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Establishment and Characterization of a Chicken Oviduct Epithelial Cell Line from Hy-Line Variety Brown

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Abstract: Chicken oviduct epithelial cells synthesize and secret most egg albumin can be a cell bioreactor or take part in preparation of intravital bioreactor, also are a good model for studying chicken *oviductal* gene expression regulation. Researchers established a chicken oviduct epithelial cell line named XYF-142, meanwhile, developed a new method, obturated interior digesting for efficiently procuring uncontaminated primary cells. The population doubling time of XYF-142 was 106.67 h. About 92.5% of 5th XYF-142 cells were diploid in chromosome analysis. The results of real-time PCR indicated that the expression level of oviductal epithelia-specific gene-SERPINB3 was higher in cells than in oviduct tissue and higher in 5th and 15th epithelia than in primary epithelia. In immunofluorescence cytochemistry detection for XYF-142, all cells expressed the marker protein-cell keratin 18. XYF-142 cells were competent to be transfected with vector DNA and transfection efficiency can reach 60%. Therefore, researchers concluded that researchers have successfully established a chicken oviduct epithelialcell line, provided a model for studying gene expression regulation and tissue-specific detection of chicken oviduct.

Key words: Oviduct epithelial cells, obturated interior digesting, chicken, cell line, egg albumin

INTRODUCTION

Transgenic animals were referred to as potential substitute sources of therapeutic recombinant proteins for human medicine (Han, 2009) and have been used as platforms for the production of human biopharmaceuticals (Harvey *et al*., 2002; Tomita *et al*., 2003; Zhu *et al*., 2005; Lillico *et al*., 2007; Houdebine, 2009). As a bioreactor, transgenic chicken have several advantages over other species, lower expense, higher prolificacy and the purification of recombinant proteins was predicted to be much easier. Most important of all, the glycosylation patterns of most chicken proteins have been reported to be more similar to those of humans (Raju *et al*., 2000). Therefore, the chicken oviductal magnum, a major region for generation of glycosylated proteins was regarded as an important target tissue for transgenesis (Kwon *et al*., 2010; Kaleri *et al*., 2011; Jung *et al*., 2011; Kodama *et al*., 2012). Further, most of eggproteins are synthesized and secreted by Chicken Oviduct Epithelial Cells (COECs) (Muramatsu *et al*., 1995). Thus, compared to intravital bioreactor, the chicken oviduct epithelial cells will be a preferred bioreactor which research expenseis cheaper, research cycle is shorter. In addition, COECs can be applied to be a model to study oviduct-specific regulation and hormonal induction of protein synthesis. Hence, it is necessary to establish a chicken oviduct epithelial cell line.

MATERIALS AND METHODS

Multiple cell cultures: The 40 week old hen used in experiments was a Hy-Line Variety Brown egg-laying hen. The oviduct was obtained and placed into physiological saline that contained antibiotics (100 U penicillin mL^{-1} and 100 μ gstreptomyc in mL^{-1}). The surrounding tissue of oviduct was trimmed off. The inner and outer of oviduct were rinsed with PBS containing antibiotics using a syringe. Then researchers used the method-OID (Obturated Interior Digesting) which researchers developed for obtaining the primary oviductal epithelial cells with few contaminants. The detailed procedures of OID were as followed. The lower end of the oviduct magnum region was tied withthread. Then oviduct magnum region was injected with 0.25% trypsin dissociation solution from the upper end which was tied as well afterwards. The sealed magnum bag was placed into incubator for 45 min digesting at 38.5°C. The digested liquid was drawn out with a syringe into a new tube and added 500 μL FBS in the tube. The primary cells were collected after centrifugalization, then resuspended in primary growth medium (DMED/F12, 25% FBS, 100 U penicillin mL^{-1} and 100 μ gstreptomycin mL^{-1}, 2 mM glutamine, 0.1 mM estrogen) in 60 mm culture dish and cultured at 38.5°C with 5% CO_2.

