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Comparative Expression Analysis of Spermatogonial Stem Cell Markers in Cattle and Sheep

Research Article

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ABSTRACT

Spermatogenesis is supported by stem cells called spermatogonial stem cells (SSCs), which are capable of transmitting information to the next generation. However, little is known on the specific markers of SSCs in farm animals. We investigated the expression of *cdh1*, *cmyc*, *bcl6b*, *plzf*, *gfra1*, *nanog*, *vasa*, *thy1* and *uchl1*, as specific markers of SSCs, in bovine and ovine SSCs. The expression of the studied genes was conducted by real-time polymerase chain reaction method. For this reason, the enzymatic digestion process for two times was used to achieve SSCs from male calves and ram lambs testes. Then, filtration and differential plating techniques increased the SSC number in the cell suspension caused by mechanical and enzymatic digestions. Sertoli cells treated with Mitomycin-C were applied to obtain the feeder layer. Culturing of the stem cells was done on Sertoli cell feeder layer. Our results revealed that the expression of *nanog* and *plzf* was similar in bovine and ovine SSCs. Unlike bovine SSC colonies, *cdh1* gene was not expressed in colony of ovine SSCs and its use as a specific marker of sheep SSCs is not suggested. The expression of *uchl*, *vasa*, *thy1* and *cdh1* genes was significantly higher in bovine SSCs and the *bcl6b* and *cmyc* expression was significantly higher in ovine SSCs compared to each other ($P<0.05$). From the results of this study *nanog*, *plzf*, *uchl1*, *vasa*, *thy1* and *cdh1 are* suggested as markers of bovine and *nanog*, *plzf*, *bcl6b* and *cmyc* genes as markers of ovine SSCs.

KEY WORDS cell specific Factor, molecular markers, pluripotency, surface markers, transcription factor.

INTRODUCTION

SSCs can be found on the basement membrane of the seminiferous tubules in testes and can differentiate and self-renew into spermatogenic cells at various developmental phases (Wu *et al*. 2022). SSCs are needed to be recognized and selectively purified before germ cell culture or transplant; however, they are not easy due to no available specific markers as well as the limited generation of them in the testis (Herrid *et al*. 2007). Moreover, due to limited information regarding surface markers in ovine SSCs, other techniques have been prevented, like fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) for SSC enrichment (Borjigin *et al*. 2010). SSCs self-renewal is controlled by many key players (Cai *et al*. 2020). For instance, promyelocytic leukemia zinc finger protein (PLZF) or ZBTB16 is a sheep undifferentiated spermatogonia (Zhang *et al*. 2014). PLZF as a transcription factor is necessary for the self-renewal and maintenance of SSCs and can be expressed by mouse undifferentiated spermatogonia. It is also expressed in a sub-population of gonocytes and SSCs/progenitors in cattle, pigs, goats, sheep, non-human primates and equids (Buaas *et al*. 2004; McMillan *et al*. 2014; Zheng *et al*. 2014a). It was used to

assess the number of the putative undifferentiated spermatogonia. However, the cells that express PLZF is not separable alive using immunologic methods because of the localization of the marker in the cell nucleus (Zhang *et al*. 2014). Molecular markers that are expressed exclusively in male germ line stem cells (MGSCs) are needed to be identified, isolated and purified. Different molecular markers are used to recognize and assess MGSCs. PLZF expression by MGSCs has been reported in pigs, rodents, bulls, sheep, humans and nonhuman primates (Zheng *et al*. 2014b).

