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Expression Study of Pluripotency Marker Genes in Gold Fish, *Carassius auratrus*

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A B S T R A C T

Aquarium fishes, like medaka, zebrafish and goldfish offer an excellent model to conduct studies on molecular mechanisms of embryonic developmental pathways, patterns of genes involved in pluripotency and cell fate decision. To determine the possible candidate gene as an ideal pluripotency marker in goldfish, the comparative study on quantitative expression of a set of genes were conducted in the mid-blastula derived inner cell mass (ICM) and adult somatic cells. Present work explored the expression pattern of four standard pluripotency genes i.e. Nanog, Oct4, Sox and Pou2 in blastocytes (ICM) as well as somatic tissues of kidney, liver, heart, brain, muscle and mature gonads of goldfish using RT-qPCR. Absolute copy number of mRNA transcripts was calculated for each gene in every tissue. Significantly high (P<0.05) mRNA transcript number of all the genes was recorded in ICM, compared to other somatic tissues. Maternally inherited genes like Nanog, Pou2 and Oct4 were found to be highly expressive in ICM as well as in oocytes. The expression profile showed that Nanog andOct4 could belong to similar group where expression is limited to ICM and gonads whereas Pou2expressing in almost all the tissues, including ICM and adult gonads. Because of the low expression level in ICM than in the ovary, Sox2 exhibited expression in several somatic tissues apart from the gonad. Therefore these genes could be characterized as stem cell marker but not a pure pluripotency marker. The results showed that although all the genes compared in this study can be considered as stem cell markers but Nanog gene could be employed as a pure pluripotency marker for ES cell lines from goldfish.

Introduction

Pluripotent stem cells can be derived from developing embryos and adult tissues. However, embryonic stem (ES) cells from early developing embryos are an excellent model for analyzing vertebrate development *in vitro*, and provide a versatile resource for induced differentiation into many desired cell types in large quantity for regenerative medicine. Establishment of embryonic stem cell lines and their characterization through

establishment and validation of molecular markers are necessary for utilizing them in aqua-disease diagnostics and therapeutic study further. Self-renewal and differentiation ability of ES-like cells is the result of gene expression program that allows them to self-renew yet remain poised to pluripotent state.

In order to carry out research on stem cells, it is important to identify these genes which act as a key transcriptional regulators within stem cells and to understand their transcriptional regulatory mechanism *viz.* how they control cell identity, their developmental potential and factors whose expression helps in reprogramming of somatic cells to a state of pluripotency, is the first critical step. Studies revealed that *Oct4* and *Nanog* proteins are central to the maintenance of ESC pluripotency in mouse.[1,2,3]

However, *Nanog* and *Oct4* sequences have recently been published in non-mammalian vertebrate species, such as *Xenopus* (only *Oct4* homologs)[4], zebrafish[5,6] and medaka[6,7] demonstrated that these key pluripotency factors are not exclusive to mammals. *Sox2* acts as a co-factor with *Oct4* to maintain pluripotency.[8,9]*Oct4*, *Sox2* and *Nanog* have a role in regulating the epigenetic network which supports embryonic stem cell pluripotency.[10,11,12,13]

These core transcription factors can positively auto-regulate their own expression and also activate transcription of a large fraction of the active genes, and bring out a poised state of lineage-specific genes; they frequently share enhancers with signaling transcription factors.[14] Their homologs/orthologs remain to be identified and characterized in lower vertebrates. *Nanog* is considered as one of the major transcription factors of the core pluripotency transcriptional network in mammals[15] and also a key regulator of pluripotency in ESC.[2,3] Among teleosts, *Nanog* was very recently discovered in medaka and found to be necessary for successful embryonic development[6]and characterized by Marandel *et al*., in Goldfish[16]. However, *Oct4* sequences have been reported in non-mammalian vertebrate species *viz. Xenopus* (only *Oct4* homologs)[4], zebrafish[5,6] and medaka[6,7] earlier.

Oct4 is found to maintain pluripotency of the inner cell mass (ICM) and survival of germ cells[1,17,18,19]*Sox1*, *Sox2* and Sox3, the three members of the *SoxB1* subgroup of transcription factors, have similar sequences, expression patterns and over expression phenotypes.[20] *Sox3* is the most common transcriptional factor in the neural development of vertebrates[21] and plays central role along with *Sox1* and *Sox2* in the maintenance of neural stem cells.[22]*Sox2* is found to be needed to maintain pluripotency in mouse embryonic stem cells.[23, 24]*Pou2* was first identified in Zebrafish.[5, 25]Goldfish *Pou2* partial cDNA and gene were isolated very recently.[26]It was found orthologous to teleost *Pou2* gene.

These genes have been identified in mammalian ES cells. The present study aimed at contributing substantially towards development of molecular markers for pluripotency and embryonic stem cell studies in Goldfish, exposing wide scope for embryonic stem cell research and its applications, which has not attracted much attention in Indian context.

