*Available online at* www.scholarsresearchlibrary.com

Scholars Research Library

**Der Pharmacia Lettre, 2016, 8 (14):140-144**
***(http://scholarsresearchlibrary.com/archive.html)***

Der Pharmacia Lettre
Scholars Research Library
**ISSN 0975-5071**
**USA CODEN: DPLEB4**

# Study of IGF-1 and OCT4 genes expression in the early stages of embryonic development by Multiplex RT-PCR

**Elham Asadi Farsani[1] and Hoda Ayat[2]**

[1] *MSc in Genetics, Shahrekord University, Sharekord, Iran*
[2]*Department of Genetics, University of Shahrekord, Shahrekord, Iran*

---

**ABSTRACT**

*Study of gene expression in multiple cell embryo* is *the most important step in developmental genetic. A large number of regulatory genes and transcription factors have been identified that are involved in early stages of embryo development. Experience has shown that impaired function and expression of these genes causes major abnormalities in embryos obtained from IVF. Because of importance IGF1 and OCT4 genes in early stage of embryo development of preimplantation . We tried to study possible differences in expression of two these genes during early embryonic stages in bovine. The study of gene expression in the preimplantation embryo of mammals is difficult because the standard procedures used to quantify mRNA generally require large amounts of starting material. Improvement of of protocols using different quantitative strategies generally involving the polymerase chain reaction (PCR) has provided new tools for exploration of gene expression in preimplantation embryos. Thus, in the first step, conditions for gene expression study was optimized. RNA was extracted from bovine embryo samples that were produced by IVF, synthesis of cDNA by utilizing of reverse primers OCT4 and Actin was done and multiplex RT-PCR was carried out. Finally, we tried for a comparative analyses of expression of OCT4 , IGF1 genes, in steps 8, 16 and 32 cells. In this study, RT-PCR on the bovine embryo was done for the first time in Iran. Results showed a distinct difference in expression of OCT4 gene in 8 cell stage compared with 32 cell stage. The expression of OCT4 gene was significantly low at the 16 cell stage. Also, our results suggest that IGF1 gene expression in 32 cell stage little more than this for 8 cell stage. It is noteworthy that in the 16 cell stage, there is no detectable expression for IGF1 and OCT4. Also media conditions in IVF embryo production can affect in gene expression.*

**Key words**: Developmental genetic, Gene expression, Multiplex RT-PCR, OCT4, IGF1.

---

## INTRODUCTION

Evaluation of gene expression in multicellular embryos is the most important step in the developmental genetics [1]. Therefore, determination of differences between mRNA levels provides a good tool to help viable oocyte and complete evolution. Many methods have been used to study gene expression that includes Real time RT-PCR, immunohistochemistry, microarray and several other methods [2]. RT-PCR method is a sensitive method for semi-quantitative comparison of gene expression between fetal provides in various stages of cleavage between ovulation and implantation [3].

Oct4 gene, Critical factor is for controlling the development of mammalian embryos. POU transcription factors are involved in transcriptional regulation during early embryonic development and cell differentiation [4]. Oct-4, a member of this family, has been shown to be under strict regulation during murine development. Interestingly, all members of the class to which Oct-4 belongs are expressed during early embryonic development [4, 5]. The Oct-4 has been found only in mammalian species. Subsequent analysis established that Oct-4 is expressed throughout the preimplantation period. Oct-4 mRNA and protein are present in unfertilized oocytes, as is typical of most mRNAs, Oct-4 mRNA levels drop dramatically after fertilization, although Oct-4 protein is detectable in the nuclei of two-cell embryos. Zygotic Oct-4 expression is activated prior to the eight-cell stage. Expression of Oct-4 mRNA and protein is abundant and uniform in all cells of the embryo through the morula stage. However, as the outer cells of the morula differentiate into trophectoderm, Oct-4 is downregulated and becomes restricted to cells of the ICM in the blastocyst [6]. The Oct-*4* gene has been found only in mammalian species. The amino acid sequence of human Oct-4 is 87% identical to that of the mouse (4). Oct-4 is the earliest expressed transcription factor that is known to be crucial in murine preimplantation development [7, 8]. Oct-4 expression is downregulated during formation of the blastocyst [9]. Oct-4 acts as a transcription factor for many genes specifically expressed in pluripotent cells [10, 11]. Insulin-like growth factor 1 (IGF1) is an important endocrine signal for regulation of early embryonic development. In the cow, for example, IGF1 can increase the proportion of preimplantation embryos becoming blastocysts and alter blastocyst gene expression [12, 13, 14]. Circulating IGF1 is synthesized and secreted primarily by the liver although it is also expressed in several reproductive tissues including, in the cow, ovary, oviduct, uterus and embryo (15,16). IGF-1 is a small peptide of 70 amino acids with a molecular mass of 7649 Dalton's (12) IGF-1 is one of the two ligands of the IGF system, which also includes two receptors, six high-affinity IGF binding proteins (IGFBPs) and IGFBP proteases [15].

