

The Impact of Two Embryo Culture Media, Synthetic Oviduct Fluid and Commercial BO, on pre-and post-Implantation Development of Cloned SAANEN Goat Embryos

Mehdi Hajian, Ph.D.^{1*}, Farnoosh Jafarpour, Ph.D.¹, Sayed Morteza Aghamiri, Ph.D.², Shiva Rouhollahi Varnosfaderani, M.Sc.¹, Mohsen Rahimi Andani, M.Sc.¹, Mohammad Hossein Nasr-Esfahani, Ph.D.^{1*}

1. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

2. Department of Clinical Studies, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Abstract

Background: Somatic cell nuclear transfer (SCNT) is an approach for the propagation of elite animals. *In vitro* condition, especially the composition of culture media has a profound effect on the developmental competency of *in vitro* derived embryos. There are limited studies evaluating the effect of culture media on SCNT outcomes. To address this gap, we compare the effect of two culture media synthetic oviduct fluid (SOF) vs. commercial bracket-oliphant (BO) on developmental competency.

Materials and Methods: In this experimental study, embryos derived from *in vitro* fertilized (IVF) and SCNT were cultured in both BO and SOF media for 7 days. In addition to the assessment of cleavage and blastocyst on day 3, and 7, the quantitative expression of 16 genes in the resultant blastocysts were assessed. The resultant SCNT blastocysts from SOF and BO groups were also transferred to the synchronized recipient for developmental competency to term.

Results: The blastocyst rate in the BO medium was significantly higher than that of the SOF medium in the SCNT group ($P < 0.05$). All of the examined genes showed increased expression levels in SCNT blastocyst in both media compared to IVF Blastocyst. In the IVF group, *Oct4*, *Bmpr1*, and *Gcn5* showed significantly higher expression in the SOF medium compared to the BO medium while *Akt*, *Fgfr4*, *Sox2* showed significantly lower expression in the SOF medium compared to the BO medium. In the SCNT group, *Fgfr4*, *Gcn5*, *Fzd*, *Ctnnb*, *Bmpr1*, and *Fgfr4* showed significantly higher expression in SOF compared to BO derived blastocyst.

Conclusion: It appears that in SCNT blastocysts, gene regulation is less controlled compared to IVF ones, irrespective of the type of medium. In addition, there are differences regarding certain genes expressions between IVF and SCNT derived blastocysts between SOF and BO, reiterating that culture composition affects developmental competency and gene expression.

Keywords: Culture Medium, Embryo Development, Gene Expression, Goat, Nuclear Transfer

Citation: Hajian M, Jafarpour F, Aghamiri SM, Rouhollahi Varnosfaderani Sh, Rahimi Andani M, Nasr-Esfahani MH. The impact of two embryo culture media, synthetic oviduct fluid and commercial BO, on pre-and post-implantation development of cloned SAANEN goat embryos. *Int J Fertil Steril.* 2021; 16(1): 23-29. doi: 10.22074/IJFS.2021.531302.1130.

This open-access article has been published under the terms of the Creative Commons Attribution Non-Commercial 3.0 (CC BY-NC 3.0).

Introduction

Extensive observation and investigation of normal physiological systems, especially the female reproductive system, have resulted in the formulation of media to improve *in vitro* culture conditions and suit the embryo's requirements (1). Based on the literature, it is possible to have a rough estimate of the performance of each media type regarding pre- and post-implantation competence (2). However, it must be borne in mind that in addition to the medium components (3) and embryo metabolism (4), factors influencing the culture medium condition (5), including air quality, temperature, and

humidity, can also influence the developmental competence (6).

Regarding human embryo culture, numerous commercial culture media are available in the market (7-9). Although the efficiency of some of these media has been assessed using a mouse model, there are a limited number of studies that have compared these commercially available media (10). A quick review of the literature showed that the introduction of *in vitro* fertilization (IVF) for the treatment of infertility was associated with single-step culture media and cell culture media, such as Ham's F10, KSOMaa, Bracket-Oliphant (BO) (6, 11-13).

Received: 29/May/2021, Accepted: 04/August 2021

*Corresponding Address: P.O.Box: 8159358686, Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Emails: mehdihajian2002@gmail.com, mh.nasr-esfahani@royaninstitute.org



Royan Institute
International Journal of Fertility and Sterility
Vol 16, No 1, January-March 2022, Pages: 23-29

However, further research resulted in the introduction of sequential culture media, that were based on the composition of human tubal fluid (14). Then, with the introduction of time-lapse technology in embryology and some new clinical trials, single-step culture media gained renewed attention and were used routinely in embryo culture media, especially when time-lapse technology was used to monitor embryos (15, 16). Single-step media can also be useful in the zona-free somatic cell nuclear transfer (SCNT) method, in which the embryos should be cultured in an undisturbed environment to support the development of SCNT embryos to the blastocyst stage.

The efficiency of SCNT in biotechnology (17), biomedicine, stem cells (18), transgenic animal production (19), conservation of endangered species, and breeding has attracted considerable attention in recent years (20). However, despite the significant improvements made to enhance the reprogramming efficiency of SCNT, the technical efficiency and high throughput of SCNT have limited its application and reduced its success rate compared to IVF (21).