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Monolayer cell cultures: These primary cells were subculturedat a 1:2 split ratio when they reached 90% confluence and cultured with growth medium (DMED/F12, 15% FBS, 100 U penicillin mL^{-1} and 100 μ gstreptomycin mL^{-1}, 2 mM glutamine, 0.1 mM estrogen) in 60 mm culture dish. Although, there were a few contaminating fibroblasts in the primary culture, the contaminants were absent after passage. The COEC line was named XYF-142.

Growth curve: The XYF-142 cells were seeded on a 12-well plate at 3.88×10^4 cells mL^{-1}. Cell density and cell numbers of each well were counted and recorded daily until the plateau phase was reached. Growth medium was changed every 2 days. Cell viability, i.e., percentage of viable cells was shown by dyeing with 0.4% trypan blue and counted under a microscope using a hemocytometer (Ku *et al*., 2012). Cell growth curves were plotted and the Population Doubling Time (PDT) was calculated based on the growth curve (Gu *et al*., 2006).

Cryogenic preservation and recovery: Prior to freezing, the culture should be maintained in an actively growing state (log phase or exponential growth) to ensure optimum health and good recovery. Harvested cells in the sealed vials by centrifugation and suspended at a density of 1×10^6 viable cells mL^{-1} in cryogenic medium (10% DMSO+90% FBS). The cryogenic tubes were placed into boxes filled with an appropriate amount of isopropyl alcohol frozen overnight at -80°C and the cells were transferred into a liquid nitrogen storage system (Guan *et al*., 2010).

For thawing, the cryogenic tubes were taken from the liquid nitrogen and quickly plugged into a 38.5°C water bath until the cells were dissolved. About 1 mL DMEM $F12^{-1}$ medium was added into these cryogenic tubes which was followed by centrifuging at 300×g for 5 min. Then, the cells were resuspended in growth medium and seeded into 60 mm culture dishes.

Chromosomal analysis: For chromosome analysis, the XYF-142 cells at 5th and 15th were used. The cells were dosed with 0.2 μg colchicine with 5% CO_2 at 38.5°C in 60 mm dish 2.5 h and harvested by centrifugal separation (300×g, 5 min), single cells were suspended in hypotonic solution of 0.075M pre-warmed KCl for 10 min at 37°C in water bath. After then 1 mL Carnoy's fixative (Ethanol: Acetic acid = 3:1) was added in cells and cells were again harvested by centrifugation. About 2 times fixing in 8 mL Carnoy's fixative for 30 min each at room temperature was performed then and harvested the cells by centrifugation. Slides had been placed in ice-water. Slides were prepared using the conventional drop-splash technique and then air dried (Freshney, 1994). Chromosomes were stained with Giemsa according to the method of Suemori *et al*. (2006). Total 40 spreads were counted under an oil immersion objective and chromosome karyotype was analyzed according to the protocol of Sun *et al*. (2006) and Kawarai *et al*. (2006).

Real-time PCR analysis: Total chicken oviduct RNA and celluar RNA of primary epithelia, 5th and 15th epithelia was isolated using TRNzolA+reagent according to the manufacturer's instructions (TIANGEN), respectively. Then, these RNA were quantified with a spectrophotometer (Thermo). cDNA was synthesized from 1 μg of total RNA using Prime Script TM RT reagent Kit with gDNA Eraser (perfect real time) (TaKaRa). RT-PCR was performed to detect the expression levels of oviduct epitheliaspecific expression gene-SERPINB3 with β-actin used as a normalization factor. The cDNA was diluted (1:5) in sterile water before use in RT-PCR. One microliter of cDNA was amplified with each of the forward and reverse primers and each test sample was run in triplicate. For SERPINB3, 40 cycles (5 sec at 95°C, 34 sec at 60°C) of amplification was performed with senseprimer (5'-GAG CAT GTT GGT GCT GTT GC-3') and antisense primer (5'-TTG AAC CTG TCG CCT CAG TG-3'), amplified a 317-bp product. For β-actin, 40 cycles (5 sec at 94°C, 34 sec at 60°C) of amplification was performed with senseprimer (5'-CTC TAT CCT GGC CTC CCT GT -3') and antisense primer (5'-GGA ATG GTG AAG GTG TCA GC-3'), amplified a 256-bp product. PCR amplification was performed as follows: 95°C for 30 sec, followed by 40 cycles at 95°C for 5 sec, 60°C for 34 sec, using a melting curve program (increase in temperature from 60-95°C at a rate of 0.11°C sec^{-1}) and continuous fluorescence measurement. A relative quantification of gene expression was calculated after normalization of the β-actin transcript and the nonspecificcontrol as a calibrator using the $2^{-\Delta\Delta Ct}$ method. For the control, the relative quantification of gene expression was normalized to the CT of the control oviduct.

