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Intrinsic rate of spermatogenesis in free-ranging feral pigs (*Sus scrofa* sp)

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ABSTRACT

The aim of this research was to evaluate the intrinsic rate of spermatogenesis in adult free-ranging feral pigs. Twelve adult male free-ranging feral pigs were captured, sedated, and orchidectomized, and then were released and observed to complete recovery and return to their natural environment. Fragments of the testes were embedded in plastic resin and used to prepare slides for histometric analysis. Characteristics investigated included cell populations in the seminiferous epithelium in stage 1 of the cycle of the seminiferous epithelium, intrinsic rate of spermatogenesis and Sertoli cell index. The efficiency coefficient of spermatogonial mitosis was 7.59, the meiotic index was 3.03, the overall yield of spermatogenesis was 23.97 and the cell loss ratio during the meiotic prophase was 1.04. Each Sertoli cell supported an average of 0.92 type A spermatogonia, 7.01 primary spermatocytes in preleptotene/leptotene, 7.30 primary spermatocytes in pachytene and 22.16 round spermatids. In conclusion, the results of the present study indicate that the supporting capacity of Sertoli cells in free-ranging feral pigs is among the greatest values reported for most domestic animals, and the overall yield of spermatogenesis is comparable to that reported in wild boars.

1. Introduction

The feral pig (*Sus scrofa* sp), also known as the Monteiro pig, originated from a domestic breed that was introduced into the Pantanal region in Brazil by Portuguese colonists in the 18th century. Many of these domestic swine escaped into the wild and formed independent groups (Cavalcanti, 1985). The pigs became feral and multiplied rapidly. The need to survive against predators and adapt to eating what was available in the Pantanal resulted in morphological changes that resulted in pigs similar to wild boars in physical characteristics (Herrera, 1995).

Feral pigs have been bred in captivity by some farmers, mainly in the Pantanal region, for commercial purposes. The meat has less fat and cholesterol compared with the meat of most domestic animals; amounts are similar to those in peccary and wild boar meat. These commercial farms usually produce javonteiros, a cross between feral pigs and wild boars (Herrera, 1995; Sollero, 2006). Despite its commercial potential, the feral pig has not been well studied, and there is little information about its physiology, behavior and management. The aim of the present research

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Table 1
Number of cell types in stage 1 of the cycle of the seminiferous epithelium in free-ranging feral pigs.

Animal	Non corrected numbers					Corrected numbers[a]				
	SC	A	PL/L	PQ	RS	SC	A	PL/L	PQ	RS
1	7.57	10.86	64.12	70.00	179.86	6.05	5.08	36.78	36.22	113.12
2	5.00	9.61	54.17	62.61	180.50	3.99	4.50	31.07	32.40	113.52
3	6.46	9.54	35.92	40.23	88.00	5.16	4.47	20.60	20.82	55.35
4	7.40	13.40	59.20	68.20	229.60	5.91	6.27	33.96	35.29	144.40
5	7.08	11.67	63.34	74.58	203.00	5.66	5.46	36.33	38.59	127.67
6	4.00	6.77	42.77	45.69	145.46	3.20	3.17	24.53	23.64	91.49
7	4.88	8.50	66.13	64.00	181.56	3.89	3.98	37.93	33.11	114.19
8	6.20	10.80	64.70	71.20	168.40	4.95	5.06	37.11	36.84	105.91
9	8.46	11.46	76.31	87.38	180.38	6.76	5.37	43.77	45.21	113.45
10	7.33	10.44	55.11	82.33	165.00	5.86	4.89	31.61	42.60	103.77
11	6.07	8.93	86.98	107.71	223.43	4.85	4.18	49.89	55.73	140.52
12	4.75	6.75	66.00	74.75	173.00	3.79	3.16	37.86	38.68	108.81
Mean	6.27	9.89	61.23	70.72	176.52	5.01	4.63	35.12	36.59	111.02
SD	1.37	1.97	13.60	17.80	36.68	1.10	0.92	7.80	9.21	23.07

SC = Sertoli cells; A = type A spermatogonia; PL/L = primary spermatocytes in preleptotene/leptotene; PQ = primary spermatocytes in pachytene; RS = round spermatids.
[a] Numbers corrected according to Amann (1962).

was to evaluate the intrinsic rate of spermatogenesis in adult free-ranging feral pigs.

