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Original Article

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

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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

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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

fluids and the samples were frozen at −80 °C until further analysis.

Cells of both oviducts and uterine horns were also scarped to obtain the epithelial cells. Scraped cells were briefly centrifuged (at 300 × *g* for 10 min) and the pellet was collected and protease inhibitor (1 mM) (Sigma) was added into cell scraping samples. Samples were frozen at −80 °C until further analysis.

2.2. Protein purification and measurement and clean up techniques

The samples were cleaned up through two different techniques. First one was the acetone precipitation procedure and the second process was based on a commercially available kit (Plus-one 2D clean up kit; Amersham Biosciences, Buckinghamshire, UK). For the acetone precipitation procedure, 100 μl of protein sample was mixed with 600 μl of ice cold (−20 °C) acetone (Sigma) and incubated at −20 °C over night. Thereafter, the sample was centrifuged at 14,000 × *g* for 5 min at 4 °C. The supernatant was discarded. The pellet was dried for 15–20 min at room temperature. The recovered proteomic sample was diluted with PBS and was stored frozen (−20 °C) until further use in experiments.

A Plus-one 2D clean up kit was used according to the manufacturer's instructions. Briefly, 300 μl of precipitant supplied in the kit was incubated with 100 μg of protein on ice for 15 min. Then, a 300 μl co-precipitant was added, mixed and centrifuged at 12,000 × *g* for five min. The supernatant was discarded and another 40 μl of co-precipitant was added on the top of the pellet for 10 s. After that, centrifuged at 12,000 × *g* for 5 min and discarded the supernatant. A 25 μl of distilled/deionised water was added on the top of pellet for 10 s then one ml of chilled buffer (−20 °C) and 5 μl wash additive supplied in kit for 45 min. Then, centrifuged at 12,000 × *g* for 5 min and discarded the supernatants and allowed the pellet to dry for 5 min at room temperature. The pellet was resuspended in 50 μl of chilled PBS (without Mg^{2+}/Ca^{2+}) stored frozen (−20 °C) until further use in experiments.

2.3. Depletion technique

To deplete proteomic samples from the most abundant proteins such as albumin and IgG proteins, a commercial kit (Blue albumin and IgG deletion kit; Sigma) was used. This kit utilises a urea-based equilibration buffer, which is superior to salt-based buffers for subsequent second dimensional electrophoresis (2 DE) analysis. Briefly, the "Depletion Medium" supplied in the kit was added to the spin column to equilibrate the column. 50–100 μl of proteomic sample was applied on top of the medium bed in the column and incubated at room temperature for 10 min. Then centrifuged at 10,000 × *g* for 1 min and re-applied the sample in the same column and centrifuged at 10,000 × *g* for 1 min. A 100 μl of equilibration buffer supplied in the kit was added and centrifuged at 10,000 × *g* to collect the remaining unbound proteins. Thereafter, the first elute with the remaining unbound proteins were pooled for final collection of depleted protein sample. The depleted sample was stored and frozen at −20 °C until further use in experiments.

2.4. Protein quantity measurement

Protein quantity was measured by Bicinchoninic acid (BCA) (Sigma) assay. Briefly, 2% (v/v) copper sulphate (Sigma) was added to BCA (sigma) solution. A 200 μl of this solution was added to 10 μl of protein sample and incubated at 37 °C in the dark for 30 min. The absorbance was read at 570 nm using a Benchmark 96 well plate reader.

