

Original Article

Effect of Serum and Serum Free Media on the Developmental Competence of OPU Derived Bovine IVP Embryo

Byung-Hyun Choi^{1,3}, Bun-Young Park³, Rami Kong^{1,3}, Mi-Ju Son³, Chan-Sang Park^{1,3}, Nyeon-Hak Shin⁴, Hye-Young Cheon⁴, Yeong-Rok Yang⁴, Jin-Woo Lee⁴, Jong-In Jin^{2,3} and Il-Keun Kong^{1,2,*}

¹Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 52828, Korea

²Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 52828, Korea

³GAST, Gyeongsang National University, Jinju 52828, Korea

⁴Gyeongnam-do Provincial Government Livestock Research Institute, Sancheong 52263, Korea

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*Correspondence

Il-Keun Kong

E-mail: ikong7900@gmail.com

ORCID

<https://orcid.org/0000-0002-1906-9898>

ABSTRACT Embryos produced with serum show the alterations in their ultrastructure, impaired compaction, abnormal blastulation, aberrant mRNA expression profiles and large calf syndrome with greater incidences of stillbirths and deaths after birth. The aim of the present study was to describe in vitro embryo production by analyzing embryo production, fetal production and pregnancy rate in free-serum medium. The OPU-IVP data used in this study from 2016. Approximately, sixteen cows (Hanwoo), which belonged to the Institute of Gyeongsang National University, were used. Two experimental group is used in this study. Serum groups were conducted in March to July and free-serum group was conducted in September to December. The recovered cumulus-oocyte complexes were morphologically classified to four grades based on the compaction of cumulus cells layers and homogeneity of the cytoplasm. The number of oocyte was significantly greater in serum groups than that in free-serum groups (29.61 ± 0.63 vs. 15.6 ± 0.62 ; $p < 0.05$). Between serum and free-serum groups indicate that average of 1st and 2nd grade oocytes were no difference (2.38 ± 1.67 vs. 2.38 ± 1.48 ; $p > 0.05$), but number of 3rd and 4th grade oocytes were greater in serum groups than that in free-serum groups (7.31 ± 7.64 vs. 5.60 ± 6.29 ; $p < 0.05$). Embryo cleaved competence was higher in rate in free-serum groups than that in serum groups (62.1% vs. 58.3 ; $p < 0.05$). However, blastocyst developmental rate was no difference between serum and free-serum groups (33.1% vs. 43.5% ; $p < 0.05$). 986 recipients were used for embryo transfer. Pregnancy rate was indicated that between serum and free-serum group was no difference (54.6% vs. 56.3% ; $p < 0.05$). In conclusion, we developed the free-serum system for production of in vitro bovine embryos in order to meet the developmental and qualitative requirements for large scale commercial use.

Keywords: embryo development, free-serum, Korean native cow (Hanwoo), OPU

INTRODUCTION

In vitro culture of bovine embryos derived from *in vitro*

maturation and fertilization (IVM-IVF) have succeeded in producing calves (Lu et al., 1987; Goto et al., 1988). An adequate *in vitro* culture system for bovine zygotes is re-