IGF-1 regulates cellular proliferation, differentiation, and survival. Mitogenic and primarily antiapoptotic effects induced by IGF-1 have been reported in preimplantation embryos of several mammalian species [13, 15]. The bovine embryonic genome is transcriptionally inactive during the first cell divisions until the embryo reaches the 8- to 16-cell stage, when the major resumption of transcription occurs [17, 18] The study of gene expression profiles in embryo development_in terms of understanding and etiology of difficulty which in IVF embryos development process faces, will help [19, 20]. Therefore, we decided_to study the possible differences in the expression of two genes OCT4, IGF1 in bovine embryos at different stages of development.

## MATERIALS AND METHODS

In this study bovine embryos produced by IVF in Center of Shahrekord University of cattle embryos, were used. Total RNA was extracted from the cells with TRIZOL following the protocol suggested by the manufacturer and treated with DNase I to remove contaminating DNA. 10 numbers embryo culture medium and placed in small vials and was added to it 10 microliters sterile water. Then freez-twu in liquid nitrogen twice. 50 µl TE buffer, PH = 7.5 was added to embryos. And then 30 ml lysis buffer was added. After mixing with pipette several times, was incubated for 5 min at room temperature. 30 µl chloroform was added to the above composition. The up and down gently for 15 to 30 seconds was mixed and 10 to 15 minutes at room temperature the mixture was incubated. Samples for 15 min at 4 ° C in 12000g and was centrifuged. Upper phase was carefully separated , 70 µl isopropyl alcohol was added. It was incubated for 10 min at room temperature. These vials at 4 ° C for 8 min in 12000g was centrifuged. After removal of the supernatant, 100 µl 70% alcohol added to the bottom sediment and at 4 ° C for 5 min in 12000g was centrifuged. After removal of supernatant was removed gently residual alcohol.

Vials with 12 µl of injection water were washed well. The resulting RNA at -70 ° C was maintained was stored.

**cDNA synthesis**

0.5 µl reverse primer OCT4 and 0.5 µl reverse primer Actin was added to 10 µl of RNA extracted from embryos and was placed in cDNA synthesis program at 70 ° C for 5 minutes, then immediately placed on ice.4 µl 5x reaction buffer, 1µl Rnasin, 2µl dNTP 10 mM added to it and was placed in per again. after 5 minutes adding it 2 ml M-Mulv RTase. One hour at 37° Cand 10 minutes at 70 ° C in order to disable the enzyme was placed. The resulting cDNA in -20 ° C is maintained.

**Multiplex RT-PCR**

PCR reaction was performed in 40 cycles. Denaturation at 94 ° C for 30 seconds, Hybridization at 56° C for 35 seconds, Extension at 72° C for 30 seconds. Reverse transcription--PCR (RT-PCR) products were separated on 8% polyacrylamide gels and visualized with silver salts.

**Table 1. primers used in this study.**

| | |
|---|---|
| **OCT4 F** | **5-GGAGCCGGGGTCGAGAGCAAC-3** |
| **OCT4 R** | **5-TCGGCCTGGGTATATCCTAGTG-3** |
| **IGF1 F** | **5-CTATCTGGCCCTGTGCTTGCTC-3** |
| **IGF1 R** | **5-GCAGTACATCTCCAGCCTCCTC-3** |
| **ACT F** | **5- AGACCTTCAACACCCCAGCC -3** |
| **ACT R** | **5-TGGGCACAGTGTGGGTGACC-3** |

## RESULTS

In order to optimize the reaction conditions, first, PCR was performed with cDNA equivalents different tissues and cells for example ovary tissue, Mesenchymal cells, bovine liver.(figure1)

