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Original Article

Optimisation of proteomic approaches to study the maternal interaction with gametes in sow's reproductive tract

Ahmed Aldarmahi [a,*], Mohammed Al-Rabia [b]

[a] College of Sciences and Health Professions, King Saud bin Abdulaziz University for Health Sciences, Jeddah, Saudi Arabia
[b] College of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

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ABSTRACT

The applications of 2DE and MS have been successfully used in many studies utilising different biological samples. The complex nature of cellular proteomes is a big challenge for proteomic technologies. Much effort has been applied to develop and improve the preparation techniques for proteomic samples to be able to detect the low abundant proteins. This is one of the major and unsolved challenges facing the proteomic analysis of biological samples. One partial remedy is to deplete the proteomic samples. In this study, we compared two techniques (acetone precipitation and commercial kit) for the cleaning and purification of oviductal and uterine horn secretory proteomes in primary cell culture system. The samples prepared from acetone precipitation and commercial kit 2-D clean up kit were compared by 2-dimentioanl electrophoresis. We found that no significant difference was observed in number of spots detected between the samples prepared by acetone precipitation technique to those prepared by commercial kit. Protein samples were run through strong cation exchange (SCX) liquid chromatography in order to fractionate samples of major proteins. Protein identification by mass spectrometry revealed a significant detection of low abundant proteins in comparing to high abundant proteins. In conclusion, acetone precipitation was found to be more efficient and cost effect technique. Depletion of proteomic samples from the most abundant protein species is strongly recommended to allow the mid and low abundant protein to be detected. A better resolution of the gels will be achieved by removing the major proteins such as albumin and immunoglobulin.

1. Introduction

Maternal communication begins from the moment of ovulation and/or semen deposition within the female reproductive tract. It continues throughout the fertilisation process and embryo implantation. The arrival of oocyte and sperm, or the formation of zygote, changes the oviduct and endometrium microenvironment to facilitate these events. This involves the alteration of oviductal genomic and proteomic profiles [1].

The study of the expression of different proteins in a cell or tissue in a temporal and spatial fashion is referred to as proteomics [2]. This technique is fairly new and has been advancing in the last decade. Since proteins dictate cellular functions to a large extent, comparative proteomics investigating various proteins in the normal and diseased samples is deemed to be an important factor in the diagnosis and treatment of diseases [3,4]. Comparative proteomic analyses could possibly aid in the identification of biomarkers for noninvasive diagnosis of female diseases and assist in the prediction of success rates for the assisted

* Corresponding author. Tel.: +966 1222 46666x45566; fax: +966 92000 8668x45566.
E-mail address: aldarmahiah@ngha.med.sa (A. Aldarmahi).

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reproduction techniques [5]. Several studies have demonstrated and characterised the proteomic profiles of the female reproductive tract [6–8].

An earlier study performed by Ellington et al. [9] investigated the effect of direct contact between the spermatozoa and oviduct. In 1991; Ellington et al. [9] reported that the bull sperm heads were attached to bovine monolayer oviductal epithelial cells within 1 h after co-culture and showed a vigorous tail motion. Using electron microscopy, they showed that the spermatozoa to oviductal epithelial cells behaved in the co-culture system similar to that from *in vivo* studies. In addition, the capacitation patterns of bull spermatozoa were shown to have changed when co-cultured with oviductal epithelial cells *in vitro* [9]. Interestingly, *de novo* protein synthesis of oviductal cells was later identified as the result of sperm presence, and attachment to the oviductal epithelial cell monolayers in equine [10] and bovine species [11].

