Review of: "Lipid profile of bovine grade-1 blastocysts produced either in vivo or in vitro before and after slow freezing process"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

This paper provided an interesting analysis of bovine lipid profiles of Holstein cattle produced with either IVD or IVP methods, before and after slow freezing and accounted for embryo sex.

The original research study presented in this manuscript was through with controlled variables, however, the authors failed to account for several factors which can attribute to bovine embryo lipid profile. While it is not expected each factor to be compared in this analysis, there should be some discussion about how not including these factors could impact outcomes of this study:

Suggested variables include:
Maternal body composition. What was the body condition score of the holstein heifers included in this study? Previous research shows maternal body composition can influence the lipid composition of oocytes and embryos.

Would it have been beneficial to perform mass spectrometry on some of the oocytes to show the maternal and oocyte influence on the embryos lipid profile? Do the oocytes and pre-freeze blastocyst lipid profiles differ significantly? If not, how can we address maternal factors to control lipid profiles in the future? This is a very important implication to this study.

Maternal Diet: What was the diet of the Holstein heifers in this study? Maternal diet can impact the lipid profile of the embryos and could be a variable influencing the lipid profile in this study. Maternal diet was referenced as a variable in the discussion but I am curious to know about the maternal diet of the test animals.

Animal breed: Cryodamage is a risk to all embryos through both slow freezing and vitrification methods. However, some animals “freeze better” than others. The Jersey industry has shown that their embryos survive the cryopreservation process at a lower rate than beef and Holstein cattle, likely due to the increased lipid content of the Jersey embryo. Discussion or comparison amongst different breeds of cattle would be interesting.

Slow freeze technique: It is suitable the authors chose an ethylene glycol slow freeze technique but future studies should compare the difference between embryos frozen with EG versus DMSO.

Stimulation and hormones used for embryo flush (IVD) and OPU. It is understandable these protocols differ, but mentioning how the stimulation protocol could potentially be a cause of differences in IVD and IVP other than just culture media would help make this analysis more thorough. Is there any literature which looks into this?

Blastocyst grade: It would be interesting to see how the lipid profiles vary amongst all blastocysts and not just the grade 1. In practical field application, most blastocysts are eligible for transfer (1 and 2 preferred) and preferred over morulas. However, in IVD embryo transfer, morulas are transferred fresh or frozen routinely. Additionally, blastocyst grade is a subjective quality score. Including all blastocysts or all grade 1 and 2 blastocysts in this study would have been interesting to compare how lipid profiles impact blastocyst quality or vary between blastocyst with the different (IVD or IVP) production systems, freezing or sex.

Sire influence was not mentioned in this study. One bull was used to control for sire variables but what was the bulls BCS, diet or breeding soundness exam results?

Introduction:
In regard to the introduction of the paper, there were some minor grammatical errors which need to be revised. The authors were inconsistent with their abbreviations. Please be consistent with the use of IVD and IVP as it was defined early in the paper. More background information about “chilling injuries” could support this paper, especially in regard to how lipid profiles can attribute to “chilling injuries”. Please provide a biological, physiological and metabolic explanation about how lipid profiles can change during the freezing process.

The objective of this study is unclear with how it is currently written (at the end of the introduction). I would suggest something like: "The objectives of this study were threefold: 1) to evaluate lipid profiles of bovine blastocysts between IVP and IVD produced embryos, 2) to evaluate lipid profiles of bovine blastocysts before and a after slow freezing and 3) to evaluate lipid profiles of embryos biopsied to determine embryo sex.

Please do not include results (Table 1) in the introduction section. These need to be in the results section.

Results: The results section was well written and thorough. Figures, tables and graphs were high quality

and easy to understand. Please consider re-arranging figures to be consistent with the results verbiage.

There is a concern with the embryo production data. “The average number of Q1 blastocysts produced in vitro was 2.1 per donor during OPU IVF session, compared to 3.4 per donor during in vivo session”. This is inconsistent with normal production outcomes. Typically, OPU IVF will produce more blastocysts than IVD. Do we know what caused the low embryo count in this study?

Overall, this paper is interesting and provides new insights to a very complex, multi-variable problem. Improving IVD/IVP techniques and freezing techniques to improve embryo cryopreservation outcomes can have major economical impact to the beef and dairy industry. Due to the complexities, it is important to address variables to determine the root cause of varying lipid profiles in effort to improve protocols in the future.