

RESEARCH NOTE

USE OF COCONUT WATER (*Cocos nucifera*, LINNAEUS) FOR *IN VITRO* MAINTANANCE OF *Tritrichomonas foetus* (RIEDMULLER, 1928)

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ABSTRACT:- MARTINS, N.E.; ASSIS, R. A.; LOBATO, F.C.F.; COSTA, G.M.; OLIVEIRA, P.R.; VIANA, J.C.; PIRES, E. M.; FERNANDES, A. A. **Use of coconut water (*Cocos nucifera*, Linnaeus) for *in vitro* maintainance of *Tritrichomonas foetus* (riedmuller, 1928).** [Uso de água de coco (*Cocos nucifera*, Linnaeus) para manutenção *in vitro* de *Tritrichomonas foetus* (Riedmuller, 1928)]. *Revista Brasileira de Parasitologia Veterinária*, v. 13, n. 2, p. 85-87, 2004. ¹Escola de Veterinária da Universidade Federal de Minas Gerais. Departamento de Medicina Veterinária Preventiva e Epidemiologia. Avenida Antônio Carlos 6627, Pampulha, Belo Horizonte, 30123-970 MG, Brasil. E-mail: proliver@vet.ufmg.br

Tritrichomonas foetus is the etiological agent of bovine trichomoniasis, responsible for transient infertility, pyometra and abortions usually during the first third of pregnancy. Considering the difficulties related to the *in vitro* maintenance and culture of *T. foetus*, the objective of the present study was to evaluate the use of coconut water, *in natura*, for *in vitro* maintenance of *T. foetus in vitro*.

KEY WORDS: *Tritrichomonas foetus*, *in vitro* maintenance, coconut water.

RESUMO

Tritrichomonas foetus é o agente etiológico da tricomonose bovina, responsável por infertilidade temporária, piometra e aborto usualmente durante o primeiro terço da prenhez. Considerando as dificuldades relatadas para a manutenção e cultivo do *T. foetus*, o objetivo do presente estudo foi avaliar o uso de água de côco, *in natura*, para sua manutenção *in vitro*.

PALAVRAS-CHAVE: *Tritrichomonas foetus*, manutenção *in vitro*, água de coco.

Bovine trichomoniasis is a reproductive disease that have significant economic impact in animal production in many parts of the world. The causative organism, *Tritrichomonas foetus*, can cause early embryonic death, abortion, pyometra and transient infertility. Abortions generally occur during the first third of pregnancy (SKIRROW; BONDURANT, 1988). *Tritrichomonas foetus* can be collected, transported, maintained and cultivated in several media. The first culture media appeared in the 30's, allowing diagnosing the disease, evaluating its behavior in experimentally infected laboratory animals and studies on physiological aspects of the protozoan. The media most commonly used for transportation, *in vitro* cultivation and maintenance of *T. foetus* are Diamond, Lactopep and modified Rieck (PELLEGRIN, 1999). However, maintenance of *T. foetus* under laboratory conditions using these media is expensive and requires constant subcultures. Coconut water has been used for culture of vegetal tissues and as a growth factor in tissue cultures for vegetal virus. It has also been used as a medium

