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Relationships Between Fertility and Some Parameters in Male Broiler Breeders (Body and Testicular Weight, Histology and Immunohistochemistry of Testes, Spermatogenesis and Hormonal Levels)

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Contents

In a farm of grandparent broiler breeder chickens, we followed the development of 350 roosters from 6 to 55 weeks of age. Data collected and evaluated from these males included body weight, testicular weight, histologic and immunohistochemistry studies of the testes, hormone levels (testosterone, estradiol and corticosterone) and sperm production. The objective was to understand the factors that affect or influence hatch loss that is commonly observed after 45 weeks of age in breeder flocksare often correlated to broiler breeder male chickens. The results of this study showed that in conjunction with the weight of the rooster, the testicle weight increases quickly after the rooster receives light stimulation. At an older age, the study showed that there is a process of testicular shrinkage, and the same effect is seen in sperm production and testosterone levels in broiler breeder roosters. From the histology evaluation, we defined 5 histologic phases that illustrate the evolution of the testicular tissue: perinatal, infantile, puberty, adult and senile. We observed that the adult males with a body weight < 3800 g were infertile or had subfertile levels and also had low levels of testosterone and high levels of corticosterone. In contrast, the heaviest males showed correct testicular vitality, high levels of testosterone and low levels of corticosterone. However, the roosters that had acquired this high body weight were also at risk of having less complete copulations because of their physical mass. The loss of uniformity of the males and the appearance of hierarchies within the flock accompany a decline in the percentages of hatches as a consequence of the poor confirmation of the males for copulation or the restriction to the access to the females. Results of this study show that the decrease in fertility from 45 weeks of age has been associated with a decline in testicular weight, sperm production and the testosterone levels in animals with a sub-par weight. Likewise, decreasing hatch in older flocks may also result from a loss of conformation, and the lack of complete copulations is possible because of animals that are grossly overweight.

Introduction

During unfavourable seasons in many wild bird populations, the testes appear to undergo a histologic change that includes regression or atrophy of the seminiferous tubules (Nicholls and Graham 1972; Lam and Farner 1976; Eroschenko et al. 1977; Ottinger et al. 1983; Rohss and Silverin 1983). The change can also produce a complete cessation of sperm production and a regression of the germinal epithelium (Humphreys 1975; Eroschenko et al. 1977). In contrast, domesticated fowl that have been selected for decades for the production of meat have lost this seasonal dependency and exhibit testicular development that is independent of climate conditions and is only limited by old age or disease problems. The genetic selection of broiler breeder lines (*Gallus gallus domesticus)* for the production of meat is primarily focused on producing broiler chickens that can obtain high slaughter weights with a reduced feed conversion ratio. However, these production successes of the final product (the broiler) sometimes are contrasted with the reproductive rates of the breeding stock as seen in a decline in fertility in the last weeks of life (Kirk et al. 1980; Vizcarra et al. 2010). From the observation of data from numerous farms, we have noted a decrease in fertility in breeding flocks starting at 45 weeks of age.

In heavy broiler breeder males, the decline in reproduction is considered a multifactorial problem. It is influenced by numerous factors including hormonal levels, the development of testicular tissue, behaviour, locomotion (mobility) and physical body composition. Mahecha et al. (2002) studied the formation of the calcium deposits in the epididymis of males with infertility and found that it was not related to the presence of infectious bronchitis virus or Chlamydia, although both of these pathogens had been previously described as a cause of urolithiasis (Dumanski et al. 1994; Ling et al. 1998; Hokama et al. 2000) and calcification of the epididymis (Panek et al. 2000). In another study, Mahecha et al. (2002) also described the consequences of the formation of calcification in the epididymis and testes and in the first stages observed a dilatation of the tubules with a desquamation of the germinal cells. Subsequently, they observed atrophy with a decreased diameter of the seminiferous tubules and only the presence of Sertoli cells or a minimal presence of spermatids and spermatagonia. In some cases of extreme regression, they observed that the tubules and interstitial tissue were fully composed of mononuclear interstitial cells.

The body weight of the roosters affects the fertility values, not only for the influence on different spermatic parameters, but also for the effect on the extremities of these birds because some of the excessive weight on the males can result in injuries (dyschondroplasia and destruction of articular cartilage that leads to rupture of tendons and ligaments), and can therefore negatively impact the hatch percentage for the flock. Studies such as those of Hocking and Duff (1989) found this correlation of fertility with the integrity of the limbs of the males to be true in animals considered to be obese

and in animals that were slightly overweight. In contrast, at the other extreme, it has also been widely reported that animals with a low body weight often have underdeveloped testes and are considered subfertile from the point of a certain threshold (Ross 2004).