Pluripotency genes are routinely used to characterize mammalian stem cell cultures at the molecular level but in Indian fishes, especially ornamental fishes, it is not yet validated. Identification of markers to differentiate stem cell from specialized cells will give an authentication to stem cell research and also provide a broad view of developmental biology in fish.

Materials and Methods

Experimental animals and sample preparation

Disease-free healthy two-year-old brooders of Goldfish (*Carassius auratus*) with an average weight of 150-200 gram (Fig.1) were used for experimental purposes, which were procured from Kurla Aquarium Shop, Maharashtra, India. They were acclimatized in a glass aquarium circular tank with optimum physico-chemical conditions for a period of 10 days and fed with 2 % of body weight with commercial goldfish pelleted feed. The brood fish were selected on the basis of size and colour pattern.

Female were usually easier to spot, as the belly of a mature female is generally plump, whereas male remains streamlined and more torpedo shaped. When males are ready for spawning, they develop breeding tubercles on the head and pectoral fins, principally along the bones of the fin rays. The sex ratio of the spawners was kept at 2:1 for male and female. Natural and induced breeding was performed following the method given by Mahmud *et al.*, [27].

Fertilized eggs of *C. auratus* were collected from the aquarium after breeding. Blastula stages embryos were observed under stereo microscope, and embryos reached mid-blastula stage within 2-4 hrs. Post-fertilization development was monitored at 25^0C in tap water for collection of eggs after mid-blastula stage was reached (Fig.2& Fig.3), within 3 to 4 hrs. Proteinase-k (enzyme) @ 10µg/ml was added and kept for 1 min to facilitate recovery of ICM which is visible as a clump of some 16 to 64 cells (Fig.4). Recovered cells were washed in sterile PBS by centrifuging at 7500x g for 5 min at 4^0C. Fishes were anesthetized using clove oil and then dissected aseptically to collect somatic tissues *viz.* liver, kidney, heart, brain, gonads and muscle. All the collected samples were either immediately processed for RNA extraction or else stored at -80˚C.

RNA extraction and RT-PCR analysis

Total RNA was isolated from all the collected samples, using the Trizol reagent basic protocol with few modifications.[28] Speed and time for initial homogenization step was adjusted according to the processed tissue. For ICM, homogenization was done for 40 sec at 10,000 rpm, whereas for tissue samples homogenization was done for 1 min at 15,000 rpm. Briefly, total RNA was reverse transcribed to first strand cDNA using an Oligo(dT) primer and M-MLV Reverse Trancriptase (MBI Fermentas, USA). To design appropriate primers for all the genes *viz.Oct4*, *Nanog*, *Sox2*, *Pou2* and Beta-actin (kept as an internal control) reported mRNA sequences were retrieved from NCBI GenBank for corresponding primer designing using gene Runner V. 3.0 software. Same set of primers were used for both standard RT-PCR as well as Real Time PCR (Table 1). All the pluripotency genes fragments were successfully amplified after optimization of their annealing temperatures (Table 2). The PCR products were separated on 2% agarose gels and documented with a MegaCapt gel documentation system.

Real-time qPCR analyses

Complementary DNA of three individuals were pooled to make a total of four pools to perform experiment in four batches. Relative quantification determines the changes in steady-state mRNA levels of a gene across multiple samples and expresses relative to the levels of an internal control RNA. This reference gene is often a housekeeping gene (β-actin) and can be co-amplified in the same tube in a multiplex assay or can be amplified

in a separate tube. Therefore, relative quantification does not require standards with known concentrations and the reference can be any transcript, as long as its sequence is known. Relative quantification is based on the expression levels of a target gene versus one or more reference gene(s) and in many experiments. Relative quantification was done by$\blacktriangle CT=2^{-\blacktriangle CT}$ and amplifications were carried out in Light Cycler Real Time PCR detection system (Roche).[29] The 30µl reaction mix volume contained 15 µl of Maxima SYBER Green qPCR Master Mix (MBI Fermentas, USA), 0.5ul of (0.3µM) each gene-specific primers and 2ul (50ng) of cDNA.

PCR cycle consisted of initial denaturation of 95^0C for 10min, followed by 40 cycles of denaturation at 95°C for 10sec, annealing and extension at 60^0C for 10sec. Melting curve analysis of the amplification products were generated at the end of each PCR reaction to confirm that only one PCR product was amplified and detected. For each sample, three-well replicates of 10ul each were used.The differences in transcript levels between treatments were tested for statistical significance using one-way ANOVA followed by Duncan's new multiple range tests using the statistical package, (SPSS). P value below 0.05 was considered statistically significant. The results expressed as the mean ± S.D.

