

# Chilled Semen of Captive Collared Peccaries (*Pecari tajacu*): Effects of Preservation at 17 °C on Semen Quality

Alexandre Rossetto Garcia<sup>1</sup>, Priscila Reis Kahwage<sup>2</sup>, Diva Anelie de Araújo Guimarães<sup>2</sup> and Otávio Mitio Ohashi<sup>2</sup>

1. Brazilian Agricultural Research Corporation/Embrapa Southeast Livestock-Rod, Washigton Luiz km 234, São Carlos, São Paulo 13560-970, Brazil

2. Federal University of Pará, Rua Augusto Corrêa n.1, Belém, Pará 66075-110, Brazil

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**Abstract:** The trade creation and conservation of wild species in Amazon, including collared peccaries (*Pecari tajacu*), may be favored by artificial insemination, but the time of semen storage may lead to reduced fertility in sperm of some wild animals. Thus, the aim of this research was to evaluate the influence of semen storage at 17 °C over time on seminal features of peccaries in captivity. Eight adult males were sedated and underwent electroejaculation. The ejaculates (n = 65) were evaluated for volume, concentration, pH, sperm motility, vigor, and cell with intact plasma membrane intact (IPM) and sperm morphology. Selected ejaculates (n = 21) were diluted (1:1) in Beltsville Thawing Solution and kept during 48 hours under controlled temperature (17 °C). Assessments were made after dilution (T0), after 24 hours (T24), and 48 hours after the onset of cooling (T48). The storage impacted on sperm survival ( $P < 0.05$ ). Semen characteristics changed throughout the storage period studied and after 48 hours storage. The decline of sperm motility was of 55.2% for 10.9%, vigor was 2.3 for 0.5 and IPM cells were of 59.0% for 15.8%. Primary defects sperm increased of 19.8% for 32.2%, secondary defects of 9.8% for 10.4% and total defects of 29.4% for 42.7%. However, within 24 hours of preservation, chilled semen peccaries presented sperm motility average rate and IMP cells levels indicative to use in assisted reproductive events. These results indicate chilled semen for 24 hours could be used in experimentally artificial insemination of peccaries, technology that still has not been performed before.

**Key words:** Sperm, collared peccary, wildlife, semen conservation, Amazon.

## 1. Introduction

According to the Food and Agriculture Organization of the United Nations, the conservation of biological diversity is important to ensure food security in economical disadvantaged populations [1], including traditional inhabitants of Brazilian Amazon, who use wild animals as an important alimentary alternative [2]. Among the most consumed species is the collared peccary (*Pecari tajacu*), a small artiodactyl that belongs to a separate family within the

Suiformes, the Dicotylidae, and most frequently hunted in Latin-America [3]. Peccaries are widespread in the American continent and are able to live in rainforests, savannas or deserts [4]. They feed on invertebrates, seeds, roots, forages, fruits and insects in natural habitat [5], but adapt to different feedstuffs in captivity [6].

The peccary (*Pecari tajacu*) is one of the Amazon native species that has a high yield potential for captive breeding. In the Eastern Amazon, reproductive activity of *Pecari tajacu* starts between 8 and 16 months old, parturitions are distributed throughout the year, with  $1.86 \pm 1.35$  newborns per female per year, the

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**Corresponding author:** Alexandre Rossetto Garcia, DVM, Ph.D., research fields: animal andrology, semen cryopreservation. E-mail: argarcia@cnpse.embrapa.br.

parturition-conception interval is  $58 \pm 103.7$  days, with mean production of  $1.03 \pm 0.73$  litter per female. Mean age at first parturition is  $639 \pm 243$  days, multiparous females have  $1.40 \pm 0.50$  parturitions and  $2.47 \pm 0.99$  piglets per year, 79.7% of parturitions are double and mean farrowing interval is  $196 \pm 104$  days [7]. The length of the estrous cycle ranges from 22 to 28 days [8], with gestation of  $138 \pm 5$  days [9] and estrus post parturition occurs between days 4 and 16 [10].

Even for wildlife species, artificial insemination (AI) can constitute an useful tool to interchange biological material, to increase genetic variability and to enhance the sustainability of animal populations, specially that raised in captivity. Consolidation of AI technique is based on frozen semen application, which depends on an adequate comprehension of chilling, in order to minimize injuries to spermatozoa and increase the fertility. Despite the progress achieved in semen freezing for many wildlife species [11-13], cryopreservation of peccaries semen still remains practically unknown and a few papers related were recently published [14-16]. Due to general similarities with the domestic pig (*Sus scrofa*), semen processing protocols [17] have been extrapolated from this species. Boar semen can typically be cooled to 15-18 °C and held for numerous days in liquid form for using in AI programs or transporting to laboratories for cryopreservation [18], but there are no reports on attempts to chill semen of *Pecari tajacu*. Therefore, this study aimed to evaluate the preservation of *Peccaries* diluted semen under refrigeration at 17 °C, and to measure the effects of storage time on motility, vigor, sperm membrane integrity and sperm morphology.

