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Comparative morphological studies of lamb and calf Sertoli cells treated with anabolic agents

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Summary. The morphological alterations caused by anabolic steroids (oestradiol and trenbolone acetate) on the Sertoli cells of testicles in animals for human consumption (lambs and calves) were studied.

The morphopathological study of the treated lambs revealed delayed development of the seminiferous tubules, which was marked by signs of immaturity and even degeneration of Sertoli cells.

The main morphopathological alterations affecting the Sertoli cells in calves occurred as hyperfunction symptoms marked by increased nuclei and smooth endoplasmic reticulum.

Key words: Lamb, Calf, Sertoli cells, Morphopathology, Anabolic steroids

Introduction

Anabolic steroids are used indiscriminately to increase the economic yield of animals with complete disregard to the injuries that such a treatment may cause them and the hazards to humans consuming their meat, (Lu Cheryle and Steinberger, 1978), or the fraud that increasing the animal's weight by having water build up in its tissues represents (Riquelme and del Campo, 1983; Oko and Hrudka, 1984; Kochakian, 1989).

This led us to monitor the morphological disturbances of the Sertoli cells in the testicle by subcutaneous implantation of oestradiol and trenbolone acetate, in order to determine the effect of the implanted anabolic steroid. Such disturbances are rather varied in nature and range from degenerative processes (Deschamps et al., 1987), to delayed testicle maturation in rats (Gaytan et al., 1986).

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Materials and methods

The experimental material used comprised of testicles from 16 Merino lambs and 6 Aberdeen Angus-Charolais calves, which had been implanted subcutaneously with oestradiol and trenbolone acetate according to the following schedule:

Lambs

	No. Animals	Date of Implantation (Age of lamb)	Date of Sacrifice (Age of lamb)
Experimental Group	12	45 days	65 days
Control Group	4		65 days

Calves

	No. Animals	1st implant	2nd implant	Sacrifice
Experimental Group	4	7 months	10 months	14 months
Control Group	2			14 months

Amount implanted

	Oestradiol	Trenbolone Acetate
Lambs	2,5 mg	17,5 mg
Calves	40 mg	200 mg

The testicle samples were fixed in a 2% glutaraldehyde solution in a phosphate buffer of pH 7.4 at 4° C. Then, they were refixed in osmium tetroxide and embedded in Araldite.

Sections were examined through a JEOL 200 CX transmission electron microscope, and studied morphometrically with a Kontron IBAS II instrument.

Results

Lambs

The morphological studies of the seminiferous tubules of the treated animals revealed that they had an average bore of 165 ± 25 μm and contained 34 ± 2 Sertoli cells on average; those of the control animal group were 180 ± 32 μm in diameter and contained and average of 23 ± 4 Sertoli cells.

The Sertoli cells of the treated animals showed clear signs of immaturity, with anomalously large nuclei (19 ± 3 μm in diameter, Fig. 1) compared with the control animals, with an average nucleus diameter of 12 ± 2 μm.

In the treated-animals, the aforesaid cells featured poorly-developed organelles —particularly their smooth endoplasmic reticulum—, with scarce, disperse cisternae; by contrast, the Sertoli cells of the control animals were arranged in clusters which spread over cytoplasmic areas of up to 5 ± 1 μm. Finally, it should be

