

## Analysis of some normal parameters of the spermogram of captive capuchin monkeys (*Cebus apella* Linnaeus, 1758)

### Análise de alguns parâmetros normais do espermograma de macaco-prego (*Cebus apella* Linnaeus, 1758)

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#### SUMMARY

Nine adult males of capuchin monkey (*Cebus apella*) were electro-ejaculated with a rectal bipolar probe under general anaesthesia with tiletamine – zolazepan association. From the obtained ejaculate, we only analysed the liquid fraction in order to avoid any kind of chemical treatment to attain dissolution of the seminal coagulum. The mean obtained volume was 0,2ml with the sperm concentration of  $56.169 \times 10^6$  sperm/ml and mean motility 68,4% and vigor 2,6. The mean percentage of morphologic normal spermatozoa was 39%. The technique was efficient for semen collection in *Cebus apella* and allowed seminal evaluation without possible sperm lesions caused by chemical treatment for coagulum dissolution.

**KEY-WORDS:** Sêmen. Electro-ejaculation. Capuchin Monkey. *Cebus apella*. Primates

#### INTRODUCTION

The reproduction of wild animals in captivity is one of the most important contribution to the conservation of endangered species. Many techniques of artificial reproduction, commonly used with domestic species, can be adapted to wild animals as a helpful tool in conservation<sup>3,6,8,17,18</sup>. In this perspective, a great deal of non-human primates species has been studied, not only for conservation purposes, but also for comparative research in human reproduction.

The majority of the information concerning basic knowledge of normal reproductive parameters is obtained from Old World monkeys as the chimpanzee (*Pan troglodytes*)<sup>4,7</sup> and rhesus (*Macaca mulatta*)<sup>11,14,15</sup>. Regarding the New World monkeys, the information comes mostly from the family Callitrichidae as the common marmoset (*Callithrix jacchus*)<sup>5</sup> and the squirrel monkey (*Saimiri sciureus*)<sup>2</sup>. In the family Cebidae there are fewer data in literature, being most of that related to two different species of capuchin monkey (*Cebus capucinus*)<sup>10</sup> and (*Cebus apella*)<sup>1,9</sup> and more recently to the muriqui (*Brachyteles arachnoides*)<sup>12,13</sup>.

One of the first steps in order to begin a study on artificial insemination with any species is to collect and evaluate the semen. It is already known that semen from primates coagulates after the ejaculation<sup>16</sup>. Some authors

recommend the enzymic liquefaction of the coagulum<sup>1,9,10</sup> while others suggest to allow for a spontaneous liquefaction after a incubation at 37°C and if it was not complete then the remainder coagulum must be dissolved by enzymic digestion<sup>7</sup>. Specifically with the semen from the capuchin monkey it was described that the coagulum must go under enzymic liquefaction because it won't dissolve spontaneously<sup>1,9,10</sup>. In that case the same authors did not evaluate the possible damage to the acrosome that could lead to a reduction of the potential capacity for fertilization as a consequence of the enzymic action. For that reason this work had the objective to collect and evaluate only the liquid fraction of the semen of the capuchin monkey (*Cebus apella*), avoiding any enzymic treatment and measuring the volume of the coagulum only for the calculation of the total volume ejaculated.

#### MATERIAL AND METHOD

Nine adult males *Cebus apella* were studied. All of them were kept under captive conditions at São Paulo Zoo for at least 2 years before the beginning of the study. They were selected according to the following criteria: good health status, based on clinical examination and normal hemogram values, phenotypical appearance with the secondary sexual characteristics like body mass, head tufts and a high level of aggressive behaviour. The highly aggressive behaviour was

also used as a desirable parameter for the males due to the well known positive correlation between testosterone levels and aggressiveness.

The adult condition was checked by the somatic development based on weight and through verification of dental eruption<sup>9</sup>.

The karyotypic study was also made in order to check the number of chromosomes described for this species,  $2n=54^9$ .

Animals were kept in individual cages in order to avoid some interference of dominant males over subordinated ones.

All the animals were anaesthetized prior to each collection procedure with the association of tiletamine hydrochloride and zolazepan, at a dose of 6 mg/Kg, IM.

Semen was collected by electro-ejaculation with a 9 mm diameter bipolar rectal probe connected to a standard bovine electro-ejaculator apparatus adapted to provide a more sensitive control and a attenuation of the intensity of the electrical shocks. The pattern of the electrical stimulation was five series of 20 stimulus each, in a progressive level of current intensity, ranging

from 50, 100, 200, 250, to 300 mA. Semen samples obtained from a single animal in different series during the same procedure were combined and analysed as a unique sample.

