References

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bta-miR-133b secreted by extracellular vesicles from the oviduct of pregnant cows could modulate signalling pathways during early embryo development

K. Cañón-Beltrán [a,b], Y.N. Cajas [c], R. Mazzarella [c], D. Gascón [c], C. Nuñez-Puente [c], M.G. Millán de la Blanca [c], C.A. Martinez [c], A. Gutiérrez-Adán [c], E. González [d], D. Rizos [c]

[a] Department of Biochemistry and Molecular Biology, Veterinary Faculty, Complutense University of Madrid (UCM), Madrid, Spain
[b] Programa de Medicina Veterinaria y Zootecnia, Grupo Kyron, Corporación Universitaria del Huila (CORHUILA), Huila, Colombia
[c] Department of Animal Reproduction, INIA-CSIC, Madrid, Spain
[d] Department of Anatomy and Embryology, Veterinary Faculty, Complutense University of Madrid (UCM), Madrid, Spain

Presenting author.
Dimitrios Rizos. E-mail: drizos@inia.csic.es

Application: This study enhances the understanding of embryo-maternal communication mechanisms to improve IVP systems and promote the commercial and economic growth of the cattle industry.
Introduction: Extracellular vesicles (EVs) are present in reproductive fluids and play an important role in cell-to-cell communication through their cargoes, especially miRNAs which regulate gene expression and signalling pathways related to cell metabolism, proliferation and differentiation during early embryo development. In a previous study, we showed that bta-miR-133b was present exclusively in EVs from oviduct fluid in pregnant cows (Mazzarella et al., 2021). Thus, we aimed to verify that miR-133b is uptaken in bovine IVP embryos by passive transfection (gymnosis) and to evaluate the effect of miR-133b supplementation on embryo development and quality.
Materials and Methods: Presumptive zygotes were cultured in SOF + 3% BSA (Control; C) or supplemented with 1 μM miR-133b (miRCURY LNA miRNA Mimics, Qiagen; 133b); or 1 μM control mimic (miRCURY LNA miRNA Mimic 5′FAM, N° 339173, Qiagen; CMimic). To confirm miRNA uptake, Day 7 blastocysts (BD7, $n = 10$/group) were fixed, stained with Hoechst 33342 and observed by a widefield fluorescence microscope. Besides, BD7 were snap-frozen in LN_2 (3 pools: $n = 10$/group) to examine the expression pattern of miR-133b by RT-qPCR using miRCURY LNA miRNA PCR Assay. Bioinformatic analyses were performed with miRWalk 3.0 and Metascape tools. Data were tested for normality and transformed by arcsine square root before One-Way ANOVA.
Results: Cleavage and blastocyst (Day 7 and 8) rates (%) were not affected (C: 85.4 ± 1.7, 30.6 ± 2.8, 36.0 ± 2.0; CMimic: 86.8 ± 1.6, 29.2 ± 2.9, 36.0 ± 2.2; 133b: 86.8 ± 1.8, 29.0 ± 2.3, 37.0 ± 1.4, respectively), while fluorescence staining showed that miR-133b can be uptaken by gymnosis and was also confirmed by RT-qPCR. Although embryo development was not affected, miR-133b is predicted to modulate signalling pathways that regulate pluripotency, including Wnt, TGF-β, and JAK-STAT. Additionally, miR-133b target genes are part of Hippo and Ras/MAPK pathways reported to modulate cell fate determination, differentiation, proliferation, and apoptosis during embryo development. These results suggest that the mother can regulate embryo development to the blastocyst stage through miRNAs. Gene ontology analysis corroborates KEGG results as it indicates the enrichment of cellular growth, reproductive and metabolic processes.
Conclusions: Our results determine miR-133b as a novel regulator of pluripotency signalling related to embryo development and quality and, highlight the importance of providing a comprehensive understanding of the interaction between miRNAs, oviduct and signalling pathways throughout early embryogenesis.
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061
Ovarian follicle flushing as a means of increasing the yield of oocytes and *in vitro* produced embryos in advanced cattle breeding

R.J. Simmons [a,b], D.A.R. Tutt [b], W.Y. Kwong [b], J.I. Baroni [b,a], L.N. Lim [c], R. Cimpeanu [d,e], A.A. Castrejon-Pita [d], M. Vatish [c,d], P. Svensson [f], U. Hagby [f], K.D. Sinclair [b], E.X. Georgiou [c,g]

[a] Paragon Veterinary Group, Dalston, Cumbria, United Kingdom
[b] University of Nottingham, Sutton Bonington, Leicestershire, United Kingdom
[c] John Radcliffe Hospital, Oxford, Oxfordshire, United Kingdom
[d] University of Oxford, Oxford, Oxfordshire, United Kingdom
[e] University of Warwick, Coventry, Warwickshire, United Kingdom
[f] Vitrolife Sweden AB, Västra Frölunda, Gothenburg, Sweden
[g] The Royal Women's Hospital, Melbourne, Victoria, Australia