However, only a limited number of studies have focused on the type of culture media and their effect on the efficiency of SCNT (22). In a previous study, we showed that sequential G-series culture media, despite resulting in a high blastocyst formation rate, had a limited effect on oocyte developmental competence compared to that of sequential synthetic oviductal fluid (SOF) media (23). In the light of the above information, this study was designed to compare the impact of SOF medium with that of commercial BO medium, commonly used for *in vitro* production of farm animals, using caprine SCNT embryos.

Materials and Methods

Ethical guidelines

In this experimental study, all animal experiments were conducted in compliance with the ethical guidelines established by the Institutional Ethics Committee of Royan Institute. For slaughterhouse ovaries, permissions were obtained from the manager of the abattoir and the Iran Veterinary Organization (IVO) (IR.ACECR.ROYAN.REC.1396.220). In this experimental study, Unless otherwise specified, Chemicals and media were purchased from Sigma (St. Louis, MO, USA) and Gibco (BRL, Grand Island, NY, USA).

Oocyte *in vitro* maturation

Briefly, the ovaries of Bakhtiari goat were obtained from a slaughterhouse in Isfahan. The slaughterhouse of Isfahan follows guidelines that comply with standards of the IVO. Following the dissection of ovaries around 2–4 pm, they were transferred to thermos flasks, containing normal saline with 2X antibiotics (0.1 mg/mL streptomycin, 100 IU/mL penicillin G potassium), and maintained at 15–17°C until they were transferred to the laboratory by 6 pm. Immediately, the ovaries were washed, trimmed, and stored at 11–12°C until the cumulus oocyte complexes (COCs) were harvested the day after at 9 am. The method

of maintaining ovaries at the aforementioned temperature has been previously published (20).

The *in vitro* maturation (IVM) was performed as described in our previous study (20). Briefly, the follicular content was aspirated from 2–6 mm follicles using a 20-gauge needle attached to a vacuum pump. Only COCs with homogeneous cytoplasm and at least three layers of compact cumulus cells were isolated for IVM. The COCs were cultured in 50 µl droplets of maturation medium, containing tissue culture medium 199 (TCM-199)+10% fetal bovine serum (FBS) supplemented with 10 µg/ml follicle-stimulating hormone (FSH, sigma F8174), 10 µg/ml luteinizing hormone (LH, sigma L5269), 100 mM 17-β-estradiol, 0.1mM cysteamine, 10 ng/ml epidermal growth factor (EGF), and 100 ng/ml insulin-like growth factor 1 (IGF1), under mineral oil for 20 hours at 38.5°C, 5% CO₂, in maximum humidified air.

Somatic cell nuclear transfer

We used a variant of SCNT technology which is called handmade cloning (HMC). After denudation of cumulus cells (using 300 IU/ml hyaluronidase) and the removal of zona pellucida with 5 mg/ml pronase for a few seconds), a manual method of oocyte enucleation with the aid of a fine pulled Pasteur pipette was used to enucleate the oocytes. Briefly, zona-free oocytes were incubated in TCM supplemented with 4 µg/ml demecolcine for 20 minutes at 38.5°C. Then, a cytoplasmic protrusion, containing metaphase II (MII) spindle, was removed by a manual pipette. For nuclear replacement, enucleated oocytes were transferred to dishes containing droplets of H-TCM supplemented with 10 mg/ml phytohemagglutinin; then, single fibroblast cells were attached to the membrane of the enucleated oocytes (24). Subsequently, the couplets in fusion buffer free of Ca⁺² and Mg⁺² (290 mOsm) were electrofused using sinusoidal electric current (1.7 Kv/cm) for 10 sec, followed by two direct currents (1.75 kV/cm for 30 µsec and 1 second delay). After 30 minutes, the reconstructed oocytes were activated with 5 µM ionomycin for 1 minute, followed by 2 hours of incubation with 2 mM 6-dimethylaminopurine (6-DAMP) (25). Afterward, the activated reconstructed oocytes were cultured inside the wells containing SOF medium or BO medium and incubated for 7 days under mineral oil at 38.5°C, 5% CO₂, 5% O₂, and humidified air (24). Grade 1 and 2 blastocysts were selected for embryo transfer.

Selection of genes set

In order to select the genes that could predominantly be involved in the regulation of early embryonic development and pluripotency, we followed the strategy described by McGraw et al. (26) due to the lack of sufficient data on the goat species. In brief, we sought the related information using gene expression databases that profiled gene expression and gene ontologies (GOs) in both human and mouse embryos and embryonic stem cells (ESCs, Table S1, See Supplementary Online Information at www.ijfs.ir). To be considered as a poten-

tial candidate, the genes had to be commonly present in ESCs and either oocyte or blastocyst and play critical roles in the transcription regulation, pluripotency, and differentiation. This survey provided a list of 16 genes, including *Akt*, *Oct4*, *Sox2*, *Bmpr1*, *Fgfr4*, *Cdc25*, *Cdx2*, *Gcn5*, *Pcaf*, *Foxd3*, *Smad5*, *Fzd*, *Lifr1*, *Ctnnb*, *Erk1*, and *Ifnt*. The main functions, GOs, and null alleles of each gene are summarized in Table S1 (See Supplementary Online Information at www.ijfs.ir). Due to the lack of any previous report or database regarding the gene sequences of many of the abovementioned genes, the primers were designed based on the conserved regions of these markers in bovine, ovine, human, and mouse sequences. Subsequently, specific primers were designed from these recognized sequences (Table 1).