To isolate and purify spermatogonia, the availability of markers establishing the identification of the spermatogonia is necessary. C-kit is one of the markers and a receptor for stem cell factor (SCF), which can be expressed by some A1–A4, Aal, In, and B spermatogonia. Its presence in SSCs has not been verified yet (Heidari *et al*. 2012). Transcription factor, *NANOG*, which is associated with the pluripotency of stem cells, is conserved however, it is a less reliable marker for undifferentiated spermatogonia related to domestic species, because of the dynamic alterations in the expression through germ cell growth (Goel *et al*. 2008). The Myc family of transcription factors are associated with the self-renewal division in several stem cell types. Myc can control the HSCs by differentiation and self-renewal balance that regulates the interplay between HSCs and their microenvironment (Kanatsu-Shinohara *et al*. 2016). The expression Nanog and C-myc showed a significant elevation in ES-like cells compared to spermatogonia cells (Nazm Bojnordi *et al*. 2013). Although Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1) or PGP9.5 can detect type A spermatogonia in ovine testis (Rodriguez-Sosa *et al*. 2006; Herrid *et al*. 2009), no report is available regarding ovine SSC markers (Borjigin *et al*. 2010). The UCHL1 expression identifies type A spermatogonia and gonocytes in the adult and neonatal bovine testis (Fujihara *et al*. 2011). ATP-dependent RNA helicase VASA or DDX4 has been shown as a preserved molecular marker for the germ cell lineage in different species, with expression in undifferentiated spermatogonia and primordial germ cells (McMillan *et al*. 2014). We do not know other germ cell proteins, like VASA can be expressed in sexual maturation in the bovine testis, although VASA expression is observed in the adult testis and fetal gonads (Fujihara *et al*. 2011). CDH1 (E-cadherin; 120 kDa) as a transmembrane glycoprotein is expressed in epithelial tissues. It has important roles in tissue architecture and embryogenesis through the formation of intercellular junction complexes and establishment of cell polarization. It is a cell-surface molecular marker for undifferentiated spermatogonia in rat and mouse testis (Zhang *et al*. 2014). THY1 (CD90 (cluster of differentiation 90)) is a preserved marker for SSCs in non-human primates, rodents, and pre-pubertal bovine (Abbasi *et al*. 2013). It belongs to the Ig super family as a glycosyl phosphatidyl inositol-anchored glycoprotein, and initially found in haemopoietic stem cells, and is the MGSC surface marker in rodents, non-human primates and bulls. Bulls showed THY1 expression limited to a small group of spermatogonia in pre-pubertal testes. Also, 64.4% of the cells in the $THY1^{+}$ fraction were supposed as undifferentiated spermatogonia. THY1 was for the first time used as a surface marker for undifferentiated spermatogonia in nonhuman primates, humans, and rodents. Reding *et al*. (2010) showed the THY1 expression in undifferentiated spermatogonia and gonocytes in cattle. Wu *et al*. (2013) and Abbasi *et al*. (2013) showed the THY1 expression in undifferentiated spermatogonia of goat testes, indicating THY1 as a conserved surface marker of goat SSCs. Glial cell line–derived neurotrophic (GDNF) and PLZF are critical extrinsic growth factors stimulating self-renewal in SSCs of rodents. GDNF can signal by the GDNF family receptor alpha 1 (GFRa1) that is found in the spermatogonia of adult human testes. It is a surface marker for early spermatogonia in pig neonates gonocytes, SSCs and testes (Cai *et al*. 2020). Bcl6b is important for SSC maintenance *in vitro* and for SSC self-renewal and validating the biological relevance of the GDNF-regulated genes recognized by microarray analysis. Bcl6b as a standard practice characterizes SSCs prior to next cell culture, manipulation or transplantation (Oatley *et al*. 2006).

There are limited identified spermatogonia phenotypic markers in domestic animals. However, a few markers are continually expressed in spermatogonia of domesticated species (Zheng *et al*. 2014a). We investigated the expression of molecular markers in bovine and ovine SSCs. Using primers *cdh1*, *cmyc*, *bcl6b*, *plzf*, *gfra1*, *nanog*, *vasa*, *thy1* and *uchl1*, the gene expression was performed through Real time-PCR method in the samples.

MATERIALS AND METHODS

Chemicals and antibodies

Unless otherwise specified, the chemical agents were purchased from Sigma (USA).

Samples preparation

Testes of a healthy male calves and ram lambs were collected quickly following slaughter and transferred to lab within 2-3 hours. In brief, the tunica albuginea was removed for the first enzymatic digestion. 6-10 g testis tissue was rinsed in deionized water 3 times and normal physiological saline 10 times then sprayed to 70% ethanol. When the scrotum and tunica albuginae were removed, phosphate buffer saline was used to wash testes, followed by cutting the testes to small pieces.

