strains had similar profiles and possessed major OMPs at molec lar weight 27,000, 31,000, 37-38,000, and 48,000; the major OM of Actinobacillus-like strain D107 were present at 33,000, a 42,000; and strain A2 possessed major OMPs at 24,000, 27,000, a 29,000. This data indicates that genetically conserved maj OMPs of strain A2 are markedly different from the other thr strains, which had phenotypic and biochemical characteristics the family Pasteurellaceae.

Strain A2 could not be identified to either genus or specie level based on the descriptions of currently classified organis in the division Gracilicutes as described in the first edition Bergey's manual of systematic bacteriology.[8,16,21]

References

1. Bagley C.V., Paskett M.E., Matthews N.J., Stenquist N.J. Prevalence and causes of ram epididymitis in Utah. J. Am Vet. Med. Assoc. 186:798-801, 1985.

2. Bailie W.E.: Ph.D Thesis, Kansas State University, Manhattan, 1969.

3. Baynes I.D., Simmons G.C.: Ovine epididymitis caused b Actinobacillus seminis, n. sp. Aust. Vet. J. 36:454-45 1960.

4. Bulgin M.S., Anderson B.C.: Association of sexual experienc with isolation of various bacteria in cases of ovine epidid mitis. J. Am. Vet. Med. Assoc. 182:372-374, 1983.

5. Bulgin M.S., Bruss M.L., Anderson B.C.: Epididymitis in rams Effect of vaccination and culling on the clinical incidenc of the disease. J. Am. Vet. Med. Assoc. 196:1110-1115, 1990.

6. Bulgin M.S., Bruss M.L., Anderson B.C.: Methods for contro of lamb epididymitis in large purebred flocks. J. Am. Vet Med. Assoc. 196:1110-1115, 1990.

7. Burgess G.W.: Ovine contagious epididymitis: A review. Vet Microbiol. 7:551-575, 1982.

8. Carter G.R.: Genus I. Pasteurella. In Bergey's manual o systematic bacteriology, ed. Sneath P.H.A., 1st ed., vol. 1 pp. 552-558, 1984. The Williams & Wilkins Co., Baltimore MD.

9. Claxton P.D., Everett R.E.: Recovery of an organism resem bling Histophilus ovis from a ram. Aust. Vet. J. 42:457-458 1966.

10. Cousins D.V., Lloyd J.M.: Rapid identification of Haemophi lus somnus, Histophilus ovis and Actinobacillus seminis usin the API ZYM system. Vet. Microbiol. 17:75-81, 1988.

11. DeLong W.J., Waldhalm D.G., Hall R.F.: Bacterial isolates associated with epididymitis in rams from Idaho and eastern Oregon flocks. Am. J. Vet. Res. 40:101-102, 1979.

12. Healey M.C., Bagley C.V., Bulgin M.S., Fitzgerald J.A., Hemenway K.: Results of a vaccine field trial designed to test the efficacy of a bacterial extract vaccine against epididymitis in ram lambs. Proc. Symp. Hlth. Dis. Small Rumin., pp. 35-45, 1991.

13. Healey M.C., Gharpure H.M., Kleinschuster S.J., Hwang H.H., Johnston A.V.: Use of monoclonal antibodies to identify outer membrane antigens of Actinobacillus species. Am. J. Vet. Res. 47:1446-1451, 1986.

14. Healey M.C., Hwang H.H., Kleinschuster S.J., Johnston A.V., Symons K.S.: Comparison and partial characterization of the protein profiles and outer membrane proteins of Actinobacillus species isolated from ram lambs with epididymitis. Am. J. Vet. Res. 49:1824-1831, 1988.

15. Healey M.C., Kleinschuster S.J., Gharpure H.M., Johnston A. V.: Production and preliminary characterization of monoclonal antibodies to Actinobacillus sp. isolated from epididymitis lesions in a ram. Am. J. Vet. Res. 46:1297-1302, 1985.

16. Healey M.C., Scanlan C.M., Dunn B.E.: Comparison of protein antigens among Actinobacillus species and other pathogenic gram-negative bacilli. Proc. Symp. Hlth. Dis. Small Rumin., pp. 65-86, 1991.

17. Kennedy P.C., Frazier L.M., Theilen G.H., Biberstein E.L.: A septicemic disease of lambs caused by Haemophilus agni (new species). Am. J. Vet. Res. 19:645-654, 1958.