Table 1: Primers used for real-time PCR experiment

Gene	Primer sequence (5'-3')	Length of PCR product	TM
<i>Akt</i>	F:CCCTTAAACAACCTTCTCTG R:GAATGACGAAGGTATTGG	98	60
<i>Oct4</i>	F:AGAAGGGCAAACGATCAAGC R:GAATGGGACCGAAGAGTACAGAGT	96	56
<i>Sox2</i>	F:GCCGCCGATGATTGTTAT R:AGAGAGAAAAGAAAGGGAGAGAA	182	54
<i>Bmpr1</i>	F:TGTTTCGTCGTCTCAT R:GGTGCTAAGGTTACTCC	116	58
<i>Fgfr4</i>	F:GCTGACTGGYAGGAAAAGG R:AGTGGCTGAAGCACATCG	193	56
<i>Cdc25</i>	F:TGGCAAGCGTGTATATCGT R:GGTAGTGGAGTTTGGGGTA	119	58
<i>Cdx2</i>	F:CCCCAAGTGAACCAG R:TGAGAGCCCCAGTGTG	144	53
<i>Gcn5</i>	F:ACTCACCTGATGAACCAC R:TGTCGCACCCTCGTAG	174	54
<i>Pcaf</i>	F:ACGAACAAGTCAAGGGCTATG R:CAGAGAACTCCGTGTATGGG	246	60
<i>Foxd3</i>	F:AGAGCCCGCAGAAGAAGC R:GGTCCAGTAGTTGCCCTTG	182	59
<i>Smad5</i>	F:ATTATGCCAAGTATATCCA R:GTCTGTGAATCCAICTAC	136	60
<i>Fzd</i>	F:ATTGCCTGCTACTTTTAC R:TTAGTCTGGTTGTTCATT	89	59
<i>Lifr</i>	F:CGGATTCGTTTCTACT R:AATGGCTTCTGTGGTTAA	117	56
<i>Ctnnb</i>	F:TGGCTATTACAACAGATT R:GGTCCTCATATATTCACCT	160	54
<i>Erk</i>	F:GCTAATTCACCTGGAGAT R:ACGATATAAGGCGAGTTG	204	58
<i>Ifnt</i>	F:AGAATCCGTCTCTACCTG R:TCAGTCAACGAGAACCAC	129	54

PCR; Polymerase chain reaction and TM; Melting temperature.

RNeasy Micro Kit (Qiagen®, Germany) was used to extract total RNA from day 7 blastocysts. The extracted RNA was used for first-strand cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Germany). The cDNA synthesis reaction contained 1 µL random hexamer primer, 1 µL RNase inhibitor, 4 µL 5x reaction buffer, 2 µL dNTP, and 1 µL M-MuLV reverse transcriptase, adjusted to 20 µL using DEPC-treated water.

The cDNA synthesis was performed at 42°C for 1 hour. Real-time reverse transcription polymerase chain reaction (RT-PCR) was carried out using a Rotor-Gene 6000 (Corbet®). Each reaction mixture contained 2 µL of cDNA, 10 µL of SYBR Premix Ex Taq II (TaKaRa, Japan), and 1 µL of forward and reverse primers (5 µM), adjusted to 20 µL using dH₂O. Expression level of β-Actin was used to normalize target genes. All measurements were performed in triplicate, and expression levels are reported as 2^{-ΔΔCt} (ΔΔCt=ΔCt (a target sample)-ΔCt (a reference sample)). The list of primer sequences are shown in Table 1.

Embryo transfer

Bakhtiari recipient goats with at least one parturition and normal appearance and health were selected by an expert veterinarian and screened for contagious diseases, including Johne's disease and brucellosis. The selected goats had a mean age of about 2-3 years and a mean weight of 35 kg. They were synchronized with the insertion of progesterone sponges, containing 40 mg fluorogestone acetate (Intervet™), and this was considered as day 0. Then, 500 IU of PMSG (pregnant mare serum gonadotropin), 250 µg of prostaglandin (estroPLAN®, Australia), and 1000 IU of human chorionic gonadotropin (hCG) were administered on days 5, 7, and 9, respectively. Sixteen days post-insertion of progesterone sponges, grade 1 and 2 SCNT blastocysts were selected from SOF or BO groups; then, two to four blastocysts were transferred to the synchronized goats using the laparoscopic embryo transfer technique. The establishment and progression of pregnancies in the recipient goats were measured using rectal ultrasound on days 28-38 and abdominal ultrasound on days 83-113 after embryo transfer, respectively. All the animals were allowed to undergo normal delivery (20). Please note embryos from the IVF group were not transferred, as our aim was SCNT outcomes and not IVF.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 20 (SPSS Science, Chicago, IL) was used for the analysis of data. The normality of the data was checked using the Shapiro-Wilk test. For descriptive results, data were expressed as mean ± SEM. Comparison of average percentages of the data related to the two media was examined using an independent sample t test. The differences were considered statistically significant at P<0.05.

Results

In vitro development of IVF, SCNT, and parthenogenetic embryos in two culture media (SOF vs. BO)

In order to assess developmental competency of IVF and SCNT embryos in SOF and BO, the cleavage and blastocyst rate were compared and the results revealed that the rate was not significantly different between the experimental groups (Fig.1), however, the blastocyst rate in the BO medium was higher than that of the SOF medium in the SCNT group (P=0.001, Fig.1).

