Ages at first egg and total egg numbers of lean and fat females. Age at first egg and EN of the lean and fat females from G_{14} to G_{18} were analyzed. As shown in Table 1, the average AFE of the lean line was 2 days earlier than that of the fat line ($P < 0.01$). The average egg production of the lean line was 13.96% higher than that of the fat line ($P < 0.01$).

Testicular weights and semen quality characteristics of lean and fat males. Testicular weights and TWP were compared in the lean and fat males of G_{18} at different stages of their growth and development (7, 14, 25, 30, 45 and 56 weeks of age). As shown in Table 2, TW and TWP of the lean males were markedly higher than those of the fat males at 7, 14 and 25 weeks of age ($P < 0.05$ or $P < 0.01$). Interestingly, we also noted that, in contrast, the fat males had greater TW and TWP ($P < 0.01$) at 56 weeks of age.

The semen quality characteristics of the lean and fat males from G_{18} at 30, 45 and 56 weeks of age were measured. As shown in Table 3, the lean males had a greater semen volume than the fat males at 45 weeks of age ($P < 0.05$). The semen pH of the fat males was higher than that of lean males at 30 weeks of age ($P < 0.01$). Larger numbers of sperm were observed in the lean males at 45 weeks of age ($P < 0.01$). We also found that the lean males had a higher percentage of motile sperm at 56 weeks of age ($P < 0.01$) and fewer sperm with an abnormal morphology at both 30 and 56 weeks of age ($P < 0.05$).

Fertility and hatchability of lean and fat lines. The fertility and hatchability of two lines were compared. As shown in Table 4, fertility and hatchability were both significantly higher in the lean line than in the fat line ($P < 0.05$ and $P < 0.01$, respectively).

Plasma reproductive hormonal concentrations of lean and fat birds

Given the differences in the reproductive traits of the lean and fat birds, the plasma concentrations of FSH, LH and E_2 of the females (Table 5) and FSH, LH and T of the males

Table 1 *Comparison of age at first egg (AFE) and egg numbers (EN) between lean and fat female broilers divergently selected for abdominal fat content*

Traits	Lean females	Fat females	RMSE	*P*-value
AFE	199.68	201.93	9.20	<0.0001
EN	55.82	48.98	14.51	<0.0001

RMSE = root mean square error.
The *P*-value refers to the significance of lean females *v.* fat females.

Table 2 *Comparison of absolute and relative testicular weights (TW) between lean and fat male broilers divergently selected for abdominal fat content*

Traits	Lean males	Fat males	RMSE	*P*-value
TW (g)				
Week 7	1.13	0.41	0.53	0.0042
Week 14	0.53	0.36	0.16	0.0043
Week 25	32.79	20.52	12.73	0.0248
Week 30	30.57	38.08	10.44	0.0598
Week 45	44.22	42.18	11.15	0.6473
Week 56	27.37	39.41	9.87	0.0024
TW/BW (%)				
Week 7	0.07	0.02	0.03	0.0027
Week 14	0.03	0.02	0.01	0.0049
Week 25	0.93	0.57	0.32	0.0085
Week 30	0.80	1.02	0.25	0.0277
Week 45	0.93	0.95	0.23	0.7973
Week 56	0.61	0.89	0.22	0.0015

RMSE = root mean square error.
The *P*-value refers to the significance of lean males *v.* fat males.

Table 3 *Comparison of semen quality between lean and fat male broilers divergently selected for abdominal fat content*

Traits	Lean males	Fat males	RMSE	*P*-value
Semen volume (ml)				
Week 30	0.26	0.24	0.10	0.4251
Week 45	0.27	0.21	0.09	0.0311
Week 56	0.16	0.16	0.05	0.9565
Semen pH				
Week 30	7.16	7.24	0.12	<0.0001
Week 45	7.04	7.07	0.05	0.5045
Week 56	7.13	7.18	0.11	0.0762
Sperm concentration ($\times 10^9$/ml)				
Week 30	1.50	1.42	0.36	0.3999
Week 45	1.88	1.21	0.24	0.0001
Week 56	1.89	1.37	0.43	0.1754
Sperm motility (%)				
Week 30	94.24	93.38	2.01	0.1823
Week 45	95.24	94.46	2.21	0.7866
Week 56	96.08	93.55	2.69	0.0049
Abnormal sperm morphology (%)				
Week 30	5.34	6.19	1.16	0.0393
Week 45	5.88	6.40	1.32	0.2271
Week 56	3.56	4.55	0.44	<0.0001

RMSE = root mean square error.
The *P*-value refers to the significance of lean males *v.* fat males.

