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Assessment of sexual maturity and testicular morphometrics in males of Andamani pigs under tropical humid island ecosystem of Andaman and Nicobar Islands

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**This manuscript is based on research conducted as part of the Doctoral Studies of Dr. Zachariah George, undertaken at ICAR-Central Island Agricultural Research Institute, Sri Vijaya Puram, Andaman and Nicobar Islands under West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal*
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Abstract

In 2024, the Andamani pig, a new Indian breed from the Andaman and Nicobar Islands, was registered. Conservation and popularization efforts for this breed are ongoing. This study aimed to assess the age of sexual maturity in male Andamani pigs and evaluate body and testicular morphometrics across age groups in the tropical humid island ecosystem. Conducted from September 2023 to September 2024 at the ICAR-Central Island Agricultural Research Institute (ICAR-CIARI), Sri Vijaya Puram, Andaman and Nicobar Islands, the study involved 48 healthy male piglets divided into eight groups (six piglets each) spanning ages 1 to 6 months (monthly intervals), 9 months, and 12 months. Results showed a steady increase in body weight, scrotal circumference, testicular parameters, and sperm concentration with age. Puberty was observed after 2 months, and sexual maturity at 6 months. Key findings such as body weight (3.47±0.08 to 71.82±2.62 kg), scrotal circumference (5.35±0.09 to 18.83±0.79 cm), total testicular weight (6.36±0.16 to 344.86±2.19 g), epididymal weight (1.11±0.02 to 112.72±1.65 g), testis-to-body weight ratio (1.83±0.01 to 4.84±0.24), and sperm concentration (0 to 3.26 billion/mL) were included across age groups. Therefore, it was concluded that the Andamani pig shows promising somatic and reproductive traits, making it suitable for conservation and promotion among farmers with improved breeding strategies.

Keywords: Andamani pig, puberty, sexual maturity, testicular morphometric, age, body weight, cauda epididymis, spermatozoa

Introduction

Among various livestock species, aside from broilers, pigs are considered efficient feed converters and a potential source of meat production (Nagaraj *et al.,* 2011). The domestic pig was one of the first livestock species to be domesticated, with its domestication dating back to the early Holocene, when Neolithic cultures across the Old World began farming, a period that saw the domestication of other principal farmyard animals such as sheep, goats and cattle. Domestic pigs hold a unique place among domestic animals. Archaeo-zoological evidence indicates the presence of at least two independent centres of pig domestication, one in the eastern fringe of Anatolia (western Asia), dated to around 8500 BC (Ervynck *et al.,* 2001; Conolly *et al.,* 2011), and a second in China, along the Yellow River valley, around 6500 BC (Jing and Flad, 2002; Cucchi *et al.,* 2011). The Andaman and Nicobar Islands have their own genetic diversity in pig populations. In 2024, a new breed of Andamani pig was registered at ICAR-National Bureau of Animal Genetic Resource, Karnal, and efforts to conserve and popularize this breed are underway at various levels. Assessment of breed-specific basic traits and characteristics is important and a prerequisite for planning further breed improvement. It is well recognized that genetics play an important and strong role in gonad development, early puberty, and sexual maturity, particularly in indigenous boars (Harder *et al*., 1995; Kumaresan *et al*., 2008). This study aimed to assess the age of sexual maturity in male Andamani pigs, based on the presence of spermatozoa in the cauda epididymis, and to derive various body and

testicular morphometrics across different age groups under tropical humid island ecosystem of Andaman and Nicobar Islands. This finding will assist in planning the timing of castration, weaning, and breeding in this breed.

Materials and Methods

Location: This study was conducted at the Pig Farm under the Division of Animal Science at ICAR-CIARI, Sri Vijaya Puram, Andaman and Nicobar Islands, India, located between 6°45′ to 13°41′ North Latitude and 92°12′ to 93°57′ East Longitude from September 2023 to September, 2024.