Using a two-step enzymatic isolation procedure, individual testicular cells were obtained according to Izadyar *et al.* (2002) with some changes. A segment of 2 g tissue was transferred into a 15-mL Corning™ falcon 50 mL conical centrifuge tubes. Dissection of the testis tissue free of the connective tissue and rete testis was done and placed in a Petri dish containing 5 mL DMEM with 50 µg/mL gentamycin was filter by 22/5 µm filter. The tissue was washed 4 times with 5 mL DMEM with 50 µg/mL gentamycin. The pellet was transferred into a 50-mL tube.

Enzymatic isolation and culture of SSCs

First step enzymatic digestion, was carried out with 1 mg/mL hyaluronidase type II,1 mg/mL trypsin (Inoclon), 5 µg/mL DNase and 1 mg/mL collagenase. Incubation was done in a shaker incubator (200 cycles/min) (37 ˚C for 45 min). The collected dispersed tissue was collected by centrifugation (1000 rpm for 2 min). The supernatant was removed from the plate. Considering the second enzymatic digestion, the pellet suspension was prepared in DMEM, including 1 mg/mL collagenase, 1 mg/mL hyaluronidase type II, and 5 µg/mL DNase. To achieve favorite cell population, cellular suspension underwent centrifugation (1000 rpm for 2 min). The digestion was discontinued by adding 3 mL DMEM of 10% fetal bovine serum (FBS).

Enrichment of SSCs

Filtration of the supernatant was done through 80-µm and then 60-µm nylon net filters for SSC enrichment. The SSCs was seeded onto lectin- bovine serum albumin (BSA) coated 60 mm culture dishes according to Jafarnejad *et al.* (2018). To prepare the lectin-BSA coated dishes 5 µg/mL lectin from *Datura stramonium* agglutinin was dissolved in DPBS and washing them by addition of 0.6% BSA in DPBS at room temperature for 2 h. After that, the dishes were rinsed using BSA and kept at room temperature for 2 hours. They were subjected to coating BSA at room temperature for another 2 h. The cultures or cells on the lectin-coated dishes underwent incubation in a CO_2 incubator (5-6 h at 37 ˚C). Using the incubation process, most of the contaminated cells can bind to lectin-BSA. The suspension was transferred to 15 mL tube, and it seemed to contain SSCs. It was predicted that the medium subsequently centrifuged at 1000 rpm for 5 min, contains SSCs and the pellet resuspension in DMEM.

Preparing the feeder layers

The Sertoli cell feeder layer was prepared as described in Jafarnejad *et al.* (2018) study with some modifications. In summary, the rest of attached cells in the dishes coated with lectin-BSA from enrichment of SSCs step were cultured at 37 ˚C in a humidified room using 5% CO_2 for two to three days. Incubation was done for growing Sertoli cells, then they were treated with trypsin-EDTA (0/25%) and used for sub-culturing a cell culture flask (50 mL) for propagation. Then, the Sertoli cells received mitomycin-C (10 g/mL) for 3 hours for inactivation. Finally, were rinsed five times in DPBS and DMEM supplemented with 10% FBS to remove any remaining mitomycin C, and were used as feeder cells for SSC culture.

Culture and characterization of SSCs

Culturing of the isolated SSCs was done on the Sertoli cells feeder layer coated culture flasks containing DMEM medium treated with 10 µg/mL GDNF, 10% FBS, penicillin (100 IU/mL) and streptomycin (50 mg/mL) and then incubation was done in an incubator using 5% CO_2 in air at 37 ˚C. Replacement of the culture medium was done every third day. SSC colonies were found following ten days and alkaline phosphatase (AP) staining was performed to characterize them. For this, washing of SSC colonies was done twice using DPBS followed by staining by an AP kit (Sigma, Catalogue No. 86 C) as instructed.

