

TESTICULAR HYPOPLASIA IN BOAR: ASSOCIATION BETWEEN SEMINAL ANALYSIS AND HISTOPATHOLOGY

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ABSTRACT

The aim of this study was to describe the occurrence of testicular hypoplasia in boar and correlate the findings of testicular biometrics and histopathological evaluation with the seminal parameters. The work was conducted at the Experimental Center of Porcine of the Federal University of Lavras when four boars were acquired. Since 190 ± 12 days of age, the boars were subjected to a training protocol for semen collection, and during 130 days, were made andrological evaluations weekly, with testicular weighing, biometrics and volume. In one animal was observed total absence of sperm in the ejaculated, since the first semen collect, despite having normal volume of semen and was also found smaller testicular volume compared to the others. When the boar completed three and half years old, a surgical castration was performed, being collected samples from both testis and epididymis for histopathological evaluation. On histological evaluation, there was a reduced diameter in the seminiferous tubules, which were formed only by Sertoli cells with rare spermatogenic lineage cells. A marked proportional increase in the number of Leydig cells in relation to the seminiferous tubules was observed, and the testis was covered by a thick layer of connective tissue, which formed trabeculae that radiated into the parenchyma. In the samples from the epididymis were not observed sperm. Bilateral testicular hypoplasia was present and could be previously detected by the testicular volume and semen analysis, being confirmed by histopathological evaluation of the testes and epididymis of the boar, association that is essential for the definitive diagnosis.

KEY WORDS: Azoospermy; Fertility; Reproduction; Testicles.

INTRODUCTION

Testicular hypoplasia is characterized as a congenital underdevelopment of the gonads, in which can be observed, by means of cytology and testicular histology, a very low number or even absence of germ cells in seminiferous tubules. It can occur in all farm animals, as in bulls (HUMPHREY; LADDS, 1975), ram (BRUERE ET AL., 1969) and pigs (HANCOCK; DAKER, 1981; SINGH; PARIHAR, 1998).

Considering the swine specie, Singh and Parihar (1998) conducted a study with 88 boars and they found that the frequency of testicular hypoplasia in these animals was 12%. According to Foster and Ladds (2007) this is a hereditary disorder caused by a pair of recessive genes with incomplete penetrance and variable expressivity. Therefore, this disorder may occur in total or partial forms, unilateral or bilateral and other combinations and degrees of involvement.

Regarding to anatomopathology the testicular hypoplasia can be divided into three types: mild (partial), intermediate and total (severe) (FOSTER; LADDS, 2007). In severe hypoplasia, all or nearly all of tubules are hypoplastic, presenting a small testis, consistent with the palpations and resistant to the cut. The animal may present oligospermia or azoospermia, in this case with high rates of pathological forms of sperm in the ejaculate. When bilateral, severe hypoplasia makes the animal sterile and the diagnosis becomes more simple, but when the effect is only partial the animal is considered sub-fertile or temporarily infertile. In this case, the diagnosis may be more difficult.

According to Rao et al (1980) testicular hypoplasia is quite similar to testicular degeneration, but can be differentiated by its irreversible character. Histologically, there are no areas of fibrosis or basement membrane thickening such as testicular degeneration. Also notes total absence of germinal epithelium, only Sertoli cells within seminiferous tubules, unlike degeneration, in which there are sperm cell lineage, but with vacuolization of the epithelium at different degrees. Testicular hypoplasia can also be confused with sexual immaturity, as the prepubertal animal clinically presents reduced gonads size and a low sperm production typical of hypoplasia; however, the realization of repeated reproductive examinations allows to observe a progressive increase in testicular size, quantitative and qualitative evolution of ejaculated, showing the delayed on the puberty (RAO et al., 1980). Therefore, an appropriate and highly detailed history is essential for the correct differentiation between testicular hypoplasia, sexual immaturity and testicular degeneration.

The study aims to report the occurrence of testicular hypoplasia in one boar and describe the findings in the examinations of testicular biometric, seminal and

histopathological evaluation correlating these characteristics with the definitive diagnosis of this anomaly.