In addition, the proteomic profile of cervio-vaginal fluid in humans was determined by applying one-dimensional SDS–PAGE and strong cation-exchange chromatography followed by LC–MS/MS approach [8]. In another study performed by Seytanglue et al. [12]; distinctive differences in the oviductal proteome profile between the luteal and follicular phases of the reproductive cycle were demonstrated [12]. Several proteins have been reported to be altered in response to the arrival of spermatozoa/oocyte or embryos in the female reproductive tract [12–15]. These proteins were involved in production, maintenance and repair, anti-oxidants and the metabolic activity of the cell [14] have observed that distinct proteins were regulated in the porcine oviduct after co-incubation with sperm and oocyte [14]. Subsequently, Georgiou et al., provided *in vivo* evidence of maternal protein regulation in the presence of sperm [13]. Furthermore, the study of protein expression induced by the early embryo within the oviductal cells has been investigated. For example, the production of Dcpp protein in the oviduct was important for creating a spatial window for maternal–embryo communication [16]. In 1986, Salamonsen et al. [17] found that changes in the protein synthesis in the endometrium plays an important role within uterine–embryo interaction development, and also in pregnancy maintenance [17]. However, the effect of these proteins on the oviduct role modulating the gametes or embryo action needs to be addressed further.

Several studies in domestic animals including cows, pigs, sheep, and rabbits have indicated that the protein concentration in serum is greater than in the oviduct. Only 5–10% of the proteins in serum are found in the oviduct. Oviductal proteins derived from the bloodstream are 95% albumin and immunoglobulin G (IgG) [18]. The transport of these proteins to oviductal lumen is dependent on the protein molecular size, thus, smaller proteins enter oviduct more easily than larger proteins. Moreover, the protein expression differs among different regions of the oviduct. Protein production appears to be greater in ampulla and infundibulum in compare to the isthmic region of the oviduct. Protein concentration in the oviduct increases prior to and at the time of ovulation and during fertilisation [19,20].

In the last decade, many technologies in the proteomic field have been developed in order to identify and determine the proteomic profiles from different biological samples. These approaches are varied based on the sensitivity, efficiency, reproducibility and reliability of the technology. Two-dimensional electrophoresis (2DE) is considered as a gold standard for protein separation and was introduced in the early of 1970s [21].

Much effort has been applied to develop and improve the preparation techniques for proteomic samples to be able to detect the low abundant proteins. Majority of proteins are found in low levels in biological samples; however, the high abundant proteins disguise protein species with low copy numbers. This is one of the major and unsolved challenges facing the proteomic analysis of biological samples. One partial remedy is to deplete the proteomic samples. This can increase the chance of detecting the low and mid abundant proteins [22]. The technology applied to remove the high abundant proteins is based on antibody interactions and the affinity of the protein to specific molecules and ligands [23–25].

In the current study, we aimed to evaluate and compare two techniques for the cleaning and purification of oviductal and uterine horn secretory proteomes in the monolayer culture system. Sample cleaning is an important prerequest for preparation of proteomic samples for 2D gel analysis. As a result of this process salts and other molecules that can interfere with 2D gel electrophoresis are removed from the samples as much as possible. We also evaluated the success of a depletion protocol in depleting oviductal and uterine horn secretory proteome from highly abundant proteins. The removing of major proteins such as albumin from the oviductal and uterine horn secretory proteomes is an important step to detect the mid and low abundant proteins. Furthermore, we will examine whether these techniques can result in sufficient protein recovery that are cleaned enough for production of high resolution 2D gels. These experiments are essential for optimising the conditions for characterisation of oviduct and uterine horn secretory proteome.

2. Materials and methods

2.1. Sample preparation and isolation

The samples were collected from sow reproductive tracts. A total of 50 oviducts and 50 uterine horns were collected for the experiment. The oviduct and the uterine horn were obtained from slaughterhouse (G Wood and sons, Mansfield, UK). The uterine horn and oviduct were tied at one end by cotton thread and filled with PBS (No Mg^{2+} or Ca^{2+}) (Gibco). These tissues were incubated at 39 °C with 5% CO_2 in air for 2 h. After incubation, all the fluids were collected and discarded. Oviductal tubes and uterine horns were re-filled with fresh PBS (Gibco) and incubated for 18 h in the same conditions. After incubation, fluids in oviduct and uterine horn were collected separately from each oviduct or uterine horn tissue by squeezing the organs. The collected fluids were centrifuged at 100,000 × *g* for 30 min to remove any debris or dead cells. 1 mM protease inhibitor (Sigma) was added into oviduct and uterine horn