Bilcik et al. (2005) also observed that the fertility level and the number of matings were greater in individual males with females than with other males, which indicates the importance of competition between roosters and the consequences that this can have on fertility.

Research has shown the influence of testosterone levels on the testicular weight (Sexton et al. 1989), the development of secondary male characteristics (Ligon and Zwartjes 1995), behaviour (Astiningsih and Rogers 1996) and reproductive success (Walsh and Brake 1997), although there is really no clear idea about its direct effect on fertility in a breeder flock (McGary et al. 2005). With respect to the process of ageing in broiler breeder males, Weil et al. 1999 observed that there was a decrease in plasma LH levels and testosterone as well as an increase in estradiol concentrations in the plasma and testes, suggesting that these hormonal systems have a role in the process of declining fertility in different breeder flocks. Other indicators of testicular function are inhibin that regulates the levels of FSH and controls testicular maturation (Sexton et al. 1989), caspase that triggers programmed cell death of the testicular germinal cells (Johnson et al. 2008) and the P-450 complex that converts androgens to oestrogens (Bourguiba et al. 2003) and may reflect feminization.

The aim of this study was to evaluate testicular development, body weight and hormonal changes in the different stages of life of the broiler breeder male and their possible relationship with the decline in hatch percentages after 45 weeks of age.

Material and methods

The study was conducted in a poultry farm of selected grandparent stock kept under environmentally controlled conditions. At the beginning of the study, the grandparent flock included 3000 'Cobb 500' broiler breeder females and 350 broiler breeder males. The birds were individually identified by numbered wing bands and were monitored daily. The health conditions and flock management were considered to be normal at the farm level, and no disease problems were identified during the flock cycle that could influence the outcome of the study.

Body and testicular weights

Eight male chickens were chosen and sacrificed (using CO_2) for further evaluation at the following ages: 1 day, 6 weeks, 19 weeks, 27 weeks, 32 weeks, 36 weeks and 42 weeks. At 55 weeks of age, a total of 40 males were euthanized. All animals were physically evaluated during the post-mortem, and any males with injuries that could affect fertility were discarded from the study. For each of the males assessed, the body weight and testicular weight were recoreded, and tissue samples and blood samples to study hormonal levels were also collected. In the group of males sacrificed at 55 weeks of age, the birds were subdivided into three subgroups: overweight (15% of the birds with the highest weight), standard (70% of the birds with an average body weight for the age group) and underweight (15% of the birds with the least body weight). In these subgroups, various parameters were studied independently.

Histologic analysis of the testes

Histologic analysis was performed on the testicular tissue of all males sacrified during the study. After tissue fixation in 10% buffered formalin (pH 7), the samples were processed in paraffin blocks and subsequently stained with the hematoxylin–eosin technique. All samples were included for the histologic analysis, and the appearance of cells present in the tubules was noted. At the same time, we utilized an ocular system to directly measure the mean diameter of the seminiferous tubules, and an average of 10 tubules per cross-section was obtained.

Analysis of sperm production

Sperm production was evaluated using the method Robb used for rats (Robb et al. 1978). However, for this study, the method was adapted for the testicle of the broiler breeder male and was performed on all sacrificed males after 24 weeks of age. Specifically for this analysis, the left testicle was always utilized and was cut transversely to obtain small fragments tissue (0.5 g in weight) from the central portion of the testicle. Samples were then frozen in liquid nitrogen, kept in a freezer at −20°C and then processed using the following technique: a fragment of the 0.5-g sample of testicular tissue was homogenized in a Waring blender semimicro with 10 ml of saline for 2 min to produce a sufficient quantity of macerated tissue to evaluate the sperm. A count of elongated sperm was then conducted using the Neubauer haematology chamber, giving the total number of sperm in the volume of the chamber from which, with a known dilution, we were able to calculate the number of sperm per gram of testicular tissue.

