Animal Reproduction

Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)

Embryology, developmental biology, and physiology of reproduction

Collection of embryos and fluid from the bovine oviduct

Vitezslav Havlicek[1], Ann-Katrin Autz[1], Carina Blaschka[2], Michael Hoelker[2], Urban Besenfelder[1]

[1]University of Veterinary Medicine, Vienna, Austria; [2]Georg-August-University Goettingen, Germany; urban.besenfelder@vetmeduni.ac.at

In contrast to in vitro culture systems which are most commonly performed on a static medium culture basis, embryo development in the oviduct is directed by a dynamic supply system. In order to get more insight into stage specific needs of a developing embryo the aim of the present study was to determine, whether embryos and the corresponding tubal fluid can be simultaneously obtained from the bovine oviduct. Fifteen Austrian Fleckvieh heifers were synchronized by two intra muscular injections of PGF2a (Estrumate, Cloprostenol, MSD Animal Health, Austria) 11 days apart and GnRH (Veterelin, Buserelin, Calier, Barcelona, Spain) 48 hrs after each PGF2a injection. Fixed time artificial insemination has been performed using fresh semen. At Day 3 after expected ovulation, oviduct flushing and embryo collection has been done using an endoscopy set for transvaginal access (STORZ, Tuttlingen, Germany). Following epidural anesthesia and genital disinfection a trocar set was placed in the tip of the vagina and introduced via dorsomedial perforation through the vagina wall into the peritoneal cavity. Prior to flushing the ovulation side has been determined and the morphology of the growing corpus luteum has been assessed. Flushing was performed in two steps: first the ampulla was repeatedly flushed using 0.5 ml PBS medium. Finally, 0.2 to 0.4 ml medium/fluid has been recovered, adjusted to 0.5 ml and transferred into an Eppendorf tube and centrifuged for estimation of total protein using colorimetric Protein Quantitation Assay (Pierce BCA Protein Assay Kit, ThermoFisher, Austria). In a second step 50 ml medium (PBS, 1% FCS) was flushed through the oviduct, collected in the tip of the uterine horn via an embryo flushing catheter which has been connected to an Emcon embryo filter. Additionally, 300 ml medium were used to extra flush the uterine horn via the flushing catheter. The medium collected in the filter was checked for the presence of an embryo using a stereo microscope. Overall, 12 heifers have been synchronized 3 times and 3 heifers 2 times (in total: 42 treatments). In 35 cases ovulation has been confirmed and oviduct fluid has been collected successfully. Additionally, 21 embryos (recovery rate 60%) have been recovered at the 4 to 8-cell stage. Protein quantitation analyses of the collected tubal fluid ranged between 0.28 and 1.4 mg/ml total protein. Taken these preliminary results together it is concluded, that is possible to repeatedly collect tubal stage embryos and the corresponding fluid as a prerequisite to analyze the stage-specific environment of the embryo. It was also assumed that the obtained total protein amount was sufficient for further examining tubal components. It is expected that consecutive studies including the collection of tubal fluid and corresponding embryos throughout the migratory phase in the oviduct will provide much more detailed information about the needs of an embryo especially during in vitro culture.

Keywords: embryo, oviduct, cattle