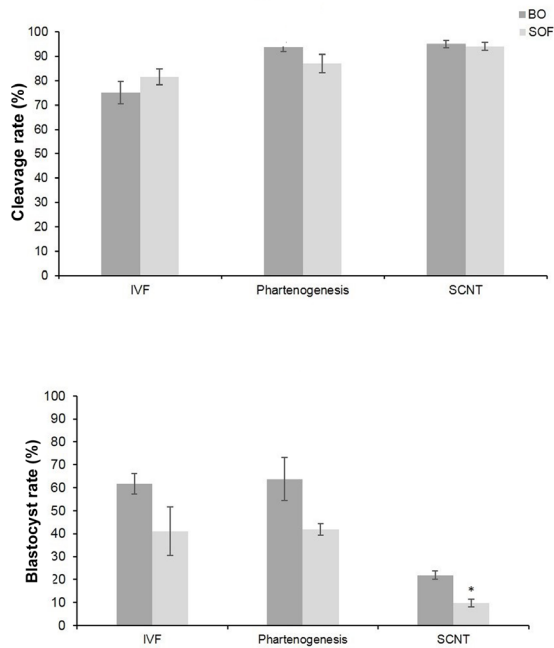


Fig.1: The *in vitro* development of IVF, parthenogenetic, and SCNT embryos in the BO and SOF media. The asterisks represent significant differences ($P < 0.05$). Five replicates and the minimum number of oocytes in each replicate were 50. IVF; *In vitro* fertilization, SCNT; Somatic cell nuclear transfer, SOF; Synthetic oviductal fluid, BO; Bracket-Oliphant IVC medium, IVC; *In vitro* culture.

Effects of embryo culture medium on the genes expression of goat SCNT and IVF blastocysts

In the IVF group, *Oct4*, *Bmpr1*, and *Gcn5* showed significantly higher expression in the SOF medium than in the BO medium. In this group, *Akt*, *Fgfr4*, and *Sox2* showed significantly lower expression in the SOF medium than in the BO medium (Fig.2). In the SCNT group, *Fgfr4*, *Gcn5*, *Fzd*, *Cttnnb*, *Bmpr1*, and *Fgfr4* showed significantly higher expression in the SOF medium than in the BO medium (Fig.3). On the other hand, all genes were overexpressed in both media, SOF and BO (Figs.4, 5).

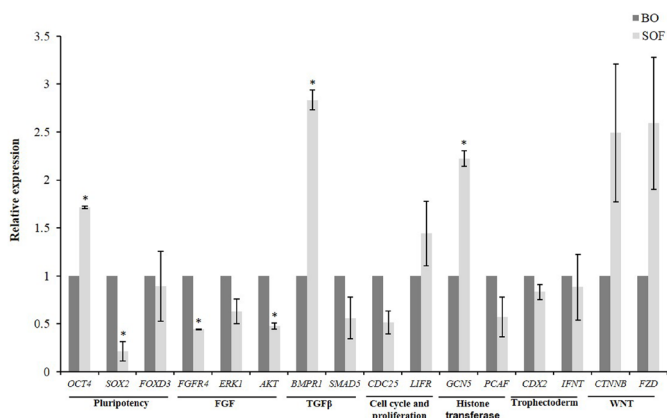


Fig.2: The genes expression of goat IVF blastocysts in the BO and SOF media. The asterisks represent significant differences ($P < 0.05$). Three replicates and the minimum number of blastocysts in each replicate were 5. IVF; *In vitro* fertilization, SOF; Synthetic oviductal fluid, BO; Bracket-Oliphant IVC medium, IVC; *In vitro* culture, FGF; Fibroblast growth factor, and TGFβ; Transforming growth factor beta.

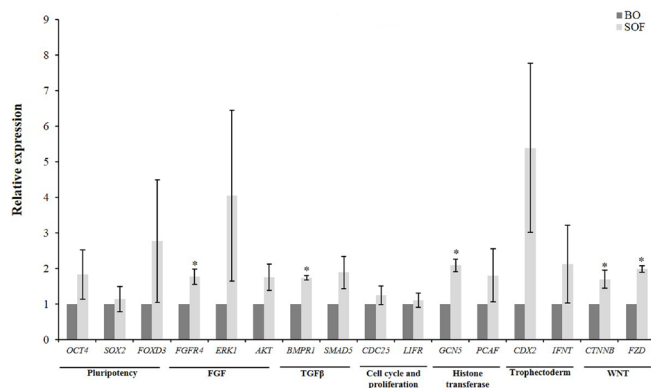


Fig.3: The genes expression of goat SCNT blastocysts in the BO and SOF media. The asterisks represent significant differences ($P < 0.05$). Three replicates and the minimum number of blastocysts in each replicate were 5. SCNT; Somatic cell nuclear transfer, SOF; Synthetic oviductal fluid, BO; Bracket-Oliphant IVC medium, IVC; *In vitro* culture, FGF; Fibroblast growth factor, and TGFβ; transforming growth factor beta.

