THE EFFECT OF CHORIOPTIC MANGE (*CHORIOPTES BOVIS*) ON RAM FERTILITY

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ABSTRACT

Chorioptic mange of the scrotum was induced in 14 of 27 rams during a study of the effects of scrotal mange on ram fertility. Scrotal lesions varied from those of only a few sq. mm in size to those which covered virtually all of the scrotum in an inflammatory exudate which was up to 5 cm thick. Beneath the scabs there were usually smaller areas of broken skin which exuded small amounts of clear fluid and scab removal usually caused petechial haemorrhages. The scrotal lesions were usually associated with similar lesions in the vicinity of the accessory digits and manipulation of chorioptic lesions in both regions often produced a characteristic nibbling response. The dermatitis, which was non specific and typical of an allergic exudative dermatitis, did not penetrate the tunica vaginalis sac nor did it involve the scrotal contents. Scrotal mange had no apparent effect on the general health of rams and limb movement was affected in only one of approximately 200 rams examined with this disease. This ram had extensive lesions on both the lower legs and scrotum. The scrotal lesions on all but one of the rams with induced mange, and many of the rams introduced with extensive scrotal mange, cured spontaneously during the observation period. In most of these cases there was no sign of a prior scrotal dermatitis after resolution of the disease while in a few there was a permanent increase in skin thickness.

In vivo mite assessment provided a simple and accurate method of detecting scrotal mites and the

technique also gave a clearer understanding on the host-mite-mange relationship.

Data collected from 24 rams with lesions of scrotal mange involving less than 10 sq. cm of the scrotum demonstrated that lesions of this extent had probably no effect on spermatozoa production. On the other hand lesions involving more than a third of the scrotum of 30 rams examined were invariably associated with seminal degenerations. Some cases of extensive scrotal mange had little effect on spermatozoa production while other relatively mild cases caused severe testicular degeneration. The degeneration varied from a mild, transient decrease in semen quality through to complete spermatogenic arrest at the spermatogonial stage of spermatogenesis. There was a close relationship between testes size, seminiferous tubule size and spermatogenic activity in rams with extensive scrotal mange. The seminal and testicular degenerations were similar to those seen in rams whose testes had been exposed to elevated temperatures.

The average testicular temperature of 11 rams with extensive scrotal mange with severe testicular atrophy was 1.8°C (range 0.6°C - 3.1°C) above that of 11 control rams and there was a similar drop in testicular temperature in 6 rams following successful treatment of the disease. As no other factor could be incriminated, it was concluded that scrotal mange caused the testicular degeneration by raising the temperature of the scrotal contents.

Scrotal mange had little if any effect on androgenic status, as assessed by changes in sexual behaviour, seminal plasma fructose, seminal vesicle weight, seminal vesicle fructose, Leydig cell numbers or Leydig cell affinity for the Sudan black stain.

In all cases examined, recovery of reproductive function followed successful treatment or spontaneous cure of scrotal mange. Recovery from mild seminal degenerations often occurred within a few weeks while cases of longstanding testicular atrophy took more than six months to recover.

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INTRODUCTION

A high reproductive performance from farm animals is essential for an efficient live stock industry. In New Zealand low fertility of the national sheep flock has caused concern in recent years. This low fertility has been attributed usually to genetic and management factors while diseases that may well be affecting fertility have received less attention. One such widespread disease is chorioptic mange. This disease, caused by the mite Chorioptes bovis, is characterised by an exudative dermatitis on the lower legs of sheep of both sexes and the scrota of rams. In New Zealand, scrotal mange caused by C. bovis is considered by many veterinarians to be the most common cause of rejection of rams for "genital unsoundness" (A.M. Day, pers. comm.; N.H. Mavor, pers. comm.; T.D. Quinlivan, pers. comm.) and in 1970, 7 1/2% of the many thousands of rams examined for genital soundness by veterinarians of the Riversdale Veterinary Club were found to have this disease (N.H. Mavor, pers. comm.)

Although it has been said for many years that scrotal mange does affect fertility (McFarlane et al., 1952; Miller and Moule, 1954; Whitten, 1968; Bruere, 1970) there is little documented evidence to support the statement. While studying the general relationship between semen quality and ram fertility Edgar (1959) found that between two examinations one of his experimental rams developed scrotal mange and during this period semen samples had deteriorated "from satisfactory to unsatisfactory". Ewes mated to this ram failed to conceive. In an extensive survey of chorioptic mange in Germany, Hiepe et al. (1968) noted that 4.2% of rams with severe foot mange exhibited "slowness of mating". Although Hiepe et al. (1968) found that 27.5% of rams examined had scrotal mange, there was no mention of scrotal mange affecting fertility. Crawford et al.

(1970) published the results of a survey in which they palpated the scrota and scrotal contents of 10,800 rams in the Gisborne district. It was noted that of 92 rams with scrotal mange, 32% had testes "smaller than normal" and 13% had testes classified as "bilateral hypoplasia or atrophy". No attempt was made to relate testes size with lesion severity nor to define if the small testes were hypoplastic or atrophic. Semen quality, testicular histology, or other estimates of reproductive function were not assessed on any of the rams. Because many of the rams with scrotal mange had testes of normal size and tone, Crawford et al. (1970) concluded that "it is unlikely that chorioptic mange of the scrotum is a cause of these abnormal testicular conditions" and suggested that scrotal mange was the result of testicular hypoplasia rather than a cause of testicular atrophy. Conclusions such as these show that there is still an almost complete lack of knowledge on the relationship between scrotal mange (C. bovis) and ram fertility.

The aim of this study, therefore, was to examine the relationship between scrotal mange and reproductive function in the ram.

PART I

PATHOGENESIS OF SCROTAL MANGE

(*CHORIOPTES BOVIS*)

Chapter 1.

PATHOGENESIS OF SCROTAL MANGE (CHORIOPTES BOVIS)

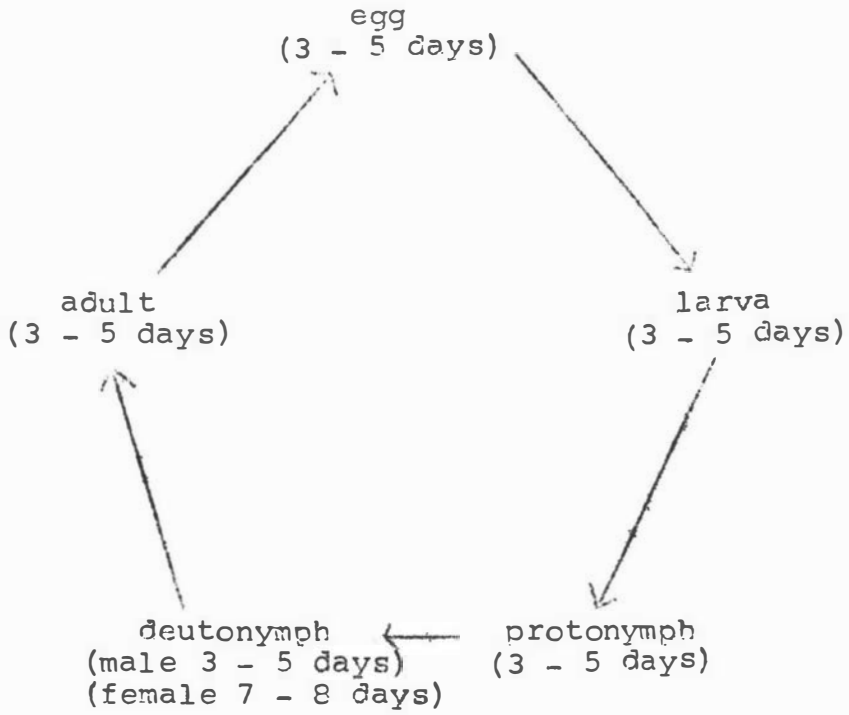
1.1 Introduction

(i) Chorioptes bovis

Sweatman (1957) showed that four species of Chorioptes (C. bovis, C. equi, C. caprae and C. ovis) had no structural differences, were able to cross breed and could be transmitted from one host species to another. Because of this and other data, Sweatman (1957) declared that the four species were synonomous and that the name of priority be C. bovis. Sweatman (1957) was also the first to rear chorioptic mange mites in vitro through sequential generations. He was able then to describe accurately the morphology and life history of the various stages (Text-fig. 1). On hatching, the larvae were translucent, with six legs and morphologically asexual. In the later stages the mites were brownish grey with eight legs and showed sexual dimorphism. There was a gradual increase in size with each stage, the adults measuring approximately 0.3 - 0.4 mm in length. The life cycle in vitro was completed in about three weeks and the egg laying females lived about the same time. Non egg laying females and adult males lived up to 7 - 8 weeks. Ovigerous females under the in vitro conditions laid their eggs singly, about one per day for 3 - 16 days.

The following classification for C. bovis was given by Baker et al. (1958): order Acarina, suborder Sarcoptiformes, family Psoroptidae, genus

Text-fig. 1: Life cycle of Chorioptes bovis under in vitro conditions (Sweatman, 1957).



Chorioptes, species bovis.

Chorioptes bovis has been recorded from most areas of the world from sheep, cattle, goats and horses as summarised in Table 1.

Most early reports of C. bovis from sheep have been in association with sporadic outbreaks of chorioptic mange, while actual surveys of mite incidence have been reported only in recent years. In one survey in the State of New York, where only 6 sheep or fewer were examined from each of an unspecified number of flocks, 80% of the flocks examined were found to be infested (Matthysse and Marshall, 1963); while another American survey showed that 57.5% of 40 flocks from 8 widely separated states to be infested with C. bovis (Roberts et al., 1964). Matthysse and Marshall (1963) and Roberts et al. (1964) both sampled from the lower legs and Roberts et al. (1964) found that at least 20% of the sheep examined had C. bovis mites on this region. A survey in Germany, of 118 flocks, showed a 100% flock incidence, 45.1% of 1,602 lower legs examined being positive for C. bovis (Hiepe et al., 1968). These results confirm the earlier report of Hiepe and Splisteser (1962) in which 62.4% of 138 sheep examined in the Berlin and Frankfurt regions carried C. bovis mites. Mites were detected far more frequently on rams than on other classes of sheep in both German surveys. Forty five of the 47 rams examined by Hiepe and Splisteser (1962) had mites on the lower legs while Hiepe et al. (1968) found that 88.9% of an unspecified number of rams in their large survey were mite carriers. Hiepe et al. (1968) suggested that housing rams all year may be the reason for a

Table 1: Reports illustrating the cosmopolitan distribution of *Chorioptes bovis* on domestic animals.

COUNTRY	SPECIES	AUTHOR
Australia	sheep	Seddon, 1951
	cattle	Stewart, 1928
	horses	Rose, 1940
Canada	sheep)	
	cattle)	Sweatman, 1957
	horses)	
	goats)	
England	sheep	Cave, 1909
	cattle)	Kirkwood and
	horses)	Littlejohn, 1970
Germany	sheep)	Zurn, 1874
	horses)	
Indonesia	goats	Oudemans, 1926
New Zealand	sheep)	
	cattle)	Helson, 1956
	horses)	
South Africa	goats	Bedford, 1932
South America	sheep	Arambillete, 1955
Switzerland	cattle	Bouvier, 1947
U.S.A.	sheep)	Matthysse and
	cattle)	Marshall, 1963
	goats	Kemper *et al*., 1952
U.S.S.R.	sheep)	Ershov, 1961
	cattle)	
	horses	Palimpestov, 1947

higher incidence of mite infestation. The same authors noted, but did not document, that mite numbers increased and lesions became more severe during the winter months. No information is available on the seasonal variation in mite numbers or lesion severity on either sheep or cattle run on pasture throughout the year.

There is virtually no information available on the incidence or distribution of C. bovis in the New Zealand sheep flock, the mite only being isolated from rams with scrotal lesions (McFarlane, et al., 1952; Crawford et al., 1970; Quinlivan and Lindsay, 1971).

Both Sweatman (1955, 1956) and Butler (1968) have made systematic studies of the mite distribution on cattle. Both authors have shown that the pasterns of cattle were commonly infested all year. Butler (1968) showed that in relatively small numbers, mites were able to "over summer" on the extremities of the legs and tip of tail. Mite numbers increased markedly in the winter months in housed cattle, mites being found over most of the body with the largest numbers centred in the escutcheon region. From his study Butler (1968) concluded, "migration of mites, beginning in February (northern winter), probably started from the mid-escutcheon and tail head, with populations moving down both the escutcheon and tail as colonies. These movements appear to be correlated with the change in temperature for these preferred areas. A reverse migration probably occurs during the Fall as mites increase in number". In sheep there have been no comparable mite distribution studies. In most sheep studies, mites have been

isolated only in association with suspected mange lesions, generally on the lower legs in all classes of sheep and also on the scrota of rams (Zurn, 1874; McKenna and Pulsford, 1947; Hiepe et al., 1968; Crawford et al., 1970; Kirkwood and Littlejohn, 1970). Mites have been found also in association with lesions on the head (Sweatman, 1957; Hiepe et al., 1968) and on the shoulder (Roberts et al., 1964). Such data has led many authors to conclude that the lower legs of sheep are the predilection site for C. bovis (Lapage, 1962; Hiepe and Splisteser, 1962; Marsh, 1965; Kirkwood and Littlejohn, 1970).

(ii) Chorioptic mange

In 1835 Keglar first associated a dermatitis with the mite now known as Chorioptes bovis (Sweatman, 1957). The first report of the mite being associated with mange on sheep was recorded in Germany by Zurn (1874) when he observed a Negretti Merino ram with chorioptic mange on the legs and on the scrotum. Since this time mange attributed to C. bovis has been recorded in most areas of the world from most breeds of sheep (Table 2).

Roberts et al. (1964) found only 2 of the 201 sheep examined or 4.7% of the mite carriers had chorioptic mange. Conversely, Hiepe and Splisteser (1962) found 54 out of 138 sheep examined or 62.8% of the mite carriers had chorioptic mange. Hiepe et al. (1968) found 77.5% of all rams examined had leg mange and 27.5% had scrotal mange. Apparently the incidence of chorioptic mange in sheep is low in Britain. "Routine clinical examination of the feet of many thousands of sheep .... revealed only one positive case" (Kirkwood and Littlejohn, 1970).

At the beginning of this study there was no

Table 2: Reports illustrating the cosmopolitan distribution of chorioptic mange in many breeds of sheep

COUNTRY	BREED	AUTHOR
Australia	Merino, Corriedale	Seddon, 1951
Canada	Suffolk	Sweatman, 1957
England	Romney Marsh	Cave, 1909
	Cheviot, Dorset Horn) Suffolk, Clun, Welsh) Mountain)	A. Kirkwood, pers. comm.
Germany	Negretti Merino	Zurn, 1874
New Zealand	Romney, Merino,) Southdown, Corriedale) Half-bred (Merino x) English Leicester)	Crawford et al., 1970
Scotland	Scottish Black Face	A.N. Bruere, pers. comm.
South America	-	Arambillete, 1955
U.S.A.	Southdown, Hampshire	Roberts et al., 1964
U.S.S.R.	-	Ershov, 1961

published data on the incidence of chorioptic mange in the New Zealand sheep flock. Recently Crawford et al. (1970) published results of a survey of 10,800 rams in the Gisborne district. They found 121 or 1.1% had scrotal mange and specifically mentioned that none of the rams had leg mange. In all other reported cases of chorioptic mange on sheep, the lesions were found on the lower legs and less frequently on other body regions. Quinlivan and Lindsay (1971) found that of 5,017 rams examined in the Northland district 274 or 5.5% had scrotal lesions. Dermatophilus species and various ecto-parasites were isolated from scrotal skin scrapings. Since no attempt was made by the authors to define the macroscopic or microscopic lesions or relate gross clinical findings with laboratory results, it is impossible to determine the number of chorioptic mange cases from their data.

Most accounts of chorioptic mange in sheep are brief descriptions from observations on rams presented with mange on their legs or scrota. Usually lesions on the feet are described as yellowish crusts or lumps of dried exudate with the underlying skin moist and reddened (Matthysse and Marshall, 1963; Roberts et al., 1964; Hiepe et al., 1968; Kirkwood and Littlejohn, 1970). McKenna and Pulsford (1947), Crawford et al. (1970) and J. G. Matthysse (pers. comm.) have described scrotal lesions similarly. Hiepe et al. (1968) and Kirkwood and Littlejohn (1970) noted that skin lesions between and below the accessory digits on some rams became thickened and Hiepe et al. (1968) suggested that this thickening was due to long term mite activity. These reports only give a scant impression of the macroscopic appearance of chorioptic mange in sheep. Hiepe et al. (1968)

have reported briefly on the histopathology of chronic chorioptic mange on the lower legs of sheep. They noted an increase in the thickness of the stratum corneum and disruption of the epidermis to the extent that the papillae in some areas were exposed to the surface. A mild infiltration of inflammatory cells was observed in the reticular layer with a more intense accumulation in the papillary layer of the dermis and there also appeared to be an increase in size of the papillae. There are no reports on the histopathology of scrotal mange. Further, there is no information on the macroscopic or microscopic development of leg or scrotal mange in the sheep and in particular nothing is known of the time interval from mite infestation to the occurrence of scrotal mange or of the time sequence of lesion development.

McEnerney (1953) gave a long description on development of chorioptic mange in cattle but his description was difficult to interpret and gave no clear picture of the gross changes that occurred. He described scabs that could be felt but not seen, early lesions as being "pimple like scabs" and large lesions as being thickened scabs of "soup plate size". In contrast Sweatman (1956) gave a short but concise description of lesion development in cattle. He noted an initial increase in the amount of scurf, followed by a small irregularly outlined patch of coalesced papuliferous nodules with exuding serum and subsequent scab formation. According to Pullin (1956) cattle skin becomes considerably thickened and wrinkled in prolonged cases of choriptic mange.

(iii) Host-parasite-mange relationship

Early workers, for example Megnin (1877), assumed that chorioptic mange was caused by the mites

actively feeding on the skin. The fact that C. bovis was not associated always with chorioptic mange was noted by Johne (1877) who found that sometimes mites infested the hind pasterns of cattle without causing lesions. Similarly McEnerney (1953) found "enormous" numbers of mites on cattle in areas without any discernable lesions and he argued that if the mites feed on the skin, such large numbers on very small areas must cause some visible reaction. From these observations McEnerney (1953) concluded that "chorioptic mites feed on the scurf and not on the skin directly". Further, Sweatman (1957) was able to maintain one population of chorioptic mites in vitro for long periods on epidermic debris. From the above data Evans et al. (1961) suggested that C. bovis mites may be simply "scavenging species rather than true external parasites". No direct microscopic observations of the mite on the host have been reported in studies investigating the host-parasite relationship.

Although many animals carry mites with no lesions, other animals with severe chorioptic mange may harbour very few mites (McKenna and Pulsford, 1947; McEnerney, 1953; Pullin, 1956; Matthysse and Marshall, 1963). Usually, lesions of chorioptic mange in cattle remain small, but occasionally in some animals they become widespread and severe within a few days (McEnerney, 1953; Sweatman, 1956). McEnerney (1953) suggested that an allergic reaction would explain these observations. In a preliminary investigation into the immunological aspects of the disease, Butler (1968) showed that two out of six cows with chorioptic mange had natural antibodies to mite antigens. Lesions resembling chorioptic mange were induced by rubbing, once only, mite extracts into skin tattoo wounds and Butler (1968) clearly equated antibodies and experimental lesion production as being indicative of a causal relationship. However, the findings of humoral

antibodies in two of six cows with chorioptic mange does not establish that chorioptic mange is an allergic response. Further, some control and affected cows produced skin reactions to mite extracts while other affected and control cows failed to respond. Thus the skin responses to mite extracts as well as the finding of humoral antibodies to mite extracts in two cows with chorioptic mange may not be indicative of an allergic reaction.

(iv) Conclusions

The following points arise from a review of the literature on chorioptic mange:

(a) No detailed description of scrotal mange, either grossly or histologically is available.

(b) There is a complete lack of information on the development of scrotal mange.

(c) Very little is known on the incidence or distribution of C. bovis in the New Zealand sheep flock.

(d) The only data available on scrotal mange in New Zealand was from a survey undertaken twenty years ago and this survey was limited to one district. No indication of the proportion of rams with small, moderate or extensive lesions of scrotal mange was given.

(e) The distribution of lesions of chorioptic mange on New Zealand sheep appears to differ from that reported in all other countries.

(f) The association between C. bovis and lesion development is still not clear.

Because of the little information available, a preliminary section of this thesis is devoted

to a description of the macroscopic and microscopic development of scrotal mange and the relationship of C. bovis to lesion development.

1.2 General Management of Experimental Animals

Experimental rams were either housed indoors or run on pasture. Rams kept indoors were housed in concrete pens (12ft x 12ft), no more than six to a pen and bedded on straw which was changed every two days. These rams were fed on high quality meadow hay and given approximately four ounces of sheep nuts daily. The sheep nuts contained sodium molybdate and calcium sulphate to prevent copper poisoning as recommended by Hogan et al. (1968).

Rams run outside were set stocked on predominantly perennial rye grass pasture, their diet supplemented with hay or sheep nuts when grass was in short supply. Natural shade was available to these rams at all times.

Prior to experimentation all rams were drenched with a widespectrum anthelmintic and vaccinated against the common Salmonellae and Clostridial diseases. The rams were shorn so that at no time did any ram have more than eight months wool growth, while the scrotum was trimmed with hand shears so that scrotal wool length was maintained at 2 - 3 cm. The hooves of all rams were checked and trimmed bimonthly and rams run on pasture were drenched for internal parasites at 1 - 2 month intervals.

1.3 Materials and Methods Used in Studying the Development of Scrotal Mange (C. bovis)

(i) Animals used

During 1969 preliminary attempts were made to reproduce extensive scrotal mange by either

infesting "mite free" housed rams with C. bovis mites or simply housing infested rams. Rams were assumed to be mite free when no mites could be found on their lower legs or scrota one month after dipping twice in 0.06% "Ciodrin" ("Novex", Tasman Vaccine Laboratories, N.Z.) or 0.06% diazinon ("Diazatos", Tasman Vaccine Laboratories, N.Z.) A total of eight 1 - 3 year old rams were studied. Five of these rams were Romneys, one a Cheviot, one a Romney-Cheviot cross and the other a Drysdale (Rae, 1969). The rams were under observation for 4 - 7 months. The scrotum of each ram was examined at 1 - 7 day intervals depending on the time of infestation and the progression of lesions. Only two of the rams developed scrotal mange and both of these cases cured spontaneously during the observation period. The following year a more extensive field trial was undertaken.

As grazing became available a total of 19 Romney ram hoggets were run together under natural farm conditions. Eight ram hoggets, dipped in diazinon in January, were obtained at the end of April 1970 and a further 13 undipped ram hoggets were added to these, five at the beginning of July and eight at the end of August. The majority of these rams were under observation until September 1971 and will be subsequently referred to as the field trial rams. Prior to introduction to the field trial experiment all rams were checked for scrotal mites and those rams without or with only a few C. bovis mites had approximately 200 transferred to their scrota. The rams were examined for scrotal lesions at fortnightly intervals and for scrotal (C. bovis) mites at monthly intervals.

Observations on rams presented with clinical scrotal mange at various stages of development will be used to supplement the discussion on the

pathogenesis of scrotal mange.

(ii) Chorioptes bovis detection, quantitative assessment, and transfer.

Rams to be examined for mites were restrained on a wooden table and their scrota scanned with a Bausch and Lomb binocular dissecting microscope (Rochester, New York) (Fig. 1). The base of the moveable arm which held the microscope was bolted to the table (Fig. 1). For routine observations a magnification of 17x was used while a magnification of 20x to 60x was used for more detailed studies. In routine studies, the scrotum of each ram was examined microscopically for five - ten minutes and from this a quantitative estimate of the total numbers of scrotal mites obtained. Rams were classified into one of six groups: 0; 1-10; 10-100; 100-1,000; 1,000-10,000 and >10,000 mites on their scrota.

The majority of mites transferred were obtained from Ram 13. This five year old ram was introduced in November 1969 and found to have >10,000 mites on the scrotum and the lower legs and was, therefore, kept as a mite donor. A large population of mites remained on the animal throughout 1970, the year when most mites were transferred. For mite transfer, the donor ram was restrained on his side in a Begg's foot trimming cradle (G.N. Begg Engineering Co. Ltd., Southland, N.Z.) (Fig. 2) while the recipient ram was restrained on the wooden table (Fig. 1). Chorioptes bovis mites were detected on the donor ram sometimes with and at other times without the aid of the dissecting microscope. Groups of mites were transferred from the scrotum of the donor ram on the tips of a pair of scissors and placed on a previously clipped area on the distal third of the scrotum of the recipient ram. The recipient ram was restrained until the majority of mites had been observed microscopically to have dispersed on the skin surface. Field trial rams without mites

Figure 1: Apparatus used to assess *Chorioptes bovis* *in vivo* and to restrain rams for mite transfer.

Figure 2: Apparatus used to restrain rams for electrical stimulation (as illustrated) and to restrain mite donor rams.

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had approximately 200 transferred at 1 - 2 month intervals (Text-fig. 2)(p.25).

(iii) Lesion development

At each examination the scrota and scrotal contents of the housed and field trial rams were carefully palpated and the wool separated and the scrotal skin examined. Lesion position, size, shape, colour and texture were recorded in detail. For simplicity in presenting results the scrotal lesions were classified according to the area of scrotum covered in either palpable active lesions or inactive caked lesions. Active lesions were defined as those which had a broken skin surface and exuded small amounts of clear fluid and sometimes blood when the dried exudate was removed. Inactive caked lesions were palpable crusts of exudate which extended down to the skin surface but on removal left unbroken but often moist skin beneath. According to this classification rams were put into one of six categories:

(1) No scrotal mange
(2) Minimal scrotal mange = < 10 sq. cm scrotum involved.
(3) Minor scrotal mange = 10 - 20 sq. cm of scrotum involved.
(4) Moderate scrotal mange = > 20 sq. cm - 1/4 of the scrotum involved.
(5) Severe scrotal mange = > 1/4 - 1/2 of the scrotum involved.
(6) Extreme scrotal mange = > 1/2 of scrotum involved.

Scrotal skin biopsies were taken from normal and affected scrota using a circular dermatome, the cutting blades having a diameter varying from 0.2 cm to 0.7 cm. Care was taken not to penetrate the sac of the tunica vaginalis with the dermatome and haemorrhage was controlled by filling the small pit in the skin with small cotton wool swabs containing

sulphanilamide powder. These swabs were removed, if they had not fallen out, at the next examination. The scrotal skin biopsies were fixed in 10% formal saline and after 24 - 48 hours were processed with the aid of a histokinette. This involved dehydrating through ascending grades of alcohol, clearing in chloroform and embedding in paraffin. Histological sections were stained routinely with haematoxylin (Ehrlich's) and eosin. Van Gieson stain was used to stain specifically collagen fibres.

(iv) Treatment of scrotal mange

After soaking the scrotum in warm soapy water, most of the chorioptic exudate was removed and the scrotum and legs dipped in 0.06% "Ciodrin" ("Novex", Tasman Vaccine Laboratories, N.Z.) or in 0.06% diazinon ("Diazatos", Tasman Vaccine Laboratories, N.Z.) The procedure was repeated at 10 day intervals until the scrotal lesions had healed completely. In one experiment (Chapter 7), the rams were totally immersed in the insecticide for the first two treatments.

1.4 Comparison of a Direct and Indirect Method for Detecting Chorioptes bovis on the Scrota of Rams

Live C. bovis mites are usually isolated by scraping the skin surface of the animal with a sharp edged object and examining the debris collected with a dissecting microscope (Pullin, 1956; Sweatman, 1956; Matthysse and Marshall, 1963; Butler, 1968).

Many rams with C. bovis mites spontaneously nibble when the infested area is manipulated (see page 35). In this study 10 rams that nibbled when the scrota was manipulated were examined. The

scrotal wool covering approximately 25 sq. cm on the distal scrotum was clipped to about 1 cm length and the area scraped lightly with a hacksaw blade. The debris was collected into a sterile petri dish and the contents systematically examined with the Bausch and Lomb dissecting microscope. This method of mite detection was compared with the direct microscopic observation of the scrota of the 10 rams for 5 - 10 minutes using the apparatus illustrated in Fig. 1.

The data presented in Table 3 shows that the indirect method will detect scrotal mites when relatively large numbers are present on the scrotum but may not detect mites when only small numbers are present. The in vivo method detected every ram that was proven positive for C. bovis while the indirect method detected only 70% of the proven carriers. In the 3 rams which were positive only with the direct method, mites were limited to a small area of the scrotum not scraped. Not only did the indirect method fail to detect scrotal mites in some cases but the method gave a relatively poor indication of the number of C. bovis mites present (Table 3).

1.5 Results of Observations on the Pathogenesis of Scrotal Mange (Chorioptes bovis)

(i) Behavioural pattern of C. bovis on the scrota of rams

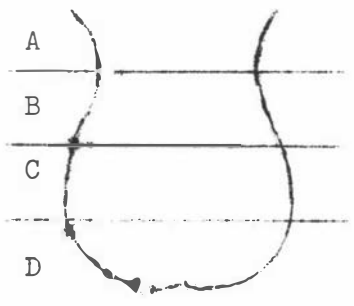
Mites were distributed unevenly on the scrota of rams. With the exception of rams with severe extensive scrotal mange, C. bovis mites tended to accumulate on the more distal aspects of the scrotum and were found usually only on the proximal quarter of the scrotum when there were extremely large numbers present (Table 4). In many rams mites were limited to the base of the scrotum while in others there was a gradient, the majority of mites being found on the base with decreasing numbers proximally (Table 4).

Table 3: Comparison of two methods for detecting *Chorioptes bovis* on the scrota of rams

Ram No.	Scrotal mange severity	Estimated number of mites observed	
		Skin scraping	Direct microscopic observation
1	"minimal"	0	1-10
2	nil	1-10	100-1,000
3	nil	0	10-100
4	"minimal"	10-100	1,000-10,000
5	"minimal"	1-10	10-100
6	nil	1-10	10-100
7	"minimal"	1-10	1,000-10,000
8	"moderate"	10-100	1,000-10,000
9	"minor"	10-100	1,000-10,000
10	"minor"	0	1-10

Table 4: DISTRIBUTION OF *CHORIOPTES BOVIS* ON THE SCROTA OF ELEVEN RAMS (30/5/71)

Position	Ram 452	Ram 214	Ram 16	Ram 435
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
D	+	+	++	++
Scrotal Mange	Nil	"Minimal"	Nil	"Minimal"
Position	**Ram 264**	**Ram 244**	**Ram 195**	**Ram 253**
A	-	-	-	-
B	-	-	-	-
C	-	-	+	+
D	+++	++++	+++	++++
Scrotal Mange	Nil	Nil	Nil	Nil
Position	**Ram 394**	**Ram 161**	**Ram 613**	
A	-	-	++	
B	-	+	+++	
C	++	+	++++	
D	++++	++++	+++++	
Scrotal Mange	Nil	Nil	Nil	

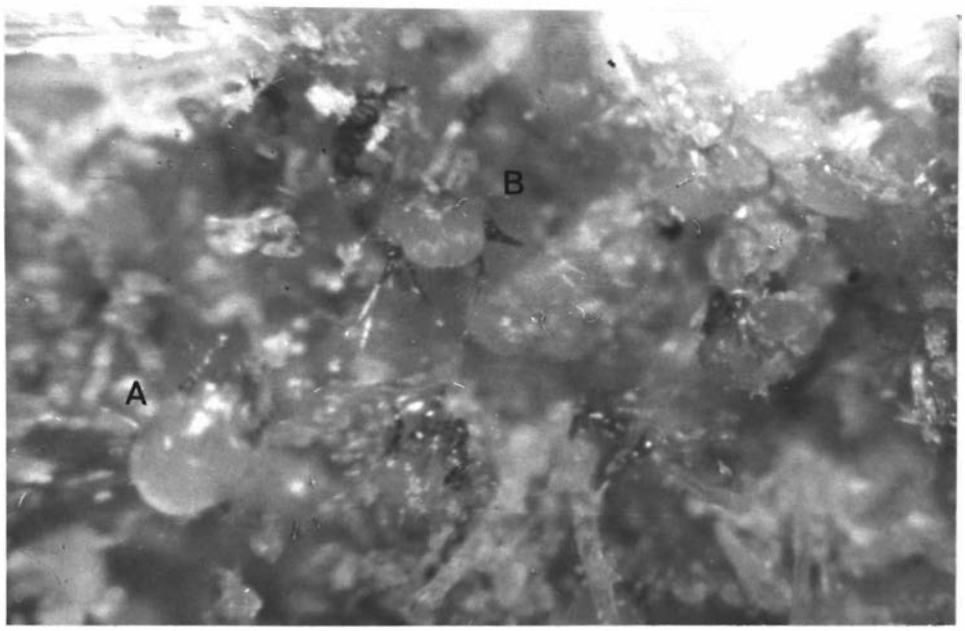


Estimated mite numbers

-	no mites
+	1-10 mites
++	10-100 mites
+++	100-1,000 mites
++++	1,000-10,000 mites
+++++	>10,000 mites

On rams without lesions C. bovis were observed only on the skin surface; on no occasion were they observed off the skin in the scrotal wool or burrowed into the skin. Commonly, the mites were found in groups, the number of mites per group varying from less than ten to more than a thousand. Although individual mites were difficult to see with the naked eye, large groups could be seen readily giving the area of scrotum involved a grey dusty appearance. The bodies of many stationary mites, both individuals and groups, were at almost right angles to the skin. Those in groups supported themselves against each other while those on their own supported themselves on the long setae on their third pair of legs (Fig. 3). Motile mites were observed to stop and push their third pair of legs downwards and as the weight was taken by the setae the body moved from being parallel to the skin surface to being almost perpendicular. Beneath groups or individual mites in the perpendicular position the skin was always dry and usually smooth and glistening. This was in contrast to the surrounding skin which was relatively dull in colour and often scaly. Occasionally, beneath individual and small groups of mites in the vertical position, there were small concave indentations in the epidermis. Sometimes minute ripples were seen on the surface of these indentations. In no instance were mites seen to cause an erosion large enough to penetrate the superficial layers of the epidermis. However, sometimes mites were observed feeding around wool follicles. The epidermal material surrounding the wool fibre was often eaten away, so that mites feeding in the mouth of wool follicles were observed just below the skin surface.

Figure 3: Two C. bovis mites on the scrotal skin surface of Ram 13. Mite A is in the normal motile position (parallel to the skin surface) while Mite B is in the typical feeding position (perpendicular to the skin surface). The setae on the hind legs of Mite B are holding the mite in the perpendicular position.



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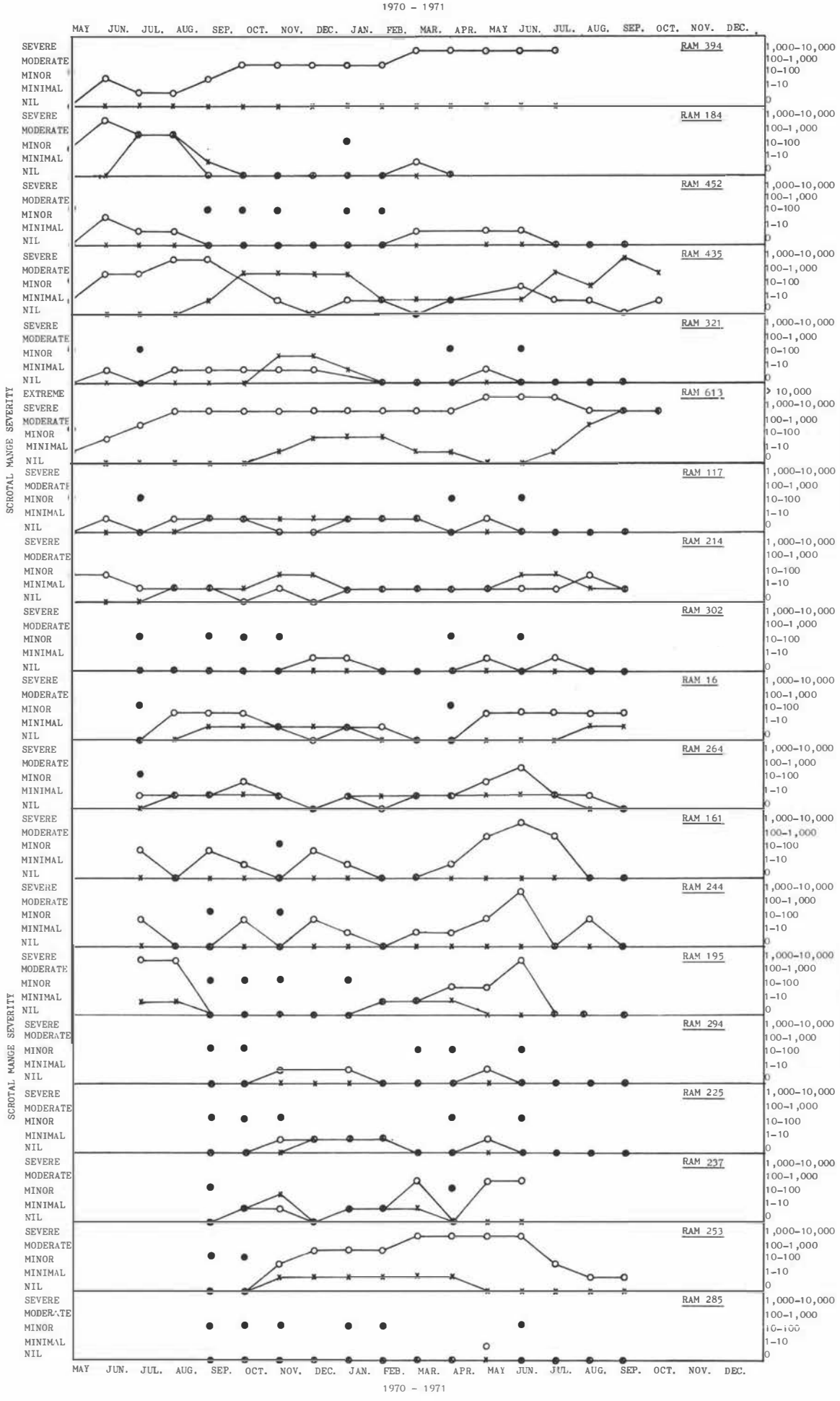
(ii) Lesion development

The experimental induction of lesions in only 2 of the 8 rams in the preliminary investigation during 1969 showed that scrotal mange was not induced readily. During 1970 many thousands of mites were placed on the scrota of field trial rams and many failed to colonize (Text-fig. 2). Not only did mites fail to colonize when transferred to some rams but the same rams failed also to become infested from mite carriers in the same flock (Text-fig. 2). Further, the colonization of the scrotum with mites does not automatically lead to scrotal mange. Rams 244 and 161 had small numbers of mites and Ram 394 had large numbers of mites for more than a year without the appearance of scrotal lesions (Text-fig. 2).

The majority of scrotal mange cases observed in this study were relatively mild. This was so for both experimentally induced scrotal mange and scrotal mange detected during routine examination of ram flocks. Usually the lesions involved only a small area on the distal eighth of the scrotum. Thus both mites and lesions attributed to them tend to occur on the base of the scrotum. In some rams scrotal mange lesions involved large areas of the scrotum and in a few rams all of the scrotum was involved.

The earliest clinical lesion attributable to C. bovis appeared as small, 1 - 3 mm, pale yellow scabs on the skin surface (Fig. 4). These small crumb like scabs gave a gritty feeling to the normally smooth skin and on removal exposed small superficial pits on the skin surface. The surface of these minute pits appeared moist and the small

TEXT-FIG. 2: RELATIONSHIP BETWEEN SCROTAL MITE (C.BOVIS) NUMBERS AND SCROTAL MANGE SEVERITY IN THE SEVENTEEN FIELD TRIAL RAMS



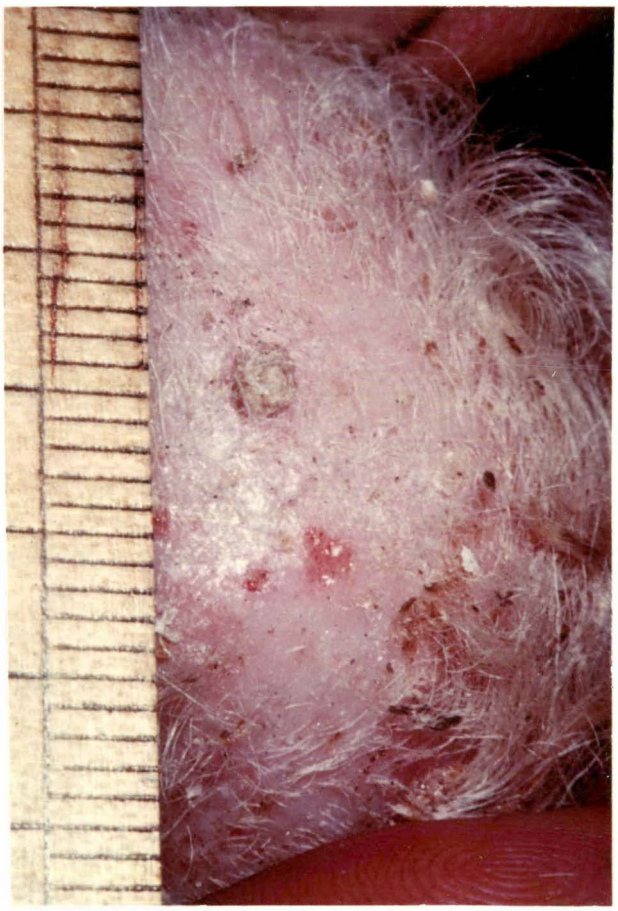
o Estimates number of scrotal C.bovis mites

● Approximately two hundred C.bovis mites transferred

x Scrotal mange severity

Figure 4: The smallest clinical lesions attributable to Chorioptes bovis on the scrotum of a ram. The small yellowish scab is intact in one lesion while two adjacent scabs have been removed exposing a broken skin surface with small amounts of clear fluid which is streaked with minute petechial haemorrhages.

(1 small division = 1 mm)



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amounts of clear fluid exuded were often streaked with minute petechial haemorrhages.

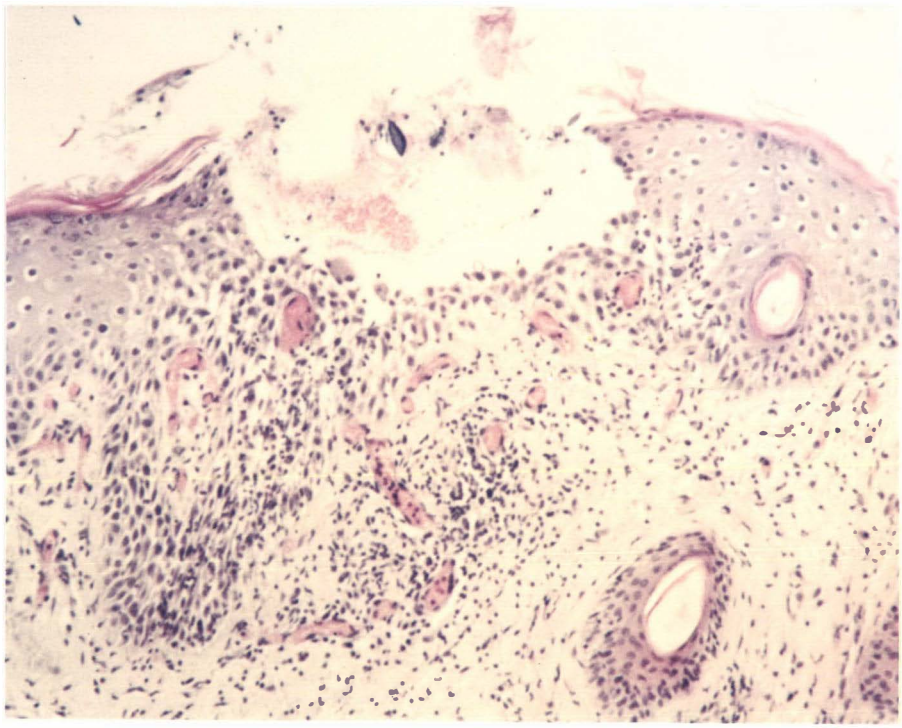
Histologically the superficial skin erosion involved most of the layers of epidermis, dermal papillae sometimes being exposed to the exterior. The epidermal cells in the vicinity of the pits were disrupted with intercellular oedema (spongiosis) and to a lesser extent by intracellular oedema (ballooning degeneration) (Fig. 6). A transition between small amounts of intercellular oedema in the periphery of lesions to microvesicle formation near the pit was frequently observed. Some of the epidermal cells in the vicinity of the pits were pycnotic and small numbers of lymphocytes usually invaded the epidermis. Beneath the skin erosions the small blood vessels in the stratum papillare were dilated (Fig. 5) and there was a mild infiltration, especially perivascularly of inflammatory cells. Apart from the occasional eosinophil, almost all of these cells were mononuclear, the majority being lymphocytes (Fig. 6). The dermal papillae were usually slightly oedematous. The reticular layer (stratum reticulare) and the deep epidermal appendages (sweat glands, sebaceous glands, wool follicles) were not involved in the dermatitis. In many cases the lesions spontaneously cured at this stage. In other rams, as some of the small lesions cured others appeared, resulting in the base of the scrotum appearing scurfy.

In some rams, the small lesions increased in size and the exudate from neighbouring lesions became confluent (Fig. 7). The lesions were typically exudative in nature, the clear exudate drying to form yellowish, crumbly to caked scabs

Figure 5: Histological picture of the earliest clinical lesion attributable to Chorioptes bovis. Note the vasodilation in the region of the epidermal pit and the mild accumulation of inflammatory cells in the stratum papillare.
(H. & E.)
(x 100)

Figure 6: High power of Fig. 5 showing epidermal spongiosis with mild infiltration of lymphocytes (L).

(H. & E.)
(x 400)



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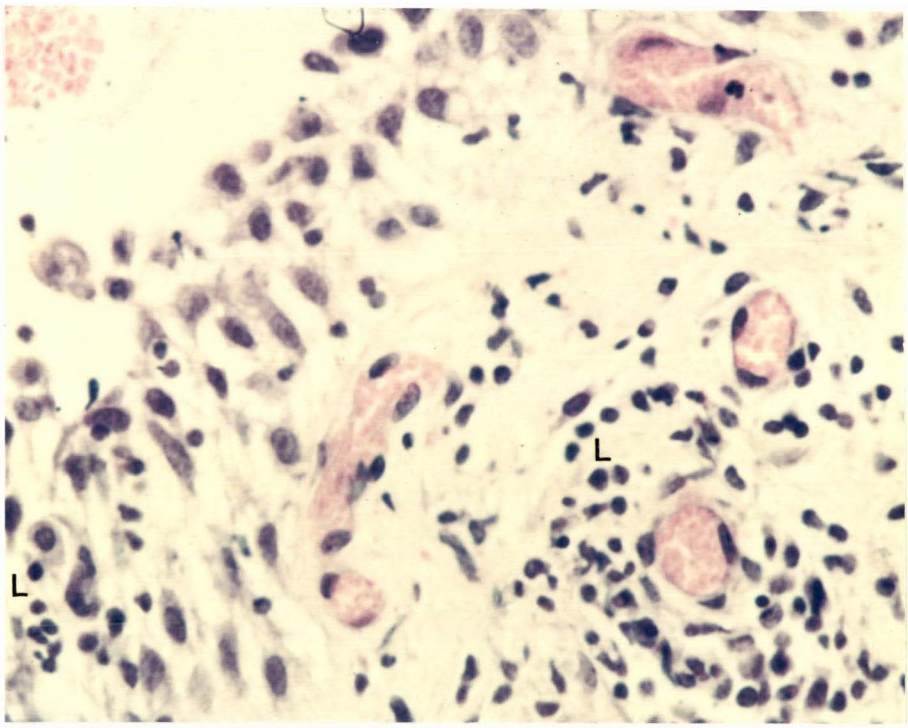

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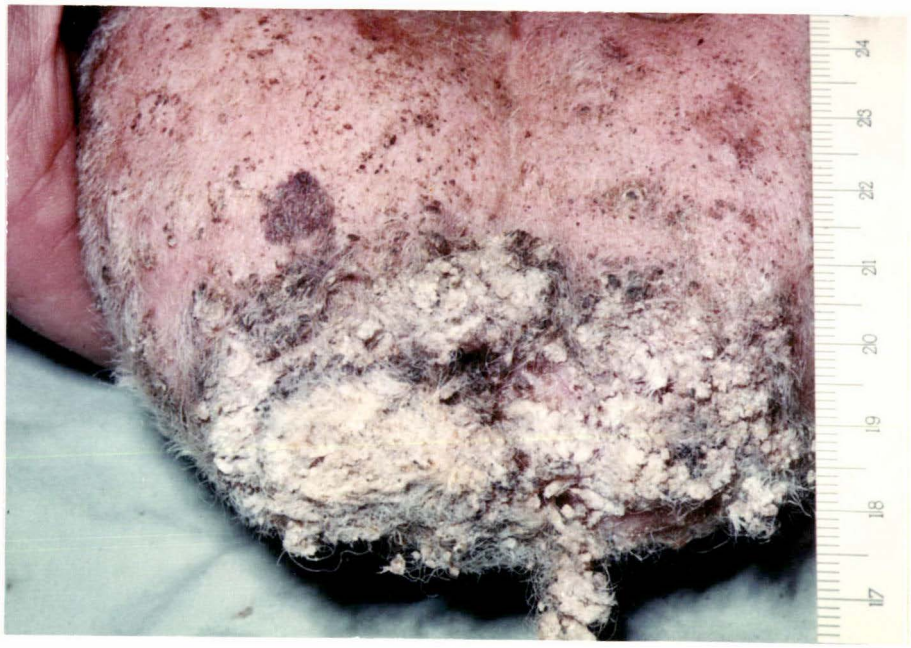
which in the early stages were as much as 1 cm thick. Smears made from the clear exudate often contained varying numbers of eosinophils. Removal of the dried exudate of acute lesions left beneath areas, usually no more than 1 sq. cm in diameter, of broken skin surface which exuded small amounts of clear fluid and sometimes small amounts of blood (Fig. 8). There was usually an increase in the number of mononuclear cells in the stratum papillare and in virtually all cases examined there was a moderate infiltration of eosinophils (Fig. 10). In some cases there was a mild, mainly perivascular, accumulation of inflammatory cells in the reticular layer of the dermis. The small dermal blood vessels were dilated and there were varying amounts of intradermal oedema. The more superficial layers of the epidermis were lacking and occasionally there was a complete lack of continuity of the epidermis, the rete pegs and wool follicles being separated by the dermal papillae, which communicated directly with the surface. The basal epidermal cells present, for example those in the rete pegs, were often disrupted and degenerating. The scabs, which stained a homogenous pink with eosin, contained clumps of eosinophils and nuclear debris. In the periphery of the active lesions the stratum malpighii was often slightly thickened (acanthosis) and the rete pegs were sometimes elongated (Fig. 9). In some rams the lesions cured spontaneously at the acute stage of lesion development while in others the lesions remained chronically active for relatively long periods, for example, 4 - 6 months. The experimentally induced chronically active lesions covered from as little as 4 sq. cm of the scrotum to approximately 30 sq. cm of the scrotum in exudate one to three cm thick. Histologically

Figure 7: Typical scrotal mange involving the base of the scrotum.

Figure 8: Above with the dried exudate removed exposing a hyperaemic skin which is broken in some areas. The broken areas are moist and there is a small amount of haemorrhage.



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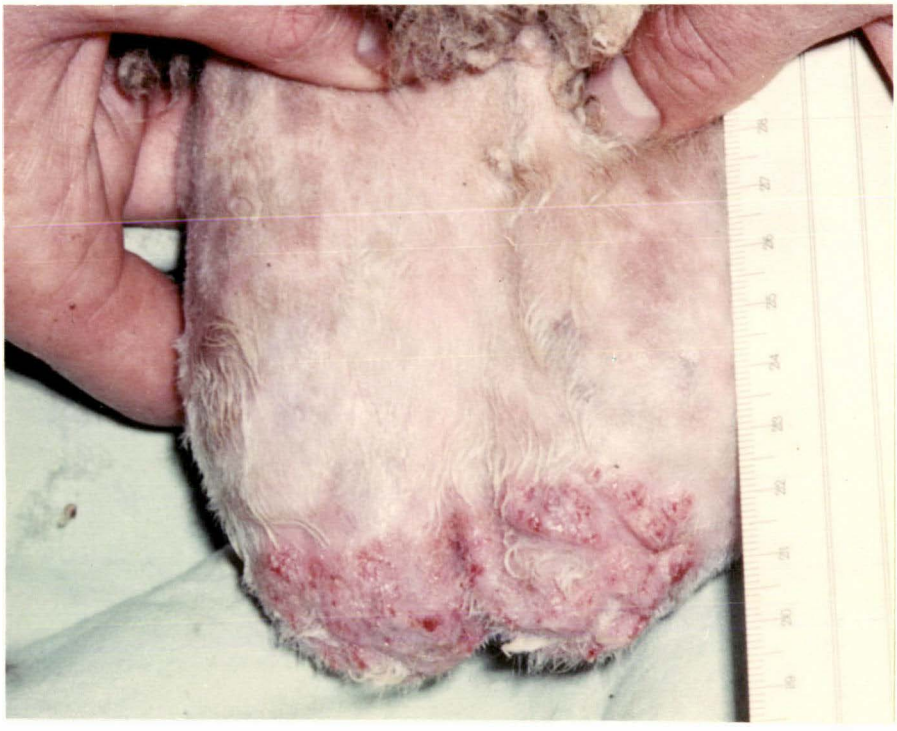

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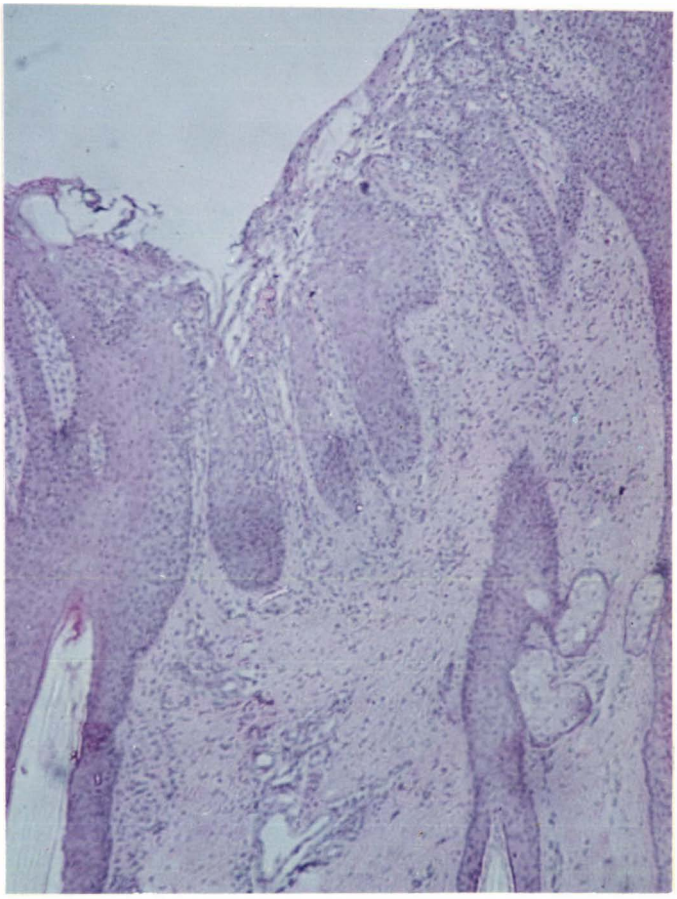
Figure 9: Section taken from active lesions of the scrotum illustrated in Figures 7 and 8. Note moderate infiltration of inflammatory cells in the stratum papillare and to a lesser extent in the stratum reticulare. The epidermis is severely disrupted and there is a moderate elongation of the rete pegs.

Figure 10: High power of Fig. 9 showing that the majority of the inflammatory cells are eosinophils (E).



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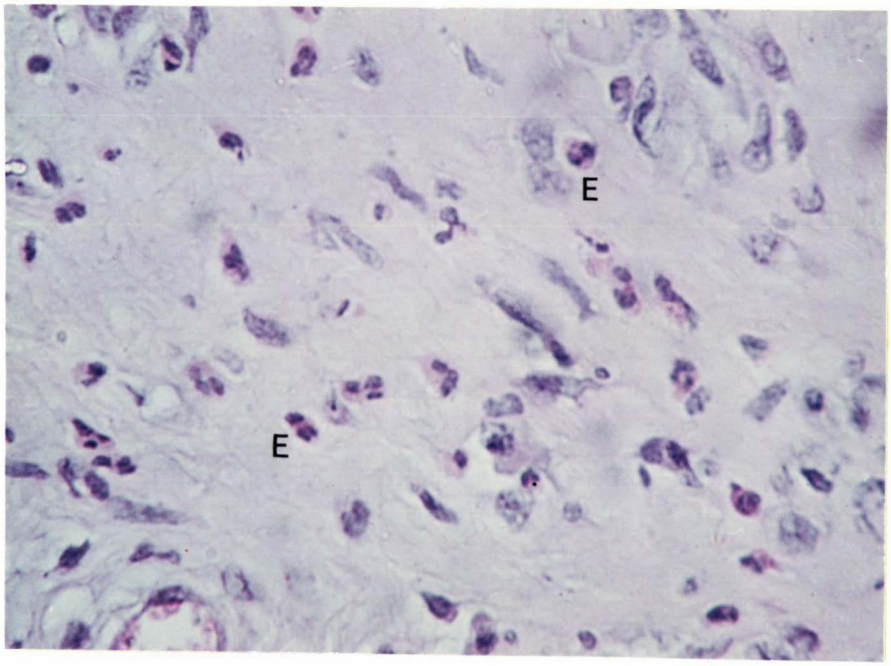

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there was little congestion of the dermal blood vessels and there was only a small amount of epidermal oedema in these chronic lesions. Cell infiltration of the dermis was often marked, with eosinophils usually predominating. The epidermis was disrupted in the vicinity of the active lesions and acanthosis and rete peg elongation was often marked. In the periphery of the active lesions there was often a mild increase in the thickness of the stratum corneum (hyperkeratosis). In some of the chronic cases there was a slight increase in the thickness of the skin in the region of the active lesions, and histologically there was a mild dermal fibrosis. Spontaneous cure of all of the experimentally induced chronic scrotal lesions occurred within 6 months of first appearing. In some rams, for example Ram 13 throughout most of 1970, as some lesions cured spontaneously others appeared. In other rams, lesions that started on the base spread proximally so that during a period of weeks more of the scrotum became involved in active lesions.