quired for large-scale embryo production by IVM-IVF and genetic improvement by means of ovum pick-up (OPU) and IVM-IVF. Until now, the major obstacle associated with the extensive use of this technology is the lack of suitable methods to preserve *in vitro* produced embryos. Culturing of embryos results in fundamentally different embryos from those produced *in vivo*, particularly for ruminants (Lonergan et al., 2001; 2003). *In vitro* culture (IVC) media plays an important role in the development of *in vitro* fertilized embryos and have diverse compositions include defined media composed of simple salt solutions or undefined complex culture media supplemented with serum and with undefined components (Lane et al., 2007). Serum has been widely added to culture media because it contains embryotrophic factors and substances beneficial for embryonic development, such as antioxidants, growth factors, and heavy metal chelators (Murakami et al., 2011), and provides nutrients necessary for cell survival and proliferation (Wirthensohn and Barth, 1985). Although the role of serum is not completely known (for review, see (Thompson et al., 2007) it has been demonstrated that serum has a biphasic effect inhibiting early cleavage divisions and accelerating the development of morula and blastocysts (Van Langendonck et al., 1997; Lonergan et al., 1999). It is also expected that serum provides energy substrates, amino-acids, vitamins, growth factors and heavy-metal chelators at concentrations that vary among batches (Pinyopummintr and Bavister, 1994). A higher development rate to the blastocyst stage is obtained from media supported with serum, but the serum effectiveness for the *in vitro* embryo production might change considerably from one batch to another (Van Langendonck et al., 1997). Furthermore, embryos produced with serum show alterations in their ultrastructure, impaired compaction, abnormal blastulation, aberrant mRNA expression profiles and large calf syndrome with greater incidences of stillbirths and deaths after birth (Van Langendonck et al., 1997; Holmet et al., 1999; Wrenzycki et al., 2004). Moreover, bovine-derived sera or proteins have recently been avoided especially in human *in vitro* production systems because of the appearance of bovine spongiform encephalopathy and a viral or prion contamination risk. In addition to increasing the sensitivity to cryopreservation, serum enhances potential sanitary risks due to viruses, prions or mycoplasma contaminations, and is suspected to contribute to the large offspring syn-

drome described in ruminants derived from IVP embryos (Jacobsen et al., 2000; Farin, 2001). It is also responsible for various alterations of embryo morphology, ultrastructure and kinetics of development (Van Langendonck, 1997; Abe et al., 1999). Moreover, serum could act as antioxidant or prooxidant, depending on its constituents, which could also affect the cryoresistance of the embryos (Rizos et al., 2003). Taken together, these data indicate that a whole free-serum system for the production of IVP embryos is desirable.

The aim of the present study was to describe *in vitro* embryo production by analyzing embryo production, fetal production and pregnancy rate in free-serum medium.

MATERIALS AND METHODS

Experimental animals

The OPU-IVP data used in this study from 2016. Sixteen Korean native cows (Hanwoo) approximately, which belonged to the Institute of Agriculture and Life Science, Gyeongsang National University, were used. Study based on 2 experimental groups. Donor is equivalent. Serum group for March to July and free-serum group for September to December.

Oocyte collection and *in vitro* maturation (IVM)

OPU derive oocyte is (TCM-199) supplemented with 10% (v/v) FBS (Gibco BRL, Life Technologies, Grand Island, NY, USA), 1 µg/mL estradiol-17β, 10 µg/mL follicle-stimulating hormone, 10 ng/mL epidermal growth factor, 0.6 mM cysteine, and 0.2 mM sodium pyruvate. Thereafter, groups of up to 50 COCs were transferred to a 4-well dish (Thermo Fisher Scientific, Waltham, MA, USA) containing 500 µL IVM medium and incubated in a humidified atmosphere of 5% CO₂ in air at 38.5°C for 22-24 h.

In vitro fertilization (IVF) and IVC

In vitro matured COCs were fertilized with frozen-thawed bovine sperm that had been previously tested for IVF as described by Mesalam et al., 2017. Semen was thawed at 39°C for 1 min, sperm were washed and pelleted in D-PBS by centrifugation at 750 × g for 5 min at room temperature, and motile sperm were recovered. The pellet was resuspended in 500 µL heparin (20 µg/mL) prepared in IVF medium Tyrode's lactate solution supplemented with 6 mg/mL bovine serum albumin (BSA),

22 mg/mL sodium pyruvate, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin] and incubated at 38.5°C in a humidified atmosphere of 5% CO₂ in air for 15 min (to facilitate capacitation). Thereafter, sperm were diluted in IVF medium (final density of 1×10^6 sperm/mL). Matured oocytes were transferred to 600 µL IVF medium containing sperm for 18-20 h. After IVF, cumulus cells were removed by repeated pipetting, and up to 50 presumed zygotes per group were washed and transferred to 4-well dishes containing 500 µL SOF-BE1 medium (Abe et al., 1999) supplemented with 4 mg/mL fatty acid-free BSA, 5 µg/mL insulin, 5 µg/mL transferrin, and 5 ng/mL sodium selenite for 3 days. Presumed zygotes were then cultured until day 8 of embryonic development (day 0 = day of IVF) in medium of the same composition (free-serum) or in which BSA was replaced by 10% (v/v) CDS FBS (serum group). Day 8 expanded blastocysts were used for embryo transfer to recipient host.

