BRIEF COMMUNICATION

EVALUATION OF A TESTICULAR BIOPSY TECHNIQUE IN THE RABBIT

A. F. McFEE* AND J. J. KENNELLY

Department of Animal Husbandry, Cornell University,
Ithaca, N.Y., U.S.A.

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Summary. A simple and convenient open-surgery procedure for testicular biopsy was tested on rabbits at 1, 3, 5 and 11 months of age. The technique yielded a representative sample of testicular material while damaging less than 1% of the remaining testicular tissue. At least two biopsies can be taken from the sufficiently separated areas of a testis at either 2- or 14-day intervals without the second sample being affected by the first.

Attempts to perform testicular biopsy in domestic animals have met with limited success. McDonald & Hudson (1960) as well as Erb, Andrews, Bullard & Hilton (1944) have reported successful biopsy of the bull while others have found that such procedures seriously damaged the testis (Sykes, Wrenn, Moore, Underwood & Sweetman, 1949; Gassner & Hill, 1955). The technique here applied to the rabbit was based on the apparently successful method of Foote & Koefoed-Johnsen (1959).

Six Dutch-Belted males in each of four age categories were randomly selected from the experimental colony maintained at this laboratory. The first biopsy was taken from the animals in these groups on the day they reached 1, 3, 5 or 11 months of age, respectively.

Light ether anaesthesia was supplemented by procaine injections about the base of the scrotum and an incision approximately 1 cm long was made in the ventral surface of the scrotum. A shorter incision in the tunica vaginalis exposed a small area of the testis. Gentle pressure on the testis held between the thumb and forefinger produced a slight bulging of a portion of the organ through this incision. A small slit was made in an avascular area of the tunica propria using the corner of a sharp razor blade. The mass of tissue which protruded from this cut was snipped off with curved iris scissors and placed in fixative. The site was then blotted for a few seconds with sterile gauze, the testis was replaced in its normal position and the scrotal incision was sutured. In the 1-month-old males the small size and incomplete descent of the testes

* Present address: University of Tennessee–Atomic Energy Commission, Agricultural Research Laboratory, 1299 Bethel Valley Road, Oak Ridge, Tennessee, 37832, U.S.A.

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made it necessary to remove the organ from the tunica vaginalis through an abdominal midline incision just anterior to the penis.

Two males in each age-group were used to test the adequacy of the biopsy in yielding a representative sample of the testis. Biopsies were taken from each testis and followed immediately by bilateral castration. Sections taken at 160 μ intervals from the biopsy and from the testis were compared using a 'hit determination' method (Chalkley, 1943) for estimating the proportion of total testicular volume falling into the following four categories: tissue within a seminiferous tubule, space within a tubule, non-tubular tissue, and non-tubular space. In these tissues taken from the 5- and 11-month-old animals, fifty randomly selected tubules in each testis and biopsy were classified as to their stage in the spermatogenic cycle (Swierstra & Foote, 1963). The diameters of twenty additional cross-sectioned tubules were measured.

The four males remaining in each group served to evaluate the effect of the biopsy on the remaining gonadal tissue. Each of the eight testes in the group was biopsied twice, the interval between operations being 2 days for half the organs and 14 days for the other half. Unilateral castrations were performed at 4, 16 or 56 days after the initial biopsy. The results of this design were two biopsies from each testis at 2-day or 14-day intervals, followed by evaluation of the remaining testicular tissue 2 days and 42 or 54 days after the second biopsy. It was expected that examination at 2 days postoperative would indicate the extent of inflammation produced by the operation while at 42 or 54 days any permanent impairment of spermatogenic function could be evaluated.

Upon castration, two pieces of the testis, each containing one of the biopsy sites, were removed and fixed. In sections taken at 200 μ intervals from these samples, the volume of tissue showing functional damage was estimated from the diameter of the affected area and the known interval of the sections. The specific gravity of similar testes was determined and used to calculate the weight of damaged tissue which was then compared with the total weight of the testis at castration.

The weight of the testis at castration was not reduced by the biopsy operation; those biopsied at 3 or 5 months of age had average weights of 1.8 and 1.8 g when removed 2 months later, and testes removed from the same number of control animals at 5 and 7 months of age averaged 1.4 and 1.9 g respectively.

Chi-square analysis of the percentage of the total 'hit determinations' falling in each classification of tissue or space showed no significant difference \((P>0.90)\) between the biopsy and testis tissues. The average tubule diameters in the biopsy tissue were not significantly different from those in the remainder of the testis \((P>0.99)\). The chi-square value for the biopsy versus testis comparison based on tubule stages lay between the 0.75 and 0.50 levels of probability. The fact that a maximum of only fifty classifiable tubules could be obtained from the biopsy samples may have influenced the probability level obtained for this comparison.

The calculations of volume of damaged tissue shown in Table 1, are necessarily based on testis weight at castration. Some increase in testis size between the time of biopsy and castration may have influenced the absolute values for the observations with the longer time lapse between biopsies, especially among
the younger animals. However, the small values obtained still indicate very little
damage as a result of the operation. A tendency towards less damage among
the testes biopsied at 1 month is obvious except in those examined 16 days
afterwards. The magnitude of the damage in this group was largely due to one
animal whose testes were unusually small when removed. An average specific
gravity of 1.035 (determined for two testes from each of four normal rabbits)
was used for these calculations.

Two and 4 days after the operation, areas of damaged tissue were usually
characterized by a moderate invasion of blood cells. Two weeks after an
operation many tubules in the damaged area contained only Sertoli cells and
spermatogonia, but many possessed 'regenerative' spermatocytes at 6 and 8
weeks. In no case was any evidence of damage seen in sections of the second
biopsy from a testis.

Inflammation and excessive bleeding into the tissues are generally blamed for
the extensive testicular damage which has followed most biopsies of domestic
animals. The success of the current technique is attributed to several factors

<table>
<thead>
<tr>
<th>Age at first biopsy (months)</th>
<th>Days after previous biopsy</th>
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<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>0.43</td>
</tr>
<tr>
<td>3</td>
<td>0.76</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
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<tr>
<td>11</td>
<td>0.56</td>
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</tbody>
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* Sections lost in processing.

which helped to reduce bleeding and inflammation of the organs. The rabbit
has a definite initial advantage over other domestic animals as a biopsy subject
because of the pattern of the blood supply to the testis. The tendency for the
surface blood vessels to run parallel to each other makes the selection of a rela-
tively avascular area for an incision much easier than it would be in the ram,
bull or boar testis. Secondly, when this technique is employed no cuts are made
below the surface of the testis, and any bleeding is almost entirely external. It
is felt that this avoidance of internal bleeding was the factor primarily respon-
sible for limiting testicular damage to the very small areas around the biopsy
site.

The tissue samples obtained were often rather small, those from the younger
males requiring special attention to avoid loss during processing. In spite of this,
the results indicate that they were representative samples of the testes from
which they came. There seems to be no reason to doubt that the developmental
and/or functional state of the testis in general can be adequately determined by
histological examination of such samples. The complete absence of damaged
tissue in second biopsies from a testis, together with the limited amount of damage found around biopsy sites, indicate that several successive samples could be taken from a rabbit testis without danger of obtaining abnormal tissue.

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REFERENCES