On first examination Rams 373 and 612 (Chapter 8) had lesions involving only the distal quarter of the scrotum and during the following weeks the lesions moved proximally resulting in most of the scrotum being covered in crumbly-caked exudate, in some areas, 4 cm thick (Fig. 11). Lesions on the base of the scrotum were chronic while the most proximal lesions were acute. The more rapidly developed lesions tended to produce exudate that caked while slowly developing lesions were more crumbly. Removal of the yellowish exudate from the scrota of Rams 612 and 373 and many other rams examined with extensive scrotal lesions, revealed

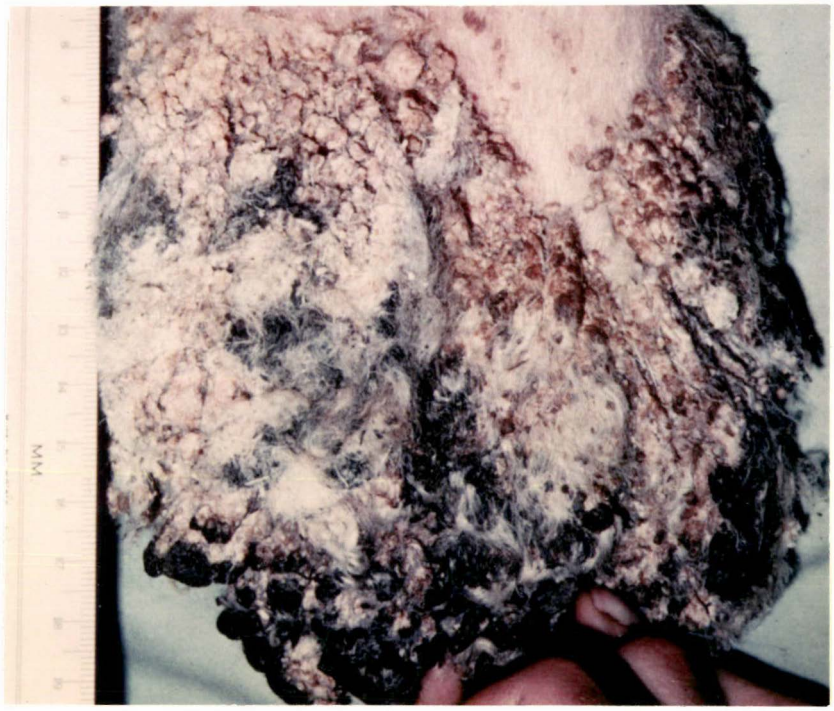
small areas of broken skin which exuded small amounts of clear fluid and blood (Fig. 12). These skin eruptions were often on top of small elevations or papules. Between the papules the skin was usually slightly reddened, and sometimes moist. If the active lesions were close together the papules became almost confluent giving the whole area a slightly thickened feeling and morocco leather appearance. Histologically there were often mild degrees of acanthosis, hyperkeratosis and parakeratosis. Usually there was a moderate to severe infiltration of cells, especially eosinophils, in the upper and to a lesser degree in the lower dermis (Figs. 13 and 14). In some rams there was also a mild dermal fibroplasia. Many of the rams examined with extensive lesions of scrotal mange underwent a partial or complete spontaneous cure while under observation (see Chapter 6).

In a few rams with extensive scrotal mange there was a rancid odour associated with the lesions and in two rams a scrotal wool break occurred, the exudate-matted wool being peeled easily off the scrotum. In a few instances the exudate close to the skin was relatively viscous and light yellow in colour and sometimes the purulent material was contained in superficial cavities (pustules). Invariably, there was a predominance of neutrophilic leucocytes in the inflammatory zone of these lesions. Cutaneous myiasis was associated with extensive scrotal mange in one ram. Although Dermatophilus species were incriminated, the organism was not isolated and there was no histological evidence of its presence in any case of scrotal mange examined.

There was no permanent damage to the epidermis

Figure 11: Extensive scrotal mange. Dried crumbly-crusty exudate approximately 4 cm thick covering most of the posterior aspect of the scrotum.

Figure 12: The anterior aspect of the above scrotum with the exudate removed, exposing broken areas of skin which are exuding small amounts of clear fluid and blood. Between the active lesions the skin, apart from being slightly hyperaemic, appears relatively normal.



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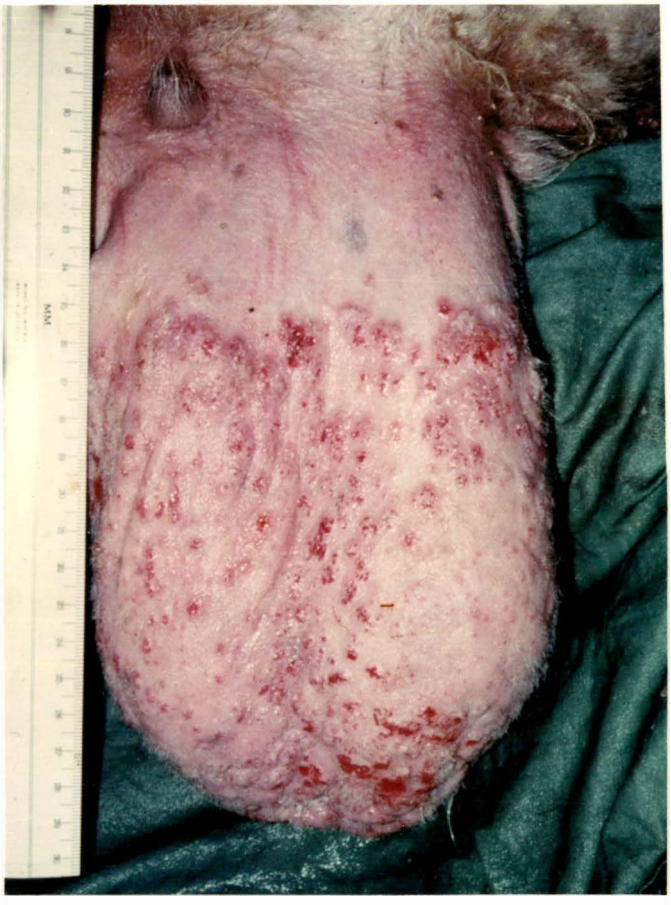

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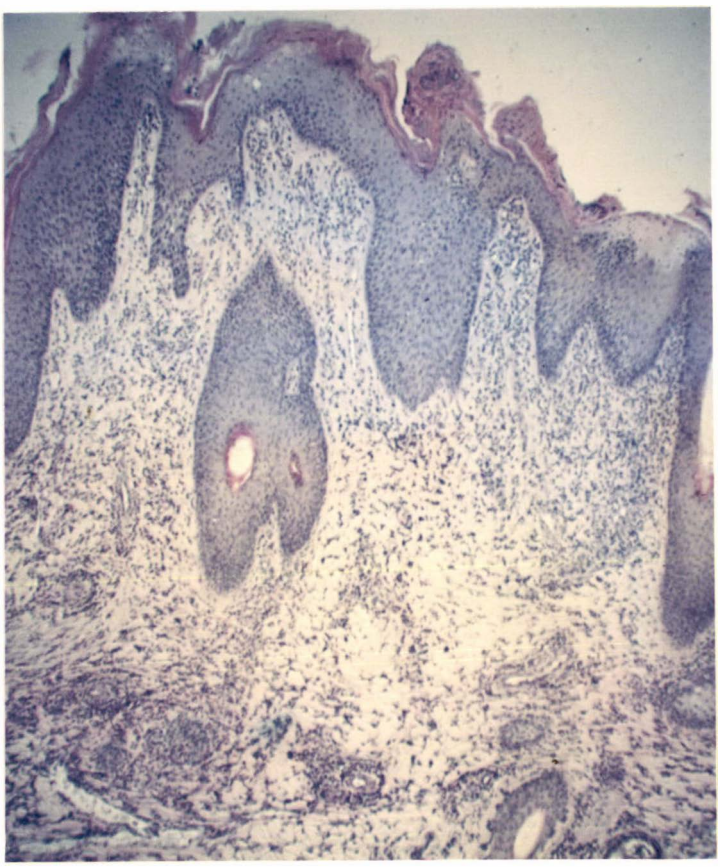
Figure 13: Severe, chronic lesion of scrotal mange. Hyperkeratosis, parakeratosis, acanthosis and an intense accumulation of cells are apparent in both the stratum papillare and stratum reticulare.
(H. & E.)
(x 40)

Figure 14: High power of above. The intense accumulation of cells in the dermis are nearly all eosinophils (E).
(H. & E.)
(x 400)



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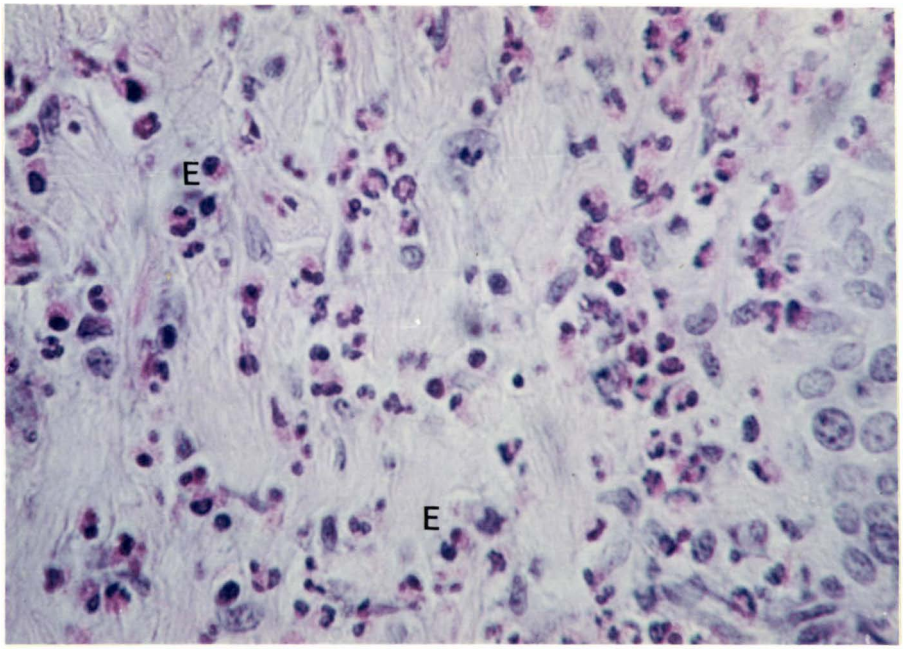

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or its appendages in any of the many rams examined with severe, scrotal mange. Most of the rams that had been successfully treated or had cured spontaneously of extensive scrotal mange had no evidence of permanent skin thickening of the scrotum while the distal aspects, especially the base, of a few rams were permanently slightly thickened. The contracted scrota of these latter rams appeared more corrugated than normal. In November, 1969 two rams were examined with chronic extensive scrotal mange. The scrota of these two rams were pendulous and slightly thickened. At the end of 1971 after both rams had been free of scrotal mange for at least six months the scrota were still pendulous, the distal aspects considerably thickened and the contracted scrotum appeared very corrugated. Further, evidence obtained at postmortem revealed that the dermatitis did not involve or penetrate the parietal layer of the tunica vaginalis, the tunica vaginalis cavity or the testicular capsule in any of the twenty rams examined with extensive scrotal mange.

(iii) Relationship between scrotal mange and *Chorioptes bovis*

Chorioptes bovis mites were associated with every case of experimentally induced scrotal mange. For example, in the field trial experiment mites preceded lesion development in 10 of the 13 induced cases of scrotal mange and at the time of lesion development in the other 3 rams (Text-fig. 2). Mites were associated with the small (1 - 3 mm) scabs in every induced case and furthermore the mites were often only detected in the vicinity of these minute crumb like active scabs. Careful microscopic examination of the small crumbs on one ram revealed a dead mite embedded in a minute

amount of dried exudate.

There was no obvious association between lesion severity or rate of lesion development and scrotal mite numbers (Text-fig. 2). However, there was a tendency for relatively extensive lesions to be produced in association with large numbers of scrotal mites while small lesions were usually associated with small numbers of mites (Text-fig. 2). Extremely large numbers of scrotal mites preceded the only observed case (Ram 13) of peracute development of extensive scrotal mange. Ram 13 had "> 10,000" mites covering all of the scrotum for more than a year and suddenly in November 1970 there was a massive outpouring of serous exudate which covered all of the scrotum within two weeks.

Although mites, in small or large numbers, were observed in close association with almost all cases of induced scrotal mange, live mites were found only on two occasions in direct contact with the moist active lesions. In one of these cases a mite was observed trapped in moist exudate, flailing its free front legs in the air. In the other case a group of mites were surrounded by clear fluid in a developing lesion. When prodded these mites showed only weak movements. Examination of the same area a week later showed many dead mites trapped in a typical chorioptic scab (Fig. 15). Occasionally mites were observed feeding on the top of, or in fissures in dried scabs, mite position, indentations and ripples in the indentations being similar to that seen on the skin surface. However, in confluent lesions the majority of live mites were observed on the boundary between the active lesions and the normal

Figure 15: Two *Chorioptes bovis* mites (A, B) trapped in the dried serous exudate of an active lesion of Ram 613.
(H. & E.)
(x 120)

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skin. Live mites were observed only on the skin surface in the exudate mass in areas where the skin surface was dry, for example, in areas where the exudate was not confluent or where fissures had developed in the caked exudate.

In most cases of relatively severe induced scrotal mange there was a decrease in live mites associated with the initial exudative phase (Text-fig. 2). On microscopic examination many dead mites were observed in the caked exudate. In cases where live scrotal mites decreased to zero, there was usually a spontaneous cure of the lesions at the acute stage of development. Spontaneous cure of scrotal mange at all other stages of development was usually also associated with a complete loss of scrotal mites or at least a loss of mites in the region of the spontaneous cure. Rams 253 and 613 (Text-fig. 2) were exceptions to this. Spontaneous cure of minimal and minor lesions of scrotal mange occurred in the presence of large numbers of scrotal mites in these 2 rams.

(iv) Relationship between scrotal mange and leg mange

Microscopic examination of the lower legs of the field trial rams showed that mites were found more commonly on the lower legs than on the scrotum, even though mites were transferred only to the scrotum. Usually rams with small numbers of scrotal mites had small or moderate numbers of mites on their front and hind legs while rams with large numbers of scrotal mites usually had large numbers on their front and hind legs. Leg mites were found most commonly on the skin at the periphery of the accessory digits and hooves. Mite numbers decreased rapidly from these positions so that

1 - 2 cm away from the coronary band or accessory digits mites were usually absent. However, in some rams mites were detected over all of the lower legs and in two rams with large numbers of leg and scrotal mites the occasional mite was observed on the woolly escutcheon region. The head was the only other area where mites were detected. An occasional C. bovis mite was seen in the region of the infraorbital fossa in one ram and on the poll of another.

In all groups of rams examined lesions of chorioptic mange were found more frequently on the lower legs than on the scrotum (Table 5). For example, 76.1% of the total rams examined in two flocks with a history of scrotal mange and the field trial rams, had leg mange while 49.6% had scrotal mange (Table 5). Further, the majority of the rams with scrotal mange had leg mange (Table 5). In most rams examined the leg lesions were mild with small amounts of crumbly exudate being palpated in the vicinity of the accessory digits. Sometimes small amounts of exudate involved the coronary band, mainly on the lateral aspect of the hoof but occasionally small amounts of exudate occurred medially. In severe cases of leg mange scabs involved most of the posterior leg from the hoof to approximately 2 cm above the accessory digits and sometimes small lesions extended round to the front of the lower leg. In a South-Suffolk ram, leg mange was observed to extend up the posterior aspect of the hind leg as far as the hock. Lesions were found slightly more frequently and often more severely on the hind legs. Limb movement was affected obviously in only one of the approximately two hundred rams examined with leg

Table 5: RELATIONSHIP BETWEEN SCROTAL AND LEG MANGE

Position of lesions	Scrotum & Feet	Scrotum Only	Feet Only	No Lesions	Total Rams Observed
Flock A	41	3	17	6	67
Flock B	8	2	10	9	29
Flock C	2	0	8	7	17
TOTAL	51	5	35	22	113

Flock A and B. Two flocks of rams with a history of scrotal mange

Flock C. Field trial rams examined on the 30/5/71.

and/or scrotal mange. This ram had scrotal lesions involving all of the scrotum and severe exudate about 1 cm thick covered most of the posterior pasterns. There was some chronic thickening of the skin in the region between the accessory digits and small raw fissures occurred in the skin. The abnormal gait of this ram consisted of a characteristic rapid and exaggerated flexion of all the hind leg joints. The gait resembled closely that of a horse suffering from stringhalt.

(v) Nibbling responses associated with Chorioptes bovis and chorioptic lesions

Many rams with C. bovis mites or typical chorioptic lesions on the scrota or lower legs characteristically extended their heads and spontaneously nibbled when these affected regions were manipulated. The response appeared to be associated with both the mites and the lesions. Some rams without lesions nibbled on manipulation of the infested regions and others nibbled spontaneously after mites were placed on the scrotum but failed to do so before mite transfer. The response was sometimes limited to a small area of the scrotum and direct microscopic observation showed that mites were usually limited to these areas. A few rams with large numbers of mites failed to give the response on manipulation of the infested areas. Other rams with typical chorioptic lesions with very few mites, or in two cases with apparently no mites (one hour direct microscopic observation of the scrotum without detecting any live mites) gave a strong nibbling response to manipulation of the lesions.

The mites and probably the lesions caused a mild disturbance to some rams without manipulation. Occasionally rams were observed biting at the lower legs or scrotum or rubbing the posterior pasterns on fence wires. However, the mites had no apparent affect on the general health of the

rams. For example, for more than a year field trial Rams 613 and 394 had large numbers of mites on their lower legs and scrota, yet the body weight and general health of these two rams was similar to or better than most of the other rams in the field trial.

1.6 Discussion on the Pathogenesis of Scrotal Mange

Direct microscopic observation of *C. bovis* on the scrotum of the ram proved to be advantageous over the usually used indirect method of mite detection and helped in the understanding of the mite-host-mange relationship. Mites were found most frequently and in the greatest numbers on the lower extremities, especially in the region of the accessory digits and above the coronary band and on the more distal aspects of the scrotum. Only occasionally were mites found on other body regions. The findings on leg *C. bovis* mites agree in general with those of Hiepe *et al.* (1968) and Crawford *et al.* (1970). There are no previous reports on the distribution of scrotal mites or the occurrence of mites on the escutcheon of rams. The rare occurrence of mites on the head of the ram is in agreement with the findings of Hiepe and Splisteser (1962) and Hiepe *et al.* (1968). Hiepe *et al.* (1968) found only 3.3% of rams examined had *C. bovis* mites on their heads while 88.9% had mites on their lower legs.

Both Sweatman (1957) and Butler (1968) have carried out experiments on the optimal temperature humidity requirements of *C. bovis* *in vitro* and Butler (1968) has investigated the microclimate of different body regions in cattle. From these experiments it is almost certain that the restriction of the majority of mites to a very small area of the body of the ram is due to these

regions having a microclimate suitable for the survival and reproduction of C. bovis.

Prolonged microscopic observations of the mite on the host show conclusively that the mite is a surface feeder as suggested by McEnerney (1953) and Evans et al. (1961) and does not penetrate the skin as suggested by Megnin (1877) and Stierwalt (1966). Direct observation shows that the mites in vivo feed on epidermal debris as suggested from in vitro studies (Sweatman 1957; Butler 1968). However, mites feed not only on epidermal debris, but also on the superficial layers of the epidermis, confirming the assumption of Butler (1968) that mites feed on the stratum corneum at times and disproving the assumption of McEnerney (1953) and Evans et al. (1961) that C. bovis is simply a "scurf feeding" "scavenging species". The ability of the mite to rise into a near vertical position, has the effect of bringing the chewing chelicerae into direct contact with the skin surface. Although this is the first report of this characteristic behavioural pattern of C. bovis on the host, the mechanism has been observed to occur in another member of the Sarcoptidae. Sarcoptes scabei has an identical behavioural pattern for positioning itself prior to chewing on the skin surface (Taylor and Murray, 1946). Unlike Chorioptes bovis, however, this mite actually burrows into the horny layer of the epidermis.

In vivo examination has allowed for the first time a direct assessment of the mite-mange relationship, removing much of the uncertainty in the literature. With relatively accurate methods of detection, C. bovis mites were detected prior to or at the time of lesion development in every case

of experimentally induced scrotal mange. This suggests that the apparent development of chorioptic mange in cattle in the absence of mites (Sweatman, 1956) was simply due to the less efficient method used by Sweatman for recovering small numbers of mites. Mites were limited to discrete areas of the scrotum in some rams and lesions were often seen to develop only at these positions. Scrotal mange has been reproduced also under controlled conditions by infesting "mite-free" housed rams with C. bovis mites. Further, in most of the rams examined, spontaneous cure of scrotal mange was associated with a loss of live mites in the region of spontaneous cure. This supports the finding of Sweatman (1956), who noted that in two heifers a "reduction occurred in the number of mites, followed by a regression of the amount of scab". Further, scrotal lesions were cured completely by removing most of the scabs and dipping the scrota and lower legs in 0.06% "Ciodrin" or 0.06% diazinon (Chapters 7 and 8). Chorioptic mange in cattle (Matthysse et al., 1967; Smith, 1967) and goats (Kemper et al., 1952) has been treated successfully with acaricides. The above data shows that in some way C. bovis causes the lesions attributed to it.

Data collected during this study would support the hypotheses of Pullin (1956) and Butler (1968) that chorioptic mange is an allergic response to C. bovis. Walton (1965) suggested and Wood (1968) agreed that there are four ways an ectoparasite may cause a skin response:

(a) Mechanical damage.

(b) Irritant, cytotoxic or "pharmacological secretions".

(c) The presence or deposition of allergens.

(d) The introduction of infective agents.

Direct observations of C. bovis on the host show that direct mechanical damage by the mite could not

account for the lesions attributed to it. The large number of mites seen on the ram (this study) and on cattle (McEnerney, 1953; Pullin, 1956) for long periods of time without causing lesions suggest that irritant, cytotoxic or "pharmacological secretions" are not directly involved in lesion production. Apart from the occasional secondary bacterial infection observed in both rams (this study) and cattle with chorioptic mange (Pullin, 1956) there was no evidence from this or other studies that C. bovis introduced infective agents. It would appear therefore, that the skin response to C. bovis is an allergic phenomenon.

The presence of large numbers of mites on the skin surface with no or little skin response for long periods of time, followed by a sudden severe exudative dermatitis in sheep (this study) and cattle (McEnerney, 1953; Sweatman, 1956), are both suggestive of an allergic phenomenon. The initial histological skin response was virtually identical to that seen in allergic delayed contact dermatitis (Fisher and Cook, 1958; Flax and Calfield, 1963; Criep, 1969) and the occurrence of relatively large numbers of eosinophils in most of the skin reactions studied is often taken to be an indication of an allergic dermatitis (Benjamin, 1961; Feingold et al., 1968). In fact Feingold et al. (1968) stated that the presence of eosinophilic leucocytes "is pathognomonic for the immediate type skin hypersensitivity". Thus there may be both delayed and immediate type allergic responses to C. bovis. Both delayed and immediate skin reactions have been shown to occur with mosquito bites (Melanby, 1946) and flea bites (Benjamini et al., 1960). When studying the skin reaction of the guinea pig to repeated flea bites, Larrivee et al. (1964) showed a sequence of delayed, delayed and immediate and finally an immediate type skin reaction. An

initial delayed type skin reaction, with almost entirely mononuclear cellular infiltration was followed by a period of immediate and delayed type reactions associated with an infiltration of eosinophils and mononuclear cells and finally a period of immediate type hypersensitivity with a cellular response of almost entirely eosinophils (Larrivee et al., 1964). These cellular responses were very similar to that seen in various stages of scrotal mange. The only other report on the histology of chorioptic mange (Piepe et al., 1968) was not detailed enough to compare the cellular responses with those described by the author.

Thus from the histological and other indirect evidence presented it seems highly probable that scrotal mange is an allergic response; further studies to investigate the immunological mechanisms involved were beyond the scope of this investigation.

The clinical features of scrotal mange reported in this study were similar to the brief descriptions of the syndrome as reported from Australia (McKenna and Pulsford, 1947), Europe (Piepe et al., 1968), U.S.A. (J.G. Matthysse, pers. comm.) and New Zealand (Crawford et al., 1970). The lesions were characteristically exudative, the yellowish exudate matting adjacent wool fibres. A few rams with severe chronic scrotal mange developed a permanent thickening of the scrotum. In most cases this thickening was limited to the base of the scrotum and the skin appeared corrugated. Piepe et al. (1968) and Crawford et al. (1970) did not mention any thickening of the skin in the many cases of scrotal mange examined while Seddon (1951) noted there was "some corrugation of the skin" in severe cases of scrotal mange.

In this investigation, the relationship between leg and scrotal mange was the same as reported from other countries. Almost every ram examined by the author with scrotal mange had leg mange. Although most of the lesions of leg mange were mild, some rams had dried exudate covering large areas of the posterior pasterns of both front and hind legs. In retrospect it is suggested that Crawford et al. (1970), who reported no cases of leg mange in over one hundred rams with scrotal mange, must have overlooked many cases of mange on this particular region of the body.

Spontaneous cure of scrotal mange, which occurred at all stages of scrotal mange development, was almost always associated with a loss of live mites in the region of the healing lesions. In some cases the loss of live mites may have occurred through unfavourable microclimatic conditions as suggested by Sweatman (1956) and by Butler (1968) in cattle. It appears also that the skin response of the host is a protective one. In many cases mites were confined to small areas of the scrotum and after a sudden serous outpouring these mites were trapped in the exudate. A similar phenomenon has been reported in cattle (McEnerney, 1953). Spontaneous cure does not mean immunity to future skin responses to C. bovis. A reoccurrence of scrotal mange has been associated with the reappearance of C. bovis mites.

Apparently, neither large numbers of mites nor severe lesions affected the general health of the many rams examined in this study. This is in agreement with the extensive survey of Hiepe et al., (1968). These authors could not establish a relationship between either C. bovis or chorioptic mange and the general health of sheep. Most other

reports on chorioptic mange in sheep do not mention general health, suggesting that it was not affected.

PART II

EFFECTS OF SCROTAL MANGE ON RAM FERTILITY

Chapter 2

REVIEW OF THE METHODS AVAILABLE FOR ASSESSING REPRODUCTIVE FUNCTION IN THE RAM

The reproductive function of the male is complex, depending not only upon the formation and ejaculation of spermatozoa but also on the sexual drive of the animal and its ability to mate successfully. The most commonly used methods for assessing the possible effects of an agent on spermatozoa production are assessment of testicular size and tone, assessment of semen quality and quantity, testicular histology and estimation of spermatozoa reserves while androgenic status can be measured directly by estimating testosterone in the testes or blood or indirectly by measuring sexual behaviour, activity of the accessory organs or testicular histology. The final criterion for normal reproductive function is the ability of a ram to successfully fertilize a high proportion of ewes.

2.1 In Vivo Assessment of Testicular Size and Tone

(i) Testis size

Several investigators (Boyd and Van Demark, 1957; Almquist and Amann, 1961; Amann and Almquist, 1961b, 1962a, 1962b) have reported that in the bull, testis weight or volume at slaughter was correlated positively to the number of spermatozoa obtained by frequent ejaculation (exhaustion tests) and with gonadal spermatozoa reserves. In the investigation of young bulls, Willet and Ohms (1957) and Hahn *et al*. (1969b) have shown a correlation of 0.92 and 0.81 respectively between testis size and

spermatozoa output per week. However, when investigating older bulls both groups of workers found that the correlation between testis circumference and spermatozoa output was much lower and Hahn et al. (1969b) concluded that testes volume was of little value in assessing the reproductive potential in bulls over 5 - 6 years.

The extensive data available for bulls has no parallel in the ram. However, in 25 rams aged from 1 - 6 years Ortavant (1952) found a correlation of 0.80 between testes spermatozoa counts and testes weights; the testes varying from 40 g to 383 g in weight. In both rams with normal testes and rams whose testes had undergone compensatory hypertrophy, Voglmayr and Mattner (1968) showed that spermatozoa numbers per gram of testis obtained from cannulated rete testes were approximately the same. The close relationship between testis size and spermatozoa production is further suggested in histological studies of the testis. Increases in testicular size during development of the ram parallel closely increases in seminiferous tubule activity (Watson et al., 1956; Skinner et al., 1968). Skinner et al. (1968) have shown a correlation of 0.95 between seminiferous tubule diameter and testis weight in the developing ram lamb and a similar correlation has been shown in the developing bull, (Hay et al., 1961). Similarly, severe seminal degeneration is associated with a decrease in testicular size and a decrease in seminiferous tubule diameter, (Setchell et al., 1965; Johnsen, 1970). Such data suggests that testis size is a reasonable guide to spermatozoa production in the ram.

Testis size can be estimated by measuring

various testicular dimensions or by comparative palpation. *In situ* measurements of testicular length x breadth, breadth and horizontal circumference have all been reported to give high correlations (greater than 0.9) with postmortem testicular volume: length x breadth - (Boyd and Van Demark, 1957); breadth - (Podany, 1964); horizontal circumference - (Willet and Ohms, 1957; Hahn *et al.*, 1969b). Willet and Ohms (1957) and Hahn *et al.* (1969b) have shown a correlation of 0.94 and 0.92 respectively between testis circumference in the live animal and testis weight. The testes of all domestic animals are ovoid in shape (Sisson and Grossman, 1963) so that testicular volume can be estimated from their length (L), breadth (B) and depth (D), according to the formulae:

$$\text{Testis volume} = 0.52 \times L \times B^2 \quad \text{(Prader, 1966)}$$

OR

$$\text{Testis volume} = 4/3 \times L/2 \times B/2 \times D/2 \quad \text{(Osman, 1970)}$$

The main problem associated with estimating testicular size *in situ* is the interference of the scrotum and epididymis when taking testicular measurements (Prader, 1966). However, using the formula $V = 4/3 \times L/2 \times B/2 \times D/2$ and allowing for skin thickness, Osman (1970) obtained a correlation of 0.96 between *in situ* testes volume and postmortem testes volume in 28 bull buffaloes. Variations in scrotal skin thickness, for example in rams with scrotal mange, would reduce the accuracy of the method.

Comparative palpation, consists of palpating the testis with one hand while model testes are palpated with the other hand. Human testis models are known as the orchidometer and usually comprise

12 models of known volume up to 25 ml (Prader, 1966). Laron and Zilka (1969) found that testis size estimated by comparative palpation "gave almost identical results" to the more tedious estimate based on measuring testis width and length. Although the orchidometer is used widely in human male fertility clinics for assessing testis size (Prader, 1966; Laron and Zilka, 1969; Johnsen, 1970; B. Lazarus, pers. comm.) the technique has not been extended to estimating testicular size in domestic animals.

(ii) Testis consistency

In 1942 Gunn et al. noted that ram testes which had undergone rapid and extensive degeneration became palpably smaller and softer. Semen analysis and histological studies showed that this softening was the result of more or less complete suspension of spermatogenesis and emptying of the seminiferous tubules. Dun (1956) made similar observations when studying an outbreak of temporary infertility caused by severe environmental stress. Galloway (1966) also found that rams with firm and elastic testes usually gave semen of good quality, while those rams with one or both testes "soft and flabby" or "firm and dull" in consistency were characterised by having semen of poor quality. However, 33% of the rams with altered testis tone had semen of good quality, suggesting that consistency changes do not necessarily give a reliable indication of testicular function. Edgar (1959) also concluded that testicular tone "appeared an uncertain criterion" for assessing ram reproductive ability. In an attempt to overcome the disadvantages of subjective assessment of testicular tone Hahn et al. (1969a) developed a tonometer for measuring testicular consistency in

bulls. The instrument gave highly repeatable results between operators and highly significant correlations (0.59 to 0.94) between semen quality parameters and testis tone. This work has not been repeated either in the bull or in other species.

2.2 Semen Examination

Semen, the final product of the male reproductive process, is easily collected from the ram without affecting the future reproductive potential (Ortavant et al., 1948; Salamon, 1962; Pepelko and Clegg, 1965). Semen assessment, therefore, provides a simple method for assessing at intervals the effect of physcial, chemical or infectious agents on the reproductive system of the ram.

(i) Semen collection

Semen is usually collected from rams by using either an artificial vagina or by electrical stimulation. Occasionally semen is collected from the vagina of ewes. The dry vagina technique involves training rams to serve ewes that are not in oestrus and the semen is aspirated from the vagina. However, contamination, dilution and failure to aspirate all of the sample are obvious disadvantages of this method (Watt, 1966). Using this technique Hulet et al. (1965) obtained semen samples from only 94 of 136 rams.

The artificial vagina technique has overcome many of the disadvantages of the dry vagina method. However, rams still need an initial period of training with a ewe in oestrus.

Gunn (1936) first recorded the satisfactory

collection of semen from rams by electrical stimulation. The method caused considerable stress to the ram and ataxia sometimes occurred after collection. The development of a single bi-polar rectal electrode (Laplaud and Cassou, 1945) led to relatively selective stimulation of the nerve complexes involved in the ejaculatory reflex. Marden (1954) developed a multipolar longitudinal electrode which he used together with a gradual raising and lowering of the electrical stimulus. This further decreased the physical stress to the animal. Experimentation with electrode design has been closely associated with varying the power to the electrodes to obtain optimal stimulation of the ejaculatory nerves. The voltage has been varied between 5.5 volts (Marden, 1954) and 54 volts (Gunn, 1936). However, there is no adequate information on the optimal voltage for ejaculation. Dziuk et al. (1954) and Dowling (1961) experimenting over a wide range of cycles found no obvious differences in its effect on ejaculation, while Marden (1954) obtained most satisfactory results using a frequency between 20 and 30 cycles per second. Marden (1954) and Dowling (1961) found that the sine-wave pulse gave optimal stimulation.

(ii) Semen assessment

Several authors have compared ram semen collected by electrical stimulation and by the artificial vagina. Generally electrical stimulation causes a greater secretion of accessory fluid resulting in a greater volume of semen and a corresponding lowering of spermatozoa concentration (Brady and Gildow, 1939; Terrill, 1940; Ortavant et al., 1948; Mattner and Voglmayr, 1962; Tilton et al., 1964). The greater amount of accessory

fluid is further reflected in a higher seminal fructose concent (Mattner and Voglmayr, 1962) and a higher concentration of sodium and potassium ions (Quinn and White, 1966). Quinn et al. (1968) have shown that semen collected by electrical stimulation is more susceptible to cold shock and this was due to the difference in seminal plasma rather than to altered characters of the spermatozoa. Brady and Gildow (1939) and Terrill (1940) suggested that there was a difference in spermatozoa activity, but more recent work has shown no differences in spermatozoa quality (Mattner and Voglmayr, 1962; Salamon and Morrant, 1963; Quinn et al., 1968).

Some reports show that the number of spermatozoa collected over a number of days with the artificial vagina and electrical stimulation techniques is similar (Dziuk et al., 1954; Mattner and Voglmayr, 1962; Tilton et al., 1964) while other reports have shown that less spermatozoa are collected by the electrical stimulation technique (Brady and Gildow, 1939; Terrill, 1940; Salamon and Morrant, 1963; Rathore, 1971). The number of spermatozoa obtained per collection appears to be more variable with semen collection by electrical stimulation (Terrill, 1940; Mattner and Voglmayr, 1962; Salamon and Morrant, 1963). Mattner and Voglmayr (1962) collected semen 2 - 3 times a week for 5 weeks and found no significant differences in the total number of spermatozoa collected by the two methods, but day to day variations in spermatozoa numbers were twice as great with semen collected by electrical stimulation.

It appears that the large variation in volume and concentration of semen obtained when using

electrical stimulation limits the usefulness of these parameters in assessing semen quantity when using this technique. Nevertheless, the spermatozoa that are obtained by electrical stimulation probably reflect the state of spermatogenesis just as accurately as spermatozoa collected with the artificial vagina.

The appraisal of semen quality is used commonly to assess the effect of diseases or experimental manipulations on the reproductive system. Various techniques of semen appraisal have been described and reviewed by Emmens and Blackshaw (1956), Salisbury and VanDemark (1961), Emmens and Robinson (1962), Melrose (1962) and Mann (1964).

In the bull biochemical tests on semen such as rate of fructolysis, oxygen uptake and methylene-blue or resazurin reduction time have been investigated as fertility predicting parameters, but not all have been studied in relation to fertility in the ram. Bishop et al. (1954) and Cummings (1954) in extensive surveys into the semen quality parameters as they were related to fertility in the bull concluded that the metabolic tests were closely related to the physical measures, and added little to the physical assessment of semen. In recent years assessment of ram semen quality has been almost entirely limited to physical parameters (Moule and Waites, 1963; Fowler and Dun, 1966; Howarth, 1969; Braden and Mattner, 1970; Rathore, 1971; Smith, 1971). The physical properties that are usually assessed are semen volume, spermatozoa concentration, motility, proportion of live spermatozoa and spermatozoa morphology.

Volume: Semen is usually collected into a

graduated centrifuge tube so that semen volume can be recorded at the time of collection.

Concentration: Spermatozoa concentration was first estimated by the counting of a diluted sample of semen mounted on a haemocytometer slide (Walton, 1927). To overcome the time consuming haemcytometer counts when large numbers of samples have to be counted, indirect methods have been developed for determining spermatozoa density. Comstock and Green (1939) developed the use of the absorptiometer to measure the optical density of ram semen, while Gunn et al. (1942) adopted a scale based on a visual estimate of semen colour. In an attempt to make the visual estimate more accurate Salisbury et al. (1943) compared the semen samples with standard barium opacity tubes. Spermatozoa concentration can also be determined by estimating the cell volume by rapid centrifugation of a portion of the semen sample in capillary tubes (Shaffner and Andrews, 1943). According to Salisbury and VanDemark (1961) this latter method is the least accurate, the correlation between spermatozoa counts and cell volume estimates being only 0.8 while all reports on the correlation coefficient between spermatozoa counts and absorptiometer or opacity tube estimates was 0.93 or higher. Comstock et al. (1943) have reported that for ram semen a standard error for duplicate counts with the haemocytometer averaged about ± 8% of the mean value, which was slightly greater than the error they obtained with the absorptiometer method. Salisbury and VanDemark (1961) have shown standard errors of ± 5 - 8% of the mean value with the absorptiometer method. Cell debris affects the accuracy of optical density measurements (Salisbury and VanDemark, 1961). To try and overcome this problem in ram semen Emik and

Sidwell (1947b) recommended calibration of the absorptiometer for five different, subjectively estimated levels of debris. An extremely large number of semen samples would be required to prepare the five standard curves and even then the method depends on an initial subjective assessment of each sample.

Motility: In most assessments of semen quality a measure is made of the physical activity of the sample. Motility estimates may be based on wave motion, the gross swirling of active semen, or on the motility of individual spermatozoa. Blom (1946) developed a standard depth "comparing chamber" which was used for assessing wave motion but could be used also for assessing motility of individual spermatozoa. Blom (1946) recommended that semen be classified into 7 grades on motility estimates.

Wave motion is dependent not only on the movement of individual spermatozoa but also on the concentration of spermatozoa in the sample and thus is of limited value as a motility index in studies where a large variation in spermatozoa concentration is anticipated. To overcome this problem semen can be diluted, for example with physiological saline (Salisbury and VanDemark, 1961) so that individual spermatozoa can be examined readily microscopically. Motility of individual spermatozoa can be assessed on the proportion that are motile and on their rate of progression. Emik and Sidwell (1947a) and Emik et al. (1948) combined these two parameters to form an "estimated motility combination". The proportion of motile cells can be calculated objectively (Brady and Gildow, 1939) using haemocytometer counts or subjectively (Gunn et al., 1942) by direct microscopic observations of diluted semen

samples. The objective method is time consuming in routine operations, and has a repeatability no higher than the subjective method when used by experienced investigators (Salisbury and VanDemark, 1961).

More objective measurements of spermatozoa velocity include the use of photo-multipliers, television scanners and cinematography (Bosselaar and Spronk, 1952; Rothschild, 1953a, 1953b; VanDemark et al., 1958). These methods are expensive and time consuming. Rothschild (1948) developed an apparatus for measuring the changes in electrical impedance in semen and showed (Rothschild 1949, 1950) that the frequency of these was closely related to motility estimates. However, the technique is dependent on spermatozoa concentration (Rothschild 1949) and is of no value in estimating motility in dilute samples.

Proportion of live spermatozoa: In 1942 Lasley et al. reported that live and dead ram spermatozoa could be differentiated by their reaction to the stain eosin, nonmotile, apparently dead spermatozoa being coloured by the dye, and live, motile spermatozoa not being stained. Opal blue was used as a background stain so that the unstained spermatozoa could be easily seen. Since this time various other vital and background stains have been used with success (Emmens and Blackshaw, 1956; Salisbury and VanDemark, 1961). The most widely used stain mixture in ram and bull semen studies is that developed by Hancock (1951) using nigrosin as a background stain and eosin as the vital stain. Dead spermatozoa stain pink and the unstained live spermatozoa are readily seen against a purple background. Hancock (1951) emphasised the necessity

of having the stain and semen at the same temperature and Campbell et al. (1956) showed the importance of keeping the staining time constant. Hypotonic staining solutions have been incriminated to increase both the proportion of dead spermatozoa (Swanson and Bearden, 1951) and the number of spermatozoa with bent tails (Bishop et al., 1954).

The repeatability of estimations made by this technique has been studied by various authors. Ortavant et al. (1952) found no difference in the percentages of dead spermatozoa in the same sample counted by two operators and also the results of counting 150 spermatozoa were not appreciably different from those obtained when 2,000 spermatozoa were counted. Campbell et al. (1956) showed that it was better to make two subsamples and count 100 spermatozoa from each than to count 200 cells from the one smear. Partially stained spermatozoa are usually considered dead (Mayer et al., 1951; Campbell et al., 1956; Salisbury and VanDemark, 1961).

Morphology: Early workers placed much emphasis on the assessment of abnormal spermatozoa in ram semen (McKenzie and Phillips, 1934; McKenzie and Berliner, 1937; Gunn et al., 1942). Blom (1948) classified spermatozoa abnormalities into those that resulted from disturbances in spermatogenesis (primary abnormalities) and those occurring after spermatogenesis was completed (secondary abnormalities). The former included pyriform, small and large heads while the latter included heads separated from their tails. Bishop et al. (1954) suggested that normally differentiated spermatozoa with the tail bent to form a loop were artifacts and should be classified into a tertiary group of abnormalities. Many spermatozoa cannot be classified readily into the

above categories so it is probably better to classify them with respect to the part of the cell affected (Salisbury and VanDemark, 1961). Although Bishop et al. (1954) counted 400 spermatozoa, Salisbury and Mercer (1945) concluded that a count of 100 cells from one slide gave a sufficiently reliable estimate of spermatozoa abnormalities.

(iii) Correlation between semen quality and fertility

Bulls in use in artificial breeding centres, and thus previously selected for semen quality, have been used mainly in experiments relating semen quality to fertility. Under these circumstances no more than about 20 - 25% of the differences in fertility can be accounted for by semen variations detected in the laboratory (Bishop et al., 1954; Melrose, 1962; Bishop, 1964). Because the fertility of these bulls tends to fall into a narrow range, the correlation between semen parameters and fertility cannot be expected to be high.

The same has been shown for the ram. After culling rams producing poor quality semen, Wiggins et al. (1953) found that no more than 18.8% of the differences in fertility could be accounted for by differences in semen quality. On the other hand, in a mating trial involving rams with a wide range of semen quality Hulet and Ercanbrack (1962) found that as much as 50% of the differences in fertility could be accounted for by simple semen parameters. By using multiple regression methods Hulet and Ercanbrack (1962) combined those semen traits that were most closely related to fertility into a fertility index. However, only a marginally improved correlation with fertility resulted when this method was used, compared with the single semen parameter most closely related to fertility. The small improvement in fertility predicting

ability of the index is outweighed by the time required to deduce the index for each semen sample. Hulet et al. (1965) observed that the same fertility occurred in rams when the percentage of motile cells ranged from 60 - 100% and when the proportion of morphologically abnormal spermatozoa ranged from 0 - 35%. This is in contrast to the early observations of McKenzie and Phillips (1934) and Gunn et al. (1942) which suggested that rams with more than 14% and 1% of morphologically abnormal spermatozoa respectively were of reduced fertility.

Edgar (1959) collected semen samples from rams by electrical stimulation at least three times and compared the semen quality of each ram with its breeding performance. Although he was able to group the rams of low fertility by semen analysis he was not able to grade the more fertile rams nor even define by semen analysis the moderately fertile rams. From his studies Edgar (1959) concluded that "the minimum requirements of a satisfactory semen sample appeared to be milky appearance, 50% motile sperm, moderate sperm motility and less than 20% abnormal sperm".

It is interesting to note that the only semen test reported to give a relatively high correlation coefficient with fertility in the bull is progressive motility after incubation at about body temperature (Buckner et al., 1954). Apparently this parameter has not been assessed in the ram.

When a toxic spermatogenic agent is applied to the ram in sufficient quantity, a marked seminal degeneration occurs with a corresponding decrease in fertilizing capacity of the semen. Examples of such agents are heat (Rathore, 1968; Braden and Mattner, 1970), chemicals (Moule and Mattner, 1961; Kreider and Dutt, 1970) and infections (Baynes and

Simmons, 1968; Swift and Weyerts, 1970). In such studies semen assessment has been a useful technique in determining whether a noxious agent affected spermatozoa production and at what stage the effects on fertility were likely to occur. Some indication of the severity of the effects can be determined from repeated assessments and observations on the recovery phase (if it occurs) made.

It seems therefore, that although semen assessment may be of limited value in predicting the reproductive potential of individual clinically normal rams, it has considerable value as a technique for studying the effects of toxic agents on spermatogenic function.

2.3 Testis Histology

Histological studies of the testis provide valuable information on the function of the seminiferous tubules. Testicular histopathology is usually described in general terms and only recently has emphasis been placed on the quantitative assessment of testis pathology. Some of the methods used for quantifying testicular histology are:

(a) A random point counting technique, permitting the calculation of the relative cell volume of the various constituent elements of the testis (Roosen-Runge, 1956).

(b) Photographing the tubules and then identifying and counting various cell types in tubules at five stages of spermatogenesis (Steinberger, 1962).

(c) Methods based on serial sections (50 - 300) of transversely cut tubules (Clermont, 1962).

(d) Counting all cell types in 20 randomly selected oil immersion fields (Clegg, 1965).

(e) Counting all cell types in 50 circular transverse fields (Mancini et al., 1965).

(f) Projection of histological sections, measuring the circumference of each tubule with a high precision map-measuring instrument and then counting and calculating spermatogonia and spermatocytes per unit wall length (Steinberger and Tjioe, 1968).

When applied to a general histopathological study of the testis all of the above methods have one or more serious limitations. Some require the presence of recognisable cellular associations for classifying tubules, others do not assess the more mature germinal cells while still others require sophisticated and expensive equipment. Most of the methods are time consuming and tedious and some do not give a clear picture of tubular heterogenicity.

Recently Johnsen (1970) described a simple quick method for quantitating human testicular counts. Each tubule of a biopsy was given a score from 1 - 10 depending on a visual estimate of the most developed cell type present. A large number of tubules can be assessed in a short period of time and this allows tissue heterogenicity to be readily evaluated. In a study of 177 cases including both normal and hypogonadal patients Johnsen (1970) obtained a correlation of 0.82 ($p<0.001$) between the mean score count and total spermatozoa counts.

Testicular biopsies or unilateral castration are required to follow directly the progressive effect of an agent on spermatogenesis in one animal.

(i) Testicular biopsy

The first report on testicular biopsies was in human fertility studies (Charny, 1940). Since this time the technique has been used widely in attempts to define the cause of human sterility. Testicular biopsy of man has been carried out usually as a diagnostic aid in cases of oligospermia or azoospermia and consequently little emphasis has been placed on the detrimental effect of the technique on subsequent spermatogenesis (Ragab et al., 1961; Girgis et al., 1969; Garduno and Mehan, 1970). Recently, however, Gordon et al. (1965) and Rowley et al. (1969) have shown that taking biopsies from normal human testes have had a detrimental effect on subsequent spermatozoa output. For example, Rowley et al. (1969) showed that the average spermatozoa count decreased by 42% and that recovery was not complete until 18 weeks after the operation.

Testicular biopsy has never been used widely in animal studies. Probably this is due to reports of detrimental effects of the technique on spermatozoa production in the bull (Byers, 1953; Hill and Gassner, 1955; Gassner and Hill, 1955; Knudsen, 1960; Veznik, 1962). Hill and Gassner (1955) and Gassner and Hill (1955) in a study on the effect of testicular biopsy on spermatozoa production in 10 bulls found the operation caused an extensive coagulation necrosis near the biopsy site and a generalised degeneration of the seminiferous tubules in all bulls biopsied. Testicular recovery did not begin until at least 2 months after surgical interference and it took from 4 - 8 months for an acceptable degree of improvement in semen quality.

(ii) Unilateral castration

Unilateral castration allows testicular

histology to be studied at two intervals during an investigation into the reproductive performance of a ram. However, removal of one testis may affect the remaining testis. Parkes (1966) summarising evidence prior to 1940 says that unilateral castration has no affect on the contra-lateral testis in the mature animal. In contrast to these early findings compensatory hypertrophy has been reported in the boar (Hauser et al., 1952) in the ram (Voglmayr and Mattner, 1968) and in the rabbit (Paufler and Foote, 1969). In the study of four adult rams Voglmayr and Mattner (1968) showed a 76% (S.E. $\pm$ 3.6%) increase in testis weight 120 days after hemicastration. No reference is made in the literature to a possible initial depressive effect of hemicastration on the contra-lateral testis.

(iii) Indirect methods of assessing spermatogenesis

In an attempt to overcome the problem associated with the removal of testicular tissue for assessment of spermatogenesis, Czerniak (1962) and Czerniak and Itelson (1967, 1970) have assessed spermatogenic activity in man indirectly by measuring the testicular uptake of previously injected radioactive phosphorus (P^{32}). However, the technique has not been adopted by other workers and has not been applied to other species.

Bishop (1968), in a preliminary report, suggested that quantitative analysis of the enzyme sorbitol dehydrogenase obtained from testicular homogenates would give an accurate assessment of spermatogenic activity.

2.4 Spermatozoa Reserves

Testicular and epididymal spermatozoa reserves have been determined in the ram by Chang (1945), Ortavant (1952) and Dott and Skinner (1967), although the descriptions of the techniques used were very brief. In contrast Amann and Almquist (1961a) have reported in detail a technique for determining the gonadal and extragonadal reserves in the bull. This technique has been used in the ram by Setchell et al. (1965) for assessing the effect of severe under nutrition on reproductive function.

2.5 Gonadal Hormones

In the mature ram testosterone is quantitatively (Skinner et al., 1968) and probably androgenically (Mann and Rowson, 1960; Mann et al., 1960; Moule et al., 1966) the main androgen.

Measuring testosterone in the peripheral blood should give the most accurate estimate of the androgenic status of the living animal. However, there is no simple technique that can be readily carried out in most laboratories. There are a number of precise methods that measure testosterone levels in the peripheral blood (see Horton et al., 1967), but they are time consuming, expensive and require specialised equipment, reagents and technical knowledge. Competitive protein binding and radio-immunoassay techniques have been recently developed to measure peripheral testosterone (Horton et al., 1967; Rosenfield, 1969; Furuyama et al., 1971); the assays being completed within 24 hours while chemical methods take 3 - 4 days. These latter techniques have not as yet become widely established

and in fact, at the beginning of 1971 there was no laboratory in New Zealand estimating blood testosterone on a routine basis.

Lindner and Mann (1960) have described a method for determining testosterone in testicular tissue. The technical problems associated with estimating blood testosterone chemically also apply to the determination of androgens in this site.

It has been known for many years that the male accessory reproductive organs are dependent on testicular androgen (Gley and Pezard, 1921; McGee, 1927). Recently, Skinner et al. (1968) showed a linear relationship between the fructose and citric acid content of the seminal vesicles and the testosterone content of the testes of the developing ram. For every unit increase in fructose, testosterone content increased by 1.69±0.17µg. Lindner and Mann (1960) have shown a similar relationship in the developing bull. For these reasons measurements of the seminal vesicles have been (Jeffries, 1931) and still are (Lostroh, 1969) a favourite assay for androgenic steriods. In the ram the weight, height of the secretory epithelium and the fructose content of the seminal vesicles have been used in assessing the androgenic status of the animal (Ortavant and Thibault, 1956; Ortavant et al., 1964; Setchell et al., 1965). However, seminal vesicle measurements can be taken only once and thus are not applicable in many studies.

In castrated rams Moule et al. (1966) showed a linear relationship between log dose of exogenous testosterone and the concentration of fructose in semen collected by electrical stimulation. This suggests that in the ram, as in other species (Mann, 1964), seminal fructose may be used as an

indicator of androgenic activity. Mann (1967) recommends that several semen samples be taken to obtain a reliable assessment of the animals androgenic status.

2.6 Sex Behaviour

Mating ability is as important as semen production for normal reproductive performance. Reviewing the sometimes conflicting evidence, Young (1961) and Parkes (1966) concluded that complete mating behaviour in male animals is dependent on testicular androgens.

Attempts to measure sex drive in the ram have been reported by many workers. Terrill (1937) and Wiggins et al. (1953) noted the number of "ejaculations" within a 30 minute period of a ram being placed with a ewe, while Pepelko and Clegg (1965) measured the total number of "ejaculations" that occurred until sexual exhaustion. Sexual exhaustion was arbitrarily defined as a period of 20 minutes with no mounts. Chang (1945) and Salamon (1964) measured the reaction time which they defined as the interval between releasing a ram in close proximity to a ewe to "ejaculation". Subjecting nine male goats to a constant routine of two semen collections every fifth day for a period of one year Fraser (1968) found that "the mean reaction time of four random observations gave an extremely reliable indication of the long-term sexual responses of the animal". Although sexual reaction times in the ram can be influenced by a variety of psychological factors (Hafez, 1951; Pepelko and Clegg, 1964, 1965), estimations of the reaction time provides a simple and reliable measure of

libido (Sharma et al., 1957).

From their extensive studies on clinically normal rams Wiggins et al. (1953) concluded that libido had a significant relation to fertility. However, the highest correlation obtained was 0.15, that is, less than 2 1/2% of the differences in fertility between rams could be accounted for by differences in libido. Further, Wiggins et al. (1953) found no differences between libido and semen production. Hulet et al. (1964) also found that semen was similar from rams with normal libido and those with poor libido. Conversely, normal libido is often seen in azoospermic rams. For example, normal libido has been associated with complete spermatogenic arrest caused by natural cryptorchidism (Zawadovsky, 1933), scrotal insulation (Glover, 1956) and Klinefelter's syndrome (A.N. Bruere, pers. comm.)

The above discussion suggests that under most conditions mating behaviour and spermatozoa production vary independently of each other and that a study of one is not likely to give a reliable indication of the other.

2.7 Mating Trial

The ultimate criterion of a rams reproductive ability is to leave a high proportion of live offspring when mated to a number of fertile ewes. Several factors tend to "overshadow" this method of assessing reproductive function in a preliminary investigation. Some of these factors are:

(a) Availability of animals, labour and facilities.

(b) Seasonal breeding pattern of ewes.

(c) Time required to carry out the experiment.

(d) Availability of other methods of assessing reproductive function (e.g. semen analysis).

(e) Cost of the experiment.

Chapter 3

MATERIALS AND METHODS USED IN ASSESSING REPRODUCTIVE FUNCTION OF RAMS WITH SCROTAL MANGE (CHORIOPTES BOVIS)

3.1 Animals Used

The general management of experimental rams is described in Part I (see page 14) and the rams used and the specific conditions applied to them are described in the introduction to each experiment.

3.2 Testis Size and Tone

Testis size and tone were assessed *in vivo*. Testes were classified into three sizes; small, medium and large. Small testes were those that were estimated to be less than 100 g in weight, medium testes 100 - 150 g and large testes greater than 150 g. Testis tone was classified as good, moderate or poor. Testes with good tone were firm and elastic (springy) while moderate toned testes were similar but had lost some resilience. Poor testes tone included testes that were soft and flabby or firm and dull.

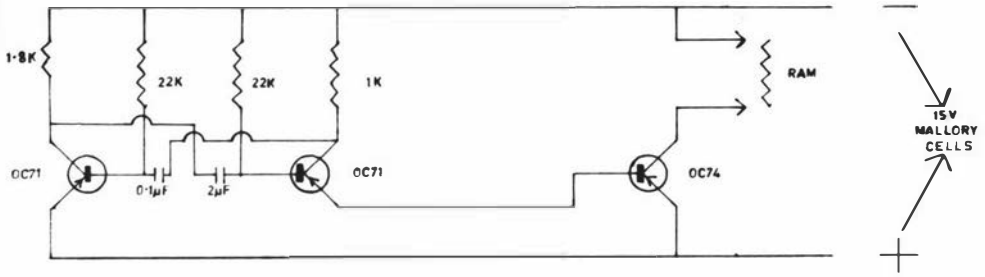
3.3 Semen Evaluation

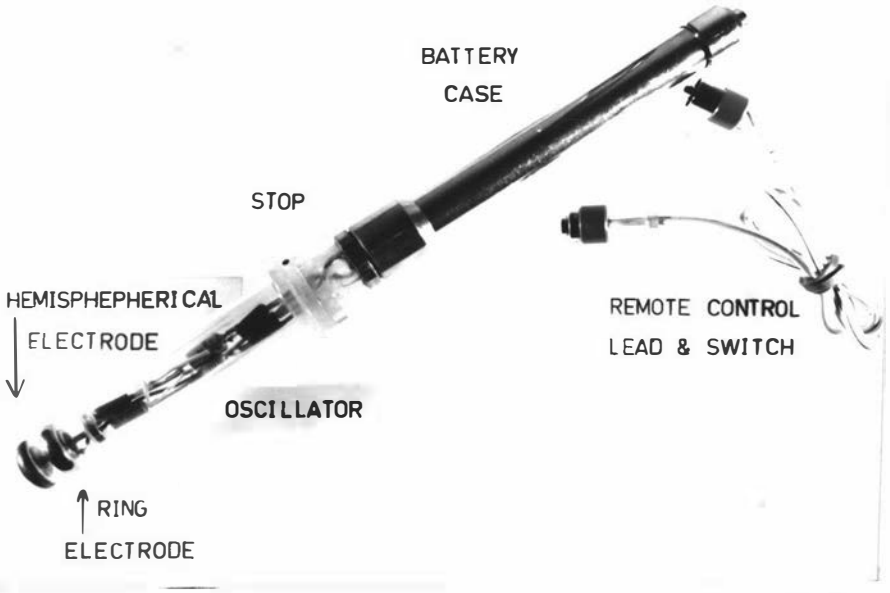
Semen samples were collected by electro-stimulation in all experiments. A transistorized bipolar rectal probe developed by Nichols and Edgar (1964) was used for electrical stimulation. The oscillatory circuit is shown in Text-fig. 3 and the complete probe in Fig. 16. The pulse frequency of the probe

Text-figure 3: Oscillatory circuit of the ram probe shown below (Fig. 16). All resistors ±10% 1/4W. All capacitors 20V d.c. minimum working voltage.

Figure 16: Transistorized rectal probe for ejaculating rams. (Ruakura Mk.IV Ram Probe, Plastic Products, Hamilton, N.Z.)

TEXT-FIG. 3


1·8K
22K
22K
1K
RAM
OC71
0·1μF
2μF
OC71
OC74
15V
MALLORY
CELLS


BATTERY
CASE
STOP
HEMISPHEPHERICAL
ELECTRODE
REMOTE CONTROL
LEAD & SWITCH
OSCILLATOR
RING
ELECTRODE

was about 60 cycles per second, the pulse width about 1/500 second and the pulse amplitude 15 V.

Rams to be electro-stimulated were restrained on their sides in the Begg's foot trimming cradle with their hind legs tied back with a leather strap (Fig. 2). The penis was extruded manually and held with a cotton gauze bandage so that the urethral process was held in the mouth of a prewarmed, 10 ml glass, graduated, centrifuge tube. An assistant lubricated the probe with "K Y Lubricating Jelly" (Johnson and Johnson, England) and inserted the probe into the rectum as far as the plastic stop (Fig. 16). The probe tip was depressed slightly into the vicinity of the seminal vasicles, prostate gland and hypogastric plexus. The probe switch was then pressed by the assistant for approximately three seconds, released for three seconds and the procedure repeated. If ejaculation did not occur within ten stimulations the ram was rested for five minutes and the procedure repeated. Semen collected was put immediately into an incubator at 35 $\pm$ 0.5° centigrade. The volume, colour, motility, morphology and the proportion of spermatozoa staining "live" were assessed on every semen sample. Motility slides, live/dead smears and morphology smears were prepared in the incubator immediately after each collection, using materials and solutions that had been prewarmed.