MATERIAL AND METHODS

The study was conducted at the Department of Veterinary Medicine (DVM) and at the Department of Animal Science (DAS) of the Federal University of Lavras, Lavras/MG since October 2006 to October 2009. Four high performance boars aged between five and six months and initial weight of 113.63 ± 3.25 kg were acquired by the Experimental Center of Porcine (DAS) for replacement of the flock of boars. From 190 days of age, were submitted to a specific training protocol for semen collection.

During 130 days since the beginning of training, when the animals had 190 ± 12 days of age were realized detailed andrological evaluations weekly, a period in which we determined the weight gain of animals, sexual behavior and monitoring of testicular development through testicular biometrics parameters. In this latter, we measured the length and the width of right testis (LRT and WRT) and left (LLT and WLT), following the model proposed by Schinckel et al. (1984) For the calculating the testicular volume was used the formula described by Owsianny et al. (1998): $T = \frac{4}{3} \times \pi \times a \times b^2$, where: T=testicular volume (mL) $\pi=3.14$, $a=1/2$ length testicular (cm) and $b=1/2$ testicular width (cm).

One of animals presented azoospermia (absence of sperm), despite the normal semen volume produced and satisfactory sexual behavior. This animal was kept on the flock just like a ruffian and subjected to the semen collection once a week just to exhaustion and volume determination, smell and semen appearance. Upon reaching 42 months of age and a total of 152 ejaculations, the animal was subjected to the surgical castration. Samples from testis and epididymis were collected for histopathological evaluation and the fragments were fixed in 10% buffered formalin and stained with hematoxylin and eosin (HE) for subsequent evaluation in an optical microscope.

The histology description of the testis and epididymis of the ruffian associated with the data of the testicular biometrics, reproductive characteristics and seminal evaluation were performed.

RESULTS

During the evaluation period the four animals evaluated showed similar average daily weight gain, which was 421 g/day for the hypoplastic animal and 415, 596 and 483 g/day for the others, being 1, 2 and 3, respectively.

During the training period and semen collection, all boars had good libido and ejaculate. However, in the hypoplastic animal was observed total absence of sperm in all ejaculates, despite presents a normal semen volume for swine specie, if compared with the other animals (Table 1). In all collections realized, the ejaculated of this animal always presented watery aspect, being this feature suggestive of oligospermia or azoospermia.

Table 1. Biometric characteristics and reproductive parameters (mean \pm standard deviation) of four boars (Hypoplastic x Normals) evaluated since 6-7 months of age for 18 weeks.

Characteristics*	Animals				Means of Normals
	Hypoplastic	Normal 1	Normal 2	Normal 3	
LLT	14.7 \pm 1.67	15.7 \pm 0.85	15.2 \pm 0.87	15.0 \pm 1.01	15.3 \pm 0.89
WLT	5.7 \pm 0.60	8.3 \pm 1.09	7.4 \pm 0.92	8.5 \pm 0.79	8.1 \pm 0.88
LRT	14.2 \pm 1.39	15.4 \pm 1.13	15.1 \pm 1.21	15.1 \pm 1.16	15.2 \pm 1.13
WRT	5.9 \pm 0.54	8.2 \pm 0.96	7.7 \pm 0.74	8.3 \pm 0.90	8.1 \pm 0.82
VLT	259.9 \pm 75.1	585.5 \pm 168.2	448.2 \pm 122.1	574.7 \pm 130.3	533.3 \pm 132.9
VRT	268.6 \pm 64.1	555.8 \pm 142.9	473.0 \pm 115.3	560.1 \pm 131.7	528.1 \pm 125.1
TTV	528.4 \pm 136.8	1141.3 \pm 302.6	921.2 \pm 234.1	1134.8 \pm 258.8	1061.4 \pm 255.3
ASV	204.2 \pm 67.5	250.3 \pm 39.8	231.1 \pm 60.2	161.9 \pm 43.3	214.4 \pm 33.1
SNC	0.0 \pm 0.0	85.9 \pm 26.0	80.3 \pm 61.6	93.9 \pm 29.8	86.7 \pm 29.9
ASC	0.0 \pm 0.0	0.429 \pm 0.130	0.402 \pm 0.308	0.470 \pm 0.149	0.434 \pm 0.150
rASV/TTV	0.402 \pm 0.136	0.233 \pm 0.066	0.263 \pm 0.095	0.148 \pm 0.044	0.211 \pm 0.052
rASC/TTV	0.0 \pm 0.0	0.178 \pm 0.066	0.206 \pm 0.217	0.323 \pm 0.167	0.213 \pm 0.101