18. Kilian M., Biberstein E.L.: Genus II. Haemophilus. In Bergey's manual of systematic bacteriology, ed. Sneath P.H.A., 1st ed., vol. 1, pp. 558-569, 1984. The Williams & Wilkins Co., Baltimore, MD.

19. Livingston C.W. Jr., Hardy W.T.: Isolation of Actinobacillus seminis from ovine epididymitis. Am. J. Vet. Res. 25:660-663, 1964.

20. Low J.C., Graham M.M.: Histophilus ovis epididymitis in a ram in the UK. Vet. Res. 117:64-65, 1985.

21. Phillips J.E.: Genus III. Actinobacillus. In Bergey's manual of systematic bacteriology, ed. Sneath P.H.A., 1st ed., vol. 1, pp. 570-576, 1984. The Williams & Wilkins Co., Baltimore, MD.

22. Piechulla R., Mutters R., Klussmeir R., Burbach S., Pohl S., Mannheim W.: Deoxyribonucleic acid relationships of "Histophilus ovis/Haemophilus somnus," Haemophilus haemoglobinophilus, and "Actinobacillus seminis". Int. J. Syst. Bacteriol. 36: 1-7, 1986.

23. Roberts D.S.: A new pathogen from a ewe with mastitis. Aust. Vet. J. 32:330-332, 1956.

24. Scanlan C.M., Healey M.C.: Antigenic and bacteriologic studies of gram-negative pleomorphs associated with epididymitis in ram lambs. Proc. Symp. Dis. Small Rumin., pp. 117, 1990.

25. Scanlan C.M., Healey M.C.: Antimicrobial susceptibility characterization of Actinobacillus seminis and Actinobacillus-like organisms. Proc. Symp. Hlth. Dis. Small Rumin., pp. 149, 1991.

26. Scanlan C.M., Healey M.C.: Characterization of a capnophilic gram-negative bacillus from the semen of a ram with epididymitis. Proc. Symp. Hlth. Dis. Small Rumin., pp. 145, 1991.

27. Scanlan C.M., Healey M.C.: The API ZYM system in the identification of facultative anaerobic gram-negative bacilli associated with epididymitis in ram lambs. Proc. Symp. Hlth. Dis. Small Rumin., pp. 46-52, 1991.

28. Scanlan C.M., Healey M.C., Torres A.R., Johnston A.V.: Cultural and biochemical characterization of Actinobacillus and Actinobacillus-like species from ram lambs with epididymitis. J. Vet. Diagn. Invest. 1:288-294, 1989.

29. Sneath P.H.A., Stevens M.: Actinobacillus rossii sp. nov., Actinobacillus seminis sp. nov., nom. rev., Pasteurella bettii sp. nov., Pasteurella lymphangitidis sp. nov., Pasteurella mairi sp. nov., and Pasteurella trehalosi sp. nov. Int. J. Syst. Bacteriol. 40:148-153, 1990.

30. Sponenberg D.P., Carter M.E., Carter G.R., Cordes D.O., Stevens S.E., Veit H.P.: Suppurative epididymitis in a ram infected with Actinobacillus seminis. J. Am. Vet. Med. Assoc. 182:990-991, 1983.

31. Stephens L.R., Humphrey J.D., Little P.B., Barnum D.A.: Morphological, biochemical, antigenic, and cytochemical relationships among Haemophilus somnus, Haemophilus agni, Haemophilus haemoglobinophilus, Histophilus ovis, and Actinobacillus seminis. J. Clin. Microbiol. 17:728-737, 1983.

32. Swanepoel M.L.: A study for the differentiation of Actinobacillus seminis, A. actinomycetemcomitans, Histophilus ovis, and Pasteurella haemolytica. Onderstepoort J. Vet. Res. 51: 41-46, 1984.

33. Van Tonder E.M., Bolton T.F.W.: Epididymitis in rams caused by Actinobacillus seminis. J. S. Afr. Vet. Med. Assoc. 39:87- 90, 1968.

34. Walker R.L., Biberstein E.L., Pritchett R.F., Kirkham C.: Deoxyribonucleic acid relatedness among "Haemophilus somnus", "Haemophilus agni", "Histophilus ovis", "Actinobacillus seminis" and Haemophilus influenza. Int. J. Syst. Bacteriol. 35:46-49, 1985.