(i) Spermatozoa numbers

The volume of semen was recorded at the time of collection. Concentration of spermatozoa was estimated in all samples at the time of collection by visual colour appraisal and in some experiments by haemocytometer counts. Semen samples were readily classified into one of the following groups; creamy, thick-milky, milky, watery-milky, cloudy or clear. Direct haemocytometer counts were made to determine

the number of spermatozoa in each category and to check the accuracy of the colour classification (Appendix 1). In semen samples with negligible debris the majority of the spermatozoa counts were included in the groupings:

creamy (cr.)	$> 20 \times 10^8$ spermatozoa/ml
thick-milky (t. mil.)	$> 10 \times 10^8 < 20 \times 10^8$ spermatozoa/ml
milky (mil.)	$> 3 \times 10^8 < 10 \times 10^8$ spermatozoa/ml
watery-milky (w. mil.)	$> 1 \times 10^8 < 3 \times 10^8$ spermatozoa/ml
cloudy (cl.)	$< 1 \times 10^8$ spermatozoa/ml

Semen subsamples for haemocytometer counts were diluted with formalin-saline (9g NaCl and 10 ml 10% commercial formalin in 1 L distilled water) so that between 50 - 150 spermatozoa could be counted on 5 secondary squares of each counting chamber of a bright lined haemocytometer, (Assistent, Germany). Each chamber was filled with a different subsample of diluted semen and allowed to settle for ten minutes before counting. Samples were counted at 400x magnification with a binocular Olympus microscope (Olympus Model E, Tokyo). Spermatozoa concentration and total spermatozoa per ejaculate were calculated from the sum of the counts in the two chambers (S), the dilution factor of the sample (D) and the volume of the ejaculate (V) according to the formula:

$$\text{spermatozoa concentration} = S \times 25{,}000 \times D$$

$$\text{total spermatozoa numbers} = S \times 25{,}000 \times D \times V$$

(ii) Motility

A drop of semen was diluted with prewarmed normal saline on a prewarmed microscope slide to

the extent that individual spermatozoa could be readily assessed microscopically. A drop of the mixture was transferred to the opposite end of the slide and covered with a coverslip. The slide was then transferred from the incubator (35 ± 0.5°C) to a warm stage (Reichert, Austria), which was maintained at 35 ± 0.5°C, and the motility assessed at a magnification of x100 with a Wild microscope (Wild Heerbrugg, Model II, Switzerland). The percentage of motile spermatozoa and their rate of progression was assessed. The percentage of motile spermatozoa were estimated in 10% steps while progressive motility was based on an arbitrary scale of 0 to 5:

0 No motile spermatozoa.
1 Majority of motile spermatozoa showing oscillating movement only.
2 Majority of motile spermatozoa showing slow progressive movement.
3 Majority of motile spermatozoa showing moderately rapid progressive movement.
4 Majority of motile spermatozoa showing rapid progressive movement.
5 Majority of motile spermatozoa showing very rapid progressive movement.

(iii) Proportion of "live" spermatozoa

The proportion of "live" spermatozoa was determined from semen stained with nigrosin and eosin (Hancock, 1951). The stain was prepared by dissolving 5 g of Y-water soluble eosin (G.T. Gurr) in 300 ml of a 10% aqueous solution of nigrosin (G.T. Gurr). One small drop of semen was mixed with 2 - 5 drops of prewarmed stain in the incubator, the number of drops depending on the concentration of the semen sample. After staining for 3 minutes

a thin smear was prepared in the same manner as a blood smear and the slide stored in the incubator. Immediately after the group of rams to be examined had been electro-stimulated, the smears were assessed at 250x magnification with a "Neopan" microscope, (Reichart, Austria). One hundred spermatozoa randomly selected from several fields throughout each smear were assessed for their affinity for eosin. Spermatozoa taking up eosin will be referred to as dead while those not staining will be referred to as live. Partially stained spermatozoa that take up eosin with similar intensity as fully stained spermatozoa will be classified as dead.

(iv) Morphology

Routine morphological assessments were made on the nigrosin-eosin smears while degenerate semen was stained also with Mayer's haemalum and eosin (see Appendix 3) using a modification of the method of Cary and Hotchkiss (Gunn et al., 1942). By this method nuclear material stained blue while the rest of the cell stained pink.

One hundred spermatozoa randomly selected from several fields throughout the smear were assessed morphologically under an oil immersion objective at a magnification of x630, with the "Neopan" microscope. Abnormal spermatozoa were classified once according to the most severe or most obvious defect seen. Morphological abnormalities were classified as:

(a) Head abnormalities (including pyriform, small, large, twin, degenerate, acrosome detachment, and irregular shaped heads).

(b) Neck abnormalities (tailless heads and

broken necks).

(c) Mid-piece abnormalities (including bent, broken, enlarged, double and filiform mid-pieces).

(d) Tail abnormalities (including coiled, broken, returned and double tails).

(e) Cytoplasmic droplets (proximal and distal).

Throughout the remainder of this thesis spermatozoa that appeared morphologically normal will be simply referred to as normal while those spermatozoa with some morphological abnormality will be referred to as abnormal. For simplicity and clarity semen quality data collected in this study was condensed into a semen quality index as proposed by Moule and Waites (1963). Each 10% live spermatozoa, 10% normal spermatozoa and 20% motile spermatozoa was given one point and these added to the scores (0 - 5) for progressive motility to give a composite score with a maximum of 30 for any sample. That is:

Semen quality index (maximum 30) = motility (% motile, maximum 5; motility rate, maximum 5) + live spermatozoa (maximum 10) + normal spermatozoa (maximum 10).

3.4 Testis Histology

Testicular samples for histology were obtained from testes that were removed either by surgery or at slaughter. Slices approximately 2 mm thick were taken from the proximal, middle, and distal ends of the testis and placed in Bouin's fixative (Culling, 1963) for 24 hours. Tissues were processed with the aid of a histokinette, by dehydrating through

ascending grades of alcohol, clearing in chloroform and embedding in paraffin. Sections were cut at 6μ and stained with Ehrlich's haematoxylin (G.T. Gurr) and eosin (G.T. Gurr), (Culling, 1963). In some studies testis tissue was also fixed in Helly's fluid overnight (Culling, 1963) and then postchromed in potassium dichromate. The tissues were then washed, dehydrated, cleared, embedded in paraffin, sectioned and treated with sudan black as described by Threadgold (1957) in his Method One.

Histological sections were examined microscopically and photomicrographs were taken with a "Leica" camera (Leitz Wetzlar, Germany) which was attached to an "Ortholux" microscope with a "micro-attachment" (Leitz Wetzlar, Germany). This latter apparatus was used also for photographing semen smears and histological sections of skin.

The diameter of the seminiferous tubules was measured with a micrometer eye-piece (Leitz Wetzlar, Germany) and a 12.5x objective, the eye-piece having been calibrated against a micrometer slide (Leitz Wetzlar, Germany). From each testis examined one hundred randomly selected tubules, which appeared round in cross-section, were measured.

A modified method of that used by Johnsen (1970), the testicular score count, was used to quantify testicular histology. One hundred randomly selected tubules from each testis were given a score of 1 - 10 according to the following criteria:

Score 10: Complete spermatogenesis with many spermatozoa and the germ cells in normal cellular associations (spermatozoa are here defined to include spermatids that

have undergone elongation).

Score 9: Many spermatozoa or spermatids present but germ cell numbers are subnormal or the germinal epithelium is disorganised (showing abnormal cellular associations).

Score 8: Only a few spermatozoa present (<5 - 10) with remainder of epithelium as for Score 7.

Score 7: No spermatozoa but moderate numbers of spermatids with subnormal numbers of other germ cells or disorganised germinal epithelium.

Score 6: No spermatozoa and only a few spermatids (<5 - 10) present.

Score 5: No spermatozoa, no spermatids but several or many spermatocytes present.

Score 4: Only a few spermatocytes (<5) and no spermatids or spermatozoa present.

Score 3: Spermatogonia are the only germ cells present.

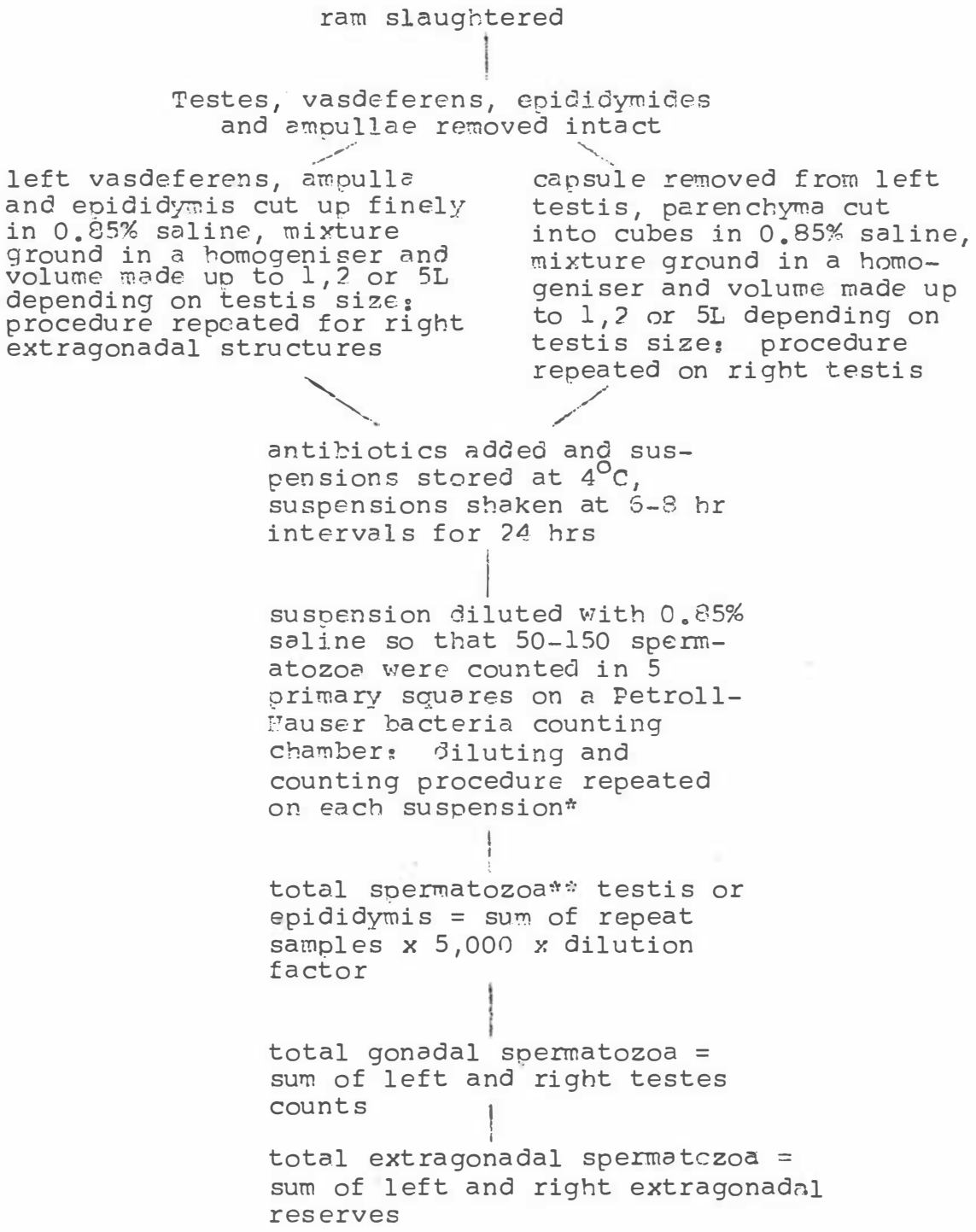
Score 2: No germ cells but Sertoli cells are present.

Score 1: No cells in tubular section.

3.5 Spermatozoa Reserves

Total testicular and epididymal spermatozoa reserves were estimated according to the procedure outlined by Amann and Almquist (1961a) for estimating spermatozoa reserves in the bull. The main steps of the procedure are shown in the flow diagram in Text-fig. 4.

Text-fig. 4: GENERAL PROCEDURE FOR ESTIMATING SPERMATOZOA RESERVES



* the procedure was repeated if the counts did not agree within 10% of each other

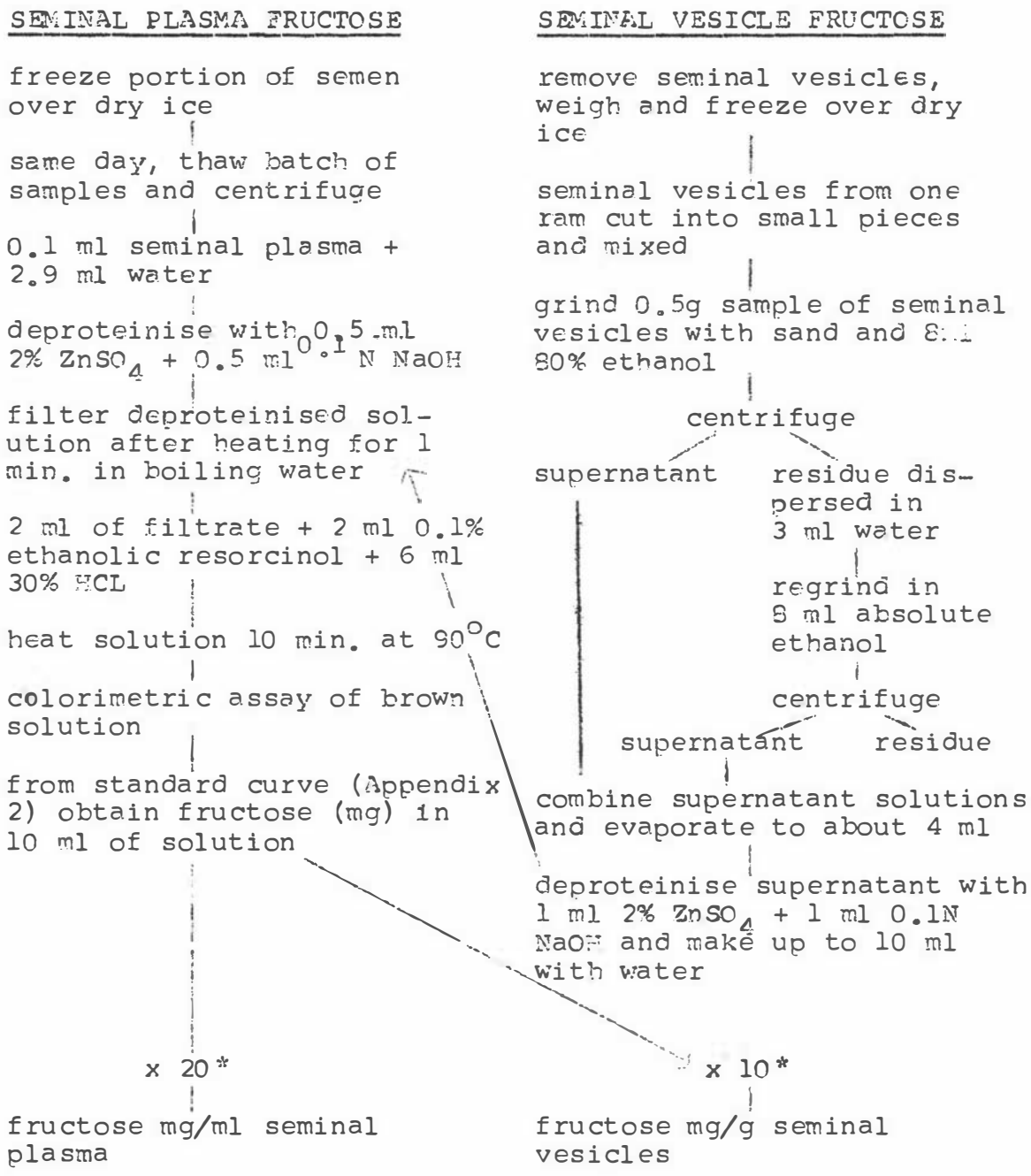
** spermatozoa are here defined to include spermatids that have undergone elongation

3.6 Androgenic status

Seminal plasma fructose and seminal vesicle fructose were both used as indicators of the androgenic status of rams. Fructose was determined by the method of Roe (1934) as adapted to semen by Mann (1948, 1964) and to seminal vesicles by Lindner and Mann (1960). The method is outlined in the flow diagram in Text-fig. 5. A SP.500 spectrophotometer (Unicam, England) with the wave length set at 540 µ was used for optical density determinations. Standard solutions for the calorimetric assay were prepared with pure D (-) fructose, after the method of Mann (1948) and the results obtained are presented in Appendix 2.

Sex drive was assessed by measuring the reaction time of each ram placed in a pen identical to that in which they were housed, with a ewe in oestrus. Ewes in oestrus were obtained during the breeding season by running a teaser ram with a mating harness with a group of ewes and those ewes strongly marked on the rump with the crayon were assumed in oestrus. During the non breeding season symptoms of oestrus were induced by injecting 20 mg of progesterone ("Progestin", B.D.H., London) intramuscularly every 2 days for 14 days and 24 hours after the last injection 100 µg of oestradiol benzoate ("Oestroform", B.D.H., London) were injected intramuscularly. All rams under experiment were exposed to the same ewe, reaction time being assessed alternatively using a ram with chorioptic mange and a control ram. After a preliminary trial to accustom rams to the procedure the process was repeated on 3 successive days and the average of the 3 reaction times taken as a measure of the ram's libido.

Text-fig. 5: GENERAL PROCEDURE FOR ESTIMATING SEMINAL PLASMA FRUCTOSE AND SEMINAL VESICLE FRUCTOSE



*10 ml of final solution is equivalent to 0.05 ml of seminal plasma or 0.1g of seminal vesicles

Chapter 4

FIELD INVESTIGATION INTO THE EFFECT OF SCROTAL MANGE (CHORIOPTES BOVIS) ON THE FERTILITY OF A FLOCK OF ROMNEY RAMS

4.1 Introduction

In an attempt to discover whether an association existed between scrotal mange and reproductive function, it was decided to examine a flock of rams where there was a natural outbreak of the disease. Such a flock was brought to the author's notice by a veterinarian in the Mid-Canterbury district.

The flock consisted of 80 fifteen month old stud Romney rams. The rams had been shorn six months previously, were uniformly well grown and all were in good health. The rams were turned over one at a time and their scrota and scrotal contents carefully examined. Scrotal lesions, provisionally diagnosed as chorioptic mange, were detected in over half of the rams. No other palpable abnormalities of the scrota or scrotal contents were detected. The provisional diagnosis was confirmed by taking single scrapings in the vicinity of scrotal lesions of 8 rams and examining the samples for live mites under the dissecting microscope. Mites, confirmed later to be C. bovis, were observed in 6 of the 8 samples. The scrotal lesions on many of the rams were mild, involving only the base of the scrotum. Thirty-seven rams with lesions involving more than 1 sq. cm of the scrotum were detected and classified according

to lesion severity into the following groups:

15 rams with minimal scrotal mange
8 rams with minor scrotal mange
6 rams with moderate scrotal mange
8 rams with severe scrotal mange

The following day semen samples were collected from the 37 rams with scrotal mange and from 13 rams picked at random from the remainder of the group. The semen samples were evaluated for volume, colour and motility immediately after collection. Live/dead and morphology smears were prepared and stored in the incubator and assessed within twelve hours of collection.

4.2 Results

One of the rams with severe scrotal mange (Ram 116) had medium sized testes with poor testes tone. The other 79 rams had large testes with tone that varied from moderate to good. There was no obvious association between lesion severity and testis tone in these 79 rams.

In general, there was a decrease in spermatozoa quality with increasing lesion severity (Table 6). Spermatozoa motility, percent live spermatozoa and percent morphologically normal spermatozoa were affected to a similar extent by scrotal mange, there being a general decrease in all three parameters with increasing lesion severity (Table 7). Thus, the composite semen score for each ram (Table 6) accurately reflects changes in the individual semen quality parameters making up the index. However, there were two exceptions. One

Table 6: RELATIONSHIP BETWEEN SCROTAL MANGE SEVERITY AND SEMEN QUALITY IN 50 RAMS

	Control	Lesion Severity			
		Minimal	Minor	Moderate	Severe
No. Rams	13	15	8	6	8
Composite Semen Score (Maximum 30)	27,29,28, 28,29,26, 23,27,28, 28,27,29, 28.	23,28,25, 19,23,28, 25,28,28, 25,30,22, 23,28,26.	16,23,16, 27,26,9, 29,24.	28,13,22, 24,13,29,	18,1,15, 10,14,14, 10,9.
Mean	27.5	25.4	21.3	21.5	11.4
Proportion of rams with inferior semen+	1/13	5/15	5/8	4/6	8/8
Corrected Chi square		1.41 N.S.	4.85*	4.64*	13.66**

N.S. $p > 0.05$ * $p < 0.05$ ** $p < 0.01$

+ Inferior semen = rams with composite semen score below two standard deviations of control group mean composite score.

Table 7: THE EFFECT OF SCROTAL MANGE ON INDIVIDUAL SEMEN QUALITY PARAMETERS

	Control	Lesion Severity			
		Minimal	Minor	Moderate	Severe
No. Rams	13	15	8	6	8
Semen quality index					
Motility (10) (± S.D.)	9.7±0.7	8.8±1.3	6.8±2.5	8.2±2.2	3.6±2.3
Live sperm (10) (± S.D.)	9.1±0.9	8.9±1.0	7.9±1.6	7.6±2.6	4.5±2.2
Normal sperm (10) (± S.D.)	8.7±0.9	7.7±2.0	6.6±3.4	5.7±2.8	3.3±2.0
Composite Score (30)	27.5	25.4	21.3	21.5	11.4

ram from each of the minor and moderate groups had relatively high motility and proportion live spermatozoa, but had a very high proportion of cytoplasmic droplets resulting in low morphology estimates.

In this survey rams were said to have inferior semen quality when their composite score was less than two standard deviations (S.D.) of the mean of the control groups composite score. Therefore, a ram with a composite score of 24 or below was said to have inferior semen quality. On this basis 8% of the control group, 33% of the minimal group, 63% of the minor group, 67% of the moderate group and 100% of the severe group had semen of inferior quality (Table 6). Using the Chi square method of analysis with Yates' Modification for small samples (Holman, 1969) the proportion of rams in the minimal group with inferior semen did not differ significantly ($p>0.05$) from the control group. However, the group of rams with minor and moderate scrotal mange did differ significantly ($p<0.05$) as did the group of rams with severe scrotal mange ($p<0.01$).

In general there was also a decrease in the semen quality index of rams with inferior semen with increasing lesion severity (Table 6). All but one of the rams in the control group and most of the rams in the minimal group produced semen of high quality, that is, had a composite score of 25 or above. One ram in the control group and 5 rams in the minimal group had slightly lowered semen quality, with immotile spermatozoa in the 30 - 50% range in 3 rams and 30 - 50% morphological abnormalities in 5 rams. Three of the 8 rams in the minor scrotal mange group had good quality semen, 2 slightly lowered semen quality and 3 (Rams 18, 234 and 47) had poor quality semen.

Ram 234 produced on electrical stimulation 1.5 ml of milky semen that contained 20% motile spermatozoa, 53% dead spermatozoa and 84% abnormal spermatozoa. Two of the rams with moderate scrotal mange had good quality semen, 2 slightly lowered semen quality and 2 poor quality semen. All of the rams in the severe group had relatively poor semen quality. Ram 116 was virtually azoospermic and this was the only ram whose semen sample contained large amounts of cellular and non cellular debris. More than 80% of the spermatozoa obtained from 4 other rams in the group were immotile and the remaining 3 rams had approximately 50% of their cells immotile. Four of the rams in the severe group had more than 75% morphologically abnormal spermatozoa, 3 had approximately 60% abnormal spermatozoa and 1 ram had 31% abnormal spermatozoa.

Tailless heads, tail abnormalities (bent, coiled and broken tails) and cytoplasmic droplets (proximal and distal) made up the majority of abnormalities in rams with inferior semen in the minimal and minor scrotal mange groups. As lesion severity increased the proportion of spermatozoa with tailless heads, tail abnormalities and cytoplasmic droplets remained similar, but there was an increase in the proportion of head abnormalities (Table 8); the majority of the head abnormalities being pyriform heads.

Apart from Ram 116 there was no apparent relationship between spermatozoa numbers, estimated from the volume and concentration of the semen samples, and lesion severity.

4.3 Discussion

A preliminary investigation into the reproductive function of a flock of rams with an outbreak

Table 8: SPERMATOZOA MORPHOLOGY IN RAMS WITH NORMAL AND INFERIOR SEMEN QUALITY

	No. Rams	Spermatozoa morphology %					
		Normal	Head Abn.	Neck Abn.	Mid-piece Abn.	Tail Abn.	Cytoplasmic drop.
Normal semen	27	89.3	0.4	3.4	0.2	4.3	2.1
Inferior semen							
Control group	1	73.0	0.0	5.0	3.0	13.0	6.0
"Minimal" S.M.	5	51.0	4.2	20.0	0.4	15.2	9.2
"Minor" S.M.	5	44.8	6.6	13.8	0.4	11.6	22.8
"Moderate" S.M.	4	37.0	15.8	18.0	0.8	12.3	16.3
"Severe" S.M.	8	33.3	17.8	13.1	1.6	21.3	12.9

Normal semen: Rams with semen quality index within 2 standard deviations of the control group mean.

Inferior semen: Rams with semen quality index below 2 standard deviations of the control group mean.

S.M., scrotal mange.

of scrotal mange suggested that scrotal lesions involving less than 10 sq. cm of the scrotum (minimal scrotal mange) has little if any effect on reproductive function. At the other extreme, rams with between a quarter and half of their scrota covered in active or insulative lesions (severe scrotal mange) are likely to have impaired reproductive function. Between these two extremes rams with apparently similar degrees of scrotal mange may have good, moderate or poor quality semen. The marked variation in semen quality in rams with minor and moderate scrotal mange may be due to one or more of the following factors:-

Different reactions of individual rams to the same degree of scrotal mange: Moule and Waites (1963) found a marked between ram variation in seminal degeneration when they exposed phenotypically similar rams to the same elevated ambient temperature. They showed that the variations between rams was due to different heat loads reaching the testes. A similar phenomenon may be occurring in rams with scrotal mange.

Erroneous classification of lesion severity: Accurate classification of scrotal mange lesions is difficult because of the marked variation in lesion pathology. For example, some rams had caked lesions with only small active lesions underneath while other rams had similar scabs with large active lesions beneath. Some rams had confluent lesions, others had scattered lesions while still others had both confluent and scattered lesions. This between ram and within ram variation in lesion pathology made a certain amount of overlap in scrotal mange classification unavoidable.

Delay in seminal degeneration: There is usually a time interval of 1 - 2 weeks before the effect of a toxic spermatogenic agent is seen in seminal ejaculates (for example, Glover, 1955; Wollrab, 1965; Rathore, 1970). This phenomenon could account

for some of the variation in semen quality in rams with similar degrees of scrotal mange.

Since the quantity of spermatozoa obtained on one collection by electrical stimulation does not always reflect spermatozoa production (Ortavant et al., 1948; Mattner and Voglmayr, 1962; Rathore, 1971) no significance can be placed on the low spermatozoa numbers obtained in some ejaculates. The large testes, the lack of seminal debris and the high spermatozoa numbers obtained in some rams with even moderate and severe scrotal mange suggests, with the exception of Ram 116, that scrotal mange was not causing widespread spermatogenic arrest. The round nucleated and non nucleated cells seen in the seminal debris of Ram 116 were similar to those described by Gunn et al. (1942) in degenerate semen samples. According to Gunn et al. (1942) these round cells originate from the germinal epithelium. Ram 116 had the smallest testes of all rams examined and there was probably a complete arrest of spermatogenesis associated with severe scrotal mange in this animal.

The general decrease in semen quality with increased lesion severity suggests, although does not prove, that scrotal mange causes testicular degeneration as assumed by Whitten (1968) and Bruere (1970) and is not simply associated with testicular hypoplasia as suggested by Crawford et al. (1970).

It was only possible to examine this flock once and thus further information on the progression and or regression of the scrotal lesions and their relationship to reproductive function was unobtainable. These factors will be examined in detail in subsequent chapters.

Chapter 5

EXPERIMENTAL INDUCTION OF SEMINAL DEGENERATION WITH SCROTAL MANGE (CHORIOPTES BOVIS)

5.1 Introduction

The rams that were housed in 1969 (see page 14) and the field trial rams (see page 15) had their testes classified for size and tone and their semen assessed prior to experimental induction of scrotal mange. Semen samples were collected from rams which developed chorioptic mange covering more than 8 sq. cm of the scrotum in active or inactive caked lesions at 1 - 3 week intervals, the interval depending on the rate of lesion development and the availability of technical assistance. At each session, the semen was assessed from at least 2 rams, one without scrotal mange and the other either without lesions or with lesions covering less than 10 sq. cm of the scrotum. Semen collections were carried out at the laboratory and immediately after collection, the samples assessed for volume, colour and motility. Live/dead and morphology smears were prepared at this time and were assessed immediately after semen had been collected from the group. Semen quality data will be expressed as the semen quality index, the individual semen quality parameters of rams with induced seminal degeneration being presented in Appendix 4.

5.2 Results

(i) Housed rams

Of the 8 housed rams introduced initially in

1969 only 1 developed obvious scrotal mange. Over a period of weeks a discrete lesion slowly developed on the base of the scrotum of Ram 148. At the height of severity this lesion covered about 10 sq. cm of the base of the scrotum. During the period of scrotal mange, testes size and tone and semen quality and quantity of this ram were indistinguishable from those of the other seven housed rams.

On the 9.10.69 two 26 month old Romney rams were housed and observed for approximately 6 months. For the previous 12 months these rams had been run together on pasture without any special attention. When first housed, one of the rams (Ram 105) had a few mites associated with a scurfy scrotum while the other ram (Ram 107) had about 50 mites associated with three chronically active lesions covering approximately 3 sq. cm of the base of the scrotum. Ram 105 did not develop scrotal mange during the observation period and, therefore, was used as a control for Ram 107 which developed lesions of moderate severity (Text-fig. 6).

During the observation period semen concentration of the control ram varied from cloudy to creamy and the semen quality index varied from 22 to 28 with a mean of 24.

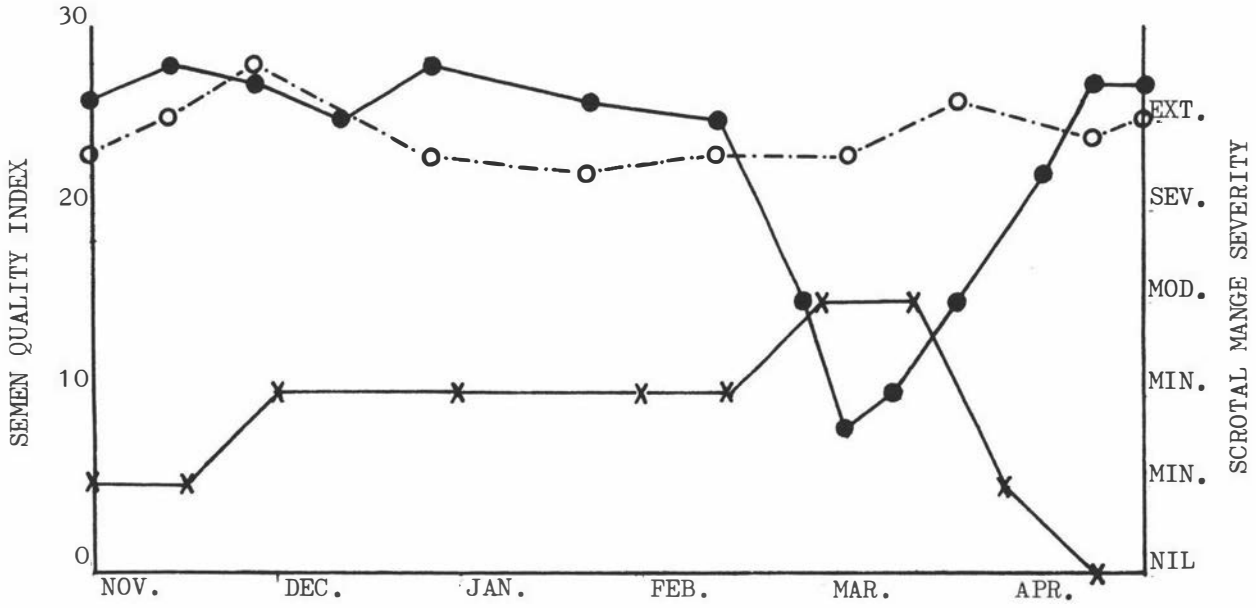
Throughout October and early November the scrotal lesions of Ram 107 did not change appreciably and semen quality and quantity were similar to the control animal (Text-fig. 6). Between 13.11.69 and 27.11.69 lesion classification changed to minor. Small lesions developed so that approximately one third of the distal third of the scrotum was covered in scattered crumbly scabs about 1/2 cm in diameter and about 1/4 - 1/2 cm thick. Lesion severity and semen quality did not

alter significantly throughout December, January and early February (Text-fig. 6). Some of the lesions cured spontaneously while others formed, lesions on the anterior aspect of the scrotum becoming slightly more severe than those on the posterior aspect. During the latter half of February there was a further rapid increase in lesion severity, scabs on the base becoming confluent with exudate as much as 3/4 cm thick. Lesion intensity decreased proximally with no lesions being found on the proximal half of the scrotum.

Associated with the development of moderate scrotal mange there was a precipitous decrease in semen quality (Text-fig. 6). At the beginning of March, less than 10% of the spermatozoa were motile, the majority of these simply oscillated with no forward progression. There was a proportionate increase in the percentage of spermatozoa staining dead and abnormals increased from 7% on 13.2.70 to 74% on 27.2.70. The majority of the abnormals observed in the first degenerate semen sample consisted of coiled tails and returned tails while an ejaculate collected a week later contained a similar number of spermatozoa with tail abnormalities and tailless spermatozoa (Fig. 17). On the 12.3.70 over half of the abnormal spermatozoa had pyriform heads and a small number had small, narrow and irregular shaped heads.

Scrotal lesions regressed slightly by the middle of March and by the beginning of April most of the scabs had begun to lift, leaving a normal skin beneath. By the end of April the only signs of a previous scrotal dermatitis was the presence of small amounts of dried yellow exudate adhering

TEXT-FIG. 6: RELATIONSHIP BETWEEN SEMEN QUALITY AND SCROTAL MANGE SEVERITY IN HOUSED RAM 107



1969 - 1970

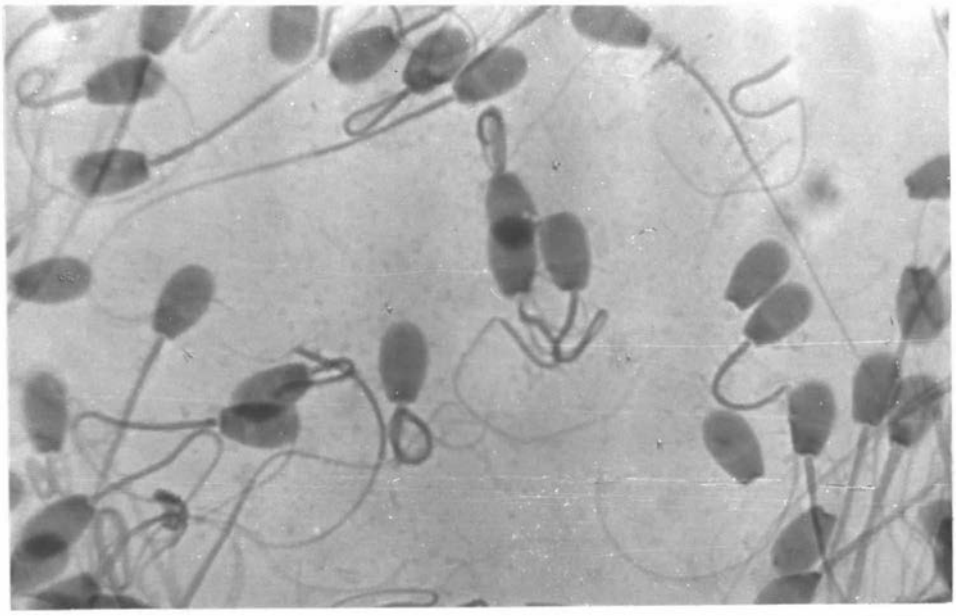
X Scrotal mange severity of Ram 107

● Semen quality index of Ram 107

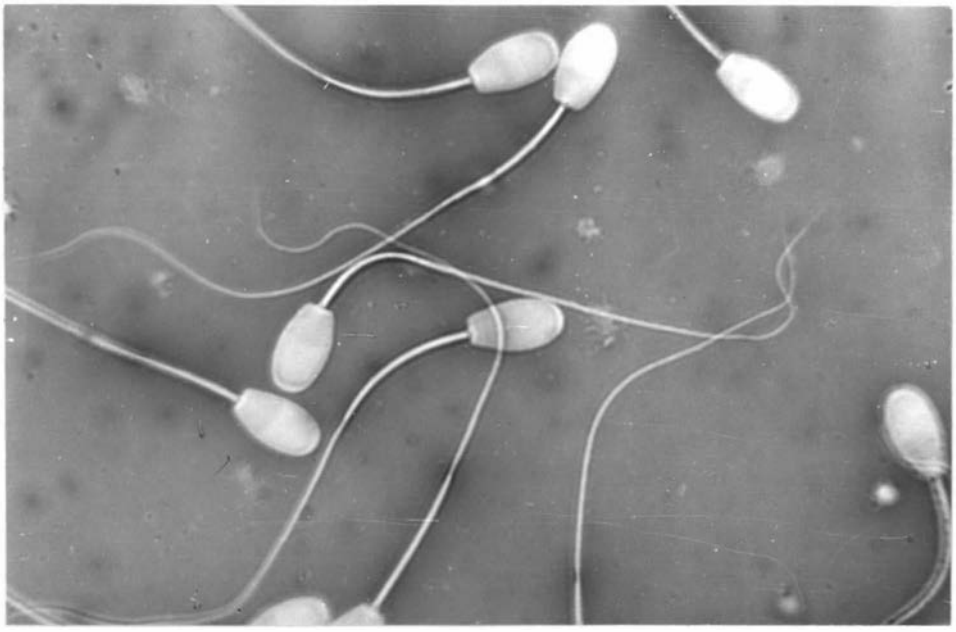
O Semen quality index of Control Ram 105

Figure 17: Semen smear from Ram 107 at the onset of seminal degeneration caused by "moderate" scrotal mange. (4.3.70). Most of the cells are morphologically abnormal. The abnormals consist of coiled, returned and kinked tails with a few tailless spermatozoa.
(Mayer's haemalum and eosin)
(x 1250)

Figure 18: Semen smear of Ram 107 on the 23.4.70 after seminal regeneration following spontaneous cure of the scrotal lesions. All of the spermatozoa are staining live and all are morphologically normal.
(Nigrosin and eosin)
(x 1250)



17



18

to wool fibres. A seminal regeneration was associated with the spontaneous cure (Text-fig. 6), semen quality returned to pre-seminal degeneration levels within a month of the first signs of regeneration (Fig. 18). There were no apparent changes in testes size or in semen quantity throughout the observation period.

(ii) Field trial rams

The semen quality index of the field trial rams without scrotal mange varied from 19 - 30 with a mean of 26.3 (Table 9). There was no apparent seasonal variation in the semen quality or quantity of these rams. The average semen quality index, and the standard deviation, of the rams with minimal scrotal mange was practically the same as rams without scrotal lesions (Table 9). Total spermatozoa numbers collected from the normal and minimal groups were also similar. Most semen samples collected from both groups had a concentration of thick-milky or creamy, while some samples were milky and a few only cloudy. As there were no differences in the semen of rams without scrotal lesions and those with minimal lesions, the semen quality indices of both groups were combined and used as controls for the rams that developed scrotal mange covering 10 sq. cm or more of the scrotum.

Large numbers of scrotal and leg mites did not affect the semen quality of the field trial rams. For example, for three months prior to July 1971 Ram 613 had ">10,000" mites on both the lower legs and the scrotum. The testes of this ram were large with good tone and on electro-stimulation on the 30.6.71 Ram 613 produced 0.7 ml of thick-milky semen. Virtually 100% of the spermatozoa were rapidly motile, 98% stained live and there were only 3% abnormals.

Table 9: RELATIONSHIP BETWEEN SEMEN QUALITY INDEX IN FIELD TRIAL RAMS WITH "MINIMAL" SCROTAL MANGE AND RAMS WITHOUT SCROTAL MANGE

Date 1970/71	Semen Quality Index		Monthly mean of both groups
	Rams without scrotal mange	Rams with "minimal" scrotal mange	
June	27, 25, 27, 28, 30.	26, 30, 30, 22.	27.2
July	29, 19.	25, 26, 30.	25.8
August	25, 26, 26, 25, 27.	23, 24.	25.1
September	28.	26, 27, 28.	27.3
October	27, 28, 27, 25.	28, 23, 22, 25, 19, 25.	24.9
November	26, 22.	26, 28, 23, 22.	24.5
December	29, 20, 27, 24, 27, 29.	22, 24, 28, 27.	25.7
January	27, 24.	26, 28, 26, 20.	25.2
February	28, 29, 27, 24.	29, 27, 28.	27.4
March	24, 26,	27, 28.	26.3
June	30, 29.	30, 27.	29
Mean ± S.D.	26.3 ± 2.5	25.8 ± 2.8	
July	30, 21, 24, 27, 25.		25.4
August	27, 29, 29, 26, 28.		27.8
September	27, 27, 26, 25, 30, 29.		27.3
October	28, 29, 28, 26, 28, 26.		27.5
November	25, 17, 27, 28.		24.8

No rams were assessed during April and May, 1971 because no ram had scrotal mange involving 10 sq. cm or more of the scrotum during this period.

Four of the field trial rams (Rams 613, 237, 321 and 214) developed minor scrotal mange during the observation period. One of these rams (Ram 613) developed severe foot rot at about the time of development of minor scrotal mange. As foot rot may affect the reproductive function of the ram (Webster, 1937; Gunn et al., 1942), no significance can be placed on the seminal degeneration observed at the time of lesion development in this ram. Approximately 4 months after successful treatment of the severe foot rot, during which time the scrotal lesions also spontaneously cured, reproductive function of Ram 613 returned to normal. The other three rams that developed minor scrotal mange had semen quality during the period of minor scrotal mange either above the average of the control group (Ram 237) or at the lower end of the semen quality range of the control group (Rams 214 and 321).

Ram 237

At the beginning of October 1970 this ram had a few small scabs on the base of the scrotum. Most of these scabs were 1 - 5 mm in diameter, with one scab approximately 1 cm in diameter. The lesions progressed so that by the end of October approximately half of a 25 sq. cm rectangular area on the posterior distal medial region of the scrotum was involved. Lesion classification changed from minimal to minor. The lesions regressed almost immediately so that by the middle of November lesion classification had changed back to minimal and the spontaneous cure was complete by the end of November. The testes of Ram 237 were large with moderate tone and semen quality remained high during the period of minor scrotal mange. For example, a semen sample collected on the 10.10.70 contained 100% motile

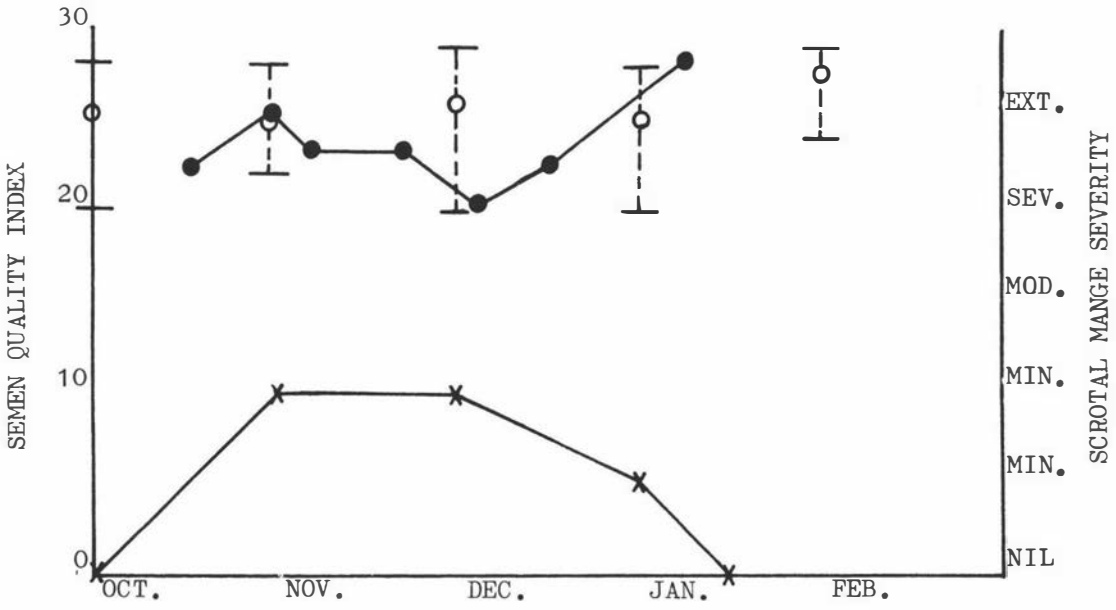
spermatozoa, 91% stained live and 93% were normal. Another sample collected on the 30.10.70 contained 80% motile, 95% live and 93% normal.

Ram 321

On 21.10.70 this ram had small active lesions of chorioptic mange covering approximately 3 sq. cm of the base of the scrotum. Over the following ten days these lesions developed further and others appeared so that approximately 15 sq. cm of the posterior distal aspect of the scrotum was covered in exudate about 1/2 cm thick, resulting in scrotal mange being classified as minor (Text-fig. 7). By the middle of November the larger lesions had spontaneously cured and the scabs had started to lift off the skin. However, small peripheral lesions had increased in severity resulting in a similar area being covered in either active or inactive lesions (Text-fig. 7). By the middle of December some of these lesions had spontaneously cured and at the beginning of January lesion classification changed to minimal and spontaneous cure was complete by the middle of January 1971 (Text-fig. 7).

Semen quality of most samples collected from Ram 321 corresponded with samples at the lower end of the semen quality range of the control rams (Text-fig. 7). Most ejaculates contained 60 - 80% motile spermatozoa, with 20 - 30% abnormals. Most of the morphological abnormalities were secondary defects, coiled and returned tails predominating. Testes size and tone and semen concentration were indistinguishable from those of the control animals during the period of minor scrotal mange.

TEXT-FIG. 7: RELATIONSHIP BETWEEN SEMEN QUALITY AND SCROTAL MANGE SEVERITY IN FIELD TRIAL RAM 321.



Ram 214

A small lesion of chorioptic mange slowly developed on the base of the scrotum of Ram 214 and by the beginning of September 1970 the scab covered approximately 4 sq. cm of the scrotal base with exudate as much as 1 cm thick. In addition, a few very small active lesions (1 - 3 mm diameter) were scattered over the distal quarter of the scrotum. Lesion severity did not change appreciably during the following month. However, a fissure developed in the exudate and by the end of September there were two distinct lesions on each side of the median raphae at the base of the scrotum. The chronically active lesions increased in size during October and by the beginning of November crumbly-caked scabs covered a total area of about 15 sq. cm, with exudate 1 - 2 cm thick.

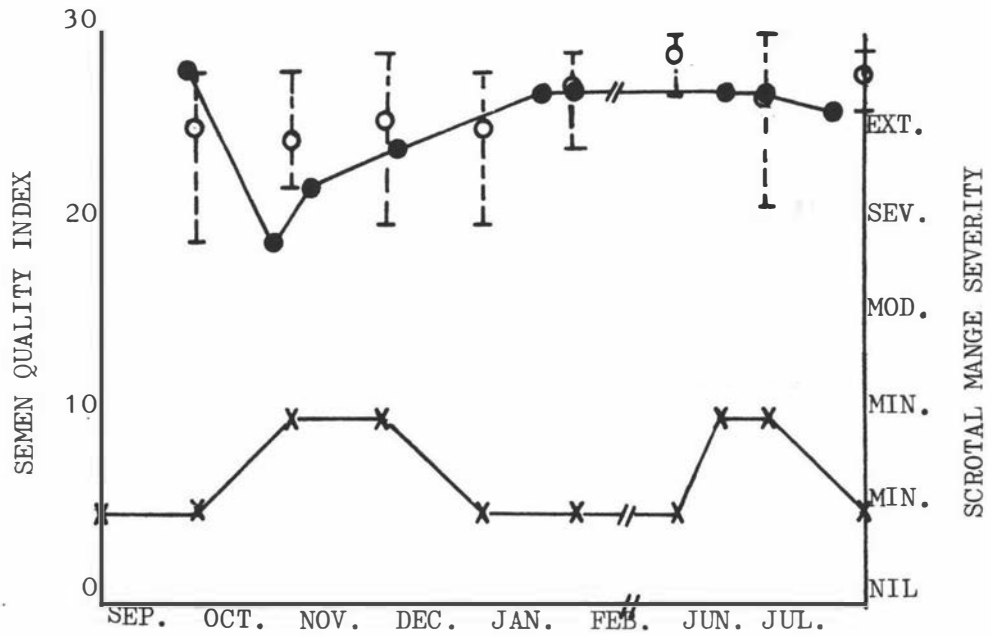
In association with the increase in lesion severity in October there was an apparent decrease in semen quality, from semen of very high quality in mid September to semen having approximately 30% immotile and 40% abnormal spermatozoa on 26.10.70. However, the semen quality index of this sample did not fall below the lowest composite score of the control rams that month (Text-fig. 8). Semen quality improved in subsequent ejaculates with greater than 90% motile spermatozoa and less than 30% abnormal spermatozoa observed in an ejaculate collected on 6.11.70. Testes size and tone and total spermatozoa per ejaculate were above the control average throughout the period of minor scrotal mange.

The chronically active lesions covered approximately 12 sq. cm of the scrotal base at the

beginning of December. These slowly regressed and by the end of March 1971 the two chronically active lesions were about 1 1/2 cm in diameter with caked exudate approximately 1 1/2 cm thick (Text-fig. 8).

During June there was again an increase in scrotal mange severity (Text-fig. 8). By the beginning of July crumbly-caked exudate 1 - 2 cm thick covered approximately 10 sq. cm of the distal part of the left posterior aspect of the scrotum. Semen concentration was above the control average during this period and virtually all spermatozoa examined were rapidly motile with only very small numbers of abnormals. There was no apparent thickening of the scrotal skin during the period of lesion involvement and testes size and tone remained above the average of the control rams.

TEXT-FIG. 8: RELATIONSHIP BETWEEN SEMEN QUALITY AND SCROTAL MANGE SEVERITY IN FIELD TRIAL RAM 214.



X Scrotal mange severity of Ram 214

● Semen quality index of Ram 214

Semen quality index of control rams (monthly mean and range)

In contrast to the rams with minor scrotal mange there was a definite seminal degeneration associated with the experimental induction of moderate, severe and extreme scrotal mange in Field Trial Rams 184, 435 and 613 respectively.

Ram 184

At the beginning of June 1970 Ram 184 had "1,000 - 10,000" mites on the basal area of the scrotum and an occasional very small (1 mm diameter) active lesion was observed microscopically in association with the mites. On the 15.6.70 readily palpable lesions had developed resulting in scrotal mange being classified as minimal. By the beginning of July scattered lesions, the largest being about 1 cm in diameter with exudate 1/4 - 1/2 cm thick, covered about a quarter of the distal two thirds of the posterior part of the scrotum and a quarter of the distal eighth of the anterior part of the scrotum. Thus relatively large areas of the scrotum were covered in small scabs, the lesions being described as moderate.

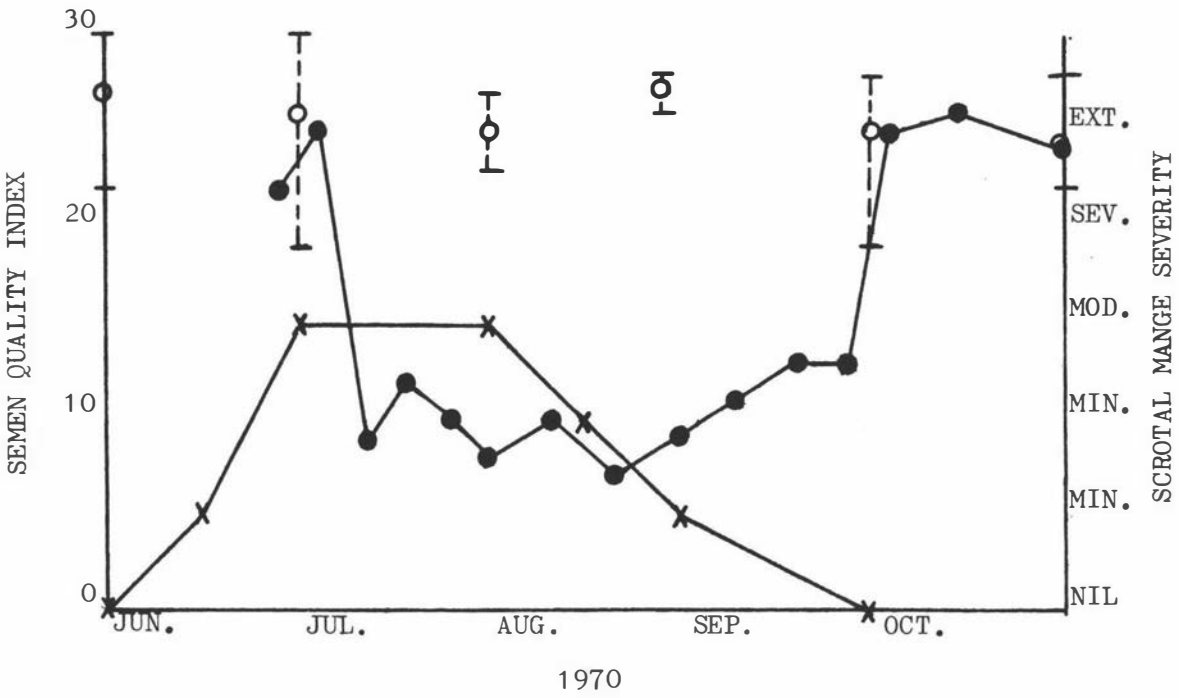
A semen sample was collected at the end of June and again on the fourth of July and both samples were indistinguishable from those of the control animals, approximately 80% of the spermatozoa being motile and more than 75% morphologically normal. During the following week, approximately a week after the scrotal lesions were first classified as moderate, there was a sudden seminal degeneration (Text-fig. 9). The first degenerate sample contained no significant increase in the number of abnormal spermatozoa but subsequent degenerate ejaculates contained 51 - 84% abnormals. A sample collected six days after the first degenerate ejaculate

contained relatively large numbers of spermatozoa with tail defects (mainly returned and coiled tails), tailless spermatozoa and spermatozoa with proximal cytoplasmic droplets. In later ejaculates as many as 44% of the spermatozoa had head abnormalities and the majority of these were pyriform heads. In some samples there was also a relatively large number of spermatozoa with abnormal acrosomes. Associated with the increase in lesion severity and decrease in semen quality (Text-fig. 9) there was also a palpable change in the testes. Testes tone changed from good to poor and testes size from large to moderate by the 24.7.70. The decrease in testes size was reflected also in the total spermatozoa numbers collected. Semen concentration fell from creamy at the beginning of July, to watery-milky in mid July, to cloudy during August. Total spermatozoa per ejaculate varied from 1.4×10^6 to 18×10^6 during August and most of these were morphologically abnormal (Fig. 19). By August some of the scattered lesions had become confluent, the largest covering approximately 8 sq. cm of the scrotum, with exudate about 3/4 cm thick. However, other lesions cured spontaneously so that lesion severity remained similar (Text-fig. 9).

By the middle of August the lesions had started to regress and a fortnight later most scabs had started to lift off the scrotum leaving only about 3 sq. cm with active or inactive lesions (Text-fig. 9). During most of September approximately 40% of the spermatozoa collected were motile and abnormals varied from 52 - 66% while samples collected at the beginning and middle of October contained approximately 80% rapidly motile spermatozoa with 25% or less, abnormal spermatozoa (Fig. 20). Total spermatozoa numbers also returned

to pre-degeneration levels, 1.5 ml of thick-milky semen being collected on the 14.10.70.