*LLT, Length of left testicle; WLT, Width of left testicle; LRT, Length of right testicle; WRT, Width of right testicle; VLT, Volume of left testicle; VRT, Volume of right testicle; TTV, Total testicular volume; ASV, Average seminal volume; SNC, Sperm number counted by

Neubauer chamber; ASC, Average spermatic concentration; rASV/TTV, Relation between Average seminal volume and Total testicular volume and rASC/TTV, Relation between Average spermatic concentration and Total testicular volume.

Regarding the testicular volume, both the left and right, presented itself inferior in the hypoplastic animal when compared to the other animals evaluated. The biometric data corroborate with the macroscopic observations of lower testicular volume in the hypoplastic animal compared to the other, evaluated during its reproductive life in the flock (Figure 1a and b).



Figure 1. Comparative macroscopic aspect of testes in two animals, being: one hypoplastic animal (a) showing a testicular volume much smaller than normal animal (b).

Relating to seminal analysis, the hypoplastic animal had seminal volume similar to the others and being considered normal for the specie (Figure 2). Considering the

normal animals at the flock, we can note that, in the course of the 18 weeks of evaluation, there was an increase of testicular volume (Figure 3).

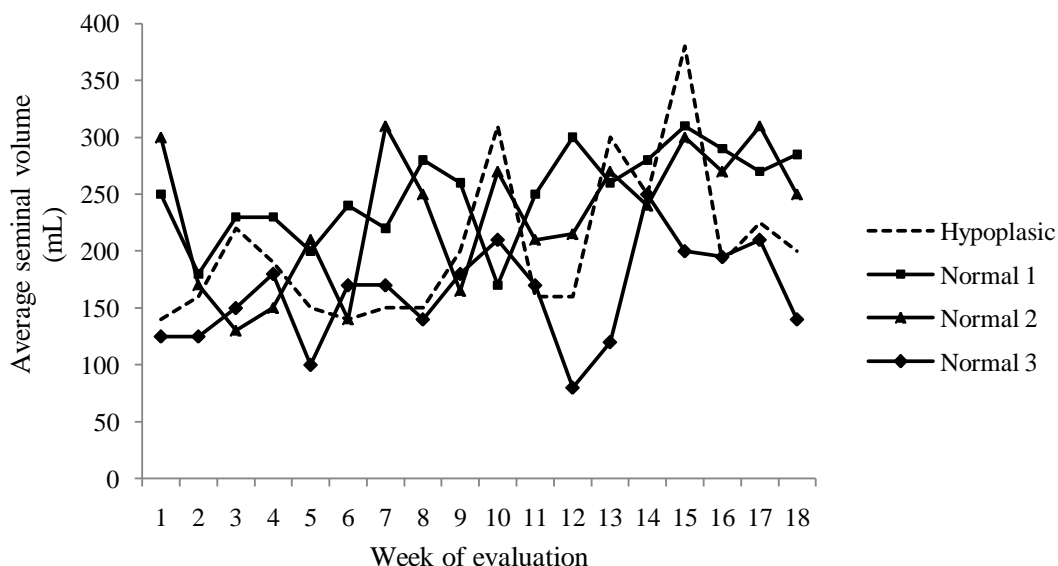


Figure 2. Average seminal volume of hypoplastic animal and normal animals during 130 days (18 weeks) of evaluation.