Immunofluorescence assay: XYF-142 cells were seeded on a 60 mm culture dish until achieving 40~60% confluence. After fixing the cells with 4% paraformaldehyde for 10 min at room temperature, the cells were rinsed with PBS 3×5 min. The cells were then in 0.5% Triton-X100 in PBS and rinsed as above. After blocking with 1% BSA in PBS for 1 h at room temperature, the cells were incubated with the primary antibody (anti-CK18, 1:50) at 4°C overnight, following which the cells were incubated with secondary antibody (Goat Anti-rabbit IgG/FITC, 1:100) for 1 h and rinsed with PBS.

Transfection of vector DNA to XYF-142 cells: The ability of XYF-142 cells to be transfected was estimated using pAcGFP1-N1 plasmid and pIRES2-EGFP plasmid. The cells were seeded at a density of 1.5×10^5 cells well^{-1} in 12-well plates individually. The same quantity of fluorescent protein vectors were transfected into the XYF-142 cells with reagent (LipofectamineTM 2000 (Invitrogen) or Calcium Phosphate Transfection Kit (Beyotime) or DNAfectin TRANSfection (Tiangen) according to the instruction. After 24 h, the non-antibiotics growth medium was removed and growth medium with antibiotics was added. The transfected XYF-142 cells were observed at 24, 48 and 72 h, respectively to estimate transfection efficiency (Guan *et al*., 2010).

RESULTS

Multiple cell cultures: The cells isolated from chicken oviduct magnum showed several shapes, e.g., polygon, spindle shape, strip shape. The cells showed various shapes in small number (Fig. 1a) but formed uniform cobblestone-like structure in large number (Fig. 1b) in passage 1. The contaminate cells, especially fibroblasts, were absent (Fig. 1b). Primary cell growth was not fast until they converged with each other and formed some islands (Fig. 1c) which made contact with each other later on (Fig. 1d). A cell monolayer formed after around 20 days, these cells were subcultured when they reached 90% confluence and the first subculture was conducted at a 1:2 split ratio. After passaging, cell population growth reached 90% confluence within 20 days in 60 mm culture dish. No microorganism was detected till 15th XYF-142 cells.

Growth curve: The PDT was 106.67 h for the cells at a seeding-density of 3.88×10^4 cells well^{-1} at 38.5°C. After slow growth, the cells proliferated rapidly and entered the exponential phase. As the cell density increased, proliferation was retarded by contact inhibition and since the 17th day, the cells entered the plateau phase and began to degenerate. After 15 days, the cells number reached 3.2×10^5 cells well^{-1}. The growth curve of the XYF-142cell line appeared as a typical 'S' shape (Fig. 2).

Cryogenic preservation and recovery: Before freezing and after thawing, the average cell viability was approximately 90 and 70%, respectively. The cells were as well cobblestone-like structureafter thawing (Fig. 1e). The cells displayed a uniform shape in the serial sub-cultivations (15th) (Fig. 1f). Results indicated that cryopreservation and recovery have a little effect on the vitality status of the cells.

