

ELECTRON MICROSCOPIC INVESTIGATIONS ON THE SERTOLI CELLS OF PHILIPPINE CARABAOS AND THEIR CROSSBREDS

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Summary

A study was conducted to compare and determine the incidence of ultrastructural alterations in the testes of Philippine carabaos and crossbred buffaloes. Thirteen Philippine carabao bulls and twenty five crossbred male buffaloes were used in this study. Testicular biopsy was used to get tissue samples which were prepared for histologic evaluation using the electron microscopy method. There was no significant difference in Sertoli cell alterations between Philippine carabaos and crossbred buffaloes. However, more crossbred buffaloes (40%) had both Sertoli cell and spermatogenic cell alterations which were significantly higher compared to the 7.7% occurrence in Philippine carabaos. Sertoli cells of crossbred buffaloes exhibited intracavitary structures and exaggerated infoldings of the nuclear envelope (36%), nuclear bleb (16%), and intracytoplasmic vacuolations (16%). Philippine carabaos exhibited few ultrastructural alterations which were mainly intracytoplasmic vacuolations in Sertoli cells (15%).

(Key Words : Sertoli Cells, Crossbred Buffaloes, Ultrastructural Alterations, Testes)

Introduction

The Philippine carabaos or swamp buffaloes (*Bubalus bubalis*) are generally raised as work animals and are particularly suited for paddy cultivation in swampy, water-logged ricefields. The river buffaloes (*Bubalus bubalis*) on the other hand, are larger and are good milkers. In many countries in Southeast Asia, the performance of the swamp buffaloes is improved by crossing them with the river type. Experience has shown that crossbreeding produces a stronger work animal which is more resistant to heat, and with a higher milk production capacity.

Reproductive consequence of crossbreeding has not been well studied in male buffaloes although information on other mammalian hybrids, particularly the deer as studied by Hrudka (1988), indicated that the Sertoli cell is the visible target of hybridization.

Sertoli cells have long been recognized as having a major influence on germ cell differentiation, development and metabolism. Experi-

ments of Dym (1973) have confirmed the presence of a blood-testis barrier in monkeys, rats, mice, hamsters, and guinea pigs. He concluded that the Sertoli cell not only functions for support and nutrition but also for maintenance of the blood-testis barrier and the compartmentalization of the seminiferous epithelium.

Basrur (1989) in her study on the effect of crossbreeding on Sertoli cells in water buffaloes noted abnormal Sertoli cells and unusual transformations of the endoplasmic reticulum. This study aimed to determine the incidence of ultrastructural alterations (intracytoplasmic vacuolations, exaggerated infoldings, nuclear bleb, electron dense membrane and inactivated mitochondria) in the testes of Philippine carabaos and their crossbreds for conclusive results by employing large number of samples.

Materials and Methods

Collection of Samples

Thirteen Philippine carabao bulls (*Bubalus bubalis*) and twenty five crossbred (Philippine carabaos × Nili-Ravi and Phil. Carabaos × Murrah) male buffaloes, F₁, with ages three years and above were used in this study. Tissue samples were taken by testicular biopsy. One testicular

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sample of 0.5 to 1.0 cm³ was collected from the testicle of each bull.

Preparation of Samples for Microscopy

The testicular sample was cut into small pieces (0.5-1 mm³) then fixed in 2.5% glutaraldehyde for two hours. It was then washed three times in 5% sucrose in pH 7.2 phosphate buffer for a few minutes then immersed in 5% sucrose pH 7.2 phosphate buffer overnight. Postfixation was in 1% osmium tetroxide for one to two hours. Dehydration at one hour each in 50%, 70%, 85%, 95% and absolute acetone followed. The samples were then placed in propylene oxide for one hour. Infiltration was for one hour each in resin + propylene oxide (1:2), resin + propylene oxide (2:1) then left overnight in 1:1 resin + propylene. Embedding was in pure araldite and then polymerized inside the oven for four days at 60°C. Two embedded tissue pieces per animal and two copper grids per embedded tissue were used. Ultra thin sections were stained with a uranyl acetate and lead citrate which are generally used for positive staining for transmission electron microscopy. Observation of ultrastructural features was by the use of a JEOL-JEM 100 U transmission electron microscope. Six sections per copper grid were counted. Electron micrographs were taken and ultrastructural alterations were noted.

Statistical Analysis

Incidence of ultrastructural alterations was analyzed by means of Fisher's exact test as described by Siegel (1956).

