Placenta 57 (2017) 113–122

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Isolation and characterization of the trophectoderm from the Arabian camel (*Camelus dromedarius*)

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ARTICLE INFO

Article history:
Received 18 April 2017
Received in revised form
15 June 2017
Accepted 15 June 2017

Keywords:
Trophectoderm
Stem cells
Embryo
Dromedary
Camel

ABSTRACT

We isolated and characterized trophoblast from *in vivo*-derived camel embryos and compared with embryonic stem-like cells. Camel embryos were flushed on day 8 post-insemination and used to derive trophectoderm and embryonic stem-like cells under feeder-free culture conditions using a basement membrane matrix. Embryos were evaluated for the expression of *POU5F1*, *MYC*, *KLF4*, *SOX2*, *CDX2*, and *KRT8* mRNA transcripts by relative quantitative polymerase chain reaction. Camel embryos grew and expanded to ~4.5 mm and maintained their vesicular shape *in vitro* for 21 days post-insemination. Trophoblast and embryonic stem-like cell lines grew under feeder-free culture conditions and showed distinct morphological criteria and normal chromosomal counts. Embryonic stem-like cells showed positive staining in the alkaline phosphatase reaction. Trophoblast cells showed a significant increase in *CDX2*, *KRT8*, *KLF4*, and *SOX2* expression compared with embryonic stem-like cells and whole embryos. Embryonic stem-like cells showed a significant decrease in *CDX2* expression and increase in *SOX2* and *KRT8* expression compared to embryonic expression. *POU5F1* and *MYC* expression showed no difference between embryos and both cell lines. We characterized embryo survival *in vitro*, particularly the derivation of trophectoderm and embryonic stem-like cells, providing a foundation for further analysis of early embryonic development and placentation in camels.

1. Introduction

Preimplantation embryo-derived stem cells hold great promise for biomedical research, genetic engineering, and as a model for studying early mammalian developmental biology [1–3]. The trophectoderm, the first cell to differentiate from the embryo, is an oligopotent stem cell that contributes to formation of the placenta and fetal membranes. The placenta is a species-diverse organ essential for conceptus development; it provides nourishment and signaling molecules important for maintaining an environment crucial to a successful pregnancy [4]. Epitheliochorial placentas are found in the strepsirrhine primates [5] and the Artiodactyla order, which includes cattle, swine, horses, camels, whales and dolphins [6,7]. Camels have noninvasive diffuse placenta in which no erosion of the uterine lining occurs and is similar to the swine placenta [8,9]. Camel placenta is characterized by a unique population of multinucleate giant cells that are apparent by day 35 of pregnancy [10]. However, the origin of these giant cells and their functions remain unclear [6]. While differentiation of the trophoblast is essential for implantation and placental formation, this process remains poorly understood in the camel because few studies have examined this critical stage of development.

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http://dx.doi.org/10.1016/j.placenta.2017.06.015
0143-4004/

Isolation of early embryo-derived cells (trophectoderm and embryonic stem cells) has been reported in species exhibiting epitheliochorial placenta, including equines [11–14], porcine [15–18], and ruminants [19–22]; however, studies involving isolation of embryo-derived stem cells from camels have not been reported because the lack of definitive information regarding cell lineages.

Xeno-free culture of embryonic cells has received a great deal of attention in both humans [23,24] and animals [25,26]. Feeder cells secrete variable nutrients or growth factors for stem cells, most of which have not been identified, limiting analysis of the mechanisms controlling cell behavior [27]. However, most cultured cells can differentiate when transferred into feeder-free culture because of the lack of growth factors secreted by the feeder cells [27]. Recently, we successfully cultured porcine trophoblast cells under feeder-free culture conditions while maintaining the trophoblast morphology and molecular markers for several passages [16].

The current study aimed to isolate and characterize camel embryo-derived cells, including trophectoderm and embryonic stem-like cells, under feeder-free culture conditions using a basement membrane matrix.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) unless otherwise stated. All equipment and media for embryo collection were purchased from (Minitube, Tiefenbach, Germany).

2.2. Camels and management

All experiments were performed following approval of King Saud University' Institutional Animal Care and Use Committee. This study was conducted during camel breeding season using three healthy, non-pregnant, and non-lactating multiparous dromedary camel females. Additionally, three fertile camel-bulls were used for natural mating. Animals were aged 7–8 years and had a mean body condition score of 2.5. Females were group-housed, while males were individually housed in yards at the experimental farm, Department of Animal Production, King Saud University, Riyadh (latitude 24° 48′ N and longitude 46° 31′ E), Saudi Arabia. The camels were fed hay and commercial pellets (14.5% CP; 2.78 Mcal ME kg-1DM) to meet their daily energy and protein requirements.

2.3. Ovarian monitoring and superovulation

Follicle development stage was assessed using a *trans*-rectal linear-array 5 MHz transducer (UST-5820-5C, SSD ProSound 2, ALOKA, Co. Tokyo, Japan). Follicle diameters were measured with electronic calipers. Ultrasound examinations were performed daily. Once minimum follicular activity was observed in both ovaries (no follicle > 0.2 cm), stimulation of the ovaries was started. A combination of equine chorionic gonadotropins (eCG; Synchropart® Ceva Sante, Animale, France) and porcine follicle-stimulating hormone (pFSH; Pluset, Calier, Barcelona, Spain; 1000 IU FSH and 1000 IU LH) was used. The eCG (3000 IU) was given as a single injection on day 1 of treatment together with the first of the twice daily injections of pFSH, followed by three more days of twice daily injections in decreasing doses of pFSH (day 1: 2 × 200 IU FSH & 2 × 200 IU FSH; day 2: 2 × 150 IU FSH & 2 × 150 IU FSH; day 3: 2 × 100 IU FSH & 2 × 100 IU FSH and day 4: 2 × 50 IU FSH & 2 × 50 IU FSH). The females were then screened and mated when most follicles had reached a mature size of 1.2–1.8 cm in diameter [28]. The females were mated again after 24 h. Although ovulation occurs in response to mating, the females received a single intravenous injection of GnRH analogue (20 mg Buserelin) at the time of the first mating to maximize the ovulation response [29].

