Proteomic analysis of oocytes retrieved from in vitro cultured ovine small antral follicles and large antral follicles cultured with amphiregulin, neuregulin-1 and tumour necrosis factor-α

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ABSTRACT

Protein components change markedly during follicular development in the ovary. In this study, a proteomics approach was used to evaluate changes in proteins during different size follicles viz small antral follicles (SAFs) and large antral follicles (LAFs) culture exposed with different growth factors. The optimum and elevated doses of amphiregulin (AREG) or neuregulin (NRG-I) or tumour necrosis factor-α (TNF-α) were used for culture of SAFs and LAFs and then oocytes were retrieved for analysis of secretory protein by SDS-PAGE. SAFs were cultured in media containing 150 ng of AREG, NRG1 and TNF-α and LAFs in media containing 50 ng of AREG, NRG1 and TNF-α. The average yield of protein from oocytes retrieved from SAFs when cultured with 150 ng of AREG, NRG-1 and TNF-α was 0.0012, 0.0016 and 0.0010 mg/ml respectively. When cultured with oocytes retrieved from LAFs in 50 ng AREG, 50 ng NRG-I and 50 ng TNF-α, the corresponding values were 0.0026, 0.0021 and 0.0014 mg/ml respectively. The SDS-PAGE profile of the oocytes retrieved from culturing of SAFs and LAFs in different doses of AREG, NRG-I and TNF-α revealed no peptide bands.

Keywords: Small antral follicles; large antral follicles; secretory protein; oocytes

INTRODUCTION

Developing a culture system for oocytes has important biotechnological implications due to its potential to produce large number of oocytes for embryo production, manipulation and transfer. Several efforts have been made to establish in vitro conditions for oocyte maturation and fertilization to improve the developmental efficacy of sheep oocytes like production of regulatory substances particularly local non-steroidal regulators and interaction of somatic and oocyte compartments at their cellular and molecular levels (Tsafriri et al 2005) for increasing reproductive efficiency. Conti et al (2012) reported that amphiregulin (AREG) and epiregulin (EREG) are necessary for the transformation of mural granulosa cells to cumulus cells. Chen et al (2013) reported that translation in oocytes is further increased by supplementing the medium with AREG. Field et al (2014) reported that source of tumour necrosis factor-α (TNF-α) is granulosa cells, theca cells and oocytes which are necessary for GC proliferation, oocytes apoptosis, follicular apoptosis and atresia. Noma et al (2011) postulated that NRG-I improved the developmental competence of oocytes during in vitro fertilization (IVF). Shiny et al (2015) opined that the protein components in the follicular fluid exhibited increase/decrease in accordance with follicle size. As a result the proteomic studies can be used to investigate changes in protein expression during oocytes maturation which can then be used to identify important regulatory proteins that are expressed during the maturation of cumulus-oocyte complexes which in turn contribute to in vitro developmental competence of oocyte. Thus the present study was planned to examine the proteome profile in oocyte cumulus complexes during growth of different stages of follicular development.

MATERIAL and METHODS

Culture of small antral follicles (SAFs) and large antral follicles (LAFs) for protein analysis

Ovine ovaries were collected from a local slaughterhouse. Ovarian sections were made and washed with follicle isolation medium and mechanical method was followed for the isolation of SAFs and LAFs by micro-dissection method using 26 G disposable needle and scalpel blade. During isolation process, isolation medium was added intermittently to avoid drying of the tissue. SAFs were cultured in media containing A1 (150 ng of AREG), A2 (150 ng of NRG1) and B1 (150 ng of TNF-α) and LAFs containing B2 (50 ng of AREG), C1 (50 ng of NRG1) and C2 (50 ng of TNF-α) in 100 µl droplets culture media without FBS or BSA. The control medium consisted of minimum essential medium (MEM) supplemented with follicle stimulating hormone (FSH-0.05 IU/ml), sodium pyruvate (0.23 mM), glutamine (2 mM), hypoxanthine (2 mM), insulin-transferrin selenium (ITS): insulin (6.25 µg), transferrin (6.25 µg), sodium selenite (6.25 µg), mercaptoethanol (10 µM/ml) and gentamicin (50 µg/ml) (Ramesh et al 2008). They were cultured under mineral oil in 35 mm Petri dish placed in CO_2 incubator (38°C, 5% CO_2 in air, 90-95% relative humidity) for 7 days. Every time the culture medium was prepared fresh and kept for incubation in CO_2 incubator for 30 minutes before using for culture.

