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

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Ninety-three semen samples obtained from bucks of several breeds were deep-frozen in French straws of 0.25 and 0.50ml and evaluated after thawing. Collection, evaluation, freezing, storage and thawing (37°C/20 sec) were proceeded according to standard techniques and are described elsewhere. Post-thawing evaluation comprised progressive motility (%), motility rate (0-5), live spermatozoa (%), extracelular concentration of aspartate aminetransferase (AST- U/ml) and alanine aminetransferase (ALT- U/ml). The type of package did not influence (P>.05) progressive motility and motility rate, which were respectively 29.19±2.16% and 2.06±0.10 for 0.25 straws and 28.84±1.71% and 2.01±0.08 for 0.50ml straws. Live spermatozoa percentage was significantly (P<.05) higher for semen packaged into 0.50ml straws (53.66±1.48) than 0.25ml (49.97±1.31). AST levels did not differ (P>.05) between straws (24.95±3.02U/ml and 29.20±2.38U/ml respectively for 0.25 and 0.50ml). On the other hand, samples packaged into 0.50ml straws showed (P<.05) lower content of ALT (5.95±0.50U/ml) than those processed into 0.25ml straws (4.61±0.43U/ml). It has been concluded that goat semen frozen in 0.25ml French straws are more vulnerable to the freezing/thawing procedure herein employed based on the fact that live spermatozoa numbers were superior and enzyme leakage was smaller for samples processed into 0.50ml.

097

IN VITRO MATURATION OF BOVINE OOCYTES FROZEN WITH GLYCEROL

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Bovine oocytes were colleted by aspiration of 3-7 mm diameter follicles from cow ovaries obtained at slaughter house. The oocytes were pooled in D-PBS 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 immediatelly 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_2. 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.

806

IN VITRO PRODUCTION OF BOVINE EMBRYOS:A YEAR STUDY

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It has been found that production of bovine embryos by in vitro fertilization (IVF) is affected by different factors. A retrospective study was designed to evaluate the efficiency of in vitro oocyte maturation, fertilization and embryo development during a 12 months period (39 batches) under the same methodology and laboratory conditions. Cross breed beef bovine ovaries were obtained at slaughter and cumulus-oocyte complexes (COC) were recovered by aspiration of follicles 2 to 6 mm in diameter. The COC (n=16554) were washed in TL-HEPES and matured (424±124 per batch) in TCM-199 supplemented with 0.5 µg/ml oFSH, 5.0 µg/ml oLH, 1.0 µg/ml oestradiol 17ß, 25 µM sodium pyruvate, 25 µg/ml gentamicin, 10% steer serum (DBS). After 24 hours in culture, COC were vortexed to remove cumulus cells and oocytes (n=15297) were inseminated with frozen/thawed semen (1x10^4 sperm/ml), capacitated with 2 ug heparin, from a single high fertility bull. After 18-20 hours of co-incubation, zygotes (n=13739) were washed in TL-HEPES and placed in a BOEC co-culture in TCM-199 plus 10% DBS, for 10 days. All cultures were done at 39° C under 5% CO_2 in air with high humidity. A sample of oocytes (5%) from each culture was fixed/stained and examined under a microscope to determine the maturation and fertilization rates. Zygotes were examined every 24 hours, from day 5 to day 10 after fertilization (day 0) and embryos forming blastocyts were counted. The total maturation and fertilization rate was 77.9±19.5% (range 25-100%) and 66.3±26.5% (range 0-100%) respectively. The total embryo development rate was 13.4±10.7% (range 0-40%). Blastocysts were found from day 6 through day 10. These results indicate that there was a great variability between batches in maturation, fertilization and embryo development rates under the same IVF conditions.

071

ÍNDICE DE PRENHEZ 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 constituido 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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