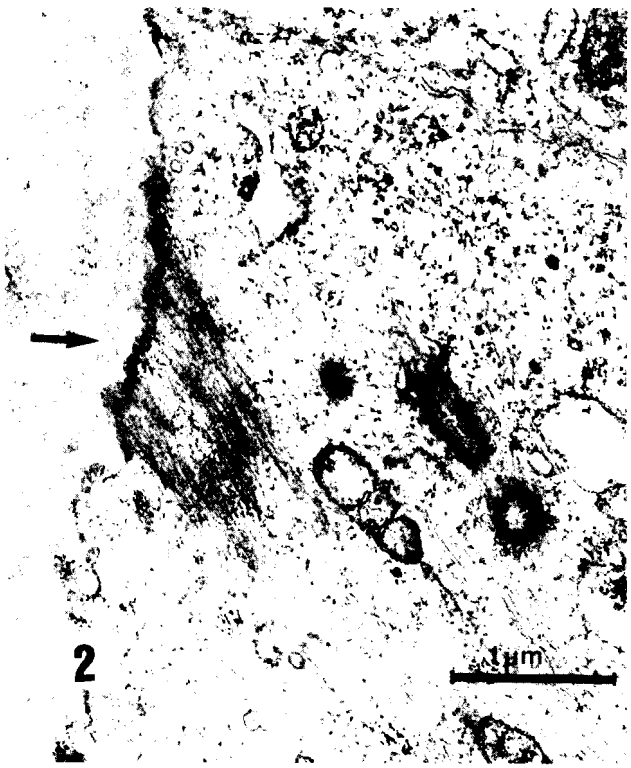


Fig. 2. Electron micrograph of the Sertoli cells in the calf testicles showing densified microfilamentous clusters in the basal zone (arrow). Bar = 1 μm.

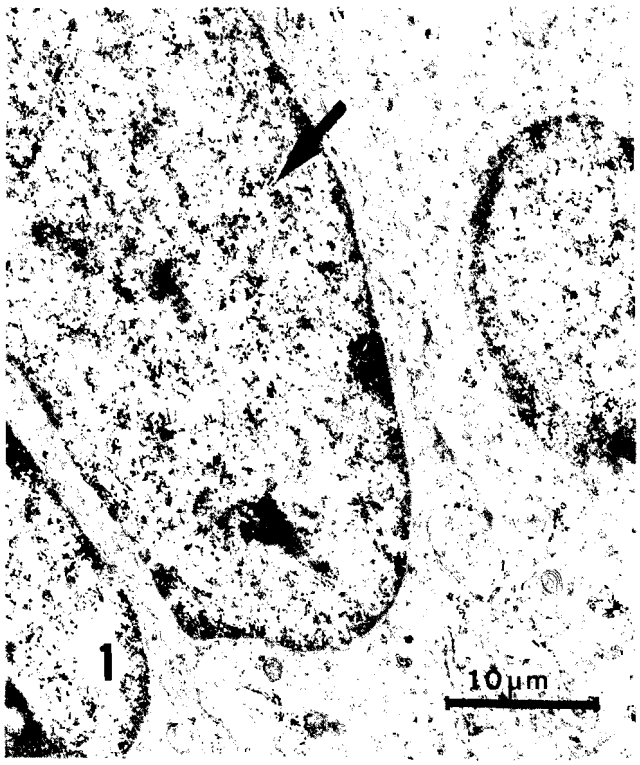


Fig. 1. Electron micrograph of the lamb testicles showing indistinctive sertoli cells with central, ovoid nuclei (arrow) and few cytoplasmic organelles. Bar = 10 μm.