Results and Discussion

RT-PCR analysis of gene expression in different tissues and ICM (inner cell mass)

To examine the expression patterns in seven representative adult somatic tissues and a critical stage of pluripotency of embryos i.e. mid-blastula derived ICM (Fig. 4). The primers used (Table 1) for expression profile study showed that out of the seven pluripotency genes screened *Nanog* and *Oct4* could belong to one group in which expression is limited to ICM and gonads whereas in second group the expression of *Pou2* gene has been seen in almost all the tissues, including ICM and adult somatic tissues because of the low expression level in ICM than ovary. The expression profile obtained through semi-quantitative RT-PCR analysis was validated by quantitative real-time PCR where mRNA transcript number of each gene in each tissue was calculated and then analysed. The mRNA transcript mean values were noted down (Table 2) and Bar Diagram (Fig.6) shows the comparative abundance of mRNA transcript number among different tissues.

Oct4

Oct4 (octamer-binding transcription factor 4) also known as Pou5F1(POU domain, class 5, transcription factor 1) is a protein that in humans is encoded by the *Pou5F1*gene.[30] In the present study, *Oct4* gene expression is the transcript was detected by qRT-PCR with beta-actin as internal control. The level of transcript number was significantly different ($P<0.05$) with control. The highest mRNA abundancewas found in the inner cell mass (ICM), followed by the ovary and testis. However, a low expression level was observed in tissues like muscle, liver, heart, kidney and brain. The presence of high number of *Oct4* mRNA transcripts in ICM and considerable amount of transcripts in the adult gonads *i.e.* testis and ovary, is reported in this study, are in accordance to many studies done earlier.[19,31] Among teleosts like medaka[32] and Zebrafish[33] have found *Oct4* transcripts which shows gonad-specific expression. Transcripts were found to show maternal inheritance and persist at a high level during early embryogenesis.*Oct4* gene mRNA transcripts were found in the ovary of medaka and little in the testis. *Oct4*gene is

reported as an ideal pluripotency marker among mammals, probably due to maternal inheritance which expresses specifically throughout the totipotent cycle, including inner cell mass (ICM) of early developing embryo, spermatogonia and oocytes in the adulthood.[19] The present findings support the previous study done on medaka shown negligible expression in somatic tissues like kidney, liver and heart however shown expressions in gonads.[32]

After activation of the zygotic genome at midblastula transition (MBT), *pou5f1* is broadly expressed in all cells.[34] While little number of transcripts in muscle justified the report of *Oct4* gene expression in adult terminally differentiated human peripheral blood mononuclear cells[35] and in adult stem cells[36] of the skin epidermis.

Nanog

Significantly high amount of *Nanog* mRNA observed in transcripts of ICM, followed by ovary. However, both were sharing different sub-sets, probably because of its maternal inheritance which agreed the earlier reports on medaka[6,32] shows gonad specific expression. *Nanog* mRNA was detected in metaphase II oocytes (i.e. unfertilized eggs) in goldfish,[16] and in other teleostsall stages observed during mid-blastula i.e. 512–1000 cells prior to the embryonic genome activation (EGA).[37]

The results obtained in teleosts are, however, in striking contrast with the expression profile of the *Nanog* transcript in other animals.[38,] mouse,[39] and pig[40]**.***Nanog* transcripts in the adult testis, probably due to due to reported DNA hypo-methylated state in sperm**.**[16] Significantly low mRNA transcripts are reported for other adult somatic tissues because of a much higher DNA methylation level (22.6%).**[16]** An extremely low level of DNA methylation (0.4%) were observed in sperm whereas much higher DNA methylation level (44%) observed in metaphase II oocytes supported present study.

Tissue specific *Nanog* expression analysed for adult flounder showed that *Nanog* mRNA transcripts were present only in the ovary and testis.[41]The absence of *Oct4/Sox2* motif in all teleosts helps to conclude that *Nanog* doesn't seem to be necessary for early lineage differentiation and pluripotency.**[6]** However, present investigation shows significant up-regulation of *Nanog* in ICM which strengthens its potential to be used as a pluripotency and germ cell marker in goldfish.

Pou2

The *Pou2* expression pattern observed highest in ICM, ovary and testis but less transcriptase level in heart, muscle, liver, kidney and brain, agreed with early reports in zebrafish,[30] medaka**,**[42] and Goldfish[26] where *Pou2* transcripts found accumulated right from the 1-cell stage to all stages preceding the embryonic genome activation (EGA) that occurs during the mid-blastula transition in teleosts[37] supporting its maternal inheritance.