Animal Selection: A total of forty-eight (n=48) healthy Andamani male piglets were selected and randomly divided into eight groups, each containing six piglets. They were managed under standard management practices until they reached their respective target ages. The target age groups were from one to six months (at monthly interval), followed by nine months and twelve months of age.

Body and testicular morphometrics: The males in the age group from one to six months were sequentially castrated upon reaching their respective ages using a scientific surgical castration method under local anaesthesia (2% Lignocaine injection) (Skade *et al.,* 2021). Before castration, body weight was measured with a weighing scale, and partial scrotal circumference was measured using thread and a measuring tape. The testes were collected along with the epididymis and transported in a thermos to the laboratory for further measurements and analysis. In the laboratory, the testes were carefully separated from the epididymis. Testicular length, width, and thickness were measured with a Vernier Calliper, while testicular and epididymal weight were measured using an electronic balance, with readings recoded. Testis: Body weight ratio (Kumaresan *et al.,* 2011) was calculated as follows: Total testicular weight (g) / Body weight (kg)

The study on reproductive maturity: The study aimed to assess reproductive maturity by observing the presence of spermatozoa in the cauda epididymis at different ages of piglets. For this purpose, epididymides were collected from piglets and boars. The cauda epididymis was punctured with a needle, and epididymal fluid was aspirated using a micropipette and examined under a microscope for the presence of spermatozoa (Kumaresan *et al.,* 2011). Spermatozoa concentrations were estimated using the haemocytometer method (Salisbury et al., 1985). Sperm samples were mixed with 1 mL NBSE (Novel Boar Semen Extender) solution and thawed at 37°C for 2 min to assess progressive motility under a microscope. The percentage of live sperm and abnormalities was determined using the Eosin–Nigrosin staining method (Campbell *et al.,* 1953).

Statistical analysis: To determine any possible difference in the observed experimental parameters with respect age groups, one-way ANOVA was applied using PROC GLM multivariate model of Statistical Analysis Software (SAS, Version 9.3.1; SAS Institute, Inc., Cary, NC, 2011) and for multiple comparison, Duncan's multiple Range test was applied. The mean values were expressed as mean ± SEM. Differences were considered significant if $P < 0.05$.

Results

The results of present study on body weight, partial scrotal circumference, testicular length, width, thickness, and weight, epididymal weights, and testis: body weight ratio are presented in table 1.

Body weight: It was observed that body weight increased with age. A significant difference ($p<0.05$) was found in body weights at month 1, 3 and from 5 to 12. However, no significant difference was observed between adjacent age groups, namely, the 1st and 2nd months, 2nd and 3rd months, as well as 3rd and 4th months. The average body weight (kg) for the respective age groups were as follows: 3.47±0.08, 7.53±0.75, 11.06±0.97, 15.83±0.87, 22.47±1.98, 29.13±1.36, 52.78±4.09, and 71.82±2.62.

Partial Scrotal Circumference: The average partial scrotal circumference were found to be 5.35±0.09, 6.53±0.48, 7.67±0.30, 10.12±0.35, 10.43±0.70, 13.08±0.46, 14.87±0.85, and 18.83±0.79 cm for 1st, 2nd, 3rd, 4th, 5th, 6th, 9th, and 12th months of age, respectively. At lower age groups, no significant differences were observed between adjacent age groups, namely the 1st and 2nd, 2nd and 3rd, 4th and 5th months. As expected, there was a general increasing trend in partial scrotal circumference with age and significant differences ($p<0.05$) were observed between other age groups.