RNA isolation and reverse transcription

Trizol reagent (Invitrogen, USA) was applied to extract total RNA as instructed. Its quantity and quality was assessed by NanoDrop spectrophotometer (Dynamica Scientific Ltd., United Kingdom) by measuring the absorbance at 260 nm, and then treatment with DNAse (Ambion Inc., Houston, Texas, USA) to remove probable contaminating genomic DNA. For each specimen, total RNA (0.5 mg) was applied for the synthesis of first-strand complementary DNA (cDNA). MMLV enzyme and oligo dT primers (Takara, Japan) was used for reverse transcription. The cDNAs were used to measure RNA abundance using quantitative real-time PCR.

Real-time PCR

The PCR reaction was fixed in a final volume of 10 µL, consisting of 0.8 µL of each primer (0.2 µM) (forward and reverse), 10 µL with 1.4 µL nuclease-free water, 5 µL Syber Green, and 2 µL template. To activate the polymerase, the thermal cycling conditions including initial denaturation (94 ˚C/15 minutes), 40 amplification cycles of denaturation (95 ˚C/10 second) and annealing specific primers based on Table 1 for 15 sec and 72 ˚C for extension for 20sec. The reactions ended by a final extension (72 ˚C/5 minutes). Also, β-actin gene was applied as the internal control to investigate the expression levels of the gene mRNA and was calculated by the (ΔΔCT) method. The used primers in are mentioned in Table 1.

Table 1 The used primers sequences (R, reverse primer; F, forward primer)

Gene	Primer Sequence (5´–3´)	Annealing temperature	Size (bp)	Accession *no.*
β-actin	F: ACCCAGCACGATGAAGATCA R: GTAACGCAGCTAACAGTCCG	62	187	U39357.1
cdh1	F: ATGTATCGGATTTGGAGGGA R: TCAAGTGTAGTCACCTGGT	58.5	483	XM_004015093.3
uchl1	F: AAGGCCAATGTCGGGTAGAT R: ATTCTCTGCAGACCTTGGCA	58.5	165	NM_001046172.2 XM_004009789.2
thy1	F: CGTCTCCAATAAGGATGTC R: GTCACAAGGAGATGAAGTC	58.5	146	NM_001034765.1
vasa	F: TCTTGGAGATTTCCGCTG R: GGCTGTGCTAACTGGCTA	58.5	219	>JX437186.1
nanog	F: CCGAAGCATCCAACTCT R: GAGACAGTGTCCGTGTCGAG	52.5	100	NM_001025344.1 XM_015094959.1
cmyc	F: AGAATGACAAGAGGCGGACA R: CAACTGTTCTCGCCTCTTC	57	227	NM_001009426.1 NM_001046074.2
gfra1	F: CCACCAGCATGTCCAATGAC R: GAGCATCCCATAGCTGTGCTT	57	101	NM_001105411.1 XM_004020339.3
plzf	F: CCTCAGATGACAATGACACG R: CGCCTTGGTGGGACTCA	57	224	
bcl6b	F: AGAGCAGGCTTTCTCCAACT R: AGCTGACACTTGTAGGGCTT	58.5	239	NM_001191366.1 XM_004012623.3

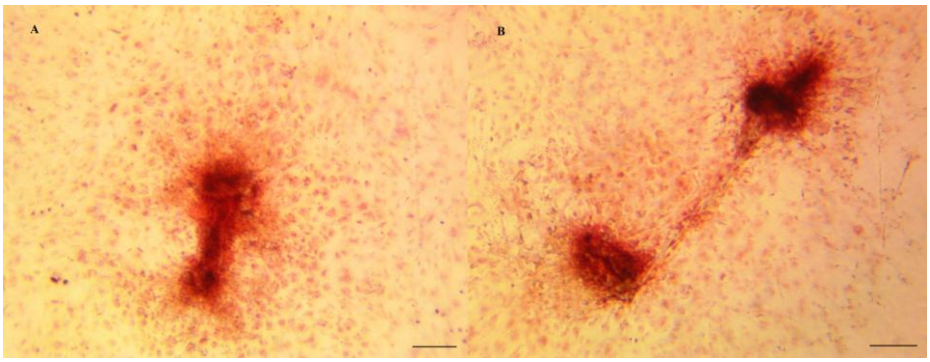

Figure 1 Alkaline phosphatase staining was used to identify SSCs. Alkaline phosphatase expressed in both of bovine (A) and ovine SSCs (B) 10 days after culture (Bar=0.5 mm)

Fold change of gene expression was calculated as a ratio of expression levels of treated groups to the expression level of the control group (Livak and Schmittgen, 2001).