Hormonal analysis

Analyses were conducted to evaluate the following hormones at 24, 26, 30, 31, 33, 34, 40, 45, 50 and 55 weeks of age: testosterone, corticosterone and estradiol. Samples were collected at the aforementioned ages from 10 broiler breeder males. Males were individually identified using numbered wing bands, and the same males were used for each blood collection. In addition, 40 blood samples were collected from randomly selected males at 55 weeks of age. The males used for this blood collection were then sacrified. In addition to the hormonal evaluation at 55 weeks, the body weight and testicular weight of these males were recorded, and they were then classified into one of 3 subgroups: overweight (15%), standard weight (70%) and underweight (15%). For the hormonal analysis, we used a centrifuge to spin the blood and a commercial ELISA kit to assess the levels of testosterone and estradiol in serum. Corticosterone analysis was performed by RIA after

centrifugation and serum removal. The commercial kits used included: DE2693 (17 beta-estradiol), DIMEDIC Diagnostic GmbH, Kiel, Germany; EIA1559 (testosterone), DRG Instruments, GmbH, Marburg, Germany; CMEIA Corticosterone EIA Kit; Masterlab Kit RIA ICN Biomedical Inc.; Corticosterone EIA Kit 900-097 Assay design.

Inmunohistochemistry

Conventional immunohistochemistry, using immunoperoxidase (PAP), was used to evaluate the paraffin sections of testicular tissue from all sacrificed males. The following commercial antibodies were used for this method: human anti-testosterone polyclonal antibody in rabbits, BioNova; human anti-17 estradiol monoclonal antibody in rabbits, BioNova; human anti-inhibin alpha monoclonal antibody in mice, Dako; anti-P450 aromatase monoclonal antibody, Chemicon; and anti-caspasa-3 Ab-4 polyclonal antibody in rabbits, BioNova. All antibodies were used according to conventional histology methods and in the dilutions recommended by the corresponding laboratories. Positive reactions were confirmed by comparison with negative controls.

Data analysis

For the analysis of the different data were used, the Student's *t*-test or Mann–Whitney *U* test for comparison of means in independent samples and chi-squared test for comparison of proportions.

Results

Body weight and testicular weight

The body weight of broiler breeder males normally doubles during the life of the flock, starting at approximately 2500 g (from 6 weeks of age as a consequence of the broilerization program used for grandparent selection) to approximately 5000 g at 55 weeks of age. However, the testicular weight undergoes an explosive increase in size between 19 and 23 weeks of age, and this increase in testicular mass (total weight of both testes) will be from approximately 1 g to more than 50 g. Later, with the occurence of testicular regresion, approximately 44% of the weight can be lost between 36 and 55 weeks of age, resulting in a final total testicular weight of 29.5 g at the end of the rooster's life. (Fig. 1a).

By differentiating between the different subgroups of males (overweight and underweight), we obtained the following result: the average weight at 55 weeks of the roosters in the overweight group is approximately 5500 g, and the total testicular weight is 59 g. However, the average body weight of the underweight group is 4500 g, and their total testicular weight is only 26 g.

Sperm production

Sperm production in broiler breeder males begins with small amounts at approximately 26 weeks of age with approximately 25×10^4 elongated sperm per gram of testicular tissue. However, by the next week of age, this amount will increase to 1000×10^4 per gram of testicular tissue. The máximum sperm production occurs at 36 weeks of age with more than $10\,000 \times 10^4$ per gram of testicular tissue, but then decreases progressively until 55 weeks of age in a manner similar to the decrease in total testicular mass (Fig. 1b). At this age (55 weeks), there was a significant difference in sperm production between the subgroups. Males in the overweight category had an average production of 7600×10^4, while the males in the underweight category produced an average of 2300×10^4 sperm per gram of testicular tissue.

Histology and immunohistochemistry

On the basis of the histologic structure, morphometry and immunohistochemistry of the testes, we have described five distinct phases in testicular development that correspond to the phases that have been described in various mammals.

Perinatal period

In this phase, we have determined that the testicular composition from birds in their first and second day of life has similar histologic features (Fig. 2a). Histologic analysis of the testes of day-old chicks showed a uniform cellular composition with seminiferous tubules lined mainly by Sertoli cells and some gonia between them. The diameter of these tubules is 0.04 nm, but no internal lumen could be seen. Of greater importance, at this age, we could see the embryonic mesonephric tissue associated with foetal Leydig cells that have a globular appearance with pale interstitial tissue.

Infantile period

This phase includes the testicles of broiler breeder male chickens beween weeks 1 and 23–24 of age (Fig. 2b). In this phase, we observed the disappearance of all foetal Leydig cells that appeared in the previous phase and the non-existence of the tubular lumen. The number of germinal cells remains low although their number may increase up to 10 cells per tubule. They are mainly found in the basal position, and the most numerous are the type A gonia. The interstitium has obviously been reduced in this phase and is primarily occupied by a fusiform complex of Leydig cells with an enlongated and dark nucleus. As seen in the previous phase, the histologic image of the testes shows a very uniform cellular structure, and the tubular diameter is 0.06 mm.