The significant down regulation of *Pou2* transcripts in differentiated somatic tissues can be justified the reports of Marandel *et al*.,[26] *Pou2* was found to have proximal regions that are hypo-methylated shortly after zygotic gene activation in the embryo and hyper-methylated in adult somatic cells**.**[43, 44]

However, the *Pou2* of goldfish verified as orthologous to the teleost *Pou2* genes. Early reports on gold fish[26] suggest that *Pou2* expression patterns were not conserved among teleosts which were further supported by the distinct expression pattern in medaka and cod. Where medaka*OlOct4* mRNAs were

detected in all stages from unfertilized egg to the fry stage.[42]

Sox2

Sox2 expression pattern study found in accordance with the previous report on gold fish[26] where the *Sox2* mRNA was first detected in goldfish embryos only after the embryonic genome activation, starting from the 75% epiboly stage. The probable reason may be due to up-regulation of mRNA transcripts for the *Sox2* in ICM. High and comparable number of transcripts in testis, ovary and muscles along with all the somatic tissue suggests that *Sox2* transcript is not maternally inherited and agreed with a previous report on zebrafish[45] and gold fish[26] but not with medaka where *Sox2* transcript is reported to be maternally inherited.[46]*Sox2* expression varies between species from distant families, suggesting different regulatory mechanisms of gene expression. During embryonic development, differences in the expression patterns between the goldfish and other teleosts, including zebrafish, were observed. This basal expression study for *Sox2*justifies the previous report on DNA methylation patterns of *Sox2* upstream sequences on gold fish[26] revealed that the studied CpG sites remained hypo-methylated at all stages of embryonic development including adult fish. The observation suggests that there is no direct involvement of DNA methylation in *Sox2* regulation in gold fish[26]possibly due to the hypomethylation of selected 5′ flanking regions in the sperm and ovum which may turn favorable at early transcriptional activity of *Sox2*after the activation of the goldfish embryonic genome.

Table.1 Primers used in this study for RT-PCR & real time PCR

Gene	Sequence	Primer Sequence	A. Temp.	Amp. Size	ACC. NO.
β-actin	Forward Reverse	GGCCTCCCTGTCTATCTTCC TTGAGAGGTTTGGGTTGGTC	57 ˚C	156 bp	HQ267516.1
Nanog	Forward Reverse	GCGCTGATTCATCGTTTTAACA TCATACGACGGTTCTGAAACCA	59 ˚C	121 bp	JF773571.1
Sox2	Forward Reverse	CCAGAGGATGGACAGTTATGC GGTCTGCGAGTTGCTCATGG	55 ˚C	184 bp	JN624313.1
Pou2	Forward Reverse	CAAACTGAAGCCGCTGCTCCAG CAACGGACGGTGCCTTCCAAAC	55 ˚C	176 bp	HQ676597.1
Oct4	Forward Reverse	CAGAGGTGGTTGAATGAGGCAG TGTTGGGTTTGAGGCACTTCAC	55 ˚C	134 bp	GU289876.1

Table.2 Relative expression level of genes in from different tissues

Tissue	Nanog	Oct4	Sox2	Pou2
ICM	4796.10	3541.20	2420.3	3365.2
Ovary	342.42	1188.3	565.28	1128.4
Testis	67.05	227.84	385.03	385.02
Kidney	21.21	52.79	66.07	66.07
Liver	18.91	16.63	75.65	98.02
Heart	17.74	15.7	46.32	114.40
Brain	17.35	12.08	49.09	16.44
Muscle	15.58	124.70	69.66	87.19

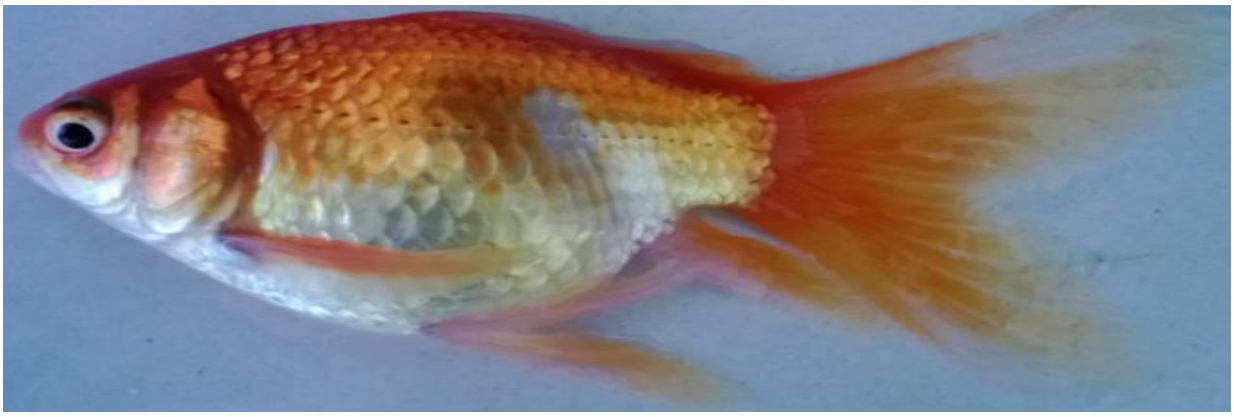
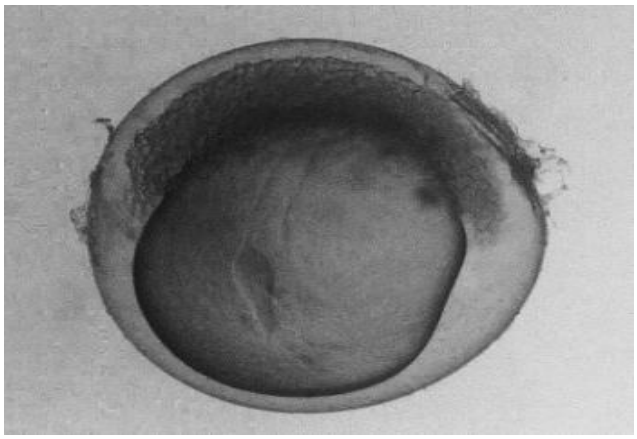