Table 4 *Comparison of fertility and hatchability between lean and fat broiler lines divergently selected for abdominal fat content*

Traits	Lean line	Fat line	RMSE	*P*-value
Fertility (%)	89.94	86.51	19.76	0.0419
Hatchability (%)	73.58	64.77	25.82	<0.0001

RMSE = root mean square error.
The *P*-value refers to the significance of lean line *v.* fat line.

Table 5 *Comparison of reproductive hormones between lean and fat female broilers divergently selected for abdominal fat content*

Traits	Lean females	Fat females	RMSE	*P*-value
FSH (mIU/ml)				
Week 7	3.94	2.20	1.71	<0.0001
Week 14	3.15	2.49	0.66	0.0059
Week 25	1.03	0.88	0.47	0.4278
Week 30	4.22	4.93	1.30	0.1431
Week 45	5.27	4.55	0.84	0.0122
Week 56	4.96	4.95	1.90	0.9794
LH (mIU/ml)				
Week 7	12.54	12.28	4.14	0.9030
Week 14	4.79	4.19	1.20	0.1243
Week 25	5.86	3.18	2.57	0.0051
Week 30	14.00	11.74	3.69	0.4699
Week 45	11.98	10.33	2.89	0.0845
Week 56	7.87	3.52	3.48	0.0161
E_2 (pg/ml)				
Week 7	21.86	22.03	6.17	0.9273
Week 14	16.02	14.92	3.87	0.3902
Week 25	356.40	324.31	61.27	0.2542
Week 30	382.96	339.19	49.80	0.0047
Week 45	392.12	320.65	66.00	0.0813
Week 56	381.52	370.74	49.73	0.6310

E_2 = estrogen; RMSE = root mean square error.
The *P*-value refers to the significance of lean females *v.* fat females.

(Table 6) in G_{18} were assayed with RIA method. The plasma concentrations of FSH were significantly higher in the lean females than in the fat females at 7, 14 and 45 weeks of age ($P < 0.05$). The plasma levels of LH were markedly higher in the lean females than in the fat females at 25 and 56 weeks of age ($P < 0.05$), as were the levels of E_2 at 30 weeks of age ($P < 0.01$). The plasma concentrations of FSH were significantly higher in the lean males than in the fat males at 25 weeks of age ($P < 0.01$). The lean males had markedly higher plasma levels of LH at 7, 14 and 45 weeks of age ($P < 0.01$) and of T at 7, 14, 25 and 56 weeks of age ($P < 0.05$) than the fat males.

Reproduction-associated gene expression in lean and fat birds

We examined the relative mRNA abundance of genes associated with reproduction in the hypothalamus, pituitary and ovary or testis tissues of the birds from G_{18} at 7, 14, 25, 30, 45 and 56 weeks of age. As shown in Figure 1a, the relative expression of *GNRH1* was significantly higher in the

Table 6 *Comparison of reproductive hormones between lean and fat male broilers divergently selected for abdominal fat content*

Traits	Lean males	Fat males	RMSE	*P*-value
FSH (mIU/ml)				
Week 7	2.46	2.40	1.06	0.8680
Week 14	2.49	2.40	0.90	0.9582
Week 25	1.20	0.71	0.72	0.0088
Week 30	5.20	5.23	1.23	0.9351
Week 45	5.24	5.32	0.90	0.7564
Week 56	4.39	4.24	1.39	0.7392
LH (mIU/ml)				
Week 7	11.04	6.79	3.04	<0.0001
Week 14	4.43	3.33	1.21	0.0060
Week 25	4.39	4.93	2.23	0.2788
Week 30	14.08	14.77	3.17	0.3876
Week 45	15.52	11.84	3.02	0.0065
Week 56	12.63	12.34	2.26	0.6909
T (ng/ml)				
Week 7	0.41	0.21	0.25	0.0385
Week 14	0.04	0.03	0.02	0.0179
Week 25	0.77	0.04	1.60	0.0002
Week 30	0.75	0.83	0.76	0.6942
Week 45	1.09	1.16	1.78	0.8769
Week 56	0.85	0.05	0.74	0.0007

T = testosterone; RMSE = root mean square error.
The *P*-value refers to the significance of lean males *v.* fat males.