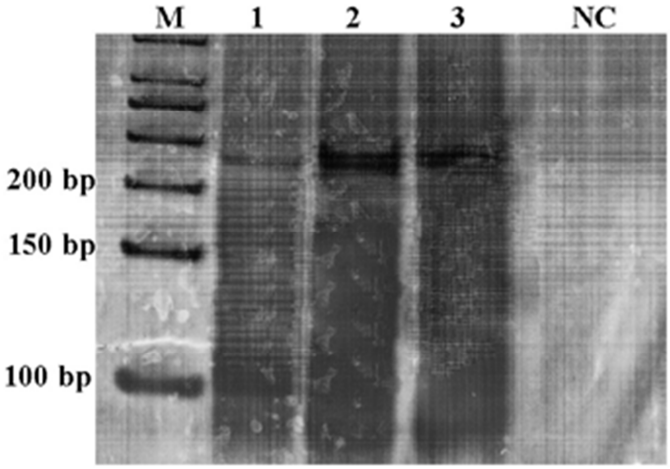


**Figure 1. RT-PCR on RNA extracted from bovine liver for IGF1 gene expression (229bp) NC: negative control. The results of three times repeat tests**.

The results of the Multiple RT- PCR reaction on embryos produced by IVF is shown in Figure 2. The findings showed that expression of IGF1 gene in the cell 32(fig. 2). The results also indicate oct4 expression in 8 and 32 cells. (Fig. 3)

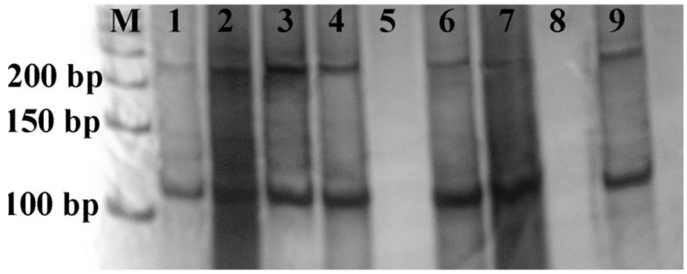


**FIGURE 2. Multiplex RT-PCR on RNA extracted from bovine embryos to study IGF1 gene expression. α-Actin gene (114bp) is used as an internal control. Embryos were used in the 32 cells for nine times reaction (Acrylamide gel 8%)**

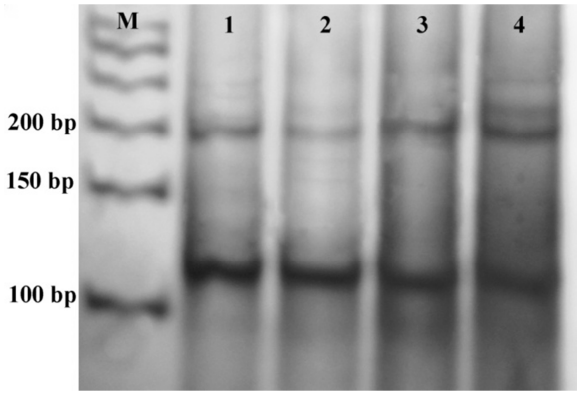


**FIGURE 3. Multiplex RT-PCR On RNA extracted from bovine embryos to study OCT4 gene expression. . *α*-Actin gene (114bp) is used as an internal control.(Acrylamide gel 8%.) Wells 1 and 2; 8-cell stage embryos.Wells 3 and 4; 32-cell stage embryos.**

## DISCUSSION

One of the important applications of methods based on molecular biology is genetic diagnosis preimplantation. Attention to the gene expression profile in the early stages of fetal development, will be realized the role of genes in important developmental events; including growth and differentiation. In mammals, the study of gene expression in the preimplantation embryo has been difficult because the standard procedures used to quantify mRNA generally require large amounts of starting material. The development of protocols using different quantitative strategies generally involving the polymerase chain reaction (PCR) has provided new tools for exploration of gene expression in preimplantation embryos [17]. Quantitative measurements of mRNA levels can be achieved using several approaches; however, some of these approaches are not suitable when working with mammalian oocytes or preimplantation embryos because of the difficulty in gathering the large amount of starting material needed to obtain enough RNA to perform these techniques [17]. In order to study comparative OCT4, IGF1 gene expression in various stages of development of preimplantation should be used standard control and internal control. Therefore, Actin as internal control to quantify RT-PCR reaction was used. 30 times in the experimental stages of development of before implantation embryo produced to IVF Showed that OCT4 gene expression in 8-cell stage is considerably. In 16-cell stage, it decreased the expression of cell and in 32-cell stage increased again. IGF1 gene expression was detectable in stages 8 and 32 cell. While the expression of the IGF1 gene was not detected in 16-cell stage. Previous studies showed that in vitro embryo production effect on different gene expression so It seems that the IVF procedure is performed in order to produce bovine embryos in vitro on the expression of two genes OCT4, IGF1 in various stages of development (1-2).Laboratory findings of this study confirmed the above issue. As was shown in previous studies, Changes in culture conditions and methods of optimizing the production of bovine embryos in vitro Similar circumstances, natural environment for the fetus can be provided [19, 20]. embryo viability in more advanced stages of development will produce. IGF1 gene.

