II Reunión Anual de la
Sociedad Argentina de Tecnologías Embrionarias

29 al 30 de Mayo de 2014, Buenos Aires, Argentina

sate Sociedad Argentina de Tecnologías Embrionarias

Relation between immature porcine cumulus oocyte complexes morphology and *in vitro* embryo development

The aim of this study was to determine the relation between COCs morphology and apoptosis, oocyte maturation and embryo development.The COCs were obtained from ovaries of slaughtered sows by aspiration of 3-8 mm follicles and classified by morphology under a stereomicroscope into six categories(A1,A2, B1, B2, C and D).Early apoptosis in oocytes was determined by Annexin V-propidium iodide technique andlate apoptosis by TUNEL assay. The A1, A2 and B1categories (complete *cumulus)* were matured *in vitro* for 44-48 h in TCM 199 medium supplemented with 10% (v / v) porcine follicular fluid, 50 mg / L gentamicin, 0.5 mg / L porcine LH and FSH.COCs were denuded (hyaluronidase 1mg/mL) and stained with Hoechst 33342 to determine nuclear maturation.A1 and A2 were pooled because they did not differ in nuclear maturation or apoptosis.*Invitro*fertilization and embryo culture were performed to A and B1 mature oocytes. Fresh fertile boar semen was centrifuged 3 times (490 x *g*, 5 min) in BO medium supplemented with 2.5 mM glucose, 10 mM Na lactate, 5 mM caffeine and 20 IU/mL heparin and diluted ½ in BO with 10 mg/mL FAF-BSA (sperm concentration= 1 $x10^6$/mL). Sperm and denuded oocytes were incubated in 100 µL drops for 30 min at 39°C, 5% CO_2 and 100 % humidity. Presumptive zygotes were washed in TALP-HEPES medium and cultured in 50 µL drops of SOF medium at 39°C, 5% CO_2, 5 % O_2 and 100 % humidity. Cleavage was determinedat day 4 and blastocysts at day 8-9. Infostat software (2011v) was used and regression tests of independence were performed to evaluate apoptosis - ANOVA for maturation and Fisher's test for cleavage and blastocysts ($p < 0.05$). Early apoptosis in immature oocytes increased with the declining quality of the COCs.However, no difference between A1 and A2 were observed. No TUNEL-positive oocytes were found in any immature COCs (n = 363). No significant differences were observed for nuclear maturation among A1, A2 and B1 (n = 106), nor cleavage and blastocyst rate between A1+A2 and B1 (n = 306). In conclusion, the presence of apoptosis in immature COCs is related to its classification under stereomicroscope by morphology.COCs containing at least one layer of *cumulus* cells completely enclosed around the oocyte (B1) have the same ability to develop *in vitro* than those with dense and plentiful *cumulus* (A1 and A2).Therefore the observation of cumulus cells in immature COCs can be useful as selection criteria of oocytes with good development potential *in vitro*.

Determinación de la hormona antimulleriana (amh) en plasma de vacas superovuladas como predictora de la respuesta

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El principal límite biológico para el desarrollo de la tecnología de transferencia de embriones (SOTE) en bovinos es la gran variabilidad en la respuesta a los tratamientos superovulatorios. Las mejoras en tratamientos y en protocolos de control de la onda folicular han mejorado las respuestas pero no han disminuido su variabilidad ni eliminado las grandes diferencias entre animales.Una prueba predictiva para la selección de vacas que pueden producir alto número de embriones transferibles representaría un gran avance en la tecnología SOTE. Recientemente se ha determinadoquela AMH brindaría información sobre el pool folicular y la respuesta a los tratamientos superovulatorios en el ganado.El presente trabajo tuvo como objetivo realizar una primera aproximación en la determinación de la concentración de AMH en suero de vacas sometidas a tratamientos superovulatoriosen establecimientos comerciales yevaluar su relación con la cantidad de embriones transferibles (ET) obtenidos. Vacas AA (34) superovuladas en el marco de un programa de mejora genética en un establecimiento privado fueron tratadas con dosis diferentes de dos hormonas comerciales (Foltropin ® y Pluset®) y la IA con semen convencional o sexado. Debido al bajo número de vacas, las muestras fueron analizadas en conjunto independientemente del tratamiento, dosis y semen utilizado.En cada vaca, el día de la colecta de embriones se tomó una muestra de sangre, se centrifugó y el plasma fue guardado a -18°C hasta su análisis. La determinación de AMH