hypothalamus of the lean females than in those of the fat females at 30 and 56 weeks of age ($P < 0.05$ or $P < 0.01$). In the pituitary gland, *FSHB* expression at 7 and 25 weeks of age was much higher in the lean females than in the fat females ($P < 0.05$ or $P < 0.01$), whereas *LHB* expression did not differ between the lean and fat females (Figure 1b). The expression levels of *FSHB* at 7, 25, 30 and 45 weeks of age, *LHB* at 14, 25, 30, 45 and 56 weeks of age, *INSR* at 7, 14, 25 and 45 weeks of age, and *ESR1* at 14, 25, 45 and 56 weeks of age were significantly higher in the ovaries of the lean females than in those of the fat females ($P < 0.05$ or $P < 0.01$) (Figure 1c). The relative hypothalamic expression of *GNRH1* was significantly higher in the lean males than in the fat males at 7 and 56 weeks of age ($P < 0.05$) (Figure 2a). In the pituitary, *FSHB* expression at 25 and 45 weeks of age and *LHB* expression at 30 and 56 weeks of age was much higher in the lean males than in the fat males ($P < 0.05$ or $P < 0.01$) (Figure 2b). In the testis, *FSHB* at 25, 30 and 56 weeks of age, *LHB* at 7, 25, 45 and 56 weeks of age, *INSR* at 14 and 30 weeks of age and *ESR1* at 14 and 25 weeks of age were markedly higher in the lean males than in the fat males ($P < 0.05$ or $P < 0.01$) (Figure 2c).

Discussion

In this study, genetically lean and fat birds with similar BWs and significantly different abdominal fat deposition were used, and they differed significantly in reproductive performance, including in their reproductive traits, sex hormones and the expression of reproductive-associated genes.

