Summary of the DVM thesis No 14187 , Factually of Veterinary Medicine, Urmia University.
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Title of thesis: Esoxomal differences before and after insemination, and in pregnant and non-pregnant Makui and Qezel ewes

Summary:
Early detection of pregnancy has economic importance in reproductive management of sheep. Current experiment was designed to evaluate the differences in SDS-PAGE gel bands, and intensity of miRNA-378-3p and miRNA-22-3p gene expression in isolated exosome from sera samples of pregnant and non-pregnant Qezel and Makui ewes (n=30). Medroxyprogesterone acetate vaginal sponges were inserted for 14 days, and the equine chorionic gonadotrophin (eCG) was injected (500 IU) at withdrawal, to synchronize the estrus and ovulation. Artificial insemination was performed via laparoscopic approach using fresh collected semen samples at 60.5± 0.5 h after eCG injection. Blood samples were collected at a day before insemination and repeated every 5 days for a total of 5 consecutive samples. Sera samples were separated using centrifugation and their exosomal contents were isolated using commercial kit. Pregnancy was diagnosed using transvaginal ultrasonographic examination at day 30 after insemination. The presence of exosomes was verified using transmission electron microscopy with negative staining. The molecular weight and sizes of exosomes were determined using DLS method. The molecular weight and sizes of exosomes were determined using DLS method. One-dimensional SDS-PAGE was used to subtract exosome protein bands, and the real time PCR was used to determine the intensity of miRNA-22-3p and miRNA-378-3p gene expression. Results indicated the greater molecular weight and sizes of exosomes in pregnant compared to non-pregnant ewes. A total of 67 common bands and 11 unique bands were detected by SDS-PAGE in non-pregnant and pregnant ewes, respectively. The expression of miRNA-22-3p and miRNA-378-3p genes were higher in pregnant compared to non-pregnant, and even in twin and triplet pregnancy compared to single pregnancy. Using the mentioned method and results, it seems that early detection of pregnancy would be accessible.

Keyword: exosome, ewe, pregnancy, SDS-PAGE, Real-time PCR.