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Cardiac Differentiation of Chicken Spermatogonial Stem Cells-A Directional Approach

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ABSTRACT

A tremendous increase in the human population has put poultry industry under an increased pressure to meet steep increase in the demand. Poultry is contributing 25% of the total world's meat production and lesser cost of investment per bird makes it more suitable for the further breeding programmes. Major poultry diseases frequently lead to cardiac damage and cause huge economic losses to poultry industry due to mortality. The *in vitro* embryonic stem cell (ESC) technology has a futuristic approach for homogeneous populace of differentiated cells, for their further transplantations. During *in vitro* conditions the differentiated cell populace can be used in grafting and transplantation processes to regenerate damaged tissues. Therefore, the current study targeted the use of spermatogonial stem cells (SSCs) in the poultry production system through cardiac regeneration. The current study will also open new boulevard for the similar kind of research in other livestock species for the management of heart diseases.

(Key words : Poultry, Embryonic Stem Cells (ESCs), Spermatogonial Stem Cells (SSCs))

INTRODUCTION

With the bombastically increase in the human population, poultry industry is facing a regular increased pressure to meet steep increase in the demand. It has been observed that during the last 10 years an overall 6 fold increase in the consumption of poultry products has been observed especially in the developing countries (Taha, 2003; Sonaiya and Swan, 2004; FAO, 2013). Poultry production in the whole world during 2011 has touched the level of 100 million metric tons with 36.5 million metric tons from Asian continent (Sodhi *et al*., 2013). Poultry products are valuable source of animal protein. 43% (meat) and 25% (egg) increase in their production has been recorded since 2000 (Best, 2011). Currently, poultry is contributing 25% of the total world's meat production and this trend is increasing continuously (Luan *et al*., 2014).

Lesser cost of investment per bird and its increased demand in the consumer market brings poultry breeding to an important aura of poultry research. The comprehensive genomic tool box of poultry provides ample opportunities to apply different types of selection procedures (Sodhi *et al*., 2013). Currently, the Asian continent is producing almost one third of the world's egg production. There are many aspects to improve the poultry production in the developing countries of Asia. Therefore, there is a dire need to develop the suitable technologies for higher poultry production to meet the increasing human demand and population (Chowdhury *et al*., 2014). Keeping emphasis on disease control, feed efficiency and increased production, a number of breeding strategies have been applied in different environmental conditions. These days use of stem cells especially spermatogonial stem cells (SSCs) is in vogue for their use in poultry production.

A survey was conducted to know about the major diseases which cause huge economic losses due to mortality in poultry industry. It was observed that the cardiac damage is one of the major causes of mortality. In the poultry birds the diseases such as listeriosis (Crespo *et al*., 2013), *leucocytozoon caulleryi* infection (Lee *et al*., 2014), spontaneous cardiomyopathy, sudden death syndrome (SDS) in growing broiler chickens (Olkowski *et al*., 2008) and congestive heart failure/ascites (Neubert *et al*., 1999) cause direct or indirect cardiac damage. Inspite of huge advancement and widen aura of knowledge, we are still not up to the mark for the poultry cardiac disorders particularly on regenerative thera-

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peutic approaches. Therefore, for the current study use of SSCs in the poultry production system through cardiac regeneration has been targeted.

SPERMATOGONIAL STEM CELLS AND THEIR ROLE IN THE DERIVATION OF CARDIOMYOCYTES

It's a well-established fact that embryonic stem cells (ESCs) can differentiate into functional cardiomyocytes. Guan *et al*. (1999a) and Boheler *et al*. (2002) reported the role of ESCs in cardiac regeneration by identifying and characterizing the expression of genes, proteins and ion channels specific for cardiomyocytes during the developmental stages. To combat with heart failure, the cardiomyocytes derived from ESCs have been examined in animal models. Even in human beings the model using cardiomyocytes derived from ESCs have been demonstrated beneficial to treat such conditions (Guan *et al*., 1999b; Kanatsu *et al*., 2004; Guan *et al*., 2006).