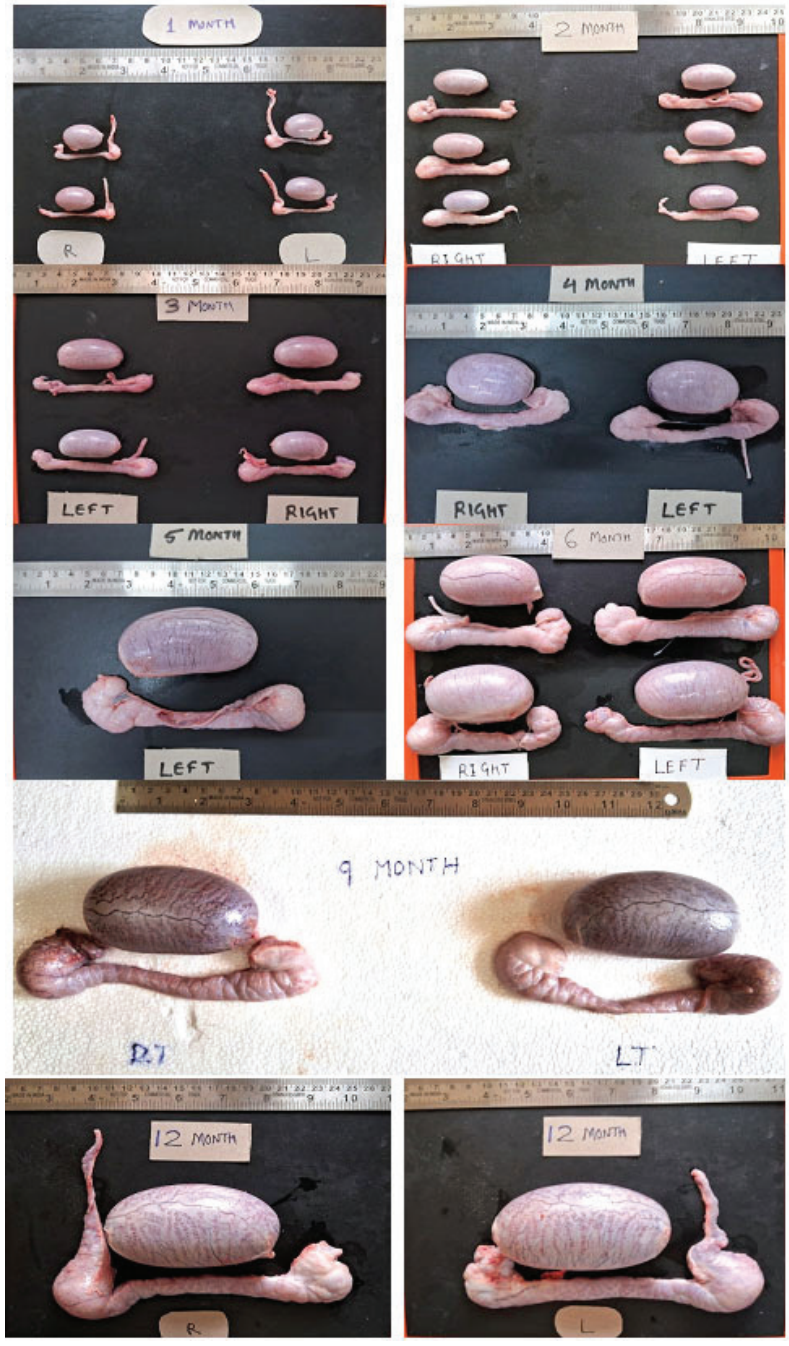
Table 1. Effect of age on scrotal circumferece, testicular parameters and sperm concentration in Andamani Pig under tropical humid island ecosystem of Andaman and Nicobar Islands (Mean ± SEM)