Pubertal period

This phase covers the period between 24 and 28 weeks of age. The testicle of the broiler breeder male begins to develop to its full potential after 29 weeks of age, so the cellular image during this period is very variable. During this phase, the tubular diameter increases slowly from 0.19 mm at 25 weeks, to 0.20 mm at 26–27 weeks and then 0.24 mm at 28 weeks of age. Another change in this phase is that 100% of the seminiferous tubules develop a tubular lumen and 80% of the animals produce sperm that are freely moving in the lumen during the puberal phase. The Sertoli cells are characterized by having an

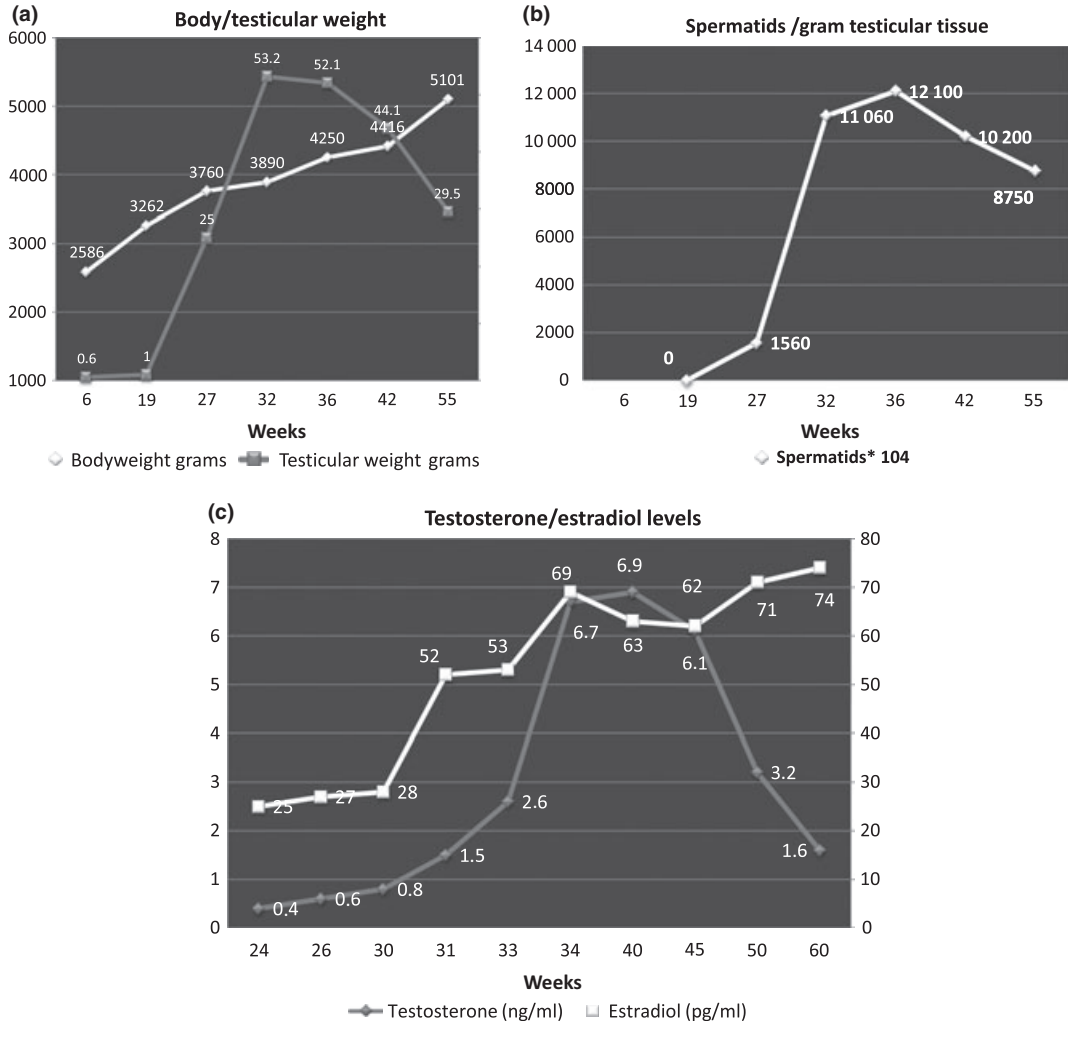

Fig. 1. (a) Evolution of the body and testicular weight between 24 and 60 weeks. (b) Number of elonged spermatids ($\times 10^4$) produced per gram of testicular tissue. (c) Testosterone and estradiol levels in broiler breeders between 24 and 60 weeks

irregular shape near the basal membrane and a small nucleus. In the germline cells, we can begin to observe cells in different stages of maturation: primary spermatocytes (at different stages of meiosis); secondary spermatocytes; round and elongated spermatids; and free sperm in the tubular lumen.