Results and Discussion

A. Incidence of Ultrastructural Alterations

Table 1 illustrates the frequency of Philippine carabaos and crossbred buffaloes with ultrastructural alterations in the testes. Among the animals sampled, a significantly low percentage (7.7%) of Philippine carabaos exhibit some testicular ultrastructure alterations compared to the 40% occurrence in crossbred buffaloes. Some animals had ultrastructural alterations only in Sertoli cells but there was no significant difference between the two groups. Electron microscopy revealed light to moderate alterations which may indicate that these are not pathologic. The present study however, was not able to find the actual level

or the extent of alterations within the testis of the animal.

TABLE 1. FREQUENCY OF ANIMALS WITH ULTRASTRUCTURAL ALTERATIONS IN THE TESTES¹

Testicular alterations	Philippine carabaos	Crossbred buffaloes
	(n = 13)	(n = 25)
 Percent of total	
Sertoli cell only	7.7 ^a (1) ² [0.021]*	16 ^b (4) [0.015]
Both Sertoli cell and spermatogenic cell	7.7 ^a (1) [0.021]	40 ^b (10) [0.019]

¹ Percentages with different superscripts between Philippine carabaos and crossbreds are statistically significant ($p < 0.05$).

² Figures in parentheses represent the number of animals with ultrastructural alterations in the testes.

* Figures in brackets are standard error of the mean.

B. Types of Sertoli Cell Alterations

Table 2 summarizes the ultrastructural alterations observed in the Sertoli cells of Philippine carabaos and crossbred buffaloes. Intracavitary structures/exaggerated infoldings (36%) are the significant ($p < 0.05$) alterations in the Sertoli cells of crossbred buffaloes. Intracytoplasmic vacuolations (16%) and nuclear bleb (16%) ranked second although these are not significantly different from those of Philippine carabaos. The cavitation in Sertoli cell nucleus as shown in figure 1 seemed to characterize the crossbred buffaloes as this was not seen in Philippine carabaos sampled.

Figure 2 shows the formation of exaggerated infoldings of the nuclear envelope which cut deep into the nucleus. Some of these infoldings have become islands inside the nucleus. The basement membrane appears to be dense. It is likely that the thick basement membrane impedes the interaction between tubules and interstitial compartments which can interfere with testicular function. A thickening of the basement membrane along with other changes, has consistently been reported to be associated with disrupted spermatogenesis in man, but only rarely in other species (de Kretser et al., 1975; Cameron et al., 1985). However, this kind of alteration was seldom

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observed in the crossbred buffaloes examined. Figure 3 shows a nuclear bleb at the site of the nuclear envelope. A bleb is a blister or a small circumscribed elevation. It is the early stage leading to eventual degeneration of the cell. Another striking feature is the formation of vacuoles inside the Sertoli cell cytoplasm (figure 4).

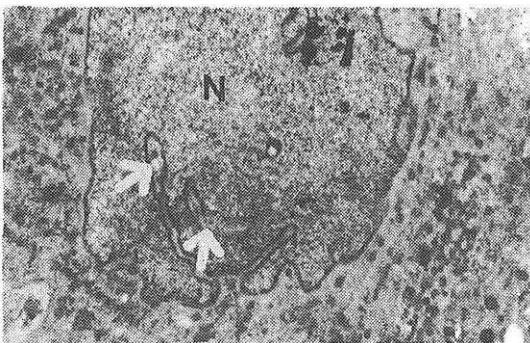
TABLE 2. ULTRASTRUCTURAL ALTERATIONS IN THE SERTOLI CELLS OF PHILIPPINE CARABAOS AND CROSSBRED BUFFALOES¹

Ultrastructures	Philippine carabaos (n = 13)	Crossbred buffaloes (n = 25)
	... Percent of total ² ...	
1. Intracytoplasmic vacuolations	15 ^a (2) [0.027]*	16 ^a (4) [0.015]
2. Inactivated mitochondria	7.7 ^a (1) [0.021]	4 ^a (1) [0.008]
3. Electron dense cytoplasm/membrane	0 ^a	8 ^a (2) [0.011]
4. Intracavitary structure/exaggerated infoldings	0 ^a	36 ^b (9) [0.019]
5. Nuclear bleb	0 ^a	16 ^a (4) [0.015]

¹ Percentages with different superscripts between Philippine carabaos and crossbreds are statistically significant ($p < 0.05$).