Statistical analysis

The experimental procedures were repeated at least three times. Data analysis was done using t-test to compare two means (SPSS, 2011). The results have arrived as the Mean ± Standard Error of Mean (SEM) and statistical significance was fixed at ($P<0.05$).

RESULTS AND DISCUSSION

The bovine and ovine SSCs suspension was achieved by two times enzymatic digestion. SSCs formed colonies seven days following culture and attachments among colonies could be observed at 10^{th} day. They were positive for alkaline phosphatase staining Figure 1. Real-time PCR was done in the removed SSCs to assess the expression of a subset of pluripotency markers, and also germ cell-specific genes. Markers used for SSC characterization in this study included: *cdh1*, *cmyc*, *bcl6b*, *plzf*, *gfra1*, *nanog*, *vasa*, *thy1* and *uchl1*. *β-actin* is considered as housekeeping gene. Results demonstrated that the expression of *nanog* and *plzf* was similar in bovine and ovine SSCs Figures 2-3. The expression of *uchl1*, *vasa*, *thy1* and *cdh1* genes was significantly higher in bovine SSCs in comparison with ovine SSCs Figures 4-7. The expression of *vasa* in ovine SSCs was very low, and *thy1* and *cdh1* was close to zero. The expression of *bcl6b*, *cmyc* and *gfra1* genes was higher in sheep than in cattle, so this difference was significant in *bcl6b* and *cmyc* genes ($P<0.05$) Figures 8-10.

Use of several methods and markers for recognition and enrichment of SSCs is important because of the low number of SSCs in testes (Abbasi *et al*. 2013).

In comparison to other species, such as rodents, there are not many known spermatogonia phenotypic markers in domestic animals. However, some markers can be consistently expressed in spermatogonia from domestic species (Zheng *et al*. 2014a). In this study expression of *cdh1*, *cmyc*, *bcl6b*, *plzf*, *gfra1*, *nanog*, *vasa*, *thy1* and *uchl1* genes were studied in bovine and ovine SSCs.

From the genes under study the expression of pluripotency gene *nanog* and germ cell-specific factor plzf was similar in bovine and ovine SSCs. Bovine gonocytes could express NANOG in the neonatal testis until the migration of gonocytes to the basal membrane (Fujihara *et al*. 2011). The similar NANOG expression patterns in various species such as pig (Goel *et al*. 2008), marmoset (Mitchell *et al*. 2008), and human (Hoei Hansen *et al*. 2005) indicate the highly conserved NANOG role in spermatogenesis in mammals (Fujihara *et al*. 2011). According to Borjigin *et al*. (2010) PLZF-positive cells in ovine testis are a Type A spermatogonia subpopulation representing the undifferentiated spermatogonia, such as SSCs that is self-renewed. Also, plzf identified Type A spermatogonia in the bovine testis and are expressed by a bovine spermatogonia subpopulation, and possibly limited to SSCs and very early spermatogonia (McMillan *et al*. 2014).

PLZF as a sequence-specific DNA-binding protein is able to repress the kit transcription, a spermatogonial differentiation hallmark leading to maintaining the source of SSCs in mice. For the first time Bahadorani *et al*. (2011) reported expressing PLZF in undifferentiated spermatogonia in sheep and goats, evidenced by positive reaction with the anti-PLZF polyclonal antibody. Thus, because positive PLZF-stained cells were merely found at the base of seminiferous tubules, PLZF is capable of expressing in undifferentiated spermatogonia.