Fig.1 *Carassius auratus* (gold fish)



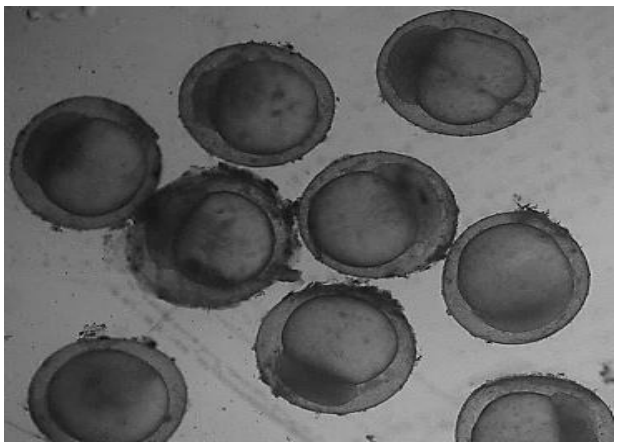


Fig.2 & Fig.3 Demonstrating embryos of *Carassius auratus* at mid blastula stage

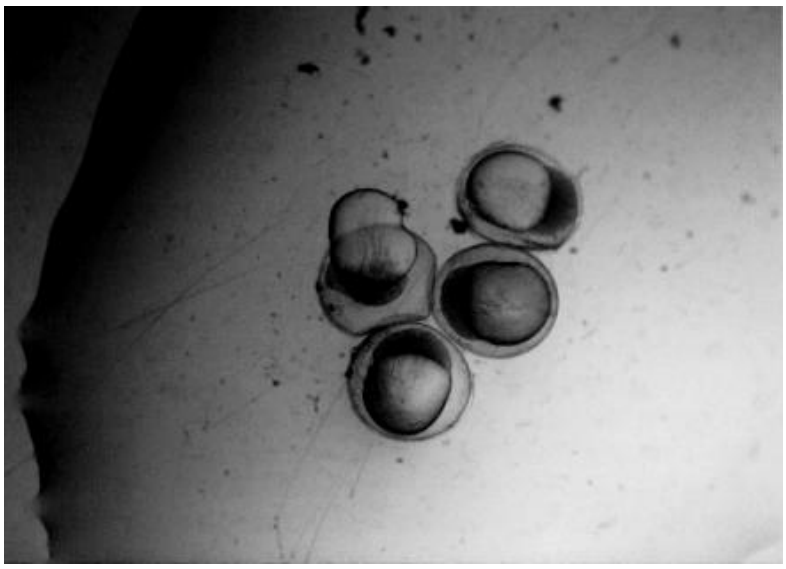


Fig.4 Demonstrating mid-blastula embryos after proteinase-k treatment

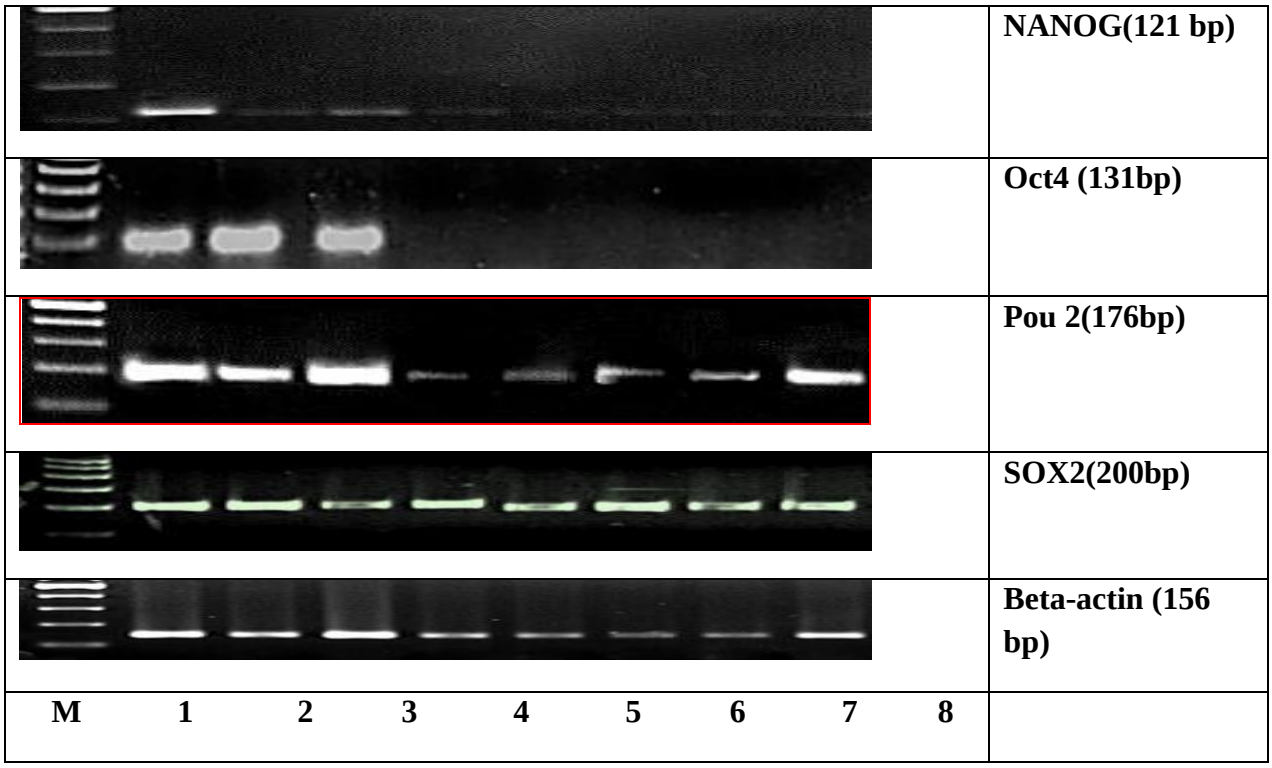


Fig.5 Expression patterns obtained through semi-quantitative RT-PCR of different genes in all the tissues. Lane M: Gene Ruler 100bp Ladder (Thermo Scientific, USA); lane 1: ICM; lane 2: Ovary; lane 3: Testis; lane 4: Liver; lane 5:Heart; lane 6: Kidney; lane 7: Brain; lane 8: Muscle

The gene expression pattern study of goldfish revealed that all the genes studied are having high expression in ICM. Hence, they are involved in the pathways defining pluripotency and presented an understanding on the cellular pluripotency. However, among candidate pluripotency marker gene in goldfish, Nanog gene could be employed as a pure pluripotency marker while developing ES cell lines from goldfish in future.

Overall, it is concluded that out of four standard mammalian pluripotency genes studied, four of them could be used as pluripotency markers in goldfish. The present study of the expression profile of genes and mechanism of pluripotencyat molecular level might open new areas to strengthen fish embryonic stem cell development and ultimately it could be used to adopt stem cell based therapy.

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References

1. Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Schöler, H., Smith, A. (1998). Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell.*, 95: 379-391.
2. Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S.(2003). Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell*., 113: 643-655.
3. Mitsui, K., Tokuzawa, Y., Itoh, H.,

Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M., Yamanaka, S.(2003). The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell.*, 113: 631–642.