2.4. Embryo collection and evaluation

Embryos were collected on the 8th day post-insemination (dpi) using a non-surgical method. Briefly, the females were placed in a restrained sitting position on the ground and sedated. The rectum, tail, and perineal region were thoroughly cleaned. A Foley catheter (20 gauge) was guided through the vagina; the cervix was then dilated manually and the catheter was inserted. Once the catheter was through the cervix, the cuff was inflated with 35 mL of saline and pulled back against the internal os cervix to seal it. The uterus was then flushed repeatedly with a total of 1000 mL of flushing medium. Flushing medium was filtered through an embryo filter until only 20 mL of the medium remained. Flushing medium was examined under a stereomicroscope for the presence of embryos. Each embryo was evaluated and graded according to developmental stage and morphological characteristics [30]. A total of 12 embryos were collected from three different females; four zona pellucida-included embryos were discarded and eight blastocysts were used for the study (Fig. 1A).

2.5. Embryo culture

Individual blastocysts (n = 5) were plated into 35-mm polystyrene-coated tissue culture dishes (Falcon, BD Biosciences, Franklin lakes, NJ, USA) and maintained for 3 successive days [31]. The culture medium was comprised of Dulbecco modified eagle medium supplemented with 10% fetal bovine serum, 0.1 mM, β-mercaptoethanol, 1% nonessential amino acids, 2 mM L-glutamine, 10 ng/mL epidermal growth factor, 10 ng/mL fibroblast growth factor (FGF-2, Miltenyi Biotec. GmbH, Bergisch Gladbach, Germany), 1% insulin-transferrin-selenium, and 1 mg/mL gentamycin. Culture was performed in a humid atmosphere of 5% CO_2 at 38.5 °C. All embryos attached on day 3 of culture and fresh medium was replaced every three days.

2.6. Isolation and culture of trophectoderm and embryonic stem-like cells

On day 12 of embryo culture, trophoblast outgrowths became clear and the vesicular morphology of the embryos was collapsed as shown in Fig. 1. Mechanical isolation of trophoblast and embryonic stem-like cells was performed as described by Strom et al. [32] with modifications. Under a stereomicroscope, a microblade was used to dislodge the ES-like cell dark clumps from the trophectoderm cell sheet and both were transferred to separate dishes and finely chopped. To maintain feeder-free conditions, each chopped cell layer (2–3 chops/well) was placed on the surface of the basement membrane matrix (known as Matrigel) that had been freshly coated onto culture dishes [16,31]. Briefly, previously cooled (−20 °C) 4-well culture plates (Nunclon Surface, Thermo Fisher Scientific, Waltham, MA, USA) were covered with 120 μL cooled Matrigel (BD Biosciences) prepared according to the manufacturer instructions. The culture medium was similar to that mentioned above and was changed every 2 days with fresh medium until ~1 mm colonies had formed. Trophoblastic vesicles and the