The spermatogonial stem cells (SSCs) are very unique germ line stem cells present in the adult testis. In male animals, throughout their life SSCs have the ability of self-renewal and keep producing efficient daughter cells which later differentiate into spermatozoa (Fig. 1) (Wang *et al*., 2010). Moreover, in earlier studies it has been reported that SSCs of mice and/or their progenitors can revert back spontaneously (without the addition of exogenous genes) to pluripotent embryonic stem-like cells (Brulet *et al*., 1980; Conrad *et al*., 2008; Golestaneh *et al*., 2009; Kossack *et al*., 2009). The regulatory molecules and signaling mechanisms have significant role in the reprogramming of SSCs during regenerative medicine (He *et al*., 2010). In addition, human SSCs are reported to produce functional cells which can be efficiently used in cell-based therapies. Kanatsu (2003) and Guan *et al*. (2006), in their respective studies on mouse reported that SSCs are able to proliferate and differentiate without tumor formation when they are transplanted into normal mouse heart even up to one month after their transplantation. Such reports supported the idea that SSCs can be a new source of unique types of cardiomyocytes for basic research and potential therapeutic application (Guan *et al*., 2007).

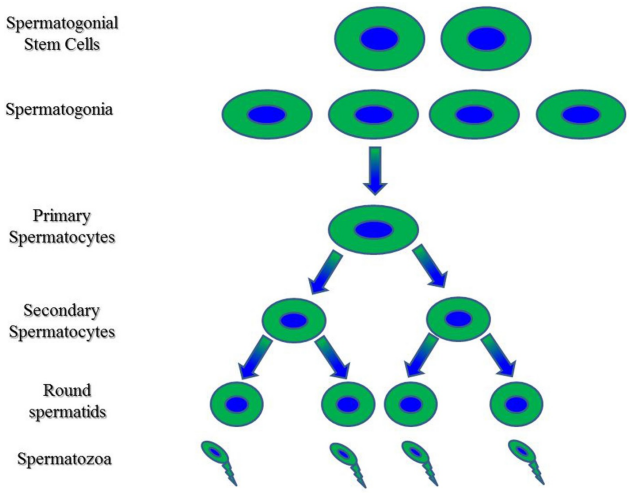

Fig. 1. Schematic diagram showing the full progress of sperm forming from spermatogonia located at basement membrane of seminiferous tubules. A clear pictorial description of transformation of spermatogonial stem cells to sperm formation has been illustrated.

In Vitro DIFFERENTIATION AND POTENTIAL OF MULTIPOTENT GERM LINE STEM CELLS FROM CHICKEN TO DIFFERENTIATE INTO CARDIOMYOCYTES

In the recent past a lot of studies on the use of SSCs from mice for cardiomyocyte development have been conducted but still meager reports regarding the use of chicken SSCs are available. Chicken SSCs are potential source of germ cells (Guan *et al*., 1999(a); Jung *et al*., 2005). To understand the concept of developmental studies chickens are an important animal model. We still lack in our knowledge about the *in vitro* differentiation potential of multipotent adult germline stem cells (maGSCs) of chicken testis along with the essential conditions for inducible differentiation. Researchers are targeting their work to analyze putative pluripotent cells from testicular cells for further culturing and characterization of those identified.

Embryonic stem (ES) cells have been broadly used in the developmental biology. Injection of ES cells into a host blastocyst may be assimilating them into the inner cell lump for the sake of the embryonic development. It has been observed that the 'donor' ES cells propagated during *in vitro* circumstances are accomplished to generate cells of all lineages. Bradley *et al*. (1984) reported their *in vivo* involvement in the development of chimeric animals. Routine cultivation of permanent ESC lines from inner cell mass (ICM) of blastocysts (Wobus *et al*., 1984; Fig. 2) have been reported by different methods. Propagation can be done either from single blastomeres of 8-cell-stages (Wobus *et al*., 1991) or from embryos at morulae stage (Eistetter, 1989).