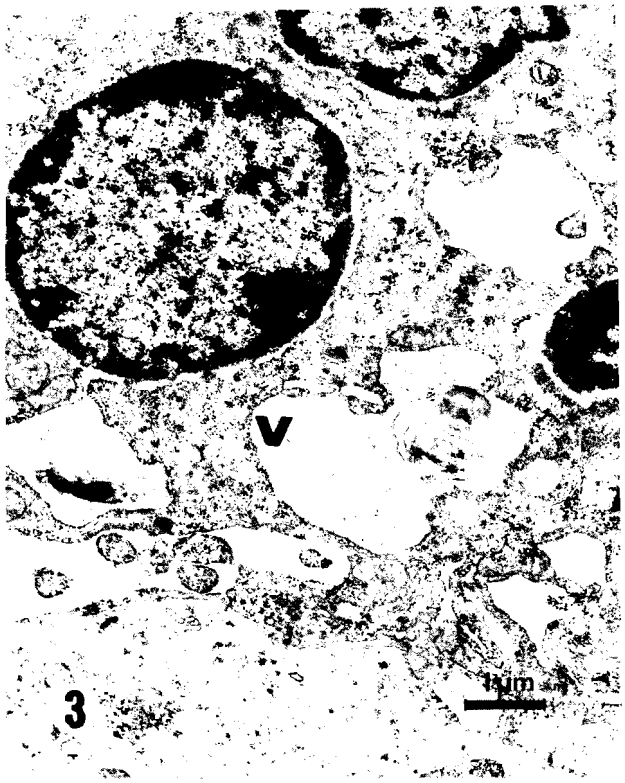
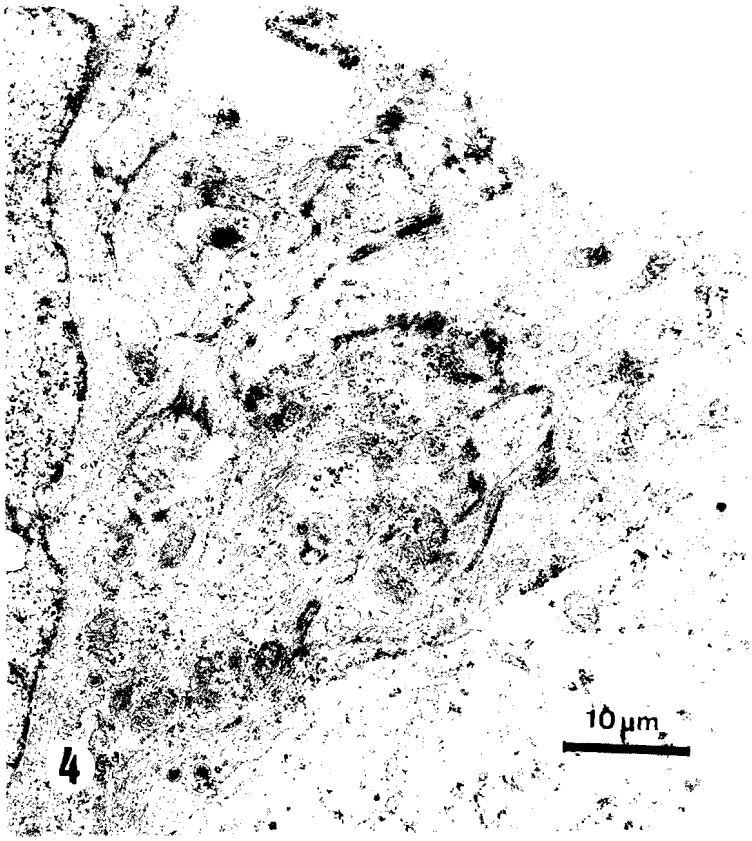


Fig. 3. Evidence of the occurrence of degenerate Sertoli cells in the lamb testicles with densified nuclei and cytoplasmic vacuolation. Bar = 1 μm.



noted that no fatty substances were found in the experimental group cells; however, they did contain microfilamentous clusters bound to the membrane and concentrating in the basal zone (Fig. 2).

The immature Sertoli cells of the experimental animals were accompanied by others undergoing degenerative phenomena such as mitochondrial tumefactions and vacuolar degeneration (Fig. 3).

Calves

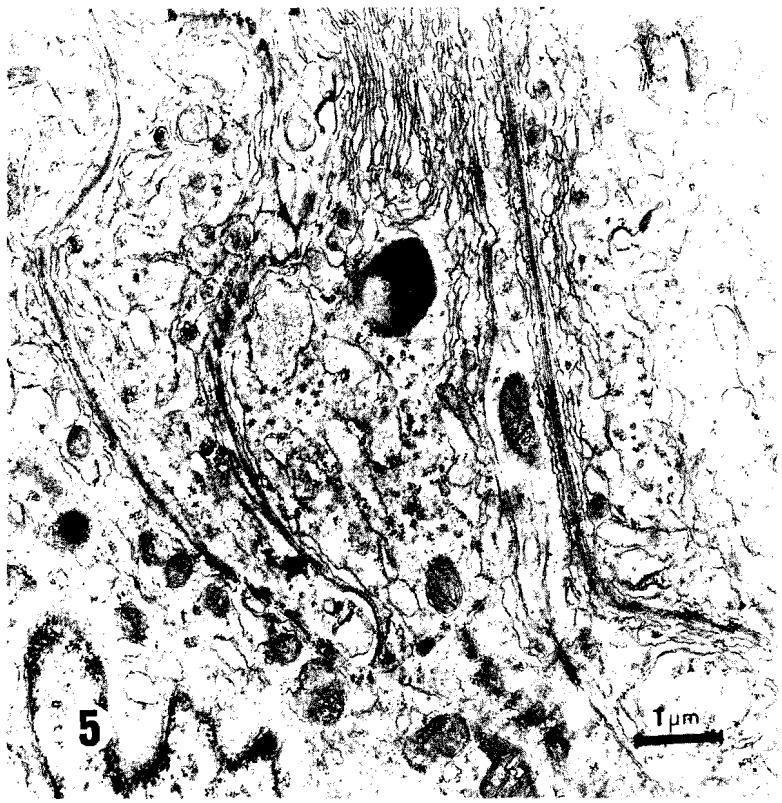
The seminiferous ducts of the treated animals had an average bore of 165 ± 15 µm and each one contained an average of 27 ± 3 Sertoli cells, while those of the control animals were 190 ± 17 µm in diameter and contained 32 ± 2 Sertoli cells on average.