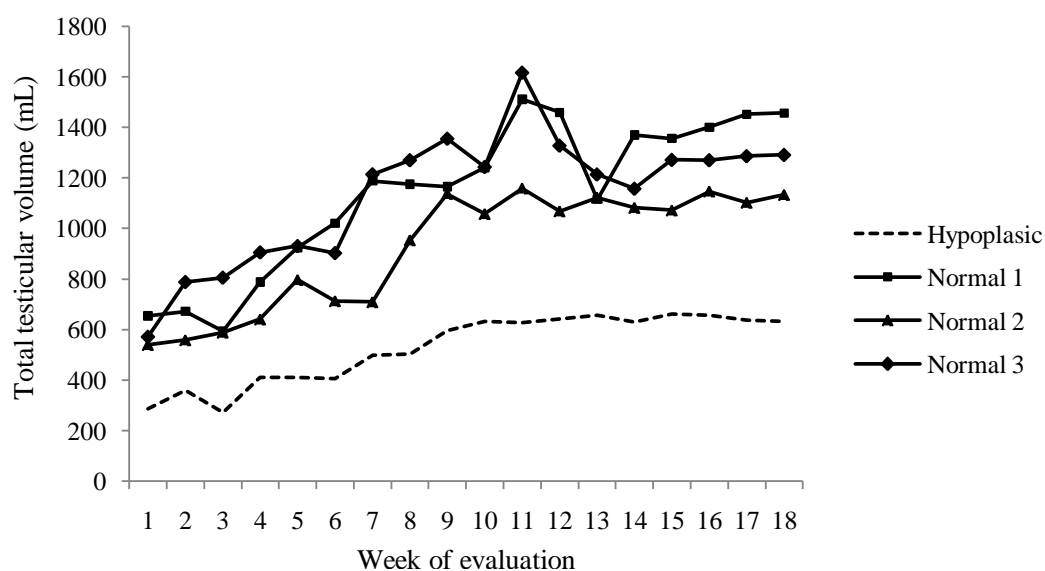


Figure 3. Total testicular volume of hypoplastic animal and normal animals during 130 days (18 weeks) of testis size evaluation.

Regarding the histological evaluation, in compare with a normal anima, the seminiferous tubules of the hypoplastic boar showed widespread small diameter (Figure 4A and B), being constituted only by Sertoli cells, with rare spermatogenic cells of the strain which also not showed mitotic figures (Figure 4C and D). There was a marked proportional increase in the number of Leydig cells in relation to the seminiferous tubules and the tunica albuginea showed thickened by connective tissue layer, forming trabeculae that radiating into the parenchyma involving the seminiferous tubules and Leydig cells. There were no observed sperm within the epididymal ducts of the cauda epididymis (Figure 4E and F).