Apart from ESCs, the embryonal carcinoma (EC) cells,

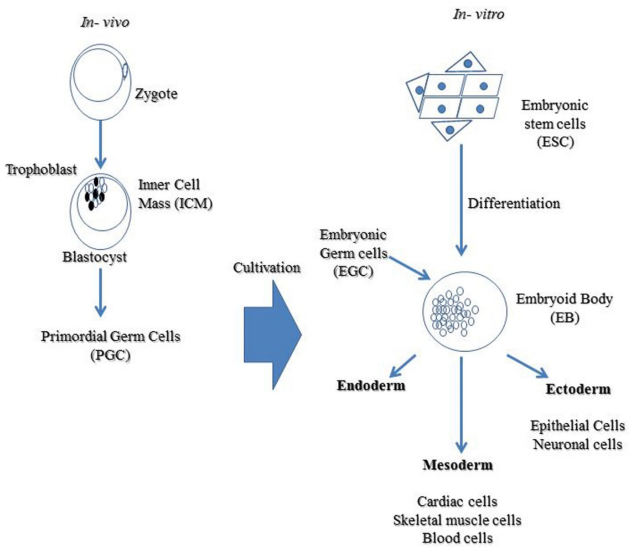

Fig. 2. Representation of *in- vitro* ESC technology, highlighting the development of inner cell mass, primordial germ cells, Embryoid body and its further differentiation into three germ layers. Schematic diagram showing the relationship between *in vivo* development of germ cells and the *in vitro* pluripotent stem cell lines that have been reported to be derived from mouse and human cells (ESC and EG).

(Martin and Evans, 1975; Stevens, 1984), and the embryonic germ cells (EGC) cultivated from primordial germ cells (PGC) are established as permanent cell lines (Fig. 2; Stewart *et al*., 1994). Both ESCs and EGCs, take part in the normal progression when injected into blastocysts (Gardner and Brook, 1997). Further, *in vitro* cultivated ESCs differentiate into 'embryoid bodies' (EBs; Fig. 2). EBs have potential to differentiate into endodermal, ectodermal and mesodermal. The pluripotent/totipotent ESCs from mesoderm ultimately differentiate into the cardiogenic cells (Wobus and Guan, 1998). Further it has been reported that the cardiomyocytes developed during *in vitro* conditions resemble attributes of atrial-, ventricle-, purkinje- and pacemaker-like cells (Hescheler *et al*., 1997).

In vitro culturing of ESCs helps in the understanding of cellular differentiation procedures during early embryonic development. It allows evaluation of the differentiation of embryonic cells via precursor cells into highly discerned and specialized cells of the cardiovascular lineages. Chicken has been successfully used to establish ESC lines and living chimaeras (Pain *et al*., 1996).

CHEMICAL INDUCTION OF HEART BEAT IN CELLS

Chemicals such as 5-aza-2-deoxycytidine, ascorbic acid, 5-azacytidine and BMP2 have been used for the induction of cardiac differentiation in mouse and human ESCs (He *et al*., 2010). Further it has been reported that differentiation of chicken mGSC into cardiac cells is pretty contrasting because of their diverse traits (Luan *et al*., 2014). Xu *et al*. (2002) reported that use of phenylephrine, isoprenaline, 3-isobutyl-1-methylxanthine (IBMX) and clenbuterol to potentiate beating of cardiomyocytes via specialized mechanisms. IBMX plays a significant role in the course of persuading stem cell differentiation into adipose cells and enhances the contraction competence of cardiac cells (Luan *et al*., 2014).

Chicken maGSCs on staining judiciously by PAS and with some specific antibodies like Oct4, SSEA1, SSEA3, SSEA4, STRA 1-60 and STRA 1-81 are capable of expressing the highly characteristic markers related to ESCs (Solter and Knowles 1978; Thomson *et al*., 1998; Sparadling *et al*., 2001; Draper *et al*., 2002; Johkura *et al*., 2004; Jung *et al*., 2005; Menard *et al*., 2005; Kim *et al*., 2008). Further it also has been reported that during *in vitro* conditions they are capable to spin-off into derivatives of the three embryonic germ (EG) layers (Brulet *et al*., 1980). It projects them to be as a source of stem cells for differentiating maGSCs into cardiomyocytes which probably divulge their functional abilities. The maGSCs are reported to differentiate into distinct cells such as contracting cardiomyocytes similar to ESCs (Luan *et al*., 2014).