TEXT-FIG. 9: RELATIONSHIP BETWEEN SEMEN QUALITY AND SCROTAL MANGE SEVERITY IN FIELD TRIAL RAM 184



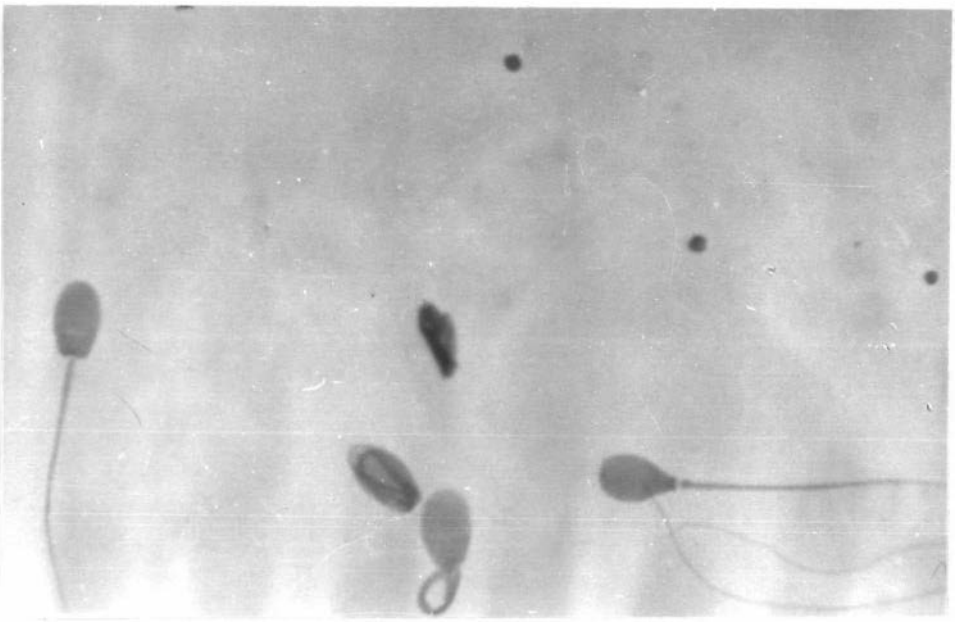
X Scrotal mange severity of Ram 184

● Semen quality index of Ram 184

Semen quality index of control rams (monthly mean and range)

Figure 19: Semen smear from Ram 184 when "moderate" scrotal mange had caused severe oligospermia (20.8.70). Most of the spermatozoa are abnormal. Note pyriform head, midpiece coiled in head, coiled tail and one normal spermatozoa.
(Mayer's haemalum and eosin)
(x 1250)

Figure 20: Semen smear from Ram 184 approximately 6 weeks after spontaneous cure of scrotal mange was obvious (3.10.70). Between the live, morphologically normal spermatozoa are "free" cytoplasmic droplets.
(Nigrosin and eosin)
(x 1250)



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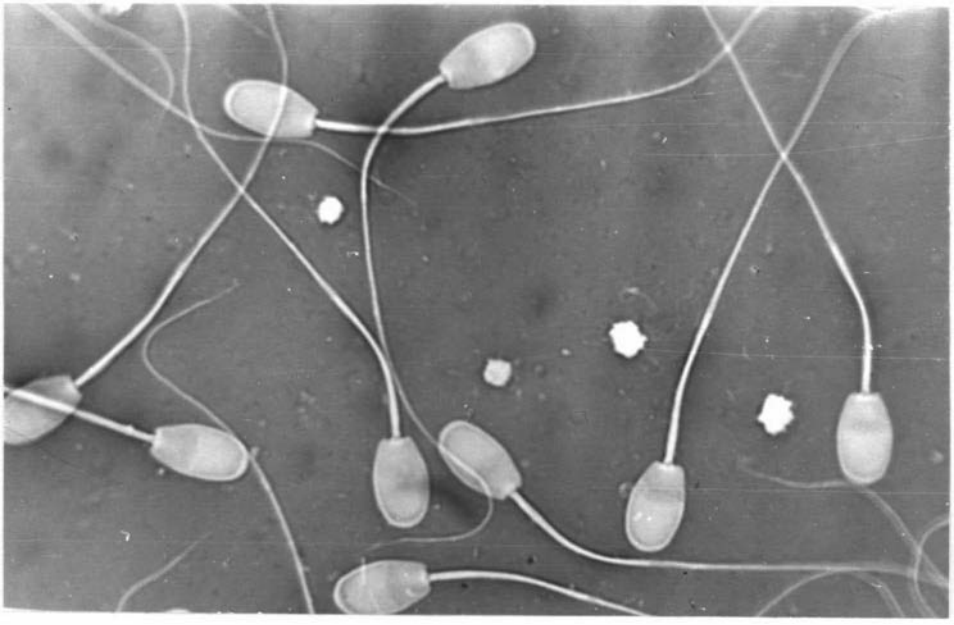

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Ram 435

Prior to lesion development more than a thousand mites were observed on the scrotum of Ram 435. On 16.8.70 a few 1 - 3 mm scabs were palpated on the base of the scrotum and by the 4.9.70 large numbers of small (1 - 3 mm diameter) scabs were palpated mainly on the distal third of the anterior aspect of the scrotum. The lesions were concentrated on the base, with about half of a 10 sq. cm area covered in exudate 1/4 - 1/2 cm thick. During the following month the lesions became more severe so that scrotal mange classification changed to moderate at the beginning of October (Text-fig. 10). At the beginning of October the dried exudate on the base was almost 1 cm thick and many of the small lesions had become confluent, the exudate covering approximately half of an 18 sq. cm area on the base of the scrotum. There were two crumbly-caked lesions covering approximately 6 sq. cm on the anterior distal quarter and two smaller lesions covering about 3 sq cm on the posterior distal third of the scrotum. By the middle of October the lesions on the base had become confluent with exudate as much as 1/2 cm thick.

The testes of Ram 435 at the beginning of October 1970 were the largest of the field trial rams and semen collected was of high quality. Spermatozoa motility dropped from 80% to 20% and abnormals rose from 18% to 79% between the 3.10.70 and 10.10.70. In the first degenerate sample the majority of abnormals were coiled tails. Although there was no apparent decrease in testes size, there was a slight decrease in testicular tone during this period. Semen quality remained low throughout October (Text-fig. 10) with a proportionate increase in the number of spermatozoa with neck abnormalities

(Fig. 21). The majority of the neck abnormalities were tailless spermatozoa with small numbers of spermatozoa with broken necks. The lesions remained active and became slightly more severe during the latter part of October and November.

There was an improvement in semen quality in November with approximately 50% of the spermatozoa being motile and a similar proportion staining live. Testes tone also improved during this period. During December and January some of the lesions cured spontaneously but the large lesion on the base still covered approximately 20 sq. cm. At the beginning of February 1971 there was a marked improvement in the semen quality index (Text-fig. 10) and by mid February virtually 100% of the spermatozoa were motile and 90% were morphologically normal.

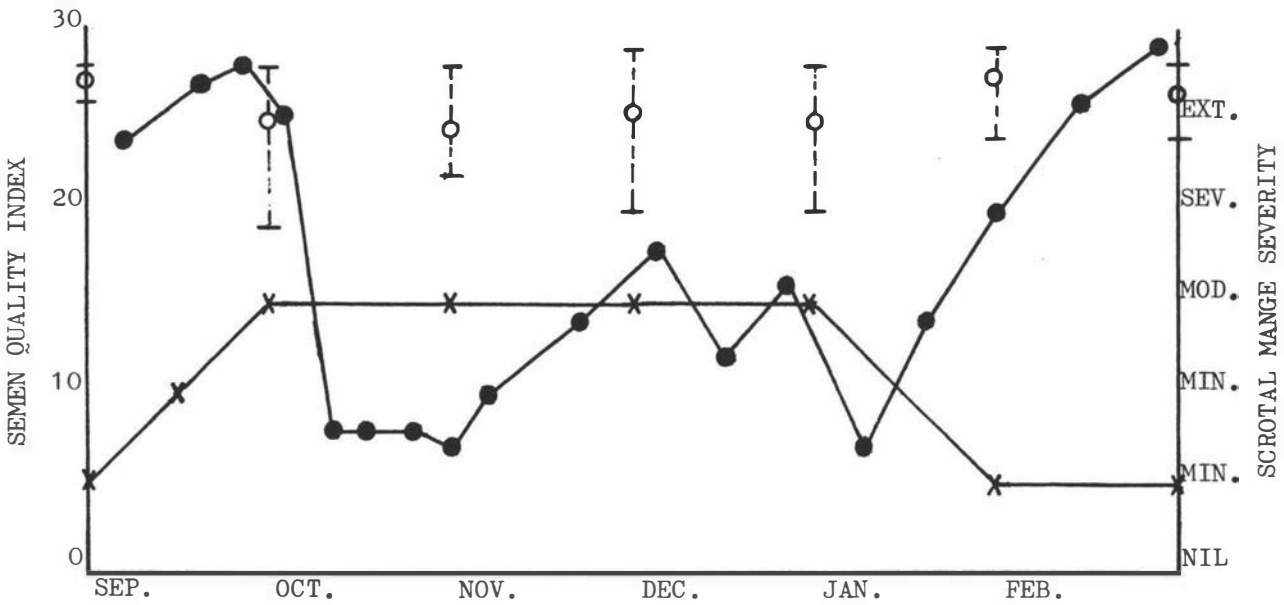
When it was obvious that seminal regeneration was occurring the large lesion on the base of the scrotum was examined more closely. The exudate was found to peel off leaving, apart from three small active lesions on the periphery of the exudate, a clinically normal skin and so lesion classification was changed from moderate to minimal.

Testes size and tone and semen quality and quantity remained high during the following four months when chronic scrotal lesions covered 2 - 5 sq. cm of the scrotum.

At the beginning of June relatively large numbers of small chorioptic scabs were palpated again on the distal half of the scrotum and by the beginning of July lesions had developed again to the moderate classification (Text-fig. 11). On the 1.7.71 a sixth of the distal quarter was covered in

scattered active lesions with exudate 1/2 - 1 cm thick. Many of the scabs were 1 cm in diameter and on the distal quarter there were two confluent lesions covering about 10 sq. cm and 4 sq. cm of the scrotum. At the beginning of July the testes were large with good tone and semen was of high quality (Text-fig. 11). Approximately a week after the reoccurrence of moderate scrotal mange a seminal degeneration was observed (Text-fig. 11). During July a slight decrease in lesion severity was noted and this was associated with an improvement in semen quality (Text-fig. 11). However, by mid August the lesions had become more severe so that most of the distal third of the scrotum was covered in exudate 1/2 to 1 cm thick. During September 1971 a moderate decrease in testes size and tone was noted and all ejaculates were less than creamy in colour. A spontaneous cure of the scrotal lesions occurred during October and November 1971; by the end of November only a few small scabs of chorioptic mange were observed scattered over the scrotum. By mid November testes size and tone were indistinguishable from the control animals and seminal regeneration was complete by the middle of December (Appendix 4).

TEXT-FIG. 10: RELATIONSHIP BETWEEN SEMEN QUALITY AND SCROTAL MANGE SEVERITY IN FIELD TRIAL RAM 435



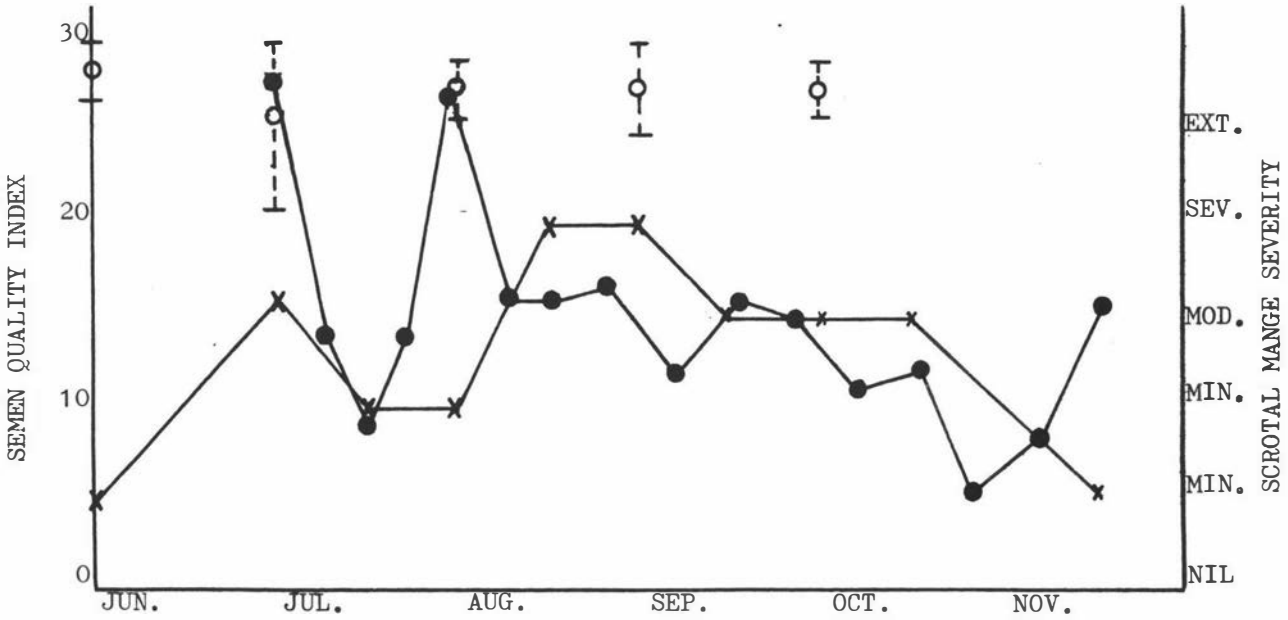
1970 - 1971

X Scrotal mange severity of Ram 435

● Semen quality index of Ram 435

Semen quality index of control rams (monthly mean and range)

TEXT-FIG. 11: RELATIONSHIP BETWEEN SEMEN QUALITY AND REOCCURRENCE OF EXTENSIVE SCROTAL MANGE IN RAM 435



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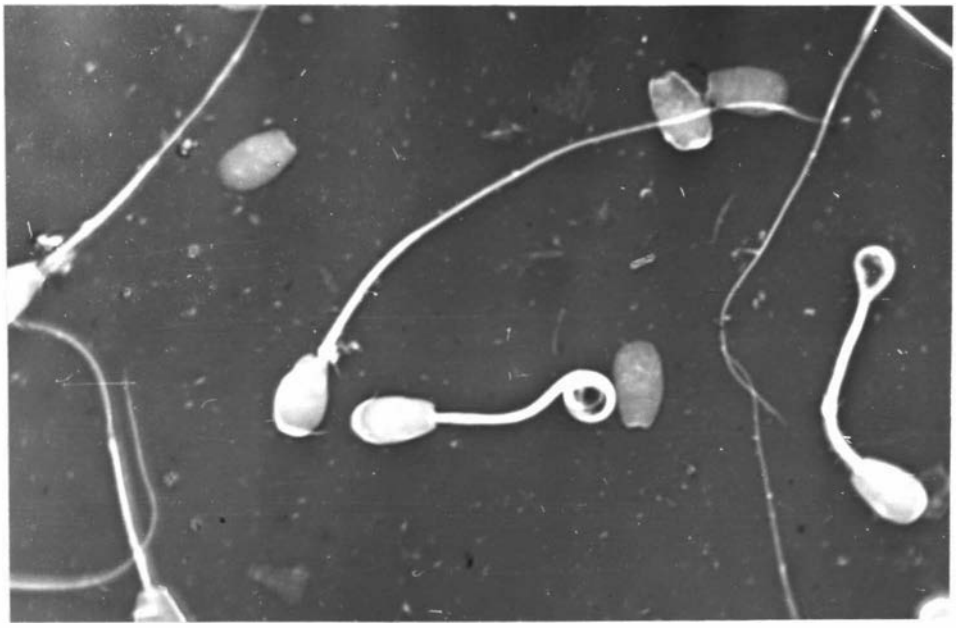
X Scrotal mange severity of Ram 435

● Semen quality index of Ram 435

Semen quality index of control rams (monthly mean and range)

Figure 21: Semen smear from Ram 435 during the seminal degeneration associated with "moderate" scrotal mange. All but one of the spermatozoa are morphologically abnormal and the tailless spermatozoa are stained dead.
(Nigrosin and eosin)
(x 1250)

Figure 22: Semen smear from Ram 435 after complete seminal regeneration following spontaneous cure of "moderate" scrotal mange (27.2.71). All the spermatozoa are live and morphologically normal.
(Nigrosin and eosin)
(x 1250)



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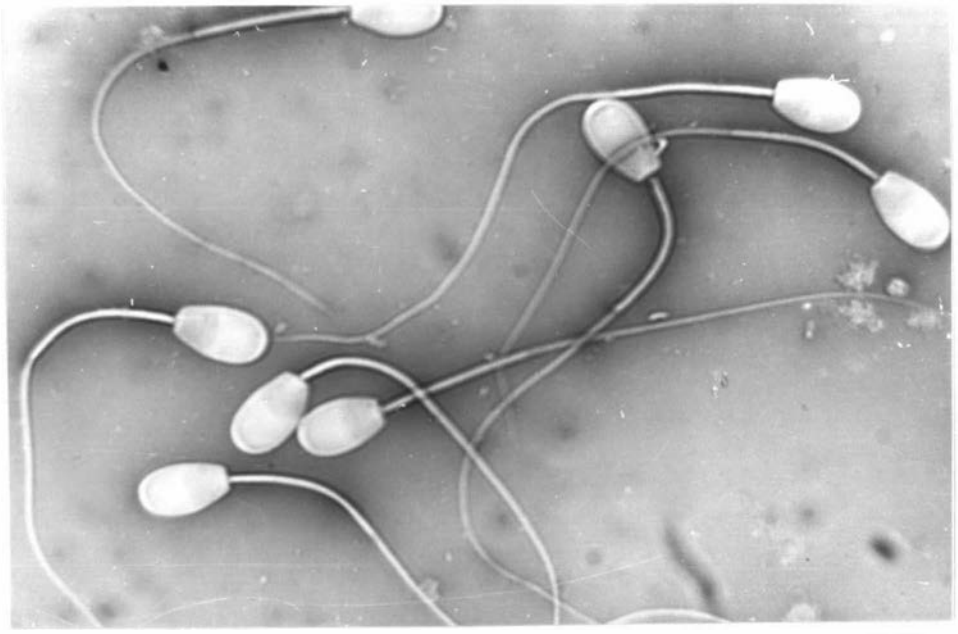

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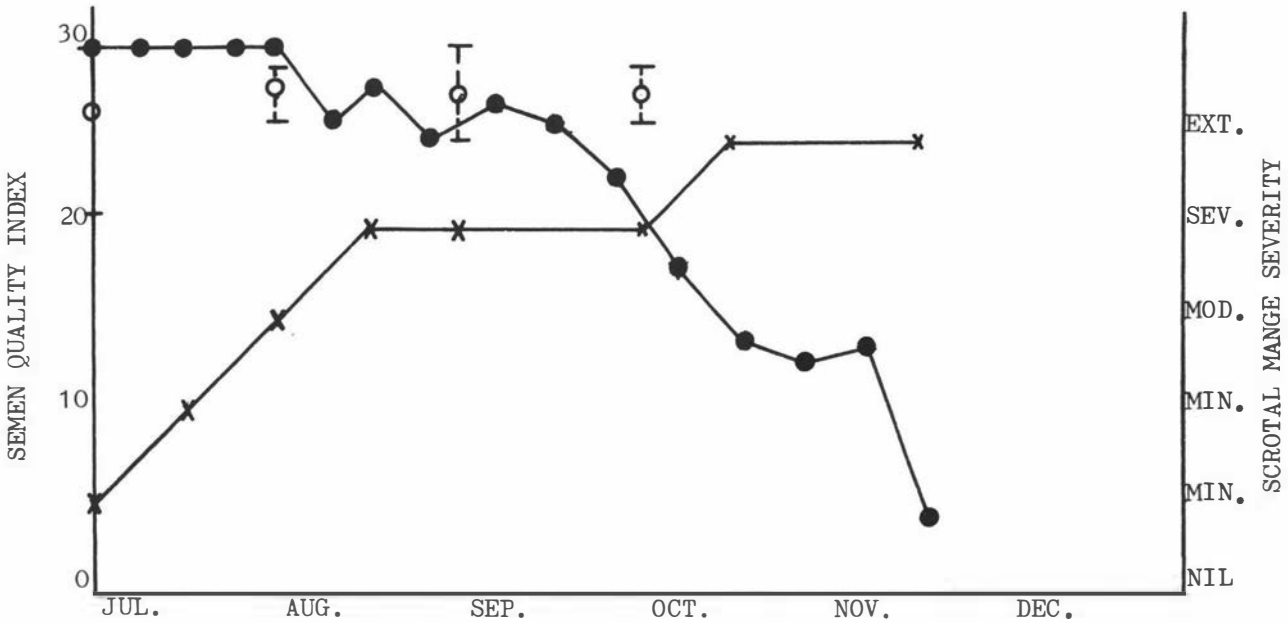
Ram 613

During the winter and spring of 1971 lesions on the scrotum of Ram 613 slowly developed from minimal at the beginning of July, to moderate at the beginning of August, to severe at the beginning of September and finally to extreme by the middle of October. Initially the scrotal lesions were limited to only the more distal aspects of the scrotum but by the end of the observation period (15.11.71) virtually all of the scrotum was covered in crumbly exudate 1 - 3 cm thick. Associated with the development of extensive scrotal mange C. bovis mites on the scrotum decreased in number from ">10,000" to "100-1,000" (Text-fig. 2).

Testes size and semen quality during the period of minimal, minor and moderate scrotal mange were above the average of the control rams (Text-fig. 12). During August and September, when scrotal mange advanced to the severe classification, there was a slight decrease in semen quality (Text-fig. 12) although the semen composite score was still within the range of the control animals and most spermatozoa were morphologically normal (Fig. 23). Towards the end of September, in association with severe scrotal mange, there was a marked decrease in semen quality (Text-fig. 12) and as the lesions became more severe over the following month the seminal degeneration became more marked (Fig. 24). Towards the end of the observation period there was a marked decrease in the number of spermatozoa collected on electrical stimulation and at the end of the observation period (15.11.71) the testes were small with moderate tone.

TEXT-FIG. 12: RELATIONSHIP BETWEEN SEMEN QUALITY AND SCROTAL MANGE SEVERITY IN FIELD TRIAL RAM 613



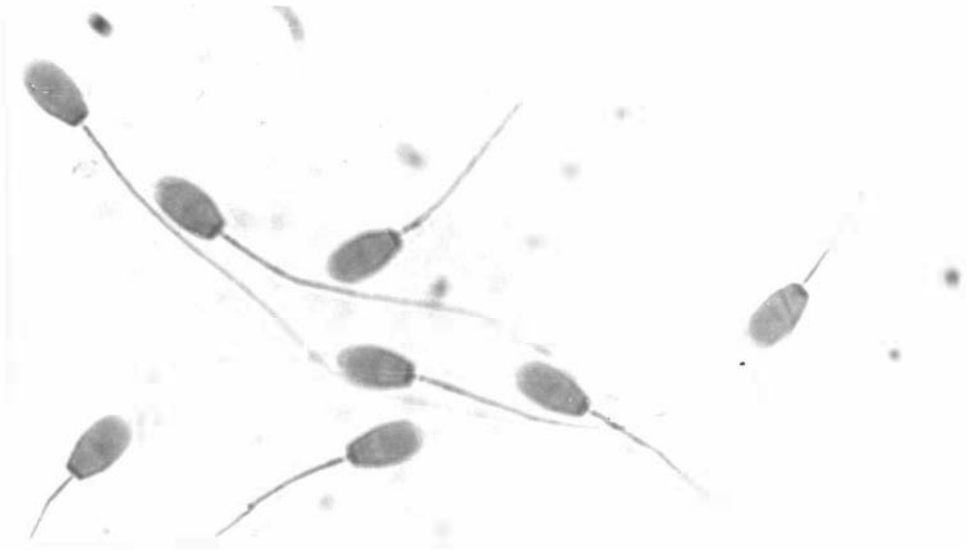
X Scrotal mange severity of Ram 613

● Semen quality index of Ram 613

T
O Semen quality index of control rams
T (monthly mean and range)

Figure 23: Morphologically normal spermatozoa associated with "severe" scrotal mange in Ram 613 on 25.8.71.
(Mayer's haemalum and eosin)
(x 1250)

Figure 24: More than half of the spermatozoa are morphologically abnormal in a semen smear from Ram 613 when scrotal mange had advanced to the "extreme" stage (26.10.71).
(Mayer's haemalum and eosin)
(x 1250)



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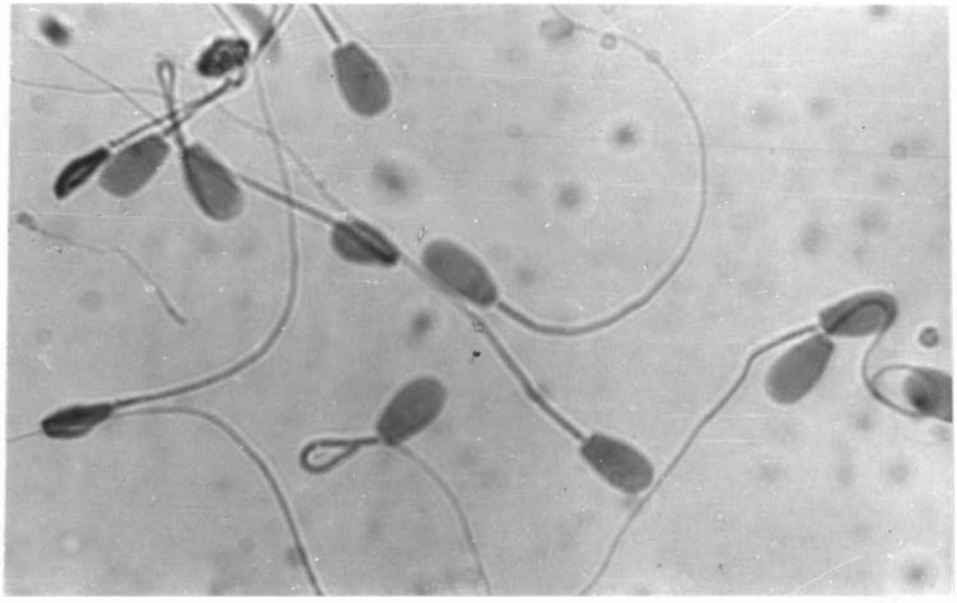

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5.3 Discussion

In general, the semen analysis data collected from rams with experimentally induced scrotal mange is comparable with the data collected from the flock of rams with an outbreak of scrotal mange (Chapter 4). Rams with experimentally induced lesions covering 8 sq. cm or less of the scrotum had semen of normal quality. One of the three rams that developed uncomplicated minor scrotal mange had semen of high quality while the other two had slightly inferior semen. There was an apparent decrease in semen quality associated with the development of minor scrotal mange in one of the rams and in the other there appeared to be an improvement in semen quality associated with the spontaneous cure. However, no significance can be placed on these two cases by reason of the small number of rams that developed minor scrotal mange and because the semen quality remained within the range of the control animals which were ejaculated during the same month. On the other hand, seminal degeneration was associated with the development of moderate, severe and extreme scrotal mange and seminal regeneration was associated with the spontaneous cure of moderate and severe scrotal mange. This confirms that scrotal mange can cause a seminal degeneration.

In agreement with the Mid-Canterbury survey, there was a wide variation in the response of different rams to experimentally induced scrotal mange. For example, some rams with relatively mild lesions had a severe seminal degeneration with severe oligospermia while another ram with extensive scrotal lesions had only a mild seminal degeneration with no marked affect on spermatozoa production. Some of this variation may be accounted for by the rate of lesion development. There was a

tendency for scrotal mange that developed slowly to have a less severe effect on semen production than similar lesions that developed rapidly.

Throughout the period of seminal degeneration, the majority of ejaculates from many rams had less than 50% motile spermatozoa and more than 60% abnormal spermatozoa. It has been shown by many workers that ram semen of this quality has reduced fertilizing capacity (Gunn et al., 1942; Edgar, 1959; Hulet and Ercanbrack, 1962; Howarth, 1969; Braden and Mattner, 1970). During various periods of the seminal degeneration in Rams 107, 184, 435 and 613 spermatozoa motility was in the 1 - 10% range and at the same time on average more than 70% of the spermatozoa were morphologically abnormal. Ram semen of this quality has been associated with complete sterility (Gunn et al., 1942; Dun, 1956; Hulet et al., 1965; Howarth, 1969). Although spermatozoa numbers were not a limiting factor, Howarth (1969) found that eight rams were infertile when their average spermatozoa motility was in the 5 - 15% range with 50 - 60% abnormals and Dun (1956) found thirteen rams were infertile with similar semen quality. As well as a severe decrease in spermatozoa quality at the time of moderate scrotal mange, one of the three rams (Ram 184) became severely oligospermic during the seminal degeneration. During August this ram produced on electro-stimulation a maximum of 18 x 10^6 spermatozoa per ejaculate. In artificial insemination a dose of at least 50 x 10^6 is usually considered a minimal number necessary for a 50% conception rate (Emmens and Robinson, 1961). Although both of these figures are only estimates of an in vivo process, it is almost certain in Ram 184 that spermatozoa quantity as well as quality would not be compatable with good fertility.

In none of these rams were there any signs of

an inflammatory involvement of the reproductive tract. For example, polymorphonuclear leucocytes were not observed in any of the degenerate semen samples obtained from the rams with extensive scrotal mange. Conversely the seminal response was typically degenerative, being very similar to that seen in heat induced seminal degenerations. The time lapse between the onset of relatively severe scrotal mange and seminal degeneration was similar to the time lag seen between the application of heat and the occurrence of seminal degeneration in the ram (Phillips and McKenzie, 1934; Gunn, 1936; Dutt and Hamm, 1957; Moule and Waites, 1963; Waites and Setchell, 1964). The rapid drop in percentage of motile spermatozoa with a similar increase in the proportion of spermatozoa staining dead and the occurrence of large numbers of tail abnormalities followed by semen samples that contained a large percentage of spermatozoa with tail abnormalities and tailless spermatozoa is also characteristic of both heat induced seminal degenerations (Dutt and Hamm, 1957; Glover, 1958; Moule and Waites, 1963; Waites and Setchell, 1964) and the seminal degenerations observed in rams with scrotal mange.

A complete seminal regeneration occurred within two months of the commencement of the regression of the scrotal mange lesions. A similar time interval for seminal regeneration is observed after heat induced degenerations (Gunn et al., 1942; Fowler and Dun, 1966; Smith, 1971). Direct evidence to support the hypothesis that the degeneration associated with chorioptic mange is heat induced is presented in Part III of this thesis.

Of the 52 rams with scrotal mange discussed in this and the previous chapter, 3 (5.8%) had

testes that could be classified as hypo-orchid ("small testes", Bruere, 1970). This is in disagreement with the findings of Crawford et al. (1970). These authors found 45% of 92 rams with scrotal mange had testes of reduced size; 32% had testes "smaller than normal" and 13% had "bilateral hypoplasia or atrophy". The relative severity of the scrotal mange of the 92 cases was not recorded, but Crawford et al. (1970) noted that "lesions of scrotal mange varied from small quantities of yellowish exudate among the wool fibres to large masses of exudate which matted the wool fibres and when torn away left the skin surface moist and papular". Crawford et al. (1970) noted that about 2% of flock rams without scrotal mange had bilateral hypoplasia or atrophy, but they failed to note the proportion of rams without scrotal mange that had testes "smaller than normal". Results presented in this and the previous chapter suggest that Crawford et al. (1970) were classifying testes as "smaller than normal" when they were actually in the normal range and/or in spite of their description of lesions were only detecting cases of extensive scrotal mange.

Chapter 6

THE EFFECT OF EXTENSIVE SCROTAL MANGE ON REPRODUCTIVE FUNCTION

6.1 Introduction

Two rams with severe scrotal mange and 3 rams with extreme scrotal mange were rejected from three stud flocks by the author during a routine presale examination in late November and December 1969. The 5 rams with scrotal mange and 1 ram without scrotal lesions from one of the flocks were brought to the University and kept indoors. A further 4 control rams were obtained from the University flock. One of the rams with scrotal mange was a Perendale and the other 9 rams were Romneys. The rams were divided into two groups of 5 and housed in adjacent pens. After a 4 month observation period the animals were slaughtered.

Testicular function was assessed by semen examination and testicular palpation in the live animal and after slaughter by estimating gonadal and extragonadal spermatozoa reserves and testicular histology. Seminal fructose and sexual behaviour were measured to assess the effects of extensive mange on the androgenic status of the live animal and the results verified by testicular histology, seminal vesicle weight and seminal vesicle fructose content.

Between group differences in fructose estimates were tested for significance by analysis of variance (Snedecor, 1956) while fructose estimates of

individual rams with scrotal mange were compared with their respective control groups using a modification of "Students" t-test (Sokal and Rohlf, 1969). Seminal plasma fructose was assessed during February 1970 and sexual behaviour in the second week of March 1970.

At least 2 of the control rams were semen tested weekly to check the effect of housing on semen quality. The majority of semen samples collected from the control rams were milky or thicker in concentration and the composite semen score did not fall below 20 in any ram except for Ram 260. This ram produced slightly inferior semen throughout January and February 1970. However, prior to slaughter all of the control rams produced at least 0.5 ml of creamy semen with an average spermatozoa quality index of 28.

The right testis of control Ram 162 was smaller than the left testis throughout the experimental period and at postmortem there was an area of approximately 3 sq. cm in the middle of the distal third of the testis that was severely atrophic. The seminiferous tubules in this region were reduced in size and the tubules contained only a single layer of cells. Tubules adjacent to the atrophic area were normal in both size and cellular activity. Semen collected from Ram 162 was above the control average in quantity and quality throughout the experiment.

There was a large between animal and within animal variation in seminal plasma fructose concentration estimates in the control group (Appendix 5) concealing any small effects that

could have been caused by scrotal mange. In an attempt to reduce the between ram variation the androgenic status experiments were repeated in November-December 1970 with a more uniform group of rams. Eight 15 month Cheviot rams, 4 with extensive scrotal mange and 4 controls, were obtained from one flock of rams that had been run together on pasture for at least the previous 6 months. The rams were brought to the University, ear tagged, and run together under natural field conditions.

The initial 5 rams with extensive scrotal mange are presented as individual case reports while in the Cheviot experiment the results are presented for the animals as a group.

6.2 Results

Case No. 1 (Ram 359)

Several rams of a flock of 15 month old Romney rams examined on the 20.11.69 had scrotal lesions diagnosed as chorioptic mange. Scrotal lesions were classified as minimal in all rams except Ram 359 which had severe scrotal mange. This ram had testes of medium size and moderate tone, was rejected as genitally unsound and was brought to the University on the 6.12.69. The scrotal lesions by this time were extreme. Except for an area of approximately four sq. cm on the base, the whole scrotum was covered in caked exudate 1 - 2 cm thick. The superficial layers of exudate were dry and crumbly but the exudate close to the skin was moist and caked. No live mites were seen in the region of moist active lesions, but 100 - 1,000

mites were seen on the dry scurfy area at the base of the scrotum. By the 15.1.71 there was 2 - 3 cm of caked exudate covering almost the entire scrotum (Fig. 25) and the testes were classified as small and soft. At the time of slaughter (23.3.70) a large amount of the proximal part of the scrotum had undergone a spontaneous cure, the dried exudate being separated from a clinically normal skin by 1 - 2 cm of wool (Fig. 26). However, active lesions and insulative exudate on the more distal portion of the scrotum resulted in a pre-slaughter mange classification of severe. The scrotum, especially distally was thickened slightly and was relatively difficult to cut with the post-mortem knife. Histologically there was an increase in the amount of fibrous tissue in the dermis. The lesions were confined to the scrotum; the cavity of the tunica vaginalis and the testicular capsule were normal with no adhesions between the visceral and parietal layers of the tunica vaginalis.

The ram was electro-stimulated for the first time on the 6.12.69 and produced 0.5 ml of milky semen which contained a total of 4.3×10^8 spermatozoa. About 1% of the spermatozoa were motile, the majority oscillating on their long axis, 92% stained dead and 72% were morphologically abnormal (Fig. 28). Thirty eight percent of the abnormalities were tailless heads, 21% tail defects (mainly bent tails) and 13% abnormal heads (mainly pyriform heads). The spermatozoa that were produced in subsequent ejaculates were all dead and numbers decreased rapidly so that by the 20.12.69 the ram was severely oligospermic, semen collected on that date yielding only 5×10^6 spermatozoa in 0.8 ml of cloudy semen. Spermatozoa numbers decreased

even further and the ram was practically azoospermic for the last two months before slaughter. Throughout this two month period the occasional spermatozoa was observed in most semen smears. About 50% of the spermatozoa collected when the ram was virtually azoospermic had tailless heads. However, the majority of the remaining spermatozoa and most of the heads of the tailless spermatozoa were morphologically normal.

At postmortem the left and right testis of Ram 359 weighed 94 g and 84 g respectively, compared with an average testis weight of 182.5 g in the control ram (Ram 162) slaughtered the same day (Fig. 27). Extragonadal spermatozoa reserves in Ram 359 were very low (Table 10) agreeing with the pre-slaughter seminal picture. There were no spermatozoa or elongated spermatids in testicular homogenates.

Histologically there was a marked decrease in the size of the seminiferous tubules, seminiferous tubule diameter being about a third less than the average of the control group (Table 10). The small seminiferous tubules were severely depleted of germ cells (compare Figs. 29, 30 with 31, 32). Primary spermatocytes were the most mature germ cell present in the majority of tubules. In the right testis a few tubules contained a small number of round spermatids (Fig. 32) while the left testis contained relatively more tubules with round spermatids and there were also more spermatids per tubule. This between testis variation was reflected in the spermatogenic score counts (Table 10). Apart from this small difference, seminiferous tubule cytology between areas and within sections was very similar; no cells beyond the round spermatid stage being seen in any of the sections (Table 10). In most tubules primary

spermatocyte numbers were reduced and they were scattered throughout the epithelium with cytoplasmic vacuoles often separating them (Fig. 32). In some tubules young and old primary spermatocytes appeared normal while others were in various states of degeneration. Some primary spermatocyte nuclei were smaller and darker staining than normal with a loss of definition (pyknosis); in other cells the nuclei were larger than normal with an apparent loss of ability to take up the basic stain (karyolysis) and in others the nuclear membrane appeared to have broken down and the chromatin was scattered as granules (karyorrhexis). In some cells the cytoplasm stained more deeply pink than normal and this was often associated with pycnotic nuclei. All tubules contained a single layer of cells around the basement membrane. Most of these were Sertoli cells with the occasional spermatogonium.

There was an apparent increase in the number of Leydig cells seen per microscopic field. However, when seminiferous tubule shrinkage was taken into consideration, there was probably no actual increase in the number of Leydig cells. Similarly, the other indicators of androgenic status of Ram 359 did not differ significantly from the control animals. Seminal plasma fructose concentration, seminal vesicle weight and seminal vesicle fructose content were similar to the average of the control group, (Table 10). When Ram 359 was placed in a pen with a ewe in oestrus, the ram immediately approached the ewe, licked and sniffed at the vulval region, elevated the head and retracted the upper lip (the Flehmen). Before actually mounting, the ram partially extruded the

penis with what appeared to be premounting thrusting movements. Reaction time with 3 different ewes on successive days was 2, 1 and 2 minutes respectively.

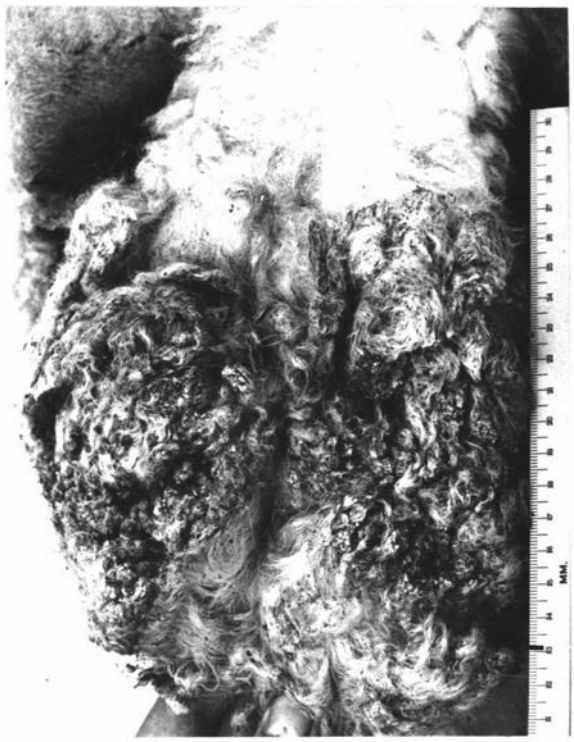
Table 10: SUMMARY OF THE REPRODUCTIVE PARAMETERS OF RAM 359 COMPARED WITH THE FIVE CONTROL RAMS

	Ram 359					Controls (Av. ± S.D.)
Body weight (kg)	58					69.4 ± 5.7
Testes	Left		Right			Left + Right/2
Testes weight (g)	94		84			194 ± 34
Testes sperm ($x10^9$)	0.0		0.0			19.57 ± 6.0
Extra gonadal spermatozoa ($x10^9$)	0.07		0.025			39.19 ± 12.65
Mean seminiferous tubule diameter (μ)	169		162			229 ± 12
Spermatogenic score count	Lt* Pr.	Lt Di.	Rt Pr.	Rt Di.	Total	Total
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	2	2	2	4	10	0
5	21	10	22	20	73	0
6	2	12	1	1	16	0
7	0	1	0	0	1	1
8	0	0	0	0	0	2
9	0	0	0	0	0	8
10	0	0	0	0	0	89
Mean score	5.1					9.9 ± 0.1
Seminal fructose (mg/100ml)	780 ± 70 (S.D.)					620 ± 314
Seminal vesicles weight (g)	9					12.24
Seminal vesicles fructose (mg)	111.6					133.4 ± 73.3
Mean reaction time (min)	1.7					2.6 ± 1.4

* Lt Pr., Left Testis Proximal; Lt Di., Left Testis Distal, etc.

Figure 25: Posterior view of the scrotum of Ram 359 on the 15.1.70. Almost all of the scrotum is covered in caked exudate 2 - 3 cm thick.
(One large division, 1 cm)

Figure 26: Spontaneous cure of the more proximal scrotal lesions of Ram 359 at the time of slaughter (23.3.70) showing caked exudate (E) being separated from a clinically normal skin (N) by approximately 1 cm of wool (W).
(One large division, 1 cm)



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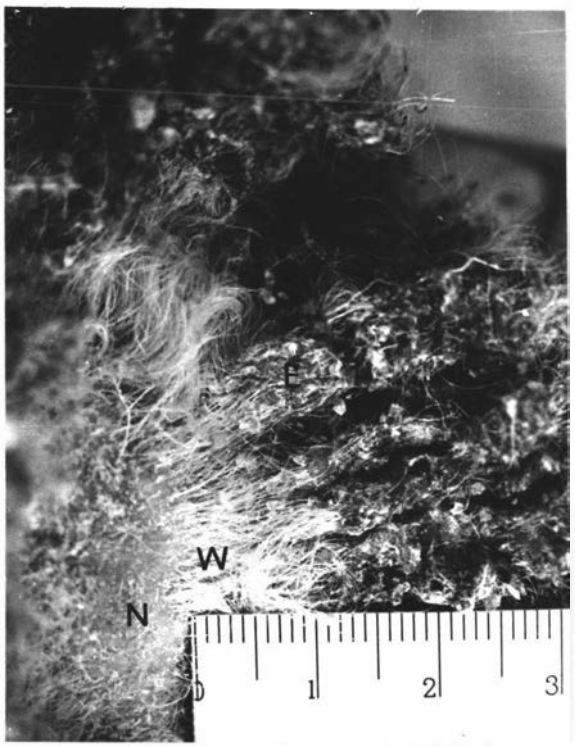

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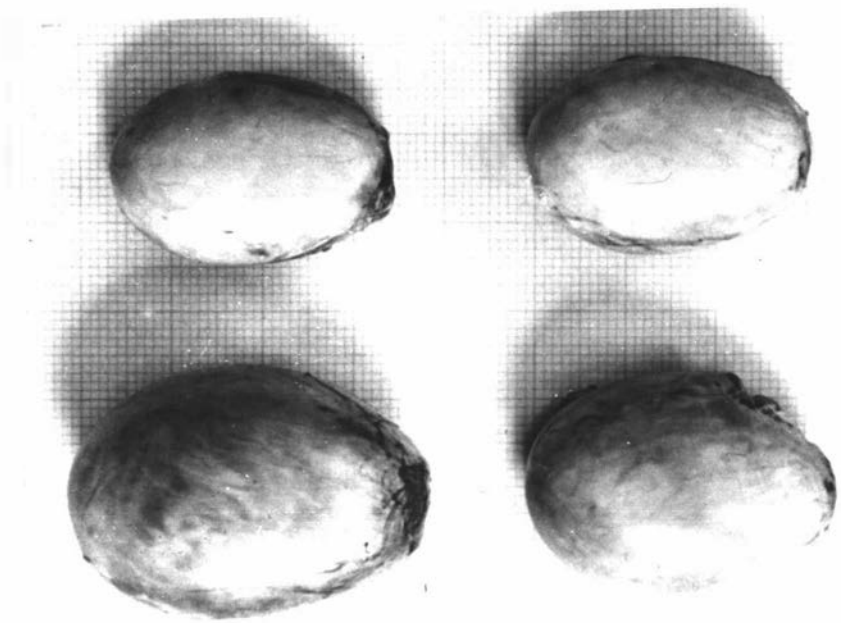
Figure 27: Testes of Ram 359 (upper) and control Ram 162 (lower).
(Large background square, 1" x 1")

Figure 28: Smear of degenerate semen sample collected from Ram 359 on the 6.12.69. The four spermatozoa shown are all morphologically abnormal.
(Mayer's haemalum and eosin).
(x1250)



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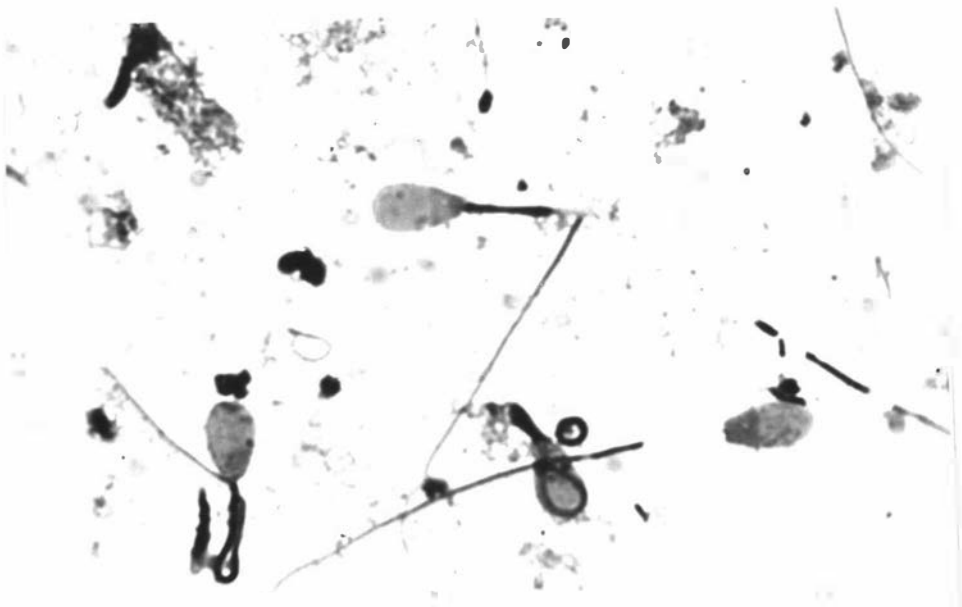

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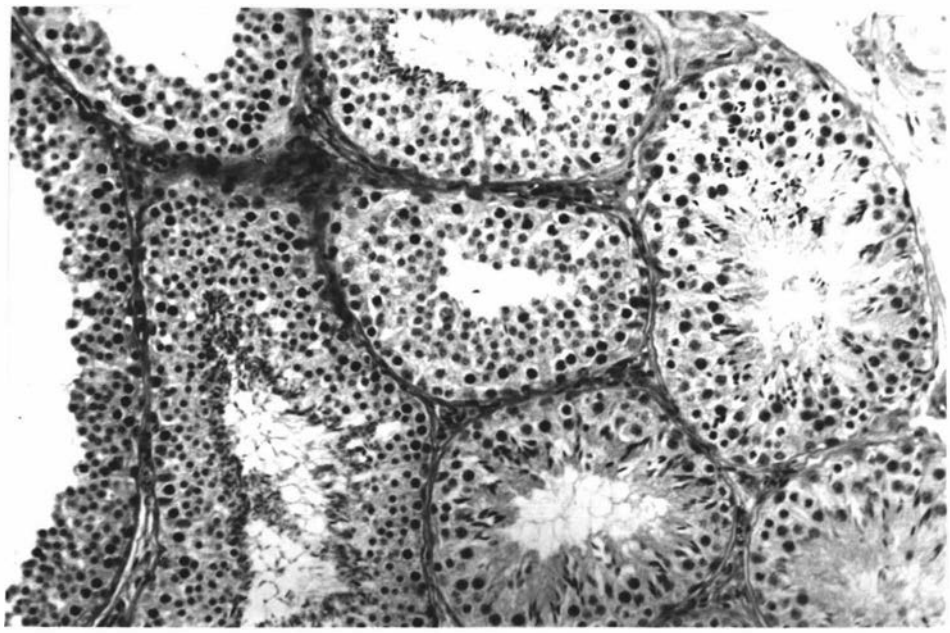
Figure 29: Section of normal testis (Control Ram 162).
(H. & E.)
(x180)

Figure 30: High power of above.
(H. & E.)
(x560)



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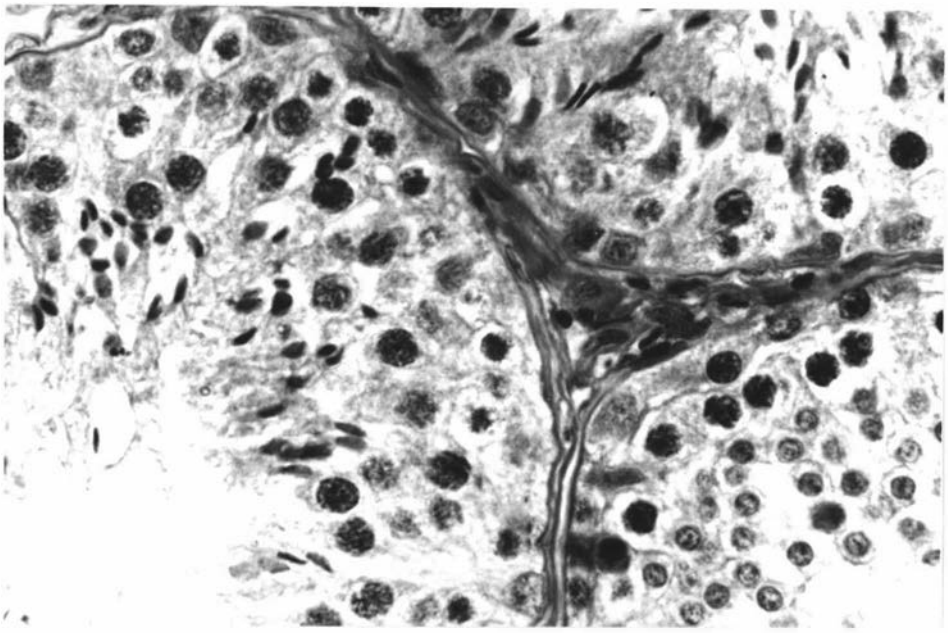

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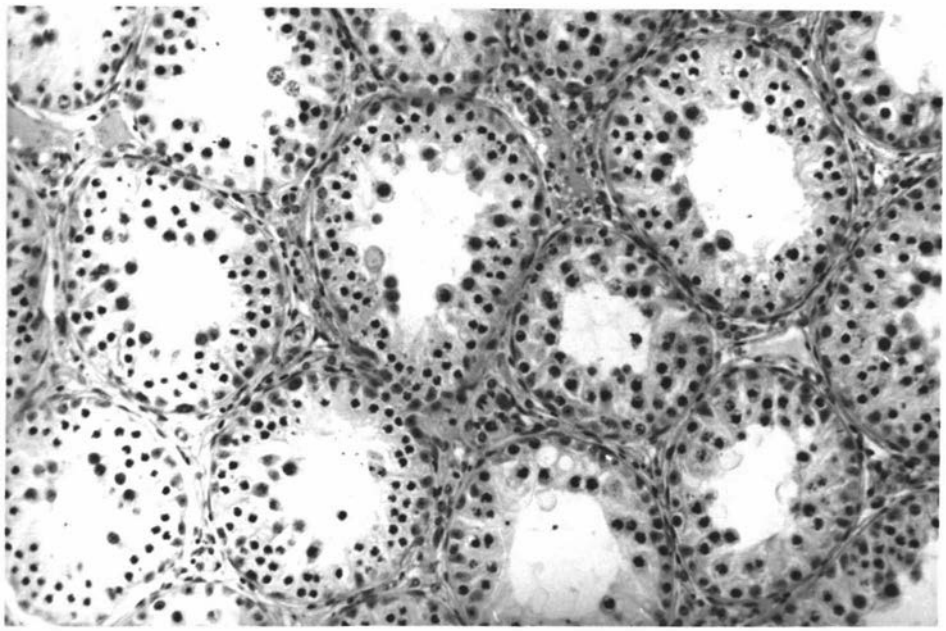

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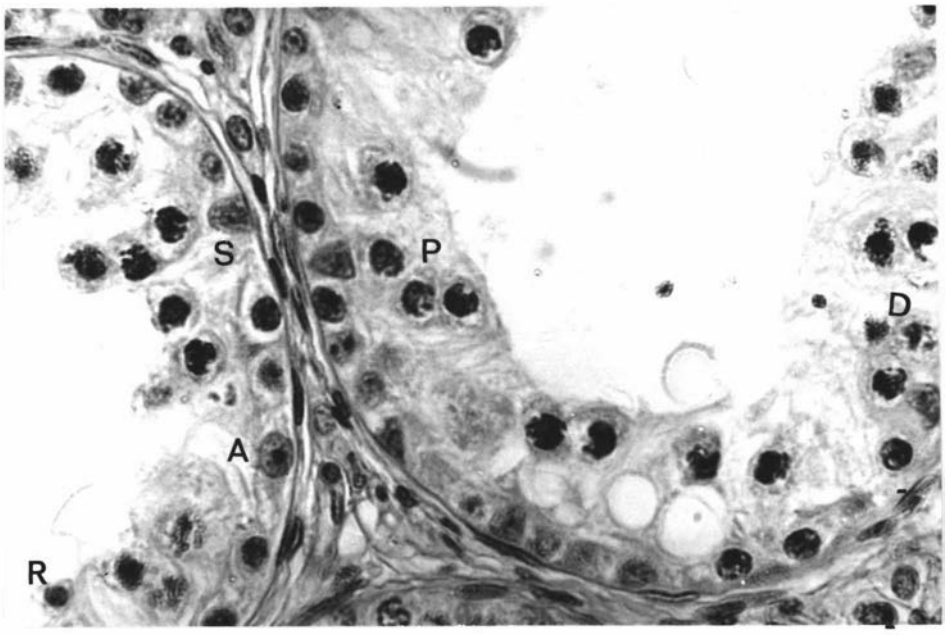

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Figure 31: Section of right proximal testis of Ram 359. Note atrophic tubules with depleted numbers of germ cells and no evidence of spermatogenesis.
(H. & E.)
(x180)

Figure 32: High power of above. The majority of the germ cells present are primary spermatocytes (P) many of which are degenerating (D). Round spermatids (R) are seen in the occasional tubule. Spermatogonia (A) and Sertoli cells (S) line the basement membrane.
(H. & E.)
(x560)

Case No. 2 (Ram 572)

Three rams with scrotal mange were detected in a flock of 65 fifteen month Perendale rams during a presale examination for genital soundness. Two of the rams had lesions classified as minimal and the other ram (Ram 572) had severe scrotal mange. Ram 572 had testes of moderate size and poor tone, while the rest of the flock had large testes of good tone. Ram 572 was classified as genitally unsound and was brought to the University on 6.12.69 and examined more closely.

The distal quarter of the scrotum was covered in active lesions with caked exudate up to 2 cm thick and there were scattered lesions over the rest of the distal half of the scrotum. Two C. bovis mites were isolated after an extensive microscopic examination of the scrotum. The lesions became progressively worse so that by the 13.2.70 eighty percent of the posterior scrotum and the lower half of the anterior scrotum was covered in crumbly-caked exudate, the exudate on the base being as much as 4 cm thick. At the time of slaughter (26.3.70) the lesions had not changed appreciably (Fig. 33).

The testes decreased in size by about 50% during the observation period and were classified as small with poor tone prior to slaughter (Fig. 34).

A semen sample collected on the 6.12.69 contained 1.2×10^8 spermatozoa in 1.6 ml of cloudy semen. Ten percent of the cells were motile and only a few of these showed progressive movement. Seventy eight percent of the spermatozoa

stained dead and 63% were morphologically abnormal. The morphological abnormalities consisted of: 14% tail defects (mainly bent tails); 4% mid-piece abnormalities (swollen mid-pieces); 10% proximal cytoplasmic droplets; 18% tailless heads and 17% pyriform heads (about half of these were also tailless).

A semen sample collected a week later contained less spermatozoa and there was a higher proportion (25%) of spermatozoa with pyriform heads; there were also a large number of cells in the sample that appeared to be microspermatozoa (Fig. 35). These cells had heads that varied from about 1/2 to 1/50 the size of a normal spermatozoa head and varied in shape from round to pyriform. Some of these cells appeared to be without nuclei, while most contained a small pycnotic nucleus. The tails of these microspermatozoa varied in length from about half to normal spermatozoa tail length. They were finer than the normal tails and there was no obvious mid-piece. No cells intermediate between the microspermatozoa and normal spermatozoa were observed. By the 20.12.69 only a few dead spermatozoa were ejaculated in a watery-milky semen sample that contained large amounts of debris. There were many round cells in the semen sample (Fig. 36) and these cells varied in size from about 1/2 to 5 times the size of a normal spermatozoa head; some contained a round nucleus while others stained a homogenous pink. Microspermatozoa were present in moderate numbers and there were also large numbers of eosinophilic filaments and some amorphous eosinophilic debris in the sample (Fig. 37). These fine filaments occasionally had a minute eosinophilic swelling at one end, and part

or all of the filaments were often coiled (Fig. 37). A further semen sample collected on the 29.12.69 contained very few spermatozoa, all of which were dead and decreased amounts of cellular debris. From this time to the time of slaughter the ram was virtually azoospermic and samples contained very small amounts of debris the semen picture being virtually identical to that seen in the latter ejaculates of Ram 359.

The scrotal skin of Ram 572 at slaughter was not appreciably thicker than that of the control ram (Ram 105) but felt more fibrous when cut with the postmortem knife. The scrotum, as in Ram 359, was non pendulous and there was no inflammatory reaction in the cavity of the tunica vaginalis, the testes or testicular capsule.

Ram 572 had the most severe testicular degeneration of the five rams in the experiment. This ram had the smallest testes, the smallest seminiferous tubules and the lowest spermatogenic mean score. Testicular histology was similar but even more uniform than the previous ram, 94% of the tubules being classified into categories 4 or 5 in the spermatogenic score counts (Table 11). No tubules contained germ cells beyond the primary spermatocyte stage and these were severely depleted in numbers (Figs. 38, 39). In a few tubules there was only a single layer of Sertoli cells and the occasional spermatogonium surrounding the basement membrane. Testicular and extragonadal spermatozoa counts were similar to the previous ram (Table 11).

There was an apparent but probably no real increase in the number of Leydig cells. The other

parameters of androgenic status of Ram 572 were similar to the average of the control rams (Table 11). Ram 572 was hesitant when first placed with a ewe in oestrus. The ram was apprehensive of people and surroundings; this probably being a breed characteristic. Once the ram became familiar with the experimental procedure he showed the normal signs of pre-copulatory behaviour and had a reaction time of 2, 2 and 1 minute respectively when placed with 3 ewes in natural oestrus on successive days 2 weeks before slaughter.

Table 11: SUMMARY OF THE REPRODUCTIVE PARAMETERS OF RAM 572 COMPARED WITH THE FIVE CONTROL RAMS

	Ram 572					Controls (Av. ± S.D.)
Body weight (kg)	65					69.4 ± 5.7
Testes	Left		Right			Left + Right/2
Testes weight (g)	68		73			194 ± 34
Testes sperm ($x10^9$)	0		0			19.57 ± 6.0
Extra gonadal spermatozoa ($x10^9$)	0.13		0.04			39.19 ± 12.65
Mean seminiferous tubule diameter (μ)	158		155			229 ± 12
Spermatogenic score count	Lt* Pr.	Lt Di.	Rt Pr.	Rt Di.	Total	Total
1	0	0	0	0	0	0
2	0	2	0	0	2	0
3	2	2	0	0	4	0
4	11	10	9	13	43	0
5	12	11	16	12	51	0
6	0	0	0	0	0	0
7	0	0	0	0	0	1
8	0	0	0	0	0	2
9	0	0	0	0	0	8
10	0	0	0	0	0	89
Mean score	4.4					9.9 ± 0.1
Seminal fructose (mg/100ml)	760 ± 120 (S.D.)					620 ± 314
Seminal vesicles weight (g)	11					12 ± 2.4
Seminal vesicles fructose (mg)	96.8					133.4 ± 73.3
Mean reaction time (min)	1.7					2.6 ± 1.4

* Lt Pr., Left Testis Proximal; Lt.Di., Left Testis Distal, etc.

