

**ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)**

Physiology of reproduction in male and semen technology

**Comparison between ram individual ejaculates and semen pool under experimental conditions****Rachel Ferreira Braga , Lucas Francisco Leodido Correia , Caroline Gomes do Espirito Santo , Cleber Jonas Carvalho de Paula , Amanda Bricio Pereira de Andrade , Viviane Lopes Brair , Felipe Zandonadi Brandão , Joanna Gonçalves de Souza-Fabjan**

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In scientific experiments, the use of semen in the sheep model is important to develop and/or optimize cryopreservation techniques, for further improvement in male genetic material dissemination and livestock reproductive increment. Semen pool is described by some researchers as a way to minimize ram individual effect and, therefore, standardize statistical parameters and the results reliability. However, it is important to assess if the pool can “hide” any important result. This study was designed to compare individual ejaculates and a pool of fresh semen. The study was approved by Ethics Committee for Use of Animals (5526080119) of Universidade Federal Fluminense. Four adult Santa Inês rams (A, B, C and D) were used for semen collection, in six replicates. The animals had their extra-gonadal reserves previously renewed and the semen was collected by electroejaculation. Immediately after each collection, the ejaculate was assessed for swirl, vigor and sperm motility (total-, progressive- and non-progressive motility); velocity (fast, medium, slow and static); average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), amplitude of lateral head displacement (ALH), beat/cross frequency (BCF), straightness (STR), linearity (LIN) and WOB, which is defined as the mean value of ratio between VAP and VCL. Computer-assisted semen analysis (CASA) was performed using SCA<sup>®</sup> system (Sperm Class Analyzer Microptic, Nikon Eclipse Ci, Tokyo, Japan). Then, all the ejaculates were pooled, homogenized, and immediately submitted to the same assessments. Values were subjected to normality (Shapiro-Wilk and Kolmogorov-Smirnov test) and parametric data were analyzed by one-way ANOVA and Tukey test. Non-parametric data were analyzed by Kruskal Wallis and Dunn’s test. When pool and ram individual ejaculates were compared, there was no significant difference in any end point, such as total motility (ram A:  $96.8 \pm 1.8$ ; B:  $98.1 \pm 0.6$ ; C:  $99.2 \pm 0.3$ ; D:  $98.5 \pm 0.4$ ; pool:  $98.7 \pm 0.7$ ). However, differences ( $P < 0.05$ ) were obtained among rams in three parameters: swirl (A:  $4.8 \pm 0.2$  vs B:  $2.8 \pm 0.7$ ), VAP (A:  $56.2 \pm 7.0$  vs C:  $80.5 \pm 3.3$ ) and VSL (A:  $37.0 \pm 4.8$  and D:  $43.0 \pm 1.0$  vs C:  $56.9 \pm 3.8$ ). When these values were compared to the pool, there was no difference ( $P > 0.05$ ) for swirl, VAP and VSL end points, respectively:  $3.7 \pm 0.2$ ;  $71.9 \pm 6.1$  and  $48.9 \pm 5.8$ . There was no difference ( $P > 0.05$ ) in other kinetics parameters. It is noteworthy that the use of pooled semen simplified manipulation, allowed to increase the total volume, making possible to propose more experimental groups and/or analyses and may be an interesting approach to be used to assess the effect of a given protocol in the sheep model. In conclusion, the use of pool allows minimizing any effect of individual variation in ram ejaculates with a homogeneous and larger volume sample. Acknowledgments: The authors thank CNPq (Project 434302/2018-0), AGIR/UFF and FAPERJ for financial support.

**ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)**

Physiology of reproduction in male and semen technology

**Prediction of *in vitro* embryo production after analysis of cryopreserved semen from Senepol bulls - Part I: Logistic regression**