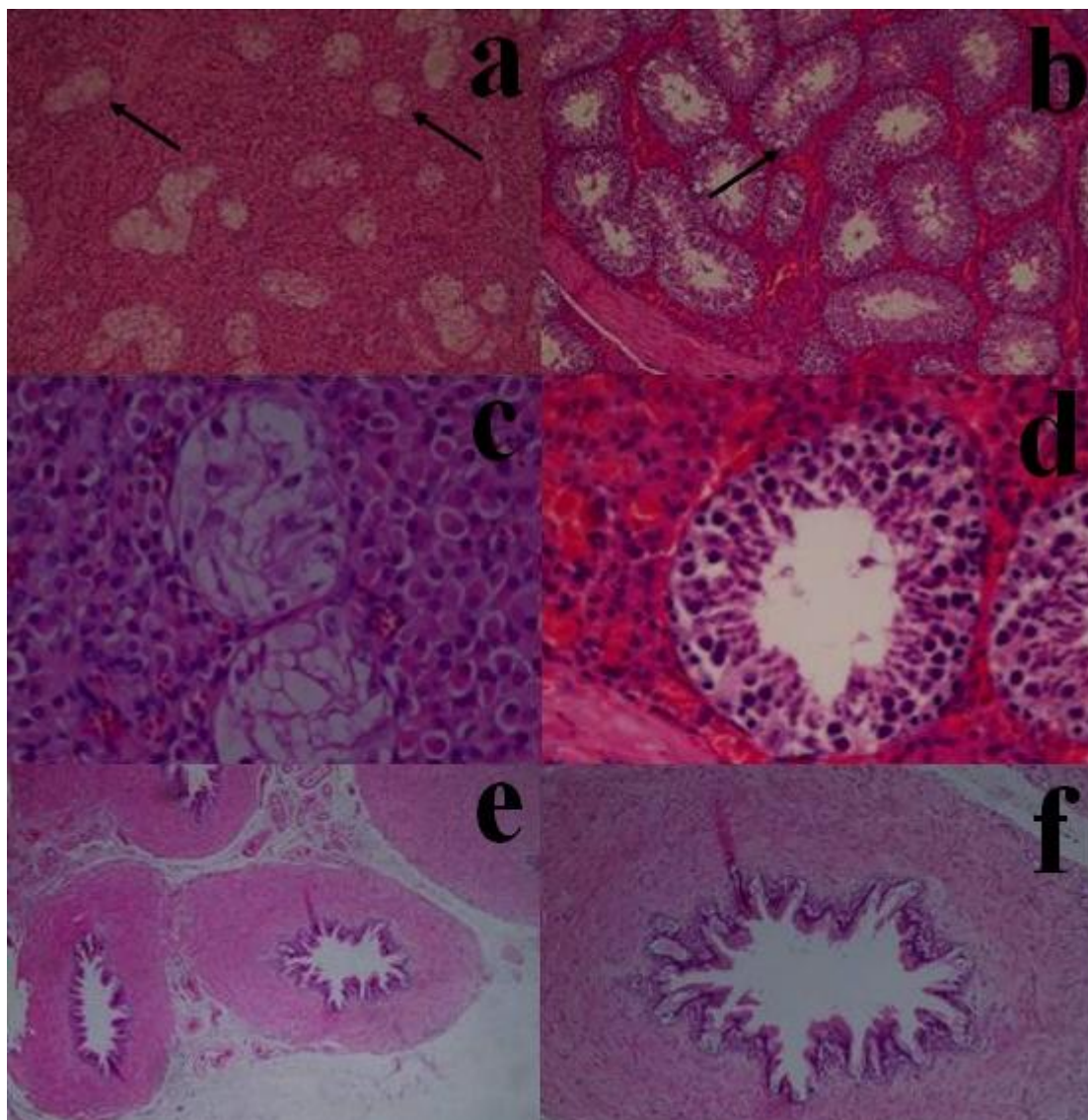


Figure 4 Comparative microscopic aspect, being (a, c, e and f) animal with testicular hypoplasia and (b and d) normal animal: (a) seminiferous tubules of small diameter (arrows); (b) testicular parenchyma showing mainly the larger size of the seminiferous tubules (arrow) (200x magnification); (c) reduced diameter of tubules, which inside consists only of Sertoli cells and absence of spermatogenic cells lineage; (d) seminiferous tubule with large amounts of the spermatogenic cells lineage inside and evident proliferative activity (400x magnification); (e) cauda epididymis, showing absence of sperm in epididymal lumen, which demonstrates a lack of sperm storage inside (200x magnification) and (f) 400x magnification.

DISCUSSION

The smaller testis volume of the hypoplastic animal when compared to normal animals is similar to the findings of Hancock and Daker (1981). In the present study, this difference was around 50% lower. Evaluating thoroughly, it was found that the left testis volume was larger than the right testicle in hypoplastic animal and similarly to the normal animals. These data are similar to those found by Owsiany et al. (1998). This result suggests that the difference in volume between the right and left testis is not a decisive factor in the diagnosis of total testicular hypoplasia.

The biometric data and lower testicular volume in hypoplastic animal found in this study are consistent with Humphrey and Ladds (1975). According to Murgas et al. (2001) the evaluation of biometrics and testicular volume measurements aid in the characterization of puberty and sexual maturity in pigs, because it shows the boar testicular development and suggest its ability to spermatid production. These data are consistent with the findings of biometric and testicular volume found in this study, being that the small testicular volume resulted in non sperm production capacity.

The seminal ejaculate volume of boars can vary of 125 to 500 mL and according to Corrêa et al. (2001) the average volume varies around 200 mL. Therefore, both the hypoplastic animal as the three normal animals showed normal semen volume for the specie. An important fact observed in this study was the similar increase of the ejaculate volume in the hypoplastic animal and sometimes even higher compared to the other animals during its body development (Figure 2), which accompanied its testicular growth (Figure 3). Was also observed that seminal volume of animals ranged of very heterogeneous way during the 18 weeks of evaluation, since the first collection and this rules out the possibility that the volume can be used as a parameter for the hypoplasia diagnosis. Moreover, although the hypoplastic animal does not produce sperm, it was produced a normal semen volume and shows that this reproductive change not affects the accessory glands of the animals and therefore, there is no impairment in ejaculate seminal volume.