Figure 33: Chorioptic lesions on the anterior aspect of the scrotum of Ram 572 at time of slaughter (26.3.70).
(One large division, 1 cm)

Figure 34: Testes of Ram 572 (upper) and control Ram 105 (lower).
(Large background square, 1" x 1")

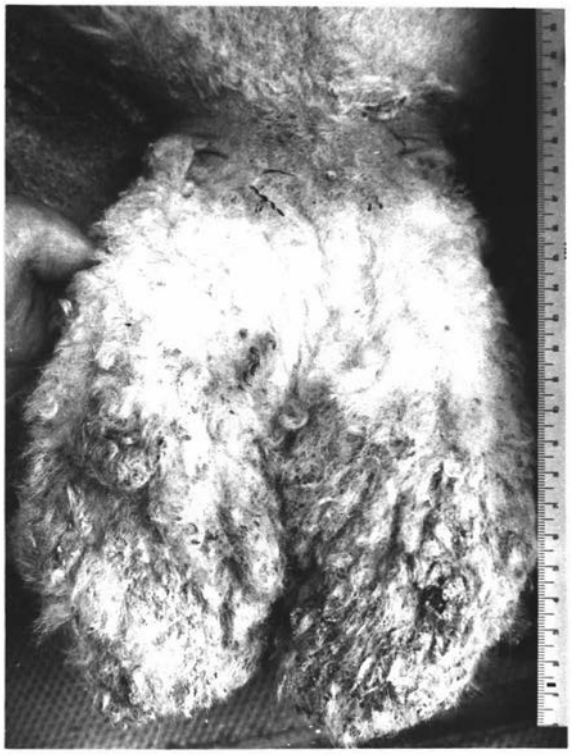

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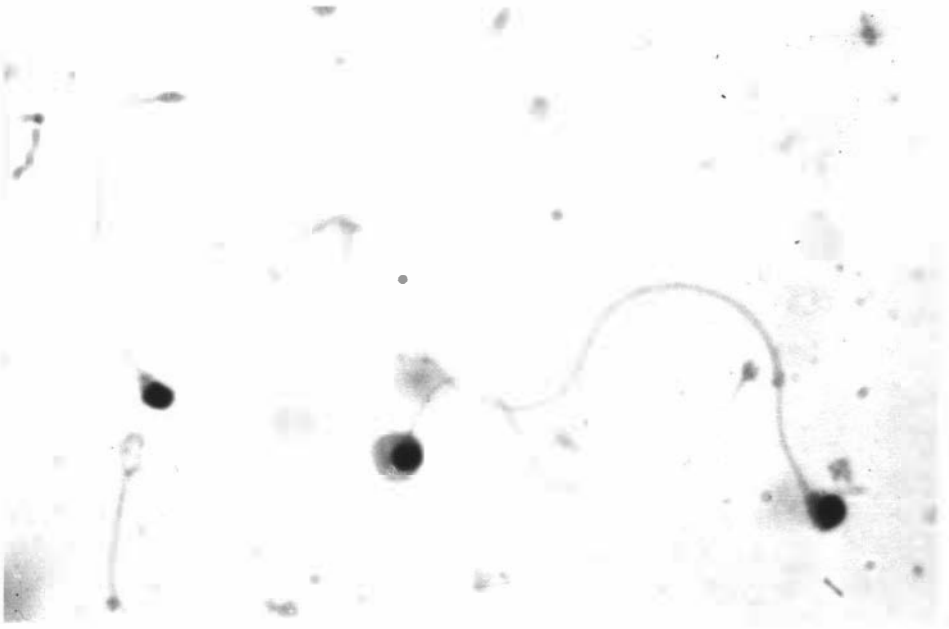
Figure 35: Microspermatozoa in semen sample collected from Ram 572 on 20.12.69. Note pycnotic spermatozoa heads and filamentous tails. Compare size of microspermatozoa with normal spermatozoon in Fig. 36 (below).
(Mayer's haemalum and eosin)
(x1250)

Figure 36: Round nucleated and non nucleated cells observed in degenerate semen sample collected from Ram 572 on the 20.12.69. Compare cell size with normal spermatozoon head.
(Mayer's haemalum and eosin)
(x1250)



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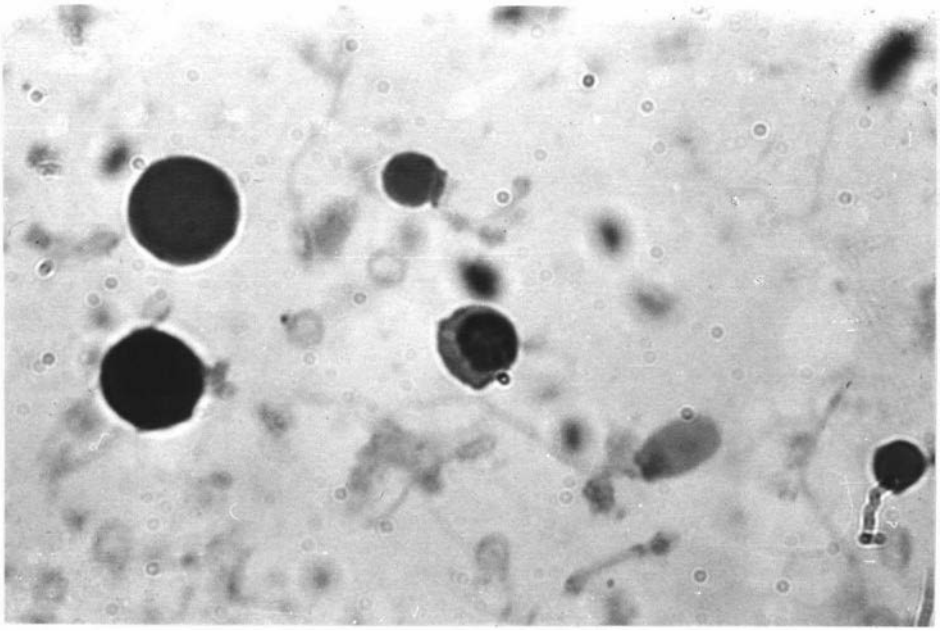

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Figure 37: Filamentous debris, both coiled and straight and amorphous eosinophilic debris in degenerate semen collected from Ram 572 on the 20.12.69. (Mayer's haemalum and eosin) (x1250)

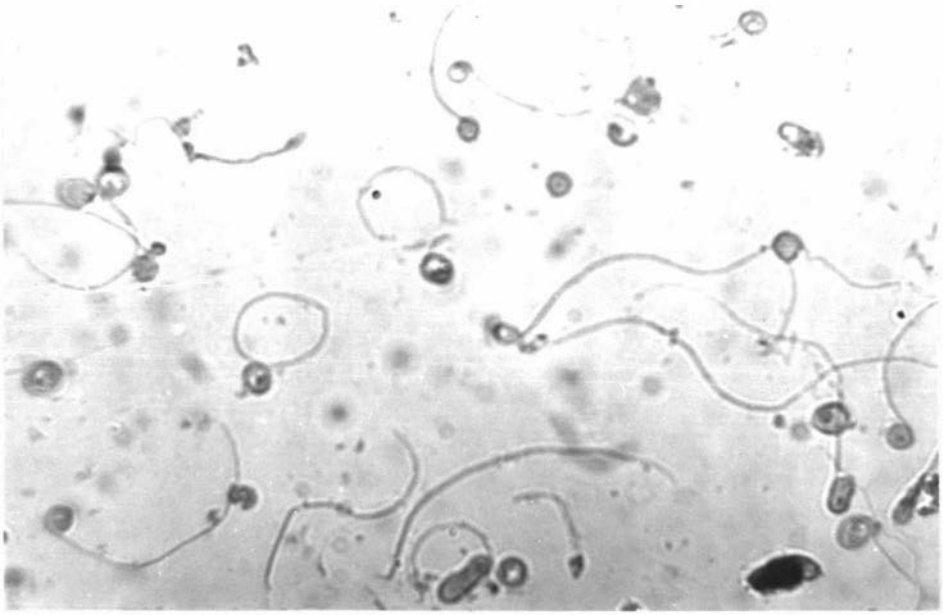

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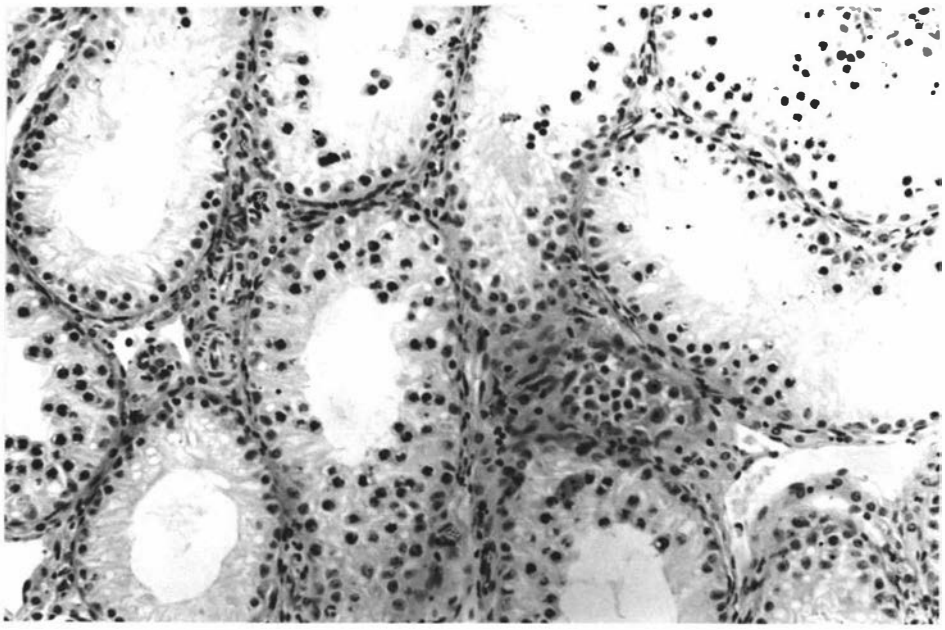
Figure 38: Section of right proximal testis of Ram 572. The seminiferous tubules are severely depleted of germ cells and the tubule lumens are virtually debris free. All tubules contain at least a basal layer of cells containing Sertoli cells and spermatogonia.
(H. & E.)
(x180)

Figure 39: High power of above, showing a few primary spermatocytes (P), many of which are degenerating (D), scattered throughout the epithelium.
(H. & E.)
(x560)



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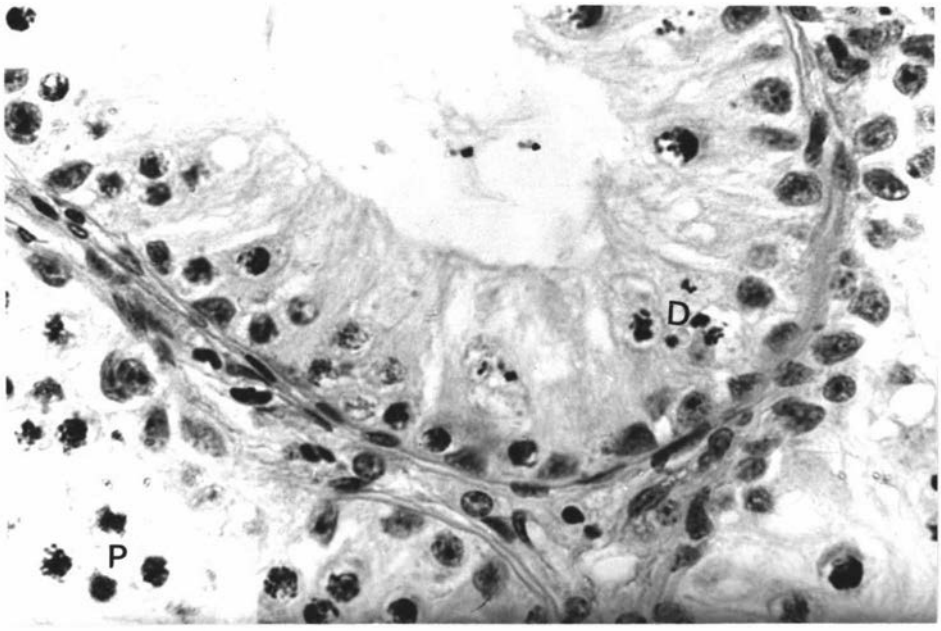

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Case Nos. 3, 4 and 5 (Rams 59, 91 and 92)

A minor outbreak of scrotal mange was detected during a routine examination of a flock of approximately 300 fifteen month old Romney rams on the 1.12.69. Most of the cases were classified as minimal and minor. However, 1 ram had severe and 2 had extreme scrotal mange. The 3 rams with extensive scrotal mange were rejected as genitally unsound and brought to the University with one genitally sound ram from the same flock on the 6.12.69.

Case No. 3 (Ram 59)

On arrival at the laboratory most of the distal two thirds of the scrotum of Ram 59 was covered in caked exudate up to 1 1/2 cm thick with active lesions beneath. Three mites (C. bovis) were isolated after an extensive microscopic examination of the scrotum. The testes were medium in size with moderate tone.

The proximal scrotal lesions cured spontaneously during the observation period, so that by the 13.2.70 only the distal half of the scrotum was affected. The lower quarter and the lower half of the left and right side of the anterior scrotum respectively were covered in active lesions with caked exudate up to 2 cm thick (Fig. 40) while the lower quarter of the posterior scrotum was covered in active lesions with exudate up to 1 1/2 cm thick. By the time of slaughter a further spontaneous cure of the more proximal lesions had occurred leaving only the base covered in chronic active lesions. The scrotal mange was classified as moderate at this stage.

There was an apparent relationship between

the more severe lesions on the right hand side of the scrotum and the size of the right testis. By the 12.2.70 there was a palpable difference between the two testes; the right testis being smaller than the left. At the time of slaughter the right and left testes were approximately one third and one half smaller respectively than the testes of the control ram and were of moderate tone.

The ram was practically azoospermic throughout December and most of January 1970 (Fig. 42). Towards the end of January increasing amounts of debris and morphologically abnormal dead spermatozoa were ejaculated (Fig. 43). By the 30.1.70, 7.3×10^6 dead spermatozoa were ejaculated and 80% of these were morphologically abnormal. The majority of the abnormals were primary abnormalities. There was present also in the sample, relatively large amounts of cellular and especially non cellular debris similar to that seen in the previous case (Ram 572). Just prior to slaughter relatively large amounts of debris and 1.6×10^7 dead spermatozoa, with morphological abnormalities similar to those seen on the 30.1.70, were collected on electrical stimulation.

The apparent association between lesion severity and spermatogenic activity was confirmed at postmortem. The right testis and extragonadal spermatozoa reserves were very low while the left testis and extragonadal spermatozoa storage structures had significantly more spermatozoa (Table 12). Differences between the two testes were also noted in the seminiferous tubule diameters and the spermatogenic score counts (Table 12). Histological sections from the

proximal and distal ends of the right testis were similar to that of Ram 572 (Case No. 2). (Compare Figs. 44, 45 with Figs. 38, 39). The seminiferous tubules in the left testis (Table 12) were larger and not so widely separated. Compared with the right testis, there was more variation in spermatogenic activity both between the positions within the left testis and between adjacent tubules within a section (compare Fig. 44 with Fig. 46; Table 12). Most tubules in the section taken from the distal end of the left testis and some of the tubules from the proximal section were similar histologically to the right testis (Table 12). However, most tubules in the proximal section contained many more germ cells in all stages of spermatogenesis. A few tubules contained the full complement of cells with normal cellular associations, while others had relatively normal numbers of cells but were disorganised so that the cellular associations were not clear. The majority of the tubules in the distal section from the left testis had tubular activity between these two extremes. The marked variation in spermatogenic activity in the left testis is reflected in the spermatogenic score count (Table 12).

Compared with the other sections and also compared with the first two cases (Ram 359 and 572), there was a relatively large amount of germinal debris in the lumen of the seminiferous tubules from the proximal section of the left testis. The tubule lumen debris was similar to that seen in semen smears prior to slaughter. Degenerating primary spermatocytes, degenerating spermatids, clumps of eosinophilic amorphous debris, non coiled filaments and the occasional

microspermatozoa were seen in the lumen of many tubules that contained relatively large numbers of germ cells.

Interstitial Leydig cells appeared in similar numbers to those in the control ram. Seminal fructose, seminal vesicle weight and seminal vesicle fructose were all slightly lower than the average of the five control rams. However, they were all within one standard deviation of the control group averages and none of the fructose estimates of Ram 59 differed significantly ($P>0.05$) from the control group. When placed with a ewe in oestrus, Ram 59 showed the normal premating behavioural pattern as described previously. However, the ram showed no partial extrusion of the penis, no pre-ejaculatory fluid dribbled from the preputial orifice and the ram made no attempt to mount ewes in oestrus.

Table 12: SUMMARY OF REPRODUCTIVE PARAMETERS OF RAM 59 COMPARED WITH THE FIVE CONTROL RAMS.

	Ram 59					Controls (Av. ± S.D.)
Body weight (kg)	74					69.4 ± 5.7
Testes	Left			Right		Left + Right/2
Testes weight (g)	135			100		194 ± 34
Testes sperm ($x10^9$)	1.43			0.12		19.57 ± 6.0
Extra gonadal spermatozoa ($x10^9$)	0.29			0.015		39.19 ± 12.65
Mean seminiferous tubule diameter (μ)	184			167		229 ± 12
Spermatogenic score count	Lt* Pr.	Lt Di.	Rt Pr.	Rt Di.	Total	Total
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	1	1	0	2	0
4	3	10	19	7	39	0
5	4	3	2	11	20	0
6	2	8	2	5	17	0
7	9	3	1	2	15	1
8	4	0	0	0	4	2
9	2	0	0	0	2	8
10	1	0	0	0	1	89
Mean score	5.3					9.9 ± 0.1
Seminal fructose (mg/100ml)	530 ± 120 (S.D.)					620 ± 314
Seminal vesicles weight (g)	10					12 ± 2.4
Seminal vesicles fructose (mg)	112.0					133.4 ± 73.3
Mean reaction time (min)	-					2.6 ± 1.4

* Lt Pr., Left Testis Proximal; Lt Di., Left testis distal, etc.

Figure 40: Chorioptic mange lesions on the anterior aspect of the scrotum of Ram 59 on the 13.2.70. Lower quarter and lower half of the left and right scrotum is covered in caked exudate about 1 1/2 cm thick. (One large division, 1 cm)

Figure 41: Testes of Ram 59 (upper) and control Ram 250 (lower).
(Large background square, 1" x 1").

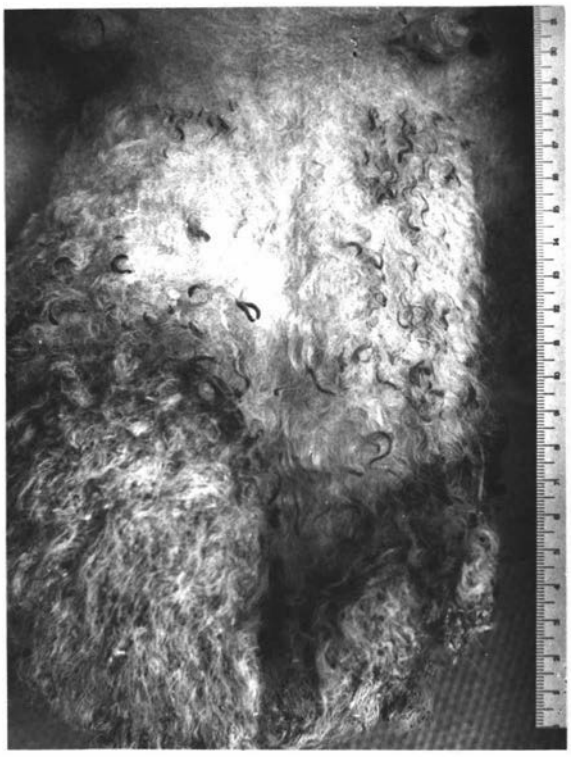

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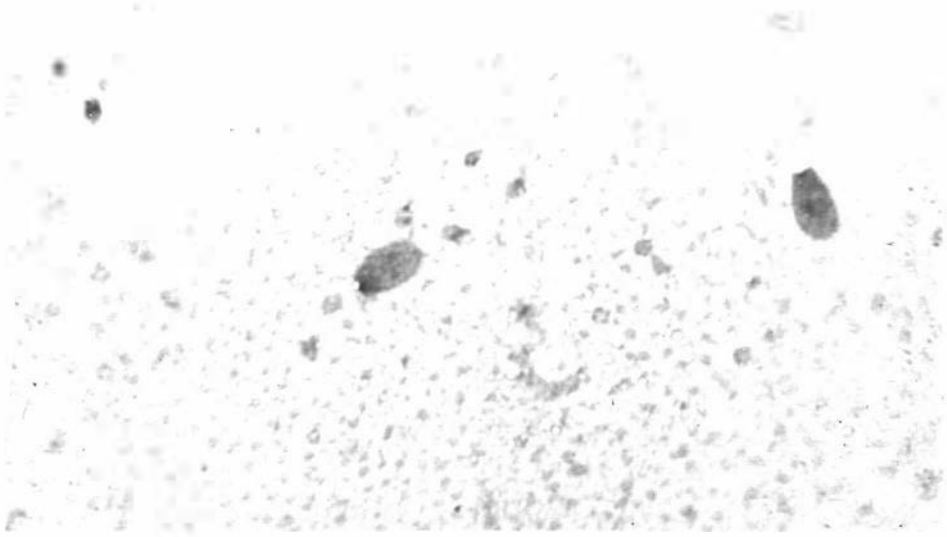
Figure 42: Tailless spermatozoa observed in the virtually azoospermic semen sample collected from Ram 59 on the 6.12.69. Note the normal morphology of both spermatozoa heads and the lack of seminal debris.
(Mayer's haemalum & eosin)
(x1250)

Figure 43: Initial signs of seminal regeneration in Ram 59 (30.1.70). A tailless spermatozoon with a pyriform narrow head (H), a spermatozoon with the mid-piece coiled in the tail (M) and a normal spermatozoon (N) are observed amongst large amounts of eosinophilic debris.
(Mayer's haemalum & eosin)
(x1250)



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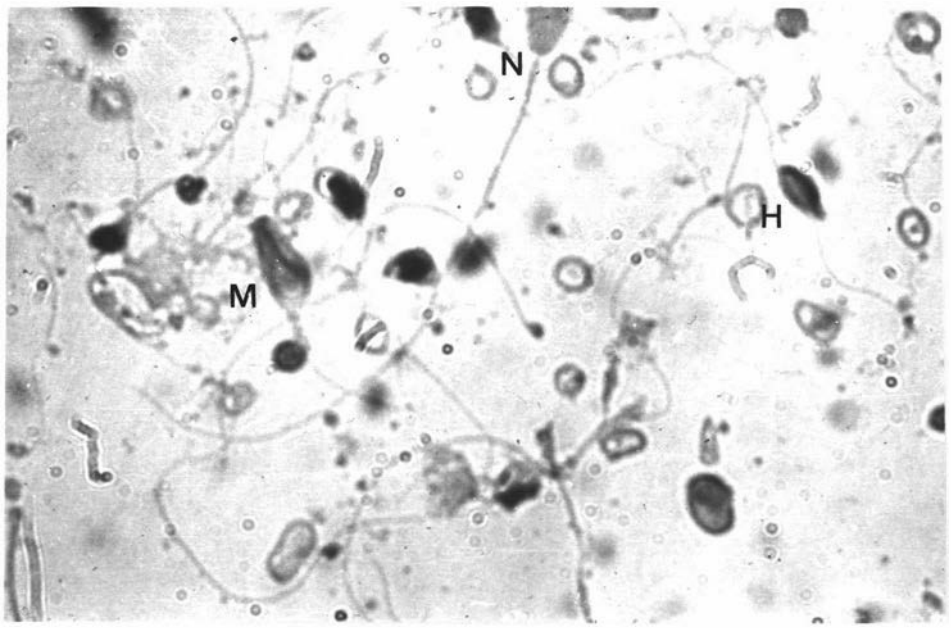

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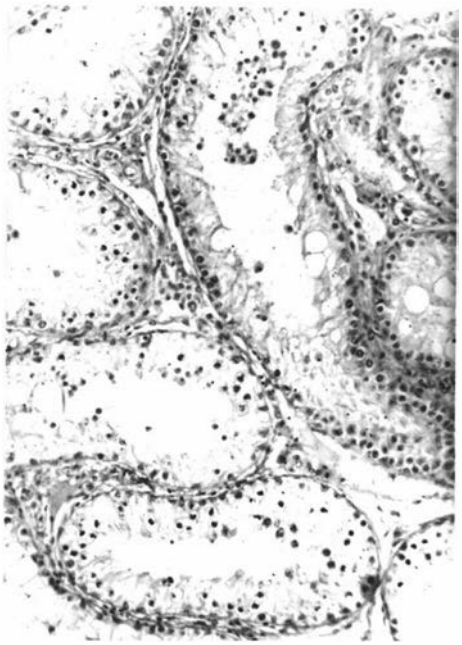
Figures 44 & 45: Section from right testis (distal) of Ram 59. All tubules are still very degenerate, none are undergoing complete spermatogenesis. Many of the primary spermatocytes (D) and round spermatids (R) are showing degenerative changes.
(H. & E.)
Fig. 44, x120
Fig. 45, x400

Figures 46 & 47: Section from left testis (proximal) of Ram 59 showing some tubules undergoing complete spermatogenesis (N) while other tubules are still very degenerate (D).
(H. & E.)
Fig. 46, x120
Fig. 47, x400



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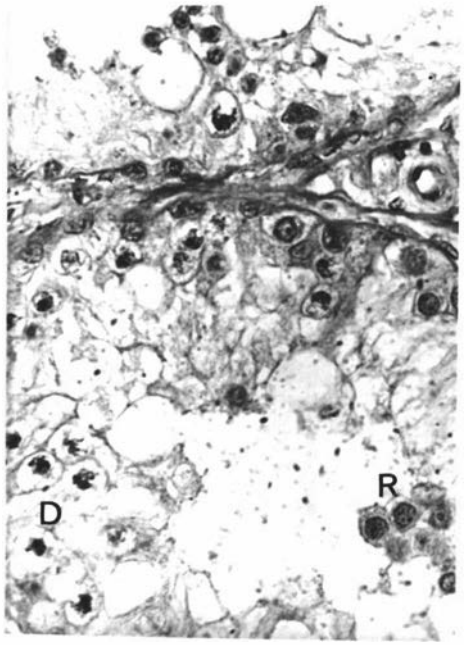

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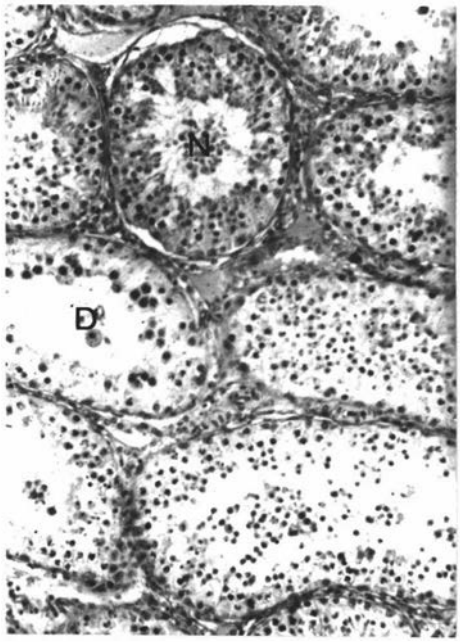

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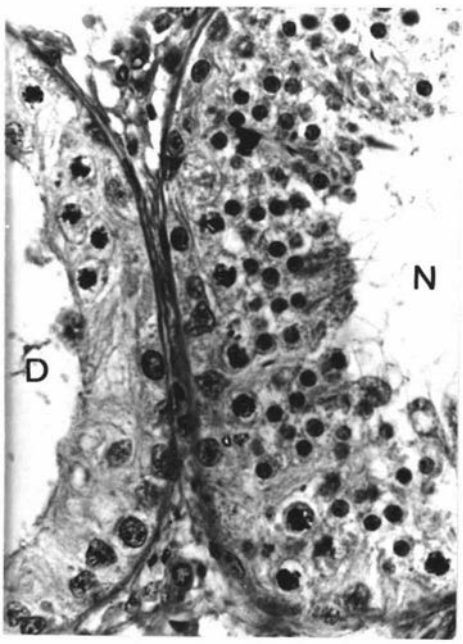

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Case No. 4 (Ram 92)

When this ram was first examined the distal three quarters of the scrotum was covered in caked exudate 1 - 1 1/2 cm thick (Fig. 48) and the testes were of moderate size and poor tone. No mites were seen on the scrotum but on the 15.1.70 a group of approximately 10 were observed. By the 12.2.70 the more proximal lesions had spontaneously cured leaving only the lower third covered in active lesions with caked exudate. The lesions, which were now classified as severe, did not change appreciably until 25.3.70 when Ram 92 and control Ram 107 were slaughtered.

The ram was severely oligospermic when first examined, 4.7×10^5 dead spermatozoa being collected in the first ejaculate. Spermatozoa numbers decreased even further so that just prior to slaughter only the occasional dead spermatozoon was observed in semen smears thickly made. Morphological abnormalities were similar to those previously described in severely oligospermic rams.

At postmortem the base of the scrotum was slightly thickened but the rest was relatively normal. The cavity of the tunica vaginalis, the testes and the testicular capsule showed no signs of involvement in the inflammatory process. The testes of Ram 92 were much smaller than those of control Ram 107 (Fig. 49).

Testicular and epididymal spermatozoa counts of Ram 92 were similar to those of Ram 359 and 572 (compare spermatozoa counts in Table 13 with those in Tables 10, 11). Seminiferous tubule cytology of Ram 92 was also very similar to that described for Ram 359 (compare Figs. 50, 51 with

Figs. 31, 32) except there were larger numbers of primary spermatocytes and round spermatids in the testes of Ram 92 (compare spermatogenic score counts in Table 13 with those in Table 10).

There was no increase in the absolute number of Leydig cells while seminal fructose, seminal vesicle weight and seminal vesicle fructose were the same or lower than in the other four rams with scrotal mange. However, none of the parameters differed significantly from the control group ($p>0.05$). Reaction time for the three successive days was 1, 5 and 9 minutes respectively. On the first day the ram showed vigorous sexual behaviour and copulated within one minute. On introduction to the second ewe Ram 92 appeared very excited in the initial stages. It kicked viciously at the ewe with the front feet, bunted the ewe and then turned away with an apparent loss of interest. After two more approaches the ram mounted the ewe and served with the characteristic ejaculatory thrust. On the third day a similar procedure was repeated with relatively long intervals when the ram showed no interest in the ewe.

Table 13: SUMMARY OF REPRODUCTIVE PARAMETERS OF RAM 92 COMPARED WITH THE FIVE CONTROL RAMS

	Ram 92					Controls (Av. ± S.D.)
Body weight (kg)	62					69.4 ± 5.7
Testes	Left		Right			Left + Right/2
Testes weight (g)	91		91			194 ± 34
Testes sperm ($x10^9$)	0.0		0.0			19.57 ± 6.0
Extra gonadal spermatozoa ($x10^9$)	0.095		0.013			39.19 ± 12.65
Mean seminiferous tubule diameter (μ)	171		169			229 ± 12
Spermatogenic score count	Lt* Pr.	Lt Di.	Rt Pr.	Rt Di.	Total	Total
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	1	0	1	0
5	14	11	10	13	48	0
6	9	11	13	8	41	0
7	2	3	1	4	10	1
8	0	0	0	0	0	2
9	0	0	0	0	0	8
10	0	0	0	0	0	89
Mean score	5.6					9.9 ± 0.1
Seminal fructose (mg/100ml)	180 ± 70 (S.D.)					620 ± 314
Seminal vesicles weight (g)	9					12 ± 2.4
Seminal vesicles fructose (mg)	61.2					133.4 ± 73.3
Mean reaction time (min)	5.0					2.6 ± 1.4

* Lt Pr., Left Testis Proximal; Lt Di., Left Testis Distal, etc.

Figure 48: Anterior view of the scrotum of Ram 92 on 6.12.69. The distal three quarters of the scrotum is covered in exudate 1 - 1 1/2 cm thick.

Figure 49: Testes of control Ram 107 (upper) and Ram 92 (lower).
(Large background square, 1" x 1").

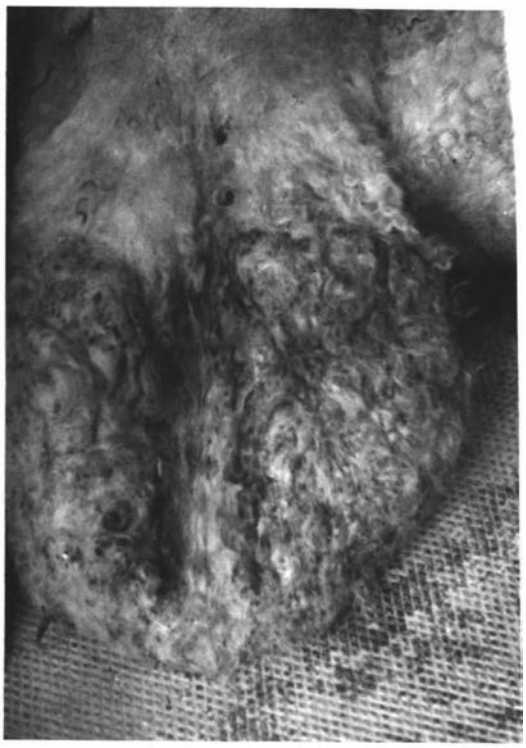

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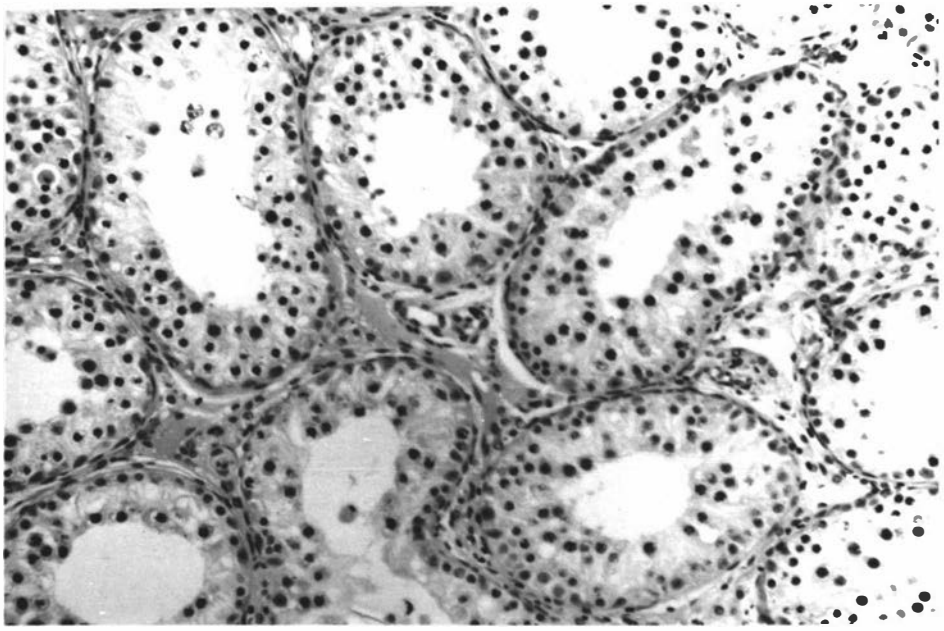
Figure 50: Section from left testis (proximal) of Ram 92 showing atrophic seminiferous tubules with little debris in the lumen.
(H. & E.)
(x180)

Figure 51: High power of above section. Primary spermatocytes, many of which are degenerating (D), are scattered throughout the epithelium. Small numbers of round spermatids (R) are seen in this tubule and numberous spermatogonia (A) line the basement membrane.
(H. & E.)
(x560)



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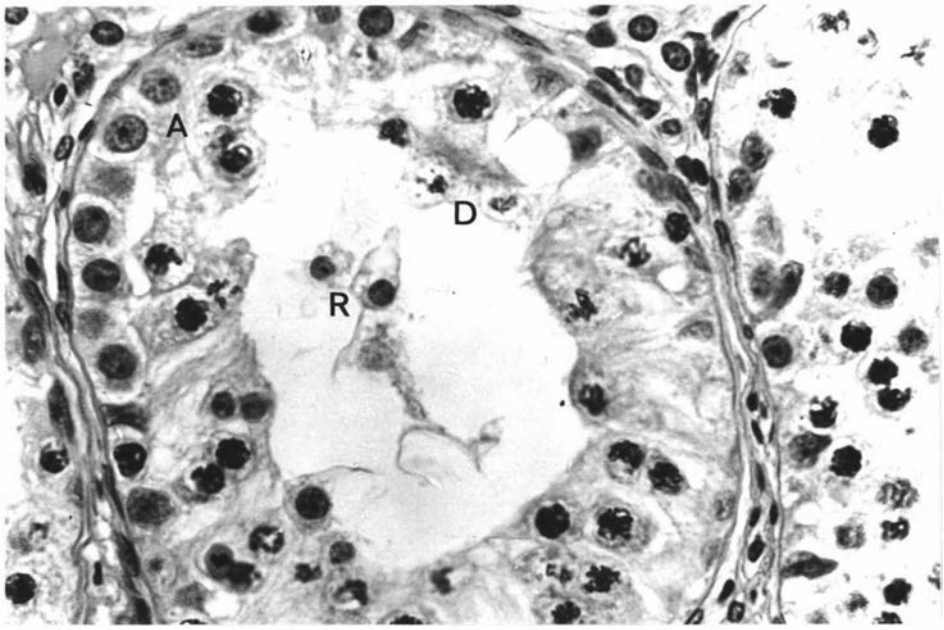

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Case No. 5 (Ram 91)

When first examined the distal two thirds of the scrotum of Ram 91 was covered in exudate up to 2 cm thick (Fig. 52) and the testes were moderate in size with poor tone. No mites were seen in the region of thick caked exudate, but 100 - 1,000 were observed mainly in groups at the junction between the scrotal lesions and the normal skin. A wool break had occurred sometime previously to the initial examination so that the matted exudate covering the distal quarter was peeling off leaving small active chorioptic scabs up to 1/2 cm thick on parts of the distal quarter of the scrotum (Fig. 53).

By the end of December the wool break had extended proximally and the old caked exudate was peeled off leaving a band of caked exudate about 2 cm wide between third and half way down the scrotum. The active lesions distal to the 2 cm band of caked exudate slowly regressed and by the 13.2.70 only about one tenth of the distal half of the scrotum was covered in scattered crumbly exudate with small active lesions. By the time of slaughter the distal half of the scrotum was virtually completely cured and wool had regrown covering the previously bare area. The 2 cm band of chronically active lesions had regressed to the stage where scrotal mange was classified as minimal. There was a marked increase in size of the testes over the last few weeks so that testes size was classified as large and tone moderate prior to slaughter.

On arrival at the laboratory Ram 91 was practically azoospermic. The ram continued to

produce clear to cloudy semen samples which contained a few dead spermatozoa and a very small amount of debris, until the end of February (Fig. 54). However, the last semen sample obtained (19.3.70) showed definite signs of seminal regeneration (Fig. 55). One ml of semen was collected which contained 3.3×10^7 spermatozoa, 5% were progressively motile and 12% stained live. Only 10% of the spermatozoa were classified as morphologically normal. A typical range of the abnormalities observed is shown in Fig. 55.

The testicular regeneration suggested by preslaughter changes in testes size and semen quality was confirmed at postmortem. This was reflected in both testicular and extragonadal spermatozoa reserves (Table 14) and testicular histology (Figs. 56, 57). Testis size and seminiferous tubule diameter were intermediate between those of rams with severe testicular atrophy and those of control rams. The spermatogenic score count shows clearly the considerable variation in testicular cytology between both the four areas sampled and between tubules within a section (Table 14). Most tubules in the section from the proximal portion of the left testis were undergoing complete spermatogenesis (Figs. 56, 57) while in the distal section many tubules only contained a small number of primary spermatocytes scattered throughout a vacuolated epithelium (Figs. 58, 59). In some tubules in the distal section all germ cell types were present in normal cellular associations while in others there was only a single basal layer of cells (Table 14). The histological picture from both the proximal and distal sections of the right testis were

similar, being intermediate between the cytological picture of the two areas sampled from the left testis (Table 14; Figs. 60, 61).

Leydig cell numbers and the histology of the seminal vesicles appeared similar to those of control animals. Seminal plasma fructose concentration, seminal vesicle weight and seminal vesicle fructose were all higher than those of the previous rams with scrotal mange although none of the parameters differed significantly from the control animals ($p>0.05$). Ram 91 showed typical behavioural signs with a ewe in oestrus and had a reaction time of 2, 3 and 2 minutes respectively when placed on successive days with three different ewes.

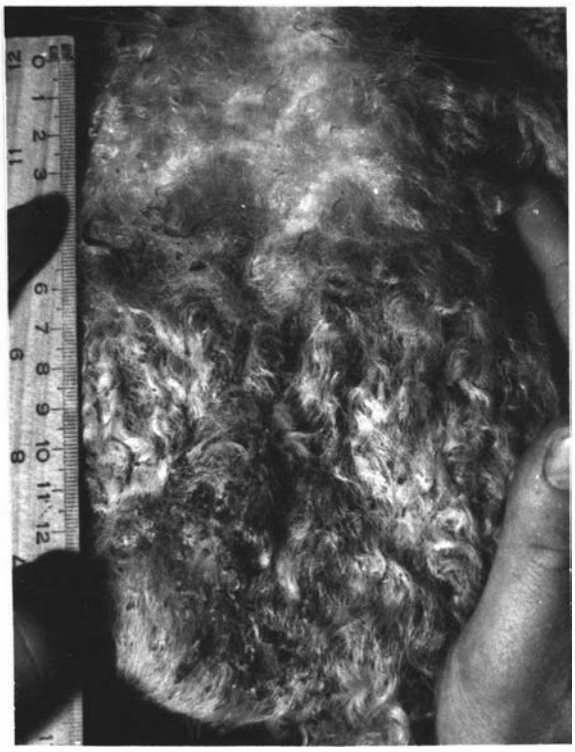
Table 14: SUMMARY OF THE REPRODUCTIVE PARAMETERS OF RAM 91 COMPARED WITH THE FIVE CONTROL RAMS

	Ram 91					Controls (Av. ± S.D.)
Body weight (kg)	74					69.4 ± 5.7
Testes	Left		Right			Left + Right/2
Testes weight (g)	141		136			194 ± 34
Testes sperm ($x10^9$)	2.99		2.85			19.57 ± 6.0
Extragonadal sperm ($x10^9$)	2.99		1.75			39.19 ± 12.65
Mean seminiferous tubule diameter (μ)	189		182			229 ± 12
Spermatogenic score count	Lt* Pr.	Lt Di.	Rt Pr.	Rt Di.	Total	Total
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	1	0	0	1	0
4	0	5	0	1	6	0
5	0	0	0	0	0	0
6	0	6	1	2	9	0
7	1	6	6	2	15	1
8	1	4	2	3	10	2
9	14	1	11	12	38	8
10	9	2	5	5	21	89
Mean score	8.2					9.9 ± 0.1
Seminal fructose (mg/100ml)	820 ± 80 (S.D.)					620 ± 314
Seminal vesicles weight (g)	17					12 ± 2.4
Seminal vesicles fructose (mg)	204					133.4 ± 73.3
Mean Reaction Time (min)	2.3					2.6 ± 1.4

* Lt Pr., Left Testis Proximal; Lt Di., Left Testis Distal, etc.

Figure 52: Anterior view of the scrotum of Ram 91 when first examined on the 6.12.69. (One large division 1 cm)

Figure 53: View of the scrotum of Ram 91 (6.12.69) showing the caked exudate (C) peeling off the base (B).
(One large division, 1 cm)



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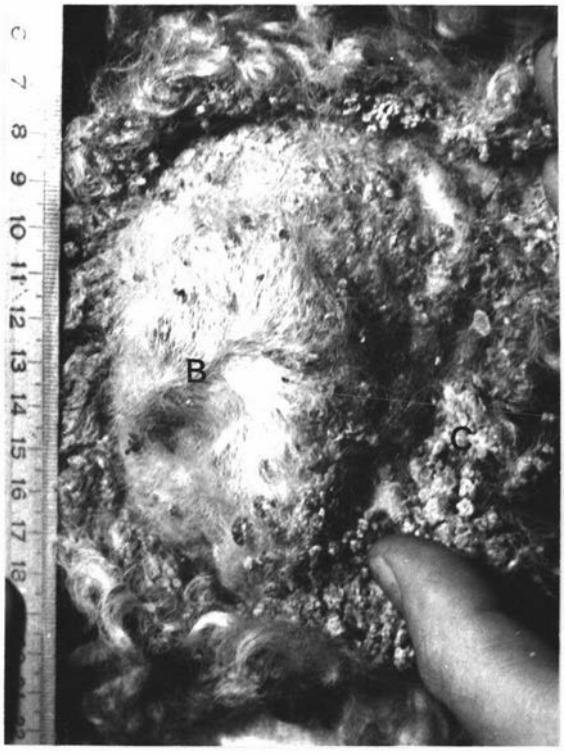

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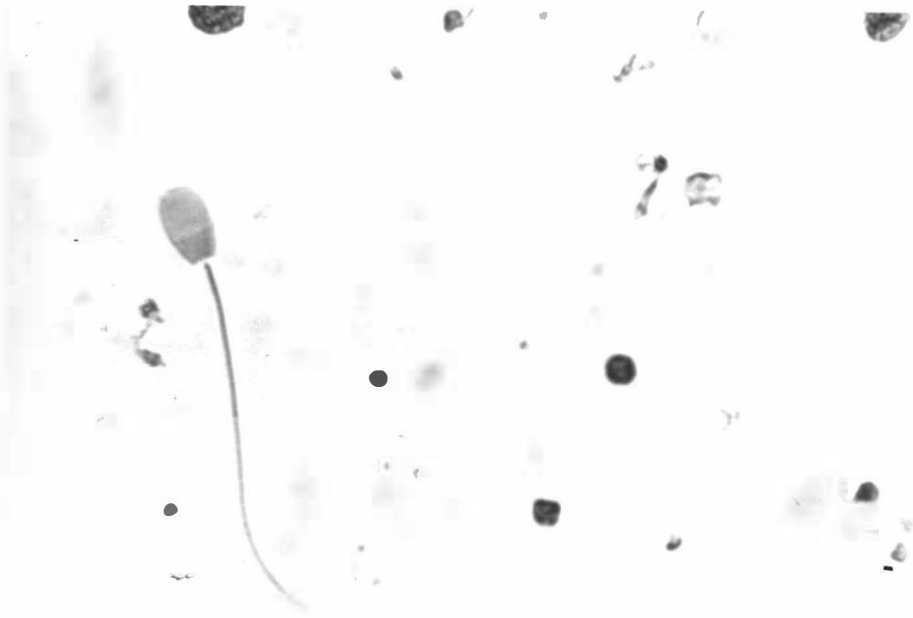
Figure 54: Morphologically normal spermatozoon and a small amount of debris observed in a semen smear when Ram 91 was virtually azoospermic (6.12.69).
(Mayer's haemalum & eosin)
(x 1250)

Figure 55: Semen smear of Ram 91 (19.3.70) showing signs of seminal regeneration. Most of the spermatozoa are morphologically abnormal. Note pyriform heads, tailless head, abaxial attachment, midpiece coiled in head, broken and thickened mid-piece and filiform tail.
(Mayer's haemalum & eosin)
(x 1250)



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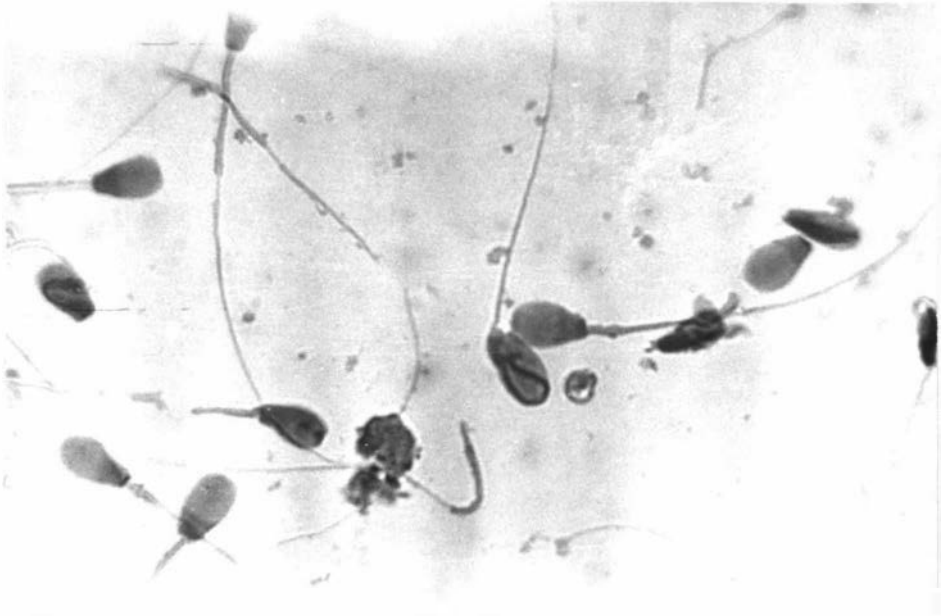

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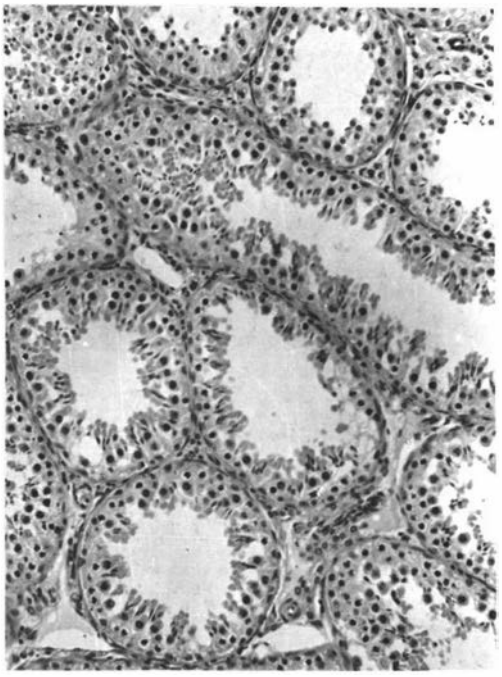
Figures 56 & 57: Section from left testis (proximal) of Ram 91 with almost all tubules showing complete spermatogenesis and normal cellular associations. (H. & E.)
Fig. 56, x120
Fig. 57, x400

Figures 58 & 59: Section from left testis (distal) of Ram 91. In contrast to the above (Figs. 56, 57) all tubules are severely depleted of germ cells, primary spermatocytes (P) being the most mature germ cell present.
(H. & E.)
Fig. 58, x120
Fig. 59, x400



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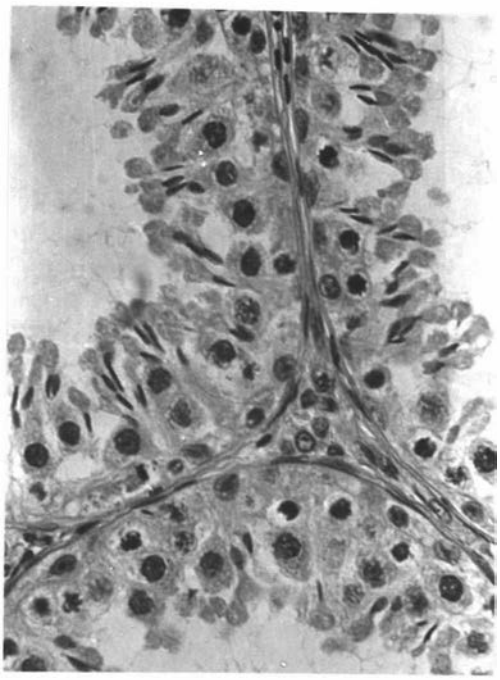

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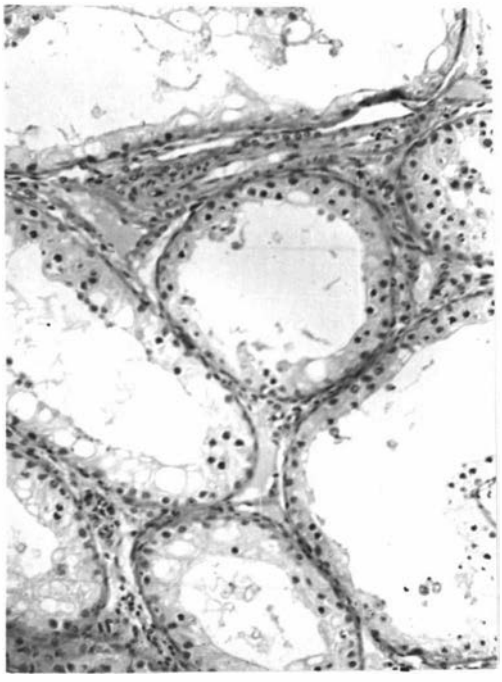

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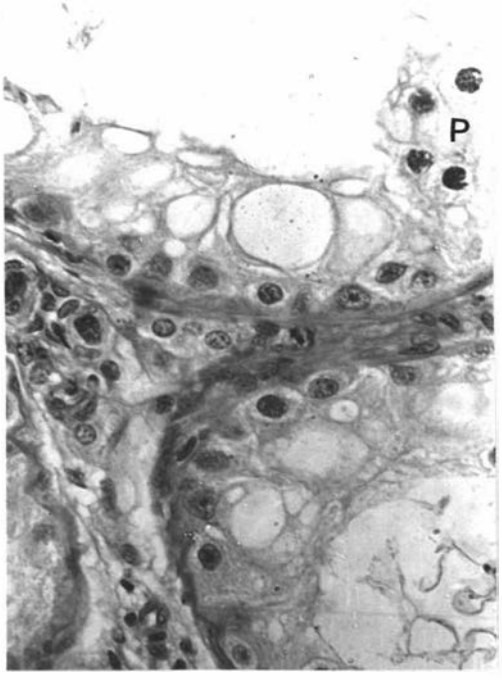

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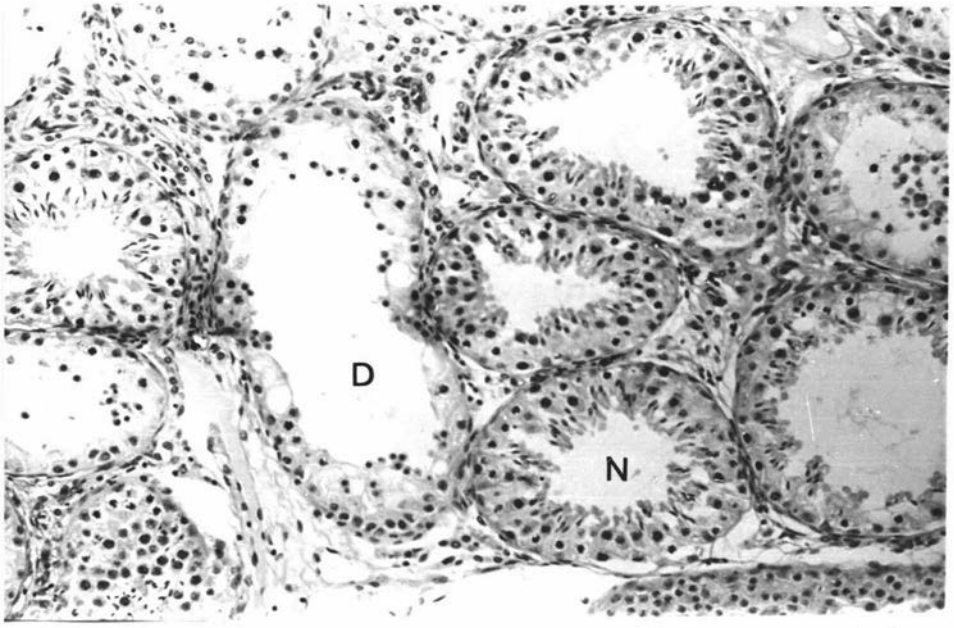
Figures 60 & 61: Section from the right testis (proximal) of Ram 91 with some seminiferous tubules showing complete spermatogenesis and normal cellular associations (N), dispersed amongst tubules with little sign of spermatogenic activity (D).
(H. & E.)
Fig. 60, x180
Fig. 61, x560



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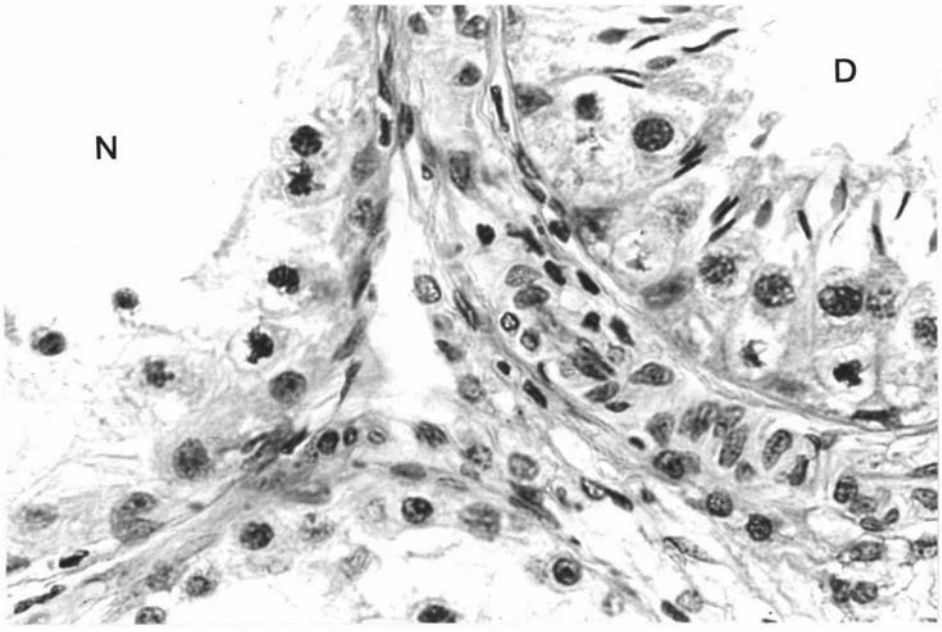

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Data from the Cheviot Rams

From approximately 1,500 fifteen month old Cheviot rams examined for genital soundness by a local veterinarian 4 with extensive scrotal mange were rejected as unsound. The 4 rams with scrotal mange and 4 control rams from the same flock were brought to the University on the 16.11.70 and run on pasture until the 12.12.70 when they were slaughtered.

A provisional diagnosis of chorioptic mange was confirmed by observing 1 - 10 C. bovis mites on the scrota of 3 of the rams. The crumbly-caked scrotal exudate was up to 3 cm thick and the lesions beneath were in most areas active. However, some of the lesions beneath the scrotal exudate of Ram 23 were inactive. There was very little thickening of the scrota of the rams and none of the scrota appeared pendulous. Rams 23 and 24 had scrotal mange classified as severe while Rams 21 and 27 had extreme scrotal mange.

On the initial examination (16.11.70) the control rams had uniformally large testes of good tone and all produced at least 0.6 ml of creamy semen with an average semen quality index of twenty six.

Rams 21 and 23 had testes of medium size with moderate tone while Rams 24 and 27 had small testes with poor tone. All of the rams with scrotal mange produced only cloudy semen samples that were severely oligospermic (less than 1×10^6 spermatozoa per ml of semen). The few spermatozoa that were seen were non motile and stained with eosin. A relatively high proportion of these

spermatozoa were tailless. However the majority of the heads of these tailless spermatozoa and many of the remaining spermatozoa were morphologically normal. The general semen picture of individual rams remained similar throughout the observation period, the last semen assessment being similar to the first.

At the time of slaughter (12.12.70) lesion severity had changed appreciably in only one ram. Some of the lesions of Ram 23 had undergone a spontaneous cure so that lesion classification changed from severe to minor.

