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Amniotic fluid TGF-β1 concentration during chick embryonic development

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ABSTRACT

Transforming growth factor- β (TGF- β) is one of the most important growth factor that plays important roles in the metazoan development. It is produced by several cell lineages and its expression serves in both autocrine and paracrine modes to control the differentiation, proliferation and state of activation of other cells. TGF- β signaling is important in the maintenance and differentiation of embryonic stem cells and somatic stem cells. The expression of TGF- β begins early in development and is observed at various developmental stages. Most marked TGF- β expression is observed in different organ undergoing morphogenetic events, particularly those involving epithelio-mesenhymal interaction or differentiation. TGF- β1 is expressed in most biological fluids including amniotic fluid (AF), serum and cerebrospinal fluid. A wide range of proteins including growth factors has been identified in AF. The importance of AF growth factors for normal embryonic development has been shown in numerous studies. In this study total protein concentration (TPC) and TGF- β1 concentration in AF samples from chick embryos were measured using a Bio-Rad protein assay and enzyme linked immunosorbent assay (ELISA). TPC decreased from days E6 to E10 and then increased from E11 to E15. However, the concentration of AF TGF- β1 was increased from day E6 to E15. It is thus concluded that TGF- β1 is a constant component of AF during chick embryonic development and its concentration changes as development proceeds.

Keywords: Amniotic fluid, concentration, TGF-beta, chick.

INTRODUCTION

Amniotic fluid (AF) is an essential component for fetal development and maturation during pregnancy. By eights week of gestation in humans, the urethra is formed and the fetal kidneys start to produce urine. Fetal swallowing begins shortly thereafter. Excretion from fetal urine,

umbilical cord, gastroentric system, respiratory system and other fetal secretions that transudation through fetal skin human are the main sources of AF in humans [1; 2]. The chick embryo begins imbibing the AF around day 13 of incubation and continues until day 19 of incubation. Hence, the embryo is exposed to and swallows the fluid containing proteins, growth factors, water, hormones, and other nutrients needed for growth and development [3]. A wide range of proteins including growth factors and cytokines has been identified in human AF [4]. Cytokines are thought to play an important role in establishing and maintaining pregnancy. They are powerful mediators of cell growth and regulators of immunological and inflammatory reactions. The gestational tissues including placenta and amnion produce several cytokines which are considered to influence the outcome of pregnancy [5].

Cytokines, growth factors and proteins present in the AF can be taken up by the fetus [6] and can diffuse through the fetal skin and can be absorbed by vascularized fetal surface of the placenta [7]. Changes in the levels of AF insulin like growth factor (IGF), vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) in the chick and avian embryos has been demonstrated [8-10].

Transforming growth factor- β (TGF- β) is a secreted protein that regulates proliferation, differentiation and death of various cell types. It is the prototype of a large superfamily of secreted signaling polypeptides with diverse functions in development of all metazoans [11]. TGF- β regulates the differentiation of neuronal, immune, mesenchymal and epithelial cell types [12]. The TGF- β expression begins early in development and is observed at various developmental stages. Most marked TGF- β expression is observed in different organ undergoing morphogenetic events, particularly those involving epithelio-mesenchymal interaction or differentiation [13]. It has been shown that TGF- β enhances the expression of adhesion molecules and extracellular matrix during development [14]. TGF- β initiate signaling by interacting with two receptor serine/threonine kinases referred to as the type I and type II receptors [15]. In this study, the total protein concentration and TGF- β1 level in chicken amniotic fluid were studied by Bio-Rad protein assay based on the Bradford dye-binding procedure and enzyme linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Antibodies and reagents

Chicken TGF- β 1 ELISA Kit (Catelog number: NB-E60030) was purchased from Novatein Biosciences.

Amniotic fluid samples

Fertile white Leghorn eggs were incubated at 38 ^{o}C in a humidified atmosphere to obtain chick embryos at different stages of development. The amniotic fluid was carefully aspirated using a pulled tip glass microcapillary pipette (Drummond Scientific Company, 20 μL) from incubated chicks embryos from day 6 to day 15 (E6 to E15).

Amniotic fluid for each analysis was collected from 22 chick embryos. The average amount of 0.5 ml amniotic fluid was collected from each embryo. To minimize protein degradation, amniotic fluid samples were kept at 4 °C during collection. Amniotic fluid samples were

centrifuged at 10000 rpm at 4 °C for 10 minutes to remove any contaminating cells. The samples that we used for analysis had no visible sign of contaminating red blood cells that we could detect under the microscope. The supernatant was frozen immediately and stored at -70 °C until used. Twenty two samples from each time point were used for analysis of total protein concentration and TGF- β1 level.

