WITH the recent development of strains of miniature swine, a new interest has been generated in the pig as an experimental animal (Bustad and McClellan, 1966). Although miniature swine are rapidly gaining acceptance in biomedical research, little information is available on the normal development of these animals. This report describes the normal pattern of testicular development in one strain of miniature pigs during the prepubertal and pubertal periods.

Materials and Methods

This laboratory maintains a herd of miniature swine of the Pitman-Moore strain which presently contains some 50 animals of breeding age. During the period covered by this experiment, a random breeding program was being followed except that matings between closely related animals were avoided. As litters of the appropriate ages became available, two males from the same litter were randomly selected for unilateral castration. Each animal's second testis was removed 2 weeks after the first. The removal of one testis was assumed, based on the work of Edwards (1940), not to affect the function or weight of the remaining organ. Under this system, testes were secured from males 1 day after birth and at weekly age intervals beginning at 1 week and continuing through 30 weeks of age. Each testis was trimmed free of its epididymis and weighed; thin transverse slices were removed from the approximate center of each organ and fixed in 1:5 acetic acid-absolute alcohol. Five-micron sections were obtained from the embedded tissues at 100-μ intervals and stained by the periodic acid-Schiff method with hematoxylin as a nuclear stain.

Microscopic examinations of testicular sections were made to compare the stage of development of their spermatogenic elements. Measurements of seminiferous tubule diameter were made on tissues from animals at 1 day and at 4, 8, 12 and 16 weeks of age, and at intervals of 2 weeks from 18 through 30 weeks of age. From each of these specimens, 40 tubules (20 from each male) were selected which had been cut in approximate cross-section as judged by their near circular appearance. The shortest diameter of each tubule was measured, using a graduated eyepiece which had been calibrated against a stage micrometer. The 40 values obtained were used to arrive at an average tubule diameter for testes of a given age.

Results and Discussion

The average testis weights and seminiferous tubule diameters plotted in figure 1 show a distinct tendency toward parallelism. Correlation calculations between these two measurements yielded an r value of .977 (P<.01). Both weight and tubule diameter increased very slowly until 18 weeks of age; both showed sharp increases between 18 and 22 weeks and a reduced growth rate thereafter. As will be described later, these increases coincided not with the onset of spermatogenic development but with the appearance of the late stages of the initial spermatogenic wave. A similar period of accelerated growth was observed by Hauser et al. (1952) among boars of domestic breeds within a few days of the same age. Phillips and Zeller (1943) compared testicular development rates in large and small type Poland China boars. Increased tubule growth began 3 to 8 weeks earlier and continued 5 to 8 weeks longer in their animals than that noted in the present study, despite the fact that miniature pigs ultimately attained a somewhat higher average tubule diameter. Phillips and Andrews (1936) presented values for tubule diameter in a limited number of boars with which the figures for miniature pigs are in good agreement.

Microscopic examination of testis sections indicated that histological development was

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somewhat more gradual and extended over a longer period of time than the increase in tubular size. Testes removed at 1 day of age exhibited a predominance of interstitial cells, with large amounts of cytoplasm, but well-formed seminiferous tubules and prominent rete tubules. Seminiferous tubules contained, along the basement membrane, a single layer of cells all of which were of the same general size and morphology. They exhibited no distinct cytoplasm and their nuclei contained a mixture of large and small chromatin granules, a condition usually typical of Sertoli cell nuclei. Gonocytes were abundant and easily recognized by their large size, filamentous chromatin and thin, but distinct, cytoplasm. They were occasionally found on the basement membrane of the tubule, although most were displaced toward the center. Except for the two or three gonocytes seen in most tubules, the central portions of the tubules were filled with a light-staining, fibrous material. Mitotic activity was not apparent in either of the cell types.

Between the ages of 1 and 8 weeks, very little change could be noted within the tubules. Most tubular cross-sections contained from three to five gonocytes which were usually displaced somewhat away from the basement membrane (figure 2a). An occasional mitotic figure could be seen among these cells but not in the Sertoli cell nuclei. At about 12 weeks of age, the first changes in general structure of the testes became apparent with the appearance of septa and the organization of tubules into lobes. This organization was accompanied by the first noticeable increase in tubule diameter and an apparent reduction in the proportion of interstitial tissue.

In 12-week-old tissues, the gonocytes tended to be located more proximal to the basement membrane than in younger samples. Accompanying this change in position was a tendency for the nuclear chromatin of these cells to change from the filamentous type of the gonocyte toward the more finely diffused condition typical of type A spermatogonia. Somewhat less than half the gonocytes in the average tubule appeared to take part in this migration and morphogenesis. An occasional cell was seen along the basement membrane with the typical characteristics of a type A spermatogonium (Clermont and Perry, 1957).

During the period between 13 and 16 weeks of age, definitive spermatogenic cells
Figure 2. Testis sections typical of miniature pigs at various prepubertal ages. (All at 400×.)

a. 5 weeks: Interstitial tissue predominates. Sertoli nuclei line the tubules while gonocytes are displaced somewhat toward the center.

b. 13 weeks: Gonocytes tend to be more closely associated with the basement membrane. The first indication of spermatogenic activity is the transformation of some gonocytes toward the morphology of primary spermatocytes.

c. 17 weeks: Most tubules have two generations of primary spermatocytes although the density of some cell types is often rather low.

d. 21 weeks: Spermatids in various stages of maturation are present in most tubules. Note the absence of primary spermatocytes from the lower left tubule indicating a disruption in its development process.
began to appear. Thirteen-week samples exhibited occasional tubules containing cells with the highly condensed chromatin characteristic of preleptotene primary spermatocytes (figure 2b). Their position in the lumen corresponded to that occupied by gonocytes which had shown no migration. The number of type A spermatogonia in such tubules seemed far from sufficient to account for the gonocytes which had disappeared, suggesting that these first primary spermatocytes were formed by direct morphogenesis from preexisting gonocytes. Increasing numbers of tubules in 14- and 15-week samples contained primary spermatocytes; during this same period, type B spermatogonia could be identified along the basement membrane of the tubule. The onset of spermatogenesis seemed to occur randomly among the tubules, there being no areas of the testicular cross-sections which appeared at any time to be more advanced than others.