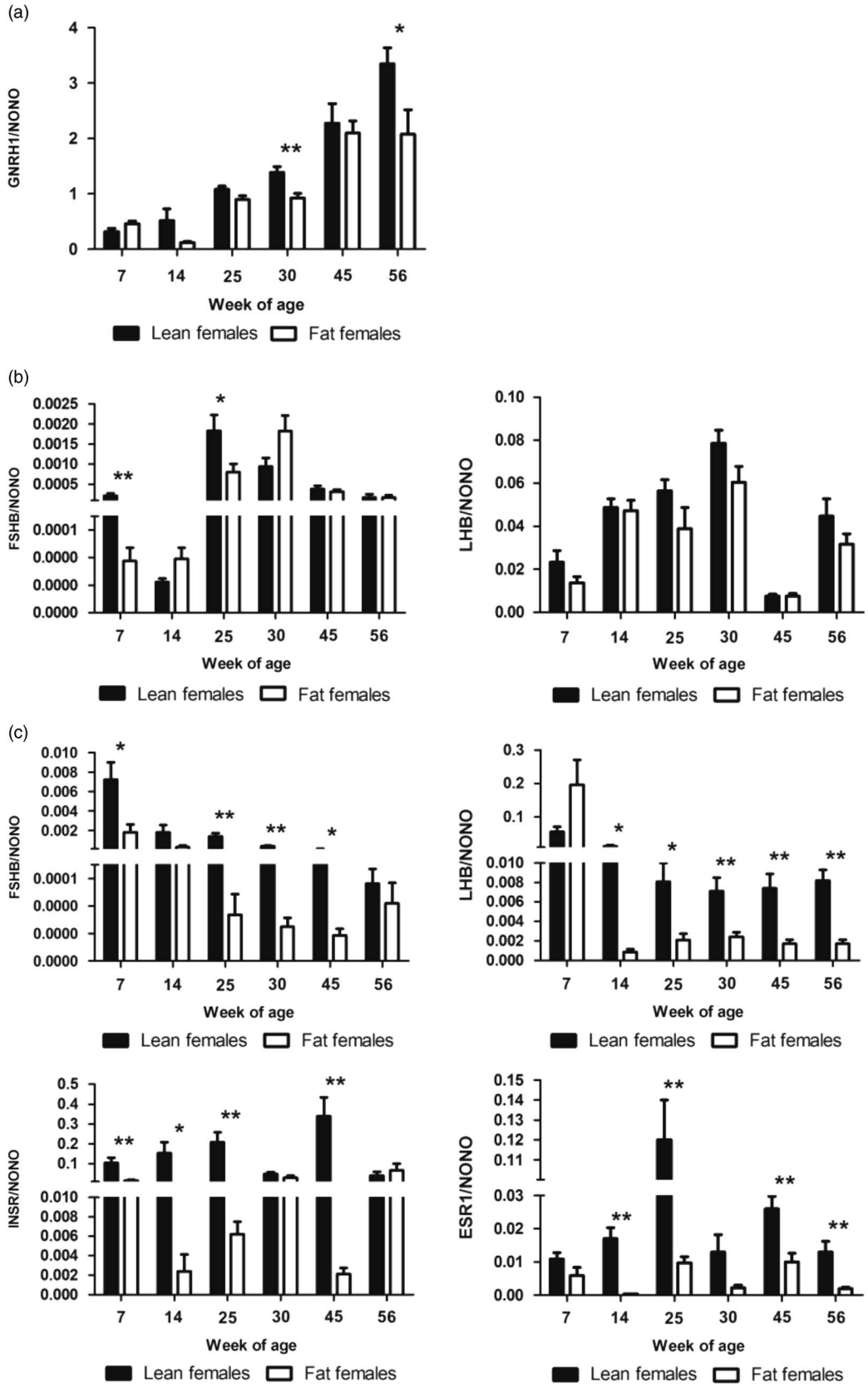

Figure 1 Comparison of the relative mRNA abundance of genes in the hypothalamus–pituitary–ovary involved in reproduction between lean and fat female broilers divergently selected for abdominal fat content. (a) Gonadotropin-releasing hormone 1 (*GNRH1*) in the hypothalamus; (b) FSHβ polypeptide (*FSHB*) and LHβ polypeptide (*LHB*) in the pituitary; (c) *FSHB*, *LHB*, insulin receptor (*INSR*) and estrogen receptor 1 (*ESR1*) in the ovary. The expression levels of each gene were determined by real-time PCR, and the expressed RNA levels were normalized to non-POU domain containing, octamer-binding gene (*NONO*). Results are given with the mean value and standard error ($n = 15$). The black columns represent the lean line and the white columns represent the fat line. Significant difference: $^{*}P < 0.05$, $^{**}P < 0.01$.

