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# Sexual maturity prediction based on hormonal profiles, testes and semen characteristics in male *Coturnix* quail (Garsault, 1764) in the Western Highlands of Cameroon

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## Abstract

With the aim to investigate age at sexual maturity in male quail in the Western Highlands of Cameroon, a study was conducted on 68 quails of 4 weeks old. Data were collected during 14 weeks on FSH, LH and testosterone levels as well as testes and semen characteristics. FSH and LH rate were similar and higher from 6 to 15 weeks old. Highest testosterone levels were recorded on 15 (2.25 ng/ml) and 18 (2.62 ng/ml) weeks old quails. Testes weight, height, diameter as well as gonadosomatic index recorded from 12 to 18 weeks old were similar but significantly higher than others. Morphometric characteristics of seminiferous tubules at 6 and 9 weeks old were similar but significantly lower than later values. Comparable sizes of seminiferous tubules circumference and germinal epithelium height were noticed from 15 weeks old. Histological analysis of testes sections showed fully developed and active seminiferous tubules from week 12 but, spermatogenic activity was more pronounced at week 15. Mass and individual motilities were comparable with quail age. However, the highest sperm count was recorded at 12 weeks old compared to later values. It has been concluded that age at sexual maturity in male quail could be situated around 12 weeks in the context of the Western Highlands of Cameroon.

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## Introduction

In domestic birds, secondary sexual characteristics (comb, wattle, pin and characteristic feathers and vocalization) have been used as external indicators of sex and sexual maturity stage (Biswas *et al.,* 2010). Growth of combs and wattles is induced by androgens especially testosterone (Sauveur, 1988; Guerin *et al.,* 2011). Comb and wattle are vestigial in quail and then, cannot be considered as an efficient indicator of sexual maturity. Androgens also induce the development of the cloacal gland which is an organ only found in the genus *Coturnix* but it appear lately when the male is already reproductively active. Age at sexual maturity refers to age at which the reproductive system achieved its complete development and at which fertility is effective (Sauveur, 1988; Froman *et al.,* 2004; Guerin *et al.,* 20011). Fertility of poultry depends on success of a number of critical steps of spermatogenesis, extra-gonadal maturation, survival and function of sperm in the oviduct (Froman *et al.,* 2011). Age at sexual maturity is also associated with the highest testes weight and highest blood concentration of testosterone and luteinizing hormone (Gilbert and Jeanine, 2005). Increase in testes weight is due to an increase in number and volume of Leydig cells (responsible of steroidogenesis) during sexual maturation (Gonzalez- Moran and Soria-Castro 2010). Mature testis has large irregular-shaped seminiferous tubules with a multi-layered germinal epithelium consisting of cells representing all stages of spermatogenesis (Johnson, 1986). This is what causes the test is to swell in size at sexual maturity and during the breeding season (Aire, 1997). In fact, Shil *et al.* (2015) recorded in Japanese quail, heaviest paired testes weight and pronounced spermatogenic activity in summer and rainy season in Bangladesh. They also observe that testes weight and diameter as well as germinal epithelium height of seminiferous tubules were positively correlated. Sperm concentration and motility are primary determinant of fertility of the domestic poultry (Froment *et al.,* 2009). It has been established that morphometric study of the testis of any breed estimating quantitative changes in testicular components and spermatogenic functions arising from factors such as age (Lin *et al.,* 1990; Al-Tememy, 2010; Okpe *et al.,* 2010; Vatsalya and Kashmiri, 2012), season (Noirault *et al.,* 2006a; Noirault *et al.,* 2006b; Akbar *et al.,* 2012; Shil *et al.,* 2015) and others factors (Ekinci and Erkan, 2012; Ozegbe and Aina, 2012; Djitie *et al.,* 2014). Testicular growth, measurements and morphohistology could be used as important indicators of age-based growth modifications (Noirault *et al.,* 2006a) and age at sexualy maturity. This study was carried out to assess testes development and activity, associated with FSH, LH and testosterone profiles that could provide additional understanding of the role of age, some hormones and testes in the development and prediction of poultry sexual maturity especially in quail raise in Western Highlands of Cameroon.