4. Morrison, G.M., Brickman, J.M. (2006). Conserved roles for Oct4 homo-logues in maintaining multipotency during early vertebrate development. *Development.*, 133: 2011-2022.
5. Burgess, S., Reim, G., Chen, W., Hopkins, N., Brand, M. (2002). The zebrafish spiel-ohne-grenzen (spg) gene encodes the POU do-main protein Pou2 related to mammalian Oct4 and is essential for formation of the midbrain and hindbrain, and for pre-gastrula morphogenesis. *Development.,* 129: 905-916.
6. Camp, E., Snchez-Snchez, A.V., Garca-Espaa, A., Desalle, R., Odqvist, L., Enrique, O., Connor, J., Mullor, J.L. (2009). *Nanog* regulates proliferation during early fish development. *Stem cells* (Dayton, Ohio)., 27(9): 2081–91.
7. Thermes, V., Candal, E., Alunni, A., Serin, G., Bourrat, F., Joly, J.S.(2006). Medaka sample (FAM53B) belongs to a family of novel vertebrate genes controlling cell proliferation. *Development.,* 133: 1881-1890.
8. Yuan, H., Corbi, N., Basilico, C., Dailey, L. (1995). The developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. *Genes Dev.*, 9: 2635-2645.
9. Remenyi, A., Lins, K., Nissen, L.J., Reinbold, R., Schöler, H.R., Wil-manns, M.(2003). Crystal structure of a POU/HMG/DNA ternary complex suggests differential assembly of Oct4 and Sox2 on two enhancers. *Genes Dev*., 17: 2048-2059.
10. Ivanova, N., Dobrin, R., Lu, R., Kotenko, I., Levorse, J., DeCoste, C., Schafer, X., Lun, Y., Lemischka, I.R. (2006). Dissecting self-renewal in stem cells with RNA interference. *Nature.*, 442: 533–538.
11. Marsun, A., Levine, S.S., Cole, M.F., Frampton, G.M., Brambrink, T., Johnstone, S., Guenther, M.G., Johnston, W.K., Wernig, M., Newman, J.(2008). Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. *Cell*., 134: 521–533.
12. Navareo, P., Chambers, I., Karwacki-Neisius, V., Chureau, C., Morey, C., Rougeulle, C., Avner, P. (2008). Molecular coupling of Xist regulation and pluripotency. *Science.*, 321: 1693–1695.
13. Wang, Y., Keys, D.N., Au-Young, J.K., Chen, C. (2009). MicroRNAs in embryonic stem cells. *J. Cell. Physiol*,. 218: 251–255.
14. Boyer, L. A., T.I. Lee., M.F. Cole., S.E. Johnstone., S.S. Levine., J.P. Zucker., M.G. Guenther., R.M. Kumar., H.L. Murray., R.G. Jenner., D.K. Gifford., D.A. Melton., R. Jaenisch., R.A. Young.(2005). Core transcriptional regulatory circuitry in human embryonic stem cells. Cell., 122: 947–956.
15. Do, J.T., Scholer, H.R. (2009). Regulatory circuits underlying pluripotency and reprogramming. *Trends Pharmacol. Sci*., 30: 296–302.
16. Marandel, L., Labbe, C., Bobe, J., Le, Bail. P.Y. (2012). nanog 5'-upstream sequence, DNA methylation, and expression in gametes and early embryo reveal striking differences between teleosts and mammals. *Gene*., 492(1): 130–7.
17. Rosner, M. H., Vigano, M.A., Ozato, K., Timmons, P.M., Poirier, F., Rigby, P.W., Staudt, L.M.(1990). A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. *Nature*., 345: 686–692.
18. Scholer, H.R.,Ruppert, S., Suzuki, N., Chowdhury, K., Gruss, P.(1990). New type of POU domain in germ line-specific protein Oct-4. *Nature*., 344: 435–439.
19. Pesce, M., Wang, X., Wolgemuth, D.J., Scholer, H. (1998). Differential expression of the Oct-4 transcription factor during mouse germ cell differentiation. *Mech. Dev*., 71(1–2): 89–98.