Sections for histology and histochemical studies were taken from the testes of the eight rams at slaughter. Rams 21, 24 and 27 all had severe testicular degeneration, a marked reduction in seminiferous tubule diameter (Table 15) and widely separated tubules. The testicular histology of these three rams was uniform, no tubule contained germ cells beyond the primary spermatocyte stage. Figures 62 - 67 clearly show that the seminiferous tubule histology and cytology of these three rams was almost identical to that previously described in rams with severe testicular atrophy associated with extensive scrotal mange.

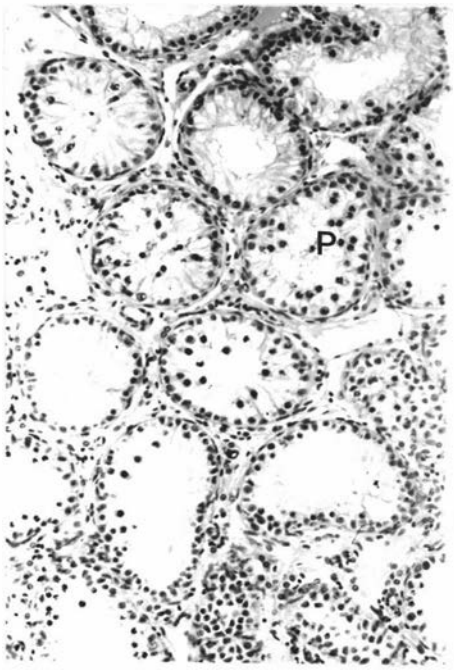
In contrast to these three rams, Ram 23 showed signs of testicular regeneration (Figs. 68, 69). The seminiferous tubules were larger than those of the other three rams and showed more cytological variation. The majority of the tubules in the sections from the left testis and the distal section of the right testis contained round spermatids and some tubules contained

Table 15: BODY WEIGHT, TESTES WEIGHT, MEAN SEMINIFEROUS TUBULE DIAMETER (M.S.T.D.) AND SPERMATOGENIC MEAN SCORE OF EIGHT FIFTEEN MONTH CHEVIOT RAMS

	RAMS WITH EXTENSIVE SCROTAL MANGE				CONTROL RAMS			
Ram No.	21	23	24	27	22	25	26	28
Body weight (kg)	60	51	47	42	50	40	47	46
Group mean		50				46		
Testes weight (g)								
Left	94	92	60	65	132	164	125	287
Right	101	78	70	62	148	174	125	287
Group mean		78				180		
M.S.T.D. (μ)	143	172	137	151	194	203	218	215
Group mean		151				208		
Spermatogenic mean score	4.4	7.2	3.9	4.5	9.9	10.0	9.9	9.7
Group mean		5.0				9.9		

Figures 62 & 63: Section from right (proximal) testis of Ram 21. All seminiferous tubules are shrunken and the most mature germ cells present are primary spermatocytes (P), many of which are degenerating (D). Many tubules have only a single basal layer consisting of Sertoli cells (S) and spermatogonia (A).
(H. & E.)
Fig. 62, x120
Fig. 63, x400

Figures 64 & 65: Section from right (distal) testis of Ram 24. Histologically this section is indistinguishable from Ram 21 (compare Figs. 64, 65 with Figs. 62, 63).
(H. & E.)
Fig. 64, x120
Fig. 65, x400



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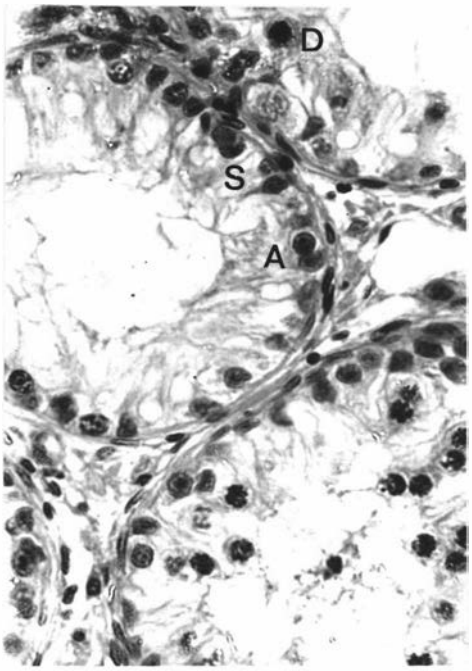

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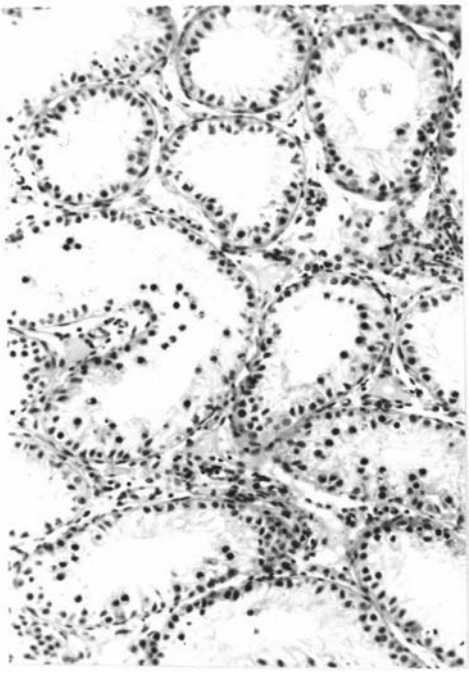

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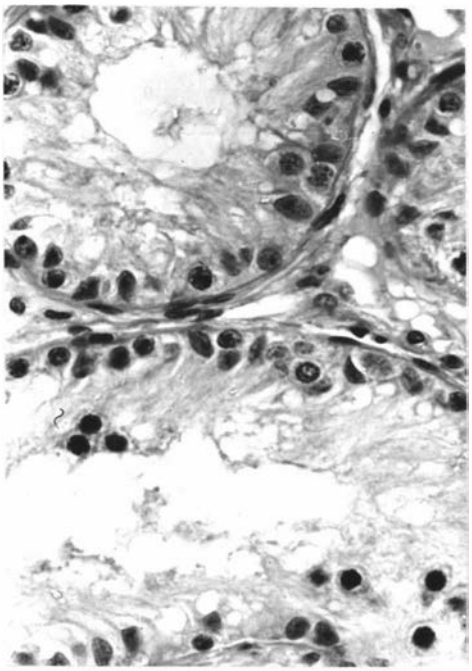

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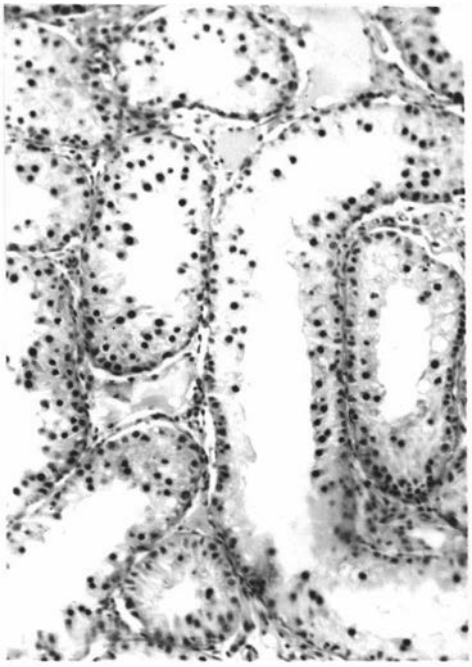
Figures 66 & 67: Section from right testis (distal) of Ram 27. Except for more primary spermatocytes (P), the section is similar histologically to Rams 21 and 24 (compare Figs. 66, 67 with Figs. 62-65).
(H. & E.)
Fig. 66, x120
Fig. 67, x400

Figures 68 & 69: Section from left (proximal) testis of Ram 23. Most tubules contain large numbers of round spermatids (R) and some tubules a few elongating spermatids (E). Note the pycnotic round spermatids (RR).
(H. & E.)
Fig. 68, x120
Fig. 69, x400



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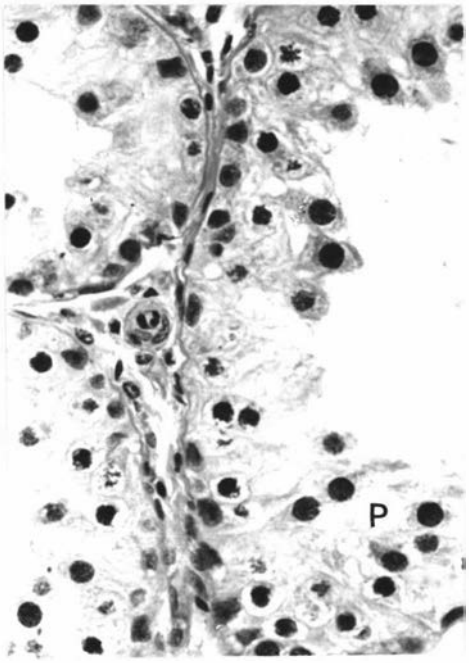

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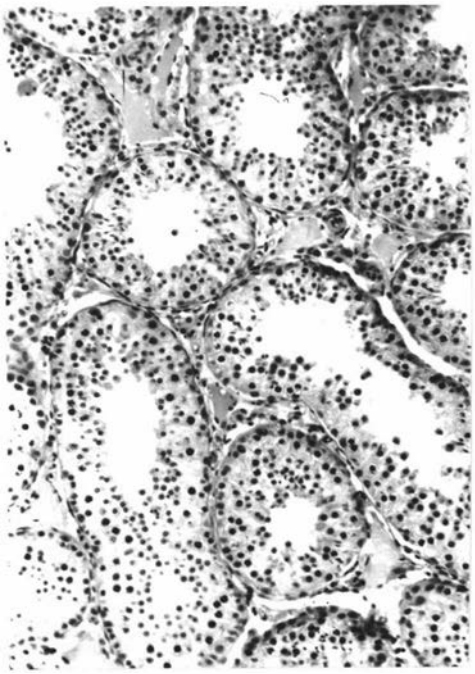

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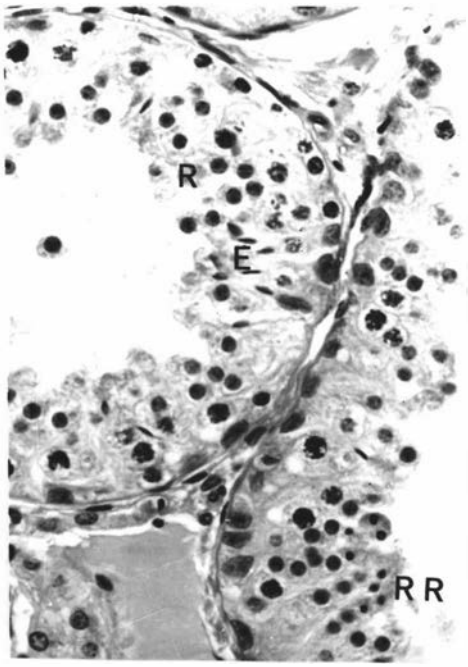

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elongating spermatids (Fig. 69). The majority of the tubules in the section from the proximal area of the right testis contained almost the normal complement of cells in all stages of spermatogenesis. Testicular regeneration appeared to be recent as there was no improvement in semen quality during the observation period.

Commencing on 16.11.70, semen samples for seminal fructose estimation were collected by electrical stimulation at four day intervals for five collections. The semen samples were collected in the field and embedded in dry ice immediately after collection. The samples were assessed for seminal fructose on return to the laboratory the same morning. There was no significant difference ($p>0.05$) between the scrotal mange and control groups in seminal plasma fructose concentration (Table 16), total seminal plasma fructose per ejaculate (Table 17), seminal vesicle weight (Table 18) or seminal vesicle fructose content (Table 18).

Seminal fructose concentration in the three rams with severe testicular atrophy was higher than many of the control rams (Table 16). However, when compared individually only Ram 27 had seminal fructose concentration significantly higher ($p<0.05$) than the control group. Ram 23 which had undergone a partial spontaneous cure of scrotal mange and was showing signs of testicular regeneration had a seminal fructose concentration and total seminal fructose content per ejaculate approximately the same or lower than all of the control rams.

Seminal vesicle weight and seminal vesicle fructose content reflected accurately the

Table 16: THE EFFECT OF EXTENSIVE SCROTAL MANGE ON SEMINAL PLASMA FRUCTOSE CONCENTRATION (mg/100ml) OF THE EIGHT CHEVIOT RAMS

	RAMS WITH EXTENSIVE SCROTAL MANGE				CONTROL RAMS			
Ram No.	21	23	24	27	22	25	26	28
DATE								
16.11.70	76	40	386	344	50	166	50	260
20.11.70	334	20	132	132	64	84	20	100
24.11.70	336	24	100	334	50	220	56	50
28.11.70	200	16	210	700	64	160	50	186
2.12.70	156	16	320	310	132	104	42	126
Mean	220	231	230	364*	72	147	44	144
Group mean	209				102			

* $p < 0.05$

Table 17: THE EFFECT OF EXTENSIVE SCROTAL MANGE ON TOTAL SEMINAL PLASMA FRUCTOSE (mg/ejaculate) OF THE EIGHT CHEVIOT RAMS

	RAMS WITH EXTENSIVE SCROTAL MANGE				CONTROL RAMS			
Ram No.	21	23	24	27	22	25	26	28
DATE								
16.11.70	0.34	0.24	3.86	2.72	0.15	1.49	0.30	2.08
20.11.70	1.34	0.10	1.06	0.40	0.45	0.59	0.10	0.60
24.11.70	1.46	0.08	0.60	1.67	0.15	2.20	0.17	0.15
28.11.70	1.00	0.06	0.84	2.80	0.38	1.92	0.50	0.74
2.12.70	0.50	0.06	2.24	0.60	0.26	0.64	0.29	0.40
Mean	0.93	0.11	1.72	1.64	0.28	1.37	0.27	0.79
Group mean	1.10				0.68			

Table 18: THE EFFECT OF EXTENSIVE SCROTAL MANGE ON WEIGHT (g), FRUCTOSE CONCENTRATION (mg/g) AND TOTAL FRUCTOSE (mg) OF THE PAIRED SEMINAL VESICLES (S.V.) OF THE EIGHT CHEVIOT RAMS

	RAMS WITH EXTENSIVE SCROTAL MANGE				CONTROL RAMS			
Ram No.	21	23	24	27	22	25	26	28
Fructose concentration								
Sample 1	2.7	1.2	4.5	8.0	2.3	6.2	4.2	4.2
Sample 2	3.7	1.7	4.7	8.0	3.0	6.0	4.3	4.4
Mean	3.2	1.5	4.6	8.0	2.7	6.1	4.3	4.3
Group mean	4.3				4.4			
S.V. weight	5.5	3.0	3.5	7.0	4.0	5.5	3.5	3.5
Group mean	4.8				4.1			
Total S.V. fructose	17.7	4.4	16.2	56.0*	10.6	33.6	14.9	15.1
Group mean	23.6				18.6			

* $p < 0.05$

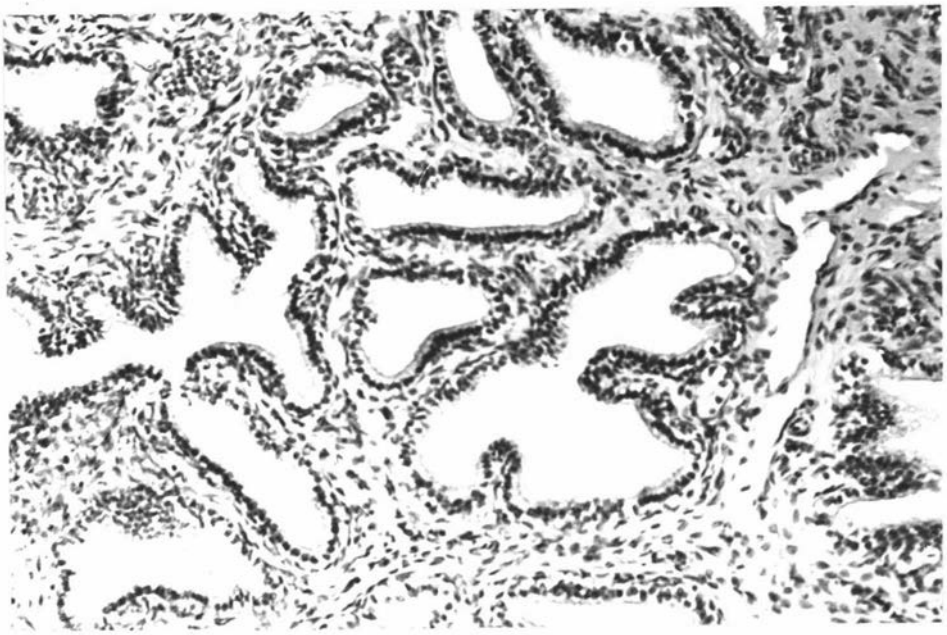
preslaughter seminal fructose picture of the individual rams. For example, of the eight rams, Ram 23 had the lowest seminal fructose, the lightest seminal vesicles and the lowest seminal vesicle fructose concentration. Conversely, Ram 27 had the highest seminal fructose concentration, the heaviest seminal vesicles and the highest seminal fructose concentration. There was no significant difference ($p>0.05$) between the individual seminal vesicle weights or seminal vesicle fructose concentration, but the total seminal vesicle fructose content of Ram 27 was significantly higher ($p<0.05$) than that of the control group.

The histology of the seminal vesicles of Rams 21, 23 and 24 was similar to that of the control rams, but that of Ram 27 was different. The alveoli of the seminal vesicles of Ram 27 were larger and more compact and the cells of the secretory epithelium higher than the other seven rams (compare Fig. 70 with Fig. 71). In the testes there was an apparent increase in the number of Leydig cells in the rams with severe testicular degeneration. However, testicular shrinkage probably accounted for the majority if not all of the apparent increase in Leydig cell numbers. Interstitial cells were clearly visible in Sudan black stained preparations from all eight rams. However, none of the cells were particularly deeply stained and those of the control rams could not be differentiated from those of the rams with severe testicular atrophy.

The sexual behaviour of the eight rams was assessed by placing them with ewes that had the

Figure 70: Section of seminal vesicle of control Cheviot Ram 22.
(H. & E.)
(x180)

Figure 71: Section of seminal vesicle of Cheviot Ram 27. There is a more compact arrangement of the alveoli and the epithelial cells are higher than the control ram. (Compare Fig. 71 with Fig. 70).
(H. & E.)
(x180)



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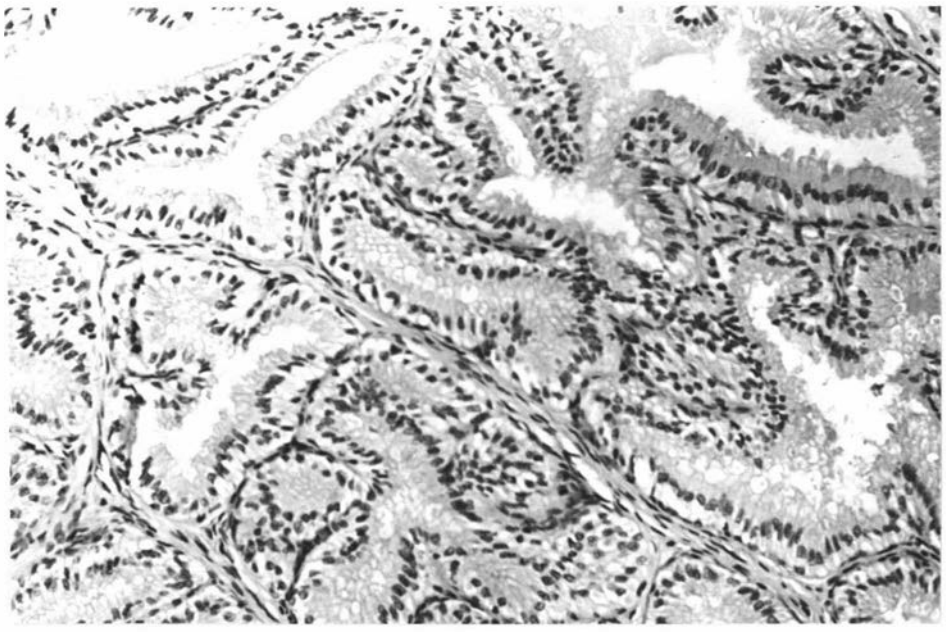

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overt signs of oestrus artificially induced. After the ewes had been used they were returned to the flock and all accepted the teaser ram.

Of the eight rams only Ram 24, which had severe testicular atrophy associated with extensive scrotal mange, showed any sexual interest in the ewes. When the rams were placed individually with a receptive ewe they all were very apprehensive and spent most of their time looking about the pen and bleating. On the second introduction Ram 24 approached the ewe and sniffed at her vulva. After about ten minutes this ram mounted the ewe but dismounted after several unsuccessful attempts at copulation. This procedure was repeated three times before intromission with the characteristic ejaculatory thrust. The other seven rams showed no interest in the ewe. On the third day the procedure was repeated, with Ram 24 having a reaction time of three minutes while the other rams still failed to show any interest in the ewe. Various attempts to provide a more natural environment for the rams did not make any difference to their sexual responses to receptive ewes. For example, two receptive ewes were run with the eight rams in a small holding paddock and observed for one hour. Ram 24 approached one of the ewes within a minute and had mated the ewe within three minutes. Apart from the occasional sniff at the vulval area of a ewe in close proximity the other seven rams continued to show no interest in the sexually receptive ewes.

6.3 Discussion on the Effects of Extensive Scrotal Mange on Ram Reproductive Function

(i) Effect on semen production

The decrease in testes size and tone and decrease in semen quality associated with a rapid increase in lesion severity in Rams 359 and 572, and the seminal regeneration associated with a spontaneous cure of scrotal mange in Ram 91 confirm the assumption of Whitten (1968) and Bruere (1970) that scrotal mange causes testicular atrophy. This immediately disproves the assumption of Crawford et al. (1970) that scrotal mange is associated with testicular hypoplasia and not a cause of testicular atrophy. Further, the partial seminal recovery of Rams 59 and 91 and the persistence of spermatogonia even in the most severely degenerated testes, suggest that testicular function of rams with severe scrotal mange may be restored after treatment. This view is contrary to that held by many veterinarians who often classify rams with hypo-orchidism ("small testes", Bruere 1970) associated with extensive scrotal lesions as "permanently unsound". An examination of seminal regeneration following treatment of extensive scrotal mange is reported in Chapter 7.

The scrotal dermatitis caused by C. bovis did not penetrate the sac of the tunica vaginalis or involve the scrotal contents in any of the nine rams examined. The testicular degeneration was a simple atrophy with no signs of inflammatory involvement. The most likely cause of the testicular degeneration is an impairment of the thermoregulatory function of the diseased scrotum. A preliminary investigation into the possibility

that scrotal mange causes testicular degeneration by interfering with the thermoregulatory function of the scrotum is described in Chapter 8.

In the developing ram (Carmon and Green, 1952; Watson et al., 1956; Skinner et al., 1968) and bull (Abdel-Raouf, 1960; Hay et al., 1961), testes size is closely related to the size of the seminiferous tubules and seminiferous tubule size is related to the spermatogenic activity within the tubules. A similar relationship between testes size, the diameter of the seminiferous tubules and spermatogenic activity was found in the nine rams with extensive scrotal mange. In general, rams with small testes had small tubules containing few germ cells and vice-versa. A r of 0.75 ($p<0.05$) was obtained between testis size and seminiferous tubule diameter and a r of 0.76 ($p<0.05$) between testes size and spermatogenic mean score count (Table 19). Thus approximately 55% of the differences in testes weight in the rams with scrotal mange could be accounted for by changes in seminiferous tubule diameter or by differences in the spermatogenic mean score. Johnsen (1970) obtained a r of 0.70 between testes size and spermatogenic mean score in normal and "hypogonadal" human subjects. He also obtained a r of 0.79 between spermatogenic mean score and mean seminiferous tubule diameter while a r of 0.85 ($p<0.01$) was obtained between these two parameters in the rams with scrotal mange. The high r obtained between spermatogenic mean score and the other parameters of testicular activity in the rams with scrotal mange confirm Johnsen's (1970) conclusions that the spermatogenic score count, which was developed by him, is a quick, accurate

Table 19: RELATIONSHIP BETWEEN TESTES SIZE, MEAN SEMINIFEROUS TUBULE DIAMETER (M.S.T.D.) AND SPERMATOGENIC MEAN SCORE (S.M.S.) IN NINE RAMS WITH EXTENSIVE SCROTAL MANGE

Ram No.	Testes weight L+R (g)	M.S.T.D. (μ)	S.M.S.
27	127	151	4.5
24	130	136	3.9
572	141	157	4.4
23	170	172	7.2
359	178	165	5.1
92	182	170	5.6
21	195	143	4.4
59	235	175	5.3
91	327	186	8.2

r (testes weight - M.S.T.D.) = 0.75 ($p < 0.05$)

r (testes weight - S.M.S.) = 0.76 ($p < 0.05$)

r (Av. M.S.T.D. - S.M.S.) = 0.85 ($p < 0.01$)

and objective method of assessing spermatogenic activity in testes with varying degrees of tubular degeneration.

The testicular histology of rams with extensive scrotal mange was similar to long standing testicular degenerations seen in rams whose testes temperature had been elevated experimentally. For example, Moore and Cslund (1924) have shown that 76 days after surgical replacement of the testes in the abdominal cavity there was a decrease of about two-thirds in testis size and a proportionate decrease in seminiferous tubule diameter. There was a severe depletion of germ cells with the majority of tubules containing only a single basal layer of cells. Phillips and McKenzie (1934) noted that the seminiferous tubules of a ram whose scrotum had been insulated for 8 weeks were severely shrunken. The tubules contained a basal layer of Sertoli cells and spermatogonia and also a small number of cells resembling primary and secondary spermatocytes. The spermatocytes were scattered throughout a vacuolated seminiferous epithelium. The tubules were shrunken and widely separated. Johnson et al. (1969) showed similar cytological changes in the testes of rams that had been exposed to elevated air temperatures (32.2^{o}C) for two weeks. Observations in this study have shown that rams with severe testicular atrophy associated with scrotal mange had some seminiferous tubules with only the single basal layer of cells, but the majority contained a few primary spermatocytes scattered throughout the seminiferous epithelium. In rams with less severe testicular atrophy there were more primary spermatocytes per tubule. In other rams spermatogenesis progressed

as far as the round spermatid stage but did not progress further. Many of the round spermatids were in the lumen of the tubules and many showed degenerative changes.

The testicular degeneration seen in rams with extensive scrotal mange was virtually identical to that described in a bull that had a dermatitis of the scrotum induced by rubbing a mild rubefacient ointment (Ichthammol) on to the scrotum twice a day for 40 days (Wollrab, 1965). The rubefacient, like scrotal mange, had no apparent effect on the general health of the animal and the inflammatory response did not penetrate the scrotum. It has been assumed, although not proven, that the Ichthammol treatment caused the testicular degeneration by raising the testicular temperature (Paufler, 1970).

Only rams with longstanding testicular degeneration due to scrotal mange have been examined and any initial changes in primary spermatocytes and B type spermatogonia, as described by Waites and Ortavant (1967, 1968) in heat induced testicular degeneration in the ram have not been observed.

The testicular histology of rams with severe degeneration was similar throughout the testis, even though lesions may have involved only the more distal parts of the scrotum. Further, when scrotal lesion severity was similar on both sides of the scrotum, histology of both testes was virtually the same. Ram 59 was the only ram that had small lesions on one side of the scrotum and extensive lesions on the other. This was also the

only ram that had one testis more severely atrophied than the other. This finding suggests that the agent causing the testicular degeneration is acting locally and not systemically. In contrast to the rams with severe testicular atrophy, rams that were undergoing testicular regeneration showed more variation in seminiferous tubule histology. Often sections from testes that were regenerating had some tubules with only a basal layer of cells while other tubules contained germ cells in all stages of spermatogenesis. The spermatogenic score count showed clearly the homogeneity of the testes of the rams with severe atrophy and the heterogenicity of the testes undergoing partial regeneration. The technique allowed the proportion of tubules at various stages of degeneration and regeneration to be readily assessed and recorded.

Rams with scrotal mange causing severe testicular atrophy were practically azoospermic. However, semen samples collected throughout the observation period from these rams contained small numbers of dead spermatozoa. These spermatozoa had probably resided in the extragonadal structures since spermatogenic arrest. This opinion was confirmed by finding a few spermatozoa in extragonadal homogenates while no spermatozoa or elongated spermatids were seen in testicular homogenates or testis sections of some rams. Small numbers of spermatozoa can reside in the extragonadal spermatozoa storage structures for long periods of time (Dunlop et al., 1963; K. L. MacMillan, pers. comm.). Dunlop et al. (1963) observed spermatozoa in semen smears from five rams that had been successfully vasectomized for

more than one year. Spermatozoa numbers per ejaculate, spermatozoa activity and spermatozoa morphology of the vasectomized rams (Dunlop et al. 1963) were virtually identical to the numbers, activity and morphology of spermatozoa collected from rams with complete spermatogenic arrest caused by extensive scrotal mange.

A considerable amount of debris was observed in semen samples of most rams with extensive scrotal mange during some stages of the observation period. The cellular debris was similar to that described by Gunn et al. (1942) in rams with degenerate semen. However, the large multinucleate cells described as occurring commonly in abnormal semen of rams (Gunn et al., 1942) and in abnormal semen of bulls (Lagerlof, 1966) were seen only rarely in the semen of rams with scrotal mange. The smaller round nucleated or non nucleated cells as described by Gunn et al. (1942) made up the majority of non spermatozoon cells seen. The occurrence in degenerate semen of large amounts of filamentous material either coiled or straight, has not been reported specifically. In rams with longstanding severe testicular atrophy there was little debris in the lumen of the seminiferous tubules and the preslaughter semen samples from these rams were almost debris free. Conversely, rams that produced large amounts of seminal cellular and filamentous debris prior to slaughter also had similar debris in the lumen of their seminiferous tubules. This suggests that most of the debris was of testicular origin.

(ii) Effect of extensive scrotal mange on the androgenic status of the ram

No obvious differences in seminal plasma fructose, seminal vesicle weight, seminal vesicle histology and sexual behaviour were detected between the two groups of rams with scrotal mange and their respective control groups.

Testicular degenerations in animals resulting from severe underfeeding (Setchell et al., 1965), orchitis (Mann et al., 1967), increased environmental temperature (Pfeiffer, 1937) and experimental cryptorchidism (Moore, 1944) have been associated with a decrease in seminal vesicle weight and/or seminal vesicle fructose content. In contrast, an increase in seminal fructose has been demonstrated in rams in which the testes temperature has been raised experimentally by either scrotal insulation (Glover, 1956) or by increasing the ambient temperature (Moule and Waites, 1963). Clegg (1960) showed an initial increase followed by a marked fall in seminal vesicle fructose in rats after experimental cryptorchidism. Thus, rams with scrotal mange may have abnormally high or low fructose levels depending on the stage of the disease when fructose estimates were carried out so that in this experiment each ram with scrotal mange was compared individually with its respective control group. On this basis only one of the rams with severe scrotal mange (Ram 27) differed significantly ($p<0.05$) from its control group. Both the seminal plasma fructose concentration and the total seminal vesicle fructose of this Cheviot ram were significantly higher than those of the control animals. However, this ram did not show any interest in ewes in oestrus while one

of the other Cheviot rams with extensive scrotal mange showed marked interest in receptive ewes.

There were large variations in fructose estimations between Romney and Cheviot experimental groups, between rams within groups and between semen samples within rams. Fructose estimations on replicate samples of seminal plasma (Appendix 5) and seminal vesicles (Table 18) varied little showing that the method of estimating fructose would not account for the large variation in fructose estimations. It was suspected that the electro-stimulation technique of semen collection was a cause of the large amount of within animal variation. However, Mattner and Voglmayr (1962) compared ram semen collected in an artificial vagina and by electro-stimulation and found that the between ram and within ram variation in seminal plasma fructose was similar for both methods of collection and they concluded that both techniques of collection were "of equal value for studies relating to seminal fructose".

The range of seminal fructose in the five Romney control rams (160mg/100ml-910mg/100ml) was similar to that obtained by Mattner and Voglmayr (1962) (197mg/100ml-1,151mg/100ml) and the proportion of the variation attributable to between rams and within rams was similar for both sets of data. Further, the average seminal fructose level of the five control rams (618mg/100ml) was similar to that recorded from normal rams by Mattner and Voglmayr (1962) - 614mg/100ml; Salamon (1964) - 596mg/100ml and Glover (1956) - 540mg/100ml.

The average seminal fructose levels of the

four Cheviot control rams (102mg/100ml) was much lower than the five Romney control rams (618mg/100ml). The differences between the two groups were probably not due to breed. Moule et al.(1966) found no differences in the seminal fructose levels between Romney and Merino rams in one experiment and between Merino and English Leicester rams in another experiment and Glover (1956) found no differences between Romney and Suffolk rams. Seasonal variations in seminal fructose may account for the differences between the Romney and Cheviot groups. Cupps et al. (1960) and Moule et al. (1966) found that seminal fructose peaked in the autumn at approximately 450mg/100ml and 800mg/100ml respectively, which was comparable to the fructose values obtained in the late summer in the Romney experiment and a trough in the spring of about 65mg/100ml and 100mg/100ml respectively which was comparable to the fructose values in the Cheviot experiment which was carried out in late spring. Seminal vesicle weight and fructose content and the variation in these two estimates in the five Romney control rams were similar to that reported previously in mature rams (Ortavant et al., 1964; Setchell et al., 1965). The lower seminal vesicle estimates in the Cheviot rams reflected the low seminal plasma levels.

The apparent increase in the number of Leydig cells in rams with severe testicular atrophy was probably a reflection of testicular shrinkage. No attempt was made to assess quantitatively the actual number of the interstitial cells, since according to earlier investigators this method is unreliable (Sand and Okkels, 1936; Hemphill et al., 1944). Sudan black was used as a specific

interstitial stain for the Leydig cells and although the cells readily stained, the sections from the rams with scrotal mange could not be distinguished from those of the control group.

In general there was no differences between the scrotal mange groups and their respective control groups in either reaction time or in general sexual behaviour. One of the ten rams in the Romney experiment failed to mount the ewes in oestrus and one of the eight rams from the Cheviot experiment mounted receptive ewes. Both of these rams had extensive scrotal mange with severe testicular atrophy. No significance can be placed on these isolated observations. The almost complete lack of sexual interest by the Cheviot rams was probably due to the fact that these were virgin animals and were exposed to receptive ewes in late spring, a time of the year when sexual drive of most breeds of rams in temperate climates is at a minimum (Chang, 1941; Glover, 1956; Cupps et al., 1960; Land, 1970). It was interesting to note that the almost complete lack of libido in the Cheviot group was associated with small seminal vesicles and low seminal plasma and seminal vesicle fructose levels. However, apart from this general association there was no relationship between fructose levels and sexual behaviour in the individual Cheviot and Romney rams. Lunca et al. (1968) specifically investigated the association between fructose levels in the semen and sexual behaviour. Although seminal fructose varied from 290-1,815mg/100ml in individual rams, Lunca et al. (1968) was unable to show a correlation between the fructose level and the intensity of sexual behaviour in 16 rams.

The lack of relationship between libido and seminal fructose reported in this and in previous studies (Glover, 1956; Lunca et al., 1968) may be due to one or more of the following: the difficulty in assessing libido; differing thresholds of susceptibility of the brain and seminal vesicles to testerone (suggested by Glover, 1956), and factors other than androgens affecting libido (Lisk, 1967; Phoenix et al., 1967) and seminal fructose (Mann, 1967).

Chapter 7

RECOVERY OF REPRODUCTIVE FUNCTION OF RAMS WITH EXTENSIVE SCROTAL MANGE

7.1 Introduction

Many veterinary surgeons in New Zealand consider that rams with small testes associated with extensive scrotal lesions are "permanently unsound" (Whitten, 1964; Quinlivan and Lindsay, 1971; K.B.R. Keene, pers. comm.). This infers that even with successful treatment, these rams will have permanently reduced fertility. However, complete seminal regeneration has been associated with the spontaneous cure of experimentally induced moderate scrotal mange (Chapter 5) and rams with severe testicular atrophy that were virtually azoospermic have shown signs of seminal regeneration following regression of scrotal lesions (Chapter 6). In contrast to veterinary opinion this suggests that reproductive function of these rams may recover with successful treatment. To examine this question more closely, five rams with extensive scrotal mange and severe testicular atrophy were studied both before and after treatment.

7.2 Animals Used and Experimental Procedure

Four mature Romney rams aged 15 months and showing relatively severe scrotal mange were obtained by the author during a routine examination of a stud flock in mid November 1970. These 4 rams plus a control ram of similar body weight were

brought to the University on the 16.10.70 and run on pasture as previously described. The rams were housed inside for approximately one month from late February when the risk of becoming affected with Facial eczema was high (Clare, 1965; Campbell, 1969). The rams were brought to the laboratory at intervals of approximately ten days throughout the observation period for semen assessment. On the 23.11.70 when the 4 rams with scrotal mange had severe testicular atrophy and were practically azoospermic the scrotal lesions were treated and the rams hemicastrated. Prior to unilateral castration all the scrotal exudate was removed and the scrotum soaked in 0.5% alcoholic solution of chlorhexidine gluconate (Hibitane, I.C.I.). After general anaesthesia was induced the left testis of each ram was removed surgically; some of the left scrotal sac was removed also and the scrotal incision sutured. The testis of each ram was weighed and sections for histology taken from the proximal and distal ends within 5 minutes of hemicastration. All five rams were given 1×10^6 units of procaine penicillin intramuscularly. The rams recovered uneventfully from anaesthesia and there were no post operative complications. When the semen quality of the rams with severe testicular atrophy had returned to normal levels, they were slaughtered, the right testis removed and samples for histology taken from both proximal and distal ends.

Ram 13, the mite donor ram, had small testes associated with extensive scrotal mange for at least 15 months. Following the spontaneous cure of the lesions in early 1971 there was a recovery of reproductive function. Details of this recovery

are reported also in this chapter.

Individual semen assessments of the five rams with scrotal mange are presented in Appendix 7.

7.3 Results

Control Ram 105

The testes of Ram 105 were large and varied in tone from moderate to good throughout the observation period. Semen concentration varied from watery-milky to creamy with most samples being at least milky in colour. The semen quality index varied from 20 to 27 with a mean of 23.3 (S.D.2.3). There was no reduction in semen quality after unilateral castration but there was a reduction in semen quantity. When removed on the 23.11.70 the left testis weighed 210g and at slaughter on the 21.5.71 the right testis weighed 285g. The spermatogenic mean score count of left and right testis was 9.7 and 9.8 respectively.

Ram 438

At the beginning of the experiment (16.10.70), Ram 438 had active mange lesions with caked exudate at least 1 cm thick covering the distal third of the scrotum. The lesions extended proximally and by 17.11.70 lesion classification had changed from severe to extreme. At unilateral castration on 23.11.70 the distal two thirds of the posterior aspect of the scrotum and the distal half of the anterior aspect of the scrotum was covered with exudate as much as 2 1/2 cm thick. Testis size and tone changed from medium and moderate on the 16.10.70 to small and soft by the end of October.

Approximately 1 ml of creamy semen, with a semen quality index of 19, was collected from Ram 438 on the 20.10.70. By the middle of November the semen quality index had fallen to zero and the ram was practically azoospermic (Text-fig. 13). The majority of the abnormals observed during the seminal degeneration were tailless spermatozoa, coiled tails and returned tails.

At unilateral castration the left testis weighed 56g and histologically the seminiferous tubules were reduced in size. The majority of tubules from both sections contained only a single basal layer of cells (Figs. 72, 73 and Table 20). A few primary spermatocytes, many of which were degenerating, were seen in a small number of tubules.

Seven weeks after initial treatment signs of seminal regeneration were observed (Text-fig. 13) and seminal regeneration was complete by the end of April (Text-fig. 13). Apart from relatively large numbers of round nucleated and non nucleated cells in the ejaculate collected on the 18.1.71 there was very little cellular or non cellular debris in the semen of Ram 438 during the observation period.

At slaughter on 24.4.71 there was no evidence of a prior scrotal dermatitis, the scrotum and its contents appearing clinically normal. The right testis weighed 221g, there was a marked increase in diameter of the seminiferous tubules and there was a repopulation of most seminiferous tubules (Figs. 74, 75). Cell numbers and cellular associations in most tubules were indistinguishable from those

seen in normal testes and the spermatogenic mean score was only slightly less than in the control ram (Table 20). However, a small number of tubules in both the proximal and distal sections of the testis had not completely regenerated, a very small number of tubules being indistinguishable from those seen in the left testis (Table 20).

TEXT-FIG. 13: SEMINAL REGENERATION IN RAM 438 FOLLOWING HEMICASTRATION AND TREATMENT OF "EXTREME" SCROTAL MANGE.

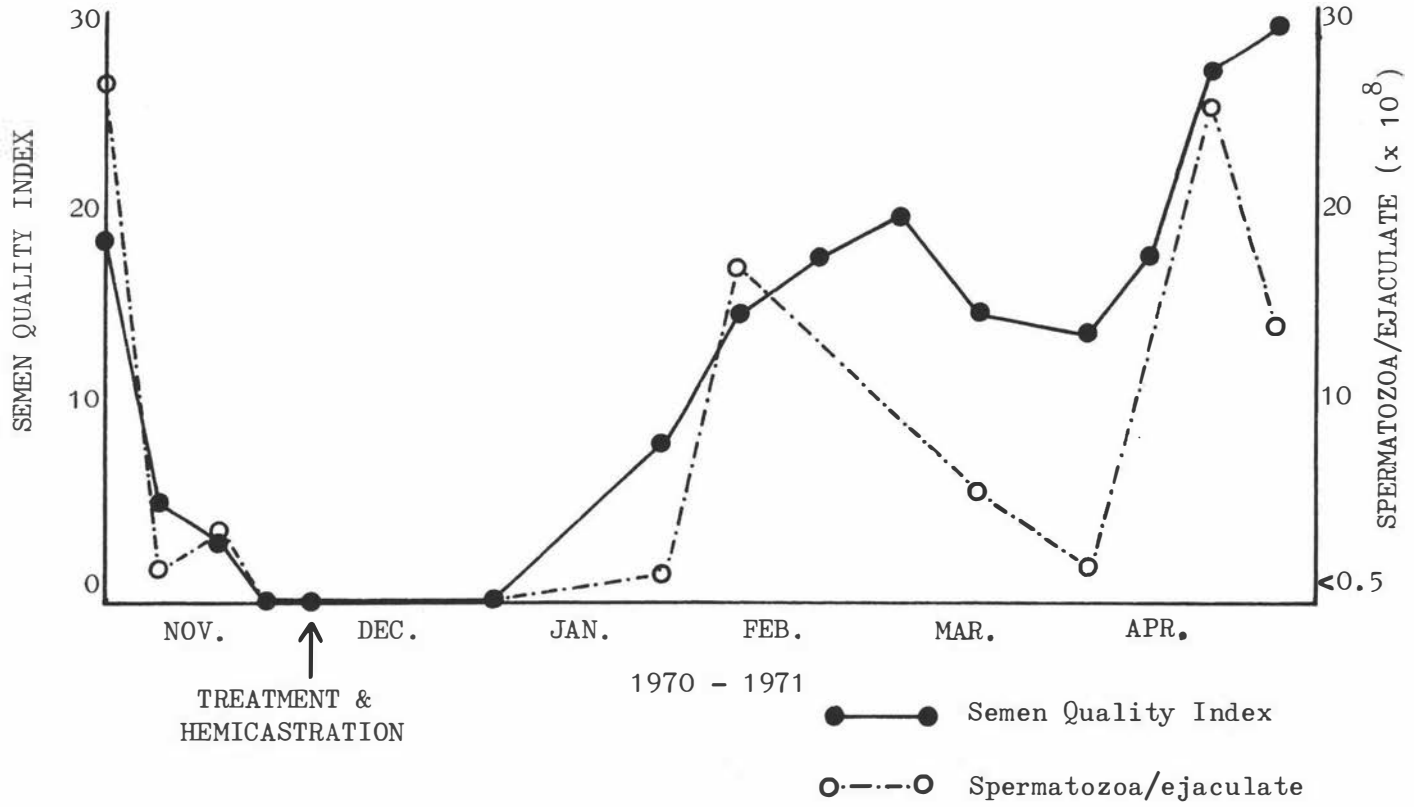


Table 20: SPERMATOGENIC SCORE COUNT OF RAM 438 BEFORE AND AFTER TREATMENT OF "EXTREME" SCROTAL MANGE

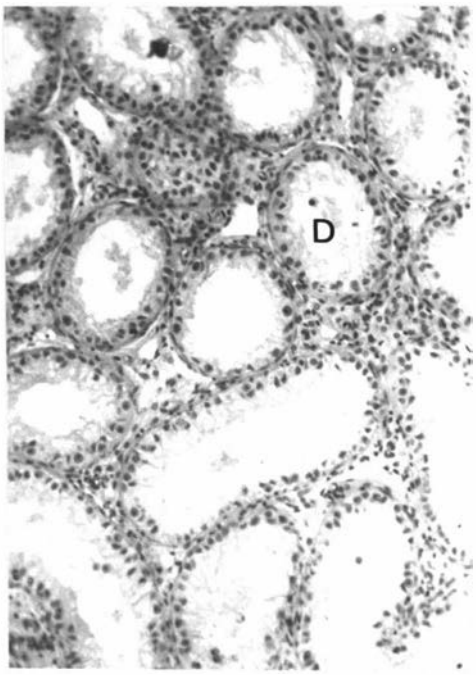
Spermatogenic score	Left testis[1]		Right testis[2]	
	Proximal	Distal	Proximal	Distal
1	-	-	-	-
2	16	16	-	-
3	25	25	1	-
4	8	8	2	2
5	1	1	1	-
6	-	-	1	-
7	-	-	1	1
8	-	-	1	-
9	-	-	6	4
10	-	-	37	43
Position mean score	2.9	2.9	9.2	9.6
Testis mean score	2.9		9.4	

1 Removed when severely atrophic

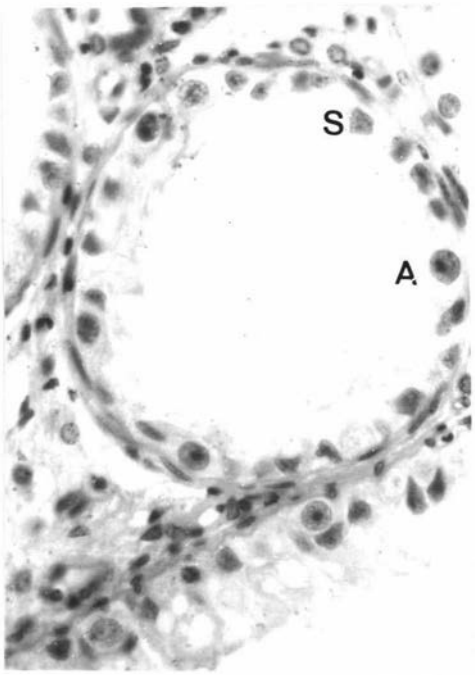
2 Removed after seminal regeneration

Figures 72 & 73: Section from proximal left testis of Ram 438 when virtually azoospermic (23.11.70). Note single basal layer of cells comprising Sertoli cells (S), spermatogonia (A) with the occasional degenerating primary spermatocyte (D). (H. & E.)
Fig. 72, x120
Fig. 73, x400

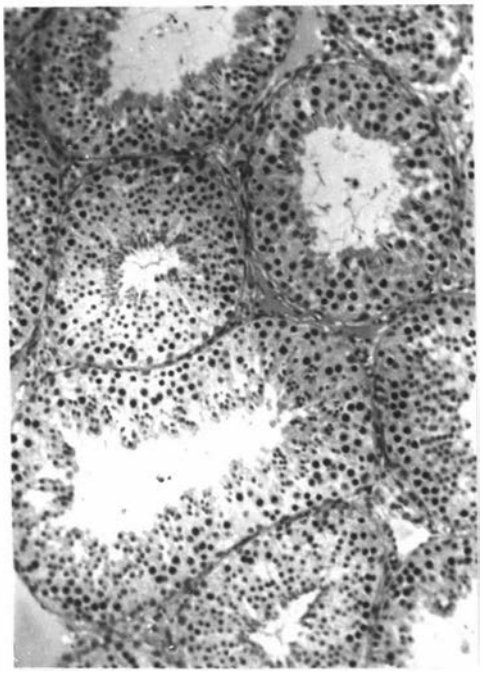
Figures 74 & 75: Section from proximal right testis of Ram 438 after seminal regeneration. Note repopulation of tubules with normal numbers of cells in normal cellular associations. (H. & E.)
Fig. 74, x120
Fig. 75, x400



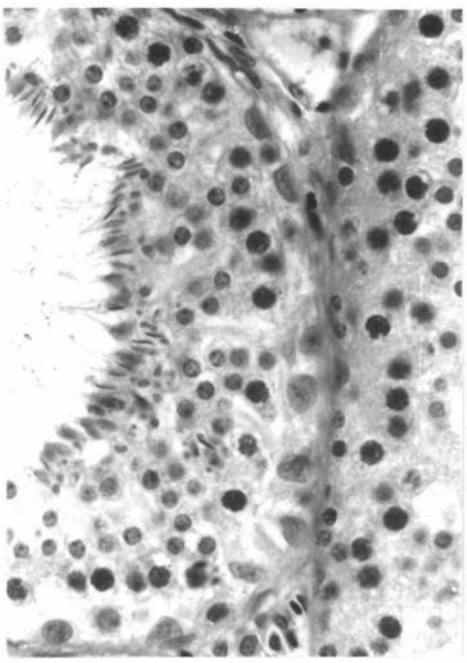
72



73



74



75

Ram 517

When first examined on the 16.10.70, Ram 517 had moderate scrotal mange. Scattered lesions 1/2 - 1 cm in diameter covered about one third of the distal third of the scrotum with exudate 1/2 - 1 cm thick. These lesions were more severe over the left testis. There were also two discrete lesions each about 3 cm in diameter, both on the area of the scrotum covering the right testis. By 17.11.70 scrotal mange classification changed to severe. Most of the scabs on the distal third had become confluent, with exudate approximately 1 cm thick. Lesion severity did not change appreciably until treatment on 23.11.70. On first examination the testes were large and with good tone but decreased to moderate size by 17.11.70 and were small and soft at the time of treatment.

One ml of creamy semen was collected from Ram 517 on the 20.10.70; 50% of the spermatozoa were rapidly motile and virtually all were morphologically normal. There was a decrease in semen quality and quantity in subsequent ejaculates (Text-fig. 14) and only an occasional live spermatozoa observed in the ejaculate collected on 30.10.70. By the 23.11.70 only very small numbers of dead spermatozoa were present in the semen collected from this ram.

When removed the left testis weighed 67g and the seminiferous tubules were severely atrophic (Figs. 76, 77). Except for a very small number of tubules which contained an occasional round spermatid (Table 21) the most mature germ cells observed were primary spermatocytes. Compared with Ram 438, more tubules contained primary spermatocytes and there were also more primary spermatocytes

per tubule (Table 21, Figs. 76, 77). A large proportion of the primary spermatocytes showed degenerative changes (Fig. 77).

By the 18.1.71 testis size had returned to large and tone to good and there were signs of seminal regeneration (Text-fig. 14). However, a secondary degeneration was observed associated with a profuse diarrhoea which was caused by accidental overfeeding of sheep nuts. Semen quality of three semen samples collected in May 1971 indicated that complete seminal regeneration had occurred. For example, on the 21.5.71 virtually all the spermatozoa were rapidly motile, stained live and were morphologically normal.

At slaughter (21.5.71), the scrotum was clinically normal and the right testis weighed 254g. There was a marked increase in the diameter of the seminiferous tubules (compare Fig. 76 with Fig. 78) and all tubules were undergoing complete spermatogenesis, tubular histology being indistinguishable from that of the control ram (compare Figs. 78, 79 with Figs. 29, 30). The spermatogenic score count clearly illustrates the virtual complete regeneration of the testis of this ram (Table 21).

TEXT-FIG. 14: SEMINAL REGENERATION IN RAM 517 FOLLOWING HEMICASTRATION AND TREATMENT OF "SEVERE" SCROTAL MANGE.

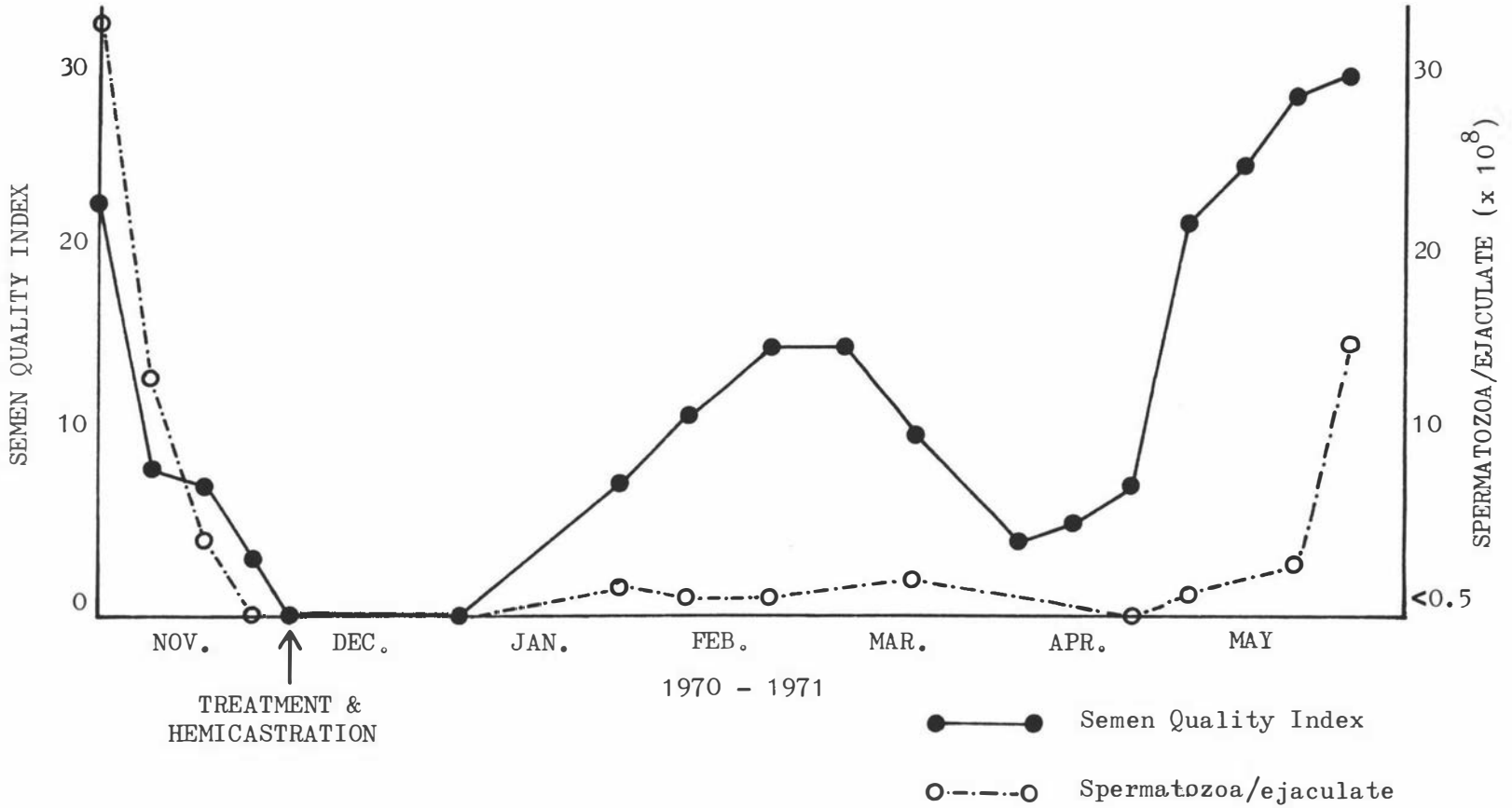


Table 21: SPERMATOGENIC SCORE COUNT OF RAM 517 BEFORE AND AFTER TREATMENT OF "SEVERE" SCROTAL MANGE

Spermatogenic score	Left testis[1]		Right testis[2]	
	Proximal	Distal	Proximal	Distal
1	-	-	-	-
2	8	9	-	-
3	12	7	-	-
4	18	17	-	-
5	12	15	-	-
6	-	2	-	-
7	-	-	-	-
8	-	-	1	-
9	-	-	4	5
10	-	-	45	45
Position mean score	3.7	3.8	9.9	9.9
Testis mean score	3.8		9.9	

1 Removed when severely atrophic

2 Removed after seminal regeneration

Ram 165

On 16.10.70 Ram 165 had moderate scrotal mange. Lesion severity increased noticeably during the following month and by 23.11.70 two thirds of the distal half of the scrotum was covered in active lesions approximately 1 cm thick. On first examination the testes were large with good tone but had become small with moderate tone by the time of treatment. The remaining testis increased in size following successful treatment and by the beginning of January 1971 was large with good tone.

Half a ml of milky semen, with a semen quality index of 8 was collected from Ram 165 on the 20.10.70. During the degenerative phase, the majority of the spermatozoa abnormalities in the two previous rams were tail defects or tailless spermatozoa. The first ejaculate collected from Ram 165 was similar. Semen quality and quantity subsequently decreased and the ram became practically azoospermic. The two ejaculates collected prior to virtual azoospermia contained 10% and 14% head abnormalities. Most of these were pyriform heads while a few had small and narrow heads.

At first treatment the distal quarter of the scrotum appeared slightly oedematous. However, as in the previous two rams the scrotal skin response did not involve the tunica vaginalis sac or the testis. At unilateral castration the left testis weighed 65g and histologically was uniformally atrophic. The most mature germ cells observed were primary spermatocytes and most tubules contained only a few of these scattered

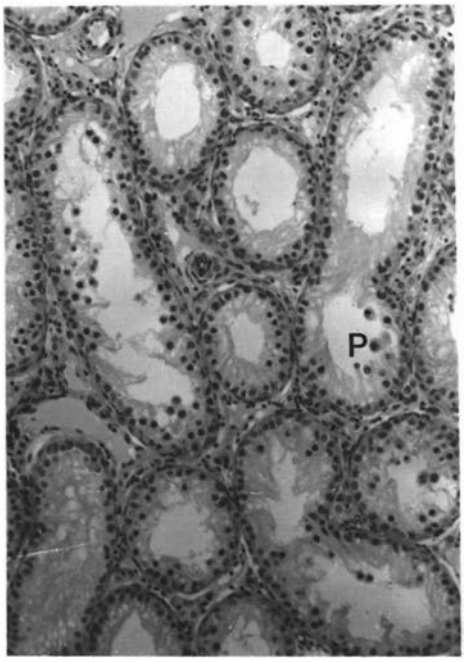
throughout the epithelium (Figs. 82, 83, Table 22).

The seminal regeneration that occurred following successful treatment of the scrotal lesions was similar to that seen in Ram 517 (compare Text-fig. 14 with Text-fig. 15). However, relatively large amounts of seminal debris occurred during the recovery phase. For example, on 18.1.71 a milky ejaculate was collected which contained only 0.07×10^8 spermatozoa per ml while a normal milky ejaculate contains approximately 5×10^8 spermatozoa per ml. The opacity of the semen sample was a reflection of the large amount of seminal debris present (Fig. 80). When compared with the other rams examined, the seminal debris of Ram 165 remained relatively high during the recovery phase. For example, on 3.5.71 small numbers of round and oval cells were still being ejaculated amongst relatively normal spermatozoa, (Fig. 81). During the recovery phase there was also a secondary seminal degeneration associated with a severe diarrhoea when the ram was accidentally overfed.

