Non-invasive Targeted Gene Knockdown of *GDF9* and *BMP15* Genes with LNA™ GapmeRs: Implications for Bovine embryo development

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In recent decades, substantial progress has been achieved by applying genetic engineering techniques to manipulate genes. RNA interference (RNAi) has been identified as a natural mechanism regulating gene expression in various higher organisms. RNAi technologies like small interfering RNA (siRNA) are widely used to down-regulate any gene expression without disrupting the expression of other genes. For delivery of these siRNAs into mammalian cells various methods have been used like microinjection, transfection reagents, and electroporation. However, there are some pitfalls in using these assisted delivery methods, such as cellular toxicity, variable transfection efficiency, and off-target effects. Hence it is necessary to deliver RNA Silencing molecules without any assisted delivery methods.

In the current preliminary study, we tested the efficiency of commercially available Antisense LNA® (Locked nucleic acids) GapmeRs (Qiagen, 339511) for the knockdown of specific target genes in bovine embryos without any assisted delivery (like transfection or microinjection). For this, we chose *GDF9* (Growth differentiation factor 9) and *BMP15* (Bone morphogenetic protein 15), two genes involved in folliculogenesis, oocyte quality and embryo development for knockdown by using GapmeRs. To do so, we targeted three exon regions of each target gene (GDF 9 and BMP15), along with negative control. We used routine *in vitro* maturation and fertilization methods but *in vitro* culture was supplemented with GapmeRs (100nM) targeting GDF9 (Group A; n = 98, 4 replicates), BMP15 (Group B; n = 99, 4 replicates), a combination (Group C n = 102, 4 replicates), a negative control (Group D; n = 102, 4 replicates), whereas culture without any supplementation served as control (Group E). The presumed zygotes (n = 501 in 4 replicates) were cultured until 8 days post-insemination (dpi), at which time blastocysts were collected and snap-frozen to further validate gene knockdown with RT-qPCR. Our results demonstrated a compromised embryo development following the gene knockdown, which was

most pronounced for BMP-15. A drop in cleavage rate was observed in group B (74%±7.06), which was significant when compared to group A (87%±8.64, p=0.045). Moreover, a significantly lower blastocyst rate was observed in Group B (26%±9.35) when compared to Group A (40%±15.83) and Group E (40%±12.64) (p=0.039, p=0.023 respectively), RT-qPCR analysis provided additional proof that GDF9 and BMP15 had a knockdown impact on blastocysts that were collected on 8 dpi. Interestingly, the knockdown of GDF9 increased the gene expression of BMP15 significantly (Fold change (FC): 4.5; $p<0.05$) compared to the control group (FC: 1.0), similarly knockdown of BMP15 induced a lower gene expression of GDF9 compared to control (FC: 0.4; 1.2, $P<0.05$ respectively). No significant gene expression changes were observed in other groups. Overall, using this unassisted Antisense oligonucleotides GapmeRs method could help in determining the functional impact of target genes (GDF9; BMP15) during bovine embryo development without using any delivery methods.