Research in Veterinary Science 117 (2018) 28–36

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Histological characteristics of the gonads of pig fetuses and their relationship with fetal anatomical measurements

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ARTICLE INFO

Keywords:
Embryology
Follicles
Morphology
Oogonia
Sertoli cells
Swine
Testis

ABSTRACT

The objective was to evaluate the histomorphometric characteristics of the testis and ovaries of pig fetuses at different gestational ages, as well as their correlation with some fetus measurements. Forty-four fetuses were separated for gender (male and female) and gestational age (50, 80 and 106 days of gestation). After slaughter, fetuses had their body length, head and thoracic perimeters measured and their gonads submitted to histomorphometric analyses. The gonadal characteristics at different gestational ages were statistically compared, correlations with the fetal measurements were performed and equations to predict the gonadal characteristics from the fetal measurements were obtained. The testis weight logarithmically increased along pregnancy, whereas ovary weight increased in a linear manner. The cordonal length and number of Sertoli cells were positively correlated with the fetal measurements, being higher at 106 days gestation, while the nuclear volume of these cells were negatively correlated. The total number of follicles was higher at day 80 and 106 of pregnancy. The number of oogonia decreased along the pregnancy, however, their nucleus size was increased. The number of follicles and volume of oogonia were positively correlated with the fetal measurements, while the number of oogonia was negatively correlated. Equations were obtained for the prediction of gonadal characteristics of fetuses. We concluded that in pigs testis cell proliferation, ovary development and histological organization was more pronounced during the final third of pregnancy. Fetal weight and size were strongly related to gonadal development, and can be used to estimate the histological characteristics of gonads.

1. Introduction

The male and female gonads contain tissues with endocrine and exocrine functions, responsible for the production of reproductive hormones and gametes, respectively. The effectiveness of these organs in adulthood is known to be directly related to reproductive function in animals. However, several factors that occur, not only at reproductive age (Jie-Ge et al., 2007), but also during fetal development (Jalali et al., 2013), can alter the efficiency of gonad function, and consequently, the reproductive capacity of the animal (Durlej et al., 2012; Hejmej et al., 2012). Thus, the effect of several factors on the gonadal development of the fetus, and their relationship to postnatal reproductive activity, have been studied (Bielli et al., 2002; Connolly et al., 2013).

During fetal life, reproductive organogenesis events are regulated by both internal and external factors. The temperature (Marco-Jiménez et al., 2014), nutrition (Rae et al., 2002) and even background noise (Jalali et al., 2013) that the fetus is exposed to during pregnancy can affect their gonadal development. In humans, decreased *in utero* development of gonads has been associated with reproductive disorders, including delayed puberty (Ibanez et al., 2000a) and a reduced size of the ovaries and uterus in adulthood (Ibanez et al., 2000b). In lambs, nutrition restriction during the second half of pregnancy reduced the number of Sertoli cells in the testis in the postnatal period (Bielli et al., 2002). These results suggest that problems that occur during pregnancy may have a detrimental effect on the reproductive performance of the offspring.

Problems in reproductive organogenesis often result from disruption of the hypothalamic-pituitary-gonadal axis, reducing the production of

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https://doi.org/10.1016/j.rvsc.2017.11.005
Received 27 September 2016; Received in revised form 14 September 2017; Accepted 10 November 2017

androgens and estrogens, and consequently, affecting the development of fetuses (Brooks and Thomas, 1995). Moreover, other important events, such as the migration, proliferation and meiosis of germ cells, in addition to folliculogenesis, may be affected. This influences the population of follicles at birth, and therefore, the number of follicles that can be recruited during adulthood (Silva-Buttkus et al., 2003). Even the reproductive behavior of animals can be influenced by low gonadal development, as the sexual differentiation processes of certain brain regions are dependent on sex hormones that are produced both before and after birth (Erhard et al., 2004; MacLusky and Naftolin, 1981). Thus, there is evidence that gonadal development during fetal life may be directly related to postnatal reproductive efficiency.

Limited studies have investigated the association between gonadal development and body development in the pig fetus during pregnancy. Therewith, the use of techniques that can estimate the fetal size *in vivo*, such as ultrasonography, could contribute to the early selection of future breeders while they are still in the uterus. Moreover, information supporting potential researches that investigate different factors that affect gonadal development in fetuses and help in the diagnosis of maternal and fetal diseases are important. Thus, the objective of this study was to evaluate the histomorphometric characteristics of testes and ovaries in pig fetuses at different gestational ages, and their correlation with fetal body measurements.

2. Materials and methods

2.1. Animals and experimental procedure

The procedures were approved by the Ethics Committee on Animal Use of the Federal University of Lavras (protocol number 079/2014). Twenty-one male and 23 female fetuses from 15 gilts with similar number of fetuses (11.7 ± 1.3) were included in the study. Gilts were primiparous DB-90 breed (Swine Genetics DB-DanBred, Patos de Minas, Brazil). They were aged approximately 240 days at slaughter, with a weight of 153.7 ± 11.9 kg. Gilts were housed in individual gestation cages (2.20 m length × 0.60 m width × 1.20 m height). The animals were artificially inseminated on the fourth day of estrus with semen from one boar (Large White) with proven fertility. Three artificial inseminations were performed on each gilt, the first of which was performed when the female presented male tolerance behavior (considered 0 h), and again 12 and 24 h after the initial insemination. The day of the final insemination was considered to be day 1 of gestation.