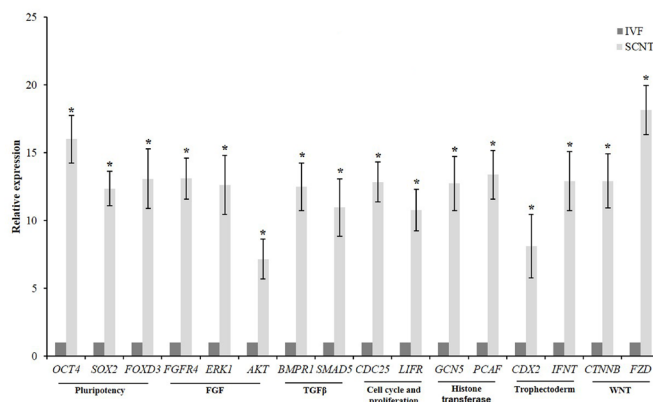


Fig.4: The genes expression of goat SCNT and IVF blastocysts in the SOF medium. The asterisks represent significant differences ($P < 0.05$). Three replicates and the minimum number of blastocysts in each replicate were 5. IVF; *In vitro* fertilization, SCNT; Somatic cell nuclear transfer, SOF; Synthetic oviductal fluid, FGF; Fibroblast growth factor, and TGFβ; Transforming growth factor beta.

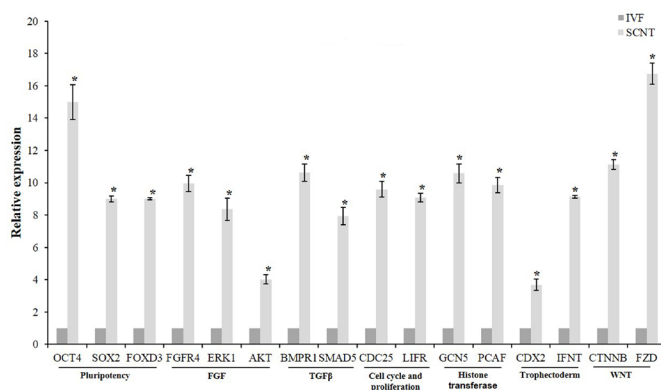


Fig.5: The genes expression of goat SCNT and IVF blastocysts in the BO medium. The asterisks represent significant differences ($P < 0.05$). Three replicates and the minimum number of blastocysts in each replicate were 5. IVF; *In vitro* fertilization, SCNT; Somatic cell nuclear transfer, BO; Bracket-Oliphant IVC medium, IVC; *In vitro* culture, FGF; Fibroblast growth factor, and TGFβ; Transforming growth factor beta.

***In vivo* development of SCNT goat embryos**

Ultrasound assessment revealed that the rate of pregnancy loss in the BO medium was lower than that in the SOF

medium, but the difference was not significant. Full-term pregnancy and SCNT efficiency based on live birth per embryos transferred to surrogate mother in the BO group were higher than those of the SOF group, but the difference was not statistically significant ($P > 0.05$, Table S2, See Supplementary Online Information at www.ijfs.ir).

Discussion

More than a century has passed since the pioneering work of Wesley Kingston Whitten (1956), the father of embryo culture medium (27), and significant progress has been made in culturing human and, to some extent, domestic species zygotes to blastocysts and then to embryos (28). However, recent studies on humans have shown that culture media composition and assisted reproductive technology (ART) procedures affect parturition (29), the weight of children at birth, and even their health after ART procedure (30). Despite these achievements in culturing human embryos, there is a need for further research, especially in culturing embryos from domestic species towards complete epigenetic patterns. The results of our previous work (23) showed that despite producing a high blastocyst rate in the IVF- and SCNT-derived from caprine embryos, the commercial G1/G2 media, containing human serum albumin (HAS)-solution, used for culturing human embryos and tested by mouse embryonic assay, had a negligible post-implantation developmental competence compared to that of the SOF medium, a homemade medium supplemented with bovine serum albumin (BSA). This study was designed to compare the SOF medium with the BO medium using a single-step medium, containing synthetic serum (31) replacement, BSA, vitamins, amino acids, and antioxidants, for culturing parthenogenic, IVF-, and SCNT-derived caprine embryos. The results showed that although there was no significant difference between the groups in terms of the cleavage rate, the SCNT blastocyst rate was significantly higher in the BO medium compared to that of the SOF medium (27). Based on the difference between the requirements of SCNT embryos and those of the parthenogenic and IVF-derived embryos, it was concluded that the BO medium was more advantageous for culturing SCNT embryos. The results regarding the post-implantation developmental competence of SCNT-derived embryos revealed that the two media had similar clinical pregnancy rates. However, the loss of pregnancy rate was lower in the BO medium compared to that of the SOF medium; consequently, the full-term pregnancy rate was higher in the BO medium, but the differences were not statistically significant and awaits further experimentation. Considering the improved blastocyst rate in BO medium, this may have important implications, like in preservation of endangered species (22) or production of adequate number of blastocysts for production of recombinant proteins in milk (23).

To assess the quality of blastocysts derived from these two media, the relative expression of sixteen genes in caprine embryos derived from the IVF and SCNT procedures cultured in the SOF and BO media were assessed

during different developmental pathways, including pluripotency, fibroblast growth factor (FGF), transforming growth factor beta (TGF β), cell cycle, proliferation, histone transferase, trophectoderm, and WNT. There were no significant differences between the two types of embryos (IVF vs. SCNT) in terms of the expression of genes involved in cell cycle, proliferation, and trophectoderm signaling. Moreover, in the IVF-derived embryos, among the genes assessed, three genes, i.e. *Akt*, *Fgfr4*, *Sox2*, which belong to the FGF and pluripotency signaling pathways were down-regulated while another group of three genes, including *Oct4*, *Bmpr1*, *Gcn5*, which belong to the pluripotency, TGF β , and histone transferase signaling pathways were upregulated in the SOF medium compared to the BO medium. On the other hand, in the SCNT-derived embryos, the relative expressions of 5 genes, i.e. *Fgfr4*, *Bmpr1*, *Gcn5*, *Ctnnb*, *Fzd*, out of the 16 genes were higher in the SOF medium compared to the BO medium.