## Materials and methods

### *Study area*

The study was carried out at Teaching and Research Farm of the University of Dschang (LN 05°26', LE 10°3'). Dschang is located about 1420m above sea level. Climate is Sudano-guinean temperated by altitude and about 2000 mm of rainfall spread over a single season from mid-March to mid-November. The average temperature is 20° C and relative humidity generally exceeds 60%.

### *Animal, diet and experimental design*

In the present work, a total of 68 male quails of 4 weeks old was divided into 4 comparable batches (as repetition) of 17 birds. Throughout the test, an iso-energetic and iso-proteic diet (Table 1) was used. Birds were housed and kept under similar environmental and managerial conditions. During the whole period of the test, birds have free access to feed and water in adapted equipment.

**Table 1.** Composition and calculated chemical values of diet.

| Ingredients (Kg) | Diet composition for 100 kg |
|---|---|
| Corn | 60 |
| Wheat bran | 14 |
| Cotton cakes | 05 |
| Soya cakes | 05 |

| Ingredients (Kg) | Diet composition for 100 kg |
|---|---|
| Fish meal | 14 |
| Bone meal | 0,5 |
| Palm oil | 01 |
| Premix 0,5%* | 0,5 |
| Total (kg) | 100 |
| Calculated chemical values | |
| Protein contents (%) | 20,11 |
| Metabolizable Energy (kcal/kg) | 2902,70 |
| Energy /Protein ratio | 144,34 |
| Fat (%) | 4,21 |
| Calcium (%) | 1,19 |
| Phosphor (%) | 0,84 |
| Lysine (%) | 1,30 |
| Methionine (%) | 0,49 |

**Premix 0,5 : mixture of vitamins A, B complex, D, K and E principally and incorporated at 0,5% in diet*

*Data collection and studied parameters*

Data were collected every 3 weeks starting from the 6th till the end of study on live body weight, FSH, LH and testosterone concentrations in serum, testes weight and measurements, gonadosomatic index, histomorphometry of seminiferous tubules and some semen characteristics.

*Semen and testes analyses*

Live body weight was recorded before the birds were slaughtered. Semen was collected immediately after bird decapitation by a slight pressure on ductus deferens from epididymis ductus till the base of the phallus. The collected semen was maintained at 38˚C - 40˚C using a water bath for sperm motility assessment. Sperm count, mass and individual motility were evaluated as described by Ngoula *et al.* (2012) and Mamun Tarif *et al.* (2013).

Testes were cleaned and weighed to the nearest milligram (mg) using a precision digital weighing scale. Testes diameter and height were measured using a digital caliper (150 MM with 0.02 mm precision). The left testicular shape index was obtained by dividing testicular diameter by testicular height. Gonadosomatic index was the percentage of paired testes weight related to the live body weight.

*Hormonal Analyses*

Serum levels of LH and FSH were determined by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) while Testosterone was evaluated by a competitive technic of ELISA. For all hormonal analyses, kits were providing by the same manufacturer (Diagnosis Automation, Inc., Calabasas, USA).

*Testes morphometry and histological analyses*

Testes tissues were fixed in Bouin's solution at 20 °C for 24 h and then, conserved in formalin (4%) till manipulation period. After cleaning with distilled water, dehydration with ethyl alcohol in increasing concentration (70-100%) and passed in two content of xylol, testes samples were embedded in paraffin, sectioned by a rotary microtome at 7µm. Slides samples were passed through decreasing concentrations (100-70%) of ethylic alcohol and in xylol stained with hematoxylin and eosin following the method described by Humason (1972).

In order to record histomorphometric data, 15 photomicrographs (10 at 10× and 5 at 40×) were taken randomly from the central zone of cross-sections of each testis using a Leica Photomicroscope. The circumferences and diameters of the seminiferous tubules, circumferences of the lumen and the germinal epithelial height were evaluated using ImageJ 1.48v software.

*Statistical analysis*

Collected data were subjected to one way analysis of variance (ANOVA) and differences were considered to be significant if P was $< 0.05$. Duncan multiple range test was used to separate means. Pearson correlation coefficient for some parameters was also performed (Steel and Torrie, 1980). IBM SPSS Statistics 21.0 was used for statistical analysis.