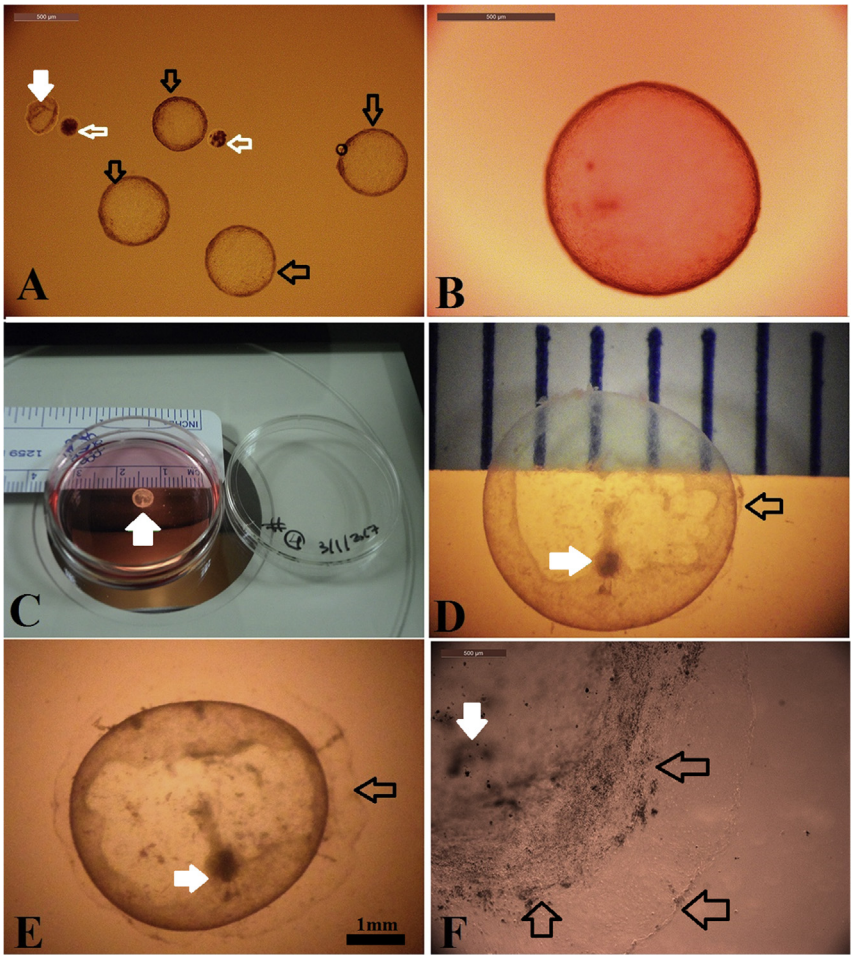

Fig. 1. ***In vitro*** **development of camel embryos**. A. Embryos were obtained by uterine flushing on the 8th day post insemination (dpi); the image shows four spherical embryos (black arrows), two zona included degenerated embryos (white arrows), and one collapsed embryo (white solid arrow) which regained its spherical shape after 24 h of culture. B. Camel embryo growth on the 11th dpi. C. Camel embryo (arrow) developed to 4.52 mm on the 19th dpi. D. Camel embryo developed on the 21st dpi and showing trophoblastic outgrowths in the periphery of the attached embryo (arrow). E. Continuation of trophoblastic outgrowths (arrow) along with embryo development on the 23rd dpi. F. On the 25th dpi, the vesicular shape of the embryos deflated with continuation of trophoblastic outgrowths. From this point, the resulting cultured embryonic cells were mechanically chopped with a sterile blade and trophectoderm cells (black arrows) were isolated from the proposed undifferentiated embryonic stem-like cells (white solid arrow) for subsequent experiments.

resulting trophoblast colonies were mechanically chopped and plated into fresh Matrigel-coated 4-well dishes. Embryonic stem-like cells were sub-cultured through trypsinization with 0.25% trypsin-EDTA, washed, and plated into fresh Matrigel-coated 4-well dishes. To form embryoid bodies (EB), the dissociated small clumps were transferred to non-coated 4-well petri dishes containing the previously mentioned culture medium for 3–4 days [33]. For both cell lines, the media were replaced with fresh media every 3 days. EBs were randomly distributed to Matrigel-coated dishes in medium with or without FGF2 to examine differentiation ability.

2.7. Alkaline phosphatase staining of cell lines

Cells were washed in phosphate-buffered saline (PBS) and fixed in acetone/citrate buffer 10 mM at pH 4.2 for 5 min at room temperature. Naphthol/fast red stain (0.2 mg/mL Naphthol AS-TR phosphate substrate/0.417 mg/mL of Fast Red) was added for 1 h at room temperature in a dark chamber. Cells were then washed and stained with Mayer's hematoxylin solution for 10 min and then microscopically examined [34].

2.8. Cytogenetic analysis with metaphase spread of cell lines

Cells were collected from the fifth passages of both trophoblast and ES-like cells. Cells were then treated with 0.15 μg/mL colcemid (Gibco, Grand Island, NY, USA) for 4 h and hypotonized with 0.075 M KCl at 37 °C for 10 min. Thereafter, the cells were fixed in ice-cold 3:1 methanol/glacial acetic acid for 10 min and dropped onto pre-cleaned chilled slides, air-dried, and stained with 5% Giemsa satin for 5 min. The slides were then washed with distilled water and air-dried; chromatids/chromosomes were examined under an oil immersion lens.

2.9. Relative quantitative polymerase chain reaction

Total RNA was extracted and eluted from whole embryos, trophoblast colonies, and ES-like cells (n = 3 each) using a Single Cell RNA Purification Kit (Norgen Biotek. Ontario, Canada)

following the manufacturer's instructions. RNA concentration and purity were estimated using a NanoDrop 2000 spectrophotometer (Thermo Fisher) by calculating the ratios of absorbance at 230, 260, and 280 nm; samples showing values of A260/A280 of ≥2.0 and A260/A230 > 2.0 were used for reverse transcription (RT). Pulsed RT was used to increase complementary DNA (cDNA) transcription efficiency [16] as follows; 50 cycles of 16 °C for 2 min, 42 °C for 1 min, and 50 °C for 1 s, followed by a final inactivation at 85 °C for 5 min. Individual RT reactions were performed using 100 ng of total RNA, random hexamer, and MultiScribe™ MuLV transcriptase (Thermo Fisher) in a 20-μL reaction volume according to the manufacturer instructions. Relative quantification of mRNA expression was determined by real-time PCR (ViiA™7, Applied Biosystems, Foster City, CA, USA). Reactions contained 100 ng of cDNA, 1 μM forward primer, 1 μM reverse primer, and 1× SYBR Green premix PCR kit (Applied Biosystems). Following normalization to the reference gene GAPDH, fold-change and relative quantification of *CDX2*, *KRT8*, *POU5F1*, *SOX2*, *KLF4*, and *MYC* transcripts was carried out using the $2^{-\Delta\Delta Ct}$ method [35]. In all assays, cDNA template-negative and reactions without RT resulted in negative amplification. Expression in whole embryo samples was set as arbitrary units to calculate the fold-change for trophoblast and ES-like cells. Thermal cycler conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 40 s. Primers sequences, annealing temperature, and approximate product size are listed in Supplemental Table 1. The melting curve for each primer was evaluated by ViiA™7 apparatus-associated software and PCR product sizes were confirmed by 1.5% agarose gel electrophoresis (Invitrogen, Carlsbad, CA, USA) with a 1 Kb DNA ladder (Invitrogen).

