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Performance, Health and Welfare of Small Ruminants under Changing Climate Scenario

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Section VII: INNOVATIONS IN SUSTAINABLE SHEEP AND GOAT PRODUCTION

Chapter 19

GOAT COLOSTRUM-DERIVED EXOSOMES: THE SHOCKING SECRETS OF CELLULAR COMMUNICATION

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Abstract

Colostrum is described as the first secretion of the mammary gland after parturition which contains a complex mixture of bioactive component. After gradual changes colostrum become to mature milk. The development of new laboratory techniques has allowed to know in deep components that are in low abundance or those which has small size. Exosomes are small membranous vesicles (20-300 nm in diameter) found in different biological fluids, such as colostrum and milk. Cell-to cell communication is the most known function, but exosomes are also noted because of their use in human cancer therapy. Furthermore, the role of colostrum-derived exosomes in immunological processes has been reported. The study of the whole transcriptome profile of goat colostrum-derived exosomes provides very interesting information about the potential roles of the genes carried by these still not well known extravesicles. To study in deep the cargo molecules contained in the goat colostrum and milk derived-exosomes is crucial to elucidate the shocking secret of these small vesicles.

Introduction

The role of colostrum on the acquisition of immunity in newborn small ruminants (goat kids and lambs) is well known (Castro et al., 2011). For that reason, colostrum intake during the first hours of life is crucial to ensure a correct passive immune transfer and protection against infections, but also to improve the future productivity rates (Morales-delaNuez et al., 2011). Colostrum is the first secretion obtained from the mammary gland after partum (Barrington et al., 2001). The composition of colostrum has been studied for years and it is known that contains a complex mixture of bioactive components (Castro et al., 2011). In the case of small ruminants several authors have focused their studies on the major components principally gross chemical composition (Hadjipanayiotou, 1995; Argüello et al., 2006) including the Immunoglobulins (Ciupercescu, 1977; Ha et al., 1986; Dos Santos et al., 1994; Csapo et al., 1994; Castro et al.; 2006). Additionally, colostrum also contains many other components such as vitamins,

growth factors, somatic cells, enzymes, and other peptides (Koldovsky 1980; Campana and Baumrucker 1995; Blum and Hammon 2000).

The development of the omics techniques allows to identify low abundance components. That is the case of the low abundance proteins in colostrum. Hernandez-Castellano et al. (2016), using proteomics, reported differences between the low abundance proteins observed in colostrum of dairy goats and sheep compared to those found in mature milk, these authors described that some of the identified proteins have immune function.

Exosomes: Isolation and roles

The immune regulatory role of exosomes in human breast milk was studied by Admyre et al. (2007). Exosomes are small membranous extracellular vesicles between 20 to 300 nm in diameter (Pegtel and Gould, 2019). They were discovered in reticulocytes of sheep in 1983 (Zhang and Yu, 2019), but nowadays it is known that exosomes can be observed in different biological fluids such as blood, urine, saliva, cerebrospinal fluid, and breast milk among others (de la Torre Gomez et al., 2018; Doyle and Wang, 2019; Sedykh et al., 2020). Several types of cargo molecules, including lipids, proteins, DNA and RNA (mRNA and miRNA) are contained in exosomes (Gurunathan et al., 2021). Cell-to cell communication is reported as the principal function of exosomes (Martellucci et al., 2020), but exosomes are also well known because they are used in the therapy of human cancer. Chen et al. (2019) observed that exosomes are involved in the intercellular transport of RNA and proteins, suggesting also a potential immunological role due to the antigen-presenting capacity of exosomes.

One of the main challenges in the exosomes studies is the isolation, which is a key factor that depends on the type of sample and species. For this reason, different isolation methods have been developed using different fluids and species (Li et al., 2017; Chen et al., 2019; Coughlan et al., 2020; Sidhom et al., 2020). The differences on the composition of the fluids containing exosomes influences the isolation which lead to a lack on a standard isolation protocol.

On the exosome studies, it is crucial to obtain a high-quality pellet after isolation. In the case of goat colostrum and milk it is very important to store the sample at -80ºC within 24 hours after collection (Simón-Betancor, 2022). In addition, Castro et al. (2022) tested several methods of isolation to obtain goat colostrum-derived exosomes, concluding that the ultracentrifugation technique described by Gao et al. (2019) is the most suitable isolation methodology for goat colostrum and milk-derived exosomes. In addition, the exosomes characterization observed using Zeta sizer device shown that the size in goat colostrum was 126 ±9.04 nm being smaller than those observed by the same authors in bovine colostrum (Castro et al., 2022).