Females were randomly assigned to three groups, according to the gestational age (50, 80 and 106 days) at slaughter. The gestational ages were randomly chosen, assuming that from the 40th day of pregnancy the fetal skeletons are calcified (Knox and Flowers, 2004) and, at 106 days, the final week of gestation (First and Bosc, 1979). The 80th day of evaluation was considered the intermediate period of evaluation.

During pregnancy, the animals were fed with a corn-soybean meal-based gestational diet, which supplied 300 g of crude protein per day and 6340 kcal of metabolizable energy, in addition to vitamins and minerals, consistent with the nutrient requirements of growing/pregnant animals (Rostagno et al., 2011). Diets were offered twice a day at 8:00 a.m. and 4:00 p.m. During the experimental period, daily monitoring of environmental temperature was performed using maximum-minimum thermometers. The average daily temperature was 23.2 ± 2.0 °C.

2.2. Slaughter and collection of fetal material

The gilts were transported to the commercial slaughterhouse at 6:00 p.m., and remained in hold stalls for 12 h without access to food. On the following day, at 7:00 a.m., the animals were slaughtered in accordance with the regulations of the Brazilian Ministry of Agriculture, Livestock and Supply (decree no. 711, November 1, 1995) (MAPA, 1995). After slaughter, the entire female reproductive tract was removed and dissected. An incision was made to the greater curvature of the uterus in the region of each uterine horn for removal of the fetuses.

Fetuses that exhibited abnormal morphological characteristics, such as a small body size compared with their peers, a whitish, greenish or blackened color, or those with softened consistency were considered to be non-viable (Okere et al., 1997), and were not included in the study.

2.3. Collection and preparation of the testes and ovaries

After removal, the fetuses were weighed using a precision scale (B400; Micronal®, São Paulo, Brazil). The body length (from the front end of the head to the base of the tail), head and chest circumference were measured using a measuring tape. The testes and ovaries were dissected and weighed to obtain the gonadosomatic index (GSI = gonad weight/fetus weight × 100). The right testis of each animal was fixed in buffered 5% glutaraldehyde solution for histomorphometric analysis.

The ovaries were fixed in Bouin solution for the subsequent production of histological slides (Van Straaten and Wensing, 1978). The slides were stained with hematoxylin and eosin, then prepared for histomorphometric analysis.

2.4. Testis morphometric analysis

Histomorphometric analyses were performed from photomicrographs, which were obtained by an image capture system consisting of a trinocular microscope (CX31; Olympus Optical do Brazil Ltda, São Paulo, Brazil), digital image capture camera (SC30; Olympus Optical in Brazil Ltda, São Paulo, Brazil), AnalySIS GetIT software (Olympus Soft Imaging Solutions GmbH, Miinster, Germany) and ocular lenses of 40× and 100× magnification. The images obtained were analyzed with Image J software (National Institutes of Health, Research Services Branch, Bethesda, MD, USA).

For the testicular evaluation, the volumetric proportions of the tubular (lumen, epithelium and tunic) and intertubular compartments (Leydig cells, blood vessels and connective tissue) were assessed from the overlap of images obtained using a graticule containing 352 intersections. For each animal, 15 fields were analyzed per testis, totaling 5280 points, following the standardization of testicular morphometric evaluations (França et al., 2006).

Thirty cross-sections of the seminiferous cords present in the most circular shape were randomly analyzed to determine the average cordonal diameter of each testis (Balarini et al., 2012). The total cordonal length (TL, in meters) of the seminiferous tubules was estimated by the formula $TL = VTS/\pi R^2$, where VTS is the total volume of seminiferous tubules (ml) in the testis and R is the tube radius (R = tube diameter/2) (Attal et al., 1963; Dorst and Sajonski, 1974). The VTS was estimated by the percentage of seminiferous tubules in relation to the testicular volume, representative of the weight of the testis without the tunica albuginea, considering that the testis density is close to 1 (1.03 to 1.04) (França, 1991).

The total number of Sertoli cells in each testis was estimated from the corrected count (CC). The CC was calculated from the number of Sertoli cell nuclei and the thickness of the histological section, also considering the average diameter (AD) of the Sertoli cells, using the formula described by Abercrombie (1946) and modified by Amann (1962), as follows:

$$CC = \text{obtained count} \times \text{slice thickness}/\text{slice thickness} + \sqrt{(AD/2)^2} - (AD/4)^2$$

The AD and the average nuclear diameter of Sertoli cells was obtained from measurements of the major and minor axes of 30 nuclei (Colenbrander et al., 1979). The nuclear volume (NV, μm^3) was obtained by the formula $NV = 4/3\ \pi R^3$, where R is the nuclear radius