Chromosomal analysis: It was shown here that most of XYF-142 cells were diploid and the modal number of chromosomes was 2n = 78 (Fig. 3), including 10 pairs of macrochromosomes and 29 pairs of microchromosomes. The chromosome number of 40 XYF-142 cells that selected randomly from each of the 5th and 15th was counted and the proportion of normal karyotin were 92.5

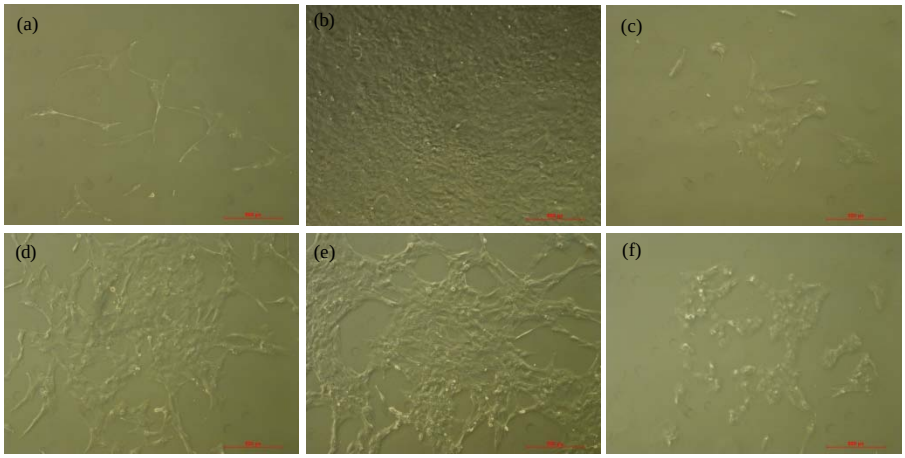

Fig. 1: Culture and morphology of epithelia: a) The cells showed various shapes in small number; b) Epithelia showed uniform cobblestone-like structure in large number and there was nearly no fibroblasts in the culture; c) Some cell islands were formed, it can be accelerated; d) Many cell islands contact with each other that indicated that the growth speed of epithelia would be faster than before; e) After freezing and thawing, epithelia were as well cobblestone-like structure after thawing; f) Epithelia displayed a uniform shape in the serial sub-cultivations (15th) (×40)

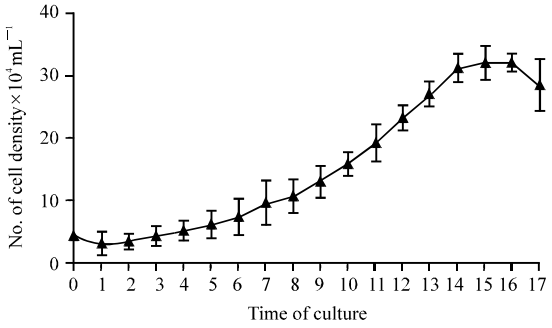

Fig. 2: Growth curve of 5th epithelia. The inoculum was 3.88×10^4 cells mL^{-1}. The growth curve appeared as a typical 'S' shape and included a long latency phase, exponential growth phase and stationary phase. The PDT was 106.67 h

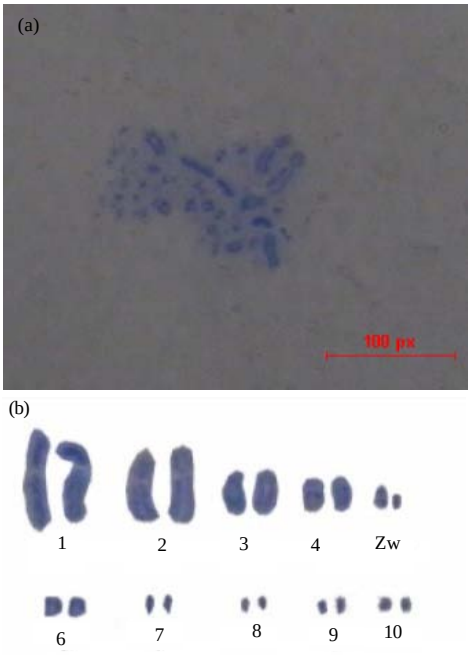

Fig. 3: Chromosomal analysis of epithelia at 15th. The modal number of chromosomes was 2n = 78 including 10 pairs of macrochromosomes and 29 pairs of microchromosomes. The type of sex chromosomes was ZW (♀). Z chromosome which was the chromosome 5 was a macrochromosome. W chromosome was heteropycnosis and the length was similar to chromosome 8

and 87.5%, respectively (Table 1). In XYF-142 cells, chromosomes 5 and 8 were Metacentric chromosomes (M), chromosomes 1 and 2 were Sub-Metacentric chromosomes (SM), chromosomes 4 and 7 were Sub-Telocentric chromosomes (ST) and the remaining