Experimental Parameters	1-month	2-month	3-month	4-month	5-month	6-month	9-month	12-month
Body weight (kg)	3.47±0.08^{a}	7.53±0.75^{ab}	11.06±0.97^{bc}	15.83±0.87^{c}	22.47±1.98^{d}	29.13±1.36^{e}	52.78±4.09^{f}	71.82±2.62^{g}
Partial Scrotal Circumference (cm)	5.35±0.09^{a}	6.53±0.48^{ab}	7.67±0.30^{b}	10.12±0.35^{c}	10.43±0.70^{c}	13.08±0.46^{d}	14.87±0.85^{e}	18.83±0.79^{f}
Right testis								
Length (cm)	2.25±0.04^{a}	2.98±0.16^{a}	3.11±0.18^{a}	4.66±0.25^{b}	5.35±0.67^{b}	7.17±0.45^{c}	8.42±0.22^{d}	9.60±0.05^{e}
Width (cm)	1.34±0.03^{a}	1.64±0.04^{ab}	1.92±0.14^{b}	2.86±0.15^{c}	3.12±0.24^{c}	3.67±0.13^{d}	4.75±0.22^{e}	5.56±0.07^{f}
Thickness (cm)	1.41±0.03^{a}	1.73±0.06^{ab}	1.98±0.14^{b}	2.81±0.16^{c}	3.08±0.21^{c}	3.80±0.13^{d}	4.81±0.17^{e}	5.72±0.04^{f}
Weight (g)	3.13±0.10^{a}	6.61±0.72^{a}	7.79±1.19^{a}	26.11±3.42^{b}	49.31±10.96^{c}	59.73±5.46^{c}	106.94±10.96^{d}	165.77±1.40^{e}
Epididymal weight (g)	0.55±0.02^{a}	2.27±0.13^{a}	2.97±0.33^{ab}	9.01±1.34^{b}	20.84±4.64^{c}	22.93±1.93^{c}	35.48±2.66^{d}	55.18±0.74^{e}
Left Testis								
Length (cm)	2.34±0.05^{a}	3.05±0.16^{a}	3.24±0.16^{a}	4.83±0.20^{b}	5.87±0.62^{c}	7.29±0.45^{d}	8.33±0.29^{e}	9.87±0.06^{f}
Width (cm)	1.28±0.04^{a}	1.71±0.05^{ab}	1.87±0.11^{b}	2.87±0.15^{c}	3.26±0.29^{c}	3.82±0.12^{d}	4.83±0.26^{e}	5.78±0.07^{f}
Thickness (cm)	1.41±0.02^{a}	1.77±0.08^{ab}	1.98±0.11^{b}	3.04±0.21^{c}	3.17±0.26^{c}	3.90±0.18^{d}	4.88±0.22^{e}	5.99±0.04^{f}
Weight (g)	3.23±0.07^{a}	6.50±0.61^{a}	8.25±1.22^{a}	26.91±3.43^{b}	50.97±11.02^{c}	63.40±5.48^{c}	106.51±11.71^{d}	179.10±2.39^{e}
Epididymal weight (g)	0.57±0.02^{a}	2.19±0.19^{ab}	3.04±0.33^{ab}	8.98±1.22^{b}	21.30±4.55^{c}	24.11±3.34^{c}	36.29±3.02^{d}	57.09±0.83e
Total testicular weight (g)	6.36±0.16^{a}	13.10±1.33^{a}	16.04±2.40^{a}	53.02±6.84^{b}	100.28±21.95^{c}	123.13±12.10^{c}	213.44±22.76^{d}	344.86±2.19^{e}
Total epididymal weight (g)	1.11±0.02^{a}	4.46±0.32^{ab}	6.01±0.65^{ab}	17.99±2.55^{b}	42.14±9.17^{c}	47.07±6.87^{c}	72.07±5.56^{d}	112.72±1.65^{e}
Testis: body weight ratio	1.83±0.01^{a}	1.73±0.28^{a}	1.45±0.16^{a}	3.34±0.33^{b}	4.46±0.71b^{c}	4.22±0.27^{bc}	4.04±0.14^{bc}	4.84±0.24^{c}
Boars with Sperm in Cauda Epididymis (%)	0	0	50	83.33	100	100	100	100
Sperm concentration ($\times 10^{9}$/mL)	0.00±0.00^{a}	0.00±0.00^{a}	0.11±0.04^{a}	0.56±0.12^{ab}	1.37±0.54^{bc}	2.12±0.22^{cd}	2.89±0.26^{de}	3.26±0.27^{e}

Means bearing different superscripts within rows (a, b, c, d, e, f, g) differ significantly ($P < 0.05$) among the different age groups