## Results and discussion

*Results*

*Testicular weight and measurements*

Testis height and diameter increased with age (table 2). Testis development is extremely high between 6th and 9$^{th}$ week old; it reduced slightly from 9$^{th}$ to 12$^{th}$ week and significantly till 18 weeks of age. Significantly (P < 0.05) lowest values of testis weight, height and diameter were recorded at 6$^{th}$ week old. Except the right testis diameter, values of the different parameters at the 9$^{th}$ week old were also significantly (P < 0.05) lower compared to comparable (P > 0.05) values obtained at 12, 15 and 18 weeks old. Left/right testis ratio was almost higher than 1 and no significant difference was recorded for this parameter during the entire period of the study. Left testis shape index increased with quail age. Although values obtained at 6$^{th}$ (0.54) and 9$^{th}$ (0.59) week were similar (P > 0.05), the 6$^{th}$ week shape index was significantly (P < 0.05) lower than those recorded from week 12 (0.65) till the end of the study.

**Table 2.** Evolution of testicular characteristics of quail cock between 6 and 18 weeks old.

| Parameters | | Age (Weeks) | | | | |
|---|---|---|---|---|---|---|
| | | 6 | 9 | 12 | 15 | 18 |
| Testis weight (g) | Left | 0,02±0,01$^{a}$ | 1,44±1,31$^{b}$ | 2,96±0,51$^{c}$ | 3,38±0,51$^{c}$ | 3,40±0,75$^{c}$ |
| | Right | 0,01±0,01$^{a}$ | 1,12±0,95$^{b}$ | 3,07±0,44$^{c}$ | 3,16±0,58$^{c}$ | 3,62±1,54$^{c}$ |
| | Total | 0,03±0,01$^{a}$ | 2,56±0,22$^{b}$ | 6,04±1,47$^{c}$ | 6,53±1,57$^{c}$ | 7,02±1,40$^{c}$ |
| Left/right testis ratio | | 1,70±1,34$^{a}$ | 1,24±1,15$^{a}$ | 0,96±0,80$^{a}$ | 1,07±0,78$^{a}$ | 0,99±0,21$^{a}$ |
| Testis height (cm) | Left | 0,42±0,74$^{a}$ | 1,86±0,53$^{b}$ | 2,54±0,22$^{c}$ | 2,52±0,27$^{c}$ | 2,47±0,29$^{c}$ |
| | Right | 0,41±0,64$^{a}$ | 1,78±0,47$^{b}$ | 2,67±0,22$^{c}$ | 2,59±0,22$^{c}$ | 2,48±0,39$^{c}$ |
| Testis diameter (cm) | Left | 0,23±0,47$^{a}$ | 1,09±0,28$^{b}$ | 1,64±0,04$^{c}$ | 1,65±0,09$^{c}$ | 1,64±0,12$^{c}$ |
| | Right | 0,21±0,46$^{a}$ | 1,02±0,17$^{b}$ | 1,56±0,14$^{b}$ | 1,60±0,18$^{b}$ | 1,59±0,14$^{b}$ |
| Left testis shape index | | 0,54±0,10$^{a}$ | 0,59±0,08$^{ab}$ | 0,65±0,6$^{b}$ | 0,66±0,60$^{b}$ | 0,67±0,10$^{b}$ |
| Gonadosomatic index (%) | | 0,015±0,01$^{a}$ | 1,28±1,12$^{b}$ | 3,20±0,21$^{c}$ | 3,28±0,40$^{c}$ | 3,28±1,28$^{c}$ |

*a,b,c Means within line with different superscripts differ significantly P < 0.05*

*Seminiferous tubules histomorphometry*

Morphometric evaluation of seminiferous tubules revealed that different parameters increased significantly (P < 0.05) with age (Fig. 1). However, values recorded at 6th and 9$^{th}$ week old were similar but significantly (P < 0.05) lower than those recorded lately. Seminiferous tubule circumferences and the germinal epithelium thickness were equally similar (P > 0.05) at 15 and 18 weeks old. Seminiferous tubules diameter and lumen circumference at 15 weeks old was on one hand, significantly (P < 0.05) higher than the value obtained at 12 weeks but on the other hand, lower than the one recorded at 18 weeks old in quail. Positive and significant correlations at the level of 0.01 also exist between all seminiferous tubules parameters in quail testis.

