Investigating the Improvement of Bull *In Vitro* Fertility Through Seminal Plasma Extracellular Vesicles

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Context:

Seminal plasma extracellular vesicles (EV) affect the biological function of sperm by enhancing their motility and fertilizing capability by transferring essential proteins, lipids, and RNAs, potentially affecting embryo development and offspring health. Using EVs derived from known high *in vitro* fertility (HF) bulls might allow for tailored IVF treatments to address specific fertility issues and improve success rates for known low *in vitro* fertility (LF) bulls.

Aim:

The main aim of this study was to test the possibility of adding seminal plasma-derived EVs from known HF bulls to our *in vitro* fertilization (IVF) system without impairing embryo development. The second aim was to see if adding seminal plasma-derived EVs from known HF bulls affects the fertilization capacity of known LF bulls in our IVF system.

Methods:

Oocytes were retrieved from abattoir-derived ovaries. All media was purchased from Stroebech Media (Denmark). *In vitro* maturation and culture were carried out using the manufacturer´s standard protocols. EVs from known HF bull seminal plasma were isolated with a size exclusion chromatography (SEC) benchtop column. Concentration and size of EVs were determined using nanoparticle tracking analysis.

During IVF, EVs in three different concentrations (A: $200x10^6$ EVs/mL, B: 400 $x10^6$ EVs/mL, and C: 600 $x10^6$ EVs/mL) were added to 100 µL IVF media and co-cultured with sperm and oocytes for 24 h under mineral oil. As a control, PBS without Ca^{2+} and Mg^{2+} was added to 100 µL IVF media. Blastocyst formation and their morphology and kinetics were recorded on days seven (D7) and eight (D8) after fertilization.

Morphological and kinetic scores were calculated similarly to Gebreyesus *et al.,* 2024. The statistical analysis was carried out using the GraphPad Prism 10 software. Blastocyst rate, kinetic, and morphological scores of different treatment groups were analyzed with one-way ANOVA followed by Tukey's multiple comparisons test with a significance value of $P \leq 0.05$.

Key results:

On D7, the highest blastocysts morphological score was recorded in group C (3.0), which was 1.1 points higher than the control group morphological score (1.9). No differences between blastocyst rates and developmental kinetics were recorded. On D8, the difference in blastocyst rates (%) between group C and the control group was 19.8 ± 2.6 and 9.4 ± 0.9, respectively, but insignificant with a $P \geq 0.05$. The morphological score of D8 in group C was the highest (2.35), 0.55 points higher than the control group score (1.8).

Conclusion:

Adding seminal plasma-derived EVs to our IVF system did not hinder embryo development. The preliminary results suggest improvement in embryo morphology and blastocyst rates when adding 600 x10^6 EVs/mL seminal plasma-derived EVs from known HF bulls to IVF media to improve the fertility capacity of known LF bulls. More research is needed to confirm and validate the benefits of adding seminal plasma-derived EVs from HF bulls to improve LF bull IVF outcomes.

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