## DEVELOPMENT OF THE EPIDIDYMIS IN MEISHAN BOARS

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## Summary

The developmental process of the epididymis was investigated in Meishan hoars from 1 to 364 days of age. Epididymal weight increased rapidly between 45 and 150-180 days of age. The diameter and epithelial area of the epididymal ducts greatly increased up to 105-120 days of age. At 1 day of age, the central and distal cauda already had a pseudostratified epithelium surrounded by smooth muscle. At 60-75 days of age, the central and distal caput, corpus, and proximal cauda revealed a well-developed structure of the epithelium. The proximal caput showed a tall, irregular and vacuolar epithelium at 105-120 days of age. PAS-positive contents in the lumen of the caput, corpus and cauda epididymides were first detected at 60-75, 45-60 and 1-30 days of age, respectively. Moreover, in the central and distal caput. PAS-positive granules appeared at 60-75 days of age, and increased until 105-120 days. These results suggest that the epididymis develops completely by approximately 120 days of age, though its weight increases rapidly up to 150-180 days. Thus, it appears that development of the epididymis occurs at an earlier age in Meishan boars than in European and American breeds. (Key Words: Meishan Boar, Epididymis, Sexual Maturity)

### Introduction

Meishan pigs, which belong to the Taihu breed of Chinese native pigs, are noted for their early sexual maturity as well as large litter size. Reports show that Meishan gilts teach puberty as early as 3 months of age (Bazer et al., 1988; Wang, 1990) and develop reproductive organs at an earlier age than European and American breeds (Miyano et al., 1990a,b,c). Previously we reported the developmental process of Meishan testis (Kato and Harayama, 1990).

The epididymis has been considered to produce a favorable environment for maturation and storage of spermatozoa by epithelial secretion and fluid reabsorption (Orgebin-Crist, 1969; Bedford, 1975), and is morphologically or histologically divided into several regions, which seem to vary functionally (Glover and Nicander, 1971). However, the developmental process and functional significance of each region remain uncertain in

Meishan boars. The objective of this study is to characterize the morphological and histological changes of the epididymis in Meishan boars after birth.

#### Materials and Methods

A total of 71 offspring from Meishan pigs imported from the People's Republic of China to our experimental farm were used. The pigs were housed as previously described (Miyano et al., 1990a). The testis-epididymides were collected after the recording of body weights at 1, 2, 7, 8, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 270, 280, and 364 days of age. Epididymides were weighted following removal from the testis and divided into eight regions (figure 1). Each region was cut into small pieces and fixed in 10% formalin in 0.1M phosphate buffer for approximately 72 hr. After the specimens were embedded in paraffin wax blocks, cut into 5 \(\rho \mathrm{m}\) thick sections, and stained with hematoxylin and eosin or with Periodic Acid-Schiff's reagent and hematoxylin. epididymal ducts were examined under light microscope. The diameter and epithelial area of the ducts with a form index (4 m × area/perimeter<sup>2</sup>) of more than 0.95 were measured using an image analyser (Mop-video plan, Kontoron Electric Group).

Data were subjected to analysis of variance.

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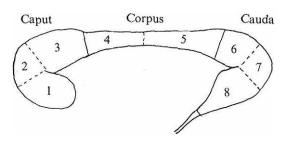


Figure 1. The eight regions of the epididymis.

1: Proxima caput, 2: Central caput,

3: Distal caput, 4: Proximal corpus,

5: Dista corpus, 6: Proximal cauda,

7: Central cauda. 8: Distal cauda

When F-test was significant, individual means were further tested by Duncan's multiple range test (Steel and Torrie, 1960).

#### Results

As shown in table 1, epididymal weight increased slowly from 1 to 45 days of age, then rapidly until 150-180 days, after which the increase showed down. The ratio of paried epididymal weight to body weight gradually increased until 150-180 days of age.

As shown in figures 2 and 3, the diameter and epithelial area of the epididymal ducts in-

TABLE 1, GROWTH OF THE EPIDIDYMIDES IN MEISHAN BOARS

Age	No. of boars	Paired weight	Ratio to body
(days)	examined	(g)	weight $(\times 10^{-3})$
1-8	6	$0.4\pm0.1^{\rm g}$	$0.3\pm0.1^{\rm f}$
30	20	$1.9 \pm 0.1^{8}$	$0.3 \pm 0.0^{\circ}$
45	4	$3.3 \pm 0.2^{18}$	$0.4 \pm 0.1e^{t}$
60	7	$8.5 \pm 0.9^{t}$	$0.5\pm0.0^{ m de}$
75	9	$14.6 \pm 0.8^{e}$	$0.6\pm0.0^{ m de}$
90	7	$19.3 \pm 1.5^{d}$	$0.7\pm0.0$ <sup>cd</sup>
105	4	$33.8 \pm 0.2^{\circ}$	$0.9 \pm 0.0^{\mathbf{ab}}$
120	5	36.0 ± 3.5°	$0.8\pm0.0$ bc
150-180	5	$60.9 \pm 1.9^{b}$	$1.0 \pm 0.1^{a}$
210-364	4	77.6 ± 2.3°	$1.0 \pm 0.1^{a}$

Values are means ± SEM.