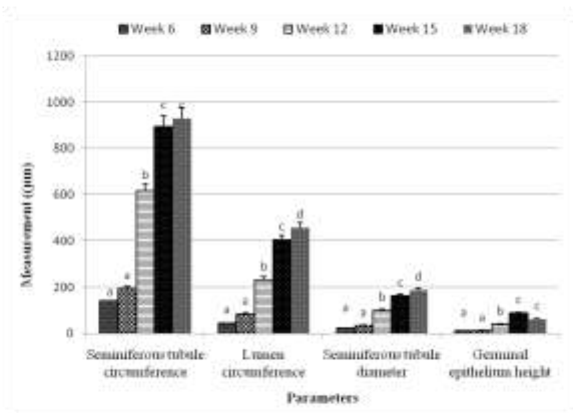


**Fig. 1.** Histomorphometrical variation of seminiferous tubules characteristics with age.

*a,b,c – for the same parameter, bars with different superscripts differ significantly P < 0.05*

Morphological analysis of seminiferous tubules (Fig. 2) revealed an increase in size of seminiferous tubules and especially, the effectiveness of spermatogenesis from 12 weeks old in quails. Spermatogenesis seem to be acute at 15th week and at 18th week, 400× magnification can revealed some large and empty seminiferous tubules lumen (8) and other lumens showing reduced size with high concentration of spermatozoa (9).