According to the results of our previous work (32, 33), during the development of oocyte, zygote, and 8-16 cell embryos to day 7 blastocysts, of the 16 genes mentioned above, the relative expressions of 11 genes, including *Oct4*, *Sox2*, *Fgfr4*, *Erk1*, *Akt*, *Bmpr1*, *Smad5*, *Cdc25*, *Lifr*, *Ctnnb*, and *Fzd*, decreased as embryos reached the blastocyst stage and only the relative expression of one gene, i.e. *Cdx2*, increased by day 7. These authors also used the same SOF composition. Therefore, those genes with reduced expression can be employed as positive selection markers to optimize the composition of the medium. In this regard, it must be noted that among the several culture media used so far for SCNT and iSCNT embryo culture in domestic and wildlife species, the SOF medium has been the most commonly used medium across different species.

Despite some specific species differences, *Nanog*, *Sox2*, and *Oct4* play a key role in the feedback loops in mammals. Therefore, any decrease in one of these factors can result in the up-regulation of one or two of this triad of factors (34). In this study, *Oct4* showed the highest expression relative to the reference genes. Therefore, the high expression of *Oct4* in the IVF group in response to the decrease in the *Sox2* expression was consistent with what has been reported in the literature, although no such difference was observed in the SCNT-derived embryos.

The segregation of cell lineages in early embryogenesis supports the establishment of pregnancy and the development of the fetus (35). The FGF4 ligand during blastocyst formation may induce some responses in neighboring cellular compartments, and the emerging trophectoderm (TE) and inner cell mass (ICM) cells may establish a close relationship. The expression of some ligand receptors, including IL6/IL6R, FGF/FGFR, TGF β /TGFBR, and BMP/BMPR, may play important roles in the coordinated development of the TE in preparation for implantation (36). In mice, the FGF4 produced by the epiblast supported the expansion of the trophoblast stem cell niche. A similar cross-talk is also operating in the pig embryo,

where FGF4 has a trophic effect during TE segregation and elongation, resulting in the formation of a one-meter-long trophoblast within a few days. In day 3.5 embryo, when the cells respond to FGF4, the expression of which is controlled by Oct4/Sox2 in ICM cells, to initiate cell differentiation into parietal endoderm (PE). The mutation in the *FGF4* gene or its cognate receptors (FGFR1/2) or the chemical inhibition of FGF/MEK signaling can result in the inhibition of PE migration in the mouse embryo (35). In summary, FGF ligand-mediated activation of FGFRs may promote a switch in the transcriptional profile of ICM from Epiblast (EPI)- to hypoblast-associated gene expression (37). FGF4 appears to be the main mediator of this segregation in mouse embryos and the lack of it can result in the enrichment of NANOG. However, this effect in bovine embryos is not mediated through FGF, and in the goat embryos, it remains to be defined. In this study, the expression of FGFR4 in the IVF-derived embryos was higher in the BO medium compared to that of the SOF medium. The opposite of this trend was observed in the SCNT-derived embryos, that is, the expression of FGFR4 was higher in the SOF medium compared to that of the BO medium. This difference highlights the difference between the two media used, the difference between the two procedures applied, and the need for a specific medium for each procedure.

The embryonic development and regulation of cell proliferation by Wnts depend on endogenous WNTs, receptors, signaling molecules, and the regulation of canonical and non-canonical pathways to fine-tuning the balance between pluripotency, self-renewal, and cell-fate commitment (38). Similar to our previous observation (32, 33), the Wnt signaling in the IVF-SOF group was repressed in the goat blastocysts; this might reflect the poised state of developmental genes in the goat embryos compared to the bovine embryos. In this regard, the maintenance of pluripotency and the inhibition of blastomere differentiation caused a decrease in the rate of blastocyst formation (39). The results showed that in the SCNT-SOF group, the expressions of *CTNNB* and *FZD* increased and the Wnt signaling was upregulated. Based on the above findings, we hypothesized that the endogenous WNTs, receptors, and signaling molecules may be different between the IVF and SCNT embryos. Moreover, it can be stated that Wnt regulation can possibly contribute to SCNT embryonic development.

One of the shortcomings of this study is that transcriptomic analysis could have revealed more light on assessing the influence of pathways involved in developmental reprogramming. In addition to the aforementioned methods, another approach for comparison of embryo quality is the assessment of total, inner cell mass, and trophectoderm cell number between blastocyst between each group, which should be considered in future studies.

Conclusion

The only difference observed between the SOF and BO media was related to the embryo development-to-

blastocyst rate, which was significantly higher in the BO medium compared to that of the SOF medium. However, no significant difference was observed between the two media in terms of post-implantation developmental competence. Given that mRNA expression of assessed genes in SOF medium and SCNT embryos was higher than BO medium and IVF embryos, respectively and in addition to higher blastocyst rate in BO vs. SOF medium, these results suggest that BO might be a more suitable choice for *in vitro* culture (IVC) of caprine embryos. However, its effect on post implantation development awaits further experimentation. This finding is in agreement with the literature, which has shown that some of these genes are down-regulated during the blastocyst stage. Considering that the expression level of any gene has a certain range and too much or too low expression may have an adverse effect on , developmental competency, suggest that further measures, like epigenetic modification, should be considered to achieve a higher reprogramming efficiency in the SCNT procedure. Therefore, further interventions are needed at different levels to optimize the application of SCNT technology in IVC media.

