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**"IN VITRO" EVALUATION OF GOAT SEMEN FROZEN IN FRENCH STRAWS OF 0.25 AND 0.50ML**

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nety-three semen samples obtained from bucks of several eeds were deep-frozen in French straws of 0.25 and 0.50ml d evaluated after thawing. Collection, evaluation, eezing, storage and thawing (37°C/20 sec) were proceeded cording to standard techniques and are described elsewhere. st-thawing evaluation comprised progressive motility (%), tility rate (0-5), live spermatozoa (%), extracellular ncentration of aspartate aminotransferase (AST- U/ml) and anine aminotransferase (ALT- U/ml). The type of package did t influence (P>.05) progressive motility and motility rate, ich were respectively 29.19±2.16% and 2.06±0.10 for 0.25 raws and 28.84±1.71% and 2.01±0.08 for 0.50ml straws. Live rmatzoa percentage was significantly (P<.05) higher for en packaged into 0.50ml straws (53.66±1.48) than 0.25ml 9.97±1.31). AST levels did not differ (P>.05) between raws (24.95±1.02U/ml and 29.20±2.38U/ml respectively for 25 and 0.50ml). On the other hand, samples packaged into 0.50ml straws showed (P<.05) lower content of ALT (.95±0.50U/ml) than those processed into 0.25ml straws (.61±0.43U/ml). It has been concluded that goat semen frozen 0.25ml French straws are more vulnerable to the eezing/thawing procedure herein employed based on the fact t live spermatozoa numbers were superior and enzyme kage was smaller for samples processed into 0.50ml.

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**IN VITRO PRODUCTION OF BOVINE EMBRYOS: A YEAR STUDY**

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has been found that production of bovine embryos by in vitro tilization (IVF) is affected by different factors. A rospective study was designed to evaluate the efficiency of vitro oocyte maturation, fertilization and embryo olopment during a 12 months period (39 batches) under the methodology and laboratory conditions. Cross breed beef ne ovaries were obtained at slaughter and cumulus-oocyte lexes (COC) were recovered by aspiration of follicles 2 to 3 in diameter. The COC (n=16554) were washed in TL-HEPES and red (424±124 per batch) in TCM-199 supplemented with 0.5 l of FSH, 5.0 µg/ml oLH, 1.0 µg/ml oestradiol 17β, 25 µM um pyruvate, 25 µg/ml gentamicin, 10% steer serum (DBS). r 24 hours in culture, COC were vortexed to remove cumulus s and oocytes (n=15297) were inseminated with frozen/thawed n (1x10<sup>6</sup> sperm/ml), capacitated with 2 µg heparin, from a le high fertility bull. After 18-20 hours of co-incubation, tes (n=13739) were washed in TL-HEPES and placed in a BOEC ulture in TCM-199 plus 10% DBS, for 10 days. All cultures done at 39°C under 5% CO<sub>2</sub> in air with high humidity. A le of oocytes (5%) from each culture was fixed/stained and ined under a microscope to determine the maturation and ilization rates. Zygotes were examined every 24 hours, from 5 to day 10 after fertilization (day 0) and embryos forming ocyts were counted. The total maturation and fertilization as was 77.9±19.5% (range 25-100%) and 66.3±26.5% (range 0- ) respectively. The total embryo development rate was ±10.7% (range 0-40%). Blastocysts were found from day 6 igh day 10. These results indicate that there was a great ability between batches in maturation, fertilization and yo development rates under the same IVF conditions.

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**IN VITRO MATURATION OF BOVINE OOCYTES FROZEN WITH GLYCEROL**

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Bovine oocytes were collected by aspiration of 3-7 mm diameter follicles from cow ovaries obtained at slaughter house. The oocytes were pooled in D-FBS supplemented with 20% FBS for evaluation. Thirty six oocytes were exposed, at room temperatura, to 0.5 M, 1.0 M and 1.5 M of glycerol in D-PBS with 4 mg of BSA, in three steps of 10 min. The oocytes were loaded into 0.25 ml straws and immediately placed in a freezing cylinder at -5°C and then cooled to -7°C and seeded. After seeding the straws were cooled at 0.3°C/min to -32°C/min and then plunged into liquid nitrogen. Following storage the straws were thawed in a 37°C wather bath for 20 sec. The cryoprotector was removed in D-PBS supplemented with 4 mg% BSA and 1.0 M of sucrose with 1.0 M, 0.5 M and 0.0 M of glycerol during 10 min. After thawing the oocytes were matured in TCM-199 supplemented with FBS, antibiotics, LH and FSH, at 38.5°C in an atmosphere of 5% of CO<sub>2</sub>. In addition, seventy three fresh oocytes were cultured simultaneously as a control group. The oocytes with the first polar body after 24 hours of culture were considered mature. The maturation rate was 13.8% (5/36) for frozen oocytes and 82.2% (60/73) for control group. The results suggested that the method used in this experiment was not suitable for freezing bovine oocytes.

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**ÍNDICE DE PREENHIZ DE ACORDO COM O INTERVALO ENTRE COBERTURA E OVULAÇÃO EM ÉGUAS PSI (PURO SANGUE INGLÊS).**

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Com o objetivo de determinar o melhor momento para a cobertura, foram utilizadas 76 éguas PSI sadias com idade de 3 a 17 anos, padreadas, por um mesmo garanhão de fertilidade comprovada, entre agosto e dezembro de 1995 na região de Boituva-SP. As éguas eram examinadas para diagnóstico da ovulação a cada 12 horas, por palpação e ultrassonografia transretal. Os animais foram acompanhados por 126 ciclos, sendo divididos em 5 grupos de acordo com o tempo decorrido entre a cobertura e a ovulação. As éguas eram reexaminadas por ultrassom, 13 dias depois, para diagnóstico de gestação. O grupo I foi constituído por 19 éguas cobertas entre 0-12 horas pós-ovulação, obtendo um índice de prenhez de 36,8%. O grupo II foi formado por 43 éguas cobertas de 0-12 horas antes da ovulação, com índice de 76,7%. O grupo III com 32 animais ovulando entre 12-24 horas pós cobertura, alcançou 75% de prenhez. O grupo IV contou com 23 éguas com ovulação entre 24-36 horas pós acasalamento e obteve 73,9% de índice de prenhez. O grupo V foi formado por 9 animais ovulando entre 36-48 horas com 22,2% de prenhez. De posse dos resultados conclui-se não haver diferenças estatísticas entre os grupos II, III e IV, sendo recomendado que a cobertura ocorra entre 0-36 horas antes da ovulação. Caso a ovulação não ocorra neste período se deve recorrer a nova cobertura. A cobertura pós ovulação seria indicada com um intervalo inferior a 6 horas.

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