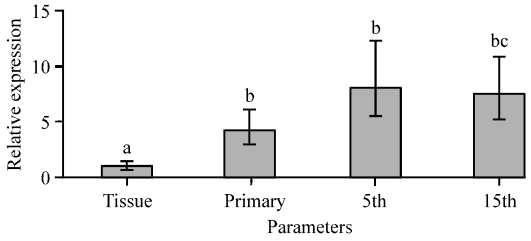

Fig. 4: Real-time PCR analysis of *SERPINB3* gene in oviduct and cells. Compared to the overall expressionlevels of SERPINB3 in oviduct tissue, the expression level was significant higher in primary epithelia and 15th cells, extremely significant higher in 5th cells. The expression level in 5th cells was significantly higher than in primary epithelia but there was no significant difference between 5th and 15th cells

Table 1: Chromosome No. of epithelia

	Chromosome No.				
Passage	Hypodiploid	Diploid	Hyperdiploid	Total cell score	Percentage of 2n = 78
5	3	37	0	40	92.5
15	3	35	1	40	87.5

The hypodiploid; diploid and hyperdiploid chromosome No. of 40 cells was counted in 5th or 15th

chromosomes were considered to be Telocentric chromosomes (T). The type of sex chromosomes was ZW (♀). Z chromosome which was the chromosome 5 was a macrochromosome. W chromosome was heteropycnosis and the length was similar to chromosome 8. These results suggested that themajority of XYF-142 cells were diploid and maintained a normal karyotype.

Real-time PCR analysis: Expression of SERPINB3 whichis an oviductal epithelia-specific gene was determined in chicken oviduct total mRNA, primary epithelia, 5th and 15th epithelia and these expression conditions was indicated in Fig. 4. Compared to the overall expressionlevels of SERPINB3 in oviduct tissue, the expression level was significant higher in primary epithelia and 15th epithelia, extremely significant higher in 5th epithelia. The expression level in 5th epithelia was significantly higher than in primary epithelia but there was no significant difference between 5th and 15th epithelia.

Immunocytochemical identification of epithelial cells: To validate the cells researchers cultured were epithelial cells, researchers examined expression of Cell Keratin18 (CK-18) by immunocytochemistry. All of the cultured cells expressed the epithelial specific intracytoplasmic CK-18 proteins (Fig. 4). The result suggested that the established cell line was an epithelial cell line (Fig. 5).

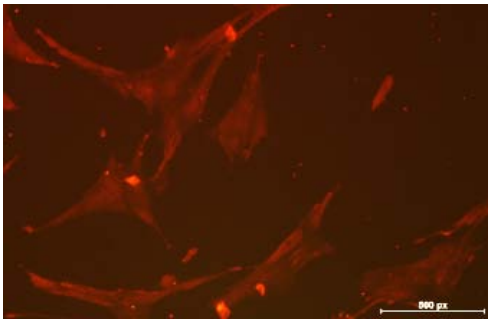

Fig. 5: Immunocytochemical identification of epithelia. The expression of intracytoplasmic markers was positive in vast majority of epithelia