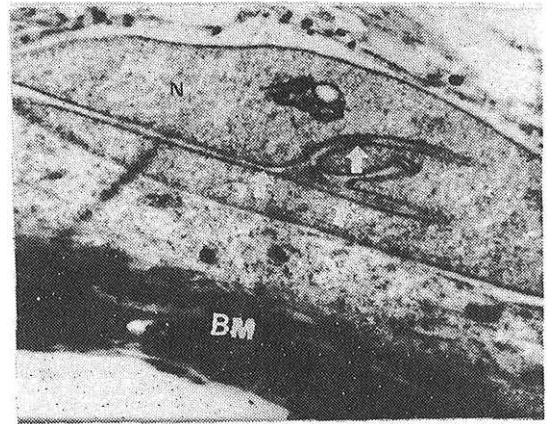
² An animal may exhibit one or more different forms of testicular alterations.

* Figures in brackets are standard error of the mean.



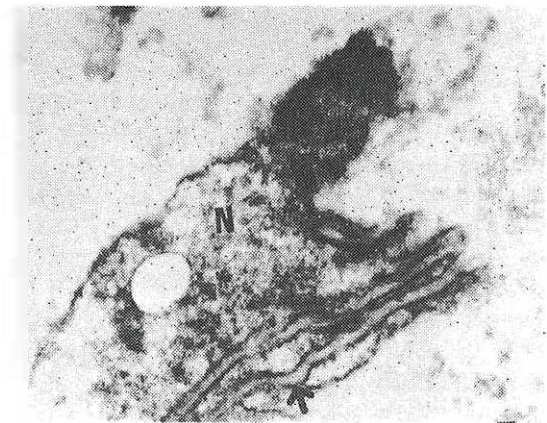
N - Nucleus Arrow - Cavitations

Figure 1. Sertoli cell of a crossbred buffalo showing cavitations ($\times 13,333$).



N - Nucleus
BM - Basement membrane
Arrow - Infoldings

Figure 2. Electron micrograph of a Sertoli cell of crossbred buffalo with exaggerated infoldings (arrows) of the nuclear envelope and dense basement membrane ($\times 9,600$).

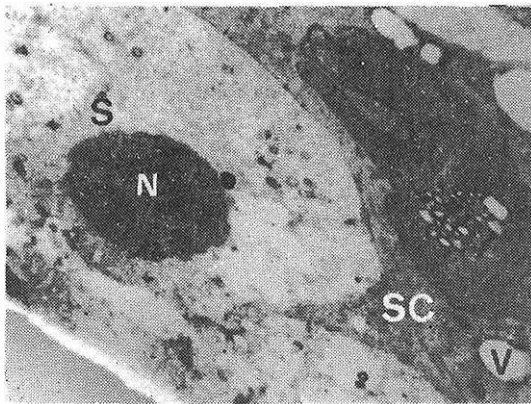


N - Nucleus Arrow - Nuclear bleb

Figure 3. Electron micrograph of a Sertoli cell of crossbred buffalo showing a bleb at the nuclear envelope ($\times 16,878$).

Ezeasor and Singh (1987) noted that in cryptorchid dwarf goats, Sertoli cell degeneration took the form of increased intracytoplasmic vacuolations and localized expansions of the intercellular space. Both Philippine carabaos and crossbred buffaloes in this study exhibited vacuolations

in the cytoplasm. Slight to moderate vacuolations may be present in healthy animals.



N	– Pycnotic nucleus of spermatogonium
V	– Vacuole
S	– Spermatogonial cytoplasm
Sc	– Sertoli cell cytoplasm

Figure 4. Section showing a Sertoli cell with vacuolations and a spermatogonium with pycnotic nucleus ($\times 10,125$).

The Sertoli cells provide mechanical support and protection for the developing germ cells and they probably participate in nutrition, regulation, coordination, and a host of other functions. The ultrastructural alterations could mean a decrease in the efficiency or failure of Sertoli cells to provide these functions. It has been reported by Soderstrom and Nikkanen (1979) that the Sertoli cells show marked morphological alterations in association with hypospermatogenesis. The ultrastructural alterations in crossbred buffalo Sertoli cells may point to the macrophage like function of Sertoli cells. Bawa (1963) and Soderstrom and Nikkanen (1979) noted that Sertoli cells have an ability to phagocytose degenerating cells of the

germ cell line. However, Dym (1973) observed that the ultrastructure of the Sertoli cell cytoplasm containing these degenerating cells is not altered in any way to suggest an active phagocytosis. The ultrastructural alteration may also imply impaired barrier function or impaired Sertoli cell function which may result to an altered micro-environment in the adluminal compartment which in turn may affect spermatogenesis. The present data suggest that the Sertoli cell is a visible target of crossbreeding in water buffaloes although the exact mechanism behind it is unknown.

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