20. Archer, T.C., Jin, J., Casey, E.S.(2011). Interaction of Sox1, Sox2, Sox3 and Oct4 during primary neurogenesis. *Developmental biology*., 350(2): 429–40.
21. Zhang, C., T. Basta, L., Hernandez-Lagunas, P. Simpson,. D.L. Stemple,. K.B. Artinger., M.W. Klymkowsky.(2004). Repression of nodal expression by maternal B1- type SOXs regulates germ layer formation in Xenopus and zebrafish, *Developmental Biology*., 273: 23–37.
22. Bylund, M., Andersson, E., Novitch, B.G., Muhr, J.(2003). Vertebrate neurogenesis is counteracted by Sox1–3 activity. Nat. *Neurosci*., 6: 1162–1168.
23. Avilion, A.A., Nicolis, S.K., Pevny, L.H., Perez, L., Vivian, N., Lovell-Badge, R.(2003). Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev., 17: 126–140.
24. Masui, S., Nakatake, Y., toyooka, Y., Shimosato, D., Yagi, R., Takahashi, K., Okochi, H., Okuda, A., Matoba, R., Sharov, A.A., Ko, M.S., Niwa, H.(2007). Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat. Cell Biol., 9: 625.
25. Belting, H.G., Hauptmann, G., Meyer, D., Abdelilah-Seyfried, S., Chitnis, A., Eschbach, C., Soll, I., Thisse, C., Thisse, B., Artinger, K.B., Lunde, K., Driever, W.(2001). spielohnegrenzen/pou2 is required during establishment of the zebrafish midbrain-hindbrain boundary organizer. *Development.*, 128(21): 4165–4176.
26. Marandel, L., Labbe, C., Bobe, J., Jammes, H., Lareyre, J.J., Le, Bail, P.Y.(2013). Do not put all teleosts in one net: focus on the sox2 and pou2 genes. Comparative biochemistry and physiology. Part B, *Biochemistry & molecular biology*., 164(2): 69–79.
27. Mahmud, Z., Faysal,A., Ghosh, A. K., Azad, Bir, J., Rahaman, S.M.B. (2012) Induced breeding, embryonic and larval development of comet gold fish (*Carassius auratus*) in Khulna, Bangladesh. *International Journal of Biosciences*, 10(2): 28-38.
28. Sambrook S. J., Russel D. W., Janssen K. A., Irwuin N. J.(2001). Molecular Cloning: *A Laboratory manual (Third Edition),* Cold Spring Harbor Laboratory Press.
29. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real- time quantitative PCR and the $2(-\Delta^{\Delta Ct})$ method. Methods 25, 402– 408.
30. Takeda, H., Matsuzaki, T., Oki, T., Miyagawa, T., Amanuma, H. (1994). A novel POU domain gene, zebrafish pou2: expression and roles of two alternatively spliced twin products in early development. *Genes Dev*., 8: 45–59.
31. Anderson, R.A., Fulton, N., Cowan, G., Coutts, S., Saunders, P.T. (2007). Conserved and divergent patterns of expression of DAZL, VASA and OCT4 in the germ cells of the human fetal ovary and testis. *BMC Dev Biol*., 7: 136.
32. Wang, D., Manali, D., Wang, T., Bhat, N., Hong, N., Li, Z., Wang, L., Yan, Y., Liu, R., Hong, Y.(2011). Identification of pluripotency genes in the fish medaka. *International journal of biological sciences.*, 7(4): 440–51.
33. Lachnit, M., Kur, E., Driever, W. (2008). Alterations of the cytoskeleton in all three embryonic lineages contribute to the epiboly defect of Pou5f1 / Oct4 deficient MZ spgzebrafish embryos. *Developmental Biology*., 315: 1–17.
34. Howley, C. &Ho, R.K.(2000). mRNA localization patterns in zebrafish oocytes. *Mech. Dev*., 92: 305–309.
35. Zangrossi, S., M, Marabese., M, Broggini., R, Giordano., M, DErasmo., E, Montelatici., D, Intini., A, Neri., M, Pesce., P, Rebulla., L Lazzari. (2007). Oct-4 expression in adult human differentiated cells challenges its role as a pure stem cell marker. *Stem Cells*., 25:1675–1680.
36. Tai, M., Chang, C., Olson, L.K., Trosko, J.E.(2005). Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis.

Carcinogenesis., 26: 495-502.
37. Kane, D.A. and Kimmel, C.B.(1993). The zebrafish mid blastula transition. *Development*., 119: 447-56
38. Saadeldin, I.M., Kim, B., Lee, B., Jang, G.(2011). Effect of different culturemedia on the temporal gene expression in the bovine developing embryos. *Theriogenology*., 75: 995–1004.
39. Hart, A.H., Hartley, L., Ibrahim, M., Robb, L. (2004). Identification, cloning and expression analysis of the pluripotency promoting Nanog genes in mouse and human. *Dev. Dyn*., 230: 187–198.
40. Kuijk, E.W., Puy, L.D., Helena, T.A., Tol, V., Christine, H.Y. O., Haagsman, H.P., Colenbrander, B., Bernard A.J. Roelen. (2008). Differences in early lineage segregation between mammals. *Dev. Dyn*., 237: 918–927.
41. Gao, J., Wang, J., Jiang, J. (2013). Identification and characterization of a Nanog homolog in Japanese flounder (*Paralichthys olivaceus*). *Gene*., 531(2): 411–21.
42. Sanchez-Sanchez, A. V. (2010). Medaka Oct4 is expressed during early embryo development and in primordial germ cells and adult gonads. *Developmental dynamics*: an official publication of the American Association of Anatomists., 239(2): 672–679.
43. Lindeman, L.C., Winata, C.L., Aanes, H., Mathavan, S., Alestrom, P., Collas, P.(2010). Chromatin states of developmentally-regulated genes revealed by DNA and histone methylation patterns in zebrafish embryos. *Int. J. Dev. Biol*., 54: 803–813.
44. Wu, S.F., Zhang, H., Cairns, B.R.(2011). Genes for embryo development are packaged in blocks of multivalent chromatin in zebrafish sperm. *Genome Res*., 21: 578–589.
45. Okuda, Y., Yoda, H., Uchikawa, M., Furutani-Seiki, M., Takeda, H., Kondoh, H., Kamachi, Y. (2006). Comparative genomic and expression analysis of group B1 sox genes in zebrafish indicates their diversification during vertebrate evolution. *Dev.Dyn*., 235: 811–825.
46. Cui, J., Shen, X., Zhao, H., Nagahama, Y.(2011). Genome-wide analysis of Sox genes in Medaka (*Oryzia slatipes*) and their expression pattern in embryonic development. Cytogenet. *Genome Res*., 134: 283–294.

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