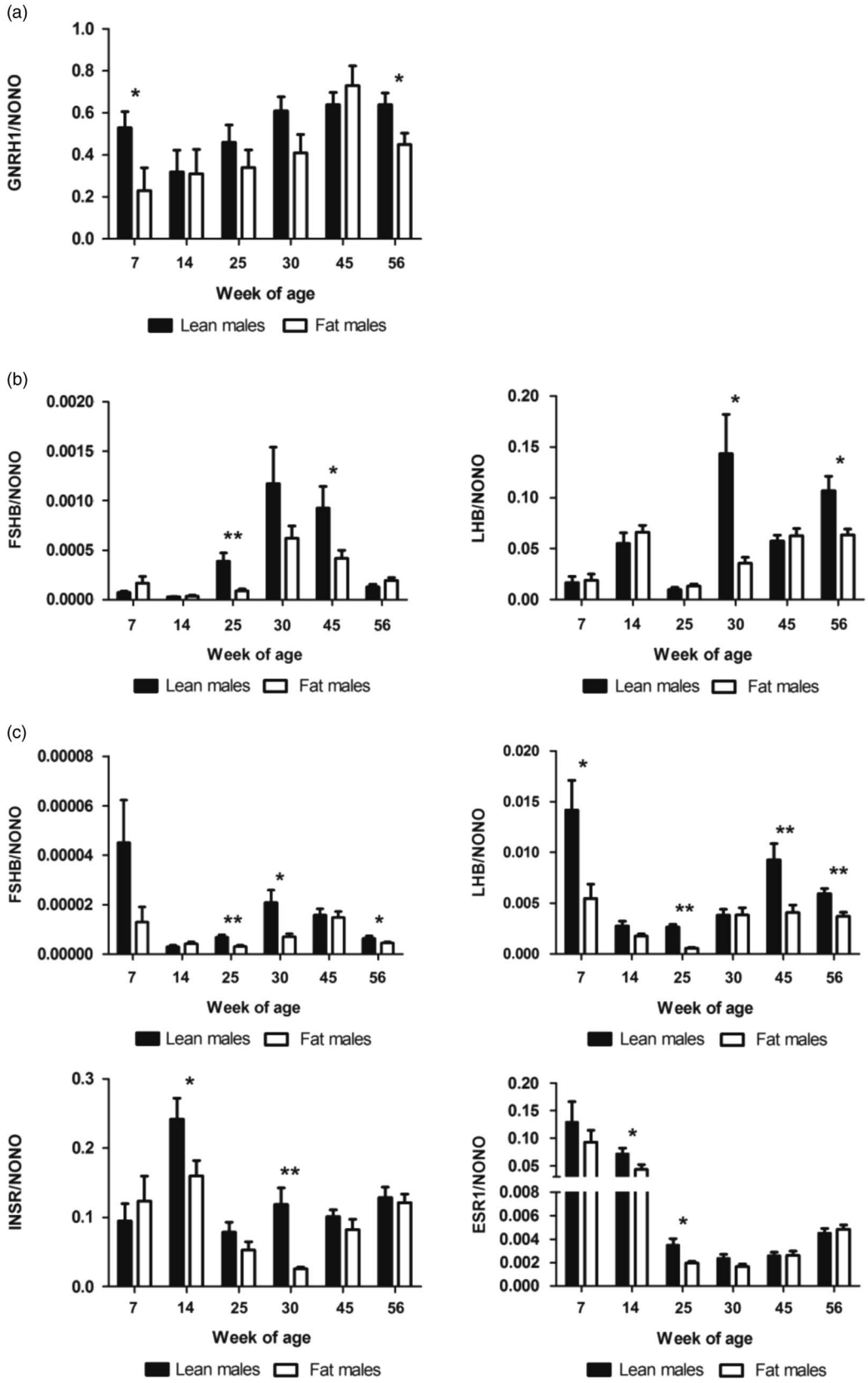

Figure 2 Comparison of the relative mRNA abundance of genes in the hypothalamus-pituitary-testis involved in reproduction between lean and fat male broilers divergently selected for abdominal fat content. (a) Gonadotropin-releasing hormone 1 (*GNRH1*) in the hypothalamus; (B) FSHβ polypeptide (*FSHB*) and LHβ polypeptide (*LHB*) in the pituitary; (c) *FSHB*, *LHB*, insulin receptor (*INSR*) and estrogen receptor 1 (*ESR1*) in the testis. The expression levels of each gene were determined by real-time PCR, and the expressed RNA levels were normalized to non-POU domain containing, octamer-binding gene (*NONO*). Results are given with the mean value and standard error ($n=15$). The black columns represent the lean line and the white columns represent the fat line. Significant difference: $^{*}P<0.05$, $^{**}P<0.01$.

Comparison of the reproductive performance between lean and fat female birds

Age at first egg and EN are important egg production traits in chicken. Our results show that lean birds had earlier onset of egg production (AFE) and larger EN than fat females. Previous studies in chicken (Bornstein *et al*., 1984) and quails (Yang *et al*., 2013) found that adequate BW and fatness were required at the onset of laying, but that obesity played a negative role in egg production. Hocking and Whitehead (1990) found that fat hens had few yellow follicles and a high rate of atresia at first egg, and Walzem and Chen (2014) reported that diet-induced obesity compromised ovarian function in hens. Obesity in humans and mice also negatively affects oocyte quality and/or maturation and the numbers of oocytes and follicles produced (Brewer and Balen, 2010; Sagae *et al*., 2012). The delayed onset of egg production observed in the fat line may be the result of slower follicular development and/or maturation caused by lower sex hormone levels and/or lower relative expression of reproduction-associated genes, which further reduce the numbers of eggs produced by fat females. The mechanisms underlying the adverse effects of obesity on egg production in chicken requires further clarification.

Fertility and hatchability are the main indicators of reproductive efficiency in chicken. We found that fertility and hatchability were higher in the lean line than in the fat line. Both paternal and maternal factors can affect fertility (Brillard, 2003). In chicken, the maternal factors of egg quality and the ability of the oviduct to store and maintain sperm after each ovulation are also important to fertility (Brillard, 2003). Based on the results of the present study, we speculate that higher levels of reproductive hormones, which affect ovulation and the development of the female reproductive tract, contributed to the higher fertility of the lean line. Hatchability is strongly influenced by incubation conditions and egg quality, which was affected by the quality of oocytes. In chicken, obesity has been shown to increase the likelihood of poor-quality oocytes (Walzem and Chen, 2014). Therefore, considering the same incubation conditions, lower levels of reproductive hormones and weaker expression of genes related to follicular development and maturation in the fat females may lead to poorer oocyte quality and thus lower hatchability in the fat line. The paternal factors affecting fertility and hatchability will be discussed in the second part of discussion.

Given the differences in the reproductive traits of the two female chicken lines, we measured the plasma concentrations of FSH, LH, and E_2 at different ages, because they play important roles in the regulation of female reproduction. Specifically, FSH, which is mainly secreted by the pituitary gland, regulates follicular development in the ovary and stimulates the secretion of E_2 and progesterone (Shanmugam *et al*., 2010). Luteinising hormone, which is also produced by the pituitary gland, promotes ovulation and E_2 secretion coordinately with FSH in the female (Norris and Carr, 2013). Unlike mammals, E_2 is mainly secreted by the theca cells in chicken, rather than by the granulosa cells , and plays an important role in the development of the oviduct and in the synthesis of egg yolk proteins by the liver (Li *et al*., 2014). In the present study, the average plasma concentrations of FSH, LH and E_2 were lower in the fat females than in the lean females, which could have impaired the functions of the gonads and resulted in poorer reproductive performance.

