

Evaluation of Animal-Free Supplements as Alternatives to Fetal Bovine Serum for In Vitro Bovine Embryo Development

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소 수정란 생산에서 FBS 대체물질로서 무동물성 배지 첨가제의 효과 평가

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Abstract

Fetal bovine serum (FBS) has been widely used in bovine in vitro embryo production (IVP), but its undefined composition, batch-to-batch variability, and association with large offspring syndrome (LOS) limit reproducibility and raise ethical concerns. In this study, we evaluated an animal-free supplement as an alternative to FBS for Hanwoo embryo culture. Oocytes were matured in Medium 199 supplemented with either animal-free supplement + FSH or FBS + FSH, fertilized with frozen-thawed Hanwoo semen, and cultured in commercial IVF/IVC media. Across four independent experiments, cleavage and blastocyst rates were comparable between groups (AOF: $48.5 \pm 13.3\%$ and $29.0 \pm 15.7\%$; FBS: $49.3 \pm 15.2\%$ and $23.8 \pm 12.6\%$), with no significant differences observed. These findings suggest that animal-free supplementation can replace FBS in supporting embryo development while reducing variability and potential risks such as LOS. Future studies will expand the sample size and evaluate other candidate supplements, including algal extracts, to further validate animal-free culture systems in bovine IVP.

1. Introduction

Bovine in vitro embryo production (IVP) systems traditionally use fetal bovine serum (FBS) as a supplement for oocyte maturation and embryo culture. Despite its widespread use, FBS poses challenges such as undefined composition, lot-to-lot variability that reduces reproducibility, and potential biosecurity risks. Furthermore, the collection of FBS raises animal welfare concerns, as it involves harvesting blood from bovine fetuses, where issues such as fetal sentience, the potential for pain perception, and compliance with welfare standards remain debated (Versteegen et al., 2021). Moreover, FBS supplementation has been associated with abnormal fetal growth, including large

offspring syndrome (LOS), raising further concerns about its application (Rivera et al., 2022; Nava-Trujillo & Rivera, 2023). Therefore, the development of animal-component-free media is essential to ensure consistency, safety, and ethical acceptability in IVP.

2. Materials and Methods

2.1 Oocyte collection

Ovaries from Korean native cattle (Hanwoo) were obtained from a local abattoir and transported to the laboratory under standard conditions. Cumulus-oocyte complexes (COCs) were aspirated from follicles and selected based on morphological criteria.

2.2 In vitro maturation (IVM)

COCs were cultured in Medium 199 (Gibco) supplemented with either an animal-free supplement and FSH (experimental group) or with HI FBS (Gibco) and FSH (control group).

2.3 In vitro fertilization (IVF)

Matured oocytes were fertilized using frozen-thawed Hanwoo semen in IVF medium (MK-BIO) under standard conditions.

2.4 In vitro culture (IVC)

Presumptive zygotes were cultured in IVC medium (MK-BIO) under standard laboratory conditions. Developmental competence was evaluated based on cleavage and morula-blastocyst formation rates.

2.5 Statistical analysis

Cleavage and blastocyst formation rates (%) were summarized as mean \pm SD across independent experiments. Group differences (animal-free vs. FBS) were tested using paired t-tests; Wilcoxon signed-rank tests were additionally performed when normality was not assumed. Two-sided $p < 0.05$ was considered statistically significant.

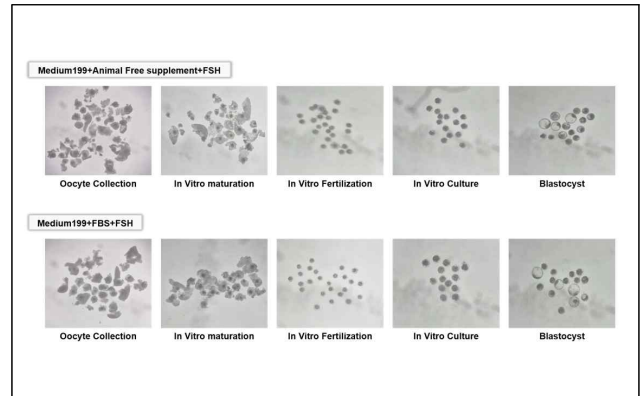
3. Results

Across four independent experiments, the animal-free supplement group and the FBS control group showed comparable developmental outcomes.

[Table 1] Developmental outcomes in Hanwoo oocytes cultured with an animal-free supplement or FBS.

Experiment	Group	Oocytes	Cleavage (%)	Blastocyst (%)
1	AOF	23	9 (39.1%)	6 (26.1%)
	FBS	22	11 (50.0%)	6 (27.3%)
2	AOF	25	16 (64.0%)	13 (52.0%)
	FBS	25	14 (56.0%)	10 (40.0%)
3	AOF	20	11 (55.0%)	4 (20.0%)
	FBS	19	12 (63.2%)	3 (15.8%)
4	AOF	28	10 (35.7%)	5 (17.9%)
	FBS	25	7 (28.0%)	3 (12.0%)

When summarized across all experiments, mean (\pm SD) cleavage rates were $48.5 \pm 13.3\%$ for the animal-free group and $49.3 \pm 15.2\%$ for the FBS group, while mean (\pm SD) blastocyst rates were $29.0 \pm 15.7\%$ and $23.8 \pm 12.6\%$, respectively. Statistical analysis confirmed no significant differences between groups ($p > 0.05$), indicating that the animal-free supplement supported embryo development at a level comparable to conventional FBS.



[Fig. 1] Representative images of in vitro-produced Hanwoo embryos.(Top: Animal-free supplement, Bottom: FBS)

4. Conclusion

In conclusion, the animal-free supplement demonstrated comparable efficacy to FBS in supporting cleavage and blastocyst development of Hanwoo embryos. These findings highlight the potential of animal-free culture systems to provide more consistent and ethically sustainable alternatives to serum supplementation, while also addressing concerns such as large offspring syndrome associated with FBS. Future work will include expanding the sample size and evaluating other candidate supplements, such as algal extracts, to further validate the robustness and applicability of animal-free media in bovine in vitro embryo production.

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