Sample preparation and SDS-PAGE

The oocytes retrieved from the culturing of SAFs and LAFs were sonicated. Protein was precipitated by adding six volumes of ice-cold acetone. The precipitates were then resuspended in lysis solution consisting of 1.0 ml of 50 Mm phosphate buffer (pH 7.4), 1 Mm EDTA, 1 Mm dithiothreitol, 0.15 M KCl and 20 µl of 0.01 per cent (w/v) phenylmethylsulfonyl fluoride (PMSF). Later the solution was centrifuged at 13,000 rpm for one hour at 4°C. The supernatant so obtained was transferred into labeled sterile 1.5 ml eppendorf tube and stored at -80°C for further analysis. The total protein content in the entire stored oocyte sample was assayed as per the Lowry's method (Lowry et al 1951). The samples were subjected to SDS-PAGE. After the completion of electrophoresis, gels were stained with coomassie brilliant blue according to Sambrook and Russell (2012). The molecular weight of the protein bands was determined in comparison with the standard protein molecular weight markers.

RESULTS and DISCUSSION

Secretory protein of oocytes retrieved from cultured SAFs and LAFs with AREG, NRG-I and TNF-α by SDS-PAGE

The data on yield of secretory protein from the oocytes retrieved from the culturing of SAFs and LAFs with AREG, NRG-I and TNF-α are presented in Table 1. The average yield of protein from oocytes retrieved from SAFs when cultured with 150 ng of AREG, NRG-1 and TNF-α was 0.0012, 0.0016 and 0.0010 mg/ml respectively. Similarly oocytes retrieved from LAFs when cultured with 50 ng of AREG, NRG-I and TNF-α, the corresponding values were 0.0026, 0.0021 and 0.0014 mg/ml respectively. Shiny et al (2015) reported that total protein concentration in follicular fluid of small, medium and large follicles did not significantly differ and remained fairly constant regardless of the size of follicles. On the other hand, Thangavel and Nayeem (2004) in their study on certain biochemical profile of buffalo follicular fluid reported that the total protein concentration decreased as the follicles grew to larger size.

Effect of AREG (150 ng), NRG-I (150 ng) and TNF-α (150 ng) on secretory protein patterns of in vitro cultured SAFs

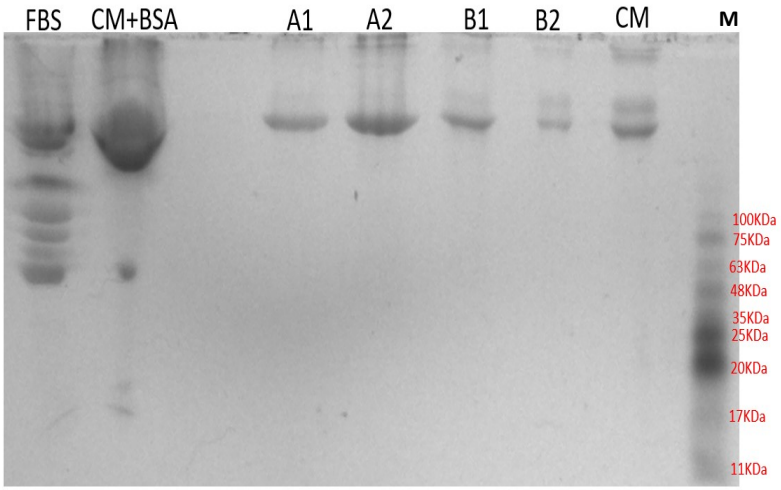
No detectable protein band was observed when subjected to SDS-PAGE using coomassie brilliant blue staining (Figs 1, 2). However proteins of molecular weight 13.8-15.1, 28.5-28.2, 56.2-53.7, 70.8-61.7, 97.7-95.5 and 114.8-112.2 kDa were present in follicular fluid (FF) of SAFs as has been reported by Shiny et al (2015). They also observed that the molecular weight ranged from 13.8 to 123.0 kDa (107.2, 42.7, 45.7, 37.2, 34.7, 23.4, 22.4, 19.5 and 17.4) in FF of small follicles only. Total of 1,401 proteins identified in the follicles where 609 were common to the three developmental stages of antral follicles and 444 were found uniquely at one of the stages (Anastacio et al 2017). Similarly Fahiminiya et al (2011) and Bijttebier et al (2009) found 113 proteins in mare FF, 53 proteins in porcine FF and 21 proteins in canine FF. However there exists no comprehensive report on secretory protein patterns of in vitro cultured SAFs with respect to different doses of AREG, NRG-I and TNF-α.