35. Walker R.L., LeaMaster B.R.: Prevalence of Histophilus ovis and Actinobacillus seminis in the genital tract of sheep. Am. J. Vet. Res. 47:1928-1930, 1986.

36. Walker R.L., LeaMaster B.R., Stellflug J.N., Biberstein E.L.: Association of age of ram with distribution of epididymal lesions and etiologic agent. J. Am. Vet. Med. Assoc. 188: 393-396, 1986.

37. Webb R.F.: Bacteriological characteristics of Histophilus ovis and its relationship to similar bacteria. Res. Vet. Sci. 35:25-29, 1983.

38. Webb R.F.: Clinical findings and pathological changes in Histophilus ovis of sheep. Res. Vet. Sci. 35:30-34, 1983.

39. Zeki S., Kumar K.G.R., Keshavamurthy B.S.: Isolation of Haemophilus agni from cases of purulent orchitis in rams. Ind. J. Comp. Microbiol. 2:30-31, 1981.

The effects of heating the testes and epididymides of rams by scrotal insulation on fertility and embryonic mortality in ewes inseminated with frozen semen.

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Proofs to be sent to Dr R Mieusset.

Summary

Fertilization rate and embryonic mortality were assessed in 636 normal ewes inseminated in each uterine horn with 50 million frozen thawed spermatozoa from 4 control rams and from 4 rams submitted to a moderate (1.4 to 2.2°C) but sustained (16 hrs/day; 21 consecutive days) elevation in their subcutaneous scrotal temperature by means of scrotal insulation. Pregnancy was assessed twice in each ewe by blood plasma concentration of progesterone at17 days and by ultrasound at 65 days after insemination. No differences were observed in the 17 day pregnancy rate between ewes inseminated with semen collected from control rams (56.0, 65.2, 66.7 and 60.3%) and from heated rams (60.6,71.8, 63.6 and 48.2%) before and on 4, 15 and 21 days of heating respectively. In contrast, the rate of embryonic mortality between 17 and 65 days after insemination was significantly higher at day 4, 15 and 21 in the heated rams (78.7, 78.6 and 93%) than in the control rams (55, 59 and 65.7%). These results indicate that a slight but sustained elevation in the subcutaneous scrotal temperature induces a significant increase in the embryonic mortality rate. As these changes were apparent already on day 4 of heating, an effect must have occured on sperm stored in the epididymis.

INTRODUCTION

It is well known that spermatogenesis in mammals with scrotal testes is susceptible to damage if testicular temperature is raised above normal levels (see Waites & Setchell, 1990). The fertility of animals subjected to an increase in whole body or in testicular temperature is also reported to be depressed or even suppressed (Waites, 1968). Decreased fertility in females mated with heat stressed males can result from a failure in fertilization (ram: Dutt & Simpson, 1957, Fowler & Dun, 1966, Howarth, 1969, Rathore, 1970, Braden & Mattner, 1970; boar: Wettemann & Bazer, 1985), from a normal fertilization but an increase in embryonic death (mice: Bellve, 1972; rabbit: Howarth et al., 1965, Burfening & Ulberg,

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1968), or from both a failure in fertilization and an increase in embryonic death (ram: Rathore, 1970; boar: Wettemann & Bazer, 1985; mice: Burfening et al., 1970; rat: Setchell et al., 1988). However, it is difficult to assess the relevance of many of these reports since in most of these studies with general heating, body temperature was increased by about 1°C due to the elevated ambient temperature but testis temperature was not measured, and in the studies using local heating, testicular temperature was raised to unphysiological levels (40 to 43°C).

In fertile men, an induced increase of 1 to 2°C in testicular temperature resulted in a marked depression in spermatogenesis (Mieusset et al., 1987a). In infertile men, at least one third were reported to have an increased scrotal temperature of about 0.5 to 1.5°C (Mieusset et al., 1987b, Zorgniotti & Sealfon, 1988), and this increase was associated with significant alterations in the exocrine and endocrine functions of the testis (Mieusset et al., 1989). Recently, an increase in the number of miscarriages was reported in women whose partner had a high frequency of hot baths (Spira, 1990).

The aim of the present study was to assess the fertilization rate and the embryonic mortality in normal female sheep inseminated with semen from rams submitted to moderate but sustained elevations in their scrotal temperature.