se realizó por el método de ELISA en sándwich por medio de un kit comercial para dosaje de AMH bovina (Bovine AMH Elisa, AL-114, AnshLbas®).El análisis de las lecturas de densidad óptica (DO) se realizó mediante regresión sobre base logarítmicaen relación con la curva standard del ensayo, la cual tuvo un R^2 de 0,996. Los coeficientes de correlación para la AMH*estructuras totales (0,62), AMH*número de estructuras fertilizadas (0,54) y AMH*número de ET (0,60)indican una correlación mediana entre la AMH y los parámetros medidos (Gráfico 1). En 28 vacas con niveles de AMH superiores a los 80 ng/ml,la producción de ET(M±DS) fue de 8,2±7,9/vaca (rango 0-37), pero 20 de ellas (71,4%) tuvieron entre 4 y 37 (11,1±7,5) y 8 tuvieron entre 0 y 2 (1,25±0,88). En las 6 restantes (niveles por debajo de 80 ng/ml), la M±DS fue 3,33±1,03 (rango 2-5). En conclusión, el método utilizadopuede ser una herramienta para mejorar la selección de las vacas superovuladas pero su precisión es aún baja para poder ser utilizado como rutina en los sistemas de SOTE sin tener en cuenta factores de variación que aquí no se analizaron.

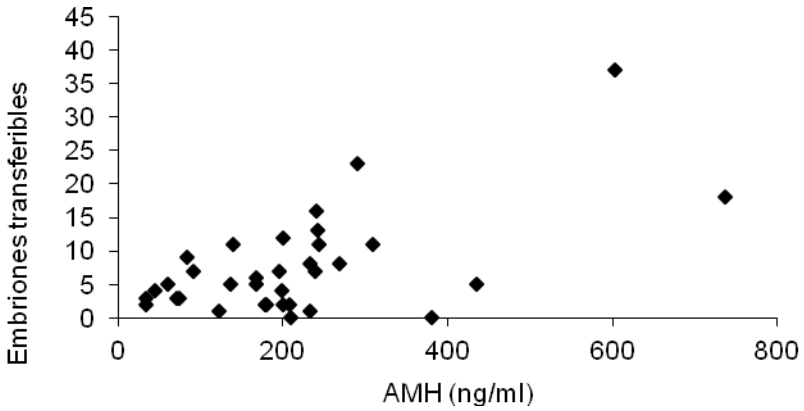

Figura 1. Relación AMH y N° de embriones transferibles

Financiado por el INTA, el Ministerio de Ciencia y Tecnología (Universidad Nacional de Mar del Plata), Instituto de Análisis Fares Taie y contribuciones de los colegas del sector privado

Antimullerian hormone (amh) in plasma as a predictor of response of superovulated cows

The major biological limit to the development of embryo transfer technology (SOTE) in cattle is the great variability in the response to superovulatory treatments. Improvements in treatments and protocols to control follicular wave have improved the responses but have not eliminated their variability nor the large differences between animals. A predictive test for selection of cows that can produce high numbers of transferable embryos would represent a breakthrough in SOTE technology. Recently it has been determined that AMH would provide information about the stock of ovarian follicles and superovulatory response in cattle. The present work was aimed to make a first approximation in determining the plasma concentration of AMH in cows subjected to superovulatory treatments in commercial establishments and evaluate their relationship with the number of transferable embryos (TE). AA cows (34),superovulated in the framework of a program of genetic improvement at a private farm, were treated with different doses of two commercial hormones (Foltropin ® and Pluset ®) and AI with sexed or conventional semen. Due to the low number of cows, the samples were analyzed together regardless of treatment, dose and semen used.The day of collection of the embryos a blood sample was taken in all animals, centrifuged and the plasma was stored at -18°C until analysis.AMH determination was performed by the sandwich ELISA method using a commercial kit for assay of bovine AMH(Bovine AMH Elisa, AL-114, AnshLbas®).The analysis of the optical densities (OD) was made by logarithmic regression based on the standard curve in relation to the test, which had a R^2= 0.996.Correlation coefficients for AMH*total structures (0.62); AMH*number of fertilized structures (0.54) andAMH*TE (0.60) indicate a median correlation between AMH and parameters measured (Figure 1). In 28 cows with AMH levels above 80 ng/ml, TE production (M±SD) was 8.2±7.9/cow (rank 0-37), but 20 of