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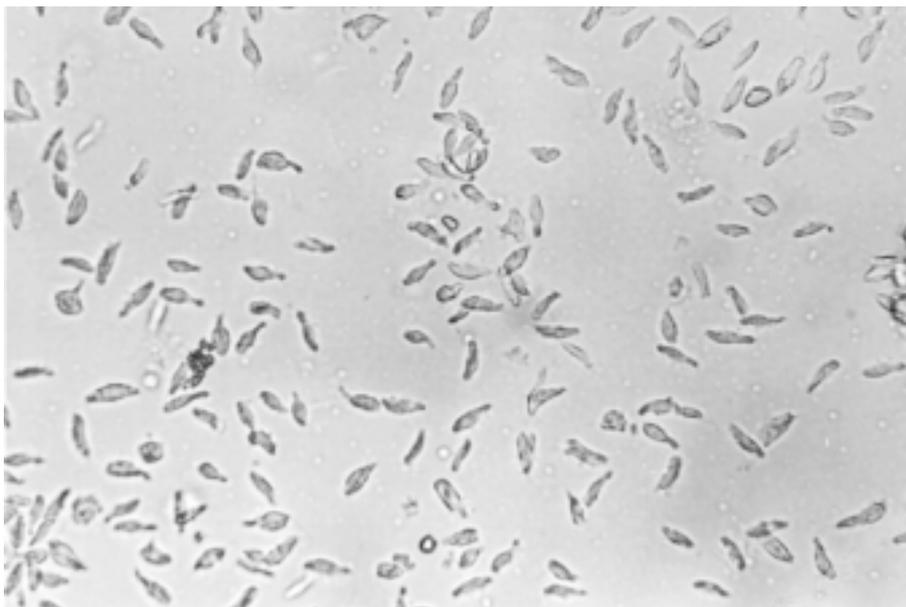


Figure 1. Five days culture of *Tritrichomonas foetus* in enriched coconut water, 416x.

for cultivation of pathogenic fungi and as a diluent of sheep, goat and pig semen (BLUME ; MARQUES, 1994).

Two strains of *T. foetus*, which had been isolated from cattle living in the States of Goiás and Minas Gerais, Brazil, were used in the experiment. The strains were isolated from prepuce washings using 0.85% Phosphate Buffer Saline (PBS) to which 2.0 g of modified Rieck medium had been added (GUIDA, 1960). In the laboratory the suspensions were sub-cultured in medium TYI-33 (DIAMOND et al. 1978) with 250 mg/ml of ciprofloxacin and 333 IU nystatin, incubated at 37°C for 48 hours under aerobiosis. Cultures were sub-cultured five times during 48h to ensure no growth of fungi or bacteria.

Supplies of coconut water used for cultures were obtained under aseptic conditions from a pool of green coconuts purchased from commercial sources. Before its use, coconut water was filtered through sterile gauze. The initial pH, ranging from 5.2 to 6.0; was as soon as corrected with NaHCO₃ at 1M for 7.2 to 7.4 and presented 330 mOsm. After this, the coconut water was supplemented with 10% equine serum, and filtered through of a sterile membrane of Millipore of 0.45µ (enriched coconut water). The pH after addition of equine serum was not measured. In order to test sterility, 0.1 ml aliquots of the solution were sub-cultured in Brain Heart Infusion (Oxoid, Basingstoke, Hampshire) and in Thioglycolate (Barcelona, Dignolab, Espanha), under aerobiosis for 48h.

Ten cc of enriched coconut water were placed into each 160 mm x 16 mm sterile tube, which were incubated at 37°C during 30 min. A 0.1 ml aliquot of *T. foetus* suspension, was inoculated into the tubes containing the enriched coconut water. The cultures were incubated in aerobiosis at 37°C for

three days, after which they were placed at room temperature protected from light for other 18 days. During the 21-day period, the cultures were monitored under phase-contrast microscopy. This assay was repeated five times (Figure 1).

The isolates evaluated showed greater quantities of live forms during the first five days of culture in enriched coconut water. After this period progressive death of *T. foetus* was observed; however both isolates had live forms up to 18 days after the beginning of culture. Similar patterns were observed in the other five subsequent assays.

It was observed that enriched coconut water allowed the maintenance of live forms up to 18 days, discarding the subcultures in short periods of time, as in cultures with conventional media, which usually require two subcultures per week.

In this study, no evidences of *T. foetus* growth or parasite reproduction was observed in the enriched coconut water. More data concerning the counting of the number of parasite and a growth curve is suggested for future trials.

These preliminary data are the first report on the use of *in natura* coconut water for *in vitro* maintenance of *T. foetus*. The use of enriched coconut water is a low cost alternative for maintenance of *T. foetus* under laboratory conditions.

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