MATERIAL AND METHODS

Animals: Eight mature Australian Merino rams aged between 3 and 5 years and weighing between 65 and 75 kg were kept in individual pens in a controlled temperature and light room, at temperatures ranging between 19 and 23°C under a 16 hour-light and 8 hour-dark regimen. Four animals were treated and 4 used as control; they were fed ad libitum with standard rations twice a day with free access to water.

Treatment: The testes and scrotum were heated for16 hours/day, beginning 4 hours before the end of the light period, for 21 successive days by means of an insulating bag made up of one layer of aluminium foil inserted between two layers of cotton cloth, and, on the outside, one layer of water proof cotton cloth (Malmgren & Larsson, 1989). The bag surrounded the scrotum without inducing any compression and was maintained in this position by four tapes tied over the back of the ram.

Preliminary assessment of the increase in temperature was performed on 5 other similar rams. Under a short anaesthesia induced with 0.4 ml of xylazine i.v. (Rompun; Bayer, Australia), a probe for recording temperature was placed into the space between the tunica vaginalis and the scrotal skin through a small incision in the skin about 10 cm above the testis. The probe was maintained in position by suturing the scrotal skin and temperatures were recorded every 5 minutes for 2 to 6 hours from the first to the fifth day after surgery, with the scrotum insulated as above. In all cases the mean subcutaneous scrotal temperature was between 1.4 and 2.2°C above control values, with a mean (±SD) increase of 1.7±0.3°C.

Semen analyses and freezing: Semen was collected twice a week by artificial vagina and analysed manually for sperm count (Improved Neubauer cell; Weber, England) and vitality (eosin-nigrosin staining; 200 spermatozoa counted). Motility characteristics were assessed by a computerized image analyser (Hamilton Thorne Motility Analyser; Daintree Ind., Australia). On days -49 before treatment and 4, 15 and 21 during treatment semen was diluted five-fold with tris-glucose-egg yolk-glycerol at 32°C with pH controlled at 7 with citric acid and pellet-frozen on dry-ice (Evans & Maxwell, 1987). The pellets were kept in liquid nitrogen until thawed in dry test tubes which were shaken in a water bath at 37°C, and the semen was used for insemination within10 min. At least one pellet from the same batch was evaluated after thawing with the Hamilton Thorne.

Insemination: 640 mature Merino ewes were randomly distributed into 8 groups of 80 ewes and their oestrus cycles were synchronized with progestagen sponges (Repromap, Upjohn, Australia), which were left in situ for 12 days, and 400 I.U. of PMSG (Folligon, Intervet, Australia) injected i.m. at the time of sponge removal. Each day 80 ewes were inseminated 50 hours after sponge removal, 40 with semen from heated rams and 40 from control rams, with the date of collection selected at random. Insemination was performed through a laparoscope by deposition of 50 million spermatozoa in each uterine horn. The same number of spermatozoa were used for both treated and control rams. As on three occasions, twice in the control and once in the heated group, no semen could be collected from one ram, 324 ewes were inseminated with semen from heated rams and 312 with semen from control rams.

Pregnancy Diagnosis : Two successive determinations of pregnancy were performed on each ewe 17 and 65 days after insemination. The first diagnosis was made by determining the plasma progesterone level at day17 (assessed in duplicate by RIA) and a pregnancy recorded when the progesterone value was at least 2 ng/ml (Robertson, 1977). The second determination was made by ultrasonic examination at 65 days (Lindahl, 1976). A female pregnant on day 17 and not on day 65 after insemination was considered to have lost its embryo.

Progesterone assay. An extraction single-antibody radioimmunoassay was used to measure plasma progesterone concentrations (D'Occhio et al., 1988). Non-radioactive progesterone was purchased from Sigma Chemicals (USA) and (1,2,6,7-tritiated)progesterone was obtained from the Radiochemical Centre (Amersham, Bucks, UK). Progesterone was extracted into heptane by vigorous vortexing and after freezing of the aqueous phase, the heptane was transferred to assay tubes and evaporated by warming to 43°C in a water bath and blowing with air. Tritiated progesterone and antiserum () were then added and the mixture was incubated overnight at 4°C. Dextran-coated charcoal was used to separate bound

and free steroid and radioactivity in the bound fraction was determined using a liquid scintillation spectrometer. The intra-assay coefficient of variation was 8.4%, and the .inter-assay coefficient of variation was 8.7%. The limit of sensitivity was 0.19 ng/ml.