Acknowledgements

The authors would like to express their gratitude to Royan Institute for financial support. The authors declare that they have no competing interests.

Authors' Contributions

M.H.N-E., M.H., F.J.; Participated in study design, data collection and evaluation, drafting and statistical analysis. Also participated in the finalization of the manuscript and approved the final draft. S.M.A., M.R.A., Sh.R.V.; Performed follicle collection and prepared oocytes for IVF and SCNT pertaining to this component of the study, transfer embryo to the recipient. M.H., Sh.R.V.; Conducted molecular experiments and RT-qPCR analysis. Drafted the manuscript, which was revised by M.H.N-E. and F.J. All authors read and approved the final manuscript.

References

1. Tribulo P, Rivera RM, Obando MSO, Jannaman EA, Hansen PJ. Production and culture of the bovine embryo. *Methods Mol Biol.* 2019; 2006: 115-129.
2. Petersen CG, Mauri AL, Vagnini LD, Renzi A, Petersen B, Matilla MC, et al. Randomized comparison of two commercial culture media (Cook and Vitrolife) for embryo culture after IMSI. *JBRA Assist Reprod.* 2019; 23(1): 33-36.
3. Takahashi Y, First NL. In vitro development of bovine one-cell embryos: influence of glucose, lactate, pyruvate, amino acids and vitamins. *Theriogenology.* 1992; 37(5): 963-978.
4. Ménézo Y, Lichtblau I, Elder K. New insights into human pre-implantation metabolism in vivo and in vitro. *J Assist Reprod Genet.* 2013; 30(3): 293-303.
5. Kleijkers SHM, van Montfoort APA, Bekers O, Coonen E, Derhaag JG, Evers JLH, et al. Ammonium accumulation in commercially available embryo culture media and protein supplements during storage at 2–8°C and during incubation at 37°C. *Hum Reprod.* 2016; 31(6): 1192-1199.
6. Simopoulou M, Sfakianoudis K, Rapani A, Giannelou P, Anifandis G, Bolaris S, et al. Considerations regarding embryo culture conditions: from media to epigenetics. *In Vivo.* 2018; 32(3): 451-460.