Analysis of amniotic fluid proteins

Total protein concentration

The total protein concentration of proteins in amniotic fluid was determined by the Bio-Rad protein assay based on the Bradford dye procedure.

ELISA

TGF- β1 in amniotic fluid was measured using the sensitive two-site ELISA and antiserum against chick TGF- β1. Microtiter plates were first coated with 80 ng primary anti- TGF- β1 antibody per well in 0.1 M Tris buffer. After overnight incubation, the plates were blocked with EIA buffer (50 mM Tris pH 7.5, 0.3 M NaCl, 0.1% Triton X-100, 1% BSA and 1% gelatin). The samples and standards were placed in triplicate wells and incubated overnight at room temperature. After washing with phosphate buffered saline (PBS) a biotinylated secondary antibody (7 ng/mL) was added to each well and incubation was carried out overnight at room temperature. β-galactosidase coupled to avidin was then added for 2 hours followed by washing. Finally, 200 μM 4-methylumbelliferyl-β-galactoside (Sigma-Aldrich, Poole, UK) in 50 mM sodium phosphate were added as well as 10 mM MgCl2 buffer and the amount of fluorescence was measured after 50 minutes incubation at 37 °C using a fluorimeter (Dynatech, Canada). All animal procedures were carried out in accordance with the Animals, Act, 1986.

All values were expressed as mean±standard error of the mean (SEM). In all experiments, a minimum of 22 measurements were made in order to calculate a mean±SEM. Statistical analysis was performed using Student's t test and only values with $P \leq 0.05$ were considered statistically significant.

RESULTS

Total protein concentration

The total protein content in AF in embryos aged E6 to E15 was determined by Bio-Rad protein assay. The total protein content decreased from day E6 to day E10 and after that the levels increased from E11 to E15 (Figure 1).

TGF- β1 concentration in amniotic fluid

The concentration of TGF- β1 in the amniotic fluid samples from chick embryos aged E6 to E15 has been analyzed by ELISA. The levels of AF TGF- β1 was increased from E6 to E15 (Figure 2).

In this study, TPC and TGF- β1 levels in the chick AF have been investigated. We studied TGF- β1 as it is one of the most important growth factor that plays a key role in the metazoan development. It has been demonstrated that signaling by TGF- β is important in the vascular morphogenesis [16] and embryonic development and homeostasis [17].