2.10. Statistical analysis

For relative quantification of mRNA transcripts, three biological and three technical replicates were used. Data were expressed as the mean ± SEM and compared by analysis of variance using the SAS program (Cary, NC, USA). Statistical significance was considered $P < 0.05$.

3. Results

3.1. Development of camel embryos in vitro

All embryos attached to the culture dish after 3 days. Embryos were maintained in culture and showed rapid growth until the 19th dpi, reached 4.56 ± 0.3 mm in 11 days, and had an average growth rate of 0.42 ± 0.85 mm/day (Fig. 1A–C). Trophoblast outgrowths appeared on the 20th dpi and became prominent on the 21st dpi (Fig. 1D and E). Along with growth of the trophectoderm layer, the vesicular shape of the embryos was deflated and the trophectoderm cell layer (characterized by cuboidal morphology with big nucleus) became easily differentiated from the inner cell mass-derived cell growth, as characterized by dark aggregation of cell masses (white solid arrows in Fig. 1D–F) on the 25th dpi. The cells were maintained until they reached a diameter of ~7 mm, after which mechanical isolation and culture of the trophectoderm in addition to embryonic stem-like cells were performed.

3.2. Culture of trophectoderm and trophoblastic vesicles

The trophectoderm cell layer was mechanically isolated and chopped into small pieces (~0.3 mm) with a sterile blade and then plated into Matrigel-coated dishes. Several trophoblastic vesicles were clearly observed on the 2nd day of culture, which had originated from the cultured trophectoderm segment (Fig. 2A, C). Trophoblastic vesicles attached and trophoblast outgrowths were observed on the third day of culture (Fig. 2B). Trophoblastic vesicles originating from the peripheral or middle cells of trophoblastic colonies were also observed (Fig. 2B, D, respectively). Formation of trophoblastic vesicles continued in subsequent mechanical cell passages (Fig. 3C). Trophoblast cells exhibited epithelium-like morphology, large and cuboidal, with a large rounded or oval

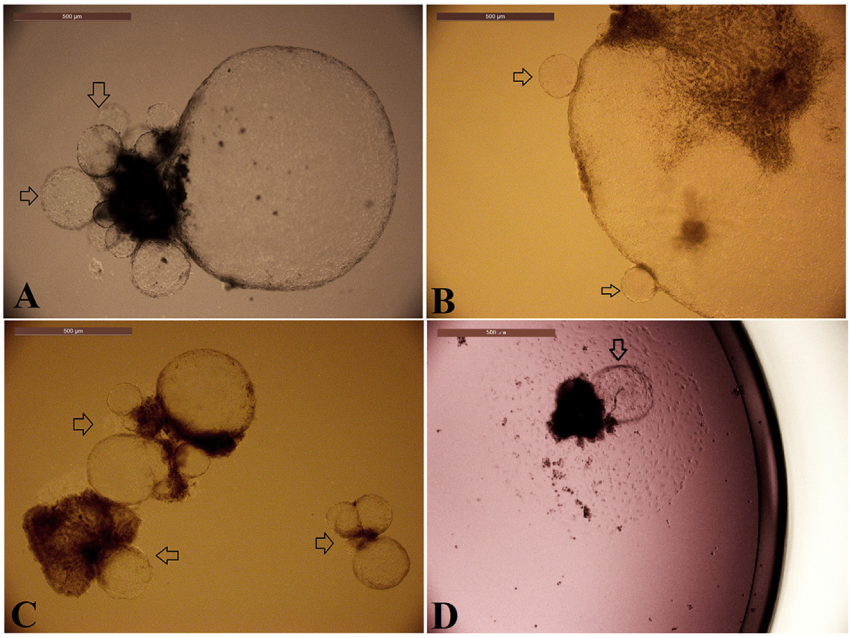

Fig. 2. Development of trophoblastic vesicles after mechanical subculture of camel embryos. A. Trophoblastic vesicles were obviously derived from the chopped pieces of primary ectoderm at 48 h after culture. B. Trophoblastic vesicles were observed on the periphery of cultured trophoblast cells (second passage). C. Trophoblastic vesicles were maintained in subsequent passages (fifth passage) and maintained even after colony formation (arrow, D). The diameter of the trophoblastic vesicles ranged from 100 to 1200 μm.