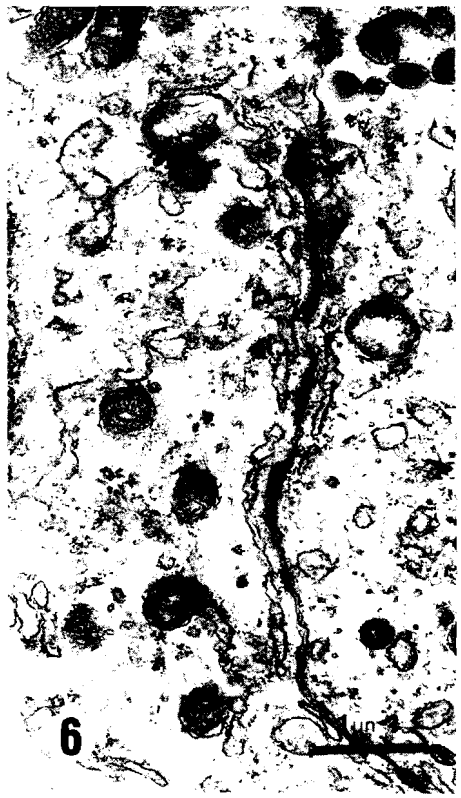
The morphological study of the Sertoli cells in the experimental group revealed significantly

Fig. 4. Magnified view of Sertoli cells in calves showing abundant cytoplasmic organelles. Bar = 10 µm.

Fig. 5. Magnified view of Sertoli cell in calves with clusters of smooth endoplasmic reticulum cisternae. Bar = 1 µm.

Fig. 6. Specialized binding complexes between Sertoli cells with ectoplasmic specializations. Bar = 1 µm.





developed cytoplasmic organelles (Fig. 4), particularly in the smooth endoplasmic reticulum, where cisternae tended to concentrate in areas of up to 12 ± 2 μm, in contrast with the 9 ± 2 μm, of the control group (Fig. 5). The nuclei of the Sertoli cells in the experimental and control groups measured an average of 23 ± 3 and 18 ± 2 μm, respectively.

An interesting morphological finding was the occurrence of complexes binding Sertoli cells in the treated calves, with numerous associated endoplasmic cisternae (Fig. 6).

Discussion

A number of authors (Riquelme et al., 1983; Oko and Hrudka, 1984; Schulze, 1988) have investigated the effects of anabolic steroids on the Sertoli cells, and consistent with their results, we found the steroids to modify this type of cell in the testicles of lambs and calves treated with oestradiol and trenbolone acetate.

The morphological results obtained for the Sertoli cells of the lambs are indicative of influence of anabolic steroids (Sakai et al., 1988), which results in delayed maturation, as found by Gaytan et al. (1986) in rats treated with the same hormone principles, and is also apparent from the differences between the different cytoplasmic organelles and nuclei of the experimental and control animals. In addition, these anabolic steroids induce degenerative processes in the Sertoli cells (e.g. those reported by Deschamps et al. (1987) and Shin et al. (1987), in the seminiferous ducts of animals treated with estrogens).

The Sertoli cells of the treated calves reacted against the anabolic implants with signs of hyperfunction judging by the great development of the principal cytoplasmic organelles of these cells or even the relatively increased nuclei found in the morphometric study (Daehlin , 1986). Such a hyperfunction can be interpreted as an attempt to diminish the serious disturbances to which the cells responsible for the production of spermatozoa are subject (Fabry et al., 1984; Holderegger and Keefer, 1986).

The outstanding differences found between the Sertoli cells in the testicles of the lambs and calves, should be emphasized. This can be accounted for on the basis of two facts. Namely: the lambs were implanted with the steroids at an early age, when their testicles were still immature and undeveloped; while the calves were implanted at a later stage, after they had reached their testicular maturity and produced spermatozoa. On the other hand, the lambs proved to be more sensitive than the calves to the anabolic steroids (O'Lamhna and Roche, 1983; Wettemann et al., 1983; Renaville and Fabry, 1987).

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