Statistical analyses

Data are presented as mean values standard deviation, except for data reporting changes during embryo development, which are presented as percentages. Each experiment was performed at least three times, and results were analyzed with SPSS (ver. 18.0, SPSS Inc., Chicago, IL, USA). Differences between means were considered significant at $p < 0.05$.

RESULTS

Follicle and oocyte dynamics

The recovered cumulus-oocyte complexes were morphologically classified into four grades based on the number of cumulus cells layers and homogeneity of the cytoplasm (Table 1). Oocytes number for session was greater in serum groups than those in free-serum groups (29.61-0.63 vs. 15.6-0.62; $p < 0.05$). There was no difference in grade 1 + 2 mean between serum and free-serum groups (2.38 ± 1.67 vs. 2.38 ± 1.48 ; $p > 0.05$), but in grade 3 + 4 mean between greater in serum group than that in free-serum group (7.31 ± 7.64 vs. 5.60 ± 6.29 ; $p < 0.05$).

Embryo development rate and the number of blastocysts

The results regarding the cleaved embryo, as well as the blastocyst development are shown in Table 2. Embryo

Table 1. Mean \pm SEM effects of individual donors on the efficiency of oocytes collected by OPU

Treatment	No. aspirated follicles	Total	No. oocytes aspirated (% Mean \pm SD)					
			Sum of grades 1 + 2	Sum of grades 3 + 4	Grade 1	Grade 2	Grade 3	Grade 4
Serum	8,262	5,445	1,341 (24.6, 2.38 \pm 1.67) ^a	4,104 (75.4, 7.31 \pm 7.64) ^b	477 (8.8, 1.72 \pm 0.08)	864 (15.9, 3.11 \pm 0.09)	3,713 (68.2, 13.36 \pm 0.38) ^b	391 (7.2, 1.41 \pm 0.08) ^a
Free-serum	4,649	2,904	867 (29.9, 2.38 \pm 1.48)	2,037 (70.1, 5.60 \pm 6.29) ^b	326 (11.2, 1.79 \pm 0.1)	541 (18.6, 2.97 \pm 0.11)	1,870 (64.4, 10.27 \pm 0.47) ^b	167 (5.8, 0.92 \pm 0.1) ^b

^{a-b}Within a column, means without a common superscript differed ($p < 0.05$).

Table 2. Mean \pm SEM effects of individual donors on the efficiency of *in vitro* embryo production

Treatments	No. of oocytes collected (%, Mean \pm SD per session)	No. cleaved embryos (%, Mean \pm SD per session)	No. of blastocyst developed (%, Mean \pm SD per session)
Serum	5,445 (65.9, 29.61 \pm 0.63) ^a	3,175 (58.3, 11.42 \pm 0.42) ^a	1,802 (33.1, 6.46 \pm 0.34)
Free-serum	2,904 (62.4, 15.6 \pm 0.62) ^b	1,802 (62.1, 9.9 \pm 0.51) ^b	1,261 (43.5, 6.78 \pm 0.41)

^{a-b}Within a column, means without a common superscript differed ($p < 0.05$).

Table 3. Effects of embryo transfer pregnancy

Treatments	No. embryo transfer	No. pregnancy	Pregnancy rate (%)
Serum	821	449	54.60
Free-serum	165	93	56.30
Total	986	542	54.97

cleaved competence rate in free-serum groups was higher than those in serum (62.1% vs. 58.3; $p < 0.05$; Table 2). But, blastocyst developmental rate in serum was no difference than that in free-serum (33.1% vs. 43.5%; $p < 0.05$).