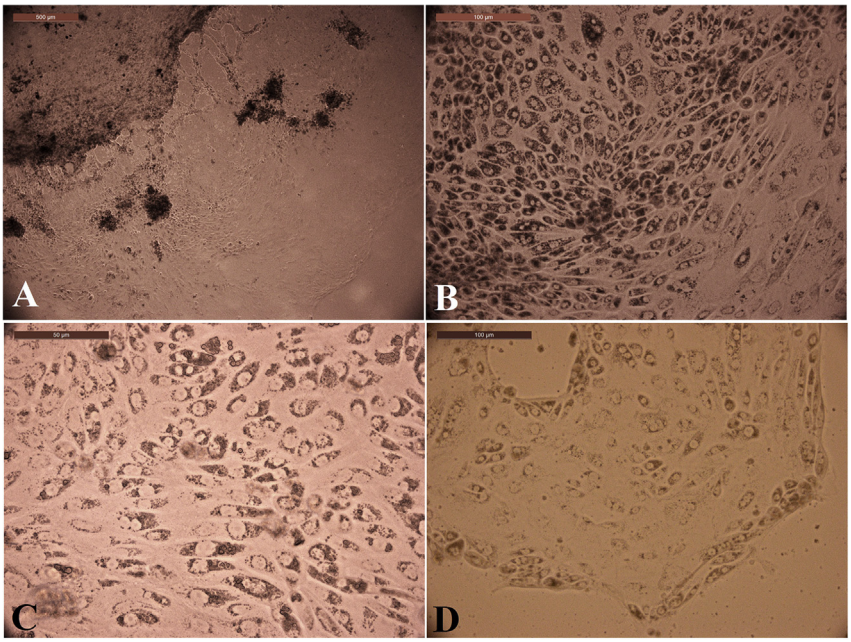

Fig. 3. Trophoblast cell line passaging. A. Trophoblast outgrowths from camel embryo on the 23rd dpi on polystyrene-coated tissue culture dish. B. Magnification of primary trophoblast outgrowth showing the size and cuboidal morphology of the trophoblast. C. Trophoblasts maintained morphology and size in the 2nd and 7th passages (D) after sub-culturing on Matrigel-coated dishes.

nucleus, dark granular cytoplasm, and well-defined boundaries (Fig. 3). This morphology was observed in the primary culture as well as first and subsequent passages (Fig. 3B–D, respectively).

3.3. Culture of embryonic stem-like cells and formation of embryoid bodies

ES-like cells were thin, elongated, had a spindle morphology, and formed 2-mm colonies within 7 days of culture (Fig. 5 A, B). Embryoid bodies, either compact (Fig. 5C) or cystic (Fig. 5E), were observed after 3 days (Fig. 5) of culture on non-coated dishes in culture medium without FGF-2. Embryoid bodies were then plated into Matrigel-coated dishes as described previously. Compact EB were attached and formed outgrowths similar to ES-like morphology (Fig. 5D), while cystic EB formed cells of different morphological features (large size, rounded, or neuron-like; Fig. 5F–I) than observed in the primary ES-like cell culture.

3.4. Alkaline phosphatase staining

Embryonic stem-like cells from the seventh passage showed positive staining of ALP characterized by the presence of red color in the cell cytoplasm (Fig. 6A); however, trophoblast cells of the same passage were negative for ALP staining (Fig. 6B).

3.5. Cytogenetic analysis

Trophoblast and ES-like cells showed normal chromosomal counts in the metaphase smear (n = 37 chromatid pairs, Fig. 6C and D, respectively) of the seventh passage.

3.6. Transcript expression in embryos and embryo-derived cells

Trophoblast cells showed significant increases in *CDX2*, *KRT8*, and *SOX2* expression compared with ES-like cells and whole embryos (Fig. 6E). Embryonic stem-like cells showed a significant decrease in *CDX2* expression and increase in *SOX2* and *KRT8* expression compared with embryonic expression. *POU5F1* and *MYC* expression showed no difference between embryos and both cell lines (Fig. 6E). Trophoblast cells showed a significant increase in *KLF4* expression compared with both embryonic and ES-like cells.

4. Discussion

This is the first report to isolate and characterize trophoblast and embryonic stem-like cells from *in vivo*-derived camel embryos. Both cell lines grew under feeder-free culture conditions on the basement membrane matrix Matrigel and showed a normal chromosomal count (n = 37 chromatid pairs).

The survival and expansion of camel embryos to this stage (~4.5 mm) is a unique finding and there have been no reports describing the growth and development of these embryos *in vitro*. In addition, embryo growth permitted clear discrimination between trophoblast and undifferentiated ES-like cells, making it easy to mechanically isolate the two different cells for subsequent culture rather than involving immuno-microsurgery using specific antisera for the inner cell mass and trophoblast cells.

Trophoblast cells were passaged and morphologically stable throughout the culture passages. We observed bi-nucleated and tri-nucleated trophoblast cells from the first passage and the subsequent passages. This finding may explain the development of giant cells that was observed previously [10,36,37]. Skidmore et al. [10] explained that multinucleate giant cells in camels are different from bi-nucleated cells in ruminants and horses. However, Gorokhovskii et al. [36] reported bi-nucleated cells in the Bactrian camel placenta. Whether these cells represent intermediates in the generation of multinucleate giant cells or constitute a unique subpopulation of trophoblasts requires further analysis.

Interestingly, the morphology of the resulting camel trophoblast exhibited a similar morphology to porcine trophoblasts either in

primary or subsequent passages observed in previous studies of others and our laboratory [15,16,38]. Camels and pigs are in the same category of the simple diffused epitheliochorial type of placentation. Moreover, the current results revealed the effectiveness of using Matrigel as a basement membrane matrix for feeder-free culture conditions in camel trophoblast culture, as we previously reported for pig [16].

One of the major differences among the two cell lines is the adherence between cells after mechanical dissociation; trophoblast cell lines were removed as a sheet and resisted trypsinization until 7 min similarly to cattle trophoblasts [19,39], whereas ES-like cells easily dissociated mechanically and after short-term exposure to trypsin (1–2 min). These results indicate the presence of tight junctions between trophoblast cells as previously reported in humans and cattle [19,40].