## 2. Materials and Methods

### 2.1 Animals and Experimental Period

The animals were originated from the experimental breeding farm of Embrapa Eastern Amazon, located in Belém, State of Pará, Brazil (01°24' S; 48°20' W). The research was developed from October/2007 to

February/2009. The experimental design was performed according to current Brazilian bioethical laws and EU directives for animal experiments. Eight sexually mature collared peccaries aged  $76.8 \pm 37.8$  months and weighing  $19.5 \pm 2.7$  kg were selected. The animals were maintained in collective paddocks of 36 m<sup>2</sup> and fed with fruits, forages, swine ration for growth and fattening with *ad libitum* water. Twenty-four hours before the experiment, the animals were isolated and went through fasting, no water or food.

### 2.2 Semen Collection

Before semen collection, adequate sedation and analgesy were obtained after the administration of acepromazine and ketamine. First, acepromazine (Acepran 0.2%®, Univet S.A., São Paulo-SP, Brazil) was applied as pre-anesthetic medication at 0.2 mg/kg IM. After five minutes, ketamine was administrated (Dopalen®, Vetbrands Saúde Animal, Jacareí-SP, Brazil) at 5 mg/kg IM. The animals were placed in lateral recumbent position and semen was collected through electroejaculator inserting 20 × 1.9 cm probe [19] connected to a piece of electroejaculation equipment with 12 V source (Boijektor 2001®, Comercial Barbôs Ltda., São Paulo-SP, Brazil) into the rectum.

The stimulation protocol consisted in the application of  $45 \pm 13$  continuous stimuli with intensity lower than 12 volts, and each stimulus was applied for every three seconds, followed by a three second rest [20]. The ejaculated samples were immediately subjected to quantitative and qualitative assessments.

### 2.3 Semen Evaluation

The ejaculates were maintained at 35 °C throughout the analysis. The semen volume (mL) was measured with analytical micropipettes. Seminal pH was measured with pH Tests® (Merck KGaA, Darmstadt, Germany). The sperm concentration was determined after semen diluting in buffered formalin, in a

proportion of 1:100, followed by cell counting in a hemocytometer, and results were expressed as sperm  $\times 10^6/\text{mL}$  [20].

Aliquots of 10  $\mu\text{L}$  raw semen were assessed for the sperm motility (%) and vigor (0-5), under light microscopy, under 100  $\times$  magnification. Semen smears were prepared to determine the integrity of plasma membrane percentage using eosin-nigrosin stain, since in spermatozoa with disrupted post acrosomal membrane, eosin penetrates in the cell, binds and stains the DNA, and appear pink, while cell with intact membrane exclude the eosin and appears white [21]. An amount of 200 cells were counted in each sample, under 1,000  $\times$  magnification, and the live spermatozoa with intact plasma membrane (IPM) were determined in percentage.

The assessment of sperm morphology was evaluated in Congo Red stained slides under light microscopy (1,000  $\times$ ) to detect abnormal cells. For each sample, an amount of 200 cells were counted, after individual classification of abnormalities that were grouped in primary or secondary defects; sperm morphology results were determined in percentage [22].

#### 2.4 Semen Conservation

The ejaculates were diluted at a ratio of 1:1 in Beltsville Thawing Solution (BTS), such as used for domestic pigs [23]. The diluted semen samples were placed in a container filled with water at 35 °C, so that they proceeded to chilling. Then, the diluted samples were kept for 48 hours under controlled temperature (17 °C) in an incubator equipped with digital thermostat, which was opened only for the assessment of samples. The adopted cooling curve was performed in two stages with different cooling rates (35 °C to 25 °C = -0.38 °C/min and 24.9 °C to 17 °C = -0.11 °C/min) [24]. Diluted seminal samples reached 17 °C in approximately 95 minutes.