Semen obtained was collected in conical glass tubes maintained inside a glass bottle with warm water at 37°C. All the glass slides and pipette tips were also kept at 37°C.

The liquid fraction of the samples was analysed immediately after the collection, in a glass slide under an optical binocular microscope Olympus BHK. The evaluation was done based on the following parameters: vigor, motility, concentration and total volume. An aliquot of semen was fixed in 10% formalin/salin solution and examined under a Zeiss phase contrast microscope for sperm pathology evaluation. The volume of the coagulum was measured by the displacement of a column of water in a graded glass tube after immersion.

## RESULTS

The results of the spermograms of the capuchin monkey are presented in Tab.1.

**Table 1**

Spermogram values and descriptive statistical analysis of nine adults capuchin monkeys (*Cebus apella*), kept under captive conditions at The São Paulo Zoo, São Paulo, Brazil, 2000.

	N°Samples	Mean	Std.Error	Range	Conf.Interv.
<b>Conc.(x10<sup>6</sup>/ml)</b>	<b>34</b>	<b>56169</b>	<b>7920</b>	<b>13400-192000</b>	<b>40056-72282</b>
Motil.(%)	34	68,4	3,1	30-90	62,2-74,6
Vigor (0-5)	34	2,6	0,1	1-4	2,4-2,9
Liq.Vol.(ml)	34	0,2	0,02	0,1-0,5	0,17-0,23
Coag.Vol.(ml)	30	0,3	0,1	0,1-1	0,3-0,5
Total Vol. (ml)	34	0,5	0,1	0,1-1,1	0,4-0,6
Major Def.(%)	29	33	3,5	3-88	25,9-40,1
Minor Def.(%)	29	28	3,8	0-74	20,2-35,8

Conc.= Concentration

Motil.= Motility

Liq.Vol.= Volume of the liquid fraction

Coag.Vol.= Volume of the coagulated fraction

Total.Vol.= Total volume of the ejaculate

Major Def.= Major defects

Minor Def.= Minor defects

## DISCUSSION AND CONCLUSION

Very few information on sperm parameters of *Cebus apella* could be found in literature and they were referred to the whole ejaculate after chemical dissolution of the coagulated fraction of the semen<sup>1,9,10</sup>. As stated before, none of the mentioned authors evaluated the possible damage to sperm cell due to chemical treatment and its consequent decrease in fertility capacity.

The results described in the literature presented some degree of variation among authors<sup>1,9,10</sup>, and when compared with our results we could find similar volumes of ejaculate in two of them (0,6 ml)<sup>10</sup> and (0,58 ml)<sup>1</sup> while the third was more than two fold bigger (1,9 ml)<sup>9</sup>. The motility was much better than in the first work (24%)<sup>10</sup> and very close to the two others (62,1%)<sup>1</sup> and (79%)<sup>9</sup>. The most remarkable difference were found in the concentration where the authors presented two to four fold higher results (161,1x10<sup>6</sup>/ml)<sup>10</sup>, (255x10<sup>6</sup>/ml)<sup>1</sup>

and (207x10<sup>6</sup>/ml)<sup>9</sup>. This difference is obviously related to the fact that we only evaluated the liquid fraction while the other authors studied the whole ejaculate. The entrapment of a great number of spermatozoa inside the coagulum was already described<sup>1</sup>.

In respect to the sperm cell pathology we found only one work in literature that reports as the most frequent defects the detached heads and tails and the twisted and bent tails<sup>1</sup>. There was no quantification of the defects.

This discussion clearly shows the lack of information on normal spermogram of the *Cebus apella*, what lead to conclude that the parameters here described are of great relevance, but further studies are imperative.

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## RESUMO

Foram submetidos a eletro-ejaculação 9 macacos-prego, adultos, pertencentes à Fundação Parque Zoológico de São Paulo, com o uso de eletrodo retal bipolar, sob anestesia geral obtida pela aplicação da associação tiletamina-zolazepan. Do sêmen assim obtido foram analisadas apenas as frações líquidas resultantes após a coagulação do ejaculado, não havendo nenhum tratamento químico para sua dissolução, apenas a separação das frações após a ejaculação. O volume médio obtido foi de 0,2ml, a concentração média de  $56.169 \times 10^6$  espermatozoides/ml, com motilidade média de 68,4% e vigor médio de 2,6. O percentual médio de espermatozoides morfológicamente normais foi de 39%. A técnica é eficiente em macacos-prego (*Cebus apella*) e estes resultados permitem a avaliação do sêmen evitando-se os danos causados pela dissolução química do coágulo seminal.

**PALAVRAS-CHAVE:** Sêmen. Eletro-ejaculação. Macaco-Prego. *Cebus apella*. Primatas.

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