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Influence of sequential culture systems on *in vitro* embryo production in sheep

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Abstract
The present study aimed to produce *in vitro* sheep embryos using different culture media. A total of 572 presumptive zygotes were cultured in three media: G1/G2 sequential media, synthetic oviduct fluid amino acid (SOFaa), and potassium simplex optimization medium (KSOM). The cleavage rates observed were 162 (84.47±3.07%), 147 (75.93±4.23%), and 140 (72.27±3.50%) for G1/G2, SOFaa, and KSOM, respectively. The cleavage rate was significantly higher in G1/G2 sequential media compared to SOFaa and KSOM. In the G1/G2 sequential media, the mean percentages of embryos progressing to the 4-8 cell, 16-32 cell, morula and advanced stages were 148 (77.15±3.04), 129 (66.39±5.61), 99 (51.77±2.86) and 86 (45.23±2.07) respectively, at different time points. In conclusion, the G1/G2 culture media demonstrated a significantly higher proportion of embryos reaching advanced developmental stages compared to SOFaa and KSOM, indicating its superior suitability for *in vitro* sheep embryo production.

Keywords: Sequential culture systems, *in vitro* embryo production and sheep

Introduction
In recent years, significant advancements have been made in reproductive biotechnologies for domestic animals. The development of *in vitro* embryo production has paved the way for the next generation of assisted reproductive techniques, Including Intracytoplasmic Sperm Injection (ICSI), transgenic animal production and somatic cell nuclear transfer. Among the factors influencing embryo development, the culture environment plays a critical role in determining blastocyst quality, regardless of the oocyte's origin (Rizos *et al.*, 2003) [2]. This study aimed to investigate the influence of different culture media on the production of *in vitro* embryos in sheep.

Materials and Methods
Ovaries from local sheep were collected from a slaughterhouse, excluding those from pregnant ewes. Oocytes were retrieved using the slicing method and only grade A, B, and C oocytes were selected for maturation. After washing, the oocytes were transferred to 50 µL droplets of *in vitro* maturation (IVM) media and incubated in a CO_2 incubator for approximately 24 hours. Oocyte maturation was assessed based on the expansion of cumulus cells and the extrusion of the first polar body.

Spermatozoa were flushed from the cauda epididymis, and the swim-up method was used to isolate the motile fraction. After washing, the oocytes were transferred into pre-incubated 75 µL droplets of *in vitro* fertilization (IVF) Tyrode's Albumin Lactate Pyruvate (TALP) medium containing heparin and cultured for 18-20 hours. The fertilized oocytes were denuded of their cumulus cell attachments by vigorous pipetting, and fertilization rates were assessed.

The fertilized oocytes were then cultured in three different media-G1/G2 sequential medium, synthetic oviduct fluid amino acid (SOFaa), and potassium simplex optimization medium (KSOM). They were incubated in a CO_2 incubator for 5-6 days, with the culture media changed every 48 hours. The developmental progress of the embryos was evaluated during the incubation period.

Results and Discussion

A total of 1,001 oocytes were harvested from 170 ovaries of slaughtered sheep using the slicing method, excluding ovaries from pregnant ewes. The average yield of good quality oocytes was 5.94±0.36 per ovary, which were subsequently used for maturation. The higher yield of good quality oocytes observed in this study could be attributed to the presence of a larger number of developing follicles in the ovaries at the time of collection. Additionally, the slicing method, associated with less physical force, likely minimized damage to the cumulus cell layers, thereby improving oocyte quality.

A total of 572 matured oocytes with good cumulus expansion (grades 1 and 2) were used for fertilization, with a mean maturation rate of 79.44±2.90 per cent. The higher maturation rates in grade A and B oocytes compared to grade C oocytes were likely due to the intact cumulus cells surrounding the zona pellucida in the former grades (Shioya *et al.*, 1988) [3]. The high maturation rate observed in this study could also be attributed to the supplementation of the culture medium with fetal calf serum (FCS), (Totey *et al.*, 1992) [5].

Following fertilization, all 572 presumptive zygotes were cultured in three different media: G1/G2 sequential media, synthetic oviduct fluid amino acid (SOFaa), and potassium simplex optimization medium (KSOM). The cleavage rates were the mean percentage of 162 (84.47±3.07), 147 (75.93±4.23) and 140 (72.27±3.50) for G1/G2, SOFaa, and KSOM, respectively, with the cleavage rate being significantly higher (p<0.05) in G1/G2 sequential media.

The developmental progress in G1/G2 sequential media showed the highest mean percentages at various stages: 4-8 cell (77.15±3.04), 16-32 cell (66.39±5.61), morula (51.77±2.86) and advanced stages (45.23±2.07%). In SOFaa, the corresponding mean percentages were 65.04±3.91, 49.39± 4.33, 35.02±3.58 and 32.47±6.75, while in KSOM, they were 53.79±3.11, 41.20±4.18, 28.83±3.03 and 19.29±2.73 per cent, respectively.

G1/G2 sequential media demonstrated superior results compared to SOFaa and KSOM at all developmental stages (p<0.05). Heindryckx *et al.*, (2001) [1] emphasized that early stage embryo culture conditions might not be suitable for post-compaction stages and vice versa. In this study, sequential media (G1.5/G2.5) were introduced to address this issue. Embryos were cultured in a simple, low-phosphate, and low-glucose medium for the first three days, followed by a medium with higher glucose content to promote the morula-to-blastocyst transition. G1.5 contained non-essential amino acids plus methionine, while G2.5 included both essential and non-essential amino acids, along with vitamins, which supported better embryo development.

SOFaa showed significantly higher developmental rates to the morula stage compared to KSOM (p<0.05). Differences in media composition likely contributed to these results. While KSOM contains glutamine, SOFaa does not, and previous studies have reported potential inhibitory and teratogenic effects of glutamine on embryos (Summers *et al.*, 2005) [4]. Additionally, SOFaa had a higher concentration of BSA compared to KSOM, which could have positively influenced embryo development.

The commercially available G1/G2 media, manufactured by Vitrolife (Sweden), performed better than the laboratory-prepared KSOM and SOFaa media. Differences in the quality of water and the presence of additional constituents in KSOM and SOFaa might have contributed to their relatively poorer performance compared to G1/G2.

Summery

The aim of this study was to produce *in vitro* sheep embryos using different culture media. The results demonstrated that the G1/G2 culture medium was superior to SOFaa and KSOM in supporting the development of sheep embryos to advanced stages.

Conclusion

Recent advancements in reproductive biotechnologies, such as *in vitro* embryo production, have revolutionized livestock breeding. This study demonstrated that the choice of culture medium significantly influences sheep embryo development, with G1/G2 sequential media showing superior results over SOFaa and KSOM. The higher cleavage and progression rates in G1/G2 highlight its effectiveness in supporting embryo growth to advanced stages. These findings emphasize the importance of optimizing culture conditions for *in vitro* fertilization and embryo development, which can enhance reproductive efficiency, facilitate genetic improvements, and contribute to sustainable livestock production systems. Continued research will further refine these techniques for practical applications.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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