Adult period

This phase, defined by the age of 30–50 weeks, correlates with the functional completeness of the testicle, which then corresponds to the hatch percentage values that are the highest for the flock (Fig. 2c). The testicular development is at the maximum potential as is the sperm production, and the tubular diameter becomes 0.27 mm, which results in a striking compression of the thin interstitium. The production of spermatids and the maximum functional development are reached at 36 weeks of age. However, the most striking histologic change seen was that 6% of the testes in this period evidence hypoplastic testicular tissue with little or no sperm production, and this finding corresponded to the lighter males with body weight below 4000 g.

Senile period

In this phase, there are more pronounced pathologic changes that correspond to 55-week-old roosters (Fig. 2d). These changes include a similar tubular diameter (0.26 mm), but a decrease in the number of sperm in the tubular lumen. Cellular characteristics and the histologic imagery of the adult testes, as discussed in the adult period, remain the same for 71.03% of the testicles studied. However, for 21.73% of the testes evaluated, the testicles showed signs typical of old age, which we normally consider to be related to subfertile or infertile males. The histologic features of these testicles, corresponding to a mild or moderate atrophy, included the following characteristics according to their frequency in appearance: failures in maturation, decreased sperm production, sloughing of immature cells of the germinal epithelium to the tubular lumen, thinning of the germinal epithelium, slight decrease in the diameter of the tubule, thickening of the interstitial space that appears to be invaded by fibroblasts and collagen fibres, hyalinization of basement membranes, appearance of multinucleated spermatids (degeneration) and calcification of the tubules. In 5.79% of the testes analysed, we found