Then we examined whether the expression of genes associated with reproductive traits differed between the two lines. Gonadotropin-releasing hormone 1 plays a critical role in regulating the secretion of FSH and LH in birds, including chicken (Dunn and Sharp, 1999). Our results showed higher expression of *GNRH1* in the hypothalamus of lean females, which may partially explain higher plasma concentrations of FSH and LH in lean female birds observed in our study.

The synthesis of FSH and LH is stimulated by GnRH through the transcriptional and posttranscriptional regulation of their β subunits (Haisenleder *et al*., 1991; Dalkin *et al*., 2001). We found gene expression levels of *FSHB* and *LHB* in the pituitary were consistent with plasma concentrations of FSH and LH in the current study.

We also found higher levels of *FSHB*, *LHB*, *INSR* and *ESR1* in the ovary of lean females. The higher expression levels of *FSHB* and *LHB* in the lean females may have positively affected their reproductive performance by promoting ovarian development. Insulin plays an important role in female reproduction, affecting steroidogenesis and ovulation, mainly mediated by *INSR*, which is widely distributed in the ovary (Poretsky *et al*., 1999). Thus, the lower expression of *INSR* may have a negative effect on the steroidogenesis and ovulation. Estrogen plays an important role in the development of the oviduct and the synthesis of egg yolk proteins by the liver (Li *et al*., 2014), and functions by binding its receptor (Liu *et al*., 2015). The higher *ESR1* expression detected in the ovaries of the lean females may lead to further effects on the reproductive performance by activating the functions of E_2.

Comparison of the reproductive performance between lean and fat male birds

The seminiferous tubules and Leydig cells are the two main functional components of the testes. Accordingly, the primary functions of the testes are sperm production and T secretion (McCullagh, 1948). Therefore, TW and TWP were measured and compared in this study. Interestingly, we found that the lean males had higher TW and TWP than the fat males in the early stages of growth (7, 14 and 25 weeks of age), but lower TW and TWP at 56 weeks of age. The relationship between testis weight and specific cell numbers in the testis has been studied. In Japanese quail, Ahmed *et al*. (2015) reported that after *P*-Nitrophenol administration, testis weight and plasma concentration of T decreased. The histology of the testes showed hypocellularity of spermatogenic cell and spermatocyte and a decrease of appearance of spermatids and spermatozoa population. Although in another study on quails exposed to clothianidin, the results showed unchanged relative testis weights with a decreased number of germ cells by histology (Tokumoto *et al*., 2013).

Combined with the results in our current study, we think lean line male birds have a better development of testes.

Semen quality is an important trait in male reproductive performance. Therefore, the semen quality in the lean and fat males was measured in this study. The values for semen volume, semen pH, sperm concentration, percentage of motile spermatozoa and percentage of spermatozoa with an abnormal morphology in the NEAUHLF lines were similar to those in previously published reports (Parker and Arscott, 1972; Kelso *et al.*, 1997). Our results showed that the lean males had higher sperm concentrations and larger numbers of motile and morphologically normal sperm than the fat males. The semen quality of male broiler breeders is reported to be affected by breed, age, feed and environmental factors, including temperature and humidity (Karaca *et al.*, 2002). When the lean and fat males were maintained under the same feed and management conditions, the poorer development of the testes and lower reproductive hormone levels and related gene expression in the fat males was probably associated with their poorer semen quality, which further negatively affected the fertility and hatchability in the fat line.

We also measured the plasma concentrations of FSH, which regulates male testicular development and promotes the growth and maturation of sperm (Franchimont *et al.*, 1975); LH, which acts on Leydig cells to promote the secretion of androgens in males (Franchimont *et al.*, 1975); and T, which is secreted by Leydig cells and is essential for the growth and maintenance of the entire reproductive system (except for the Leydig cells themselves) (McCullagh, 1948) at different ages. In this study, the average plasma concentrations of FSH, LH and T were higher in the lean males, which may have better promoted their testis development, spermatogenesis and steroidogenesis. Similar results have been reported in obese men (Jensen *et al.*, 2004) and mice (Swerdloff *et al.*, 1976).

The mRNA expression of these genes was also determined in the male birds. The expression of *GNRH1* in the hypothalamus and *FSHB* and *LHB* in the pituitary gland was higher in the lean males, which may have led to the higher plasma concentrations of FSH and LH in the lean males.