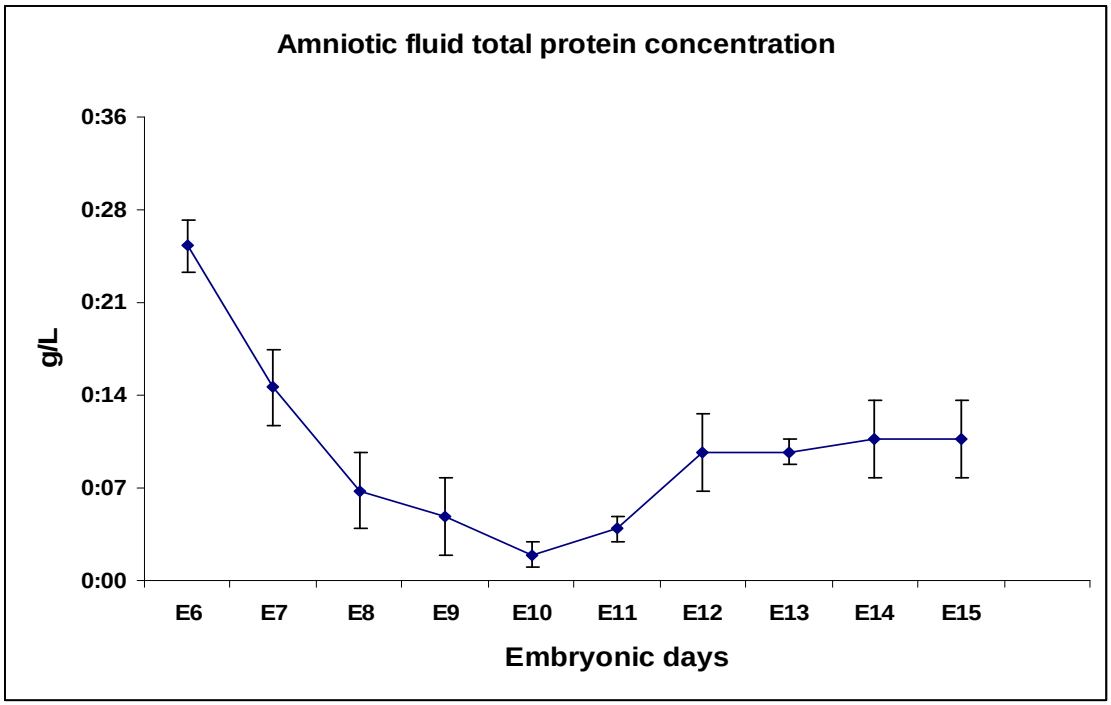

Figure 1. Total protein concentration in the amniotic fluid samples from days E6 to E15 (g/L). (n=22 at each time point)

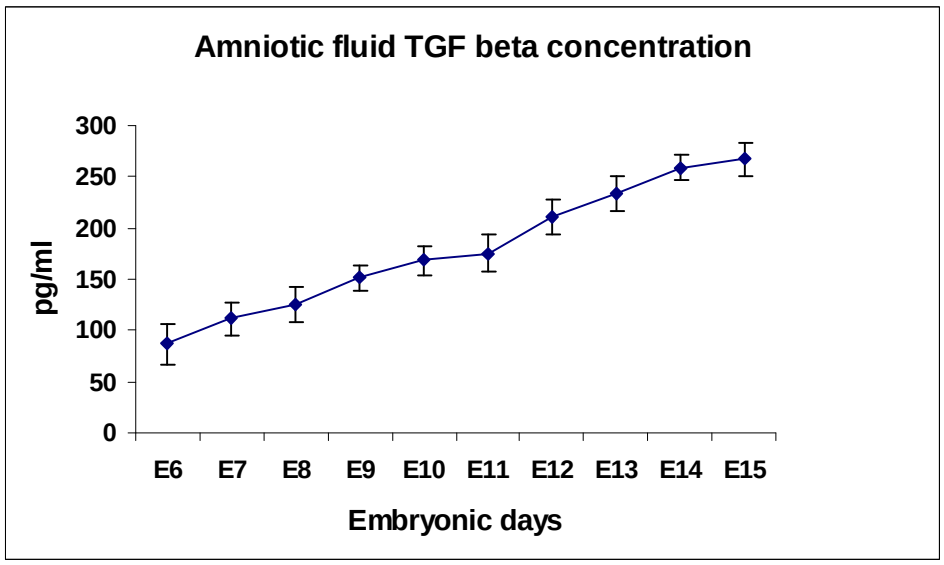

Figure 4. TGF- β1 concentration in the amniotic fluid samples from days E6 to E15 (pg/ml). (n=28 at each time point).

It has also been shown that the TGF- β might be involved in regulating development of the skin at embryonic stage and also in wound healing [18]. In vertebrates, TGF- β superfamily ligands play roles in the morphogenesis of most organs. TGF- β family members are well known for their ability to induce epithelial-mesenchymal transition (EMT) [19]. It was shown that TGF- β is an important in the maintenance and differentiation of embryonic stem cells and somatic stem cells [20].

To our knowledge, this is the first study to investigate the levels of TGF- β1 in the chick AF. AF is important in the fetal health because it forms a protective sac around the fetus that prevent mechanical and thermal shock, assists in acid/base balance, contains nutritional, growth factors and cytokines [21; 22]. A wide range of proteins has been identified in AF [2; 23; 24]. Maternal urine tissues, amniotic fluid cells, fetal urine, umbilical cord and other fetal secretions that include transudation through fetal skin are the main sources of proteins in AF [2; 25; 26]. The concentration of proteins in the AF changes during development [2; 9; 10]. In this study we have shown that AF TPC changes during chick embryonic development. We have also demonstrated that AF TGF- β1 concentration increases as development proceeds.

It has been shown that AF growth factors plays important role in the normal embryonic development [27]. In this study we demonstrated that TPC and TGF- β1 concentrations in the AF changes during chick embryonic development. It has been shown that TGF- β1 may be temporally and spatially expressed during chicken embryogenesis [28]. Both the type II and type III TGF- β receptors are co-expressed during chick embryogenesis in the developing heart, lung and sys [29]. Changes in the AF TPC and TGF- β1levels during chick embryonic development may be associated with organ development. As AF is in contact with developing embryo and the embryo swallow AF, changes in the AF TGF- β1 levels may affect the developing embryo. It is thus concluded that TGF- β1 is a constant component of AF during chick embryonic development and its concentration changes as development proceeds.

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