Camel ES-like cells maintained a spindle morphology and could develop into either compact or cystic EBs, which is a major criteria of pluripotent stem cells [41]. Solid EB behaved differently compared with cystic bodies; the latter differentiated into irregular cells when the culture medium was depleted of FGF-2. This result agrees with those of Kulinski et al. [42] who recently highlighted the differences between solid and cystic EB, particularly in gene expression, epigenetic hallmarks, and *in vitro* differentiation. Embryoid body systems are extremely valuable for investigating embryonic stem cells differentiation and embryonic development *in vitro* because it is challenging and time-consuming to isolate early-stage cells from developing embryos, as reviewed in Höpfl et al. [43]. In addition, ES-like cells showed positive ALP staining (Fig. 6A), which is a marker of pluripotency [44], while trophoblasts were negative for ALP staining, which is associated with differentiation [45].

The current results represent variable differences in transcript expression between trophoblast and ES-like cells (Fig. 6E). As expected, *CDX2* was significantly increased in trophoblast cells compared to in ES-like cells. *CDX2* is a crucial gene required for trophectoderm differentiation with variable interaction patterns with different transcription factors in early preimplantation embryos in different mammalian species, including mouse, pig, cattle, horse, and human [14,19,46–51]. For example, *CDX2* represses *OCT4* (*POU5F1*) expression for segregation of the inner cell mass and trophectoderm in mouse and pig [46,52], while *OCT4* expression was observed together with *CDX2* in the primary and early trophoblast lineage of cattle and human [19,50,51,53].

Interestingly, the results showed that *SOX2* expression was increased in trophoblast cells. This result is in accordance with those of Keramari et al. [54] who showed that *SOX2* facilitated establishment of the trophectoderm lineage in the preimplantation mouse embryo. Moreover, Roberts et al. [55] showed that *SOX2*, in a synergistic manner with *OCT4*, is crucial for trophoblast proliferation in mouse.

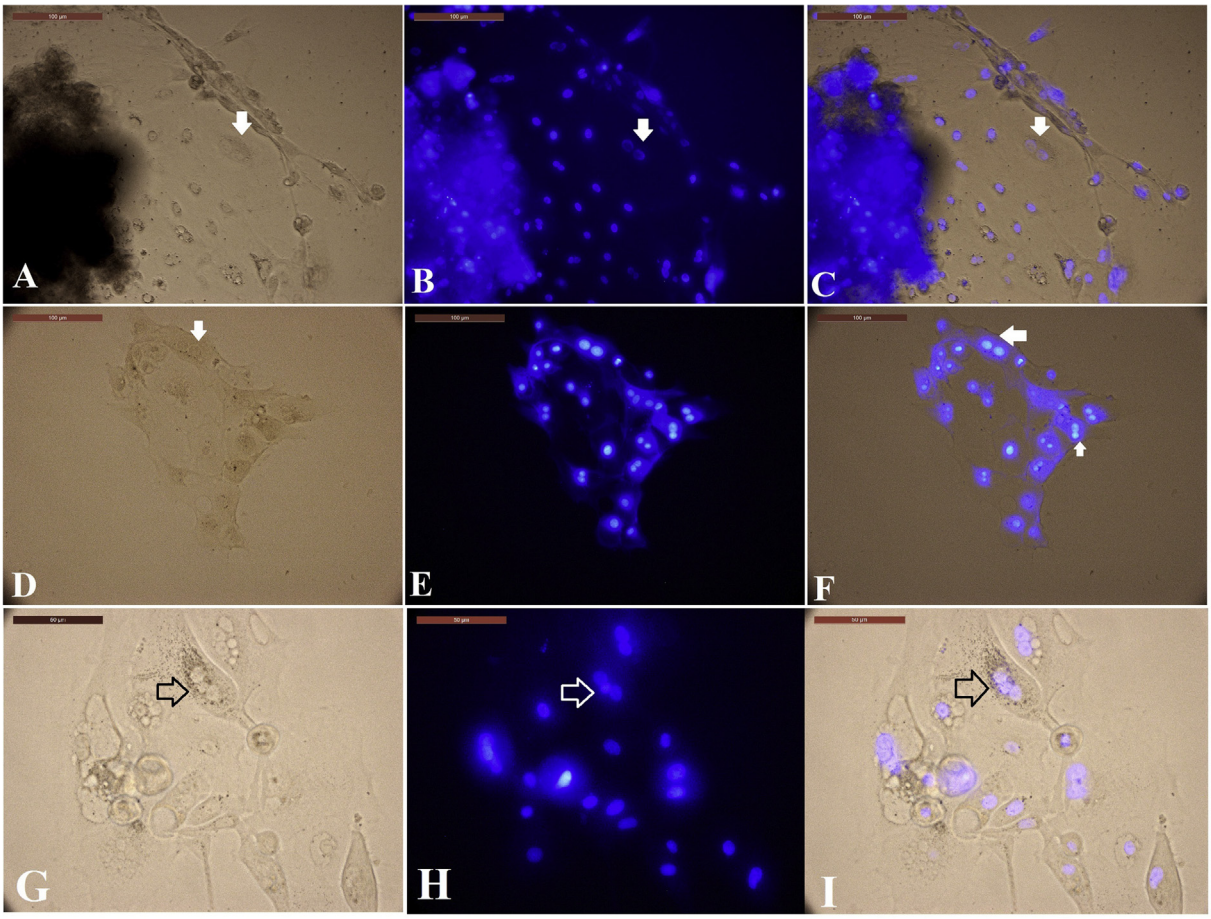

Fig. 4. Staining trophoblast cell lines with Hoechst stain. Trophoblast colonies were stained with Hoechst stain and showing; A. Presence of bi-nucleated cells (arrow) in the 1st passage of trophoblast cells. D. Bi-nucleated cells were maintained and relatively increased in numbers (arrows). G. Tri-nucleated cell (arrow) in the fifth passage of trophoblast culture. A, D, and G images were captured after exposure to visible light. B, E, and H images were captured after exposure to UV rays. C, F, and I are merged from visible light and Hoechst staining of the same line images.