At 15 to 17 weeks of age, some tubules possessed two distinct generations of primary spermatocytes although cell numbers were often quite low (figure 2c). As tubule diameter began to increase rapidly, the interstitial cells occupied proportionately less space. Although there was no evidence of necrosis among these cells, the amount of cytoplasm per cell decreased as the testis approached maturity. A similar morphogenesis of the Leydig cells has been described in domestic swine by Erickson (1964). Tubules containing spermatids were first encountered in 17-week testes, and these cells were present in many tubules by 19 weeks of age. Those tubules with spermatids were the first to exhibit a patent lumen and disappearance of the fibrous material which filled the lumina in younger tissues. Most tubules from animals at 19 through 21 weeks of age contained spermatids in various stages of elongation (figure 2d), but not until 22 weeks were mature sperm ready to be shed into the lumen. Sperm and spermatid numbers were noticeably reduced in many such tubules.

Hauser et al. (1952) found the first primary spermatocytes in tubules of inbred and crossbred boars at an average age of 19 weeks. These were followed 2 weeks later by secondary spermatocytes and spermatids with spermatozoa appearing at an average age of 25 weeks. Phillips and Zeller (1943) likewise found spermatozoa in some tubules of all their males at 25 weeks of age. In testes evaluated by Erickson (1964), spermatocytes first appeared at about 14 weeks and spermatids at 18 weeks.

Several strains of miniature swine are

| Table 1. Proportion of Seminiferous Tubules at Various Stages of Development in Testes of Miniature Swine of Prepubertal Ages |
| --- | --- | --- | --- |
| Age (wk.) | Primary spermatocytes | 1 generation | 2 generations | Spermatozoids |
| | Pre-mordial | % | % | % | % |
| 13 | 95.0 | 0.0 | 0.0 | 0.0 | 1.0 |
| 15 | 53.0 | 37.0 | 0.0 | 0.0 | 0.0 |
| 17 | 27.0 | 61.0 | 0.0 | 0.0 | 0.0 |
| 19 | 4.0 | 40.0 | 30.5 | 25.5 | 0.0 |
| 21 | 2.0 | 1.0 | 12.5 | 84.5 | 0.0 |
| 23 | 0.0 | 0.5 | 2.0 | 97.5 | 0.0 |

* Tubules classified on the basis of the most advanced cell type present in their germinal epithelium; 100 tubules scored in each of 2 males at each age.

** Gonocytes and/or spermatogonia.

such an indistinct transition that attempts to separate them are impractical. Only rarely were type B spermatogonia encountered as the most advanced cell type, since they rapidly undergo division to produce primary spermatocytes.

During the first few weeks after maturity, the spermatogenic process seemed to be quite susceptible to disruption. Conditions similar to that shown in the lower left tubule of figure 2d were sometimes seen. The presence of spermatids and type B spermatogonia, but complete absence of primary spermatocytes from this tubule, indicate that the spermatogenic process had been interrupted for a period of time but subsequently resumed. Many tubules with maturing sperm exhibited a disorderly arrangement of these cells rather than the oriented "bundles" of cells characteristic of the normal mature tubule. Necrosis of spermatids was also prevalent, especially in those tubules just prior to shedding mature sperm into the lumen. Sperm and spermatid numbers were noticeably reduced in many such tubules.

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Several strains of miniature swine are
presently in use and considerable variation obviously exists among them, some of which is certainly related to the feeding regime under which they are reared. Under a system of limiting feed intake, Weaver and McKean (1965) kept the weights of their Pitman-Moore pigs to about 22 kg. at 6 mo. of age and 35 kg. at 1 year. Our system allows free feed consumption and produces pigs which average 9, 22, 33 and 46 kg. at 2, 4, 6 and 8 mo., respectively. Variations in sexual development obviously occur also, since German workers have used pigs which “often reach sexual maturity by 8 to 10 weeks of age” (Haring et al., 1966). The present results indicate that boars of the Pitman-Moore strain reach comparable stages of testicular development an average of only 2 weeks earlier than males of the standard domestic breeds, and that the small size of miniature swine should not be taken to imply early sexual maturity.

Summary

The growth rate and histological development of the testis during the prepubertal and pubertal periods were studied in organs taken at weekly age intervals from males of the Pitman-Moore strain of miniature swine. The periods of most rapid testicular growth and increase in seminiferous tubule diameter occurred at approximately the same age (17 to 21 wks.) in these pigs as in the standard domestic breeds, while comparable stages of histological development were attained some 2 weeks earlier by the miniature pigs. Seminiferous tubules contained only gonocytes and Sertoli cells until about 12 weeks of age. Definitive spermatogenic cells began to appear between 13 and 16 weeks, and spermatids in various stages of maturation were present in 19- to 21-week tissues. All animals more than 23 weeks old possessed tubules with mature sperm.

Literature Cited

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