At slaughter (12.5.71), the semen of Ram 165 was of high quality (Text-fig. 15, Fig. 81). The scrotal skin, especially the base, was slightly thickened but the sac of the tunica vaginalis and its contents were clinically normal. At slaughter the right testis weighed 213 g. Histologically the right testis was similar to the right testis of Ram 438 (compare Table 22 with Table 20). Although the majority of the tubules were undergoing spermatogenesis with normal cell numbers and cellular associations, there were a small

Figures 76 & 77: Section from distal end of the left testis of Ram 517 (23.11.70) showing severe tubular atrophy with many tubules containing a small number of primary spermatocytes (P) many of which are degenerating (D).
(H. & E.)
Fig. 76, x120
Fig. 77, x400

Figures 78 & 79: Section from distal right testis of Ram 517 after complete seminal regeneration (21.5.71). Note normal cell numbers with normal cellular associations.
(H. & E.)
Fig. 78, x120
Fig. 79, x400



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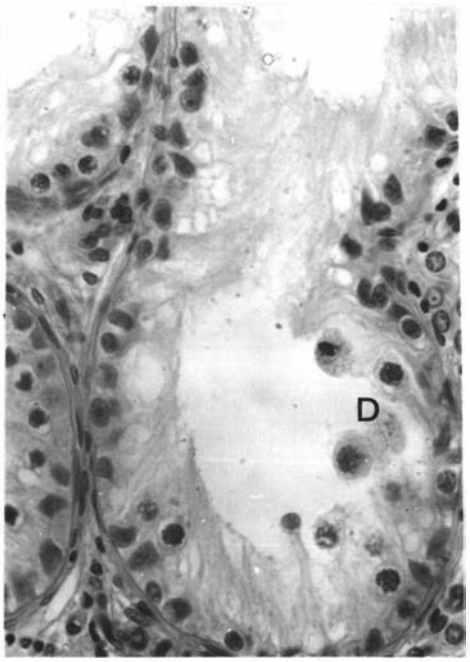

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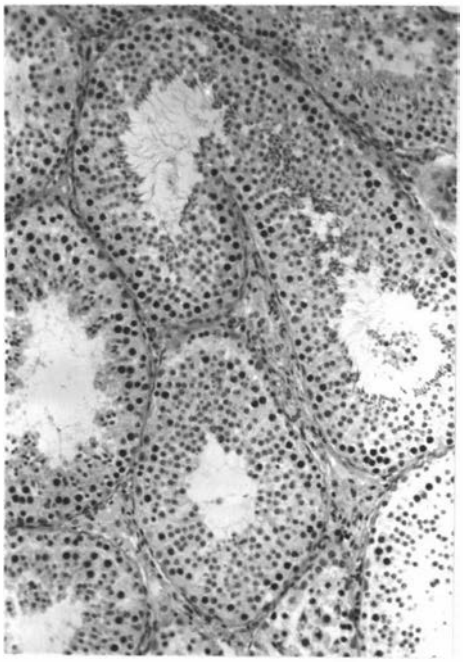

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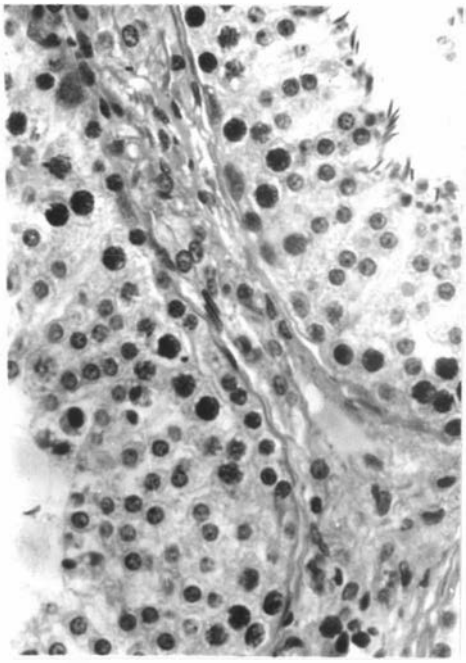

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number of tubules that had not completely regenerated. Most of these tubules had approximately normal cell numbers but abnormal cellular associations while a small proportion had severely depleted cell numbers. The occasional tubule was indistinguishable histologically from those of the left testis which was removed prior to treatment (compare Fig. 84 with Fig. 82). Similar to the previous rams there were no histological differences between the two sample areas of the right testis. Table 22 illustrates clearly the similarity between the two areas and the proportion of tubules with normal and atrophic tubules.

TEXT-FIG. 15: SEMINAL REGENERATION IN RAM 165 FOLLOWING HEMICASTRATION AND TREATMENT OF "SEVERE" SCROTAL MANGE.

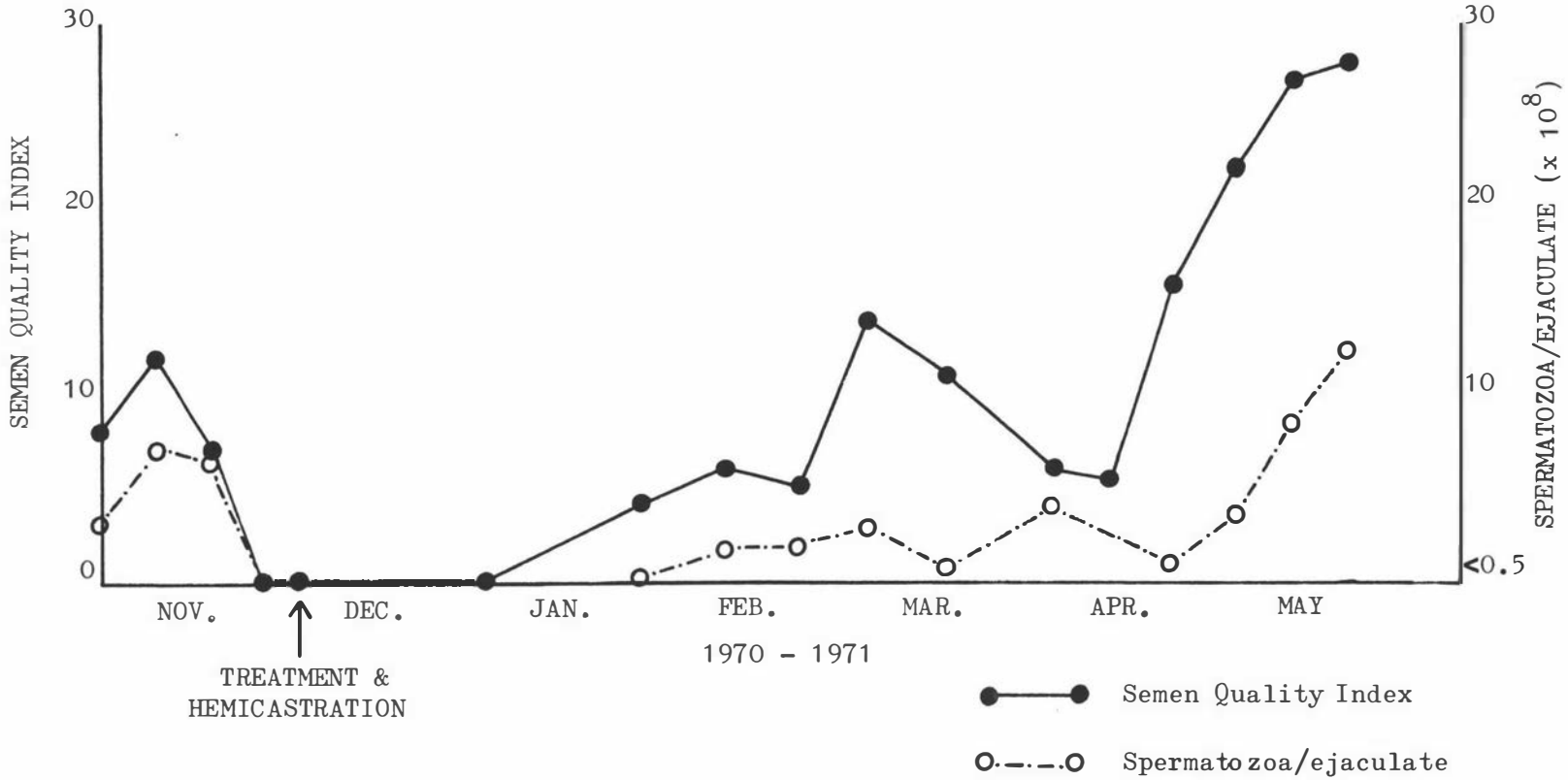
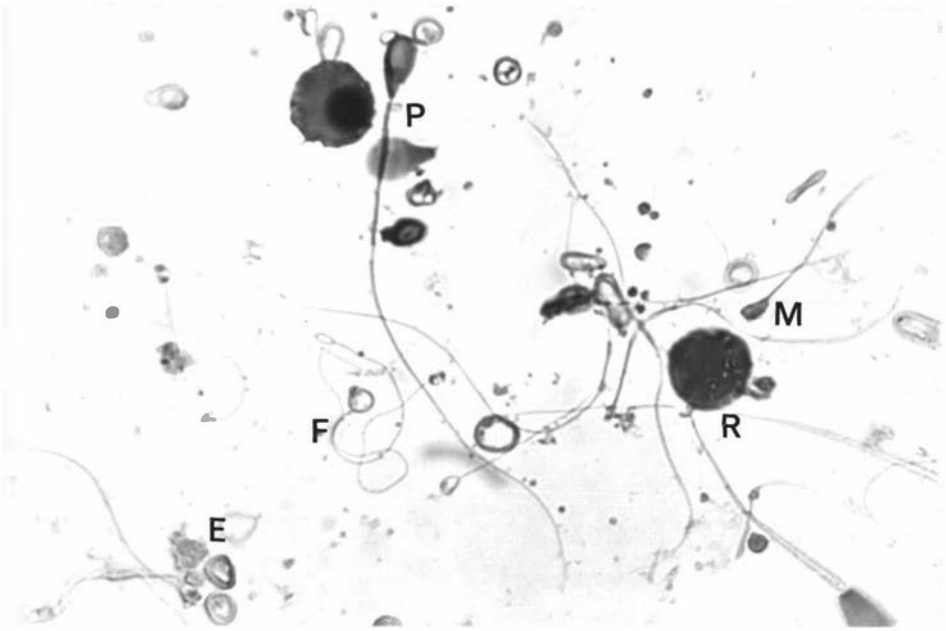


Figure 80: First sign of seminal regeneration in Ram 165 (18.1.71). A few small pyriform spermatozoa (P) are seen amongst large amounts of seminal debris. Coiled and uncoiled filaments (F), microspermatozoa (M), round cells (R) and eosinophilic debris (E) are seen.
(Mayer's haemalum & eosin).
(x1250)

Figure 81: Oval nucleated cell (O) amongst relatively normal spermatozoa in the semen of Ram 165 in the latter stages of seminal regeneration (3.5.71).
(Mayer's haemalum and eosin).
(x1250)



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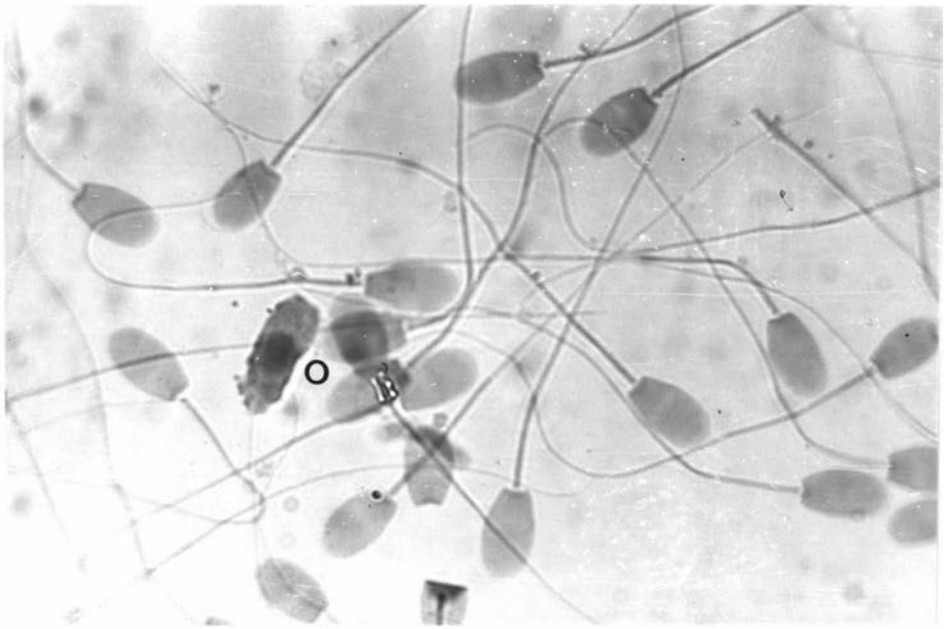

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Table 22: SPERMATOGENIC SCORE COUNT OF RAM 165 BEFORE AND AFTER TREATMENT OF "SEVERE" SCROTAL MANGE

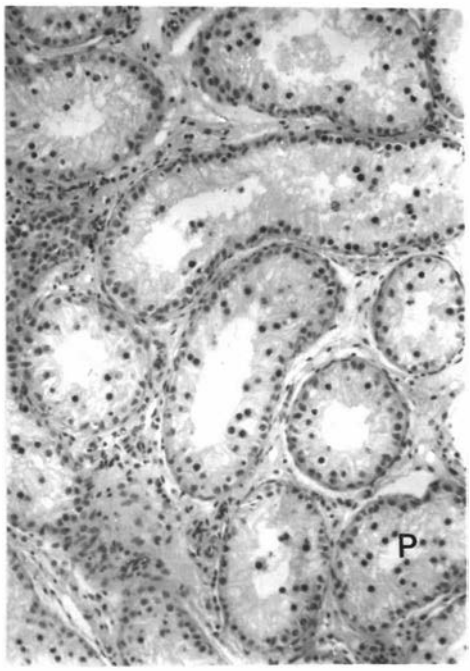
Spermatogenic score	Left testis[1]		Right testis[2]	
	Proximal	Distal	Proximal	Distal
1	-	-	-	-
2	1	1	-	-
3	1	1	-	-
4	9	18	-	1
5	39	30	3	1
6	-	-	2	-
7	-	-	1	1
8	-	-	2	1
9	-	-	6	5
10	-	-	36	41
Position mean score	4.7	4.5	9.3	9.6
Testis mean score	4.6		9.5	

1 Removed when severely atrophic

2 Removed after seminal regeneration

Figures 82 & 83: Section from proximal area of left testis of Ram 165 (23.11.70) showing shrunken tubules with basal layer of cells and primary spermatocytes (P), many of which are degenerating (D). (H. & E.)
Fig. 82, x 120
Fig. 83, x 400

Figures 84 & 85: Section from right testis of Ram 165 after seminal regeneration (12.5.71). Most tubules have normal cellular structure (N) while a few are still degenerate (D).
(H. & E.)
Fig. 84, x 120
Fig. 85, x 400



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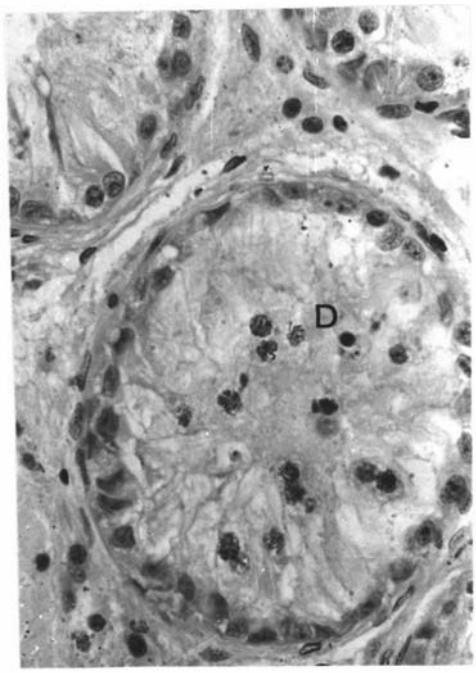

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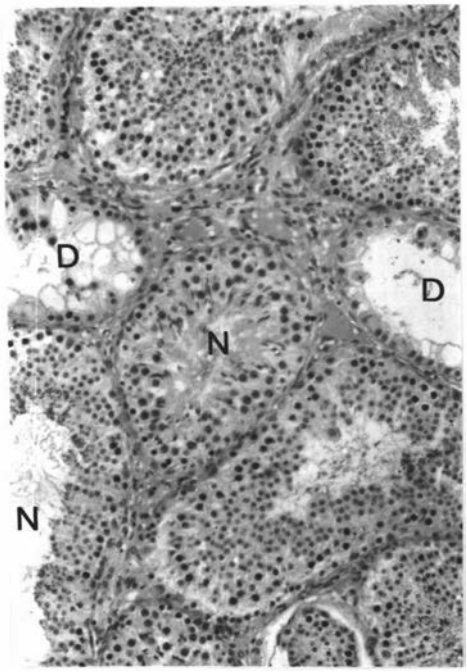

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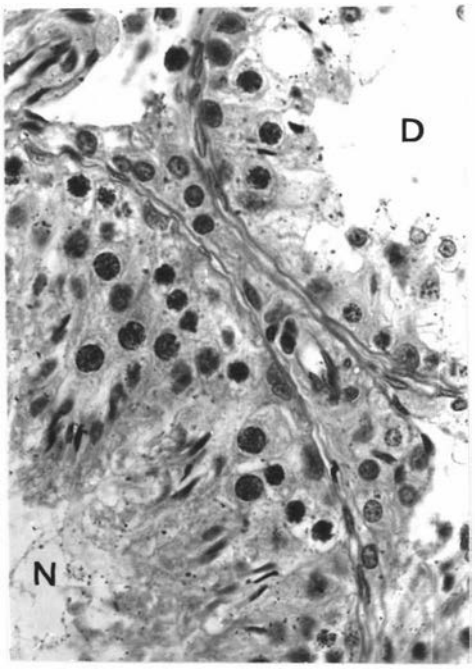


85

Ram 427

Scrotal lesions of Ram 427 were classified as severe when first examined and the classification did not change during the observation period. On the 16.10.70 the distal quarter of the scrotum was covered in caked exudate 1 - 2 cm thick and a few small scabs were scattered over the remainder of the distal half of the scrotum. The lesions increased only slightly in severity during the following month and by the time of slaughter the dermatitis covered the distal third of the scrotum.

On initial examination the testes were small with poor tone and they remained so until the middle of May 1971. From first examination Ram 427 was practically azoospermic, a maximum of 2.4×10^6 spermatozoa per ejaculate being obtained in fifteen samples collected between the 20.10.70 and 12.5.71. During this period there was very little seminal debris.

The left testis was removed surgically on 23.11.70 and weighed only 45g. Testicular atrophy was uniform both within a section and between the two samples. In many tubules there was only a single layer of cells while in some tubules a few degenerating primary spermatocytes were observed (Figs. 86, 87).

Whereas, successful treatment of the lesions of the previous three rams occurred within three weeks of the initial treatment, the lesions on the scrotum of Ram 427 were slightly more persistent. Treatment for five weeks was required before the lesions healed completely. The right testis remained very small from December, 1970 to April,

1971 although by the beginning of February testis tone was good. By 12.5.71 the remaining testis appeared to have increased in size and a semen sample collected the same day showed signs of seminal regeneration (Text-fig. 16), about 5% of the spermatozoa collected showed weak oscillatory motion. There were also small numbers of coiled and uncoiled filaments and microspermatozoa observed in the sample. Testis size changed to medium on the 21.5.71 and to large a month later. Seminal regeneration, compared with that of the previous three rams, was slow and the percentage of normal spermatozoa recovered more slowly than did the percentage of motile and the proportion of live spermatozoa. From the semen samples collected during August 1971 it appeared that seminal regeneration was complete (Text-fig. 16).

At postmortem the scrotum and scrotal contents appeared clinically normal and the right testis weighed 184g. Histologically the right testis appeared very similar to the right testis of the previous rams. However, there were more tubules that were very degenerate containing only a basal layer of cells with or without a few primary spermatocytes. The variation in testicular histology of the regenerating testis is illustrated clearly in Figs. 88 and 89 and in the spermatogenic score counts (Table 23).

TEXT-FIG. 16: SEMINAL REGENERATION IN RAM 427 FOLLOWING HEMICASTRATION AND TREATMENT OF "SEVERE" SCROTAL MANGE.

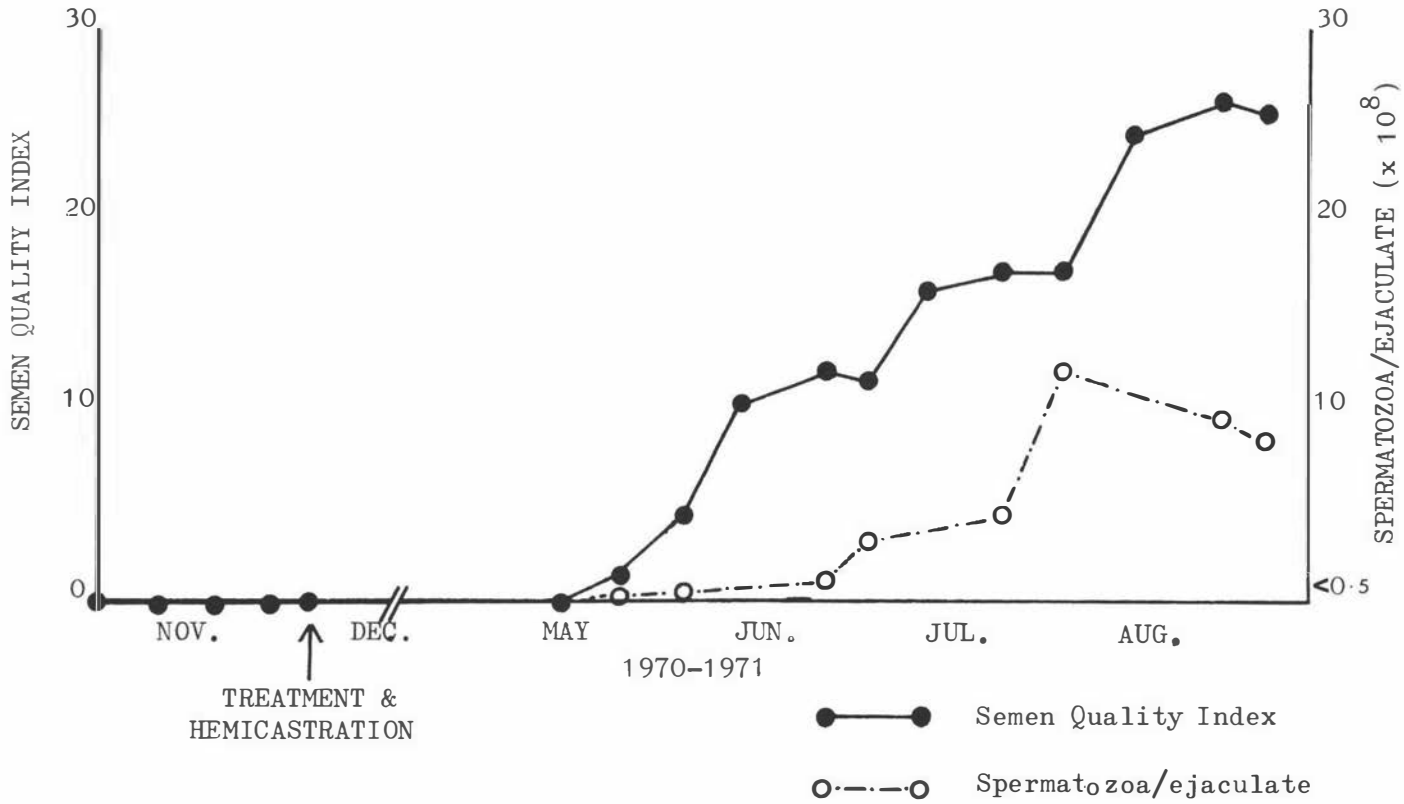


Table 23: SPERMATOGENIC SCORE COUNT OF RAM 427 BEFORE AND AFTER TREATMENT OF "SEVERE" SCROTAL MANGE

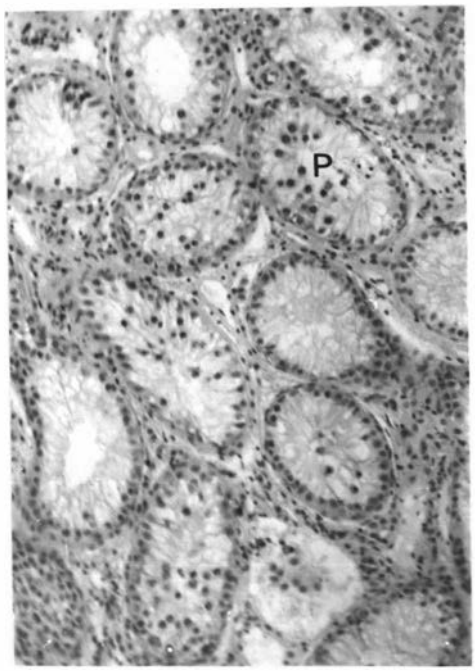
Spermatogenic score	Left testis[1]		Right testis[2]	
	Proximal	Distal	Proximal	Distal
1	-	-	-	-
2	5	6	-	-
3	15	13	4	1
4	24	19	3	5
5	6	12	1	2
6	-	-	1	2
7	-	-	2	1
8	-	-	1	2
9	-	-	5	7
10	-	-	33	30
Position mean score	3.6	3.7	8.6	8.6
Testis mean score	3.7		8.6	

1 Testis removed when severely atrophic

2 Testis removed after seminal regeneration

Figures 86 & 87: Section from the left proximal testis of Ram 427 prior to treatment of severe scrotal mange. Note severe testicular atrophy with relatively widely separated seminiferous tubules. The most mature germ cells present are primary spermatocytes (P) while many tubules have only a basal layer of Sertoli cells (S) and spermatogonia (A).
(H. & E.)
Fig. 86, x 120
Fig. 87, x 400

Figures 88 & 89: Section from right proximal testis of Ram 427 after seminal regeneration following treatment of scrotal mange. Note one degenerate tubule (D) amongst normal tubules (N).
(H. & E.)
Fig. 88, x 120
Fig. 89, x 400



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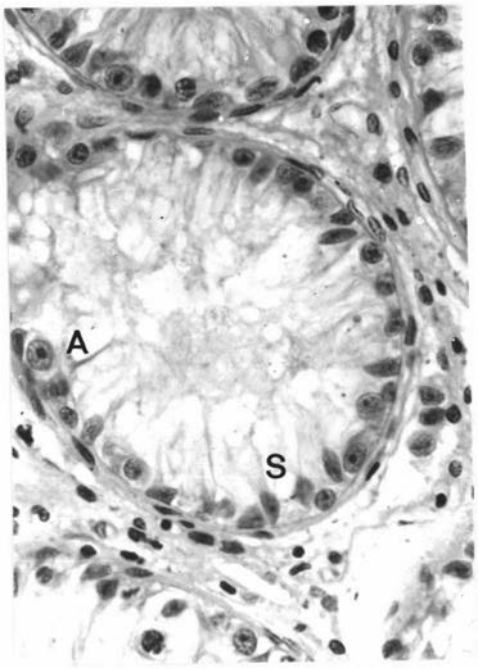

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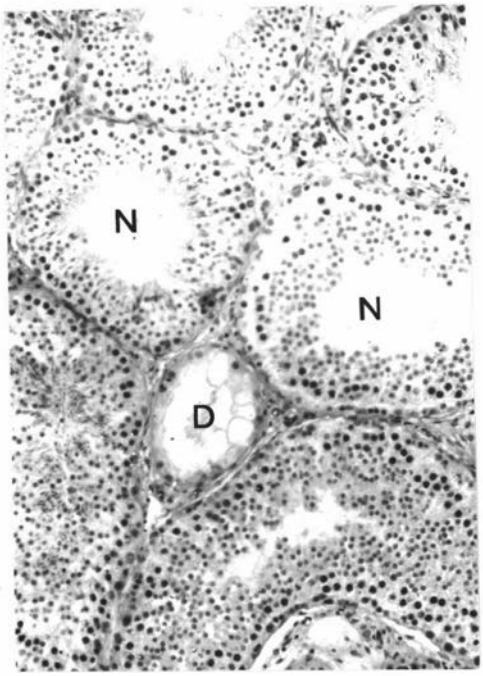

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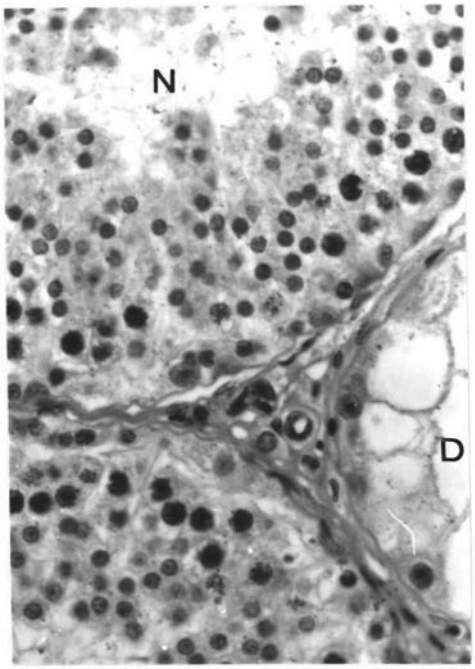


89

Ram 13

In November, 1969 this five year old ram was brought to the University and had severe scrotal mange associated with a thickened pendulous scrotum and testes of moderate size and poor tone. At first examination Ram 13 was severely oligospermic with few motile spermatozoa. Extremely large numbers of C. bovis mites were observed on the legs and scrotum so that the ram was kept as a mite donor. Throughout 1970 and early 1971 the ram was run on pasture without special attention and its semen was not assessed. The lesions which were scattered over all the body of the scrotum, remained severe throughout most of 1970. Some of the lesions cured spontaneously while others formed and still others remained chronically active and the testes remained small. In November, 1970 there was a massive outpouring of serous fluid which covered all the body of the scrotum in typical chorioptic exudate about 3 cm thick. Testis size decreased even further during this period and each testis was estimated to weigh not more than 50g. A gauge 12 needle with embedded thermistor was inserted into each testis during the extreme scrotal mange stage.

Early in January, 1971 a spontaneous cure of the lesions on the body of the scrotum was apparent and by the beginning of March all of the exudate was beginning to lift, leaving a clinically normal skin surface. During the spontaneous cure of the lesions on the body of the scrotum small lesions appeared on the neck of the scrotum so that at the beginning of March 1971 lesions were classified as minimal. The scrotum had become more thickened and pendulous during 1970 and early 1971, and remained thickened and pendulous

until 23.8.71 when the ram was slaughtered. In early May 1971, an improvement in testis size and tone was observed. By this stage lesions had begun to reappear on the body of the scrotum, in association with the reappearance of large numbers of mites on the body of the scrotum. The scrotal lesions which were now classified as minor were treated and signs of testicular regeneration assessed by semen analysis.

A semen sample collected on the 13.5.71 contained 0.8×10^8 spermatozoa of which approximately 10% stained live and 46% were morphologically normal. The majority of abnormalities were pyriform heads, neck abnormalities and tailless spermatozoa. During May, June and July there was a gradual improvement in semen quality and quantity (Text-fig. 17). Three samples collected in August 1971 showed that semen quality had returned to an acceptable level (Text-fig. 17).

At the time of slaughter (23.8.71) the testes were large with moderate tone. The scrotum was extremely pendulous (compare Fig. 90 with Fig. 91) and the scrotum itself was extremely thickened (compare Fig. 92 with Fig. 93) and appeared corrugated. The increase in thickness was reflected in the weight of the scrotum. The scrotum of Ram 13 weighed 850g while those of 3 normal rams of similar body weight to Ram 13 and slaughtered the same day weighed 170g, 210g and 240g. The epidermis and epidermal appendages, including the sweat glands, of Ram 13 appeared histologically normal but there was a large increase in the dermal and subdermal fibrous tissue which accounted for the increased skin thickness. Apart from a small

amount of fibrous tissue on the tunica vaginalis propria where the large needle had been inserted into the left testis, there were no signs of an inflammatory involvement of the tunica vaginalis sac or scrotal contents. The left and right testis weighed 165g and 178g respectively and histologically there were no differences between the two areas sampled within each testis or between the two testes. However, within a section there was a marked variation in seminiferous tubule cytology. Some tubules contained apparently only Sertoli cells, others were in various stages of regeneration while many had relatively normal germ cell numbers in normal cellular associations (Figs. 94 - 97). The spermatogenic score counts in Table 24 illustrate the variation and proportion of tubules at varying stages of regeneration. Compared with the four previous rams there were more tubules, that contained germ cells in all stages of spermatogenesis but with severely reduced numbers, disorganised cellular associations or with relatively large numbers of degenerating cells (Table 24).

TEXT-FIG. 17: SEMINAL REGENERATION IN RAM 13 FOLLOWING SPONTANEOUS CURE OF "EXTREME" SCROTAL MANGE.

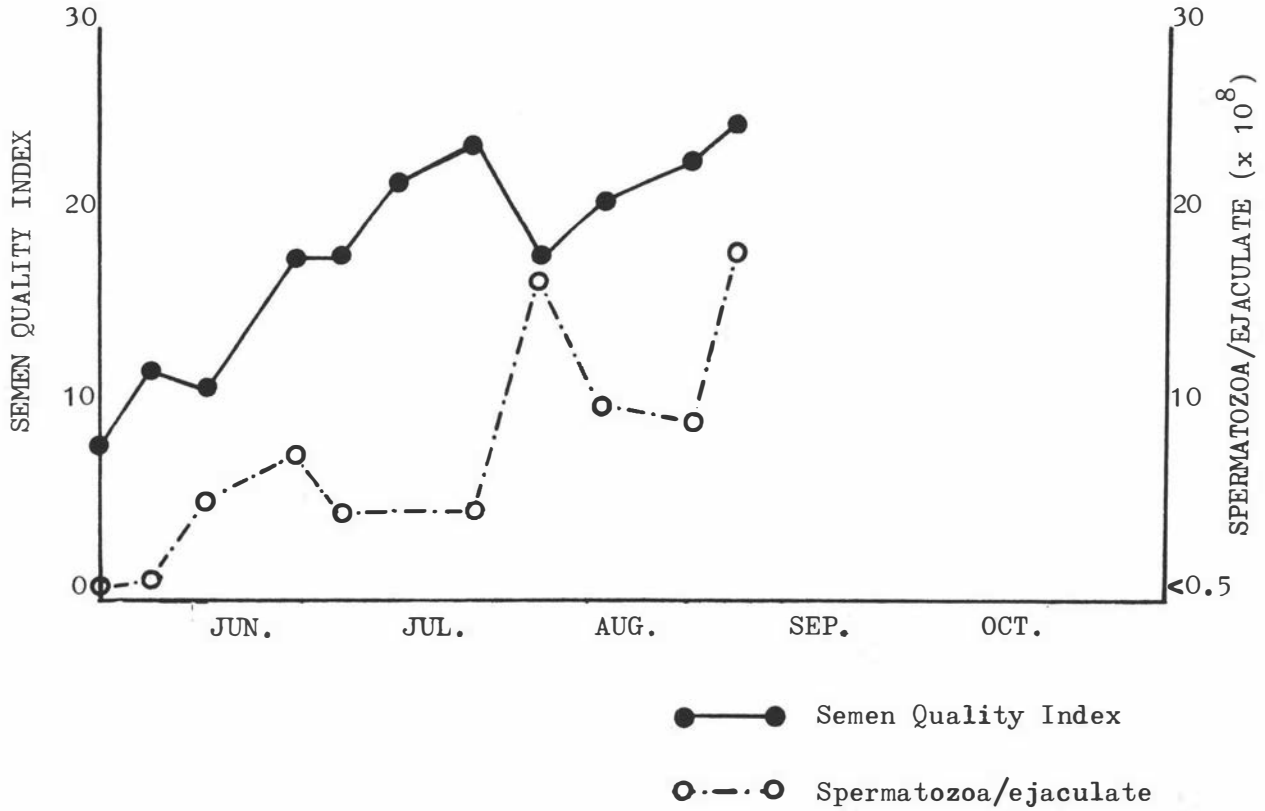


Table 24: SPERMATOGENIC SCORE COUNT OF RAM 13 FOLLOWING SPONTANEOUS CURE OF "EXTREME" SCRTOAL MANGE

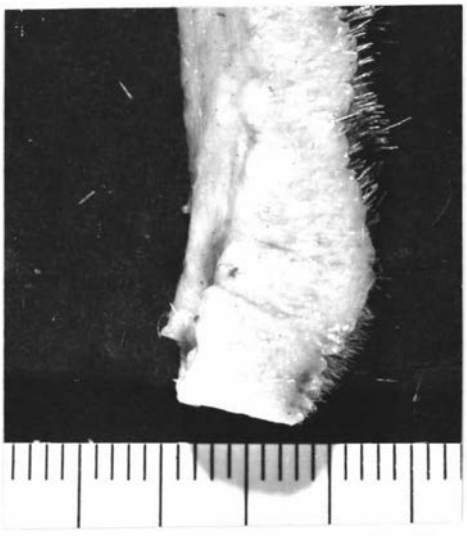
Spermatogenic score	Left testis[1]		Right testis[2]	
	Proximal	Distal	Proximal	Distal
1	-	-	-	-
2	1	3	0	1
3	2	2	3	1
4	5	3	7	8
5	1	2	-	3
6	1	2	4	-
7	-	-	2	1
8	3	4	3	2
9	10	15	8	17
10	27	19	23	17
Position mean score	8.5	8.1	8.0	8.0
Testis mean score	8.3		8.0	

1 Left testis removed when severely atrophic

2 Right testis removed after seminal regeneration

90

91



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Figures 90 & 91: Compare the pendulous scrotum of Ram 13 (Fig. 90) just prior to slaughter with a normal ram photographed the same day (Fig. 91).

Figures 92 & 93: Compare the thickness of the section of skin taken from the distal third of the scrotum of Ram 13 (Fig. 92) and from the distal third of the scrotum of a normal ram (Fig. 93).
(1 small division, 1 mm)

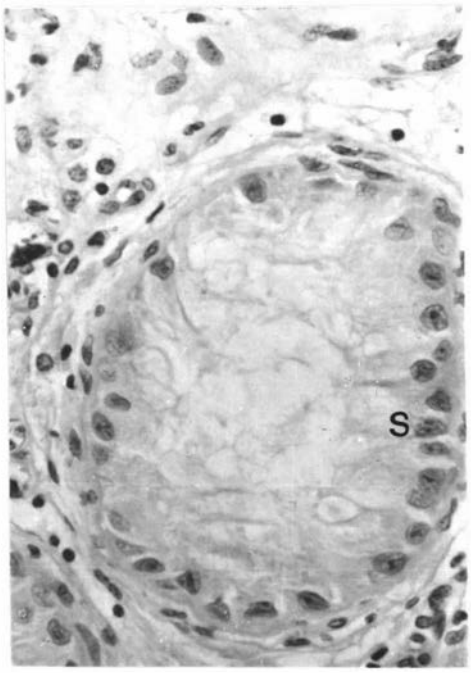
Figures 94-97: Section taken from the left distal testis of Ram 13 illustrating the degree of variation in seminiferous tubule cytology after semen quality had returned to acceptable levels.

Figure 94: Seminiferous tubule with a single basal layer of Sertoli cells (S) without any spermatogonia.
(H. & E.)
(x 400)

Figure 95: Tubule containing a few elongating spermatids (E). Cell numbers are still very reduced and many of the germ cells are degenerating (D).
(H. & E.)
(x 400)

Figure 96: Tubule containing many elongating spermatids. However, cell numbers and cellular associations still not normal.
(H. & E.)
(x 400)

Figure 97: Tubule with approximately normal numbers of germ cells in all stages of spermatogenesis and in normal spatial relationship.
(H. & E.)
(x 400)



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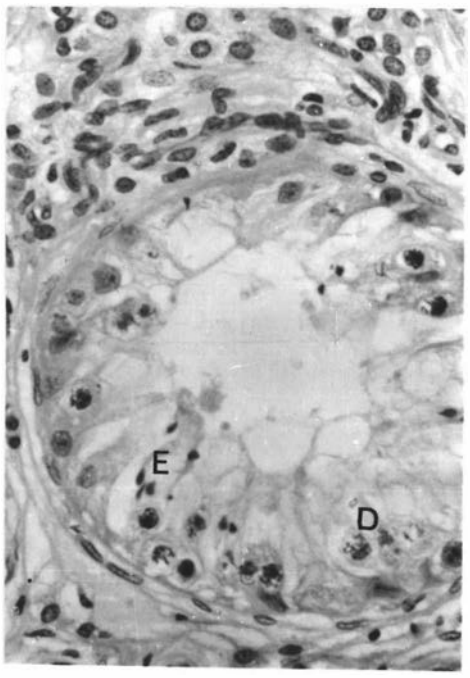

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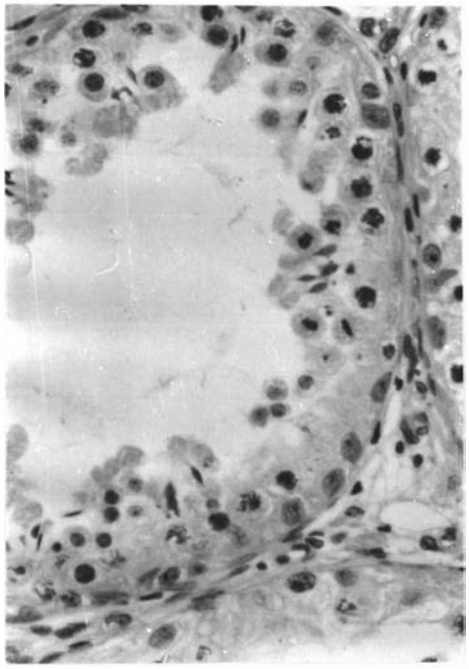

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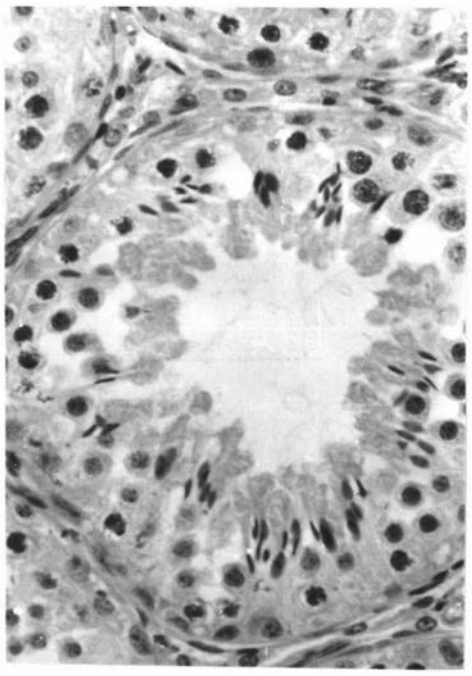

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7.4 Discussion

It would appear that seminal recovery from very severe scrotal mange in rams does take place. Even Ram 13 whose scrotum was considerably thickened and pendulous, due to long term chronic scrotal mange, finally produced semen of acceptable quality after spontaneous cure of the scrotal lesions.

There is little comparable information available on the recovery of reproductive function following simple scrotal dermatitis. Gunn et al. (1942) noted a severe seminal degeneration associated with both an arsenical dermatitis of the scrotum and cutaneous myiasis of the scrotum. A seminal regeneration occurred in all rams which was complete within four months. However, the seminal degenerations associated with both arsenical dermatitis of the scrotum and cutaneous myiasis of the scrotum were probably due to systemic effects as well as local effects of the two agents (Gunn et al., 1942), and therefore, may not be comparable with the effects of scrotal mange. Recently, Wollrab (1965) produced a scrotal dermatitis by rubbing Ichthammol ointment into the scrotum of 2 bulls twice daily for 40 days. The bulls were practically azoospermic twenty-three days after the beginning of treatment. The testicular histology of one bull slaughtered at the end of treatment was very similar to that seen in rams with extensive scrotal mange. In the other bull a seminal regeneration occurred after discontinuing treatment and was more or less complete within four months.

It has been known for a long time that the stem cells of the spermatogenic series, the

spermatogonia (Fuki, 1923; Moore and Oslund, 1924; Young, 1927) or at least some classes of A type spermatogonia (Oakberg, 1959; Waites and Ortavant, 1967, 1968; Dym and Clermont, 1970), are very resistant to noxious stimuli and that on removal of the noxious stimuli these cells will give rise to a new series of germ cells in normal cellular associations (Young, 1927; Moore, 1926; Nelson, 1951; Dym and Clermont, 1970). Similarly the spermatogonia, or at least some spermatogonia, appear to be very resistant to the effects of scrotal mange (C. bovis). Spermatogonia were observed in the majority of seminiferous tubules of even the most severely affected ram. Further the testicular regeneration that occurred in the remaining testis shows that these cells are functionally competent.

In one of the rams examined in this section, testicular regeneration was complete while in the others, especially where the testes had been atrophic for some time, a proportion of seminiferous tubules had not completely regenerated. A small number of these tubules were indistinguishable cytologically from those seen in the left testis removed prior to treatment, while many were in various stages of regeneration. Similarly, there was a wide range in seminiferous tubule activity in rams that were slaughtered during the early stages of testicular regeneration following spontaneous cure of extensive scrotal mange (see Chapter 6).

A similar phenomenon, that is, apparently uniform spermatogenic arrest followed by uneven tubular regeneration, has been observed in guinea

pigs whose testes have been exposed to high temperatures for short periods (Young, 1927), rats made experimentally cryptorchid (Nelson, 1951) and bulls with experimentally induced scrotal dermatitis (Wollrab, 1965). In the case of the rams with scrotal mange, some seminiferous tubules were still in a state of regeneration when seminal regeneration appeared to be complete. A very small number of tubules were indistinguishable from those of the left testis observed prior to treatment. These tubules may be permanently atrophic. Young (1927) noted a similar phenomenon in guinea pigs whose testes were exposed for a short period (15 min) to a high temperature (47^{o}C). Although most of the seminiferous tubules returned to normal following treatment a very small number remained permanently atrophic.

Not only was there a variation in tubular regeneration in individual testes following treatment of extensive scrotal mange, but there were also large differences between rams in the onset of seminal recovery. In general, rams that had severe testicular atrophy for some time took longer to recover than rams that had apparently similar testicular degenerations but of shorter duration. Signs of seminal regeneration were apparent within two months of treatment in the three rams that were undergoing testicular atrophy during the month prior to treatment while the fourth ram of the group, which had severe testicular atrophy for more than a month did not show signs of seminal recovery until six months after the beginning of treatment. This ram also had the largest proportion of degenerate tubules at slaughter. Nelson (1951) demonstrated a similar

phenomenon in rats that had been made experimentally cryptorchid for varying periods. The longer the testes were retained intra-abdominally the longer was the period required for testicular regeneration and less tubules recovered completely.

It is generally accepted that the time taken for spermatogenesis in most species is a "biological constant" (Ortavant, 1959), the process taking approximately 49 days in the ram (Ortavant, 1956). Further, spermatozoa have been shown by various techniques to take 1 - 2 weeks to traverse the epididymis in the ram (see Rowley et al., 1970). Therefore, the large between ram and between tubule within ram variation in seminiferous tubule activity cannot be accounted for by changes in the process of spermatogenesis or in the rate of passage of spermatozoa.

Recent evidence suggests that variations in spermatogenic activity may account for the variations observed in this and other studies. It has been shown in the rat (Clermont and Bustos-Obregon, 1968) and the monkey (Clermont, 1969) that there may be two general classes of A type spermatogonia; the "renewing" stem cells and the "reserve" stem cells. According to these authors the "renewing" stem cells give rise to the differentiating spermatogonia (e.g., A_2, Intermediate, B type spermatogonia) and more "renewing" stem cells while the reserve stem cells participate "little if at all" in the routine production of spermatocytes (Clermont and Bustos-Obregon, 1968; Clermont, 1969). The same group of workers have recently shown that X-irradiation (300r) of the rat testis caused a severe depletion of the "renewing" stem cells but

had relatively little effect on the "reserve" stem cells. After treatment the "reserve" stem cells became mitotically active and largely contributed to the spermatogonial repopulation (Dym and Clermont, 1970). The complete process of stem cell renewal took about forty days.

Although the differentiating spermatogonia of the ram (A_2, Intermediate, B type spermatogonia) are relatively susceptible to the effects of heat (Waites and Ortavant, 1967, 1968) there is no evidence of "reserve" and "renewal" populations of germ cells in this species. However, indirect evidence suggests that such a phenomenon may occur in the bull (Hochereau, 1968). A study of the spermatogonial population in the normal ram, in the ram at the height of testicular degeneration, and in the ram during the early stages of testicular recovery, may explain why some tubules and some testes take much longer than others to recover from apparently similar degrees of testicular degeneration. Such a study would probably also give a better understanding into the marked resistance of at least some germ cells to the long term effects of scrotal mange.

Spermatozoa produced after complete seminal regeneration following a period of severe seminal degeneration are of normal fertilizing capacity (Young, 1927; Gunn et al., 1942; Bowler, 1967; Braden and Mattner, 1970). In this study seminal regeneration of three rams with scrotal mange was complete in the Autumn. Semen quality of these rams prior to slaughter was high, virtually 100% of the spermatozoa being rapidly motile with practically no morphological abnormals. Spermatozoa

produced by these rams, therefore, is almost certain to have been of high fertilizing capacity. The other two rams were slaughtered in the early spring, a time of the year when reproductive function of many breeds of rams is slightly reduced (McKenzie and Berliner, 1937; Starke, 1949; Dun et al., 1960; Ortavant et al., 1964). Although the semen quality of these two rams was slightly inferior compared with the first three rams, the semen quality was still within the limits of normal fertility as shown by Edgar (1959) and Hulet et al. (1965).

PART III

CAUSE OF THE TESTICULAR DEGENERATION ASSOCIATED WITH EXTENSIVE SCROTAL MANGE

Chapter 8

HEAT AS A CAUSE OF THE TESTICULAR DEGENERATION OBSERVED IN RAMS WITH EXTENSIVE SCROTAL MANGE

8.1 Introduction

It is well known that the scrotum provides a suitable thermal environment for normal spermatogenesis. Evidence for this has been derived from many experiments. For example, Moore and Quick (1924) found the temperature inside the tunica vaginalis was several degrees cooler than inside the body cavity. Transplanted testes produced spermatozoa only if they were put in the scrotum (Moore, 1923, 1924b, c) or in a site with a similar temperature to the scrotum, for example, the anterior chamber of the eye (Turner, 1938). When the scrotum of a ram was insulated for eighty days, all seminiferous tubules were damaged to various degrees and "the animal sterilized itself with its own body heat because of the interference with the local regulatory capacities of the scrotum" (Moore and Oslund, 1924a).

In many studies the body-testes temperature gradient of the ram has been shown to be in the vicinity of 5°C (Phillips and McKenzie, 1934; Harrison and Weiner, 1948; Foote et al., 1957; Waites and Moule, 1961; Johnson et al., 1969). Waites and Moule (1961) confirmed the earlier proposal of Harrison and Weiner (1949) that most of the temperature drop occurred in the spermatic cord, the heat being transferred from the internal

spermatic artery to the venous blood in the pampiniform plexus. Waites and Moule (1961) found the temperature of the blood in the internal spermatic artery where it first passes on to the testis surface was approximately 5°C cooler than the blood entering the same artery from the aorta, while blood leaving the pampiniform plexus was about 5°C warmer than the venous blood on the testis surface. However, the vascular heat exchange is not autoregulatory; the mechanism only serves to cool the testis when the returning venous blood from the testis is cooler than the arterial inflow. This relationship can be maintained only if heat is being lost through the scrotum (Waites and Moule, 1961). Thus the countercurrent heat exchange, which is the main mechanism for cooling the testis, is dependant on the normal functioning of the scrotum.

The scrotum has many features which facilitate heat transfer. The scrotal skin of the ram is thinner than the skin covering most body regions (Kozlowski and Calhoun, 1969); there is usually a low density of wool fibres (Lyne and Hollis, 1968); there is a lack of subcutaneous fat and relatively large scrotal sweat glands (Waites and Voglmayr, 1962) capable of producing more sweat than the glands on the body (Brook and Short, 1960). Further, on leaving the spermatic cord the spermatic artery passes over the testis surface to the ventral pole where it divides and the branches from it ramify under the tunica albuginea before penetrating the testis surface, so that the major testicular blood vessels are in close proximity to the scrotum, allowing maximal heat exchange between testis and scrotum. These anatomical

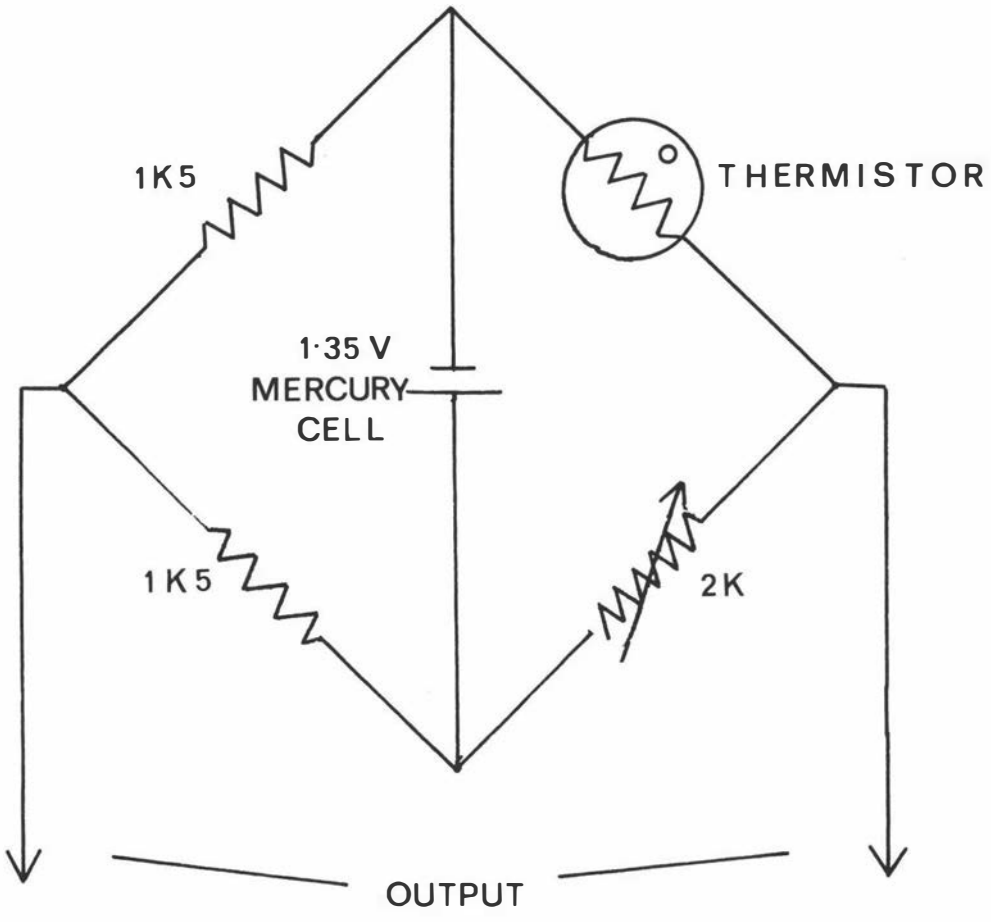
factors suggest that changes in scrotal temperature would be reflected by changes in testicular temperature. Waites and Moule (1961) applied warm and cool air to the scrotum and found that "the temperature of the venous outflow from the testis is always similar to the temperature beneath the scrotum." The increased venous blood heat was rapidly transferred to the whole testis via the countercurrent heat exchange mechanism, so that the temperature of the testicular tissue approached that of the inside of the scrotal skin (Waites and Moule, 1961).

It is likely that extensive scrotal mange interferes with the normal thermoregulatory function of the scrotum, resulting in an increase in testicular temperature which would cause the severe testicular degeneration observed. To test this hypothesis subscrotal, intratesticular and body temperatures were recorded before slaughter from both control and affected rams. In other rams temperatures were recorded from the left side prior to treatment of scrotal mange and from the right side after the scrotal lesions had been cured and there were signs of testicular recovery.

8.2 Materials and Methods

Temperatures were recorded with a Philips (E205CE/P1K5) thermistor which was embedded with araldite in a 3 inch, 12 ga. S.W.G. hypodermic needle with the sensory tip exposed. The thermistor was placed in a Wheatstone Bridge Circuit (Text-fig. 18) and a potentiometer was used to balance the bridge at 33°C. The output of the bridge was fed into a Beckman Linear Chart Recorder (model 93507),

TEXT-FIG. 18: WHEATSTONE BRIDGE CIRCUIT WITH THERMISTOR (PHILIPS E205CE/P1K5) INCORPORATED.



Thermistor embedded with araldite in 12ga.S.W.G. hypodermic needle with sensing tip exposed. 1K5 ohm resistors high stability types. 2K ohm wire wound potentiometer used to balance bridge at 33°C. Output of bridge fed into a Beckman Linear chart recorder (model 93507).

which had a 100mV span covering 5 inches of chart. The chart was graduated from 0 - 100. The apparatus was calibrated against a clinical thermometer (G. H. Zeal, London) which complied with British Standards 691, including accuracy to within 0.1°C. The clinical thermometer was checked for accuracy by Tasman Vaccine Laboratory, New Zealand. The clinical thermometer was marked from 95 - 110°F (35 - 43°C) being above the minimum temperature (33°C) required. Therefore, the instrument was also calibrated against an accurate mercury in glass thermometer with a range of 0 - 50°C (G. H. Zeal, London. B.S. 1365/80 N/F ACN/321). Details of the calibration of the apparatus are recorded in Appendix 8. The simple regression equation was:

$$y = 33.0 + 0.117x,$$

where y = °C and x = number of chart divisions. The recording equipment was checked against the clinical thermometer at 35°C and 40°C before and after each experimental session. The average of the two chart recordings was always within the 95% confidence limits of the calculated regression line. Air temperature during the various recording sessions varied from 16°C to 22°C.

All rams were shorn two weeks prior to recording testicular, subscrotal and jugular vein temperatures. The scrotum of each control ram was shorn to approximately the same length as that of the affected ram recorded in the same session. The scrotal wool length varied from 1cm to 4cm. For recording temperatures the rams were restrained in a standing position in the Begg's foot trimming cradle. The thermistor needle was inserted, from the posterior aspect of the scrotum, so that the needle tip was near to the centre of the left

testis and the temperature recorded. The needle was then withdrawn until the sensitive tip lay between the testis and scrotum and the subscrotal temperature recorded.

Temperatures were recorded from eleven pairs of rams in the first experiment. One of each pair had either severe or extreme scrotal mange and the other was a control animal. Affected and control rams were alternated for first temperature recording and each pair of rams were recorded within a half hour period. At the time of temperature recording all rams with scrotal mange had severe testicular degeneration with no signs of seminal or testicular regeneration. Conversely, all the control animals had milky to creamy semen with semen scores of twenty or more.

In the second experiment six rams had their testicular, subscrotal and jugular vein temperatures recorded when extensive scrotal mange was causing severe testicular atrophy and again after successful treatment of the scrotal lesions, when signs of testicular regeneration were apparent. Because of possible complications following the use of the 12 ga. needle, temperatures were recorded prior to treatment from the left testis and left subscrotum and from the right side after treatment.