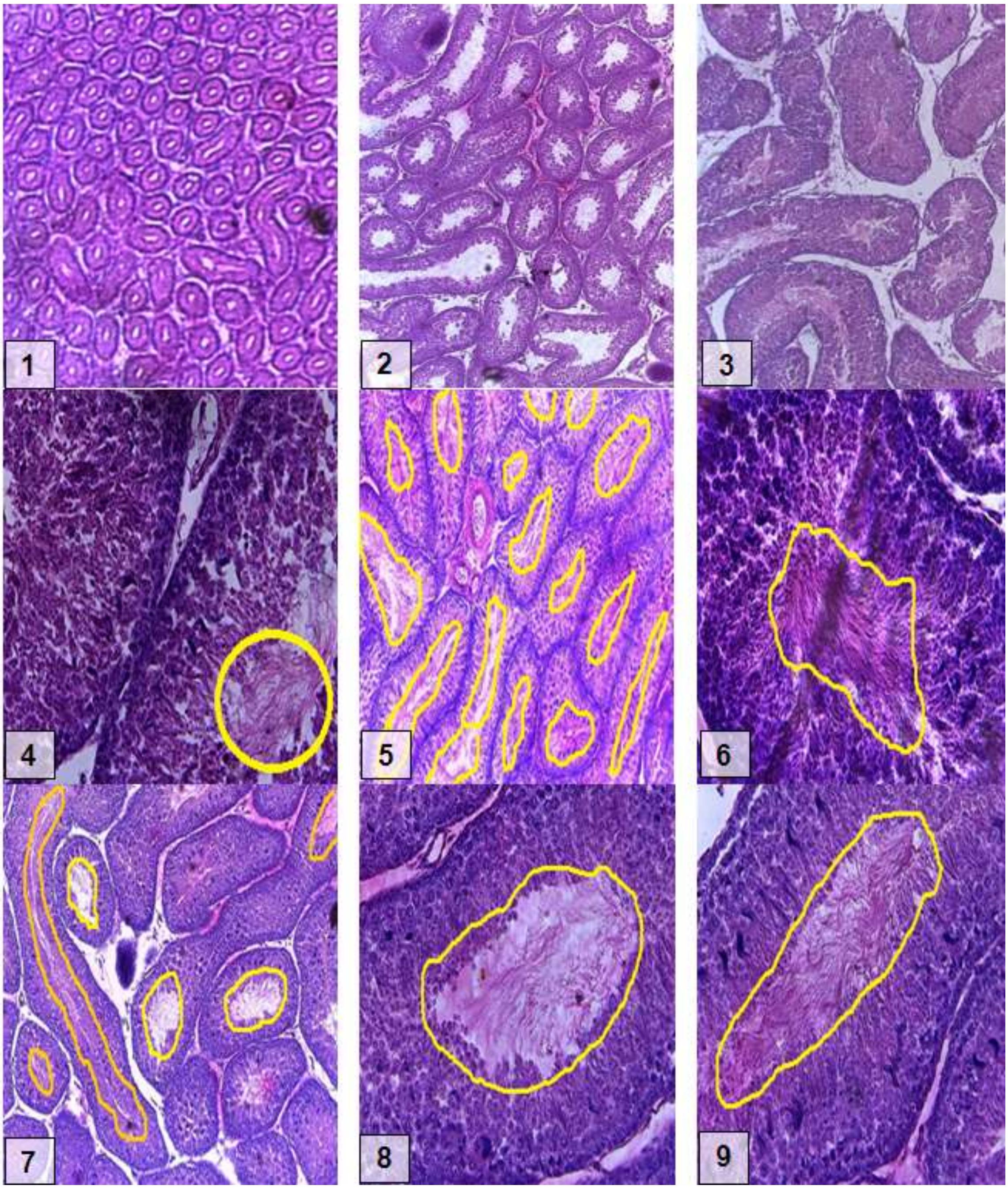


**Fig. 2.** Histological evolution of seminiferous tubules of quail testes sections from 6 to 18 weeks old.

**1.** Growing and nonfunctional seminiferous tubules (Week 6, 100×), **2.** Seminiferous tubules initiating spermatogenesis (Week 9, 100×), **3.** Active seminiferous tubules (Week 12, 100×), **4.**Spermatozoa in the seminiferous tubules lumen (Week 12, 400×), **5.** Generalized and pronounced spermatogenic activity (Week 15, 100×), **6.** Abundant spermatozoa in lumen (**15** week, 400×), **7.** Cross testes section at 18 weeks old displaying high (orange) and reduced (yellow) activity levels (100×), **8.** Seminiferous tubule at 18 weeks old presenting large and relatively empty lumen (400×), **9.** Seminiferous tubule at 18 weeks old showing pronounced spermatogenic activity (400×).

*FSH, LH and testosterone profiles*

As presented in fig. 3, significantly ($P < 0.05$) highest LH concentration was recorded at 15$^{th}$ week (1.04 ng/ml) and the lowest (0.28 ng/ml) at the 18$^{th}$ week old. LH values recorded between weeks 6 and 15 were similar. Comparable values were also obtained at week 9 and 18.

FSH concentrations varied from 0.14 (week 18) to 0.92 (week 12) ng/ml during the present study. FSH concentrations were similar between weeks 6 and 15 but were also significantly ($P < 0.05$) higher than the value recorded at 18 weeks of age.

Testosterone concentrations were similar between age of 15 (2.25 ng/ml) and 18 (2.62 ng/ml) weeks but were significantly ($P < 0.05$) higher than values obtained between 6 and 12 weeks. Testosterone concentrations at week 6 and 12 were also comparable and significantly ($P < 0.05$) lower than value obtained at week 9.

*Semen characteristics*

Effect of age on semen characteristics presented in table 3 shows that, mass and individual motilities varied from 3 to 3.17 and from 2.67 to 3.17 respectively. However, the two parameters were not significantly ($P < 0.05$) affected by age. Sperm counts per ml and per gram of testis were significantly ($P < 0.05$) higher at week 12 compared to values recorded later and whose comparable values were also recorded.

**Table 3.** Variation of semen characteristics in quail cock between 6 and 18 weeks old.

| Parameters | Age (Weeks) | | |
|---|---|---|---|
| | 12 | 15 | 18 |
| Mass motility | 3,00±0,63$^{a}$ | 3,00±0,00$^{a}$ | 3,17±0,41$^{a}$ |
| Individual motility | 2,67±0,82$^{a}$ | 2,67±0,52$^{a}$ | 3,17±0,41$^{a}$ |
| Sperm count ($10^9$/ml) | 8,42±2,81$^{b}$ | 4,17±1,31$^{a}$ | 4,03±2,29$^{a}$ |
| Sperm count ($10^9$/g testis) | 3,42±0,81$^{b}$ | 1,37±0,74$^{a}$ | 1,89±1,26$^{a}$ |

*a,b Means within line with different superscripts differ significantly $P < 0.05$*