To verify the effect of storage time on semen parameters, aliquots were evaluated in different

moments. The sperm motility, sperm vigor, morphology and IPM cells were assessed immediately after dilution (Time 0 = T0), 24 hours (Time 24 = T24) and 48 hours after the onset of chilling (Time 48 = T48). Samples were gently homogenized before each assessment in order to disperse toxic factors resulting from the spermatozoa metabolism [25]. Each aliquot of semen was removed from refrigerated incubator and it was preheated during five minutes before the assessments, so that the hipobiotic cells resumed their normal metabolism [26].

#### 2.5 Statistical Analysis

The results were subjected to analysis of variance, using the method of least squares and the effects of time immediately after dilution and storage periods (T0, T24 and T48) were tested. The *F* test was performed and the means were compared using the Tukey test in order to determine the significance of the effects of the factors studied. The level of probability adopted was 5% and the results were analyzed using the NTIA Software, Version 4.2.2 [27].

### 3. Results and Discussion

#### 3.1 Raw Semen Features and Individual Variation

The characteristics of collected ejaculates and sperm parameters of raw semen on peccaries are shown in Table 1. The mean results were similar to previous data [28, 29] or superior to some published information [20]. Considering that *Pecari tajacu* is a wild species and the males used were not previously selected for fertility, the mean raw semen features suggested feasible storage.

There were individual variations in the sperm production, quality and preservation among the males. The most of individuals selected presented motility range from 45.0%  $\pm$  27.8% to 62.1%  $\pm$  24.1%, spermatozoa with intact plasm membrane from 45.9%  $\pm$  23.7% to 66.0%  $\pm$  23.3% and total morphological defects from 22.5%  $\pm$  4.9% to 28.0%  $\pm$  11.6%. One of the semen donor presented high quality semen, with

**Table 1 Raw semen characteristics of adult *Pecari tajacu* collected by electroejaculation (65 samples).**

| Variable                                | Mean ± SD     |
|---|---------------|
| Volume (mL)                             | 0.8 ± 0.8     |
| Concentration (×10 <sup>6</sup> spz/mL) | 138.1 ± 154.0 |
| pH                                      | 7.92 ± 0.73   |
| Motility (%)                            | 52.8 ± 29.1   |
| Vigor (0-5)                             | 2.1 ± 0.8     |
| IPM (%)                                 | 55.4 ± 28.6   |
| Primary defects (%)                     | 22.6 ± 13.1   |
| Secondary defects (%)                   | 9.6 ± 7.2     |
| Total defects (%)                       | 31.9 ± 13.5   |

IPM = Spermatozoa with intact plasma membrane.

82.2% ± 5.6% of motility, 83.1% ± 5.8% of IPM cells and 22.5% ± 4.9% of total defects. On the other hand, there was one male presented lower seminal traits, such as 18.2% ± 20.5% of sperm motility, 20.6% ± 23.2% of IPM cells and 50.9% ± 14.3% of total defects. Differences among individuals and among ejaculates from the same animal remained during sperm conservation and highlighted the individual effect on semen preservation during storage, concerning the maintenance of fertilizing ability and cryoresistance [30].

### 3.2 Effect of the Storage Period on Sperm Survival

During the storage period, there was an expected reduction in sperm motility ( $F = 94.5$ ), in vigor ( $F = 123.05$ ), in the percentage of IPM cells ( $F = 94.4$ ) and

increase in sperm defects (major defects,  $F = 78.3$  and total defects,  $F = 89.6$ ) (Table 2). The decline of sperm motility, vigor and IPM cells after 48 hours of storage was of 55.2% for 10.9%, 2.3 for 0.5 and 59.0% for 15.8%, respectively, associated with an increase of total defects from 29.4% to 42.7%, and indicated that preservation by cold induced structural changes which compromised cell integrity and functionality. These results corroborate the findings of decrease in motility, viability and reduced acrosome integrity after cooling in swine sperm [31].

Cooling rate is a critical variable influencing sperm cryosurvival and thus it is a prerequisite for an optimal sperm preservation protocol [32]. Despite the adoption of the optimal cooling rate for boar spermatozoa [33] in the used curve, spermatozoa from different species respond differently to chilling process. However, results demonstrated that spermatozoa of *Pecari tajacu* presented sensitivity to the induced cold in a very similar way to swine spermatozoa, which are considered one of the most sensitive when compared to other domestic species [34]. Nevertheless, other authors have described a superior cryotolerance to *Pecari tajacu* spermatozoa [35].