Some more recent studies, described the miRNA and mRNA transport into exosomes, concluding the role as mediators of cell-to-cell communication via signal transduction between cells (Sedykh et al., 2020; Narang et al., 2022). Likewise, studding the profiling of the microRNA, Ma et al. (2023) described similar miRNA expression in goat colostrum and milk, identifying miRNA which play important roles in cell proliferation, bone homeostasis and neuronal network formation in newborn.

In our research group, the total transcriptome profile of goat colostrum and mature milk-derived exosomes was analysed. The whole transcriptome profiling showed a total of 132 identified genes that were significantly different ($P<0.05$) in goat colostrum than in mature milk. From that 66 were upregulated and 66 downregulated in colostrum compared to mature milk ($P<0.05$). Focusing on the upregulated genes 32 of them were at least 5-fold more expressed in colostrum than in

mature milk. It is remarkable that 7 of them were *LOC* related genes whose function is not known yet. The two more expressed genes were *ARHGEF28* and *CCND1*, whose function is described in the human libraries. In addition, some of the other more expressed genes observed play several important roles in human. This is the case of *GYPC*, involved in in regulating the mechanical stability of red cells. *AQP5* that plays a role in the generation of saliva, tears and pulmonary secretions and *TOP2A*, gene that encodes a DNA topoisomerase, an enzyme that controls and alters the topologic states of DNA during transcription. (unpublished data).

From the 66 downregulated genes, 6 were observed as the less expressed in colostrum compared to mature milk. Noteworthy is the case of *PNPLA3* observed as the less expressed. This gene that encodes a protein involved in the triacylglycerol hydrolysis in adipocytes. Moreover, *C5* was also downregulated in goat colostrum, this gene encodes a component of the complement system (unpublished data). The complement system is an important component of the innate immunity in small ruminants. Moreno-Indias et al. (2012) reported that Factor H isolated in goats are like the human counterparts and is involved on the alternative pathway activity, which is the predominant pathway in goats. In addition, Castro et al. (2008) observed that the complement system in goat kids was activated by goat milk intake.

Conclusion and Future Perspectives

Despite the well-known colostrum bioactive components, the goat-colostrum derived exosomes have shown to carry transcripts involved in several important physiological functions. The omics techniques (transcriptomics and proteomics) are crucial tool to improve the knowledge in this field. Further studies about the proteins encoded by the identified genes in goat colostrum and mature milk would necessary, as well as to investigate the role of the goat-derived colostrum exosomes in the newborn goat kid.

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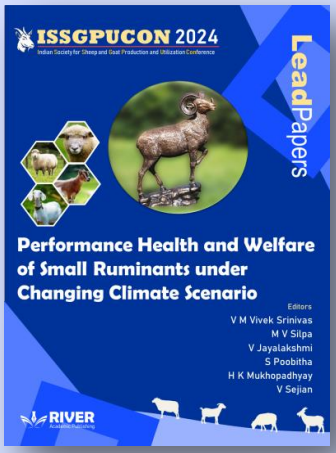
Among the livestock species, small ruminants play a vital role in contributing towards Indian economy and provides livelihood to nearly two-third of the rural community. Sheep and goat farming supports the agrarian economy especially in areas where crop and dairy farming are not economical. Furthermore, in the current global climate change scenario with the alarmingly rising human population and urbanization, small ruminant farming may play a significant role in ensuring economic security to the poor and marginal farmers. Having said so, farming and research developments in small ruminant farming is relatively lesser when compared to other livestock species like cattle.



This book thereby is a compilation of the vital concepts and some of the recent advancements and innovations in the field of sheep and goat production. This volume can serve as a preamble for students, lecturers, researchers, scientists and policy makers in India and across the globe. This volume consists of chapters authored by eminent researchers from India and across the world having vast experience in small ruminants. The volume is an exemplary compilation of 34 chapters which can be broadly categorized into 11 sections. Most importantly the book covers areas on climate resilient small ruminant production, novel approaches in small ruminants feeding and nutrition. The book also focusses on the clinical advancements like recent trends in reproductive management, contemporary diagnostic techniques in small ruminants, advances in small ruminant therapeutics and ethno veterinary practices. Particular emphasis was also given to cover the area on omics approaches for re-defining breeding strategies in small ruminants, innovations in sustainable sheep and goat production and prospects for value addition in small ruminant products. Lastly, this volume also provides detailed description of newer perspectives in sheep and goat welfare. Overall this volume would be of great interest to varied policy makers as it contains sections on role of small ruminants in socio-economic upliftment and policies and marketing strategies for small ruminant sector. Thus, this book can be a vital source of reference for all stakeholders on livestock farming system, consisting updated information in varied aspects of small ruminant production.

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