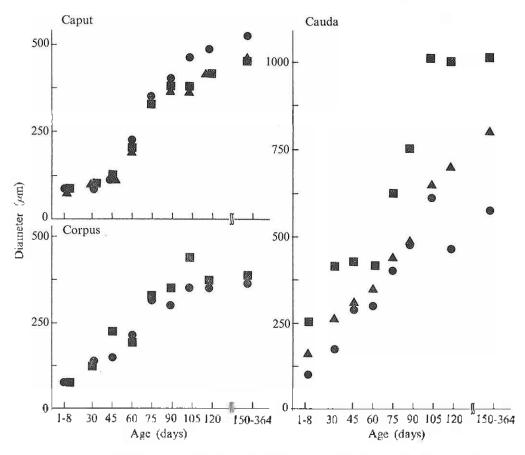
creased slowly in the caput until 45 days of age, followed by a rapid increase. On the other hand, these parameters steadily increased in the corpus and cauda after 1-8 days of age. After 105-120 days of age, however, no significant increase was observed in the diameter and area in any region of the epididymis.

At I day of age, the epididymal ducts in the caput, corpus and proximal cauda were characterized by a low: and simple epithelium (figure 4, 7 and 10), while in the central and distal cauda, the ducts already had a well developed epithelium surrounded by smooth muscle (figure 13). As the age increased, the epithelium of each region increased in height. The central and distal caput, corpus and proximal cauda first showed a pseudostratified epithelium at 60, 7-30 and 7-8 days of age, respectively (figure 5, 8 and 11), and their

structure was histologically established at 60.75 days of age (figure 6, 9 and 12). At 60-75 days of age, the proximal caput showed an irregular and pseudostratified epithelium (figure 14) and an increased number of large vacuoles in the principal cells up to 105-120 days. After 75 days of age, spermatozoa were present in the lumen of the epididymal duct from all regions, though their number was less in the proximal caput (figure 15) than in any other region (figure 6, 9 and 12).

PAS positive reactions were first detected in the luminal contents and stereocilia in the central and distal caput, corpus and cauda epididymides at 60-75, 45-60 and 1-30 days of age (figure 16, 17 and 18), respectively. In the proximal caput, PAS-positive stereocilia were observed at 60-75 days of age (figure 19), although luminal contents

<sup>\*\*</sup> Values within the same column with different superscript letters differ significantly (p < 0.05).



●: The proximal region, ▲: The central region, ■: The distal region

Figure 2. Age-related changes in the diameter of the epididymal ducts in Meishan boars.

were negative or faintly positive. Moreover, at the same age, PAS positive granules appeared in the epithelium of the central and distal caput (figure 16), and increased until 105-120 days of age (figure 20). In addition, all of the PASpositive materials were still observed even after digestion by salivary amylase.

### Discussion

In this study, epididymal weight increased rapidly during the period between 45 and 150-180 days of age, when the testis greatly developed (Kato and Harayama, 1990). According to histological and histochemical observations, marked development of the ducts in the caput occurred after 45-60 days of age. In the other regions, epididymal ducts showed a pseudostratified epithelium at an earlier age than the caput and increased steadily until 105-120 days. These results

are similar to those reported for the ram (Carreau et al., 1984). It has been considered that the maintenance of structure and function of the caput epididymides in a mature animal is dependent upon androgen, androgen binding protein and/or unknown factors originating from rete testicular fluid (Gustaffson, 1966; Cooper and Waits, 1974; Brooks, 1977; Moniem et al., 1978; Fawcett and Hoffer, 1979; Jones et al., 1981, 1988; Harayama et al., 1988). It has been reported that the testis begins releasing this fluid at the first appearance of spermatids in the seminiferous epithelium (Setchell, 1970). In Meishan boars, spermatids first appeared in the epithelium between 45 and 60 days of age (Kato and Harayama, 1990), when the caput epididymides began a rapid development. These events indicate that a factor (s) from rate testicular fluid plays an important role in the development of the caput epididymides.

Biochemical (Mann and Lutwak-Mann, 1981)

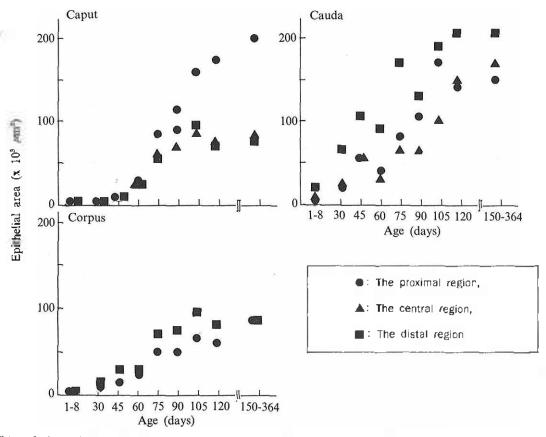


Figure 3. Age-related changes in the epithelial area of the epididymal ducts in Meishan boars.