The resistance of spermatozoa to cold shock differs among species, depending on the chemical and molecular composition of plasma and mitochondrial

**Table 2 Effect of time on motility, vigor, spermatozoa with intact plasma membrane (IPM) and frequency of primary, secondary and total defects observed in the semen of *Pecari tajacu*, during storage to 17 °C.**

| Seminal features      | Storage time                 |                              |                              |
|-----------------------|------------------------------|------------------------------|------------------------------|
|                       | T0                           | T24                          | T48                          |
| Motility (%)          | 55.2 ± 19.8 a<br>(10.0-80.0) | 22.5 ± 25.9 b<br>(0-80.0)    | 10.9 ± 22.0 c<br>(0-70.0)    |
| Vigor                 | 2.3 ± 0.5 a<br>(1-3)         | 1.1 ± 1.0 b<br>(0-3)         | 0.5 ± 0.7 c<br>(0-2)         |
| IPM (%)               | 59.0 ± 19.4 a<br>(15.0-85.0) | 30.4 ± 25.2 b<br>(0-80.0)    | 15.8 ± 23.1 c<br>(0-71.0)    |
| Primary defects (%)   | 19.8 ± 10.1 a<br>(5.5-54.0)  | 29.5 ± 11.9 b<br>(7.5-66.0)  | 32.2 ± 12.5 c<br>(9.5-69.0)  |
| Secondary defects (%) | 9.8 ± 4.4 a<br>(3.0-21.5)    | 9.7 ± 5.1 a<br>(2.0-23.5)    | 10.4 ± 5.3 a<br>(2.5-27.0)   |
| Total defects (%)     | 29.4 ± 10.9 a<br>(13.5-63.0) | 39.3 ± 12.7 b<br>(19.5-74.0) | 42.7 ± 12.9 c<br>(22.0-77.0) |

T0 = immediately diluted semen, T24 = 24 hours of storage, T48 = 48 hours of storage.

a, b, c Values within a row with different superscript letters are significantly different at  $P < 0.05$ .

Values are expressed as mean and standard deviation; maximum and minimum in parentheses.

membranes, especially fatty acids and phospholipids [36]. The swine spermatozoa sensitivity may be related to the low ratio of cholesterol: phospholipid (0.12) presented on the plasma membrane, which is lower than that observed in bulls (0.38) or rams (0.36), and this characteristic predisposes the inner layer of the plasma membrane vulnerability to cold shock [37]. Nowadays, no reports about chemical composition and molecular plasma membrane of spermatozoa of peccaries are published.

The storage period impacted on sperm survival, because during the time, structural and functional changes occurred, probably due to physical and chemical stresses. Functional changes are related to alterations in mitochondria, flagellum, acrosome, plasma membrane and chromatin [38, 39]. Furthermore, during the thermal shock, an accumulation of intracellular  $\text{Ca}^{2+}$ ,  $\text{Na}^{+}$  and  $\text{Zn}^{2+}$  occurs, and there is a loss of  $\text{K}^{+}$  and  $\text{Mg}^{2+}$ . Also, other structural damages occur, such as a detachment of the acrosome, spoliation of enzymes and proteins, a loss in midpiece and tail plasma membrane, and a change in the internal arrangement of mitochondria [38]. These injuries are incompatible with fertility, and lead to a drop in sperm motility, a reduction of metabolic activity and a loosening of cellular components [40, 41].

Although the parameters have markedly declined after 48 hours of storage, the decreases in the first 24 hours were less intense. After 24 hours of conservation, sperm motility, vigor, IPM cells and total defects were 22.5%, 1.1, 30.4% and 39.3%  $\pm$  12.7%, respectively. Individual effects were observed during storage period. After 24 hours of conservation, some males presented interesting values for sperm motility ( $> 35\%$ ), for IPM cells ( $> 40\%$ ) and for total defects ( $< 30\%$ ), which can be considered acceptable features for using the semen in assisted reproduction biotechnologies. Since it was the first attempt to chill *Pecari tajacu* semen, it is clear that technical advances and more essays are necessary, in order to improve the results. Nevertheless, based on published data on

domestic swine [42], it would be supposed that peccaries short-term stored chilled semen might present potential fertility to achieve satisfactory conception rates in artificial insemination programs. According to these authors, fertility rate of 71% was observed in sows inseminated with cooled semen presenting 30% sperm motility and 35.5% of cells with normal acrosome.