2. Material and methods

2.1. Animals

Twelve adult male free-ranging feral pigs were captured using a rope loop in a farm in Pantanal do Rio Negro, Mato Grosso do Sul, Brazil (IBAMA license for collection #1916054), during June and July, 2009. The animals' weight ranged from 45.5 to 76.6 kg, with a mean body weight of approximately 57 kg and the mean testis weight was about 121 g. After capture, the animals were sedated with intramuscular azaperone (Stresnil® Janssen Animal Health) 1.0 mL/20 kg combined with 10.0 mg of intramuscular diazepam and were orchidectomized. They were then released and observed to complete recovery and return to their natural environment.

2.2. Histological processing

Immediately after castration, the testes were separated from the epididymis, and the testicular artery was cannulated for infusion with 0.9% saline containing heparin (Liquemine® Roche) 5000 IU/L, at least 15 min at room temperature. Immediately after this procedure, the tissue was perfused with a fixative solution of 4% glutaraldehyde (Vetec Quimica Fina) in 0.05 M phosphate buffer, pH 7.2, for 20 min, also at room temperature (Costa et al., 2007). Testicular fragments approximately 3.0 mm thick, 5.0 mm wide and 8.0 mm long were collected and immersed in glutaraldehyde (Vetec Quimica Fina) 4% in 0.1 M phosphate buffer, pH 7.4, for 24 h and stored at 4 °C in phosphate buffer until processing.

The collected material was embedded in a solution of glycol methacrylate, using routine techniques. Sections 3 μm thick were stained with toluidine blue–1% sodium borate solution (Costa et al., 2007). Finally, images were obtained using a digital camera (Leica DFC400) attached to a light microscope (Leica DM 2500) at 400× magnification, and these images were analyzed with the aid of morphometry software ImageJ 1.34 (Rasband, 1997–2009).

2.3. Cell population of seminiferous tubules

The number of each cell type in seminiferous tubules was estimated by counting nuclei of germinative cells and nucleoli of Sertoli cells, in 30 cross sections of seminiferous tubules of symmetrical outline in stage 1 of the cycle of the seminiferous epithelium (CSE), classified using the tubular morphology method (Ortavant et al., 1977; Costa et al., 2004; Costa and Silva, 2006; Costa et al., 2007). The following cell types were considered: type A spermatogonia (A), primary spermatocytes in preleptotene/leptotene (PL/L), primary spermatocytes in pachytene (PC), round spermatids (RS) and Sertoli cells (SC). Numbers were corrected for mean nuclear diameter and slice thickness, using the Abercrombie (1946) formula modified by Amann (1962).

Mean nuclear or nucleolar diameter was determined for at least 15 nuclei in each type of germinative cell or 15 nucleoli in Sertoli cells, in CSE stage 1, in each animal. Because type A spermatogonia have ovoid or slightly elongated nuclei, means of the largest and smallest nuclear diameters were used.

Cell populations were determined by counting each cell type with the aid of morphometry software ImageJ 1.34 (Rasband, 1997–2009), using digitized microscopic images. Measurements of nuclear diameters of germ cells and Sertoli cell nucleoli were also performed with the aid of this software.

2.4. Intrinsic rate of spermatogenesis

The intrinsic rate of spermatogenesis was estimated from the ratio between corrected cell numbers, determined in stage 1 of the CSE. The following ratios were determined: efficiency coefficient of spermatogonial mitosis (PL/L:A), meiotic index (RS:PC), overall yield of spermatogenesis (RS:A) and cell loss during the meiotic prophase (PL/L:PC).