and morphological (Nicander and Malmqvist, 1977; Goyal, 1985) studies have established that the epididymal epithelium adds various secretions to the luminal fluids. Glycoproteins in the fluid have been considered to play essential roles in modifications of spermatozoa associated with their maturation (Hammerstedt and Parks, 1987; Eddy, 1988). In this study, PAS-positive contents first appeared in the lumen of the central and distal caput, corpus, and cauda at 60-75, 45 60 and 1-30 days of age, respectively. The appearance of the materials at an earlier age in a lower region indicates that they originate from the epididymal epithelium. Thus, these observations suggest the age when epithelial secretions of PAS-positive materials begins in each region. In the central and distal caput regions, PAS-positive granules were first found at the appearance of the PAS-positive luminal contents and then greatly increased as the augmentation of matured spermatozoa in the cauda epididymides (Harayama et al., 1990). Moreover, the luminal contents and

granules were still observed even after digestion by salivary amylase, indicating that they are not glycogen. It is possible that these granules are glycoproteins secreted from the epididymal epithelium into the lumen. Such glycoproteins might bind to the surface of spermatozoa and be associated with sperm maturation.

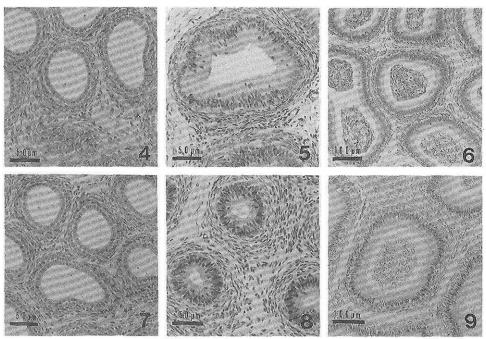
Our observation that luminal spermatozoa are greatly concentrated within the caput epididymides agrees with the results obtained with bulls (Crabo, 1965; Goyal, 1985; Amann, 1987), camels (Tingari and Moniem, 1979) and mice (Abe et al., 1982), and indicates that the absorption of water from luminal fluid by the caput epithelium is very active. From 60-75 to 105-120 days of age, vacuoles which seemed to include materials from luminal fluid increased in the proximal caput, suggesting that the function of epithelial absorption probably develops during this period.

In Meishan boars, rapid increase in epididymal weight occurred from 45 to 150-180 days of age and the epithelial structure of the epididymal

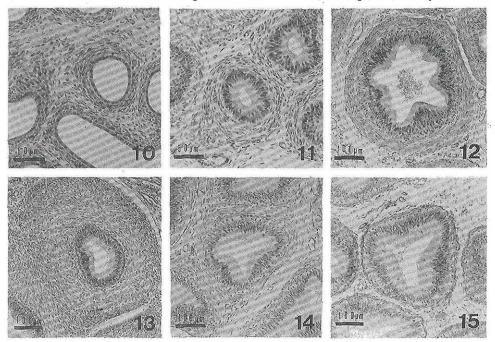
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Figures 4-20. Histological sections of the epididymides from Meishan boars.

Sections were stained with hematoxylin and eosin (figures 4-15), or with periodic acid-schiff's reagent and hematoxylin (figures 16-20).

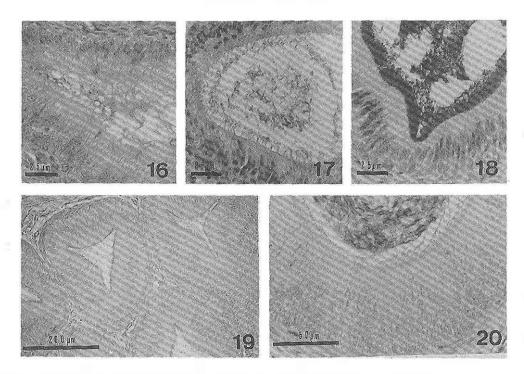


Figures 4, 5 and 6: The distal caput region at 1, 60 and 75 days of age, respectively. Figures 7, 8 and 9: The proximal corpus region at 1, 30 and 75 days of age, respectively.



Figures 10, 11 and 12: The proximal cauda region at 1, 7 and 75 days of age, respectively. Figure 13. The central cauda region at 1 day of age.

Figures 14 and 15: The proximal caput region at 60 and 105 days of age, respectively.



Figures 16 and 20: The distal caput region at 60 and 105 days of age, respectively.

Figure 17: The proximal corpus region at 45 days of age.

Figure 18: The distal cauda region at I day of age.

Figure 19: The proximal caput region at 75 days of age.

ducts in all regions were well developed by 105-120 days. In Yorkshire (Ito and Niwa, 1946; Godinho et al., 1979), Berkshire (Niwa and Mizuho, 1954a), Large White (Niwa and Mizuho, 1954b) and Poland China (Niwa and Mizuho, 1954b), rapid increase in epididymal weight has been observed between approximately 4 and 7-8 months of age. In Landrace boars at 120 days of age, the epithelium of the caput and corpus was still undeveloped or developing (unpublished data). Thus, it appears that development of the epididymis occurs at an earlier age in Meishan boars than in European and American breeds.

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