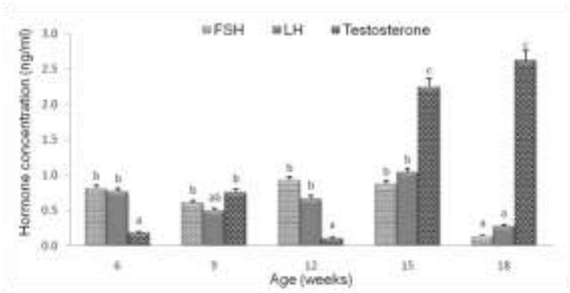


**Fig. 3.** Variation of FSH, LH and testosterone profiles with age.

*a,b,c - bars with the same design and different superscripts differ significantly $P < 0.05$*

**Discussion**

Testes weights recorded in this work are higher than those observed at 52 days by Vatsalya and Kashmiri, (2012) as well as Saqib *et al.* (2001). This could be justified by the fact that weights recorded by these authors, were collected from younger birds compared to ours. In fact, weight and testicular volume generally increase with age until puberty and during breeding season where maximum values are reached (Sauveur, 1988). This work showed that the weight of the left testis is almost always higher than the right testis weight. This confirms observations of other authors (Johnson, 1986; Sauveur, 1988; Vatsalya and Kashmiri, 2012) in birds. In fact, studies have shown in quail (Vatsalya and Kashmiri, 2012), rooster (Moller, 1994; Yu, 1998; Vatsalya and Kashmiri, 2012), Muscovy duck (Sauveur and de Carville, 1990), Mullard duck (Denk and Kempenaersle, 2005) and turkey (Burke, 1973) that there is a dimorphic weight and volume in favor of the left testicle. However, at 12 and 18 weeks old, weight of the right testis was slightly higher than that of the left testicle. Similar observations were made by Vatsalya and Kashmiri, (2012) for three weeks in quail also. According to Deviche *et al.* (2011), this asymmetry in growth seems to be likely due to low sensitivity of the least developed testis to gonado-stimulating factors. However, cellular basis of this potential difference have not yet been investigated (Sauveur, 1988; Deviche *et al.,* 2011). The rapid testicular growth up to 12 weeks is due to the high proliferation of Sertoli cells. Their increase in size results in the development of the seminiferous tubules leading to a gradual increase in testicular weight and volume (Denk and Kempenaersle, 2005; Sauveur, 1988).

Gonadosomatic indexes recorded between 9 and 18 weeks of age are higher than value (0.01%) obtained by Djitie *et al.* (2015) on 7 weeks old quail. Between 12 and 18 weeks old, gonadosomatic indexes were close to 3,68% recorded by Lanna *et al.* (2013) on 60 days old Japanese quail in Brazil. Regarding these results, it is clear that age at sexual maturity could be affected by geographical area and strain of quail since in the present study, a gonadosomatic index of 1.1% has been recorded at 63 days and is very low compared to 3,68% recorded by Lanna *et al.* (2013) on 60 days old Japanese quail.

In general, testicular height and diameter significantly increased with age. Unlike the left testis diameter that has been relatively higher than value of the right testis throughout this study, the left testis height shows same observation only between 6 and 9 weeks. The increase in these measurements is due to the increase in height and diameter of seminiferous tubules, as well as in Leydig and interstitial cells number (Braun, 2004; Sauveur, 1988). In fact, during the prepubescent period, testicular development is highly correlated with the number and size of Sertoli cells while during puberty; it is better correlated with germ cells (Kirby *et al.,* 1996).

Seminiferous tubules showed a diversity of form but the majority has circular shape. The circumference of seminiferous tubules significantly increased and reached 931 microns at 18 weeks old. Same observation was done for the lumen circumference (457 microns) at the same period. Seminiferous tubules circumference value corroborates those obtained by Shil *et al.* (2015) in the rainy season and summer with Japanese quail in Bangladesh. However, seminiferous tubules diameter and germinal epithelium height were higher in their work compared to ours. Birds age could explain this difference since those used in the context of our study were relatively young compared to theirs which have been for long in reproduction. Seminiferous tubules diameter obtained in our study at 15 and 18 weeks (164 and 187 microns respectively), were higher than those (155 microns) reported by Baraldi Artoni *et al.* (2007) in partridge during reproduction in Brazil. These differences could be justified by weight and testicular measurements which vary considerably not only with bird species and strains, but also with environmental parameters such as the season. In fact, testicular growth is significant during breeding season. Its growth rate is in close relationship with an increase of volume, diameter and length of seminiferous tubules (Hien, 2002). Testicular regression in non-breeding period could be in some cases, the justification of low values of these parameters (Kirby and Froman, 2000; Aire and Ozegbe, 2007; Deviche *et al.,* 2011).