The expression of the *FSHB*, *LHB*, *INSR* and *ESR1* genes in the testis was determined. The expression of these genes was lower in the fat males than in the lean males, which may have contributed to their poorer semen quality by affecting their testicular development. In males, *INSR* plays a critical role in the regulation of male reproduction, probably by modulating spermatogenesis (Walters *et al.*, 2012). The lower expression of *INSR* mRNA in the fat males suggests that excessive fat deposition impairs spermatogenesis and further reduces sperm concentrations, partly through an *INSR*-mediated mechanism. It is well documented that spermatogenesis is regulated by gonadotropins and T, the effects of which are modulated by complex interrelationships with other factors, including E_2 (Chimento *et al.*, 2014), which is also a receptor-mediated hormone. Estrogen receptor 1 is a classical E_2 receptor and its deletion (as in ESR1-knockout animals) reduces fertility, possibly by affecting spermatogenesis (Hess *et al.*, 1997). The lower expression of *ESR1* mRNA in fat males may be associated with their poorer semen quality via the dysregulation of spermatogenesis.

In summary, lean birds showed better reproductive performance, and because unique divergent chicken lines were used in this study, these results imply that selecting against abdominal fat deposition positively affects the reproductive performance of birds, possibly by influencing the expression of reproductive hormones and the genes associated with reproduction. We hope that these results will provide useful information for current broiler breeding programs and improve our understanding of the relationship between obesity and reproduction in birds.

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Supplementary material

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References

Ahmed E, Nagaoka K, Fayez M, Abdel-Daim MM, Samir H and Watanabe G 2015. Suppressive effects of long-term exposure to P-nitrophenol on gonadal development, hormonal profile with disruption of tissue integrity, and activation of caspase-3 in male Japanese quail (*Coturnix japonica*). Environmental Science and Pollution Research International 22, 10930–10942.

Arnett DW, Holland GL and Totusek R 1971. Some effects of obesity in beef females. Journal of Animal Science 33, 1129–1136.

Astiz S, Gonzalez-Bulnes A, Perez-Solana ML, Sanchez-Sanchez R and Torres-Rovira L 2013. *In vitro* release of ovarian progesterone is decreased during the estrous cycle and pregnancy of swine with obesity/leptin resistance. Reproduction in Domestic Animals 48, 44–48.

Bornstein S, Plavnik I and Lev Y 1984. Body weight and/ or fatness as potential determinations of the onset of egg production in broiler breeder hens. British Poultry Science 25, 323–341.

Brewer CJ and Balen AH 2010. The adverse effects of obesity on conception and implantation. Reproduction 140, 347–364.

Brillard JP 2003. Practical aspects of fertility in poultry. Worlds Poultry Science Journal 59, 441–446.

Chimento A, Sirianni R, Casaburi I and Pezziv V 2014. Role of estrogen receptors and g protein-coupled estrogen receptor in regulation of hypothalamus-pituitary-testis axis and spermatogenesis. Frontiers in Endocrinology 5, 1–29.

Dalkin AC, Burger LL, Aylor KW, Haisenleder DJ, Workman LJ, Cho S and Marshall JC 2001. Regulation of gonadotropin subunit gene transcription by gonadotropin-releasing hormone: measurement of primary transcript ribonucleic acids by quantitative reverse transcription-polymerase chain reaction assays. Endocrinology 142, 139–146.

Dorgan JF, Fears TR, McMahon RP, Friedman LA, Patterson BH and Greenhut SF 2002. Measurement of steroid sex hormones in serum: a comparison of radioimmunoassay and mass spectrometry. Steroids 67, 151–158.

Dunn IC and Sharp PJ 1999. Photo-induction of hypothalamic gonadotropin releasing hormone-ImRNA in the domestic chicken: a role for oestrogen? Journal of Neuroendocrinology 11, 371–375.

Franchimont P, Chari S and Demoulin A 1975. Hypothalamus-pituitary-testis interaction. Journal of Reproduction and Fertility 44, 335–350.

Goldsmith AR and Follett BK 1983. Avian LH and FSH: comparison of several radioimmunoassays. General and Comparative Endocrinology 50, 24–35.

Guo L, Sun B, Shang Z, Leng L, Wang Y, Wang N and Li H 2011. Comparison of adipose tissue cellularity in chicken lines divergently selected for fatness. Poultry Science 90, 2024–2034.

Haisenleder DJ, Dalkin AC, Ortolano GA, Marshall JC and Shupnik MA 1991. A pulsatile gonadotropin-releasing hormone stimulus is required to increase transcription of the gonadotropin subunit genes: evidence for differential regulation of transcription by pulse frequency *in vivo*. Endocrinology 128, 509–517.