Testicular Morphometric: Figure-1 showed photographs of testis and epididymis of different age groups studied. The result revealed that, as expected, the length of right and left testes increased with age. These were found to be 2.25±0.04 and 2.34±0.05, 2.98±0.16 and 3.05±0.16, 3.11±0.18 and 3.24±0.16, 4.66±0.25 and 4.83±0.20, 5.35±0.67 and 5.87±0.62, 7.17±0.45 and 7.29±0.45, 8.42±0.22 and 8.33±0.29, 9.60±0.05 and 9.87±0.06 cm for the 1st to 6th, 9th and 12th months of age, respectively. There was no significant difference between age groups until the month 3; however, significant ($p<0.05$) differences became more prominent from the 6th month of age onwards.

Figure 1. The plates showing testis and epididymis of different age groups studied



Similar trends were observed for both right and left testicular width and thickness. The respective values for right and left testicular width were 1.34±0.03 and 1.28±0.04, 1.64±0.04 and 1.71±0.05, 1.92±0.14 and 1.87±0.11, 2.86±0.15 and 2.87±0.15, 3.12±0.24 and 3.26±0.29, 3.67±0.13 and 3.82±0.12, 4.75±0.22 and 4.83±0.26, 5.56±0.07 and 5.78±0.07 cm for the 1st to 6th, 9th and 12th months of age. The corresponding value for right and left testicular thickness for the same age groups were 1.41±0.03 and 1.41±0.02, 1.73±0.06 and 1.77±0.08, 1.98±0.14 and 1.98±0.11, 2.81±0.16 and 3.04±0.21, 3.08±0.21 and 3.17±0.26, 3.80±0.13 and 3.90±0.18, 4.81±0.17 and 4.88±0.22, 5.72±0.04 and 5.99±0.04 cm, respectively.

The above trends were also observed in the weights of the right and left testes and epididymis. The average weights of the right and left testes were found to be 3.13±0.10 and 3.23±0.07, 6.61±0.72 and 6.50±0.61, 7.79±1.19 and 8.25±1.22, 26.11±3.42 and 26.91±3.43, 49.31±10.96 and 50.97±11.02, 59.73±5.46 and 63.40±5.48, 106.94±10.96 and 106.51±11.71, 165.77±1.40 and 179.10±2.39g, respectively. Similarly, the weights of the right and left epididymis were recorded as 0.55±0.02 and 0.57±0.02, 2.27±0.13 and 2.19±0.19, 2.97±0.33 and 3.04±0.33, 9.01±1.34 and 8.98±1.22, 20.84±4.64 and 21.30±4.55, 22.93±1.93 and 24.11±3.34, 35.48±2.66 and 36.29±3.02, 55.18±0.74 and 57.09±0.83g, respectively.

Total testicular weight: The total testicular weight was calculated by adding the weights of the left and right testes. The result revealed a steady increase in testicular weight with age. No significant differences were observed in testicular weight between 1st to 3rd months and the 5th and 6th months. However, the 4th, 9th and 12th months showed significant differences compared to all other age groups studied. The average total testicular weights for the respective age groups were 6.36±0.16, 13.10±1.33, 16.04±2.40, 53.02±6.84, 100.28±21.95, 123.13±12.10, 213.44±22.76, and 344.86±2.19 g.

Total epididymal weight: A relatively similar trend was observed in the total epididymal weight, showing an overall increase with age. The average total epididymal weights were 1.11±0.02, 4.46±0.32, 6.01±0.65, 17.99±2.55, 42.14±9.17, 47.07±6.87, 72.07±5.56, 112.72±1.65 g for the respective sequential age groups.

Testis: body weight ratio: The testis: body weight ratio showed significant ($p<0.05$) differences between the age groups of the 3rd, 4th, and 5th months, while no significant differences were observed between adjacent age groups. The average values for the testis: body weight ratio were 1.83±0.01, 1.73±0.28, 1.45±0.16, 3.34±0.33, 4.46±0.71, 4.22±0.27, 4.04±0.14, and 4.84±0.24, respectively, for the age groups under study.