The severity of the effects observed after 48 hours of conservation may be related to the cooling rate and absence of prior incubation temperature, since the very rapid cooling leads to thermal shock and this, in turn, causes severe damage to sperm [43]. Swine sperm acquires resistance to cold when incubated at 15 °C and, therefore, it increases the rates of spermatozoa survival when they are subjected to conservation [44]. In this study, the cells showed sensitivity throughout the storage period, since there was a reduction in motility and in vigor within 48 hours. Procedures related to dilution, cooling and rewarming lead to occurrence of sperm abnormalities, mainly those related to tail and acrosome structures, which lead to changes in motility [13, 45], sperm capacitation and acrosome reaction [46]. Sperm cells stored under refrigeration at 17 °C do not have the fully reduced cellular metabolism, and it causes them to have greater susceptibility to morphofunctional changes [34].

### 3.3 Sperm Defects Detected during Storage Period

Absolute increases of 12.4% for primary defects and 13.3% of total defects were detected during storage time, while no difference was observed for secondary defects in T0, T24 and T48 ( $F = 0.57$ ). The most frequent primary defects observed were tightly bent or coiled tail (TBCT), acrosome defects (ACROS) and proximal cytoplasmic droplets (PCD), as showed in Table 3. Secondary defects found in semen diluted and stored were simple bent tail (SBT), abaxial insertion (AB) and distal cytoplasmic droplets (DCD). The incidence of distal cytoplasmic droplets was

**Table 3** Sperm abnormalities more frequently observed in *Pecari tajacu* semen, during storage temperature 17 °C.

| Primary defects                     | Storage time |        |        |
|-------------------------------------|--------------|--------|--------|
|                                     | T0           | T24    | T48    |
| Tightly bent or coiled tail (TBCT)  | 10.14%       | 14.64% | 15.31% |
| Acrosome defects (ACROS)            | 6.69%        | 9.01%  | 10.53% |
| Proximal cytoplasmic droplets (PCD) | 3.68%        | 4.78%  | 4.44%  |
| Secondary defects                   | T0           | T24    | T48    |
| Distal cytoplasmic droplets (DCD)   | 6.48%        | 5.76%  | 4.91%  |
| Abaxial insertion (AB)              | 2.93%        | 3.25%  | 3.41%  |
| Simple bent tail (SBT)              | 2.6%         | 3.81%  | 2.89%  |

decreased from T0 to T48, and there was no significant effect of storage time on the frequency of cells presenting simple bent tail or abaxial insertion.

Incidence of TBCT and ACROS may be related to chilling procedures or to the rapid reheating of the samples, which leads to reduced motility and lower fertilizing ability of spermatozoa [47, 48]. Approximately 20% of spermatozoa submitted to the cooling to 8-12 °C presented curvature on the flagellar region or in the region before the midpiece, which originates a typical 180 °arc damaged-flagellum [49]. This damage is directly related to structural changes of the lipid layer of the plasma membrane, and results in changes in sperm tail, with negative effect on sperm motility [50].

The low level of PCD incidence is considered physiological, but when it occurs with high incidence (> 30%) it may be associated with abnormal spermatogenesis in adult males [51], but no effect of cooling process is related to this kind of abnormality. The abaxial insertion of midpiece was identified, but at a low frequency (2.5%) in spermatozoa of peccaries. The sperm head of peccaries is wider (2.4 µm) than sperm cells from other species [52] and it could favor the decentralized implantation of the midpiece. The spermatozoa head size can be related to the sperm vulnerability to the cold shock. Spermatozoa with larger and flatter heads, such as swine sperm cells, could be more sensitive than smaller and more compact head sperms [34]. Since the spermatozoa of peccaries present broad and flatten head, similar to the swine sperm, it could have contributed to their

sensitivity observed in this study.

Despite the reduction in sperm features observed during the storage period, the semen of peccaries should not be considered impracticable for fertilization after chilling and 24 hours storage. The maintenance of the *Pecari tajacu* in captivity has recently emerged as an economic alternative in response to demands for sustainable development. Thus, many questions about their reproductive aspects, behavior, nutrition and basic health need to be clarified. Therefore, the success rates observed, although being bellow the domestic animals standard, should not discourage further research on peccaries semen conservation and artificial insemination.

#### 4. Conclusions

The semen of peccaries presented sensitivity to cold and decreased quality in 48 hours storage. Additional research in this field is necessary to improve the technique, but the semen characteristics observed after 24 hours of storage are indicative of potential fertilizing ability. Thus, short-term chilled semen could be used in experimentally artificial insemination of peccaries, technology that still has not been performed before. Additionally, more studies concerning the chemical composition, molecular and ultrastructural sperm of peccaries are critical to determine suitable methods for longer conservation.