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To obtain more accurate prediction of ejaculate fertility in cattle, different sperm attributes should be evaluated, associated to the knowledge about sperm subpopulation, which is heterogeneous in ejaculate. Therefore, the aims of the present study were to derive data on sperm subpopulations of Senepol cryopreserved bull semen, employed for *in vitro* fertilization protocol, and to determine a model to predict *in vitro* embryo production (IVEP) based on sperm subpopulations analyzed with other sperm quality parameters. Thirty-eight Senepol bull semen batches, chosen from 386 retrospective IVEP routines, were used. Samples were thawed in a 37 °C water bath for 45 seconds and, after thawing, the concentration was adjusted to 25 x 10<sup>6</sup> sperm/mL. All samples were submitted to a semen washing protocol, based in two semen washes at low centrifugation force. Afterwards, all samples were evaluated by the Computer-Assisted Sperm Analysis system for identification and characterization of sperm subpopulations, by flow cytometry for evaluation of plasma and acrosomal membranes integrity, mitochondrial potential, oxidative status and chromatin resistance, and analysis of sperm morphology by wet preparation. Because of the dependency structure contained in the variables, multivariate statistical techniques of grouping and main components were applied to obtain sperm subpopulations. After categorization of IVEP in high, medium and low embryo yield rate (30 to 60%, 20 to 29.99% and 19.99% to 0, respectively), logistic regression analysis was applied to associate the sperm subpopulations and the other variables of sperm quality with IVEP. Three subpopulations of spermatozoa were characterized: SBP1 (fast and progressive movement), SBP2 (hyperactivated movement) and SBP3 (slow and non-progressive movement). An equation was generated: Embryo yield = 0,1563 + 0,0328(SBP1) + 0,0173(SBP2), where SBP1 and SBP2 represents the absolute value of the percentage of subpopulations in semen. If the calculated value (by this equation) is close to 1, the embryo yield is low; if is close to 2, is medium; if is close to 3, is high. It was concluded that, in our experimental conditions, SBP1 and SBP2 variables had effect on IVEP with cryopreserved semen from Senepol bulls. Acknowledgements: FAPESP (2016/24107-7).

## ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

Physiology of reproduction in male and semen technology e biotechnology

**Percoll® gradient induces sperm capacitation detected by intracellular Zinc signaling****Julia de Lima Simões , Camilla Mota Mendes , Thais Rose dos Santos Hamilton , Adriano Felipe Perez Siqueira , Raphaela Gabrielle Brito Sousa , Carlos Alonso Paco Nagaki , Marcilio Nichi , Mayra Elena Ortiz D'Ávila Assumpção**

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For the success in each step of IVP process (IVM, IVF and IVC) some requisites are needed. Concerning the sperm, cells need to be capacitated for fertilization by functional and structural alterations resulting in permeability changes in cell membrane, promoting the influx of calcium. Recent studies described zinc signatures as a method to assess sperm capacitation (Kerns et al., Nat Communi, 9:2061, 2018). Although sperm needs to be capacitated to fertilize, a premature capacitation is harmful. The manipulation, freezing/thawing, sperm preparation for IVP and cell sorting are involved with this premature capacitation. It is not yet clear if the submission of semen to a Percoll® gradient promotes complete sperm capacitation, reflecting on the success of the *in vitro* embryo production. The aim of this study was to evaluate if Percoll® gradient induces sperm capacitation, and its correlation with IVP rates. For that, semen of 10 Nelore bulls, used on in-vitro embryo production where submitted or not to Percoll® gradient. Sperm motility was evaluated by computer assisted sperm analysis (CASA) and sperm acrossomal/plasmatic integrity (FITC/PI), mitochondrial membrane potential (JC1) by Flow Cytometry. Sperm capacitation was evaluated by chlortetracycline fluorescence assay (CTC) (Ward and Storey., Develop Bio, 2:287-6, 1984) and zinc assay with fluorescence probe Fluozin™-3 AM (FZ3). A correlation test (PROC CORR SPEARMAN RANK) was done in attempt to correlate the sperm capacitation variables with *in vitro* embryo production (cleavage rate: total of cleavage structures/oocytes, blastocyst rate: blastocyst/oocytes and embryo development rate: blastocyst/cleavage structures). Sperm zinc signature in samples submitted or not to Percoll® gradient were: signature 1 (non capacitated): 14.11% and 6.21%, signature 2 (undergoing capacitation): 45.32% and 33.38%, signature 3 (capacitated sperm): 1.95% and 4.86%, and signature 4 (remodeled plasma membrane): 38.62% and 55.55%, respectively. Semen not submitted to Percoll® gradient presented higher percentage of zinc signature 3 4.86%,  $p = 0.025$ , higher percentage of capacitated spermatozoa with reacted acrosome by CTC 82.98%,  $p = 0.002$ , and higher percentage of sperm rectilinearity 80.40%,  $p = 0.041$  when compared to samples submitted to Percoll® gradient. In addition, sperm submitted to Percoll® gradient, had a higher percentage of non-capacitated cells in CTC evaluation 20.64%,  $p = 0.003$ , when compared to sperm not submitted to Percoll® gradient. We observed a relative correlation between sperm Zinc signature 3 (capacitated cells) and in-vitro embryo development rate ( $\rho$ : -0.650,  $p$  0.041), showing that the more capacitated cells, less embryos are produced. Percoll® gradient seems to remove sperm already capacitated from samples, confirmed by the presence of more non-capacitated cells after centrifugation. This condition seems to be important for bovine in-vitro fertilization and success of bovine IVP.

**ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)**

Physiology of reproduction in male and semen technology

**Case-study: Immunocontraceptive Impact on the reproductive physiology and behavior in alpha male capybara (*Hydrochoerus hydrochaeris*)****Derek Rosenfield, Cristiane Schilbach Pizzutto**

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Human-Wildlife-Conflicts are globally on the rise, thus, the demands for wildlife and feral population control. However, managing free-ranging animals is a complex and challenging task, as strategies are as diverse as environments and species. A non-lethal fertility control method should offer characteristics such as: being highly effective and long-lasting, perhaps reversible without any pathological and adverse behavioral impacts; applicable to both genders and preferably by remote delivery, without the need to prior capture; and most importantly, an “one-shot” administration, with no need for a booster application; in addition to being non-polluting and not entering the food chain. The concept of immunocontraceptives has a twenty-year proven track-record in controlling wildlife populations in several species, presenting nearly all afore-mentioned characteristics. Capybaras, the world-largest semi-aquatic rodent, is highly proliferative and resistant to environmental influences. Specifically, in Brazil, they lack natural predators and invading agricultural and urban areas with overabundant food availability. Caused by quick propagation into pest-like superpopulations, human- “capybara”- conflicts (traffic accidents; crop destruction; promoting tick-spread zoonotic diseases) are triggered. In this work, we aimed to investigate the effects of a single-dose anti-GnRH vaccine in alpha-male (n=1) to prompt an auto-immune response, subsequently inhibiting sex hormone synthesis, and consequently, causing infertility. Data on reproductive history; agonistic dominance behavior; body weight; testicular biometry/histopathology, and computer-assisted semen analysis were collected prior to treatment. Afterward, the male was inoculated with a single-dose anti-GnRH vaccine (IM/1000 $\mu$ l). Our findings, respecting a capybara-specific spermatogenic cycle of 70 days, indicated an alteration of the reproductive physiology that rendered the male infertile. This was demonstrated by absent offspring, testicular (n=2) atrophy (pre-immunization 62,63gr vs. post 26,63gr), and oligospermia: (pre-immunization  $146.25 \times 10^6$  spz/mL vs post  $1.08 \times 10^6$  spz/mL, which would be considered inadequate fertility. The testicular parenchyma was distinctively altered (irregular/deformed seminiferous tubules, enlarged lumen, incomplete spermatogenic stages, lack of mature spermatozoa, germ cell arrest, presence of vacuoles). No agonistic behavioral changes were observed. The findings suggest that the contraceptive treatment was efficient to make the alpha male infertile, without altering the male's dominant status, which is considered a crucial aspect to maintaining the group's social integrity, thus, effectively managing capybara's population.

## ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

Physiology of reproduction in male and semen technology

**Effect of equilibration time in plasmatic membrane integrity and motility of boar spermatozoa****Matheus Pasini Martins<sup>1</sup>, André Furugen Cesar de Andrade<sup>1,3</sup>, Marina Passareli<sup>1</sup>, Ana Carolina Pedrosa<sup>1</sup>, Frederico Ozanam Papa<sup>2</sup>, José Antônio Dell'Aqua-Jr<sup>2</sup>, Marco Antônio Alvarenga<sup>2</sup>, Simone M.M.K. Martins<sup>3</sup>**

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Among the techniques with the potential to decrease cryodamage in spermatozoa is the equilibrium time (ET). ET is defined as the period during which sperm cells are kept in contact with cooling/freezing media components at a temperature of 5°C, as this may provide a more proper osmotic balance between the intra and extracellular environment. An alternative that has already proved efficient in improving the cryotolerance of sperm from other species. Therefore, the experiment aims to evaluate the influence of the ET in the cryopreservation protocols of boar semen. For this, five boars of a commercial hybrid line are used. Four ejaculates of each animal (n= 20) were collected by the gloved-hand technique. After collection, semen samples were analyzed for total motility (TM) and Progressive motility (PM) (Computer-Assisted Sperm Analysis, SCA<sup>®</sup>) and sperm concentration in the Neubauer chamber. Only samples with more than 80% TM were cryopreserved. Posterior to the analysis, samples were destined for the two-step cryopreservation procedure. Each sample was extended 4-fold with Beltsville Thawing Solution. Semen was maintained at 17°C/24 hours and centrifuged (2100xg/3min), sperm pellets were extended in cryopreservation medium without cryoprotectant (BotuSui Fração A<sup>®</sup>, Botupharma, Botucatu-SP). Subsequently cooling to 5°C, samples were remaining under this temperature for periods of 0, 2, and 4 hours of ET. Then samples were diluted with cryopreservation medium (BotuSui Fração B<sup>®</sup>, Botupharma, Botucatu-SP - 6% glycerol and 6% methylformamide) to 1x10<sup>9</sup> sperm/mL. Extended sperm were packed into 0.5mL straws. The freezing curve from 5°C to -120°C was -20°C/min, using a controlled-rate freezer (TK 3000<sup>®</sup>; TK Tecnologia em Congelação Ltda., Uberaba-Brazil). Samples were finally plunged into liquid nitrogen at -196°C and stored. So, four straws were thawed at 37 °C for 30 seconds for analyzes of TM and PM in CASA and integral plasma membrane (MI) by flow cytometry (Accuri C6<sup>®</sup>; Becton Dickinson and Company; San Jose, CA, USA) using the probes SYTO-59 (S10341, Molecular Probes Inc., Eugene, Oregon, EUA) and propidium iodide (PI, L0770, Sigma-Aldrich Co., Saint Louis, Missouri, EUA). The original or transformed data were submitted to PROC MIXED using the SAS program. We expected that the equilibration time would be beneficial to TM, PM, and MI. However, our results are contrary to expected, showing no changes in the value of the samples. There was no difference (p<0.05) for TM (15.32%±1.54 - 0h, 17.72±2.06 - 2h, and 15.97±1.67 - 4h) and for PM (6.26±0.87 - 0h, 6.57±0.90 - 2h and 6.26±0.84 - 4h). Concerning MI, the population of the treatments 2 and 4 hours (32.30±1.99 and 31.90±2,93, respectively) presents no statistical difference between the treatments (p<0.05). The implementation of equilibration time for periods of 0, 2, and 4 hours not shown to be beneficial for TM, PM and MI. Financial support by FAPESP - 2017/20796-5.

**ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)**

Physiology of reproduction in male and semen technology

**Effects of high intensity interval training in the seminiferous epithelium and sperm motility of hypertensive rats****Margarete Jardimetti de Oliveira<sup>1</sup>, Evellin Heloisa Paulineli Pereira<sup>1</sup>, Lauren Chrys Soato Marin Schaffer<sup>1</sup>, Kátia Cristina de Melo Tavares Vieira<sup>1</sup>, Aline de Oliveira Santos<sup>1</sup>, Francis Lopes Pacagnelli<sup>1</sup>, Caliê Castilho<sup>1</sup>, Ana Paula Alves Favareto<sup>1</sup>, Leonardo Oliveira Mendes<sup>1</sup>, Giovana Rampazzo Teixeira<sup>2</sup>, Ines Cristina Giometti<sup>1</sup>**<sup>1</sup>UNOESTE - Universidade do Oeste Paulista (Rodovia Raposo Tavares, Km 572, Bairro Limoeiro, Presidente Prudente (SP), Brasil.); <sup>2</sup>Unesp - Universidade Estadual Paulista (Rua Roberto Simonsen, 305 - Centro Educacional - P. Prudente/SP - CEP 19060-900).