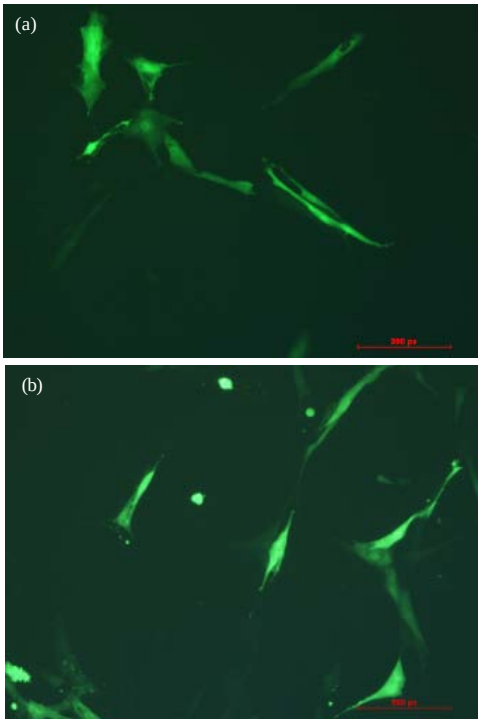

Fig. 6: Expression of *GFP* gene and distribution of GFP in epithelia at 48 h after transfection with vector DNA: a) Transfection with pAcGFP1-N1, the transfection efficiency was approximately 60%; b) Transfection with pIRES2-EGFP, the transfection efficiency was approximately 47%

Transfection of vector DNA to XYF-142 cells: Out of the 3 transfection reagents researchers used, Lipofect TRANSfection Reagent was the only one that got the transfection efficiency >10%. The 2 fluorescent protein genes pAcGFP1-N1 and pIRES2-EGFP were all highly expressed in most cells. At 24 h after transfection, researchers could observe the GFP expression in XYF-142 cells. The number of positive cells increased significantly afterwards and reached the maximum at 48 h and the fluorescence intensity enhanced at 72 h and then gradually faded, mostly disappearing 2 weeks after transfection. The fluorescent signal of a few cellscan maintain 1 month indicating that the exogenous genes in XYF-142 cells can be transcribed and translated. The viabilities of cells transfecting with pAcGFP1-N1 and pIRES2-EGFP were 60%, 47%, respectively (Fig. 6a, b).

DISCUSSION

COECs can be a model for research of oviduct-specific regulation be applied as a cell bioreactor or have important functionon preparation of intravital bioreactor. No matter what kind of application, the COEC line is essential. A successful cell line is dependent up on several factors: No microorganisms, no contaminants and the proliferation potential of cells (Drewa *et al*., 2006, 2009). The most common contaminate in epithelial culture is fibroblasts. Many alternative methods had been suggested for obtaining more primary cells which however, followed by an increase of amount of fibroblasts (Kasperczyk *et al*., 2012), such as the most approved method, magnum mincing (Liu *et al*., 2001; Pang and Li, 2005). Another wildly used method was magnum scrapping which was time-consuming and laborious, leading slightly increasing of primary epithelial cells without eliminating contaminates. For as much as that was crucially necessary to purify the epithelial cells by passage again and again for those methods. Researchers developed a method IOD for obtaining purer primary cells which can efficientlylessencontaminants of epithelial cells. The digestion time, a factor that was easier to control, mainly determined type and quantity of the cells in the method. In Kasperczyk's reserch, serum-free medium was suggested as the optimal medium because of good attachment of cells and few fibroblasts (Kasperczyk *et al*., 2012). In contrast, researchers used a more common medium-DMEM/F12 1:1 which can achieve good attachment of XYF-142. In another word, if OID was adopted it can be easier, faster and practical to obtain qualified chicken primary epithelial cells and a conventional growth medium could be used.

In addition to the earliar, researchers were also concerned about cell preservation. XYF-142 cells viability of thawed cells reduced to 70% after cryopreservation in liquid nitrogen. The viability of XYF-142 cells was lower than chicken fibroblasts after freezing and thawing (Guan *et al*., 2010). The reason of the different results may due to the different cell type in the same species. Because

there was always a loss of some cells during XYF-142 cells passages (approx. 10-15%) which suggested that trypsin and low temperature had a little impact on XYF-142 cells.

The karyotype of chicken was consisted of 2M+2SM+2ST+32T (Ladjali-Mohammedi *et al*., 1999). A large measure of XYF-142 cells had 2n = 78 chromosomes. And in XYF-142 cells, the karyotype of identifiable chromosomes is the same to Ladjali-Mohammedi's results (Ladjali-Mohammedi *et al*., 1999). However, the different ratio of normal karyotype between 5th and 15th passages shown that the number of cell chromosome was time-varying, accompanied by more frequently occurring of hypodiploid and hyperdiploid. Hence, cell application is the earlier the better.