Boars with spermatozoa in cauda epididymis: It was observed that, at month 1 and 2 there was absence of spermatozoa in cauda epididymis of the piglets. However, 50.00 and 83.33 percent piglets were having spermatozoa at month 3 and 4, respectively. From 5th month onwards every males were having spermatozoa in their cauda epididymis.

Spermatozoa concentration: There was an increase trend in concentration of spermatozoa in the cauda epididymis fluid from 3rd month onwards. Although there was no significant difference between adjacent age groups from 3rd to 9th month but other non-adjacent age groups showed significant difference ($p<0.05$). The sperm concentration for respective age groups from 3rd to 12th months were 0.11, 0.56, 1.37, 2.12, 2.89 and 3.26 billion sperms per mL.

Discussion

The Andamani pig was registered as a new breed in 2024, and multifaceted conservation and popularization efforts are being carried out at ICAR-CIARI, Sri Vijaya Puram, through the ICAR-funded AICRP on Pig project. It is, therefore, important to determine the precise age of puberty and sexual maturity of boars to educate farmers and researchers and to formulate standard management and breeding practices for this breed.

Body weight is a good indicator of piglet health and is influenced by nutritional status, management practices, environmental factors, and genetic makeup. The body weight of the Andamani pig increased with age, as expected, during the study period. A significant difference ($p < 0.05$) was observed in body weights at month 1, 3,

and from 5 to 12 of age. However, no significant difference was observed between adjacent age groups until the month 4. Kumaresan et al. (2008) reported that Large White Yorkshire (LWY) and Hampshire pigs had slightly higher body weights at month 2 and 4 compared to the Andamani pig in this study. However, at month 6, the Andamani pig exhibited a slightly higher body weight than LWY (29.13 kg vs. 26.3 kg) and was comparable to Hampshire pigs. In contrast, the body weights of non-descript local pigs, as reported by Kumaresan *et al.* (2008), were lower than those of the Andamani pig at month 2, 4, and 6. The average body weight at month 9 in this study was lower than the average reported by Kundu *et al.* (2020) for the Andamani pig. This difference may be attributed to the fact that the boars in this study were sexually active and undergoing training for semen collection using a dummy. Regarding partial scrotal circumference, no significant differences were observed between adjacent age groups at lower ages. Partial scrotal circumference is a reliable indicator of testicular weight (Duane and Robert, 1977). As expected, there was a general increasing trend in partial scrotal circumference with age, and significant differences were observed between other age groups.

At the onset of puberty, testicular size increases rapidly as seminiferous tubules expand in diameter, thereby occupying a greater proportion of the testis (Ford and Wise, 2010). In our study, the length of the right and left testes increased with age. No significant differences were observed between age groups until the month 3; however, significant differences became more prominent from the month 6 onwards. Similar trends were noted for the right and left testicular width, thickness, weight, and epididymis measurements. The total testicular weight also steadily increased with age. No significant differences in testicular weight were observed between the month 1 and 3 or between the month 5 and 6. However, the month 4, 9 and 12 showed significant differences compared to all other age groups. This suggests that significant testicular growth begins after 3 months of age. These findings differ slightly from those of Patra *et al.* (2021), who characterized testicular changes in Tenyi-vo, a miniature pig breed from the North East Himalayan region, and found that testicular weight and volume were comparable between 2 to 5 months but significantly heavier than 1.5 months. Karunakaran *et al.* (2008) reported comparatively higher testicular weight, length, and breadth in Naga boars at day 53 and 85 than observed in our study. In contrast, the total testis weight of LWY, Hampshire, and non-descript local pigs, as reported by Kumaresan *et al.* (2008), was lower than that of Andamani pigs in our study. This difference may be attributed to genetic variation and the effects of different climatic zones on rearing practices. Genetic constitution plays a crucial role in influencing gonadal development, puberty, and sexual maturity (Harder *et al*., 1995).