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## References

- [1] Food and Agriculture Organization of the United Nations, Agriculture and environmental challenges of the twenty-first century: A strategic approach for FAO [online], 2009, [http://www.fao.org/unfao/bodies/coag/coag21/index\\_en.htm](http://www.fao.org/unfao/bodies/coag/coag21/index_en.htm).
- [2] R.E. Bodmer, J.F. Eisenberg, K.H. Redford, Hunting and the likelihood of extinction of Amazonian mammals, *Conservation Biology* 11 (1997) 460-466.
- [3] J.G. Robinson, K.H. Redford, Neotropical Wildlife Use and Conservation, University of Chicago Press, Chicago, 1991.
- [4] R.M. Nowak, J.L. Paradiso, Walker's Mammals of the World, 4th ed., Johns Hopkins University Press, London, 1983.
- [5] L.A. Deutsch, L.R.R. Puglia, Os Animais Silvestres: Produção e Manejo, Globo, Rio de Janeiro, 1988, pp. 69-72.
- [6] H. Liva, L.F.D. Moraes, S.G. Nogueira Filho, A. Lavorenti, Aspectos da alimentação do caititu (*Tayassu tajacu*) em cativeiro. Anais, Congresso Paulista de Iniciação Científica, I, Piracicaba, 1989.
- [7] P. Mayor, D.A.A. Guimarães, I. Le Pendu, J. Da Silva, F. Jori, M. López-Béjar, Reproductive performance of captive collared peccaries (*Tayassu tajacu*) in the Eastern Amazon, *Animal Reproduction Science* 102 (2007) 88-97.
- [8] R. Mauget, F. Feer, O. Henry, G. Dubost, Hormonal and behavioral monitoring of ovarian cycles in peccaries, in: Proceedings of the First International Symposium on Physiology and Ethology of Wild and Zoo Animals II, 1997, pp. 145-149.
- [9] P. Mayor, F. Lopez-Gatius, M. Lopez-Bejar, Integrating ultrasonography within the reproductive management of the collared peccary (*Tayassu tajacu*), *Theriogenology* 63 (2005) 1832-1843.
- [10] L.K. Sowls, The Peccaries, The University of Arizona Press, Tucson, USA, 1984.
- [11] S.A. Hussain, C. Lessard, M. Anzar, Quantification of damage at different stages of cryopreservation of endangered North American bison (*Bison bison*) semen and the effects of extender and freeze rate on post-thaw sperm quality, *Animal Reproduction Science* 129 (2011) 171-179.
- [12] A.E. Domínguez-Rebolledo, M.R. Fernández-Santos, A. Bisbal, J.L. Ros-Santaella, M. Ramón, M. Carmona, et al., Improving the effect of incubation and oxidative stress on thawed spermatozoa from red deer by using different antioxidant treatments, *Reproduction Fertility and Development* 22 (2010) 856-870.
- [13] M.A. Stoops, M.W. Atkinson, E.S. Blumer, M.K. Campbell, T.L. Roth, Semen cryopreservation in the Indian rhinoceros (*Rhinoceros unicornis*), *Theriogenology* 73 (2010) 1104-1115.
- [14] A.L.P. Souza, T.S. Castelo, J.P.A.F. Queiroz, I.O. Barros, V.V. Paula, M.F. Oliveira, et al., Evaluation of anesthetic protocol for the collection of semen from captive collared peccaries (*Tayassu tajacu*) by electroejaculation, *Animal Reproduction Science* 116 (2009) 370-375.
- [15] P.R. Kahwage, A.R. Garcia, D.A.A. Guimarães, O.M. Ohashi, R.S. Luz-Ramos, H.L.T. Dias, et al., Biometria testicular, eletroejaculação e características seminais de caititus, *Tayassu tajacu* Linnaeus, 1758 (Mammalia, Artiodactyla, Tayassuidae) mantidos em cativeiro na Amazônia Oriental, *Acta Amazonica* 40 (2010) 771-778.
- [16] T.S. Castelo, F.S. Bezerra, G.L. Lima, H.M. Alves, I.R. Oliveira, E.A. Santos, et al., Effect of centrifugation and sugar supplementation on the semen cryopreservation of captive collared peccaries (*Tayassu tajacu*), *Cryobiology* 61 (2010) 275-279.
- [17] E. De Mercado, A. Rodríguez, E. Gómez, E. Sanz, Cryopreservation of Iberian pig spermatozoa, comparison of different freezing extenders based on post-thaw sperm quality, *Animal Reproduction Science* 118 (2010) 54-61.
- [18] C.X. Zou, Z.M. Yang, Evaluation on sperm quality of freshly ejaculated boar semen during *in vitro* storage under different temperatures, *Theriogenology* 53 (2000) 1477-88.
- [19] A.R. Garcia, O.M. Ohashi, D.A.A. Guimarães, R.S. Luz-Ramos, P.R. Kahwage, H.L.T. Dias, et al., Eficiência da eletroejaculação em Caititus (*Tayassu tajacu*), in: Proceedings of the XXVIII: Congresso Brasileiro de Reprodução Animal, Belo Horizonte, MG, Brazil, 2009, p. 462.
- [20] D.S. Costa, T.A.R. Paula, Coleta e avaliação de sêmen de catetos (*Tayassu tajacu*), *Biota Neotropica* 5 (2005) 131-136.
- [21] E. Mocé, J.K. Graham, *In vitro* evaluation of sperm quality, *Animal Reproduction Science* 105 (2008) 104-118.
- [22] E. Blom, On the evaluation of bull semen with special reference to the employment for artificial insemination, Ph.D. Thesis, Copenhagen, Carl F. Mortensen, 1950.
- [23] L.D.S. Murgas, M.G. Zangerônimo, A.G.O. Santos, S.L.

