**Promoters**

Prof. dr. Ann Van Soom  
Faculty of Veterinary Medicine, UGent

Prof. dr. An Hendrix  
Faculty of Medicine and Health Sciences, UGent

Prof. dr. Dawit Tesfaye  
Department of Animal Breeding and Husbandry, Uni Bonn

**Dr. Kelly Tilleman**  
Reproductive medicine, Ghent University Hospital

Prof. dr. Wim Van Den Broeck  
Department of Morphology, Faculty of Veterinary Medicine, UGent

Prof. dr. Catharina De Schauwer  
Department of Virology, Parasitology, and Immunology, Faculty of Veterinary Medicine, UGent

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**Members of the examination committee**

Prof. dr. Luc Peelman (Chairman)  
Department of Nutrition, Genetics, and Ethology, Faculty of Veterinary Medicine, UGent

Prof. dr. Alireza Fazeli  
Department of Oncology & Metabolism, Reproductive and Developmental Medicine, The University of Sheffield

Prof. dr. Bart Gadella  
Department of Biochemistry and Cell Biology, Biochemistry, Faculty of Veterinary Medicine, University of Utrecht

Prof. dr. Jo Leroy  
Department of Veterinary Sciences, University of Antwerpen

Dr. Karen Goossens  
Institute for Agricultural and Fisheries and Food, Animal Husbandry, ILVO

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**Curriculum Vitae**

Krishna Chaitanya Pavani was born on July 16th, 1989 in Ongole Town, Andhra Pradesh, India. He completed his Bachelor of Technology in Biotechnology (2010) in India. In 2012 he obtained his master’s degree in Molecular Biology at the University of Skövde, Sweden. He got his Doctoral degree with distinction grade from the University of the Azores, Portugal in 2018 with specialization in Animal Reproduction.

In 2016, he was selected as an Early Stage Researcher (ESR) in Rep-Biotech Joint Doctoral Project (which is an MSCA-ITN funded by the European Horizon 2020) at Faculty of Veterinary Medicine at Ghent University (Belgium).

Krishna Pavani is author and co-author of 13 publications in international peer-reviewed journals. His work has been presented at various national and international conferences.

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**Invitation**

Oral defense of the doctoral thesis of

**Krishna Chaitanya Pavani**

28th October 2019  
Department of Reproduction, Obstetrics and Herd Health
Summary of the thesis

Efficient communication between cells and tissues is necessary for many physiological processes, including embryo development. Usually, an embryo is able to promote its own development, either in the presence or absence of the maternal genital tract. Preimplantation embryos cultured in group are able to promote their own development in vitro by the production of autocrine embryotropins. Recently, extracellular vesicles (EVs) gained more interest as possible players in intercellular communication. Extracellular vesicles are membrane-bound vesicles found in all kinds of biological fluids and culture media conditioned by cells and embryos (Chapter 1). We hypothesized that these nano-sized vesicles could act as possible embryotropins released by embryos during culture. Besides, there is only limited information available on the EVs isolated from culture medium conditioned by bovine embryos. The goals of the thesis are detailed in Chapter 2.

In Chapter 3 we compared two different culture media by culturing the presumed zygotes in synthetic oviductal fluid supplemented with either bovine serum albumin (BSA) or polyvinylpyrrolidone (PVP) along with their ultracentrifuged counterparts to avoid protein or nanoparticles that could possibly interfere with quantification of EVs. Embryo development was recorded at 7 dpi and 8 dpi and blastocyst quality was assessed by differential staining. We were able to identify a suitable culture medium for isolating EVs, as we observed a higher apoptotic cell ratio and lower total cell number in both protein-free media. So, we chose ultracentrifuged BSA medium (for EVs quantification and identification analysis) to avoid possible contamination of proteins or EVs. Besides, embryos cultured in ultracentrifuged BSA had similar embryo development and quality as those in BSA medium.

In Chapter 4, we assessed the suitability of different EV isolating methods for the search of high quality EVs with maximum purity. Three methods were checked: Differential ultracentrifugation (DU), OptiPrep™ density gradient (ODG), and Size exclusion chromatography (SEC) methods. Separated EVs were characterized by western blot, electron microscopy, and Nanoparticle tracking analysis to assess the yield and purity. OptiPrep™ density gradient (ODG) centrifugation outperforms differential ultracentrifugation (DU) and size exclusion chromatography (SEC) in terms of purity, lack of contaminating proteins such as ribonuclease protein (Argonaute-2) and Lipoproteins (ApoA-i). The ODG method was superior for identifying EV-enriched components and improving our understanding of EV function in embryo development.

In Chapter 5, we isolated and characterized EVs from bovine embryos cultured in group and verified whether these EVs were functionally active. Bovine embryo-derived EVs were sizing between 25-230 nm with an average concentration of 2.37±0.12×10^10 particles/mL. Moreover, PKH67 EV pre-labeling showed that embryo-secreted EVs were uptaken by zona-intact bovine embryos, proving embryo-embryo crossstalk. After individual culture in BSA medium enriched with EVs derived from group culture, embryos showed higher blastocyst rates at day 7 and 8 and a lower apoptotic cell ratio. This was evidence that EVs are embryotropins released by bovine embryos.

In Chapter 6, we determined the relationship of embryo-secreted EVs with embryo development stage. We were able to isolate EVs with size exclusion chromatography (Izon’s™ qEV single column) from culture medium conditioned by bovine embryos that developed to the blastocyst stage and embryos that were not able to reach the blastocyst stage (non-blastocyst). We demonstrated that blastocysts released larger size of EVs, whereas smaller size vesicles were released by embryos that did not reach the blastocyst stage. Furthermore, we could identify five miRNAs (bta-miR-378, bta-miR-186, bta-miR-371, bta-miR-184, bta-miR-192) that can serve as possible biomarkers for embryo quality and implantation. This research opens possibilities in the search of biomarkers for human embryo implantation.

The general discussion and the conclusions of this thesis are presented in Chapter 7. In UC BSA medium both embryo quality and the purity of the culture medium for extracellular vesicles isolation was also high. OptiPrep™ density gradient (ODG) ultracentrifugation method is an effective method for EVs isolation compared with Differential ultracentrifugation (DU), and Size exclusion chromatography (SEC) methods. However, ODG had some drawbacks such as the fact that it is an expensive and time-consuming method. Embryos in group culture release EVs and these EVs are internalized by embryonic cells, providing strong evidence for the existence of embryo-embryo crossstalk, and they have a positive influence on embryos cultured individually. Finally, we identified five potential miRNAs, present as cargo in extracellular vesicles, that can be used to differentiate blastocysts from non-blastocysts and may serve as a marker for embryo implantation.