In order to detect the expression of oviductal epithelia-specific gene, researchers choose *SERPINB3* gene as the maker gene. SERPINB3 mRNA was most abundant in the glandular epithelia of magnum and isthmus, as well as luminal epithelia of the infundibulum in chicken oviduct (Lim *et al*., 2012). The expression levels in cells were significant higher than in oviduct tissue, because the proportion of epithelia is higher in cells, the different between primary and 5th epithelia was probably also for this reason. As the result of no significant difference between 5th and 15th epithelia, there were probably no differece. In addition, *SERPINB3* gene may bedown-regulated *in vitro* culture. It needs to detect more passages toprovide more evidence.

In the case of transfecting vector DNA, XYF-142 is not an easy to transfect cell line (only one of three transfection reagents was available). The result was similar to bovine oviduct epithelial cells. The sameness may be a character of oviduct epithelial cells. Although, the XYF-142 cells were selective to the transfection reagent if the appropriate transfection reagent was applied, the efficiencies for DNA of over 60% are completely satisfied.

CONCLUSION

This result indicated that the appropriate transfection reagents for XYF-142 are cationic liposomes rather than complexes of calcium phosphate-DNA. Thus, XYF-142 had the potential to be a research tool of exogenous genes *in vitro*. As to whether the XYF-142 could be applied as a cell bioreactor, it depends on its capacity of secreting biologically activity proteins.

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REFERENCES

Drewa, T., K. Szmytkowska, Z. Wlodarczyk and C. Kierzenkowska-Mila, 2006. Does the presence of unwanted dermal fibroblasts limit the usefullness of *Autologous epidermal* keratinocyte grafts? Transplantation Proc., 38: 3088-3091.

Drewa, T., R. Joachimiak, A. Kaznica, V. Sarafian and J. Sir, 2009. Primary cultures from rat vibrissae as a potential cell source for *in vitro* construction of urinary bladder wall grafts. Transplantation Proc., 41: 1932-1935.

Freshney, R.I., 1994. Culture of Animal Cells: A Manual of Basic Techniques. Wiley, New York, pp: 387-389.

Gu, Y., H. Li, J. Miki, K.H. Kim and B. Furusato *et al*., 2006. Phenotypic characterization of telomerase-im m ortalized primary non-malignant and malignant tumor-derived human prostate epithelial cell lines. Exp. Cell. Res., 312: 831-843.

Guan, W., X. He, L. Li, H. Liang, Q. Zhao, Y. Pu and Y.H. Ma, 2010. Establishment and biological characterization of fibroblast cell line from the Langshan chicken. Cell Proliferation, 43: 157-163.

Han, J.Y., 2009. Germ cells and transgenesis in chickens. Comp. Immunol. Microbiol. Infect. Dis., 32: 61-80.

Harvey, A.J., G. Speksnijder, L.R. Baugh, J.A. Morris and R. Ivarie, 2002. Expression of exogenous protein in the egg white of transgenic chickens Nat. Biotechnol., 20: 396-399.

Houdebine, L.M., 2009. Production of pharmaceutical proteins by transgenic animals. Comp. Immunol. Microbiol. Infect. Dis., 32: 107-121.

Jung, J.G., W. Lim, T.S. Park, J.N. Kim, B.K. Han, G. Song and J.Y. Han, 2011. Structural and histological characterization of oviductal magnum and lectin-binding patterns in Gallus domesticus. Reprod. Biol. Endocrinol. (Electr. Resour.), Vol. 9.

Kaleri, H.A., S.Y. Xu and H.L. Lin, 2011. Generation of transgenic chicks using an oviduct-specific expression system. Genet. Mol. Res., 10: 3046-3055.

Kasperczyk, K., A. Bajek, R. Joachimiak, K. Walasik, A. Marszalek, T. Drewa and M. Bednarczyk, 2012. *In vitro* optimization of the Gallus domesticus oviduct epithelial cells culture. Theriogenology, 77: 1834-1845.