7. Mohd-Fazirul M, Nor-Ashikin MNK, Kamsani YS, Sharaniza Ab-Rahim, Norhazlin JMY, Wan-Hafizah WJ, et al. Comparison of the effects of three commercial media on preimplantation mouse embryo development and morphological grading. *Biomed Res*. 2015; 26(3): 477-484.
8. Morbeck DE, Baumann NA, Oglesbee D. Composition of single-step media used for human embryo culture. *Fertil Steril*. 2017; 107(4): 1055-1060. e1.
9. López-Pelayo I, Gutiérrez-Romero JM, Armada AIM, Calero-Ruiz MM, Acevedo-Yagüe PJM. Comparison of two commercial embryo culture media (SAGE-1 step single medium vs. G1-PLUS™/G2-PLUS™ sequential media): influence on in vitro fertilization outcomes and human embryo quality. *JBRA Assist Reprod*. 2018; 22(2): 128-133.
10. Hemkemeyer SA, Schwarzer C, Boiani M, Ehmcke J, Le Gac S, Schlatt S, et al. Effects of embryo culture media do not persist after implantation: a histological study in mice. *Hum Reprod*. 2014; 29(2): 220-233.
11. Soto-Heras S, Menéndez-Blanco I, Catalá MG, Izquierdo D, Thompson JG, Paramio MT. Biphasic in vitro maturation with C-type natriuretic peptide enhances the developmental competence of juvenile-goat oocytes. *PLoS One*. 2019; 14(8): e0221663.
12. Deng M, Liu Z, Chen B, Wan Y, Yang H, Zhang Y, et al. Aberrant DNA and histone methylation during zygotic genome activation in goat cloned embryos. *Theriogenology*. 2020; 148: 27-36.
13. Menéndez-Blanco I, Soto-Heras S, Catalá MG, Piras AR, Izquierdo D, Paramio MT. Effect of vitrification of in vitro matured prepubertal goat oocytes on embryo development after parthenogenic activation and intracytoplasmic sperm injection. *Cryobiology*. 2020; 93: 56-61.
14. Yao T, Asayama Y. Human preimplantation embryo culture media: past, present, and future. *J Mamm Ova Res*. 2016; 33(1): 17-34.
15. Sfontouris IA, Kolibianakis EM, Lainas GT, Petsas GK, Tarlatzis BC, Lainas TG. Blastocyst development in a single medium compared to sequential media: a prospective study with sibling oocytes. *Reprod Sci*. 2017; 24(9): 1312-1318.
16. Kij B, Kochan J, Nowak A, Nizański W, Prochowska S, Fryc K, et al. Using time lapse monitoring for determination of morphological defect frequency in feline embryos after in vitro fertilization (IVF). *Animals (Basel)*. 2020; 10(1): 3.
17. Salilew-Wondim D, Tesfaye D, Hoelker M, Schellander K. Embryo transcriptome response to environmental factors: implication for its survival under suboptimal conditions. *Animal Reprod Sci*. 2014; 149(1-2): 30-38.
18. Song H, Li H, Huang M, Xu D, wang Z, Wang F. Big animal cloning using transgenic induced pluripotent stem cells: a case study of goat transgenic induced pluripotent stem cells. *Cell Reprogram*. 2016; 18(1): 37-47.
19. Wan Y, Zhang Y, Zhou Z, Jia R, Meng L, Huang R, et al. Effect of potassium simplex optimization medium (KSOM) and embryo screening on the production of human lactoferrin transgenic cloned dairy goats. *Afr J Biotechnol*. 2013; 12(49): 6887-6893.
20. Hajian M, Jafarpour F, Aghamiri SM, Varnosfaderani SR, Esfahani MH. Effects of ovary storage temperature and embryo vitrification on somatic cell nuclear transfer outcomes in goats. *Reprod Fertil Dev*. 2020; 32(4): 419-424.
21. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997; 385(6619): 810-813.
22. Cordova A, King WA, Mastrodonato GF. Choosing a culture medium for SCNT and iSCNT reconstructed embryos: from domestic to wildlife species. *J Anim Sci Technol*. 2017; 59: 24.
23. Hosseini SM, Hajian M, Ostadhosseini S, Forouzanfar M, Abedi P, Jafarpour F, et al. Contrasting effects of G1. 2/G2. 2 and SOF1/SOF2 embryo culture media on pre- and post-implantation development of non-transgenic and transgenic cloned goat embryos. *Reprod Biomed Online*. 2015; 31(3): 372-383.
24. Hosseini SM, Moulavi F, Nasr-Esfahani MH. A novel method of somatic cell nuclear transfer with minimum equipment. *Methods Mol Biol*. 2015; 1330: 169-188.
25. Lan GC, Han D, Wu YG, Han ZB, Ma SF, Liu XY, et al. Effects of duration, concentration, and timing of ionomycin and 6-dimethylaminopurine (6-DMAP) treatment on activation of goat oocytes. *Mol Reprod Dev*. 2005; 71(3): 380-388.
26. McGraw S, Vigneault C, Sirard MA. Temporal expression of factors involved in chromatin remodeling and in gene regulation during early bovine in vitro embryo development. *Reproduction*. 2007; 133(3): 597-608.
27. Nielsen JMK, Wrenzycki C, Hyttel P, Poppicht F, Strøbech L. New IVF media affect blastocyst development and gene expression levels in in vitro produced bovine embryos. *Reprod Fertil Dev*. 2015; 27(1): 206-207.
28. Penitente-Filho JM. Improved in vitro production systems for bovine embryos. *CAB Reviews*. 2015; 10(048): 1-4.
29. Chiavarini M, Ostorero A, Naldini G, Fabiani R. Cancer risk in children and young adults (offspring) born after medically assisted reproduction: a systematic review and meta-analysis. *J Multidiscip Sci*. 2019; 2(4): 430-448.
30. Hargreave M, Jensen A, Hansen MK, Dehlendorf C, Winther JF, Schmiegelow K, et al. Association between fertility treatment and cancer risk in children. *JAMA*. 2019; 322(22): 2203-2210.
31. Holm P, Booth PJ, Schmidt MH, Greve T, Callesen H. High bovine blastocyst development in a static in vitro production system using SOFaa medium supplemented with sodium citrate and myo-inositol with or without serum-proteins. *Theriogenology*. 1999; 52(4): 683-700.
32. Hosseinia P, Hajian M, Tahmoorespur M, Hosseini SM, Ostadhosseini S, Nasiri MR, et al. Expression profile of developmentally important genes in preand peri-implantation goat embryos produced in vitro. *Int J Fertil Steril*. 2016; 10(3): 310-319.
33. Hosseinia P, Hajian M, Jafarpour F, Hosseini SM, Tahmoorespur M, Nasr-Esfahani MH. Dynamics of the expression of pluripotency and lineage specific genes in the pre and peri-implantation goat embryo. *Cell J*. 2019; 21(2): 194-203.
34. Naddafpour A, Zadegan FG, Hajian M, Hosseini SM, Jafarpour F, Rahimi M, et al. Effects of abundances of OCT-4 mRNA transcript on goat pre-implantation embryonic development. *Anim Reprod Sci*. 2020; 215: 106286.
35. Alberio R. Regulation of cell fate decisions in early mammalian embryos. *Annu Rev Anim Biosci*. 2020; 8: 377-393.
36. Hajian M, Hosseini SM, Ostadhosseini S, Nasr-Esfahani MH. Targeting the transforming growth factor- β signaling during pre-implantation development in embryos of cattle, sheep and goats. *Growth Factors*. 2016; 34(3-4): 141-148.
37. Moradi M, Riasi A, Ostadhosseini S, Hajian M, Hosseini M, Hosseinnia P, et al. Expression profile of FGF receptors in preimplantation ovine embryos and the effect of FGF2 and PD173074. *Growth Factors*. 2015; 33(5-6): 393-400.
38. Morris SA, Guo Y, Zernicka-Goetz M. Developmental plasticity is bound by pluripotency and the Fgf and Wnt signaling pathways. *Cell Rep*. 2012; 2(4): 756-765.
39. Denicol AC, Block J, Kelley DE, Pohler KG, Dobbs KB, Mortensen CJ, et al. The WNT signaling antagonist Dickkopf-1 directs lineage commitment and promotes survival of the preimplantation embryo. *FASEB J*. 2014; 28(9): 3975-3986.