Pregnancy of embryo transfer

The results regarding the embryo transfer, the number of pregnancy, as well as the pregnancy rate are shown in Table 3. Embryo transfer for 986 recipients. Based on high quality embryo, recipients were allocated into two groups: Group 1, embryo production from serum treatment; Group 2, embryo production from free-serum treatment. Pregnancy rate were result of serum was no difference than that in free-serum (54.6% vs. 56.3%; $p < 0.05$).

DISCUSSION

This study aimed to evaluate the suitability of a serum-free culture system for OPU derived bovine embryos. Blastocyst quality is crucial factor to ensure the optimal pregnancy rates following transfer (Rizos et al., 2003). Serum in culture medium plays a role positive embryotrophic factors and inactivation of emryotoxic agents and lead to improve the embryo development, but their mechanism was not determined (Bavister et al., 1995). The effect of serum on lipid metabolism can be due to three mechanisms: (a) lipoproteins present in the serum can be internalized by the cells, increasing the intracellular lipid content (Sate et al., 1999; Abe and Hoshi, 2003), (b) the presence of serum can alter mitochondria and thus b-oxidation of lipids, leading to increased storage of

intracellular lipids (Crosier et al., 2001; Abe et al., 2002) and (c) serum could increase neosynthesis of triglycerides by the embryo (Razek et al., 2000). Hatching rate was able to consider to some extent as a measure of embryo quality. To reproduce the culture conditions usually observed after OPU, we compared the impact of the serum-free culture media and serum media on embryos culture. We investigated the development rate of blastocysts, grade of oocytes, recovery of oocytes and number of follicles according for OPU-derived embryo production. The mean number of oocytes obtained by OPU session varies from 7.2 to 20.9.7 (Galli et al., 2001; Su et al., 2012; Jin et al., 2015; Roth et al., 2018), these data agree with our results reported that the average embryo production per donor were 29.6 and 15.6 embryos at sessions, respectively. However, large variations were observed between breeds and donors. Whatever the culture medium, no difference was observed on embryo development. These results are in agreement with two previous reports (Donnay et al., 1997; Ward et al., 2000). The results of embryo transfer were not different between serum and serum free culture medium. In present results indicate that the free-serum culture media was suitable to use for OPU derived embryo production.

CONCLUSION

In conclusion, we developed the serum-free system for production of *in vitro* bovine embryos in order to meet the developmental and qualitative requirements for large scale commercial use.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR'S AFFILIATION, POSITION AND ORCID NO.

- BH Choi, Gyeongsang Nat'l Univ., Researcher,
<https://orcid.org/0000-0001-9768-1962>
- BY Park, Gyeongsang Nat'l Univ., Researcher,
<https://orcid.org/0000-0001-7313-7858>
- R Kong, Gyeongsang Nat'l Univ., Researcher,
<https://orcid.org/0000-0003-4575-1994>
- MJ Son, Gyeongsang Nat'l Univ., Researcher,
<https://orcid.org/0000-0003-1580-6974>
- CS Park, Gyeongsang Nat'l Univ., Researcher,
<https://orcid.org/0000-0002-3906-1674>
- NH Shin, Gyeongnam-do Provincial Government Live-stock Research Institute, Ph.D.,
<https://orcid.org/0000-0002-9069-0601>
- HY Cheon, Gyeongnam-do Provincial Government Live-stock Research Institute, Researcher,
<https://orcid.org/0000-0003-0814-6277>
- YR Yang, Gyeongnam-do Provincial Government Live-stock Research Institute, Researcher,
<https://orcid.org/0000-0002-7699-0923>
- JW Lee, Gyeongnam-do Provincial Government Livestock Research Institute, Director,
<https://orcid.org/0000-0001-9001-7858>
- JJ Jin, Gyeongsang Nat'l Univ., Ph.D.,
<https://orcid.org/0000-0002-5790-7637>
- IK Kong, Gyeongsang Nat'l Univ., Professor,
<https://orcid.org/0000-0002-1906-9898>

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