Kawarai, S., K. Hashizaki, S. Kitao, S. Nagano and H. Madarame *et al*., 2006. Establishment and characterization of primary canine *Hepatocellular carcinoma* cell lines producing á-fetoprotein. Vet. Immunol. Immunopathol., 113: 30-36.

Kodama, D., D. Nishimiya, K.I. Nishijima, Y. Okino and Y. Inayoshi *et al*., 2012. Chicken oviduct-specific expression of transgene by a hybrid ovalbumin enhancer and the Tet expression system. J. Biosci. Bioeng., 113: 146-153.

Ku, J.L., K.H. Kim, J.S. Choi, S.H. Kim and Y.K. Shin *et al*., 2012. Establishment and characterization of six human gastric carcinoma cell lines, including one naturally infected with Epstein-Barr virus. Cell. Oncol., 35: 127-136.

Kwon, S.C., J.W. Choi, H.J. Jang, S.S. Shin and S.K. Lee *et al*., 2010. Production of biofunctional recombinant human interleukin 1 receptor antagonist (rhIL1RN) from transgenic quail egg white. Biol. Reprod., 82: 1057-1064.

Ladjali-Mohammedi, K., J.J. Bitgood, M. Tixier-Boichard and F.A. Ponce de Leon, 1999. International system for standardized avian karyotypes (ISSAK): Standardized banded karyotypes of the domestic fowl (*Gallus domesticus*). Cytogenet. Genome Res., 86: 271-276.

Lillico, S.G., A. Shearman, J.M. McGrew, C.D. Robertson and J. Smith *et al*., 2007. Oviduct-specific expression of two therapeutic proteins in transgenic hens. Proc. Natl. Acad. Sci. USA., 104: 1771-1778.

Lim, W.1., S.E. Ahn, W. Jeong, J.H. Kim and J. Kim *et al*., 2012. Tissue specific expression and estrogen regulation of SERPINB3 in the chicken oviduct. Gen. Comp. Endocrinol., 175: 65-73.

Liu, Y.B., M.Z. Yu and X.Z. Shen, 2001. The establishment of a temporary expression system in chicken oviduct Epithelium. Dev. Reprod. Biol., 10: 13-19.

Muramatsu, T., H. Hiramatsu and J. Okumura, 1995. Induction of ovalbumin mRNA by ascorbic acid in primary cultures of tubular gland cells of the chicken oviduct. Comp. Biochm. Physiol. B Biochem. Mol. Biol., 112: 209-216.

Pang, Y. and Q.W. Li, 2005. GFP Reporter gene under the direction of chicken ovalbumin gene promoter expressed in the CHO cell and in the primary cell cultures of chicken oviduct. Chin. J. Biotechnol., 21: 154-158.

Raju, T.S., J.B. Briggs, S.M. Borge and A.J. Jones, 2000. Species-specific variation in glycosylation of IgG: evidence for the species-specific sialylation and branch-specific galactosylation and importance for engineering recombinant glycoprotein therapeutics. Glycobiology, 10: 477-486.

Suemori, H., K. Yasuchika, K. Hasegawa, T. Fujioka, N. Tsuneyoshi and N. Nakatsuji, 2006. Efficient establishment of human embryonic stem cell lines and long-term maintenance with stable karyotype by enzymatic bulk passage. Biochem. Biophys. Res., Commun., 345: 926-932.

Sun, Y.L., C.S. Lin and Y.C. Chou, 2006. Establishment and characterization of a spontaneously immortalized porcine mammary epithelial cell line. Cell Biol. Int., 30: 970-976.

Tomita, M., H. Munetsuna, T. Sato, T. Adachi and R. Hino *et al*., 2003. Transgenic silkworms produce recombinant human type III procollagen in cocoons. Nat. Biotechnol., 21: 52-56.

Zhu, L., M.C. van de Lavoir, J. Albanese, D.O. Beenhouwer and P.M. Cardarelli *et al*., 2005. Production of human monoclonal antibody in eggs of chimeric chickens. Nat. Biotechnol., 23: 1159-1169.