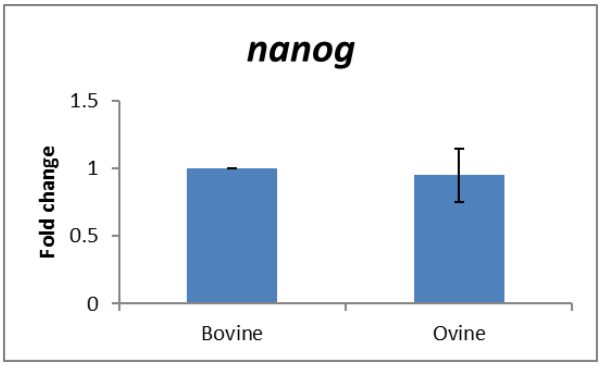

Figure 2 Comparison of *nanog* gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

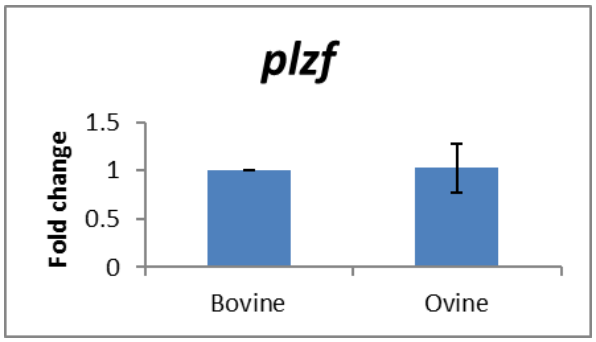

Figure 3 Comparison of *plzf* gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

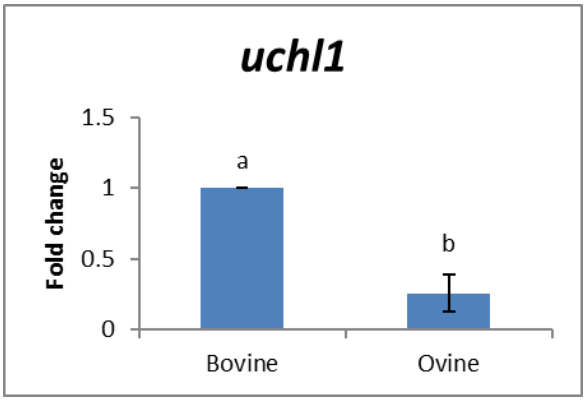

Figure 4 Comparison of *uchl1*gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

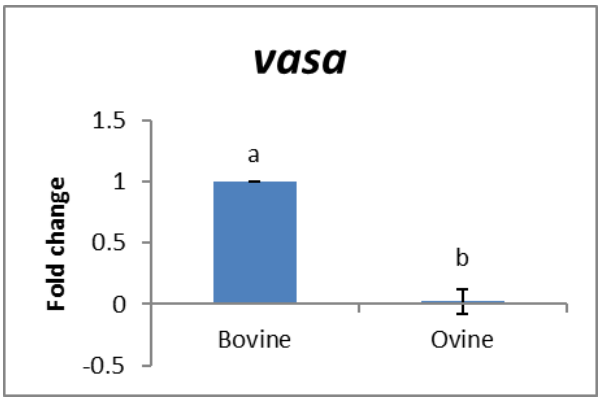

Figure 5 Comparison of *vasa* gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

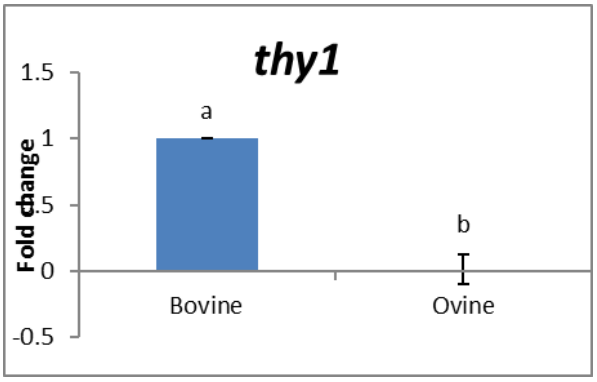

Figure 6 Comparison of *thy1*gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