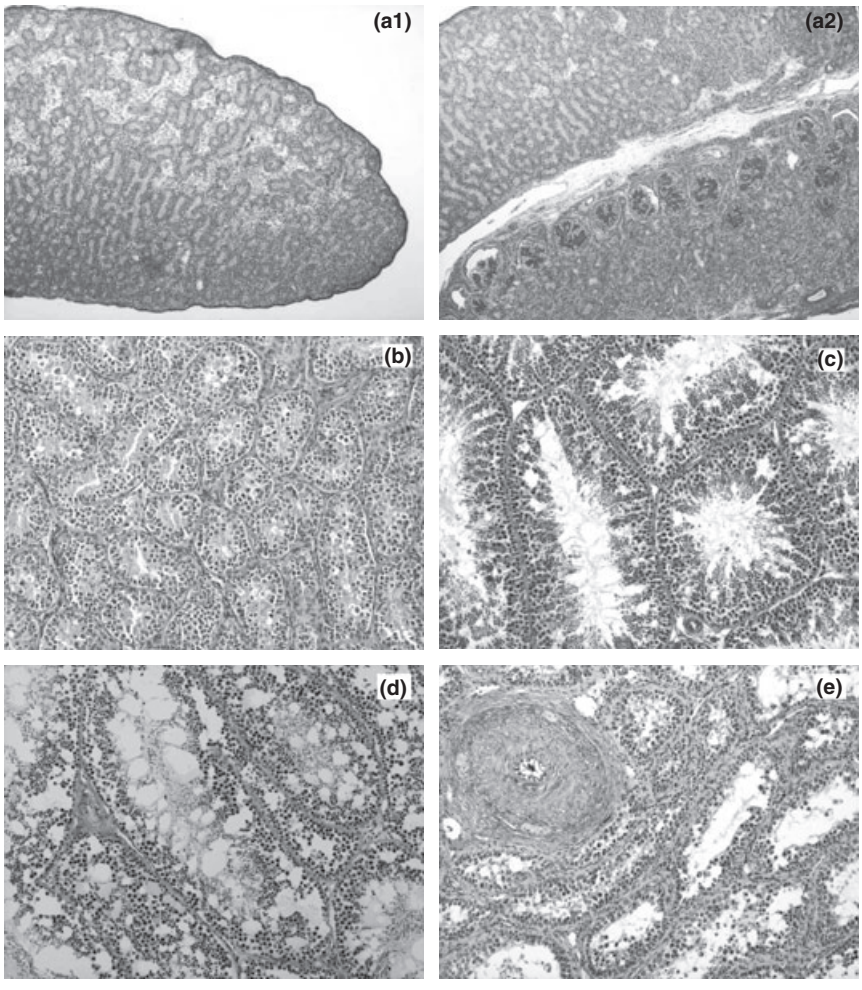

Fig. 2. (a1, a2) Perinatal testes [(a1: H&E × 20); (a2: H&E × 40)]. (b) Infantile testes (6 weeks) (H&E × 200). (c) Adult testes 33 weeks (H&E × 200). (d) Senile period (55 weeks) (H&E × 200). (e) Only Sertoli cell syndrome (Masson trichomic × 100)

histology features similar to those of the infantile phase that included hypoplasia and the absence of sperm production. In these testes, the seminiferous tubule is lined by Sertoli cells, there are few immature germ cells, and the diameter of the tubular lumen is reduced. These testicles corresponded to roosters who had lighter weights throughout the production stage, and the overall histologic analysis is compatible with testicular hypoplasia. During this stage, 1.44% of the roosters studied were completely infertile based on the testicular analysis. The lesion seen in these males was characteristic of the Sertoli syndrome (Fig. 2e), in which there was a complete absence of sperm production; a total absence of germ line cells and the seminiferous tubules was lined by Sertoli cells. In the cytoplasm of the Leydig cells, we saw a brown pigment similar to lipofucsin (a pigment associated with wear or waste). Males with this injury had body weights in the overweight category during much of the study; however, the testicular weights of these males actually had the lowest values.

Immunohistochemistry

Testosterone

The immunohistochemistry method with anti-testosterone in the infantile stage of the testicle (between 1 and 23 weeks of age) showed a positive diffusion of luminal area of the tubular epithelium with a moderate staining of the luminal areas of the cytoplasm of Sertoli cells and few gonia. We also observed a moderately positive reaction in the Leydig cells that had a flattened appearance; however, the interstitial cells with their globular appearance did not show any positivity. In the adult-phase testicles, the Leydig cells, germ cells and elonged spermatids showed strong positivity, but the germinal epithelium had variable positivity, with some cellular associations appearing less marked than others.

Estradiol

The immunomarking technique for the anti-estradiol was positive in Sertoli cells in testicles evaluated from the infantile and adult phases, and the number of positive cells increased with the age of the males. We also observed a positive reaction of the germinal epithelial cells of the adult roosters, but this was not the case in the testicles of younger males that were negative.

Aromatase

With respect to the location of the staining of cells producing the P450 enzyme aromatase, we noted that

the interstitial space and the tubules in the infantile testicles did not show any marked cells. However, in the adult testes, there were many positive elements: the Leydig cells in the adult phase showed strong positivity, whereas the Sertoli and germ cells showed positivity with varying degrees of intensity, with a higher intensity in the case of the gonia cells.

Inhibin

The cells that showed the most positivity to inhibin were the Sertoli cells, regardless of the age of the rooster, and these elements were positive in the testicles of roosters from the infantile and adult phases (Fig. 3). In the adult-phase testicles, we also observed a moderate positive reaction in the basal areas of the tubular epithelial cells and in some germ cells. The remaining testicular cells were negative in all animals studied.

Caspase

The positivity of caspase was observed mainly in the nuclei of regressing germinal cells in testicles from males in the infantile phase. In the case of adult animals, we observed an anti-caspase expression that was evident in the nucleus of primary spermatocytes and was especially intense in the testicles from senile-phase males with numerous factors in decline.

Hormonal analysis

Testosterone levels increased progressively from week 24 to week 33 of age from a level of 0.4 ng/ml to 6.7 ng/ml and remained at this higher level until approximately 40 weeks of age. From this point, they began to decline significantly and at 55 weeks of age measured 1.7 ng/ml. Likewise, estradiol levels increased from week 24 to week 33, an increase of 2.5 ng/ml to 7.3 ng/ml. However, unlike testosterone, the estrongen levels were maintained during the adult phase, and levels at 55 weeks of age were similar to those of a younger male (Fig. 1c). Estradiol levels showed no significant differences in the groups of overweight and underweight males at 55 weeks, but testosterone levels were much lower in the lighter weight males (2.7 vs 1.2 ng/ml testosterone) (Table 1).