- Oliveira, Oxitocina no sêmen suíno heterospermico resfriado a 15 °C, *Ciência Animal Brasileira* 3 (2002) 33-40.
- [24] E.F. Nascimento, V.R. Vale Filho, J.M. Silva Filho, J.K.F. Nascimento, Efeito da taxa de resfriamento sobre a motilidade, sobrevivência e morfologia espermática de varrões, *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 50 (1998) 691-694.
- [25] A.N.M.A. Vasconcelos, G. Moraes, I. Moreira, L.P. Rigolon, E.M. Martins, Características espermáticas de sêmen resfriado de suíno e conservação em diferentes diluentes, *Revista Brasileira de Zootecnia* 30 (2001) 394-401.
- [26] M.J. Estienne, A.F. Harper, J.L. Day, Characteristics of sperm motility in boar semen diluted in different extenders and stored for seven days at 18 °C, *Reproductive Biology* 7 (2007) 221-223.
- [27] Embrapa, Ambiente Software NTIA, Versão 4.2.2: Manual do Usuário, first ed. Embrapa-Centro Nacional de Pesquisa Tecnológica em Informática para Agricultura, Campinas, 1997.
- [28] R.L. Lochmiller, E.C. Hellgren, L.W. Varner, L.W. Greene, M.S. Amoss, S.W.J. Seager, et al., Physiological responses of the adult male of collared peccary *Tayassu tajacu* (Tayassuidae) to severe dietary restriction, *Comparative Biochemistry and Physiology* 82 (1985) 49-58.
- [29] E.C. Hellgren, R.L. Lochmiller, M.S.J.R. Amoss, S.W. Seager, R.J. Magyar, K.P. Coscarelli, et al., Seasonal variation in serum testosterone testicular measurements and semen characteristics in the collared peccary (*Tayassu tajacu*), *Journal of Reproduction and Fertility* 85 (1989) 677-686.
- [30] J. Roca, M. Hernandez, G. Carvagal, J.M. Vazquez, E.A. Martinez, Factors influencing boar sperm cryosurvival, *Journal of Animal Science* 84 (2006) 2692-2699.
- [31] C. Dube, M. Beaulieu, C. Reye-Moreno, C. Guillemette, J.L. Bailey, Boar sperm storage capacity of BTS and Androhep Plus: Viability, motility, capacitation, and tyrosine phosphorylation, *Theriogenology* 62 (2004) 874-886.
- [32] R. Großfeld, B. Sieg, C. Struckmann, A. Frenzel, W.M.C. Maxwell, D. Rath, New aspects of boar semen freezing strategies, *Theriogenology* 70 (2008) 1225-1233.
- [33] R.V. Devireddy, B. Fahrig, R.A. Godke, S.P. Leibo, Subzero water transport characteristics of boar spermatozoa confirm observed optimal cooling rates, *Molecular Reproduction and Development* 67 (2004) 446-457.
- [34] P.F. Watson, The causes of reduced fertility with cryopreserved semen, *Animal Reproduction Science* 60-61 (2000) 481-492.
- [35] T.S. Castelo, F.S. Bezerra, A.L. Souza, M.A. Moreira, V.V. Paula, M.F. Oliveira, et al., Influence of the thawing rate on the cryopreservation of semen from collared peccaries (*Tayassu tajacu*) using Tris-based extenders, *Theriogenology* 74 (2010) 1060-1065.
- [36] W.V. Holt, A. Medrano, L.M. Thurston, P.F. Watson, The significance of cooling rates and animal variability for boar sperm cryopreservation: Insights from the cryomicroscope, *Theriogenology* 63 (2005) 370-382.
- [37] F.E. De Leeuw, B. Colenbrander, A.J. Verkleij, The role membrane damage plays in cold shock and freezing injury, *Reproduction in Domestic Animals Suppl.* 1 (1991) 95-104.
- [38] L.A. Johnson, K.F. Weitze, P. Fiser, W.M.C. Maxwell, Storage of boar semen, *Animal Reproduction Science* 62 (2000) 143-151.
- [39] Z. Nur, B. Zik, B. Ustuner, H. Sagirkaya, C.G. Ozguden, Effects of different cryoprotective agents on ram sperm morphology and DNA integrity, *Theriogenology* 76 (2010) 1267-1275.
- [40] D.L.K. Baudi, K. Jewgenow, B.S. Pukazhenthii, K.M. Spencoski, A.S. Santos, A.L.S. Reghelin, et al., Influence of cooling rate on the ability of frozen-thawed sperm to bind to heterologous zona pellucida, as assessed by competitive *in vitro* binding assays in the ocelot (*Leopardus pardalis*) and tigrina (*Leopardus tigrinus*), *Theriogenology* 69 (2008) 204-211.
- [41] A. Kumaresan, G. Kadirvel, K.M. Bujarbaruah, R.K. Bardoloi, A. Das, S. Kumar, et al., Preservation of boar semen at 18 °C induces lipid peroxidation and apoptosis like changes in spermatozoa, *Animal Reproduction Science* 110 (2008) 162-171.
- [42] J.A.C. Gonsales, J.C. Deschamps, M.N. Corrêa, T.J. Lucia, A.C. Machado, D.C. Rech, Fertilidade do sêmen suíno preservado a 5 °C em diluente contendo trealose, *Revista Brasileira de Agrociência* 5 (1999) 233-236.
- [43] P.F. Watson, J.M. Plummer, The response of boar sperm membranes to cold shock and cooling, in: *Proceedings of International Conference on Deep Freezing of Boar Semen*, Swedish University of Agriculture Sciences, Uppsala, Sweden, 1985, pp. 113-127.
- [44] V.G. Pursel, L.A. Johnson, L.L. Schulman, Effect of dilution, seminal plasma and incubation period on shock susceptibility of boar spermatozoa, *Journal of Animal Science* 37 (1973) 528-531.
- [45] R. Muiño, A.I. Peña, A. Rodríguez, C. Tamargo, C.O. Hidalgo, Effects of cryopreservation on the motile sperm subpopulations in semen from Asturiana de los Valles bulls, *Theriogenology* 72 (2009) 860-868.
- [46] G.J. Morris, K. Faszer, J.E. Green, D. Draper, B.W.W. Grout, F. Fonseca, Rapidly cooled horse spermatozoa: Loss of viability is due to osmotic imbalance during