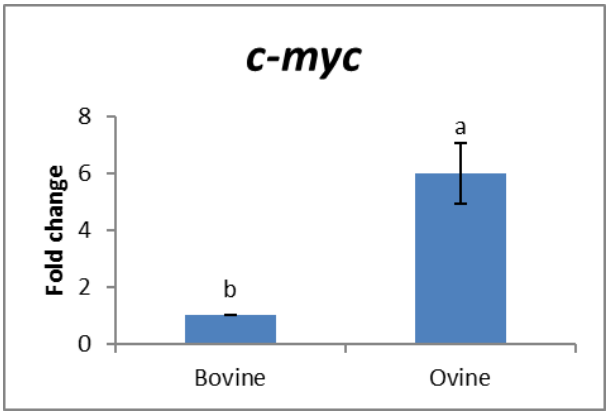

Figure 9 Comparison of *c-myc* gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

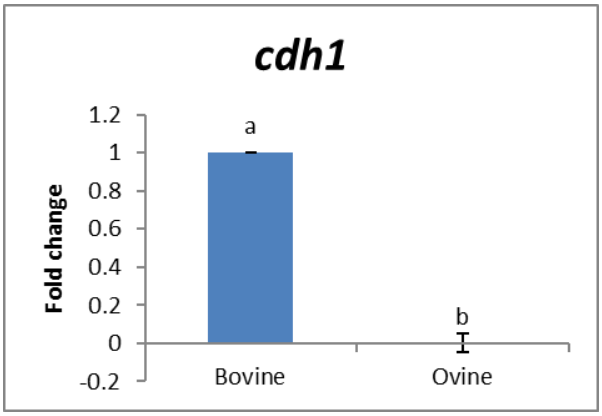

Figure 7 Comparison of *cdh1*gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

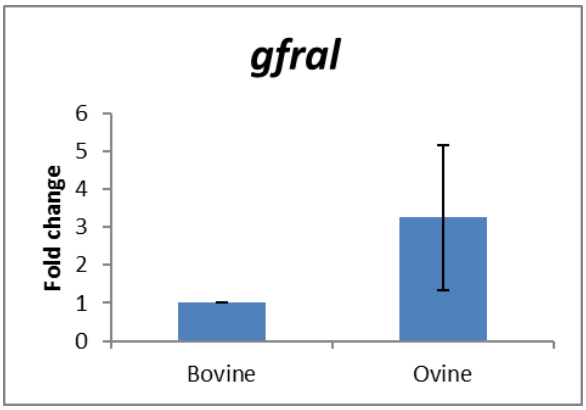

Figure 10 Comparison of *gfral* gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

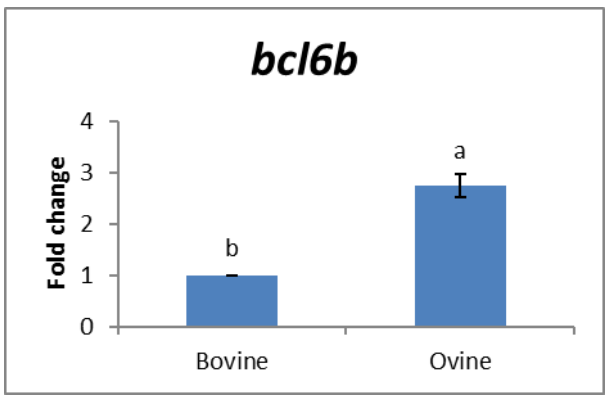

Figure 8 Comparison of *bcl6b* gene expression levels between bovine and ovine SSCs. Relative fold change determined by quantitative real-time PCR analysis. The data were normalized with β-actin expression and given as relative to the control (bovine SSCs)

Our results showed that *cdh1* gene only expressed in bovine SSCs and *uchl1*, *vasa* and *thy1* genes were more expressed in bovine SSCs in compare to ovine SSCs. Inconsistent results are available on the CDH1 expression and the localization of CDH1-positive cells in testes of rodents. CDH1 was not observed in the testis, whereas many recent papers have declared opposite results. Tokuda *et al*. (2007) reported CDH1 expression in some germ cells in the testes of mice. There were limited number of CDH1-positive cells in the adult mouse testis, indicating the reason why CDH1 staining has been missed in some studies. In contrast to our results in ovine SSCs, Zhang *et al*. (2014) found that CDH1 was expressed in undifferentiated spermatogonia of sheep by immune histochemistry examination of frozen section of seminiferous tubules of sheep testis. Yu *et al*. (2014) reported CDH1 expression by some spermatogonia, and

CDH1 localization was limited to the single and paired spermatogonia basement membrane. Zheng *et al*. (2014a) and others reported the UCHL1 expression in pre-pubertal testes of buffalo, pigs, cattle, sheep and, goats (Abbasi *et al*. 2013; Yu *et al*. 2014).