Corticosterone levels also experienced a progressive increase from week 24 to week 33, going from 1.6 to 8.1 ng/ml and were then maintained until 55 weeks of age. However, at this older age, there were significant differences between the smallest and the heaviest roosters. The males with the lowest body weight had corticosterone levels that were much higher than the overweight roosters (14.1 ng/ml vs 6.4 ng/ml) (Table 1).

Table 1. Testosterone and corticosterone levels in overweight & underweight subgroups of 55-week-old roosters

55-week-old Roosters	Testoterone ng/ml	Corticosterone ng/ml
15% Overweight	2.7 [a]	6.4 [A]
15% Underweight	1.2 [b]	14.1 [B]

Different letters show statistically significant differences.

Discussion

The decrease in the percentage of hatch values of broiler breeder males may be associated with various diverse and individual factors, but to understand the correct cause of this decline, it is best to deal with joint characteristics such as body weight and conformation, testicular development and spermatogenesis, hierarchal relationships established between roosters and their hormonal levels. The relationship between these parameters and testicular mass was investigated by Pizzari et al. (2004). The relationship between physical parameters and fertility has also been studied by McGary et al. (2005).

We observed a regression in testicular weight of 44.54%, which was accompanied by a decrease in sperm production and an increase in body weight between 36 and 55 weeks of age. These findings were similar to those observed by Vizcarra et al. (2010). The five phases described by the histologic characteristics (perinatal, infantile, pubertal, adult and senile) are equivalent to those of other animal species. However, it is important to emphasize the shortened period of the pubertal phase for roosters, as well as the appearance of spermatogenesis that occurs later in meat-type strains relative to other animals of the same species because of the management conditions that are used for selection of grandparent poultry breeding stock, Satterlee et al. (2006).

We, unlike Mahecha et al. (2002), did not find any calcifications in the seminiferous tubules, epididymis or efferent ducts that could cause infertility. In some of the broiler breeder males in our study, we saw a severe or marked testicular regression by 55 weeks of age. We also observed that some roosters had testicular weights < 12 g (approximately 6 g per testicle), which is consid-

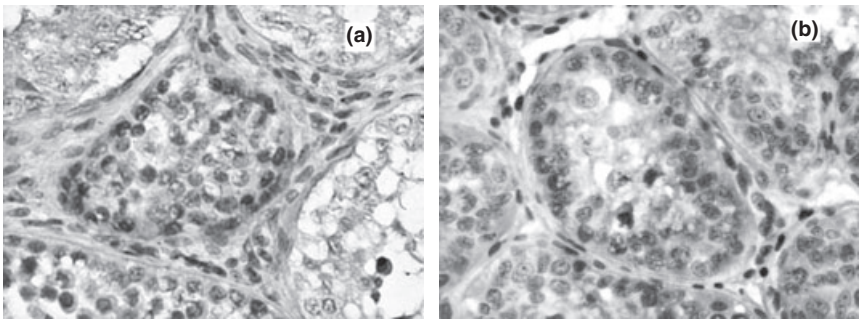

Fig. 3. PAP positive reaction in the basal areas of the tubular epithelial cells, on the left (a) we can observe some positive Sertoli cells and gonia in adult testes and on the right (b) more diffuse reaction (Human alphainhibin × 400)

ered to be subfertile (Ross 2004), and had hypoplastic testes when evaluated histologically. Generally, testicles that weigh < 5 g correspond to infertile testes (atrophic or hypoplastic); testicles weighing between 6 and 10 g are considered to be subfertile; and testicles larger than 10 g are considered to be functional. Taking into account all of the data corresponding to the weight of the testicle, in our study, the weights mentioned are the total testicular weight, so we should take 50% of the weight mentioned to have individual testicular weights. The roosters that we saw with the extremely low testicular weights corresponded to animals that possibly never reached their optimal body weight at any stage of their development and weighed < 3800–4000 g. Immunohistochemistry of the testicle showed estradiol as the marker that was most accentuated with age of the rooster, but the inverse happened with testosterone. In partial accordance with the findings of Bilińska et al. (2006), we also found a positive immunohistochemistry reaction for testosterone in Leydig cells, Sertoli cells and germinal elements. These authors also found positivity in Leydig cells for testosterone, but they did not see positivity in the germinal epithelium as we did.