The total epididymal weight showed an overall increase with age, following a trend similar to other parameters. The testis-to-body weight ratio displayed significant ($p<0.05$) differences between the age groups of the month 3, 4, and 5, while no significant differences were observed between adjacent age groups. The average values for total epididymal weight and the testis-to-body weight ratio of the Andamani pig were higher than the values reported by Kumaresan *et al*. (2008) for LWY, Hampshire, and non-descript local pigs.

Sexual maturity in the boar can be defined as the presence of sperm in an ejaculate, ability to mate and impregnate females. Depending upon the breed, nutrition, environmental and social cues sexual maturity occurs at variable age (Andersson, 1999). The sperm get matured and acquires its fertilizing capacity in epididymis and remains stored at cauda epididymis until ejaculation (Einarsson *et al*., 1979; Barth and Oko, 1989). The appearance of first physiologically normal spermatozoa in the cauda epididymis may be an indication of the onset of puberty in males (Kumaresan *et al.,* 2011). Our study showed that till 2nd month there was no spermatozoa in cauda epididymis of the Andamani piglets. However, some piglets were having spermatozoa at 3rd month (50%) and 4th month (83%) of age. From 5th month onwards every males were having spermatozoa in their cauda epididymis. The concentration of spermatozoa in the cauda epididymis fluid increased from 3rd month onwards. Although there was no significant difference between adjacent age groups from 3rd to 9th month but other non-adjacent age groups showed significant difference ($p<0.05$). Earlier studies also indicated the same trend in

domestic pigs. They reported that, the domestic pig having wild boar as ancestor, reaches puberty at about month 5 and thereafter attain sexual maturation from about 7 to 9 months of age, depending on the breed (Andersson *et al*., 1998; Cheon *et al*., 2002; França *et al*., 2005; Fraser *et al*., 2016). Franc *et al.* (2000) reported that puberty in Piau pigs occurred between 4 and 5 months of age and sexual maturity attained from 7 to 8 months of age. According to Umesiobi (2006) total sperm, sperm motility and normal acrosome morphology were correlated with testis weight and the same was observed in Andamani pigs. Patra *et al.,* (2021) concluded in their study that Tenyi-vo male pigs attained puberty at the earliest by 60 days of age with a body weight of around 4 kg which was earlier than Andamani pig observed in this study. Karunakaran *et al.* (2008) through their study confirmed that Naga boar had higher semen concentration in cauda epididymis at 85 days compared to 53 days of age. Kumaresan *et al.,* (2011) also reported that the sperm concentration in the cauda epididymal fluid of local boars of NEH at 2, 3 and 6 months of age to be 2255 ± 186.6, 3685 ± 103.8 and 4325 ± 146.2 million/mL respectively. Our study indicated same trend although from 3 month onwards and with lower sperm concentration. These variations in result may be attributed to variation in breed, climatic zones, nutritional and management practices between the study areas. Our study indicated that after 2 months of age the Andamani male piglets slowly attained puberty which indicates that the castration of males should be done at or before 2 months for meat production as well to avoid undesirable breeding in the farm. The study also indicated that the sexual maturity in Andamani pig (boars) was attained at 6 months of age.

Conclusion

The study indicated that body weight, partial scrotal circumference, testicular parameters, and sperm concentration in the cauda epididymis showed an increasing trend with age. The Andamani male piglets gradually attained puberty after two months and reached sexual maturity at six months of age. The growth rate and testicular parameters were comparable to exotic breeds and superior to non-descript local breeds of the NEH region. Hence, it can be concluded that the Andamani pig possesses promising somatic and reproductive attributes and should be conserved both *in situ* and *ex situ*. Furthermore, it should be promoted among farmers with improved breeding plans for further enhancement.

Ethical Permission

Ethical permission for animal experiment was got approved from Institutional Animal Ethics Committee (IAEC) of ICAR-CIARI, Sri Vijaya Puram.

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