Hypertension is a cause of low fertility in men and exercises are indicated to decrease blood pressure and improve overall health. The objective of this study is to verify the germinal epithelium area and sperm motility of spontaneously hypertensive rats (SHR) submitted to high intensity interval training (HIIT). Wistar-Kyoto rats, male, adult, with and without spontaneous hypertension were distributed in 3 groups: K-G (control of Wistar-Kyoto rats without hypertension, n=5); SHR-G (group of SHR rats, n=9); and SHR-HIIT-G (group of SHR rats submitted to HIIT, n=9). The treadmill HIIT training was realized for 5 days/week for 8 weeks, for 50 minutes approximately, based on the maximum exhaust speed, with active rest intervals. The lactate threshold analysis was performed to determine the training speeds. The animals initially underwent an adaptation to the HIIT training, which consisted of running on the treadmill for 2 minutes at 0.5 km/h, followed by 5 minutes of rest and 3 minutes at the speed of 0.7 km/h, increasing 0.2 km/h every 3 minutes until it reached 1 mmol/L of lactate above the initial test. After the adaptation period HIIT was performed with 5 minutes of heating in at 40% of the lactate threshold. After warming up, the training was started with 3 minutes at 60% of the lactate threshold followed by 4 minutes of 85% of the lactate threshold, which was repeated seven times each session. After the death of the animals, the vas deferens with the sperm were collected. The evaluation of sperm motility was performed immediately at the time of euthanasia. The areas of testicular seminiferous epithelia were measured after hematoxylin-eosin staining using MOTIC<sup>®</sup> software. The results were analyzed by analysis of variance (ANOVA), followed by the Tukey test ( $P < 0.05$ ). The SHR-G presented higher number of immobile sperm ( $16.95 \pm 1.30$ ) than the K-G ( $10.90 \pm 0.62$ ) and SHR-HIITG ( $12.22 \pm 1.16$ ;  $P < 0.05$ ). No difference was observed among the groups in the area ( $\mu\text{m}^2$ ) of germinal epithelium ( $13066.88 \pm 456.56$ ,  $13923.18 \pm 424.43$  and  $14362.96 \pm 312.75$ , for K-G, SHR-G and SHR-HIIT-G, respectively). It is concluded that HIIT prevents the decrease of motile sperm and that hypertension do not alter the area of testicular seminiferous epithelium in SHR rats. Financial support by FAPESP (process number: 2018/22682-0) and PIBIC-EM (CNPq).

**ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)**

Physiology of reproduction in male and semen technology

**Testicular morphometry of Wistar rats supplemented with quercetin and submitted to a high calorie diet**

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The aim of this study was to verify the action of quercetin supplementation on the testicular morphometry of wistar rats submitted to a high calorie diet. For that, the experiment used 48 Wistar rats in 6 experimental groups: CT (rats fed with commercial feed and supplemented with 0 mg / day of quercetin gummy); Q10 (rats fed commercial fed and supplemented with 10 mg / day of quercetin gummy); Q20 (rats fed with commercial feed and supplemented with 20 mg / day of quercetin gummy); HC (rats fed with high calorie feed and supplemented with 0 mg / day of quercetin gummy); HCQ10 (rats fed with high calorie feed and supplemented with 10 mg / day of quercetin gummy); and HCQ20 (rats fed with high calorie feed and supplemented with 20 mg / day of quercetin gummy). At the end of the experiment, the rats were anesthetized and killed by exsanguination, the testicles were harvested and placed in a Methacarn fixative solution for 24 hours and then washed, cut and conditioned in 70 % alcohol. The testicles were embedded in paraffin and the histological slides were made using the hematoxylin-eosin staining method. The slides were photographed and the areas of tubules, seminiferous lumens and germinal epithelium were checked. The results passed the Shapiro-Wilk normality test and the statistical analysis used was ANOVA followed by Tukey. No differences were found in the areas analyzed between the different groups ( $P > 0.05$ ). The means and the standard deviations of areas ( $\mu\text{m}^2$ ) of germinal epithelium were: CT =  $11787.17 \pm 2013.12$ ; Q10 =  $11889.89 \pm 1599.31$ ; Q20 =  $12685.80 \pm 1976.57$ ; HC =  $12328.82 \pm 2447.88$ ; HCQ10 =  $12681.65 \pm 1364.25$ ; and HCQ20 =  $13393.76 \pm 2606.15$ . It is concluded that the hypercaloric diet or quercetin supplementation does not alter the germinal epithelium area of the testicles of Wistar rats. Financial support CAPES (process # 88887.373968/2019-00)



## ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

Physiology of reproduction in male and semen technology

**Bovine chilled semen by 24h or 48h in two different commercial extenders for fixed-time artificial insemination in beef cattle****Juliana Corrêa Borges Silva<sup>1,4</sup>, Márcio Ribeiro Silva<sup>2</sup>, José Francisco Martinelli Massoneto<sup>3</sup>, Pablo Storari Loro<sup>3</sup>, Ivo Augusto Cavalieri Alves<sup>3</sup>, Eriklis Nogueira<sup>1,4</sup>, Luiz Orcirio Fialho de Oliveira<sup>4</sup>, Urbano Gomes Pinto de Abreu<sup>1</sup>, Daniel Barros Marinho<sup>3</sup>**

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Chilled semen showed an increase in pregnancy compared to cryopreserved semen, in the ejaculate, of the same bulls (J. C. Borges-Silva et al., *Reproduction Fertility and Development*, on line, 2015). However, it is unknown how long chilled semen remains with satisfactory pregnancy rates, in different commercial extenders. Thus, the aim study was to evaluate the pregnancy rate after fixed-time artificial insemination (FTAI) using chilled semen at 5°C, for 24 and 48 hours using, two commercial extenders (B - Botubov and I - Inra 96), totaling four treatments (24B, 24I, 48B and 48I). Ejaculates were collected from five genetically superior Nellore bulls and were used on two farms (1 and 2) on the Pantanal - MS. The 0.25 mL straws ( $30 \times 10^6$  sperm) were chilled in four Botutainer boxes, two for each farm (24 and 48 hours), until the FTAI moment. All semen was in CBRA standards (70% motility minimum and 30% total defects maximum). The median proportion of extender:semen used to diluted liquid semen was 5:1. Nellore cows (n = 946) submitted to FTAI received 2mg estradiol benzoate (EB, RICBE<sup>®</sup>, im, Tecnopec-Agener União, Brazil), and an intravaginal progesterone device (Primer<sup>®</sup>, Tecnopec-Agener União, Brazil) on D0 which remained for 8 d, and 150µg d-cloprostenol (Prolise<sup>®</sup>, im, Arsa, Argentina) and 1mg EB (RIC-BE<sup>®</sup>) on D8. On D10, 44 h after implant withdrawal, the cows were randomly inseminated using cooled semen of the different treatments. The pregnancy diagnosis was performed 40 days after FTAI by ultrasound (DP-2200 Vet<sup>®</sup>, Mindray, China). The statistical analysis was accomplished by the SAS program (SAS/STAT<sup>®</sup> 9.2, SAS Institute Inc., USA), using the variance analysis by Tukey and Chi-square test (P < 0.05). There was no difference in pregnancy per AI (P/AI) using chilled semen by 24 (49.3% [319/646]) or 48 hours (49.6% [149/300]), and there was no difference among extenders (P > 0.05) (24B - 49.5% [161/326], 24I - 49.3% [158/320], 48B - 52.7% [78/148], 48I - 46.6% [71/152]), although one bull differed (P < 0.04) for extender B (58.6%) in relation to I (34.1%), there was no difference among bulls (P > 0.05), but there was a difference between farms 1 (54.5% [239/438]) and 2 (45.0% [229/508]) (P = 0.03). In conclusion, fertility rates were equal when samples were chilled by 24 and 48 hours in both extenders. Therefore, it is possible to use for more long time chilled semen (until 48h at least) when performing a FTAI program. Acknowledgments: Agener União Saúde Animal e Ema Pantanal Ltda. Keywords: pregnancy rate, semen process, sperm viability.