Further, successful cardiac differentiation has been proclaimed in mouse and human ESCs using diverse induction chemicals in both species, such as 5-aza-2-deoxycytidine, ascorbic acid, 5-azacytidine, BMP2, etc. (He *et al*., 2010). However, differentiation into cardiac cells from chicken maGSC is quite contrasting due to their distinct traits. Xu *et al*. (2002) reported the use of phenylephrine, isoprenaline, 3-isobutyl-1-methylxanthine (IBMX) and clenbuterol to potentiate the beating process in cardiomyocytes via precise mechanisms. IBMX has been reported to have a significant role in the mechanism of induction of stem cell differentiation into adipose cells and potentiates the contraction ability of cardiomyocytes. It is further reported to be used as an auxiliary stimulant to differentiate cardiac like cells from chicken maGSC because of its ability to control the calcium ion channels through biological processes via a cAMP-dependent pathway. Recently, Jasmin *et al*. (2010) also reported the use of DMSO to induce cardiac differentiation in P19 cells.

THERAPEUTIC APPLICATION OF SSCS IN CARDIAC REGENERATIVE MEDICINE

The chicken has been successfully used to study the

developmental biology. They have come up as a well-established animal model for the research purpose. They are among the top food producing animals of the world (Jordana *et al*., 2014). The cardiomyocytes derived from ESCs have been tried for the treatment of heart failure in animals (Guan *et al*., 1999(b); Passier *et al*., 2008). They project a new option for the repair of cardiac/myocardial injuries in animals. mGSCs provide a plenty of cell types for the screening of drugs and cell therapy (Thomson *et al*., 1998). Cardiomyocytes derived from ESCs can multiply indefinitely in culture during an undifferentiated state. Antigens acting as surface markers such as stage-specific embryonic antigens (SSEA) and expression of transcription factor OCT-4 are reported to aid in their characterization (Draper *et al*., 2002; He *et al*., 2010). These traits have been demonstrated to be useful for estimating the culture condition of SSCs.

Disease such as congestive heart failure/ascites (Neubert *et al*., 1999), Leucocytozoon caulleryi infection (Lee *et al*., 2014), listeriosis (Crespo *et al*., 2013), spontaneous cardiomyopathy and sudden death syndrome (SDS) in growing broiler chickens (Olkowski *et al*., 2008) directly or indirectly lead to cardiac damage in birds. Meager information is available regarding the regenerative therapeutic approaches to deal with cardiac disorders.

Ascites is one of the major causes which lead to huge economic losses to the farmers. The potential of mGSCs derived from chicken testis to get differentiated into cardiomyocytes projects that these cells may open a new avenue in the cardiogenic research to enhance the chances of cardiac repair. It is also recommended that maGSCs from chicken testis have the ESC like properties and are capable of differentiating into cardiomyocytes. The cardiomyocytes derived from mGSC have been tested for their competence to refurbish the function of injured hearts in the diseased birds.

It has been reported that cardiomyocytes derived from maGSC express in sarcomeric alpha actinin, which is known to be explicit for alpha-cardiac actinin (Luan *et al*., 2014). Cardiac- specific troponin- T and Conexin-43 a protein expressing in the gap junctions in cardiac clusters has been reported to show their significant expression during the cardiac differentiation of the SSCs (Luan *et al*., 2014). The gap junctions are thought to have an important role in the synchronized contraction of the heart during embryonic development. Moreover, cardiac troponin T, which is the tropomyosin binding subunit of the troponin complex, is reported to control muscle contractions in response to changes in the intracellular calcium ion concentration (Kanatsu *et al*., 2004; Guan *et al*., 2006; Guan *et al*., 2007). These findings suggest that maGSCs cater a new source of a noticeable type of cardiomyocyte for elemental research and are promising in regenerative medicine therapies.

CONCLUSION

In the future, the *in vitro* ESC technology poses a futuristic approach to have homogeneous populace of differentiated cells, for their further transplantations. ESCs differentiated into cardiac ventricular cells (Wobus *et al*., 1997b) can be used as a unique source of cells for somatic therapies and transplantation. The development of efficient vector systems and systematic planning will widen the horizon for *in vivo* gene expression methods (Koh *et al*., 1995; Rust *et al*., 1997). *In vitro* transgenic ESC lines carry tissue-specific promoters along with choose-able marker genes differentiate into the definitive clans. Followed by *in vitro* selection, the differentiated cell population can be used in grafting and transplantation processes to regenerate defective tissues. The current study will also open new boulevard for the similar kind of research in other livestock species for the management of heart diseases.

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