Effect of AREG (50 ng), NRG-I (50 ng) and TNF-α (50 ng) on secretory protein patterns of in vitro cultured LAFs

No detectable protein band was observed when subjected to SDS-PAGE using coomassie brilliant blue

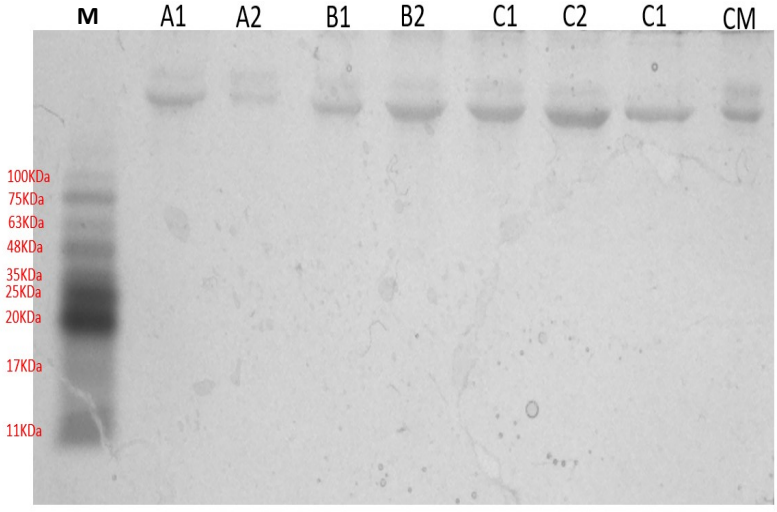
Table 1. Yield of protein in oocytes retrieved from cultured SAFs and LAFs

Oocytes	Groups	Yield of protein
Oocytes retrieved from SAFs	AREG: 150 ng	0.0012 mg/ml
	NRG1: 150 ng	0.0016 mg/ml
	TNF-α: 150 ng	0.0010 mg/ml
Oocytes retrieved from LAFs	AREG: 50 ng	0.0026 mg/ml
	NRG-I: 50 ng	0.0021 mg/ml
	TNF-α: 50 ng	0.0014 mg/ml



FBS: Fetal Bovine Serum **CM+BSA: Control media+Bovine Serum Albumin**
A1: CM+150ng AREG **A2: CM+150ng of NRG-I**
B1: CM+150ng TNF-α **B2: CM+50ng AREG** **CM: Control media**

Fig 1. SDS-PAGE profile of oocytes retrieved from cultured SAFs and LAFs in media containing different growth factors



M: Marker A1: CM+150ng AREG A2: CM+150ng of NRG-I B1: CM+150ng TNF-α

B2: CM+50ng AREG C1: CM+50ng NRG-I C2: CM+50ng TNF-α CM: Control media

Fig 2. SDS-PAGE profile of oocytes retrieved from cultured SAFs and LAFs in media containing different growth factors

staining (Figs 1, 2). According to Rodgers and Irving-Rodgers (2010) blood-follicle barrier played a central function in determining the composition of follicular fluid by regulating the transfer of proteins with a molecular weight <500 kDa. Shiny et al (2015) reported molecular weight ranging from 13.8 to 123.0 kDa (107, 81.3, 79.4, 77.6, 70.8, 47.9, 42.7, 37.2 to 34.7, 32.4, 26.3 and 19.5) expressed in the LAFs of buffaloes. However there exists no comprehensive report on secretory protein patterns of in vitro cultured LAFs with respect to different doses of AREG, NRG-I and TNF-α.

CONCLUSION

No detectable protein band was found from oocytes isolated from small antral follicles and large antral follicles cultured either with amphiregulin or neuregulin-1 or tumour necrosis factor-α. These findings may help researchers to better understand some of the mechanisms that govern ovine oocyte maturation.

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