UCHL1 expression was observed in pre-meiotic male germ cells and showed an affinity for somatic cells, making it a good marker for spermatogonia in domestic testes (Zheng *et al*. 2014a). VASA expression was observed in bovine gonocytes in the neonatal testes and its expression continued in spermatogonia until migration to the basement membrane (Fujihara *et al*. 2011). VASA expression was detected in the adult bovine testis, in differentiated germ cells, like round spermatids and spermatocytes, but not in spermatogonia. The same VASA expression patterns were reported in mouse (Toyooka *et al*. 2000), human (Castrillon *et al*. 2000), and pig (Lee *et al*. 2005) adult testes.

Accordingly, besides mice, cattle, and rats, VASA antibody is applicable to recognize germ cells in goats and sheep (Bahadorani *et al*. 2011). In agreement with results of Bahadorani *et al*. (2011), the VASA protein belonging to the ATP-dependent RNA helicase of the DEAD-box family protein, has a conserved structure; thus, the PCR was applied to verify this point across many species from *Caenorhabditis elegans* to humans (Zeeman *et al*. 2002). In contrast to PLZF and VASA as respectively cytoplasmic and nuclear markers, THY1 has been reported as a surface marker. THY1 is a GPI (N-glycosylated, glycophosphatidyl inositol; 25-37 kDa) anchored protein that was initially detected as a thymocyte antigen and was found in some stem cell sources, such as SSCs (Rege and Hagood, 2006). THY1 expression as a conserved surface marker of undifferentiated spermatogonia is a common phenotype of SSCs in many mammals and showed conservation in other livestock (Reding *et al*. 2010).

Our results indicated that the *bcl6b*, *cmyc* and *gfra1*genes were more expressed in ovine SSCs. BCL6B expression was upregulated by GDNF and suppressed in the AKT presence (Protein Kinase B) inhibitor (Yu *et al*. 2014). Oatley *et al*. (2006) declared that BCL6B has a significant role in SSCs maintenance *in vitro*, but it is a crucial transcription factor for SSCs proliferation self-renewal, and survival. *c-myc* gene as a pluripotency marker, code for important transcription factors in self-renewal division and the cell cycling of many stem cell types (Bojnordi *et al*. 2017). Yamanaka's transcription factors, including c-Myc (OSKM factors), Oct3/4, Klf4 and Sox2 are associated with the somatic differentiated cell reprogramming into induced pluripotent stem cells (iPSCs) (Aoi *et al*. 2008). The KLF4 and c-Myc showed higher expression levels in SSCs than ES cells (Corbineau *et al*. 2017). GDNF maintained goat SSC self-renewal and GDNF could up-regulate c-Myc expression by the PI3K/Akt pathway to increase SSC proliferation in goats (Niu *et al*. 2016). Gfra1 is a surface marker for undifferentiated spermatogonia in the testes of mice (Buageaw *et al*. 2005), and can be expressed in a subpopulation of gonocytes in porcine testes of neonates (Lee *et al*. 2013).

CONCLUSION

Our results revealed that the expression of *nanog* and *plzf* was similar in bovine and ovine SSCs. Unlike bovine SSC colonies, *cdh1* gene was not expressed in colony of ovine SSCs and its use as a specific marker of sheep SSCs is not suggested. The reason for the lack of expression of *cdh1* gene in ovine SSCs can be the difference in the expression of this gene in single and paired cells in comparison with the colony stage. The results of the present study showed that *uchl1*, *vasa*, *thy1,* and *cdh1*genes and *bcl6b* and *cmyc* genes were more expressed in bovine and ovine SSCs, respectively.

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