The data from individual rams with scrotal mange and their respective control rams in the first experiment and data from the left and right side of each ram in the second experiment were compared on a paired basis using "Student's" t-test (Snedecor, 1956).

8.3 Results

(i) Temperature recordings from rams with extensive scrotal mange and from control animals

There were no significant differences ($p>0.05$) between the mean jugular vein temperatures of the scrotal mange and control groups while there were highly significant differences ($p<0.001$) between both intratesticular temperatures and subscrotal temperatures from the two groups. All the affected rams had testes temperatures above those recorded from the control rams on the same day (Table 25). The temperature increase varied from $0.6^{o}C$ to $3.1^{o}C$, with an average increase of $1.8^{o}C$ (Table 25). There was no apparent difference between varying degrees of scrotal mange severity and testis temperature, the average testis temperature of the rams with severe scrotal mange being similar to the average temperature of the rams with extreme scrotal mange. Subscrotal temperatures of affected rams were from $0.4^{o}C$ to $2.8^{o}C$ (average of $1.6^{o}C$) higher than those recorded from the control rams on the same day (Table 25). The individual subscrotal temperatures were similar to the testis temperature in each ram, although many of the individual subscrotal temperatures were slightly higher. In retrospect, this was probably due to the position of the needle within the tunica vaginalis sac. The hypodermic needle containing the thermistor was relatively long and heavy and often, though unintentionally, the heat sensitive tip was directed towards the proximal pole of the testis where the temperature, compared to the lower parts of the scrotum is relatively high. Fowler (1968) has shown that the subscrotal temperature at the point where the neck

Table 25: TEMPERATURE RECORDINGS (°C) IN 11 PAIRS OF RAMS: ONE WITH EXTENSIVE SCROTAL MANGE AND ONE CONTROL

Ram Pair	Left or Right Side	Testes			Subscrotal			Jugular vein		
		Mange	Cont.	Dif.	Mange	Cont.	Dif.	Mange	Cont.	Dif.
1	L	36.2	35.2)	+1.0	37.0	34.7)	+2.1	39.0	38.4	+0.6
	R	36.3	35.4)		36.9	35.1)				
2	L	35.2	33.8)	+1.7	35.0	34.3)	+0.9	38.9	38.9	0.0
	R	35.7	33.6)		35.4	34.3)				
3	L	36.3	33.4)	+3.1	35.7	33.8)	+2.1	38.5	38.7	-0.2
	R	36.5	33.2)		36.4	34.1)				
4	L	34.9	34.2)	+0.6	34.5	35.1)	+0.4	38.7	38.9	-0.2
	R	34.9	34.4)		35.7	34.2)				
5	L	35.9	33.7)	+2.0	37.6	34.3)	+2.3	39.2	38.9	+0.3
	R	35.7	33.9)		35.7	34.3)				
6	L	36.1	33.8)	+2.4	36.6	33.8)	+2.8	38.8	38.5	+0.3
	R	36.5	33.9)		36.8	34.1)				
7	L	36.3	34.3	+2.0	36.5	34.6	+1.9	38.9	38.7	+0.2
8	L	35.4	34.4	+1.0	35.6	35.0	+0.6	38.7	38.8	-0.1
9	L	36.4	34.5	+1.9	37.1	35.7	+1.4	39.4	38.5	+0.9
10	L	36.4	34.4	+2.0	36.4	35.1	+1.3	38.9	39.5	-0.6
11	L	36.9	35.4	+1.5	37.6	35.4	+2.2	38.5	39.0	-0.5
Mean		36.0	34.2	+1.8	36.3	34.7	+1.6	38.9	38.8	+0.1

Pair No. 1, (M = 359, C = 162); No. 7, (M = 155, C = 134);
No. 2, (M = 92, C = 107); No. 8, (M = 373, C = 439);
No. 3, (M = 604, C = 106); No. 9, (M = 612, C = 860);
No. 4, (M = 985, C = 253); No.10, (M = 18, C = 66);
No. 5, (M = 572, C = 105); No.11, (M = 149, C = 148).
No. 6, (M = 13, C = 32);

M = Ram with extensive scrotal mange; C = Control ram.

Rams 359, 92, 604, 985 and 155 had "severe" scrotal mange while Rams 572, 13, 373, 612, 18 and 149 had "extreme" scrotal mange.

of the scrotum starts to increase in diameter is $2^{o}C$ higher than the testicular temperature in the proximal pole. Further, subscrotal temperature recordings could be varied as much as $2^{o}C$ by simply redirecting the needle tip in the scrotal sac. This would account for the relatively large variation between subscrotal temperatures from the same ram compared with the small variation in intratesticular temperatures (Table 25).

(ii) Temperature recordings before and after treatment of extensive scrotal mange

There was no appreciable difference in the mean jugular vein temperatures of the six rams before ($38.9^{o}C$) and after ($38.8^{o}C$) successful treament of extensive scrotal mange. However, the testes and subscrotal temperatures of all six rams were lower after successful treatment (Table 26). Differences in temperature from the left testis (before treatment) and from the right testis (after treatment) varied from $0.6^{o}C$ to $2.3^{o}C$ while differences in subscrotal temperature varied from $1.3^{o}C$ to $3,2^{o}C$. Average testes temperature dropped from $36.3^{o}C$ to $34.7^{o}C$ while the average subscrotal temperature dropped from $36.6^{o}C$ to $34.6^{o}C$. These differences were highly significant, ($p<0.001$).

8.4 Discussion on the Cause of the Testicular Degeneration Associated with Extensive Scrotal Mange

The mean testis temperature of the control rams ($34.2^{o}C$) and of the rams that had been cured of scrotal mange ($34.7^{o}C$) were similar to the average testicular temperatures recorded from control animals by many authors. For example, Phillips and McKenzie (1934), Waites and Moule (1961) and Johnsen

Table 26: TESTICULAR, SUBSCROTAL AND JUGULAR VEIN TEMPERATURES (°C) IN 6 RAMS, BEFORE AND AFTER TREATMENT OF EXTENSIVE SCROTAL MANGE

Ram No.	Mange	Testis			Subscrotal			Jugular vein		
		Before treat. (L)	After treat. (R)	Dif.	Before treat. (L)	After treat. (R)	Dif.	Before treat. (L)	After treat. (R)	Dif.
83	S	36.3	34.3	+2.0	36.4	34.4	+2.0	39.1	38.9	+0.2
155	S	36.3	35.6	+0.7	36.5	35.2	+1.3	38.7	39.0	-0.3
18	E	36.4	34.2	+2.2	36.4	34.4	+2.0	38.8	38.3	+0.5
149	E	36.9	35.1	+1.8	37.6	35.2	+2.4	38.5	38.5	0.0
612	E	36.4	34.1	+2.3	37.1	33.9	+3.2	39.4	38.9	+0.5
373	E	35.4	34.8	+0.6	35.6	34.3	+1.3	38.7	39.0	-0.3
Mean		36.3	34.7	+1.6	36.6	34.6	+2.0	38.9	38.8	+0.1

S = "severe" scrotal mange E = "extreme" scrotal mange

et al. (1969) recorded average testes temperatures of 34.9°C, 34.2°C and 34.4°C respectively. Moule and Knapp (1950) recorded a testis temperature of approximately 1°C higher than obtained in this study while Fowler (1968) recorded an average testicular temperature of approximately 1°C cooler. The testis temperature variation between control rams (20% C.V.) was greater than that obtained by Waites and Moule (1961) (11%C.V.), similar to that obtained by Fowler (1968) (17% C.V.) and below that of Johnson et al. (1969) (26.6% C.V.).

The average increase of 1.8°C in testicular temperatures of rams with extensive scrotal mange is sufficient to cause the severe testicular degeneration seen in these animals. Phillips and McKenzie (1934) found that testicular degeneration caused by experimental scrotal insulation was associated with an average increase in testes temperature of 2.1°C. Moule and Knapp (1950) measured testicular temperatures from three rams and found that experimental scrotal insulation increased testes temperatures by approximately 1.5°C. Johnson et al. (1969) exposed rams to a controlled environmental temperature of 32.2°C for 1 - 2 weeks and found that the resulting severe testicular degeneration was caused by a 1.3°C rise in testes temperature.

Testicular histopathology of rams with extensive scrotal mange was similar to that seen in rams where testicular temperature was increased by other means (see Chapter 6). Further, the scrotal mange lesions were limited to the scrota of all rams examined; the tunica vaginalis sac, the testicular capsule and the testes showed no signs

of inflammatory involvement (see Chapter 6). The rams examined in this, and previous sections of this study showed no signs of general ill health. Blood temperatures, bodyweight, appetite, liveliness and general appearance were apparently unaffected even in rams with the most extensive scrotal lesions.

The evidence presented supports the hypothesis that severe scrotal mange causes testicular degeneration by increasing the temperature of the scrotal contents and that other factors, for example toxic agents from the local inflammatory reaction, are probably not involved.

The second experiment demonstrates clearly that the testicular and seminal regeneration associated with successful treatment of scrotal mange is accompanied by a return of testicular and subscrotal temperature to normal levels. Ideally subscrotal temperatures should have been recorded during various phases of scrotal mange development and not simply at the height of scrotal lesion development and after testicular regeneration. However, repeated scrotal temperature recordings may have led to the introduction of pathogenic bacteria from the scrotal skin and possible rupture of blood vessels resulting in haemorrhage into the tunica vaginalis sac.

The increase in testis temperature caused by extensive scrotal mange was probably due to both the mechanical insulation effect of the exudate and to the actual dermatitis. The proportion of the temperature increase attributable to each factor probably varies from ram to ram depending on the severity of the dermatitis and the resultant

exudate formation. In some rams, caked exudate matted the wool together over most of the scrotum so that simple insulation by the caked exudate would probably be sufficient to cause the associated testicular degeneration. In other rams the dermatitis *per se* could have accounted for the testicular degeneration. For example, a non exudative scrotal dermatitis, experimentally induced in a bull, has produced a testicular degeneration almost identical to that seen in rams with extensive scrotal mange (Wollrab, 1965).

Chapter 9

GENERAL DISCUSSION AND CONCLUSIONS

9.1 Chorioptic Mange

From the available evidence it would appear that C. bovis causes the lesions of chorioptic mange and that these are almost certainly the result of an allergic reaction to the mite or its products.

As reported previously (Matthysse and Marshall, 1963; Roberts et al., 1964), small and sometimes extremely large numbers of mites were seen on sheep without lesions. Direct microscopic observation of the skin surface of rams has shown that the great majority of mites are found on the lower legs and on the scrotum with only small numbers very occasionally being observed on other body regions. The most common site for the mites on the lower legs was in the region of the accessory digits and above the coronary band of the hoof while on the scrotum, mites tended to accumulate on the base. The mites soend all their life on the skin surface, often in groups, feeding on skin debris and the superficial layers of the epidermis.

The scrotal lesions caused by C. bovis varied in size from covering a few sq. mm to the whole of the scrotum. Some lesions developed slowly, others very rapidly, while still others remained small and discrete for many months. The lesions were limited to the more superficial layers of the scrotum, the inflammatory process neither

involving the tunica vaginalis sac nor the scrotal contents.

Spontaneous cure of scrotal mange was observed at all stages of lesion development. This was associated usually with a loss of mites in the vicinity of the lesions. In sheep (this study) and cattle (McEnerney, 1953) large numbers of mites have been observed trapped in the serous exudate suggesting that the host skin response is a protective one. In most instances there was no sign of a prior scrotal dermatitis in rams that had spontaneously cured or had been treated successfully for scrotal mange. In a few severe longstanding cases there was permanent skin thickening, especially of the more distal aspects of the scrotum, and when contracted the scrotum appeared very corrugated.

As all previous reports on chorioptic mange in sheep have been brief, accurate diagnosis of scrotal mange was difficult. Observations in this study on some aspects of the pathogenesis of scrotal mange show that the main features by which the syndrome is identified are:

(a) The type of skin response.
(b) The presence of lesions on the lower legs as well as on the scrotum.
(c) Position of the lesions on the lower legs and scrotum.
(d) The isolation of C. bovis mites.

The skin response: The skin response is typical of an exudative allergic dermatitis. The exudate is straw coloured and when dry has a crumbly texture. In very small lesions the exudate may

be only 1 - 2 mm in diameter while in severe cases the crusts may coalesce so that almost all of the scrotal wool is matted with exudate 4 - 5 cm thick. Removal of the exudate reveals smaller areas of broken skin which exude small amounts of clear fluid. Scab removal causes usually petechial haemorrhages which streak the clear fluid. The histological picture reflects the exudative nature of the dermatitis, for example, one of the noteable features in the acute stage being extensive spongiosis. The initial cellular response is mild with almost all of the cells being mononuclear, the majority of these being lymphocytes. However, polymorphs predominate in most clinical cases of scrotal mange and the majority of these are eosinophils.

Lesions on the feet as well as the scrotum: In nearly all rams with chorioptic mange of the scrotum there were similar lesions, although often only mild, on the lower legs.

Position of the lesions: Lesions were found most frequently on the scrotum and lower legs where mite numbers were highest, that is, on the base of the scrotum and between and below the accessory digits.

Oral response to manipulation of the lesions: Manual manipulation of the scrotal and leg lesions of chorioptic mange resulted often in a characteristic nibbling response from the ram. The response appeared to be associated with both the mites and the lesions. Although the nibbling response has been associated only with C. bovis mites or chorioptic mange it cannot be regarded as pathognomonic for either.

Detection of C. bovis: Chorioptes bovis mites have been observed prior to or at the time of development of every case of experimentally induced scrotal mange. However, in agreement with other reports there was little relationship between lesion severity and scrotal mite numbers. In many cases of extensive scrotal mange only the occasional mite was observed.

After taking 60 - 70 scrapings from a ram with typical extensive lesions of chorioptic mange of the scrotum, McKenna and Pulsford (1947) detected only three mites. Therefore, negative scrapings from a ram with scrotal lesions cannot be taken as indicative of the lesions being caused by something other than C. bovis. Direct microscopic observations show that live mites are most likely to be detected on the dry skin surface at the periphery of exudative lesions. Occasionally dead mites can be detected in the exudate itself.

If the above features are considered, there should be no difficulty in differentially diagnosing chorioptic mange of the scrotum from other reported causes of scrotal dermatitis (Table 27).

Of the rams examined with scrotal mange nearly all were Romneys, but since more than 80% of the sheep in the North Island of New Zealand are Romneys, it cannot be concluded that this breed of sheep is particularly susceptible to scrotal mange. However, the high incidence of scrotal mange in the few flocks examined by the author suggests that scrotal mange is relatively common in this particular breed. A noticeable characteristic of the New Zealand Romney and the Merino, the only

Table 27: REPORTS OF AGENTS OR SYNDROMES, APART FROM *CHORIOPTES BOVIS*, CAUSING OR IN ASSOCIATION WITH SCROTAL LESIONS IN THE RAM

AGENT OR SYNDROME	REFERENCE
Contagious ecthyma	Ohman, 1941
Arsenic	Gunn *et al.*, 1942; Crawford *et al.*, 1970
Cutaneous myiasis	Gunn *et al.*, 1942; Morley, 1948
Trauma	Gunn *et al.*, 1942
Scrotal oedema	Miller and Moule, 1954; Voglmayr and Setchell, 1968
Psoroptic mange	Blood and Henderson, 1963
Sun burn	Whitten, 1964
Dermatophilus spp.	Crawford *et al.*, 1970
Linognathus pedalis	Crawford *et al.*, 1970
Haemaphysalis spp.	Quinlivan and Lindsay, 1971

other breed of sheep with a reported high incidence of scrotal mange (Hiepe and Splitteser, 1962; Hiepe et al., 1968), is that both have relatively woolly scrota. Further, the four Cheviot rams introduced with extensive scrotal mange, compared with the rest of the flock from which they had come, also had very woolly scrota.

An extensive survey of the incidence and severity of scrotal mange of various breeds and perhaps strains within breeds of sheep is required to provide information on breed susceptibility to scrotal mange. For valid comparison, these trials should be carried out in flocks under similar mangement and environmental conditions. As well as surveys on breed susceptibility to scrotal mange other surveys are required to provide information on the incidence and severity of C. bovis mite infestation and chorioptic lesions in the national sheep flock.

From the limited amount of data collected from the field trial rams there was no clear evidence of a seasonal variation in the occurrence of scrotal mange. However, there was a suggestion that mite numbers may be highest in the Autumn. The only month when mites were observed on all of the field trial rams was May 1971 and scrotal mite numbers were highest in many of the rams at this time. High numbers of mites can be found on the scrotum and lower legs all the year so that more extensive trials over a number of years would be necessary to show seasonal fluctuations in C. bovis numbers or the incidence of scrotal mange.

In agreement with the extensive survey of

Riepe *et al.* (1968), no relationship could be shown between mite numbers or scrotal mange and general health. However, *C. bovis* mites and chorioptic mange caused severe irritation to a small number of rams. These animals spent long periods trying to bite and rub the affected parts, getting up and lying down and appeared generally very disturbed. Under extreme circumstances, as in the case reported by Roberts *et al.* (1964), the mites or lesions of chorioptic mange may affect the general health of sheep.

9.2 The Effect of Scrotal Mange on Reproductive Function

(i) Effect on spermatozoa production

Semen samples were collected readily from all rams by electrical stimulation. Under most conditions assessment of semen quality proved to be a valuable method for assessing the affect of scrotal mange on reproductive function. However, in one flock examined with an outbreak of scrotal mange, semen quality from the clinically healthy rams varied from good to very poor. Because of these findings and previous reports of the susceptibility of the N.Z. Romney to apparently mild stresses (Webster, 1937, 1952), care was taken to manage experimental rams under as optimal conditions as possible. For example, they were shorn regularly, had access to shade and shelter and diet was adjusted to avoid extremes in body condition. Under these conditions semen quality of the control rams did not vary greatly so that the only difficulty in incriminating scrotal mange as a cause of poor semen quality was in rams with

only slightly inferior semen. Apart from this, semen quality analysis showed at what stage scrotal mange affected reproductive ability, the degree to which it was affected, the length and time of the effect, when recovery commenced, and when reproductive function had recovered fully. A number of semen collections from any one ram gave a reasonable indication of gross changes in spermatozoa production, although there was a marked week to week variation in spermatozoa numbers collected by electrical stimulation.

A high correlation was obtained between testis size and spermatogenic activity in both this study and a previous study of the ram (Ortavant, 1952). It is suggested that semen quality assessment in conjunction with estimates of testicular size will give a reliable indication of the effect of an agent on the reproductive function in the ram. In the future it is hoped that an orchidometer, as suggested by Bruere (1970), can be made so that testes size in rams with scrotal mange can be assessed more accurately. Before such an instrument could be used with certainty the accuracy of the orchidometer would have to be assessed. Rams to be slaughtered at an abattoir would provide ideal material for such an experiment.

Gonadal and extragonadal spermatozoa counts added little information to that obtained from semen data, testis size and testicular histology and, therefore, would not be recommended for future studies on the effect of scrotal mange on reproductive function.

In contrast, the modified method of Johnsen

(1970), for quantitating testicular histology, proved to be a simple means of obtaining an accurate overall picture of the spermatogenic activity of the testis and more particularly for illustrating variations in activity between seminiferous tubules. However, the method is dependent upon classifying the most mature germ cell present in a tubule, so may be of limited value in assessing the early affects of agents (e.g. X-rays) that affect, at least initially, earlier stages of spermatogenesis.

Unilateral castration proved valuable for assessing spermatogenesis before treatment and again after seminal regeneration was complete. Biopsies taken at various stages of lesion development and regression would have given a more accurate picture of the effect of scrotal mange on spermatogenesis, but a proven safe method for testicular biopsy in the ram has not been described. Recently Galina (1971) found that a modified testicular biopsy technique, involving the use of a 12-gauge Vim-Silverman needle, had no deleterious effects on spermatozoa production in three rams. However, a more thorough investigation of this technique with larger numbers of rams and assessing reproductive function more thoroughly is required before the method could be safely recommended.

Data presented in this thesis shows that active or inactive caked lesions of scrotal mange that involve less than 10 sq. cm of the scrotum probably had no effect on spermatozoa production in the ram. In contrast all rams with active or inactive caked lesions of scrotal mange involving

more than a third of the scrotum showed definite signs of seminal degeneration. Between these two extremes some rams with relatively severe scrotal mange had high quality semen while others with relatively minor scrotal mange had semen of poor quality. Therefore, apart from rams with small scrotal lesions (involving less than 10 sq. cm) and those with extensive scrotal lesions (involving more than a third) it was almost impossible to predict if scrotal mange was affecting spermatozoa production. The variation was probably due to a number of factors, namely: difficulty in accurately classifying the lesions of scrotal mange, varying responses of individual rams to the same degree of scrotal mange and varying rates of lesion development. For example, from the few cases of induced seminal degeneration it appeared that rams with rapidly developing lesions were more likely to be associated with a seminal degeneration than where similar lesions developed over a longer period.

Scrotal mange did not have an all or none effect on spermatozoa production. The effects varied from a transient decrease in semen quality with no apparent effect on semen quantity or testes size, through to virtual azoospermia associated with severe testicular atrophy.

In general, the more extensive the lesions the more severe the effect. For example, most rams with reproductive function affected by minor or moderate scrotal mange had semen of decreased quality while semen quantity was not obviously affected. Conversely many rams with extensive scrotal mange had decreased semen quantity and quality and severe testicular atrophy. However,

there was a wide variation. Some rams with moderate scrotal mange had severe testicular atrophy while others with extensive scrotal mange had only a moderate decrease in semen quality.

In most cases seminal and testicular degeneration occurred rapidly while occasionally the degeneration progressed relatively slowly. In most instances the rate of the degenerative process was related to the rate of lesion development. The testicular degeneration was a simple atrophy with no signs of inflammatory involvement.

In relatively mild cases of seminal degeneration there was a moderate decrease in spermatozoa motility, proportion of live spermatozoa and proportion of abnormal spermatozoa. In the mild cases the majority of abnormal spermatozoa had either bent or coiled tails or were tailless. In slightly more severe cases of degeneration there was an increase in primary abnormalities, for example, spermatozoa with abnormally shaped heads. In relatively severe cases spermatozoa numbers decreased markedly and there was often an increase in seminal debris. The debris consisted mainly of round nucleated and non nucleated cells of varying sizes, coiled and uncoiled eosinophilic filaments and amorphous eosinophilic material. Most of this seminal debris was of testicular origin.

In cases of mild seminal degeneration associated with scrotal mange there was no palpable change in testis size while severe cases were associated usually with a decrease in testis size. In most rams with severe testicular atrophy associated with scrotal mange, spermatogenesis did

not proceed beyond the primary spermatocyte stage and in nearly all cases there was a marked reduction in the number of these cells. In some cases there were a small number of tubules that contained round spermatids. In even the most severe testicular atrophy there was still the occasional tubule with primary spermatocytes and all tubules had a single basal layer of cells. This layer was composed of Sertoli cells with occasional spermatogonia. Further, there was no sign of seminiferous tubule collapse in any of the rams examined and no evidence of interstitial fibrosis. Another feature was the uniform histology throughout the atrophic testis.

In all rams examined there was a recovery of reproductive function associated with the spontaneous cure or treatment of scrotal lesions. Rams with a mild seminal degeneration and with testes of good size recovered within a few weeks while rams with severe testicular atrophy of long duration took many months to recover. It would be necessary to study a large number of animals with varying degrees of seminal and testicular degeneration to show accurately the actual length of time for recovery of reproductive function. In most cases, recovery of reproductive function was considered to be complete when semen of high quality and quantity was produced and virtually all seminiferous tubules were undergoing complete spermatogenesis. It is important to note that recovery of testis size to a clinically acceptable level often preceded the return of semen quality to an acceptable level. The histological studies indicated that in rams whose testes had been atrophic for a considerable time, a small number

of tubules may become permanently atrophic. In spite of this fact these rams ultimately produced semen of acceptable quality.

(ii) Effect of scrotal mange on androgenic status

In contrast to the marked effect on spermatozoa production, no differences were detected in androgenic status between control animals and groups of rams with extensive scrotal mange and severe testicular atrophy. There were no group differences detected in sexual behaviour, seminal vesicle weight, seminal vesicle fructose, seminal plasma fructose, or Leydig cell numbers. Also Leydig cells from both groups stained similarly with Sudan black. The negative results may have been due to either scrotal mange having no effect on the androgenic status or the changes in androgenic status may have been too small to be detected by the methods used.

Large variations in seminal plasma fructose, seminal vesicle weight and fructose content were observed in the control rams, even in those of the same age and breed that had been run together since lambs. Because of the large variation in the fructose estimates of the control rams, scrotal mange would need to have a marked effect on these parameters before differences could be detected between control and scrotal mange groups. Recent evidence from human studies also puts doubt on the use of seminal fructose as an estimate of androgenic status. Moon et al. (1970) have shown that there was no relationship between peripheral blood testosterone and seminal fructose levels. They also showed that testosterone blood levels below the expected normal range were still capable

of maintaining normal seminal fructose. This study of 11 oligospermic men is the only report where peripheral blood testosterone has been compared with seminal fructose levels. Because of the large variation between control animals and the lack of relationship between peripheral testosterone and seminal fructose (Moon et al., 1970) it must not be concluded that seminal fructose is necessarily a sensitive indicator of androgenic status as suggested by Moule et al. (1966) and Mann (1967).

Further, sex drive may not give a reliable estimate of the androgenic status of the mature ram. According to Young (1961) differences in behaviour are not related necessarily to the quantity of androgen, provided a certain minimal amount is present. Even castration of the mature ram does not lead to an immediate loss in sexual behaviour (Phillips and McKenzie, 1934; Banks, 1964; Clegg et al., 1969), some rams showing ejaculatory responses a year after castration (Clegg et al., 1969).

From the above discussion it appears that the indirect methods used for assessing androgenic status may not have been sensitive enough to detect small alterations in testosterone production. With the recent development in this University of a competitive protein binding assay for assessing peripheral blood testosterone (W. Torrey pers. comm.), it is hoped that a more accurate assessment of the effect of scrotal mange on the androgenic status of the ram will be undertaken.

Because of testicular shrinkage it is

impossible to subjectively assess the effects of scrotal mange on Leydig cell numbers. Recently Heller et al. (1971) described a method using the Sertoli cell as a point of reference. As Sertoli cell numbers are not effected by scrotal mange, the method could be used to quantitate the effect of scrotal mange on Leydig cell numbers. However, testosterone production may alter without a change in Leydig cell numbers (Heller and Leach, 1971) so that there is probably little use in quantitating these cells in a clinical study.

Apart from the survey of Crawford et al. (1970) there is virtually no other information available on the effect of scrotal mange on ram fertility. In fact, there is very little information generally on scrotal dermatitis and its association with reproductive function. In man, thickening of the scrotum as a result of elephantitis has been associated with testicular atrophy (Crew, 1922). Scrotal dermatitis caused by arsenic and cutaneous myiasis have been associated with seminal degeneration in the ram (Gunn et al., 1942). However, neither seminal response is comparable strictly to scrotal mange as both agents may affect reproductive ability systemically as well as locally.

The dermatitis produced by rubbing Ichthammol ointment on to the scrota of two bulls (Wollrab, 1965), is the only report of a scrotal dermatitis that can be readily compared with the results obtained in this study. Both Ichthammol ointment and scrotal mange had virtually identical effects on reproductive function in the bull and ram respectively. Ichthammol treatment caused spermatogenic arrest at the primary spermatocyte stage, and

the remaining bull showed a complete recovery of reproductive function. The treatment had no effect on seminal fructose levels.

Paufler (1970) assumed that Ichthammol-induced scrotal dermatitis caused testicular degeneration by raising the temperature of the scrotal contents. Indirect evidence collected during this study suggested also that scrotal mange caused testicular degeneration by raising the temperature of the scrotal contents. This assumption was shown to be correct by recording intratesticular temperatures, scrotal temperatures and jugular vein temperatures from eleven rams with extensive scrotal mange and from eleven control rams. Scrotal mange had no effect on body temperature but the average testicular temperature of the rams with extensive scrotal mange was raised by 1.8^{o}C, sufficient to cause the testicular degeneration observed. Successful treatment of lesions of extensive scrotal mange was associated with a similar drop in testes temperature. No other factors could be incriminated as contributing to the testicular degeneration. For example, the disease had no apparent effect on the general health of the rams, did not involve the scrotal contents directly and did not increase the deep body temperature.

The results of the effect of scrotal mange on reproductive function should be comparable with those of other studies that involve temperature elevation of the scrotal contents. In general, the seminal degeneration observed during development of scrotal mange was similar to that observed after the scrota of rams had been insulated (Moore and Oslund, 1924; Phillips and McKenzie,

1934; Glover, 1956), the exposure of rams to elevated ambient temperature (Dutt and Simpson, 1957; Simpson, 1960; Moule and Waites, 1963) and the circulation of warm water around the scrotal contents (Waites and Setchell, 1964; Braden and Mattner, 1970). The histopathology of testes of rams with extensive scrotal mange was also similar to that seen after scrotal insulation (Moore and Oslund, 1924; Phillips and McKenzie, 1934; Gunn, 1936) raised ambient temperature (Simpson, 1960; Johnson et al., 1969) and experimental cryptorchidism (Moore and Oslund, 1924). Further the wide range of seminal degeneration seen in rams exposed to the same elevated ambient temperature (Moule and Waites, 1963; Fowler, 1968; Smith, 1971) was seen in rams with similar degrees of severity of scrotal mange. Moule and Waites (1963), Fowler (1968) and Smith (1971) have shown that the between ram variation is due to the heat load reaching the testis rather than a variation in the response of the testis to the same amount of heat. The same is probably true for the heat induced degenerations associated with scrotal mange.

Both rams with scrotal mange and rams whose scrotal contents have been exposed to elevated temperatures by scrotal insulation (Glover, 1955), circulation of warm water around the scrotal contents (Waites and Setchell, 1964; Braden and Mattner, 1970) or increased ambient temperature (Gunn et al., 1942; Dutt and Hamm, 1957; Simpson, 1960; Moule and Waites, 1963; Fowler and Dun, 1966; Braden and Mattner, 1970) have shown a complete seminal recovery after treatment. Most of these studies have involved raising the

temperature of the scrotal contents for short periods. However, in studies of experimental cryptorchidism in the dog (Wangensteen, 1927) and the rat (Nelson, 1951) where testicular temperature was elevated for long periods there was evidence of permanent testicular atrophy. It appears that the longer the testes are held at a temperature above normal the higher the proportion of tubules that suffer permanent damage. Therefore it is conceivable that a small number of rams with long-standing, extensive scrotal mange may become permanently sterile.

Although there is general agreement on the effect of elevated temperature on spermatogenesis the same is not true for the effect of temperature on the androgenic status. For example, Glover (1956) and Moule and Waites (1963) obtained an increase in seminal fructose after scrotal insulation and increased ambient temperature respectively while Smith (1971) showed a significant decrease in mean fructose concentration with increasing duration of heat treatment. Clegg (1960) has shown an increase followed by a decrease of seminal vesicle fructose in rats made experimentally cryptorchid. Most studies agree that moderate increases in testes temperature have little if any effect on sexual behaviour of the ram (Phillips and McKenzie, 1934; Gunn et al., 1942; Glover, 1956; Dutt and Hamm, 1957; Howarth, 1969).

Recently in a study on rats Amatayakul et al. (1971) have shown a 50% decrease in serum testosterone within seven days of experimental cryptorchidism and the testosterone level remained at the depressed level for the five week observation

period. Further, the absolute content, but not the concentration of testicular androgen, was reduced about ten times in the unilateral cryptorchid testis of the ram, bull and stallion (Skinner and Rowson, 1967, 1968b). It is hoped that as the methods for measuring peripheral testosterone become more widely used, there will be a better understanding of the effect of elevated temperature, both short and long term, on the androgenic status of the ram.

Now that the effect of scrotal mange on reproductive performance has been defined more clearly an extensive survey needs to be undertaken to find what effect scrotal mange has on the reproductive performance of the New Zealand sheep flock. Surveys would need to be carried out just prior to or preferably during the mating season.

In stud flocks ewes are exposed to one ram at a time and the likely effect of extensive scrotal mange on conception rate is obvious. However, in most commercial flocks in New Zealand ewes are mated to groups of rams and the effect of varying proportions of subfertile or sterile rams on the proportion of ewes becoming pregnant is difficult to assess. In fact, until recently there was no documented evidence on this subject. However, recent evidence from Australia (Fowler and Jenkins, 1970) suggests that when 50% of rams mated to ewes are sterile, even when there were sufficient normal rams for high fertility, the flock reproductive performance is likely to be affected. Fowler and Jenkins (1970) mated 8 rams (4 normal and 4 vasectomized) to 233 ewes for 6 weeks and found that 70.7% of the ewes became

pregnant while 92.1% of the control ewes (100 ewes mated to 3 rams) became pregnant. They noted also that there were 30% fewer pregnancies amongst ewes that had mated with one fertile ram and one or two infertile rams compared with ewes that had mated only once with a fertile ram.

During this study several flocks of rams were examined where there were outbreaks of scrotal mange with a relatively high proportion of rams with extensive lesions of this disease. The preliminary findings of Fowler and Jenkins (1970) would suggest that under such conditions reproductive performance could be affected. However, more information on flocks varying the proportion of sterile and fertile rams and the percentage of rams run with ewes is required before any accurate predictions can be made.

According to the definition of soundness as laid down by the 1969 seminar on "Breeding Soundness in the Ram" (Quinlivan, 1970) any ram for sale with active or inactive extensive lesions of chorioptic mange should be classified as "temporarily unsound". It is important to remember that many of these rams may be of high fertility. Before the rams can be passed as genitally sound the chorioptic lesions must be successfully treated and the rams re-examined. Treatment should include removing most of the exudate and soaking at least the legs and the scrotum in a suitable acaricide. The procedure is repeated at 10 day intervals until recovery is complete. The lesions of chorioptic mange appear to be an allergic response, therefore, immediate regression of the lesions cannot be expected. If scrotal mange is present in more than a small

proportion of the flock all the rams should be treated. Owing to the lack of information available on the treatment of C. bovis in sheep and since the eggs of the mite are relatively resistant to acaricides (Sweatman and Pullin, 1958) the treatment should be repeated after ten days. Although C. bovis is probably susceptible to many acaricides when used in sufficient concentration, overseas studies on cattle suggest that "Ciodrin" (Shell Chemical Co.) is the drug of choice (Matthysse and Marshall, 1963; Matthysse et al., 1967; Smith, 1967). However, a detailed study of the efficiency of various acaricides against the mite C. bovis on sheep under New Zealand conditions is urgently required. In vivo mite detection using a dissecting microscope mounted on a moveable arm would make such a study relatively simple.

Re-examination of rams after successful treatment of active or inactive caked lesions covering more than 10 sq. cm of the scrotum should include estimates of testes size and if just prior to the mating season semen analysis. Significance of estimates of reproductive function in rams that have had scrotal mange should be based on similar assessments made on clinically healthy rams from the same flock.

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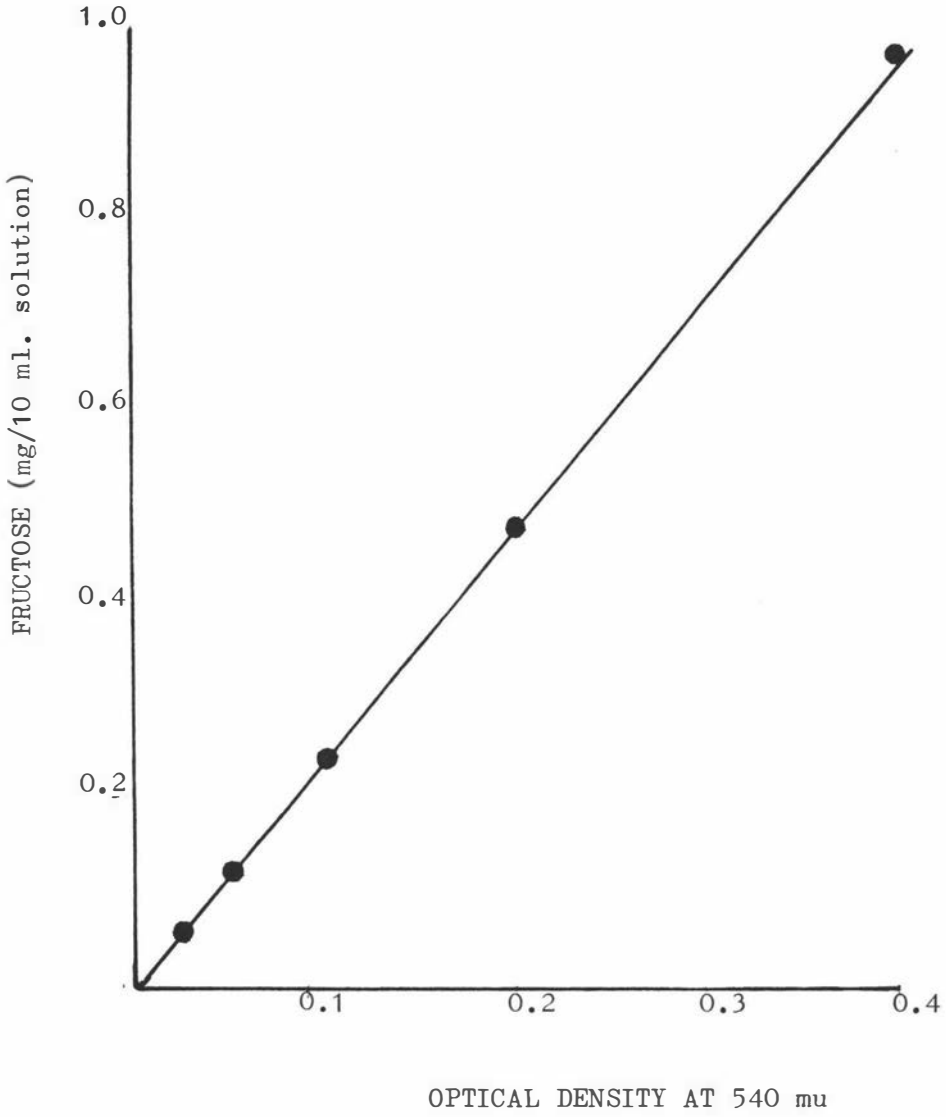
Appendix 1

SPERMATOZOA NUMBERS (x 10^8) ESTIMATED BY DIRECT HAEMOCYTOMETER COUNTS IN SEMEN SAMPLES CATEGORISED ACCORDING TO COLOUR

Cr.	t.mil.	mil.	w.mil.	Cl.
38.0	16.1	9.0	2.7	0.60
27.0	12.2	11.8	1.0	0.05
28.3	11.9	3.4	1.6	0.31
37.8	12.8	9.6	2.4	0.52
31.5	16.8	7.7	2.6	0.37
26.8	14.5	6.9	3.8	0.07
23.3	17.5	6.7	1.6	0.01
20.5	23.0	6.8	1.3	0.77
23.0	17.3	4.2	2.9	0.18
19.5	11.3	7.0	2.8	0.02
39.8	12.0	10.6	1.6	0.55
25.4	11.0	5.7	1.8	0.04
26.8	8.5	6.5	1.2	0.04
21.8	14.3	5.5	1.5	0.29
26.0	20.0	8.8	1.8	0.72
18.0	13.8	2.7	2.4	0.83
23.7	17.5	10.2		
25.0	15.3	8.0		
34.5	11.0	7,2		
33.3	24.0	5.0		

APPENDIX 2

SPECTROPHOTOMETER READING OF STANDARD SOLUTIONS OF PURE D(-)FRUCTOSE.



Appendix 3

MAYER'S HAEMALUM AND EOSIN STAIN AS ADAPTED TO SEMEN BY GUNN ET AL. (1942)

(1) Fix semen smears with methyl alcohol for 2 minutes.

(2) Rinse in water.

(3) Stain in acidified solution of Mayer's haemulum for 6 minutes.

(4) Wash in water for 3 minutes.

(5) Counter-stain with acidified, saturated alcoholic solution of eosin (G. T. Gurr) for 2 minutes.

(6) Rinse in water.

(7) Air dry.

Appendix 4

SEMEN QUALITY AND QUANTITY OF FIELD TRIAL RAM 107 DURING THE PERIOD OF "MODERATE" SCROTAL MANGE

Date 1969/70	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
9.10	0.7	cr.	100	5	80	68	-	10	-	20	2
20.10	1.3	cr.	100	5	93	92	-	2	-	6	-
30.10	2.0	cl.	100	5	90	74	-	5	-	14	7
13.11	1.0	cr.	90	5	88	90	-	2	-	3	5
27.11	1.1	cr.	80	5	88	90	-	2	-	3	5
11.12	1.0	cl.	70	3	91	86	4	3	-	2	5
26.12	0.8	cr.	90	4	98	88	-	9	-	2	1
22.1	1.2	cl.	100	5	80	82	-	5	-	7	6
13.2	0.4	mil.	80	4	84	93	-	1	-	4	2
27.2	0.4	cr.	50	4	47	26	-	15	-	58	1
4.3	0.5	mil.	10	1	15	38	-	27	-	35	-
12.3	0.2	cr.	10	2	36	34	36	6	6	10	8
23.3	0.8	w.mil.	40	3	71	34	1	27	2	32	4
7.4	0.4	mil.	30	2	88	86	-	8	-	6	-
15.4	0.8	cr.	80	5	94	91	-	5	1	3	-
23.4	0.5	cr.	90	4	90	93	-	-	-	7	-

Appendix 4 (Cont'd)

SEMEN QUALITY AND QUANTITY OF FIELD TRIAL RAM 321 DURING THE PERIOD OF "MINOR" SCROTAL MANGE

Date 1970/71	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
16.10	0.4	mil.	60	4	80	71	-	8	-	9	12
30.10	0.5	mil.	60	4	90	87	-	3	-	10	-
6.11	0.5	mil.	60	4	91	69	-	5	-	26	-
21.11	1.0	t.mil.	70	5	60	81	-	2	-	15	2
4.12	0.5	mil.	60	3	61	69	-	2	-	28	1
15.12	0.5	mil.	80	3	82	67	-	11	-	21	1
8.1	0.8	cr.	90	5	91	85	-	2	-	11	2

Appendix 4 (Cont'd)

SEMEN QUALITY OF FIELD TRIAL RAM 214 DURING THE PERIOD OF "MINOR" SCROTAL MANGE

Date 1970/71	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
19.9	0.8	cr.	80	4	97	100	-	-	-	-	-
26.10	0.3	mil.	70	4	47	60	-	27	-	11	2
6.11	1.0	cr.	90	5	50	72	-	23	-	5	-
4.12	1.0	cr.	90	5	80	62	-	33	-	5	-
19.1	1.0	cr.	80	5	86	94	-	-	-	6	-
1.2	1.0	cr.	80	4	89	98	-	-	-	2	-
18.6	0.7	cr.	90	4	91	94	-	3	-	3	-
30.6	1.2	cr.	90	4	87	94	-	1	-	5	-
22.7	0.9	t.mil.	90	4	84	87	-	3	-	8	2

Appendix 4 (Cont'd)

SEMEN QUALITY AND QUANTITY OF FIELD TRIAL RAM 184 DURING THE PERIOD OF "MODERATE" SCROTAL MANGE

Date 1970	Volume (ml)	Colour	Motility (%) (rate, 0-5)	% live	Spermatozoa morphology (%)					
					Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
27.6	0.7	cr.	80 3	70	78	1	14	1	6	-
4.7	0.3	cr.	80 5	76	76	-	6	-	11	7
11.7	0.5	cr.	1 1	2	74	-	6	4	5	11
17.7	0.5	w.mil.	10 2	49	39	10	8	1	21	21
24.7	0.6	w.mil.	10 3	35	24	28	8	3	4	33
30.7	0.4	cl.	1 2	38	16	44	12	6	16	6
10.8	1.0	cl.	10 2	24	49	5	17	3	22	4
20.8	0.6	cl.	10 1	25	23	25	20	8	23	1
30.8	0.8	cl.	10 2	34	29	10	24	5	4	28
9.9	0.6	cl.	30 3	25	34	7	17	6	9	27
19.9	0.6	cl.	40 2	41	48	10	16	1	7	18
26.9	0.5	cl.	40 3	45	34	14	23	3	13	13
3.10	0.6	w.mil.	80 4	93	75	2	3	2	5	13
14.10	1.5	t.mil.	80 5	82	88	-	4	2	6	-
1.11	1.4	t.mil.	70 4	80	75	-	15	2	8	-

Appendix 4 (cont'd)

SEMEN QUALITY AND QUANTITY OF FIELD TRIAL RAM 435 DURING THE PERIOD OF "MODERATE" SCROTAL MANGE

Date 1970/71	Volume (ml)	Colour	Motility (%) (rate, 0-5)		% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
7.9	0.8	cr.	70	4	87	70	-	14	9	1	6
19.9	0.6	cr.	90	5	75	87	-	5	-	8	-
26.9	0.8	cr.	100	5	95	79	-	11	-	10	-
3.10	0.8	cr.	80	4	85	82	-	12	-	5	1
10.10	0.2	cl.	20	4	11	21	-	7	-	67	5
16.10	0.4	mil.	1	2	8	54	-	28	-	18	-
24.10	0.4	mil.	10	2	13	44	-	30	3	22	1
30.10	0.2	mil.	10	2	38	12	7	49	10	17	5
6.11	0.5	cr.	50	2	8	44	-	22	-	34	-
21.11	0.5	mil.	50	2	50	36	3	17	4	29	11
4.12	1.0	mil.	70	3	63	54	-	25	-	10	11
15.12	0.8	t.mil.	30	3	46	17	-	48	8	19	8
20.12	0.4	t.mil.	40	3	47	58	1	21	-	15	5
8.1	0.8	mil.	20	2	11	33	2	9	-	54	2
19.1	1.0	t.mil.	50	2	51	41	4	13	7	24	11
1.2	1.0	mil.	60	4	57	66	-	19	4	8	3
14.2	1.6	w.mil.	90	3	91	90	-	10	-	-	-
27.2	0.8	cr.	80	5	96	97	-	2	-	1	-

Appendix 4 (cont'd)

SEMEN QUALITY AND QUANTITY OF FIELD TRIAL RAM 435 FOLLOWING THE REOCCURRENCE OF "EXTENSIVE" SCROTAL MANGE

Date 1970/71	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
30.6	0.5	t.mil.	90	5	96	75	-	5	-	20	-
8.7	0.6	t.mil.	50	3	44	35	-	35	-	26	4
15.7	1.0	t.mil.	20	3	33	19	21	32	-	28	-
22.7	1.0	cr.	30	4	50	33	-	32	1	32	2
29.7	1.5	t.mil.	90	4	95	77	-	5	-	16	2
9.8	1.2	cr.	40	3	31	82	-	4	1	13	-
16.8	0.8	t.mil.	30	3	55	49	-	1	1	48	1
25.8	1.0	cr.	80	3	62	35	-	10	-	54	1
6.9	1.5	t.mil.	30	3	45	24	-	41	-	34	1
16.9	1.0	t.mil.	60	3	62	42	4	17	5	32	-
26.9	1.3	t.mil.	60	3	59	30	1	20	1	47	1
6.10	1.0	mil.	20	3	24	53	2	15	4	24	2
16.10	0.9	mil.	10	2	31	55	2	22	2	19	-
26.10	0.8	w.mil.	1	1	16	22	11	28	3	18	18
5.11	1.0	mil.	10	2	37	6	-	76	-	18	-
15.11	0.8	mil.	50	3	52	35	1	42	-	22	-
29.11	1.1	t.mil.	80	4	78	78	2	12	-	6	2
15.12	0.8	cr.	90	5	93	96	-	2	-	2	-

Appendix 4 (cont'd)

SEMEN QUALITY AND QUANTITY OF FIELD TRIAL RAM 613 DURING THE PERIOD OF "EXTREME" SCROTAL MANGE

Date 1970/71	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
30.6	0.8	t.mil.	100	5	99	100	-	-	-	-	-
8.7	1.2	cr.	100	5	98	100	-	-	-	-	-
15.7	0.5	cr.	90	5	95	98	-	-	-	2	-
22.7	0.5	cr.	100	5	100	97	-	-	-	3	-
29.7	1.1	cr.	90	5	97	97	-	1	-	2	-
9.8	1.0	cr.	80	4	86	93	-	3	-	4	-
16.8	1.1	cr.	90	5	88	89	-	3	-	8	-
25.8	0.8	cr.	80	4	76	94	-	3	1	2	-
6.9	0.8	t.mil.	90	5	94	77	-	1	-	7	15
16.9	0.8	cr.	80	5	94	84	-	2	-	5	9
26.9	1.0	mil.	80	4	78	69	-	4	3	17	7
6.10	0.5	w.mil.	70	3	51	60	2	6	1	13	18
16.10	1.0	cr.	30	3	44	46	4	12	4	26	8
26.10	1.0	mil.	30	3	46	32	14	18	1	32	3
5.11	1.3	mil.	40	3	51	36	8	15	3	24	14
15.11	1.0	w.mil.	0	0	0	44	16	15	9	12	4

Appendix 5

EFFECT OF EXTENSIVE SCROTAL MANGE ON SEMINAL PLASMA FRUCTOSE CONCENTRATION (mg/100ml)

GROUP	SCROTAL MANGE					CONTROL				
Ram No.	59	91	92	572	359	260	162	32	105	574
DATE										
7.2.70	460	870	80	840	800	740	870	200	1060	840
11.2.70	610	700	150	600	710	640	1220	90	470	380
15.2.70	400	860	270	790	700	320	860	50	660	830
19.2.70	550	750	240	850	850	830	950	160	660	860
23.2.70	720	880	200	860	770	490	830	80	220	730
27.2.70	440	870	150	600	850	860	750	360	750	760
Mean	530	820	180	760	780	650	910	160	640	730

Mean of Scrotal Mange Group = 614mg/100ml.

Mean of Control Group = 618mg/100ml.

Appendix 6

SEMINAL PLASMA FRUCTOSE CONCENTRATION (mg/100ml) OF FOURTEEN REPEAT SEMINAL PLASMA ESTIMATES

Date	Ram No.	Sub Sample 1	Sub Sample 2
7.2.70	59	430	480
7.2.70	91	860	870
7.2.70	162	900	840
7.2.70	32	200	190
11.2.70	359	660	750
11.2.70	260	650	630
11.2.70	574	380	380
15.2.70	92	270	260
15.2.70	105	650	660
27.2.70	92	130	160
27.2.70	572	590	600
27.2.70	105	680	810
27.2.70	91	860	870

Appendix 7

SEMINAL REGENERATION IN RAM 438 FOLLOWING HEMICASTRATION AND TREATMENT OF "EXTREME" SCROTAL MANGE ON THE 23.11.70

Date 1970/71	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
20.10	0.8	cr.	50	3	68	62	-	8	-	30	-
30.10	0.4	w.mil.	1	2	4	29	-	44	-	26	1
9.11	0.7	mil.	0	0	3	29	1	48	-	10	12
17.11	0.3	cl.			severe oligospermia, total sperm/ejac. 1.8×10^4						
23.11	0.5	cl.			severe oligospermia, total sperm/ejac. 1.5×10^4						
22.12	0.5	cl.			severe oligospermia, total sperm/ejac. 2.1×10^4						
18.1	0.5	mil.	20	2	11	39	1	12	-	40	8
1.2	0.8	t.mil.	40	3	57	42	3	15	4	22	14
14.2	0.5	w.mil.	50	2	83	48	25	5	8	6	8
25.2	1.0	w.mil.	70	3	81	46	-	13	1	14	26
8.3	1.0	mil.	20	3	38	68	3	12	-	12	5
25.3	0.5	mil.	10	3	25	72	2	10	6	3	7
4.4	0.6	w.mil.	70	3	68	37	4	7	-	16	36
14.4	1.1	cr.	100	5	92	92	-	5	-	3	-
24.4	1.1	t.mil.	100	5	95	95	-	1	-	2	2

Appendix 7 (cont'd)

SEMINAL REGENERATION IN RAM 517 FOLLOWING HEMICASTRATION AND TREATMENT OF "SEVERE" SCROTAL MANGE ON THE 23.11.70

Date 1970/71	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
20.10	1.0	cr.	50	5	48	98	-	-	-	2	-
30.10	0.6	cr.	1	2	4	63	-	28	-	8	1
9.11	0.5	mil.	5	1	20	35	2	26	-	37	-
17.11	0.5	w.mil.	0	0	0	30	2	43	4	21	-
23.11	0.5	cl.	0	0	severe oligospermia, total sperm/ejac. 1.3×10^5						
22.12	0.5	cl.	-	-	azoospermia						
18.1	0.5	w.mil.	5	2	4	51	-	12	5	30	2
1.2	0.6	w.mil.	20	2	25	46	14	6	8	20	6
14.2	0.6	cl.	40	2	76	30	10	30	4	1	25
25.2	0.7	w.mil.	60	2	62	42	3	10	-	3	42
8.3	1.0	w.mil.	20	2	11	60	17	7	7	3	6
25.3	0.6	mil.	1	1	0	44	18	25	-	8	5
4.4	0.6	cl.	5	1	3	43	6	31	11	9	-
14.4	0.4	cl.	5	2	16	28	6	30	20	10	6
24.4	0.2	mil.	80	4	81	57	2	7	-	19	15
3.5	0.4	mil.	80	3	85	92	-	3	-	1	4
12.5	0.8	mil.	100	4	97	97	-	1	-	2	-
21.5	1.0	t.mil.	100	5	100	98	-	2	-	-	-

Appendix 7 (cont'd)

SEMINAL REGENERATION IN RAM 165 FOLLOWING CHEMICASTRATION AND TREATMENT OF "SEVERE" SCROTAL MANGE ON THE 23.11.70

Date 1970/71	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
20.10	0.5	mil.	20	2	14	38	4	24	-	28	6
30.10	1.0	mil.	30	2	28	46	10	26	-	13	5
9.11	0.5	mil.	1	1	10	51	14	8	-	17	10
17.11	0.5	cl.	0	0	(severe oligospermia, total sperm/ejac. 3×10^5)						
23.11	0.6	cl.	0	0	(severe oligospermia, total sperm/ejac. 8×10^4)						
22.12	0.8	cl.	0	0	(severe oligospermia, total sperm/ejac. 7×10^3)						
18.1	0.5	mil.	1	1	12	16	74	4	-	6	-
2.2	1.4	w.mil.	1	2	2	36	12	12	16	20	4
14.2	1.3	w.mil.	1	1	2	37	23	12	7	9	12
25.2	1.0	w.mil.	60	2	58	31	25	19	7	14	4
8.3	0.5	cl.	10	2	39	36	9	25	5	7	18
25.3	0.8	w.mil.	5	2	1	41	18	20	1	7	13
4.4	0.2	cl.	5	1	4	38	20	29	2	4	7
14.4	0.4	w.mil.	60	3	54	50	1	9	1	9	30
24.4	1.0	mil.	90	4	84	51	5	14	-	10	20
3.5	1.0	mil.	100	5	88	84	-	10	-	2	4
12.5	0.8	t.mil.	90	5	94	88	-	5	3	4	-

Appendix 7 (cont'd)

SEMINAL REGENERATION IN RAM 427 FOLLOWING HEMICASTRATION AND TREATMENT OF "SEVERE" SCROTAL MANGE ON THE 23.11.70

Date 1970/71	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
20.10 to 3.5	0.2 to 1.0	cl.	0	0	(total sperm/ejac. varied from 0 to 2.4 x 10^6)						
12.5	0.4	cl.	5	1	(severe oligospermic, total sperm/ejac. 6.4 x 10^4)						
21.5	0.8	cl.	10	2	5	10	12	50	14	14	-
1.6	0.5	cl.	30	2	20	36	13	15	9	17	10
14.6	1.1	cl.	30	2	21	58	4	5	17	8	8
21.6	0.6	w.mil.	40	2	19	46	21	14	7	7	5
1.7	0.7	w.mil.	40	3	50	59	10	7	4	6	14
12.7	0.8	mil.	70	3	58	41	9	19	-	23	8
22.7	0.8	mil.	80	4	45	44	-	45	-	8	3
2.8	0.5	mil.	80	5	76	71	-	13	-	4	12
16.8	1.0	mil.	80	5	83	88	-	2	-	6	4
23.8	0.9	mil.	80	5	74	90	-	3	-	2	5

Appendix 7 (cont'd)

SEMINAL REGENERATION IN RAM 13 FOLLOWING SPONTANEOUS CURE OF "EXTREME" SCROTAL MANGE

Date 1971	Volume (ml)	Colour	Motility (%)	(rate, 0-5)	% live	Spermatozoa morphology (%)					
						Normal	Head abn.	Neck abn.	Midpiece abn.	Tail abn.	Cyto. drop.
13.5	0.6	w.mil.	10	2	5	46	16	25	2	10	1
21.5	0.6	w.mil.	30	3	42	31	15	38	1	4	11
1.6	1.0	mil.	30	3	24	35	7	49	-	5	4
14.6	1.0	mil.	70	4	44	56	6	23	2	11	2
21.6	1.5	w.mil.	70	4	55	36	1	17	-	40	6
1.7	0.6	mil.	70	5	80	47	1	8	1	35	8
12.7	1.0	mil.	90	4	77	74	-	2	2	22	0
22.7	0.7	t.mil.	80	4	71	34	-	33	2	28	3
2.8	0.7	t.mil.	80	4	83	50	1	21	-	18	10
16.8	1.2	mil.	80	5	74	74	-	15	-	5	6
23.8	1.0	t.mil.	80	5	83	78	2	6	-	6	8

Appendix 8

THE CALIBRATION OF TEMPERATURE RECORDING APPARATUS

The accurate centigrade thermometer (see text) and the needle containing the thermistor were held in a clamp above a large vacuum flask which contained water at approximately 30°C. The heat sensitive tips of the two instruments were kept within one cm of each other and were immersed in the water. Hot water was added in small amounts and the water mixed thoroughly by stirring briskly for about twenty seconds. Chart recordings were marked at 1°C steps from 33°C to 40°C. The procedure was repeated with the clinical Fahrenheit thermometer with chart recordings marked from 35°C, the thermometers lowest marking, to 43.3°C. The results are presented in table form and graphically. The regression line and 95% confidence limites of it were calculated after the method described by Snedecor (1956).

The simple regression equation was:

$$y = 33.0 + 0.117x$$

where y = temperature in °C and x = number of chart divisions. Before and after each temperature recording session the temperature recording apparatus was checked for accuracy at 35°C and 40°C with the accurate Centigrade thermometer. The average of the two recordings at each temperature was always within the 95% confidence limits of the calculated regression line. That is:

$$35^{\circ}C = 17 \pm 2 \text{ chart divisions}$$

$$40^{\circ}C = 60 \pm 2 \text{ chart divisions}$$

Appendix 8 (cont'd)

RESULTS OBTAINED IN CALIBRATING THE TEMPERATURE RECORDING APPARATUS USING TWO ACCURATE MERCURY IN GLASS THERMOMETERS

Thermometer	Thermometer recording (^{o}C) (y)	Chart divisions (x)
C	33.0	0
C	34.0	8
F	35.0	15
C	35.0	17
F	35.6	20
C	36.0	26
F	36.7	30
C	37.0	34
F	37.8	40
C	38.0	43
F	38.3	44
C	39.0	51
F	40.0	59
C	40.0	59
F	41.1	67
F	42.2	77
F	43.3	88

C = "Centigrade" thermometer F = "Fahrenheit" thermometer

APPENDIX 8 (Contd.)

RELATIONSHIP BETWEEN TEMPERATURE AND THERMISTOR RECORDINGS.