## REFERENCES

[1] S J Kimber, S F Sneddon, D J Bloor, A M El-Bareg, J A Hawkhead, A D Metcalfe, F D Houghton, H J Leese, A Rutherford, B A Lieberman and D R Brison. *Reproduction*; **2008**; 1470–1626.
[2] Koichi Ushizawa, Chandana B Herath, Kanako Kaneyama,Satoshi Shiojima, Akira Hirasawa, Toru Takahashi, Kei Imai,Kazuhiko Ochiai, Tomoyuki Tokunaga, Yukio Tsunoda,Gozoh Tsujimoto and Kazuyoshi Hashizume. *Reproductive Biology and Endocrinology* **2004**, 2:77.
[3] Jalali, S. A. H. Nikbakht, G, Mahboobi Soofiani, N.Jalali, S. M. A. *Iranian Journal of Fisheries Sciences;* **2011**; 10(4) 749-752.
[4] Guangming Wuand Hans R Schöler. *Cell Regeneration*; **2014**, 3:7.
[5] Lei Ying, Jason A. Mills, Deborah L. French, and Paul Gadue. *Stem Cell Reports*; **2015**; 490–498.
[6] Kay Kotkamp, Rebecca Mossner, Anita Allen, Daria Onichtchouk, Wolfgang Driever. *Developmental Biology* 385(**2014**)433–447.

[7]Abdulrahim A.Sajini,LucasV.Greder,JamesR.Dutton,JonathanM.W.Slack.*Developmental Biology* 371(2012)170–179.
[8] Wu G, Gentile L, Fuchikami T, Sutter J, Psathaki K, Esteves TC, Arauzo-Bravo MJ, Ortmeier C, Verberk G, Abe K, Scholer HR: *Development* **2010**, 137:4159–4169.
[9] Peter James Hansen, Anna Carolina Denicol, Kyle Bradley Dobbs. *Turk J Vet Anim Sci* (**2014**) 38: 589-598.
[10] Guang Jin Pan, Zeng Yi Chang, Hans R. Scher, Duanqing Pet. *Cell Research* (**2002**); 12(5-6):321-329.
[11] Osorno R, Tsakiridis A, Wong F, Cambray N, Economou C, Wilkie R, Blin G, Scotting PJ, Chambers I, Wilson V: *Development* **2012**, 139:2288–2298 **.**
[12] Hiromi Ochiai, Shoko Okada, Akiko Saito, Kazuto Hoshi, Haruto Yamashita, Tsuyoshi Takato. *The Journal of Biological Chemistry*. , June 29, **2012** ; Vol. 287, No. 27, pp. 22654–22661.
[13] Anastassios Philippou, Maria Maridaki, Spiros Pneumaticos, and Michael Koutsilieris. *MOL MED*. 20 : 202-214 , **2014**.
[14] Georgios D. Agrogiannas, Stavros Sifakis, Efstratios S. Patsouris and Anastasia E. Konstantinidou **.** *Molecular Medicine Reports* 10: 579-584, **2014 .**
[15] Yaseen MA, Wrenzycki C, Herrmann D, Carnwath JW & Niemann H . **2001**, *Reproduction* 122 601–610.
[16] Kowalik A. , H-C Liu, Z-Y He. , C. Mele, L. Barmat and Z. Rosenwaks. *Molecular Human Reproduction*, **1999**,861-865 **.**
[17] Claude Robert, Serge McGraw, Lyne Massicotte, Marco Pravetoni, Fulvio Gandolfi, and Marc-Andre Sirard **.** *Biology Of Reproduction* ,**2002**, 1465–1472.
[18] Memili E, Dominko T, First NL. *Mol Reprod Dev* **1998**; 51:36– 41
[19] Rizos D, Lonergan P, Ward F, Duffy P, Boland MP. *Mol Reprod Dev* **2002**;61:234-248.
[20] Hakan S., Muge M., Abdullah K., Neal L F, John J P,Erdogan M. *Society for Reproduction and Fertility*;**2006**;895-904