The present study showed an irregular evolution but comparable values of FSH concentrations till 15 weeks and a significantly lower value at week 18. FSH in birds as in mammals, mainly involved in the regulation of gonadal growth and their secretory activity (Jones and Lin, 1993; Deviche *et al.,* 2011). In fact, as demonstrated by Tsutsui and Ishii, (1978) in Japanese quail, FSH and Testosterone act synergistically to increase testicular weight by inducing Sertoli cell hypertrophy. Blood concentration of FSH is high during the prepubescent phase when testicular growth is rapid. However, it declines at the end of puberty when testis are fully developed (Sauveur, 1988). Results of the present study suggest that quail testis could be fully developed and active around age of 12 weeks. In fact, this period corresponds at the moment where semen collection was possible and where spermatozoa were present in seminiferous tubules. Highest FSH concentration and sperm production at week 12 confirms observation of Deviche *et al.* (2011) who reported that spermatogenesis initiation requires high FSH concentration in blood. FSH value at 12 weeks in the present study was close to value recorded by Kobayashi and Ishii (2002) on Japanese quail; but was smaller than value obtained by Vizcarra *et al.* (2010) on broiler breeders. Giving these results, differences could be attributed to genetic material and management.

The present study revealed an irregular evolution of LH level during the whole period of the study and then, corroborate findings of de Revier and William (1978) on rooster between 2 and 42 weeks old. These authors noticed that changes in plasma level of LH were characteristic of the prepubescent phase. They coincide with the proliferation of Sertoli cells but do not result in any change in plasma testosterone level which is very low at that time. The period of 6 to 12 weeks may be considered in quail as prepubescent period. In fact, if females are generally characterized by a precocity in egg laying, sexual maturity of males arrives lately compared to females (Sauveur, 1988). Although LH evolution tendency was similar to de Revier and Willam (1978) as well as Vizcarra *et al.* (2010), LH values in the present study were smaller than those recorded on male quail (Wilhems *et al.*, 2005 ; Li *et al.,* 2006) at 6 to 9 weeks of age as well as on Guinea fowl (bacon *et al.,* 2000). However, they were close to values recorded at 12 weeks old on Japanese quail by Kobayashi and Ishii (2002). According to these observations, it could be suggest that blood LH concentration vary with specie and age as reported by Vizcarra *et al.,* (2010) during their work on broiler breeders.

In birds, testosterone is responsible for the development of the male genital tract, secondary sexual characteristics and specific behavioral expression. It also plays a role in spermatogenesis. From results of the present study, it appears that testosterone level was low until the 12$^{th}$ week. However, it increased significantly from the 15$^{th}$ week. This increase suggests that quail could have reached puberty around 12 weeks. In fact, according to several authors (Johnson, 1986; Sauveur, 1988; Penforld *et al.,* 2000; Guerin *et al.,* 2011), testosterone levels increase gradually then sharply and reach an average of 2.5 ng/ml during the breeding season. That comes into close conformity with the values obtained between 15 and 18 weeks and could correspond in this case to the end of the pubescent phase.

Evaluation of semen characteristics is an important step for reproduction management. Sperm motility test are among the fastest and efficient way to know sexually matured males and semen quality. No significant effect of age on mass and individual motilities between 12 and 18 weeks old was observed. Mass motility values were similar to those obtained by Tadondjou *et al.,* (2014) who also not noted any significant effect of age on this parameter among Cameroon village barred roosters between 20 and 40 weeks old. Unlike us, these authors noticed a significant effect of age on individual sperm motility. Differences could be explained not only by the genetic material that was different, but also by sperm collection frequency and method. In fact, abdominal massage was used for semen collection in their case while in the present study, semen was directly collected from the ductus deferens recognized for their ability to store maturating sperm that do not yet have full motility of fertilizing potential (Millan, 1997; Kirby and Froman 2000).

**Conclusion**

It has been concluded that age at sexual maturity in male *Coturnix* quail could be situated around 12 weeks in agro climatic conditions of the Western Highlands of Cameroon.

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