Presenting author.
Kevin Sinclair. E-mail: kevin.sinclair@nottingham.ac.uk

Application: Ovarian follicle flushing can improve the efficiency of cattle embryo production within genetic improvement programmes.
Introduction: Maximising the yield of high-quality oocytes is key to the success of transvaginal follicular aspiration (Ovum Pick-Up; OPU) and *in vitro* embryo production (IVP). Follicle flushing (FF) is used widely in human OPU but not in cattle. A novel double-lumen needle (OxIVF) was designed in which the flushing fluid flows perpendicular to that of aspiration. Here we report on three studies that assessed the merits of this needle, and FF strategies for oocyte recovery, on IVP success.
Materials and Methods: Study 1 recovered oocytes from 189 abattoir-derived ovaries to compare FF with a standard 16G × 455 mm double lumen needle and the OxIVF needle (matched dimensions but flushes perpendicular to the needle shaft 7mm from the tip). Subsequently, 12 Holstein heifers underwent two stimulated cycles of OPU in a cross-over design (i.e., six flushed with OxIVF, six with the standard needle (Cycle 1); then swapped between needles (Cycle 2)). Net flow rates were 15 mL/minute for flush and aspiration in follicles ≥7 mm in diameter. In Study 3, 11 Holstein heifers underwent two stimulated cycles of OPU in a similar cross-over design, however both treatments used the OxIVF needle. One treatment flushed ≥7 mm follicles only, the other flushed follicles ≥7 mm followed by aspiration of 5–6 mm follicles. There then followed three cycles of conventional (single-lumen needle) follicle aspiration. Oocytes in both cross-over studies underwent standard IVP. Proportions were analysed by logistic regression; donor formed the random effect. Data are presented as means ± SEM.
Results: Oocyte recovery in the Study 1 was proportionately greater ($P = 0.034$) for the OxIVF than the Control needle (0.741 ± 0.0209 vs 0.670 ± 0.0223), as was Grade I oocytes ($P < 0.001$) (0.273 ± 0.0271 vs 0.122 ± 0.0216). In Study 2, oocyte recovery was proportionately greater ($P = 0.045$) for the OxIVF than the Control needle (0.891 ± 0.0298 vs 0.796 ± 0.0347). By Day 6 of culture, embryo yields were greater ($P = 0.017$) for the OxIVF than Control needle (0.872 ± 0.0438 vs 0.676 ± 0.0673). In Study 3, oocyte recovery was 0.821 ± 0.0506 vs 0.742 ± 0.0491 vs 0.662 ± 0.0348 for FF vs FF plus aspiration vs aspiration alone ($P = 0.033$). By Day 6 of culture, embryo yields were similar for FF and FF plus aspiration (0.672 ± 0.0879 vs 0.693 ± 0.0763).
Conclusions: Ovarian FF leads to high yields of quality oocytes contributing to IVP success in stimulated cycles of OPU in cattle.
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Dose- and time-dependent effects of interferon tau on bovine endometrial gene expression

A.K. Talukder [a], J.A. Browne [a], G. Charpigny [b], P. Lonergan [a]

[a] School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland
[b] INRA, Biologie du Développement et Reproduction, Jouy en Josas, France

Presenting author.
Anup Kumar Talukder. E-mail: anup.talukder@ucd.ie

Application: The improved understanding of the fundamentals of pregnancy establishment during the pre-implantation period, when a significant proportion of embryo loss occurs, could help to minimise early embryonic death and thus improve pregnancy rate in dairy cattle.
Introduction: Conceptus-derived interferon tau (IFNT) is responsible for maternal recognition of pregnancy (MRP) in cattle by blocking luteolytic pulses of prostaglandin F2alpha, thereby maintaining progesterone output by corpus luteum. We have demonstrated that short conceptuses fail to induce a large number of interferon-stimulated genes (ISGs) in the bovine endometrium that are altered by both IFNT and age-matched long conceptuses, suggesting insufficient IFNT production is a major contributing factor for lower survival of such conceptuses (Sánchez et al., 2019). The threshold level of IFNT required to establish pregnancy in cattle remains unknown. However, the transfer of conceptuses up to Day 16 of the oestrous cycle can establish pregnancy (Betteridge et al., 1980) suggesting that the signalling effect is quite acute. Thus, we aimed to test the hypothesis that there is a dose- and time-dependent relationship between IFNT and the endometrial expression of key genes involved in the signalling cascade leading to MRP, which might be associated with successful pregnancy establishment in cattle.
Materials and Methods: Bovine endometrial explants collected at the late luteal stage of the oestrous cycle were cultured in RPMI medium without (control) or with IFNT (1, 10, 100 ng/mL) for 6h. In parallel, endometrial explants were cultured in medium containing 100 ng/mL IFNT for different time periods (15 min, 30 min, 1 h, 3 h, 6 h). Endometrial explants from the same uterus ($n = 8$) were used for both dose- and time- dependent experiments in order to minimise variation. Gene expression was analysed by RT-qPCR. The treatment effect was considered significant at $P < 0.05$.
Results: The ISGs (*ISG15, OAS1, MX1* and *MX2*) were significantly ($P < 0.05$) up-regulated in the endometrial explants by 1 ng/mL IFNT, and the intensity of such changes were increased with higher concentrations (10 and 100 ng/mL) ($P < 0.05$). IFNT at 100 ng/mL significantly ($P < 0.05$) stimulated *ISG15, OAS1, MX1* and *MX2* in endometrial explants when cultured for 1, 3, or 6 h, but not shorter (15 min and 30 min, $P > 0.05$). The analysis of other target genes is currently on-going.
Conclusions: These results suggest that IFNT acts on the uterus in both a dose- and time- dependent manner in cattle that might be associated with successful pregnancy establishment.
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