Since the beginning of the evaluations the hypoplastic animal showed a total testicular volume (TTV) much lower when compared with the other animals and since the nine week of analysis, the testicular volume has not grow sharp as in the other boars,

remaining almost constant. These data support the hypoplastic testicular occurrence associated with non-sperm production by the hypoplastic animal.

The increase in testicular volume of normal animals during the 18 weeks of evaluation agree with Murgas and Zangeronimo (2004) which mentioning that with increasing age, there are the animals body development, the major testicular development and, consequently, higher spermatic production ability until the animal reaches its full sexual maturity. According to these authors, the spermatic production and the total sperm reserves are directly related to testicular size, which can also be demonstrated in this study, being these dimensions lower in the azoospermic animal. According to Valenzuela (1982) the testicular and epididymis dimensions are highly correlated with age, development and body weight. According to Allrich et al. (1983) an increase in testicular size is due to the increase in diameter and width of the seminiferous tubules and the increase in the Leydig cells number.

The testicular development is a parameter widely used in breeding programs due to be highly correlated with seminal volume and spermatic characteristics of boars. Thus, the evaluation of biometric and testicular volume may help in the choice of males to be used in the pig farming.

In young boars, the testicular measures are also positively correlated with the spermatogenic activity (SCHINCKEL et al., 1983) and with serum levels of testosterone (SCHINCKEL et al., 1984). There are indications that the testicles size may be genetically determined (TOELLE et al., 1984; YOUNG et al., 1986). Rathje et al. (1995) observed increasing of semen production, in a selection of nine generations, in relation to testicular weight and linear measurements of the testicles.

The higher relation between seminal volume with testicular volume of the hypoplastic animal during the evaluation period of the animals, can be explained by the hypoplastic animal presents normal seminal volume despite having a small testicular volume compared to the others. According to Corrêa et al. (2001) a gradual increase in the total ejaculate volume and total number of sperm occurs between seven and twelve months old of the boar, being that maximum levels are reached around the three years of age. In this study, in the end of the evaluation period, the animals were in the age of 320 ± 12 days. Thus, the variation in the seminal volume can be considered normal due

to the animals had not reached full sexual maturity that would result in a more homogeneous standard.

Relating to spermatic concentration, we observe the development of hypoplastic animal compared to the others during the 18 weeks of evaluation of reproductive development. Was observed a total absence of sperm throughout the period, featuring the total bilateral testicular hypoplasia. Relating to the normal animals, there is mixed trend of this parameter, which can be explained by the animals age (MURGAS; ZANGERONIMO, 2004).

All findings on histopathological evaluation are consistent with Foster and Ladds (2007) who report that, in hypoplastic animals, the most or all of the seminiferous tubules are small diameter, often surrounded only by Sertoli cells, and perhaps, a layer basal cells or spermatogonia without mitotic activity to the severe form of testicular hypoplasia.

The absence of sperm within the epididymal ducts of the cauda epididymis demonstrates the non-occurrence of spermatogenic activity and sperm storage. According to Foster and Ladds (2007) the definitive diagnosis of testicular hypoplasia is confirmed by clinical findings and, especially, the histological examination, which reveals partial or total absence of spermatogenic lineage cells associated with the absence of sperm in the epididymis.

CONCLUSION

The bilateral testicular hypoplasia was present and it was previously detected by testicular volume, semen analysis and confirmed by histopathological examination of the boar testicles and epididymis, being this association essential for the definitive diagnosis of total bilateral testicular hypoplasia.

ACKNOWLEDGMENTS

This work was supported in part by FAPEMIG (Research Support Foundation of the State of Minas Gerais), CNPq (National Council for Scientific and Technological

Development), CAPES (Coordination of Improvement of Higher Education Personnel), Veterinary Medicine and Animal Science Graduate Program and Graduate Directory of the Federal University of Lavras, Brazil.

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