In the testicles from males in the infantile phase, almost all of the cells were negative for anti-P450 aromotase antibody. Conversely, in the adult-phase testicles, we saw a reaction in the germinal cells, Leydig cells and Sertoli cells, and these findings are similar to part of the those observations made by Levallet et al. (1998) in rats in which positivity was found in Leydig cells in mature testicles and also in the Sertoli cells and germinal epithelium of immature testicles. With respect to the germinal epithelium, Carreau et al. (2001) and Bilińska et al. (2006) observed positivity in mammalian pachytene spermatocyes, spermatids and flagella. Kwon et al. (1995) detected a positive reaction in roosters in pachytene spermatocytes, rounded spermatids, elongated flagella of spematids and sperm.

The positive reaction of inhibin in Sertoli cells that we have described was already observed by Au et al. (1986) in foetal and prepubertal testicles, although these authores also described a reaction in germinal cells and in the tubular lumen. With regard to the caspase reaction, we observed a positive reaction in testicular cells in apoptosis that has been observed in young testes whose spermatogonia is in the process of regression and in senile adults in which the primary spermatocytes are in a fase of regression as noted by authors Bernal-Mañas et al. (2005). In their study, they observed greater positivity in the testicles with intense apoptosis with the degeneration of tubules associated with age in hamsters, as well as the basal germinal cells.

The daily number of copulations is influenced by the libido of the roosters which in turn is determined by their hormone levels, especially testosterone. Although the maximum values were found between roosters of 33 to 40 weeks of age, a gradual decrease was seen until reaching minimum values at the end of life. Our observations are consistent with those of numerous authors, although in constrast to Vizcarra et al. (2010), we noted that this decline is much sharper than he noted in his study. This decline also coincides with the poor results in hatch percentages that are reported from most breeding farms. With regard to the estradiol levels in blood, as noted in the study by Weil et al. (1999), we found a steady increase until 55 weeks of age, although it is difficult to think that the levels achieved are excessive and have a repercussion on the libido of the males because there is no siginficiant difference between males in the overweight category or the underweight category.

The hierachies established in a poultry barn also have a direct influence on the testicular develoment of the roosters as seen in the results of the corticosterone analysis. Clearly, the animals with lower testicular weight have corticosterone levels superior to those with great testicular weight, while the opposite effect is true for testosterone levels in these same males. This behaviour of sexual agression and stress level was described by McGary et al. (2005) and can be determined by ranking the individuals in a population as determined by the ratio of testosterone to corticosterone.

We concur with the study by McGary et al. (2005) in which the levels of testosterone and corticosterone determine the testicular development of the roosters and their behaviour, but as noted by other authors (Hocking and Duff 1989), it is difficult to establish a direct relationship with fertility because it is influenced by other factors such as the aggressiveness of the flock, body weight of the birds, testicle weight and the condition of the joints of the rooster.

In conclusion, the progressive ageing of the animal's physiologic status as well as the decline in sperm production and testosterone levels and the effect of body weight gain can cause a decrease in the fertility within a breeding farm, and this effect can be worsened if there are hierarchal relationships that can produce multiple consequences for the flock with regards to those animals that are outside the standard weight range (overweight and/or underweight roosters). On one extreme, we can have animals with good testicular development, sperm production and libido, but they may be unable to perform a complete mating with the hen because of their excess body weight. On the other extreme, roosters with a lower body weight, poor testicular development and low sperm production may exist within the flock and have a low level of sexual activity because of their unfavourable social position and subordination. As a result, this leads us to the conclusion those roosters in either subcategory (overweight or underweight) can lead to a decreased fertility on the farm. Therefore, for the best fertility of the flock, we must try to avoid situations in which animals fall into these two categories during the development and maintenance of the breeding flock. Various management techniques are being used to try to eliminate these groups within breeding flocks: isolation techniques for males with low body weights to allow them to return to a standard weight; removal of defective males (triage or culling); addition of new males (also known as spiking) to break the hierarchy of the flock. With these methods, some improvements in fertility have been achieved in the last third of the production phase of breeder farms.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

The individual contribution to this research and its publication is as follows: J Sarabia has designed the study and performed experiments (This paper is a part of the work that he obtained his PhD in Veterinary Science). M. Pizarro was the scientific director and performed the laboratory analysis. J C Abad has performed experiments and directed the works of field. P Casanova has performed experiments. A. Rodriguez has performed necropsies and immunohistochemistry, and K Barger has written the manuscript. All authors have contributed to the analysis of data and the discussion of the results.

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