Statistical analysis : The numbers of pregnant females were compared by chi-square test. Between group comparisons of the semen parameters were done by unpaired Student's t-test, and within ram comparisons at different times by paired Student t-test.

RESULTS

Semen characteristics

Before beginning the scrotal insulation, no difference between control and heated rams was observed in the total sperm count, the number of dead spermatozoa and in the percentages of motile and of rapid spermatozoa (Table 1). On day 4 of heating, the only difference between the two groups was a reduced percentage of rapid spermatozoa ($p < 0.05$) in the heated rams. On day 15 of heating, the mean number of dead spermatozoa in the heated rams was significantly higher than in the control rams on the same day ($p < 0.01$) and than in the heated rams before treatment ($p < 0.05$). On day 21 of heating, the number of dead spermatozoa was higher ($p < 0.01$), and the percentages of motile ($p < 0.01$) and of rapid ($p < 0.01$) spermatozoa were lower in the heated than in the control rams, while the total sperm count did not differ between the two groups. However, in the heated group the total sperm count and the percentages of motile and of rapid spermatozoa were lower at 21 days of heating ($p < 0.05$) than the values observed in the same rams before treatment.

Pregnancies at 17 and 65 days after insemination.

No statistically significant difference was observed in the pregnancy rate at 17 days between the females inseminated with semen from control rams and those with semen from heated

rams collected before treatment (56% vs 60.6%), at day 4 (65.2% vs 71.8%) and at day 15 (66.7% vs 63.6%) of treatment (Table 2). At day 21 of heating, pregnancy rate from heated rams (48.2%) was slightly but not significantly less than from control rams (60.3%).

The percentage of females still pregnant did not differ between the control (22.7%) and heated (25.2%) groups 65 days after insemination with semen collected before treatment (Table 2). But the percentage of females still pregnant 65 days after insemination was significantly less in the heated than in the control rams for semen collected at day 4 (15.3 vs 29.3%, chi square= 4.989, p<0.03), at day 15 (13.6 vs 27.3%, chi square= 4.471, p<0.04), and at day 21 (3.5 vs 20.6%, chi square= 10.98, p<0.001) of treatment.

Embryonic loss

Embryonic loss was expressed as a percentage of the number of females pregnant 17 days after insemination minus the number of females pregnant 65 days after insemination divided by the number of females pregnant 17 days after insemination (Fig. 1). Before treatment, embryonic loss was similar in control (28/51) and heated (25/40) rams. Embryonic loss was significantly increased at day 4 (chi square = 7.67, p<0.006), at day 15 (chi square = 4.45, p<0.04) and at day 21 (chi square = 8.83, p<0.003) of treatment in heated (48/61, 44/56 and 38/41, respectively) than in control rams (33/60, 26/44 and 25/36, respectively).

DISCUSSION

As in the present study there was no difference in the fertilization rate of females inseminated with semen from control and heated rams , it can be concluded that an increase of about 2°C in subcutaneous scrotal temperature for 16 hours per day does not induce modification in the fertilizing capacity of the spermatozoa after 4, 15 and 21 days of treatment. These results are apparently in contradiction with those reported in previous studies dealing with heat effect on fertilization rate in rams (Braden & Mattner, 1970, Dutt & Simpson, 1957, Fowler & Dun,

1966, Howarth, 1969, Rathore, 1968,1970), boars (Wettemann & Bazer, 1985) and rats (Setchell et al., 1988). Besides a difference in the heating procedure, with testicular temperature elevated to 40 to 43°C (Braden & Mattner, 1970, Setchell et al., 1988), or with general modifications induced through a whole body increase in temperature (Dutt & Simpson, 1957, Fowler & Dun, 1966, Howarth, 1969, Rathore, 1968,1970, Wettemann & Bazer, 1985), in some of these studies problems of methodology could have been involved such as a small number of control rams (Braden & Mattner, 1970, Rathore, 1968,1970) or of mated females (Fowler & Dun, 1966). But more important is the fact that the reduction in the fertilization rate reported in some of these studies was obtained from animals which semen characteristics were drastically altered by a long exposure to heat, ranging from 45 to 80 days (Dutt & Simpson, 1957, Wettemann & Bazer, 1985).