Hess RA, Bunick D, Lee KH, Bahr J, Taylor JA, Korach KS and Lubahn DB 1997. A role of oestrogens in the male reproductive system. Nature 390, 509–1210.

Hocking PM and Whitehead CC 1990. Relationship between body fatness, ovarian structure and reproduction in mature females from lines of genetically lean or fat broilers given different food allowances. British Poultry Science 31, 319–330.

Jensen TK, Andersson AM, Jorgensen N, Andersen AG, Carlsen E, Petersen JH and Skakkebaek NE 2004. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. Fertility and Sterility 82, 863–870.

Karaca AG, Parker HM and McDaniel CD 2002. Elevated body temperature directly contributes to heat stress infertility of broiler breeder males. Poultry Science 81, 1892–1897.

Kelso KA, Cerolini S, Speake BK, Cavalchini LG and Noble RC 1997. Effects of dietary supplementation with alpha-linolenic acid on the phospholipid fatty acid composition and quality of spermatozoa in cockerel from 24 to 72 weeks of age. Journal of Reproduction and Fertility 110, 53–59.

Li J, Leghari IH, He B, Zeng W, Mi Y and Zhang C 2014. Estrogen stimulates expression of chicken hepatic vitellogenin II very low-density apolipoprotein II through ER-α. Theriogenology 82, 517–524.

Liu L, Li D, Gilbert ER, Xiao Q, Zhao X, Wang Y, Yin H and Zhu Q 2015. Effects of monochromatic light on expression of estrogen receptor (ER) and progesterone receptor (PR) in ovarian follicles of chicken. PLoS ONE 10, e0144102.

McCullagh PE 1948. Testicular dysfunction. Bulletin of the New York Academy of Medicine 24, 341–363.

Norris DO and Carr JA 2013. Vertebrate Endocrinology, 5th edition. Academic Press, Waltham, MA, USA.

Parker JE and Arscott GH 1972. Obesity and fertility in a light breed of domestic fowl, *gallus domesticus*. Journal of Reproduction and Fertility 28, 213–219.

Peters SO, Shoyebo OD, Ilori BM, Ozoje MO, Ikeobi CON and Adebambo OA 2008. Semen quality traits of seven strain of chickens raised in the humid Tropics. Bulletin of the New York Academy of Medicine 7, 949–953.

Poretsky L, Cataldo NA, Rosenwaks Z and Giudice LC 1999. The insulin-related ovarian regulatory system in health and disease. Endocrine Reviews 20, 535–582.

Sagae SC, Menezes EF, Bonfleur ML, Vanzela EC, Zacharias P, Lbaczeuski C, Franci CR and Sanvitto GL 2012. Early onset of obesity induces reproductive deficits in female rats. Physiology & Behavior 105, 1104–1111.

Schmittgen TD and Livak KL 2008. Analyzing real-time PCR data by the comparative C_T method. Nature Protocols 3, 1101–1108.

Shanmugam M, Pandita S and Palta P 2010. Effects of FSH and LH on steroid production by buffalo (*Bubalus bubalis*) gannulosa cells cultured *in vitro* under serum-free conditions. Reproduction in Domestic Animals 45, 922–926.

Swerdloff RS, Batt RA and Bray GA 1976. Reproductive hormonal function in the genetically obese (ob/ob) mice. Endocrinology 98, 1359–1364.

Tokumoto J, Danjo M, Kobayashi Y, Kinoshita K, Omotehara T, Tatsumi A, Hashiguchi M, Sekijima T, Kamisoyama H, Yokoyama T, Kitagawa H and Hoshi N 2013. Effects of exposure to clothianidin on the reproductive system of male quails. Journal of Veterinary Medical Science 75, 755–760.

Walters KA, Allan CM and Handelsman DJ 2012. Rodent models for human polycystic ovary syndrome. Biology of Reproduction 18, 1383–1394.

Whitehead CC, Armstrong J and Herron KM 1990. The growth to maturity of lean and fat lines of broiler chickens given diets of different protein content: body composition, plasma lipoprotein concentration and initial egg production. Animal Production 50, 183–190.

Walzem RL and Chen SE 2014. Obesity-induced dysfunctions in female reproduction: lessons from birds and mammals. Advances in Nutrition 5, 199–206.

Yang S, Suh Y, Choi YM, Shin S, Han JY and Lee K 2013. Loss of fat with increased adipose triglyceride lipase-mediated lipolysis in adipose tissue during laying stages in quail. Lipids 48, 13–21.