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INTERVIEW

The people behind the papers – Priscila Ramos-Ibeas and Pablo Bermejo-Álvarez

Symmetry breaking in ungulates occurs in a flat embryonic disc similar to that of humans, making it a potential model for studying early human development. However, it has previously been impossible to develop ungulate blastocysts *in vitro* to the symmetry-breaking stages. Now, a new paper in Development describes culture conditions that allow ungulate embryos to be developed *in vitro* through to early gastrulation. We caught up with corresponding authors, Priscila Ramos-Ibeas, Científico Titular at El Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), and Pablo Bermejo-Álvarez, Científico Titular at INIA, to find out more about their research and their future plans.

Pablo Bermejo-Álvarez (L) and Priscila Ramos-Ibeas (R)

Pablo, can you give us your scientific biography and the questions your lab is trying to answer?

PB-A: My scientific career has been focussed on different aspects of mammalian preimplantation development. I obtained my DVM degree at Universidad Complutense de Madrid in 2005 and conducted my PhD (2006-2010) at Alfonso Gutiérrez-Adán laboratory at INIA (Spain) studying sexual dimorphism in bovine embryos. During this time, I visited the labs of Pat Lonergan, Keith Campbell, and Jerry Yang and Cindy Tian, where I learned diverse embryo manipulation techniques in multiple species. During my first postdoc (2010-2012) at Cheryl Rosenfeld and Michael Roberts laboratories at University of Missouri, I assessed the long-term effects of metabolic alterations in preimplantation embryos. Afterwards, I moved to Bhanu Telugu's laboratory at University of Maryland/USDA (2012-2014), where I was involved in induced pluripotent stem cell (iPSC) experiments and started using CRISPR to generate genome-edited mice. In 2014, I gained a tenure-track (Ramón y Cajal) position at INIA to establish my own laboratory and was tenured (Científico Titular) in 2018. As an independent researcher, my career was initially focussed on developing CRISPR technology for genome editing in livestock species. Nowadays, we are routinely using it to answer fundamental questions in reproductive and developmental biology by means of knockout (KO) models in multiple mammalian species. The main line of research of my lab aims to understand ungulate conceptus elongation. Our research is currently funded by a Starting Grant from the European Research Council, and one of the three main objectives of the project was to establish an *in vitro* system for post-hatching embryo development in ungulates. I find the study of conceptus elongation particularly exciting, because it is largely unexplored, it serves to revisit dogmas in mammalian developmental biology established based on mouse KO models, and generates applicable knowledge for livestock reproductive management and human assisted reproduction.

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Priscila, can you share your scientific biography, the questions your lab is trying to answer and how you came to collaborate with Pablo on this work?

PR-I: Pablo and I have known each other for a long time, and we have always shared common research interests. Both of us gained our DVM degrees at Universidad Complutense de Madrid, although at different periods (myself in 2009). We first met in 2010 when I was starting my PhD at INIA in the same lab where he was just finishing his PhD. In 2012, I did a research stay at the University of Maryland (USA) in the same lab in which Pablo was a postdoc and we collaborated in a project using iPSC and CRISPR approaches to understand the erasure of genomic imprinting marks. Then, after completing my PhD (2014), I worked as a postdoc with Giovanna Lazzari (Avantea, 2015-2016) and Ramiro Alberio (University of Nottingham, 2016-2018). During my postdoc with Ramiro, we drew a transcriptional map of pig preimplantation development by single-cell RNA-seq, which highlighted pluripotency pathways similar to humans and divergent to the mouse model. In 2018 I returned to INIA, initially funded by a Talent Attraction Postdoctoral Fellowship in Alfonso Gutiérrez-Adán's lab. Later, in 2020 I was granted a tenure-track (Ramón y Cajal) position to pursue an independent career, and in 2021 I was tenured (Científico Titular). Since 2020, I have established my own lab, in which we investigate the mechanisms directing embryo development and lineages specification in the ovine model. At INIA, Pablo and I re-started our prior collaboration as we shared an interest in the developmental aspects of ungulate preimplantation development.

Can you give us the key results of the paper in a paragraph?

PR-I & PB-A: We have developed a fully *in vitro* culture system that allows the development of ovine embryos up to the beginning of gastrulation, starting from slaughterhouse material. Under this system, ovine embryos recapitulate most developmental landmarks achieved by their *in vivo* counterparts, providing a platform to explore developmental processes in a pre-gastrulating mammalian

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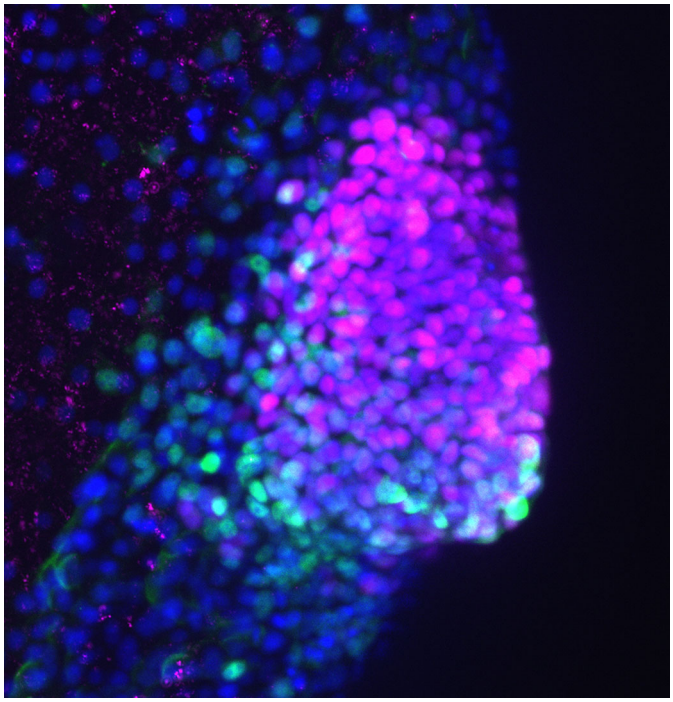

