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PRELIMINARY REPORT ON THE USE OF COCONUT WATER *(Cocos nucifera)* AS A DILUTER OF BUFFALO SEMEN

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Abstract

Coconut water obtained from (***Cocos nucifera***) was used for the development of a new diluter for buffalo semen. According to previous research done in other domestic species like caprine and swine, the substance present in the coconut water is the indole-3-acetic acid (IAA) which appeared to be the main sperm protective substance present in this vegetable. The utilization of this new diluter was tested in the laboratory, when diluted and frozen semen were submitted to the evaluation of motility and vigor, according to the methodology described by (1) and (2), as well as the thermo resistance test (TRT) of 30 minutes and 1 hour, live and dead sperm and acrossome damage. For fresh semen, the average for wave motion, motility and vigor were 3,1, 71,1 per cent and 3,8, respectively; for diluted semen the average for motility and vigor were 72,2 per cent and 4,1, respectively and for frozen semen motility and vigor were 44,4 per cent 3,3, respectively. Frozen semen was tested through the use of T.R.T. for 30 minutes. The motility and vigor were 38,8 per cent 3,0 and for T.R.T. through 1 hour were 30,0 per cent 3,1, respectively. Also the frozen semen was tested for live and dead sperm and acrossome damage and an average of 39/61 and 10,4 per cent were obtained, respectively.

Key words: coconut water, artificial insemination, buffalo

Introduction

The use of vegetal extenders for preservation of live cells has been reported for a long time.

Different types of vegetable products such as tylose, sodium alginate, agar-agar, semi-hydrolyzed starch, dextrin, flax and quince seed (3), as well as soybean milk (***Glycine soja***) which was one of the first vegetable used as semen extender it was

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reported as an good alternative as an extender for bovine semen (4). Some vegetables derivate like coconut water and tomato juice seem to have some properties to protect bovine and caprine sperm cells. Coconut water has showed to be an excellent alternative for conservation of bovine and swine semen (5), (6) as well as caprine semen due the presence of phytohormones. This substance protects the semen of this species against the deleterious effect of the enzyme phospholypase A, secreted by the Cowpers glands and present in the ejaculated semen, (Nunes et al., 1996). Moreover, the same authors reported that ***indole-3-acetic acid*** (IAA) appeared to be the main sperm protective substance present in the coconut water. The present work has the objective to find out an alternative method for buffalo semen preservation, using a new dilutor for buffalo semen.

Material and Methods

Eleven ejaculations from 4 buffaloes 3-4 years of age of Murrah and Mediterranean breed were used for this purpose, Table 1. The semen was collected in an artificial vagina at 44-45 ° C. Each collection consists of two ejaculations - first and second, collected within an interval of minimum 30 minutes. False mounts were performed to increase the quality of the ejaculation. Immediately after the collection the ejaculate must be assessed for volume, color, wave motion, vigor, concentration, live and dead and acrossome lost, according to the recommendation of (1) and (2) with a final concentration maintained at 30 million live sperm/dose (0.25 ml), using the CEBRAN-1 diluter, Table 2, as the current routine performed in CEBRAN's laboratory for deep freezing semen. High temperature viability tests - Thermo Resistance Test (TRT) were performed with frozen semen according to the methodology described by (8).
The coconut fruit must be a new one, and have a fine jelly layer and must be filtered. The final solution must have an osmolarity to 320 milliosmoles and pH = 6,8.

Table 1. - Descriptive data on semen processed as well as evaluation processed using CEBRAN-1 diluent.