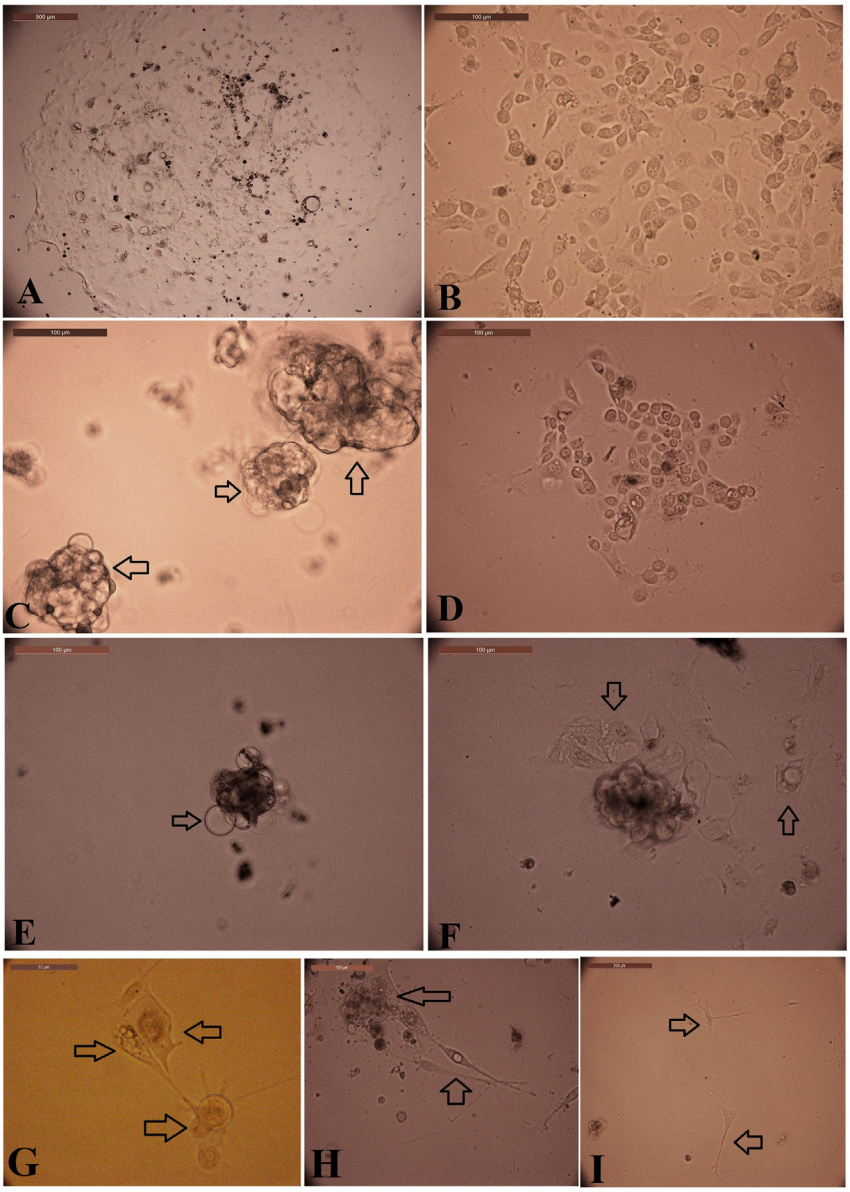

Fig. 5. Culturing embryonic stem-like cells from camel embryos. A. Isolated cells were cultured and formed colonies (first passage) and the morphology was maintained in the fifth passage (B). Cells aggregated and formed embryoid and cystic embryoid bodies (C and E). Embryoid bodies maintained the ES-like cell morphology when the medium was supplemented with FGF-2 (D), while the cells differentiated into different cell morphology after removing FGF-2 from the culture medium; large sized, rounded (F,G), long spindle-shaped, and neuron-like (H,I).

Notably, the current results revealed increased expression of *KRT8*, a widely used marker of trophoblasts [56], in trophoblast cells, which agrees with the findings of Telugu et al. [57] who observed expression of *KRT8* in the human early trophoblast lineage and with Saadeldin et al. [16] who observed its expression in porcine trophoblasts. In our study, *KRT8* showed increased expression in ES-like cells which is in accordance with Maurer et al. [58], who showed that *KRT8* is highly expressed in human ESCs compared to in mouse ESCs.

Surprisingly, *KLF4* showed increased expression in trophoblast cells, which agrees with the results of Li et al. [59] who showed that *KLF4* was highly expressed when human cytotrophoblasts differentiated into syncytiotrophoblasts. This result may explain the association between *KLF4* and the formation of bi-nucleated or tri-nucleated cells (Fig. 4) in the resulting trophoblast cells [60].

Remarkably, *POU5F1* and *MYC* showed similar patterns of expression in both trophoblasts and ES-like cells, suggesting that numerous factors potentiate the stemness of the resulting trophoblast cells [61]. Early work established that these two transcription factors work together to regulate genes required for the self-renewal and pluripotency of stem cells [62,63]. Collectively, the expression of *POU5F1* and *MYC* along with *SOX2*, *KLF4*, *CDX2*, and *KRT8* suggests that common factors promote self-renewal in both trophoblast and embryonic stem cells [64] and indicates unique features of camel trophoblast transcript expression.