Ovine embryonic disc fully developed *in vitro* (day 14) initiating gastrulation, as evidenced by the expression of brachyury (T, green). Epiblast cells are stained for SOX2 (magenta) and nuclei are labelled with DAPI (blue).

embryonic disc. The system is also useful to understand pregnancy losses in farm ungulates caused by developmental failures at these stages.

Why did you choose to focus on the development of ovine embryos?

PR-I & PB-A: The advance in the knowledge of conceptus elongation in ungulates entails a dual interest. On one side, it constitutes the most susceptible period for reproductive losses in ungulates, which include the four most relevant mammalian livestock species worldwide (sheep, cattle, goats and pigs). Embryonic losses exert a great economic impact on farming and understanding the basis of such failures is crucial to develop strategies to minimize them. On the other side, ungulate embryos undergo gastrulation in a flat embryonic disc, similar to that found in humans and contrasting to the three-dimensional egg cylinder developed in mouse embryos. This similarity, together with a closer developmental timing and the possibility of generating embryos without using experimental animals, makes the sheep an excellent mammalian model to understand early human development. To that aim, the main advantages of sheep compared with other ungulate models are a smaller body size and the availability of protocols to generate embryos *in vitro* at a high efficiency.

Your use of N2B27-supplemented media was key for the survival of the epiblast cells; why was this media so crucial for ovine cells compared with human or mouse epiblasts?

PR-I & PB-A: To our surprise, the medium employed to achieve peri-gastrulating stages in mouse and human embryos failed to support epiblast survival in ovine embryos, probably due to the lack of essential components present in N2B27. We considered trying N2B27 because it had been used to derive and maintain embryonic stem cells, the *in vitro* equivalent to the epiblast. It would be certainly interesting to test whether human embryos develop in N2B27, but we cannot perform such research.

Do you think that it will be technically possible to get cultured embryos to elongate and advance through gastrulation *in vitro*?

PR-I & PB-A: That is our next goal and it should be possible, especially given the free-floating nature of these embryos, the development of which through gastrulation solely relies on the components of the uterine fluid. To that aim, we are analysing uterine fluid composition, attempting to pinpoint candidate molecules that could be added to improve N2B27. Advancing through gastrulation would be truly ground-breaking as it would grant access to more complex developmental events that cannot be studied in human embryos due to the 14-day rule.

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When doing the research, did you have any particular result or eureka moment that has stuck with you?

PR-I: Yes, I really enjoy imaging embryos at the fluorescence microscope and I remember the moment when I found mesoderm cells in an *in vitro* embryo for the very first time. It was amazing that the embryonic disc could have developed that far *in vitro*, and I was very happy to be the first one to see it.

And what about the flipside: any moments of frustration or despair?

PR-I: There are always frustrating moments in an embryology lab. For example, when all our embryos were arresting and it took us several weeks to find out that a specific oil batch that we regularly used for embryo culture up to the blastocyst stage suddenly started to be toxic.

Where will this story take your labs next?

PR-I: We are currently studying the mechanisms driving lineage specification and post-blastocyst embryo development by genome editing tools and using our *in vitro* culture system to bypass the need for experimental animals.

PB-A: The system can be used to identify the transcription factors required for lineage commitment or signalling pathways involved in pre-gastrulation embryo development using KO ungulate models. Without this system, the study of any developmental event beyond first lineage differentiation requires the transfer of the edited embryos to recipient females, so being able to observe more advanced stages *in vitro* definitely speeds up our research and makes our life easier (and that of the experimental animals). We are also using the system to envision possible nutritional or pharmacological strategies to alleviate embryonic losses in farm animals, by testing the developmental outcomes of the addition of specific compounds to N2B27 medium. Another application is in determining the developmental potential of blastocysts (also known as ‘embryo quality’). Such an application is very useful to validate advances in

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artificial reproductive techniques, and we are currently collaborating with a research and development company using bovine embryos as a model to test novel procedures for human IVF. The next step would be to attain complete ungulate gastrulation *in vitro*, a very ambitious yet seemingly achievable goal.

Finally, let's move outside the lab – what do you like to do in your spare time?

PR-I: I like doing sport, hiking with my partner and my dog, and exploring international cuisine, although nowadays I just enjoy my time looking after my newborn son.

PB-A: I love outdoors, at home or abroad: for example, when traveling to scientific meetings, I always find some fishing spot, mountain wilderness or swamp to spend an extra day. In more civilized environments, I enjoy discussing life with my partner, and I am a Mahou (Madrid's local beer), porras and tapas fan, which I enjoy with family and friends.

Reference

Ramos-Ibeas, P., González-Brusi, L., Torres Used, M., Cocero, M. J., Marigorta, P., Alberio, R. and Bermejo-Álvarez, P. (2022). *In vitro* culture of ovine embryos up to early gastrulating stages. *Development* **149**, dev199743. doi:10.1242/dev.199743