Name	Date	N.	Fresh semen			Diluted semen		Frozen semen		T.R.T. 3 0 min		T.R.T. 1 h		L/D	Acrossom
		doses	W. M.	M.	V.	M.	V	M	V	M.	V	M.	V	(%)	damage (%)
Dubak	30.01.97	68	3	80	4	80	4	40	3	30	3	20	2	29/71	6
"	03.02.97	19	3	70	4	80	4	50	3	30	3	20	3	41/59	14
"	08.05.97	23	2	80	3	60	3	40	3	40	3	40	3	31/69	15
Vidrado	30.01.97	12	4	70	4	70	4	40	4	40	3	30	3	52/58	9
"	17.03.97	25	2	80	4	80	4	50	3	40	3	30	3	44/56	14
Vulcão	23.01.97	68	3	60	4	80	5	50	5	40	3	30	3	41/59	5
"	17.03.97	35	3	70	4	80	4	60	3	60	3	40	3	45/55	8
"	03.04.97	77	4	70	4	40	5	30	3	30	3	40	6	42/58	11
Dardo	08.05.97	23	4	80	4	80	4	40	3	40	3	20	2	34/66	12
x=	-	-	3.1	71.1	3.8	72.2	4.1	44.4	3.3	38.8	3.0	30.0	3.1	39/61	10.4

W.M.= wave motion; Mot.=Motility; T.R.T.=Thermo Resistance Test; L/D=live and dead.

Table 2. - Composition of CEBRAN-1 diluter.

EXTENDER	
- Stock solution II	93,0 ml
- Glycerol	7.0 ml
- Penicillin potassium G	1.000 UI/ml
- Streptomycin sulfate	2.0 g/100ml
- Adjust pH (6.8 - 7.0 with 10 % NaOH sol.)	
STOCK SOLUTION I	
- Coconut water	50,0 ml
- Bi-destilated water	25,0 ml
- Sodium citrate 5%	25,0 ml
- q. s. p.	100,0 ml
STOCK SOLUTION II	
- Stock solution I	90, 0 ml
- Egg yolk	10,0 ml
- q. s. p.	100,0 ml

Results and Discussion

The results obtained through the use of the diluent CEBRAN-1, Table 1 and 2, show the feasibility of its use routinely for deep freezing buffalo semen.

The use of organic compounds, vegetable or animal for semen dilution and conservation have been reported for a long time (3), (4), (5) and (9). For caprine species (6) and (7) have found excellent results in deep frozen semen and artificial insemination. Diluted and frozen semen were submitted to the evaluation of motility and vigor, acceding to the methodology described by (1) and (2), as well as to the Thermo Resistance Test (TRT) 30 minutes and 1 hour, live and dead sperm and acrosome damage (8). For fresh semen, the average for wave motion, motility and vigor were 3,1, 71,1 per cent and 3,8, respectively; for diluted semen the average for motility and vigor were 72,2 per cent and 4,1, respectively and for frozen semen motility and vigor were 44,4 per cent 3,3, respectively. Frozen semen was tested through the use of T.R.T. for 30 minutes the motility and vigor were 38,8 per cent 3,0 and for T.R.T. through 1 hour were 30,0 per cent and 3,1, respectively. Also the frozen semen was tested for live and dead sperm and acrossome damage and an average of 39/61 and 10,4 per cent were obtained respectively.

The new diluter based in coconut water shown the maintenance of a higher motility when the semen was diluted in the solution, results which can be compared for others traditional diluters used for bovine and buffalo or in the case of TRIS (hydroxy-methyl-amino-methan) and TES (hydroxy-methyl-amino-ethan) buffered extender, with 7% of glycerol (10), (11), (12), (1) and (2). It can also be pointed out that through TRT that the diluter showed the same characteristic previously observed for other diluters used for buffalo semen as well as the acrossome integrity (8).

It was found that there was a significant increase in sperm motility as soon as the diluter is mixed with the ejaculate; however, a significant decrease was observed in post-thawing which was similar to those observed in other diluters like TES and TRIS buffered extender. Inseminations were performed and there are some pregnant females nevertheless, a large number of buffaloes need to be inseminated under controlled conditions to further fertility results. In view of the present preliminary study it can be inferred that coconut water is an excellent alternative for deep freezing buffalo semen.

The coconut fruit must be a new one, and have a fine jelly layer and must be filtered. The final solution must have an osmolarity adjusted to 320 milliosmoles and pH=6,8

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