In the present study, embryonic mortality was calculated from the difference in the number of ewes pregnant at 17 and 65 days after insemination. Such an evaluation underestimates the rate of embryonic death, as a ewe can be pregnant with two embryos at 17 days and with only one fetus at 65 days.

The high embryonic mortality rate (55 to 68%) in the ewes inseminated with frozen-thawed semen from control rams confirmed the previous reports of the interference by the surgical technique used in the intra-uterine insemination of ewes (Maxwell et al., 1984). Nevertheless, embryonic death was significantly increased in ewes inseminated with semen from the heated rams as early as day 4 of treatment. The lack of modification in the fertilization rate associated with an increase in the embryonic death observed in the present study is in agreement with the results reported in mice (Bellve, 1972, Burfening et al., 1970) and rats (Setchell et al., 1988) when the females were mated with males immediately after heat treatment, and in the rabbit when spermatozoa were heated before insemination (Burfening et Ulberg, 1968, Howarth et al., 1965). However, as implantation of the embryo takes place in the ewe between 15 and 24 days after fertilization (Robertson, 1977), and as

the first diagnosis of pregnancy was performed on day 17 after insemination, it cannot be deduced from the present study if the embryonic loss occured before or after the implantation.

The epididymis is currently thought to be resistant to heat from the lack of abnormal spermatozoa or of modification in the motility of spermatozoa in the ejaculates of rams or boars until about 14 days after heating (Glover, 1955, Moule & Waites, 1963, Waites & Setchell, 1964, Wettemann & Bazer, 1985). Such a resistance was also confirmed in rams by the lack of change in fertilization rate within the first week after heating in some studies using a small number either of males or of females (Braden & Mattner, 1970, Howarth, 1969). However, in the rat, mouse and bull, the lower temperature of the cauda epididymidis has been recently shown to facilitate the storage of sperm by enhancing oxygen availability (Djakiew & Cardullo, 1986). As in rams the passage of spermatozoa through the epididymis requires a period of about 11 to 14 days (Ortavant, 1954), the increase in the embryonic death at day 4 of heating in the present experiment indicates an effect of heat on the epididymal spermatozoa. Such an epididymal effect of heat on embryonic survival was already present, although the authors do not comment on this point, when mice were mated during the first week after heating (Bellve, 1972, Burfening et al., 1970).

Different mechanisms can be evoked for the decreased capacity of the spermatozoa to induce a viable embryo: a developmental retardation of the embryo before implantation (Bellve, 1972); a reduced capacity to induce implantation sites, either as a direct effect of heat, or as a result of the slower division rate of the embryo through desynchronisation with the uterine environment. Such a desynchronisation could be also involved in post -implantation embryonic death. As Fournier-Delpech & al. (1979,1981) showed that embryo mortality was increased when ova were fertilized with immature epididymal spermatozoa, epididymal maturation of the spermatozoa could be affected by the higher temperatures; however, this cannot explain the early increase in embryonic mortality observed on day 4 of heating.

Furthermore, it was recently reported that exposure of mice to an environment of approximatively 35°C for 2 to 5 days caused dissociation of the X-Y chromosome bivalent in diakinesis-metaphase primary spermatocytes as well as formation of multinucleated giant cells in the testis (Waldbieser & Chrisman, 1986). If, as suggested by these authors, cell aberrations resulting from these anomalies could be involved in the decreased fertility found after subjecting scrotal mammals to high temperatures, again this cannot explain the increase in embryonic mortality observed as early as on day 4 of heating the scrotum. Whatever the mechanism, the alterations induced in the spermatozoa by such a heating used in the present study must manifest themselves at a later stage than fertilization which proceeds normally.

The results of the present experiment show that increasing the subcutaneous scrotal temperature of about 2°C for16 hours per day in rams induced as soon as day 4 of treatment an increase in embryonic mortality without any modification in the fertilization rate at least until 21 days of heating, at which time the quality and, to a lesser extent, the quantity of the spermatozoa in the ejaculates are beginning to be affected. Thus the earliest effect of heat seems to be on the epididymal spermatozoa. From these results, and from those already reported in the literature, we suggest that the sequence of the heat effect on spermatogenesis could be firstly a reduction in the capacity of the spermatozoa to produce a viable embryo, secondly a decrease in the capacity of the spermatozoa to fertilize the ovum and finally a decrease in the number of spermatozoa produced. More detailed analyses of the characteristics of the spermatozoa are required to establish a relationship between the semen parameters and this sequence.

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