- thawing, not intracellular ice formation, *Theriogenology* 68 (2007) 804-812.
- [47] J.B. Siqueira, J.D. Guimarães, E.P. Costa, M. Henry, C.A.A. Torres, M.V. Gualberto, et al., Relação da taxa de gestação com sêmen bovino congelado e testes de avaliação espermática *in vitro*, *Revista Brasileira de Zootecnia* 36 (2007) 387-395.
- [48] A.M. Petrunkina, G. Volker, K.F. Weitze, M. Beyerbach, E. Töpfer-Petersen, D. Waberski, Detection of cooling-induced membrane changes in the response of boar sperm to capacitating conditions, *Theriogenology* 63 (2005) 2278-2299.
- [49] W.V. Holt, G.J. Morris, G. Coulson, R.D. North, Direct observation of cold-shock effects in ram spermatozoa with the use of a programmable cryomicroscope, *Journal of Experimental Zoology* 246 (1988) 305-314.
- [50] C.C. Love, Relationship between sperm motility, morphology and the fertility of stallions, *Theriogenology* 76 (2011) 547-557.
- [51] A.D. Barth, R.J. Oko, *Abnormal Morphology of Bovine Spermatozoa*, 1st ed., Iowa State University Press, Ames, 1989.
- [52] A.D. Meisner, A.V. Klaus, M.A. O'Leary, Sperm head morphology in 36 species of Artiodactylans, Perissodactylans, and Cetaceans (Mammalia), *Journal of Morphology* 263 (2005) 179-202.