In conclusion, we isolated and characterized camel trophoblast and ES-like cells derived from *in vivo*-fertilized camel embryos on feeder-free culture and maintained these cells for several passages.

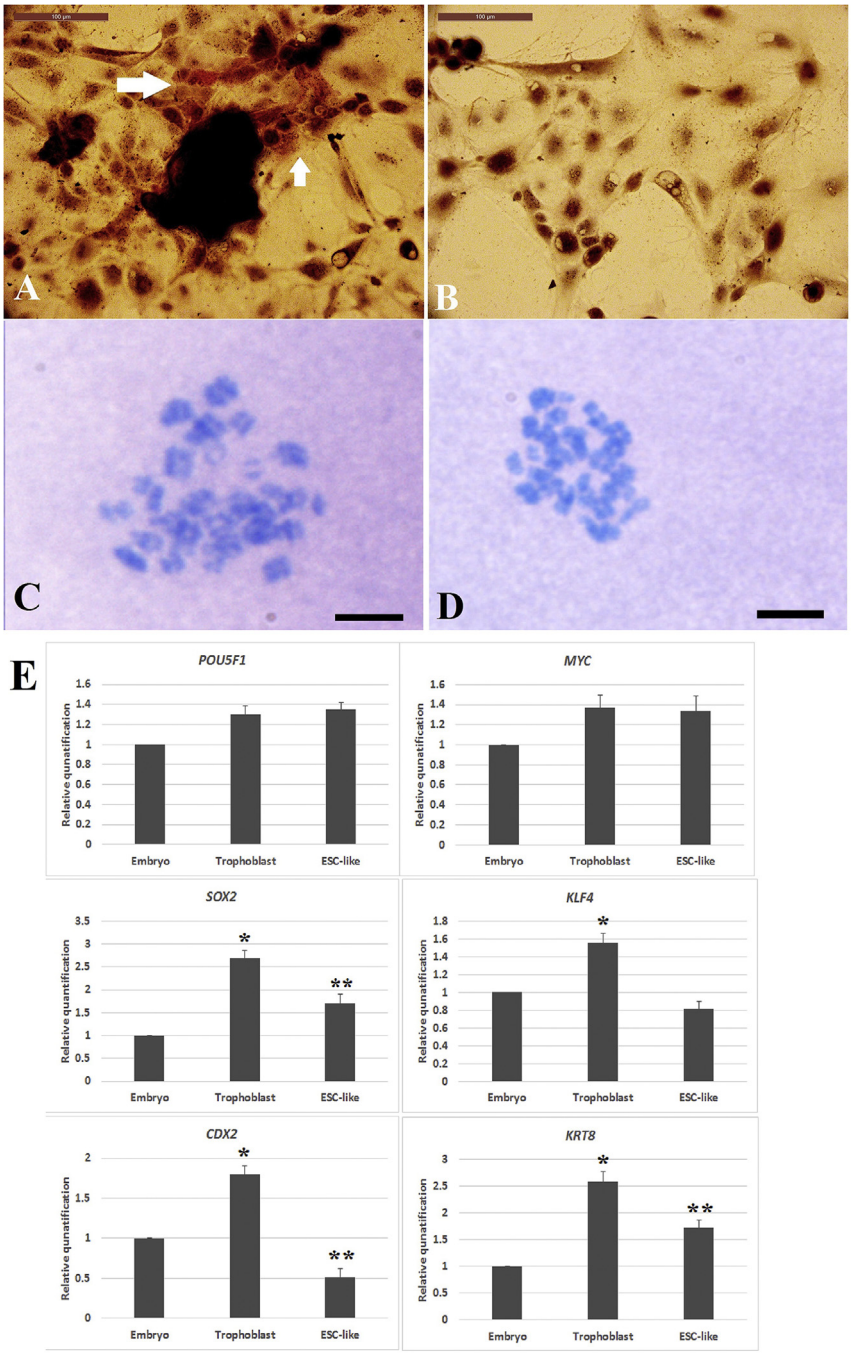

Fig. 6. Alkaline phosphatase, metaphase staining and relative quantification of *POU5F1*, *MYC*, *SOX2*, *KLF4*, *CDX2*, and *KRT8* mRNA transcripts in trophoblast and ES-like cell lines. A. Alkaline phosphatase-positive staining of ES-like cells showing red color-stained cells. B. Trophoblast cells showing negative staining for ALP because of differentiation. C and D representing metaphase spread of ES-like and trophoblast cells, respectively. Both cells showing 37 double-chromatids (n = 74) in the seventh passage, scale bar = 5 μm. E. The relative abundance of each mRNA transcript was normalized to *GAPDH* levels. The mRNA expression in the whole embryo at the 8th dpi was arbitrarily set as one-fold. Asterisks (*, **) indicate significant differences between columns at $P < 0.05$.

The current results provide a foundation for further analysis of early embryonic development and placentation in camels.

Grant support

The authors extended their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group No (RG-1438-018).

Acknowledgment

We would like to thank Sami Almalki, the graduate student at College of Food and Agricultural Sciences, King Saud University, for his technical assistance.

Appendix A. Supplementary data

Supplementary data related to this chapter can be found at

http://dx.doi.org/10.1016/j.placenta.2017.06.015.

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