2.5. Second-dimensional gel electrophoresis: isoelectric focusing (IEF)

The rehydration solution containing 350 μg of the protein sample with 0.5% (v/v) immobilised pH gradient buffer (IPG) for pH 3–10 (Amersham Biosciences), dithiothrietol (DTT) and urea (Sigma) were added to give a final concentration of 40 mM DTT. 0.002% (w/v) bromophenol blue (Amersham Biosciences) was also added to this mixture. The prepared sample was loaded into the Immobiline™ Drystips (pH 3–10, 18 cm) linear (Amersham Biosciences) in the platform according to the manufacturer's instruction. The strips were rehydrated overnight. Proteins were resolved in the first dimension by isoelectric focussing using Ettan IPGphor Isoelectric Focusing system (Amersham Biosciences). The Step and Hold voltages used for pH 3–10 linear strips were 500 V (0.5 kVh), 1000 V (0.8 kVh), 8000 V (13.5 kVh) and 8000 V (12 kVh). The Ettan IPGphor II system Control Software Version 1.1 was used to monitor the actual voltage passing through the strips.

Following the IEF, the second-dimensional SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed. SDS–PAGE is used to separate the polypeptides according to their molecular weights (MW) after being focused by their charges. Homogenous 12.5% polyacrylamide gels (25.5 × 19.6 cm, 1 mm thick) were made by a special reusable precast gel cassette (Amersham Biosciences). Prior to application of the strips into the 2-DE, the focused strips were equilibrated in order to saturate the Immobiline DryStrip gel with SDS buffer. The equilibration solution contained 50 mM Tris–HCl (1.5 M, pH 8.8), 2% SDS (w/v), 30% (v/v) 87% glycerol, 6 M urea, and 200 μl (1 μl/ml) bromophenol blue in distilled deionised water. 1% (w/v) of DTT (Sigma) was added and gently rocking on a shaker (Stuart See-Saw Rocker SSL4, USA) for 15 min. The free cysteine residues of protein were alkylated with 2% (w/v) iodoacetamide (sigma) for another 15 min to prevent reformation of disulfide bonds.

Thereafter, the equilibrated strip gels were applied into a homogeneous 12.5% polyacrylamide SDS–PAGE and the gels were performed vertically in EttanDalt (DALTA*six*) (Amersham Biosciences) at 25 °C. A molecular weight marker was used for protein standard and also to obtain best results. The gels were run for 30 min (step 1) at 5 Watts per gel and for step 2 at 15 Watts per gel for 4 h.

2.6. Gel staining and image analysis

As soon as the gel run was finished, gels were placed in the fixation solution containing 7% glacial acetic acid in 40% (v/v). The gels were stained with Brilliant Blue G-Colloidal (Sigma) or Coomassie with 20% (v/v) methanol for 2 h. Then, the gels were destained with 10% acetic acid in 25% (v/v) methanol for 1 min and rinsed with 25% (v/v) methanol and further destained in 25% (v/v) methanol for overnight.

For the image capturing step, gels were scanned by using an Image Scanner II flatbed scanner and LabScan software (Amersham Biosciences). The gel spots were detected and analysed by ImageMaster 2D Platinum v6.0 software (Amersham Biosciences). Manual detection and modification was also performed. Moreover, the minor differences in protein loading among the same group of gel were normalised correctly. The volume of spot in each gel was normalised by adjusting the intensity of each spot against the total of intensity volumes of the detectable spots in the gels. These spots were excised and prepared for identification step.

2.7. Strong cation exchange (SCX) liquid chromatography and identification of protein by mass spectrometry (MS) reduction, alkylation, and digestion

Protein samples were run through strong cation exchange (SCX) liquid chromatography in order to fractionate samples of major proteins. Samples were reduced by addition of reducing agent (10 mM DTT, Sigma) in 50 mM ammonium bicarbonate (ABC) (BDH Laboratories Supplies, Poole, UK). The reducing buffer was added to the samples for 60 min at 60 °C followed by addition of alkylation buffer (10x of iodoacetomide in 55 mM ABC) to the samples. The samples were incubated in a dark place for 30 min at room temperature. Thereafter, samples were digested (1:50) by 20 ng/μl of sequencing grade modified trypsin (Promega, Southampton). Later on, 10% acetonitrile (ACN) (BDH) was added to activate the trypsin digestion binding and the samples were incubated overnight at 37 °C. Digested samples were then dried in a vacuum centrifuge (miVac DNA concentrator, Barnstead Genevac, Ipswich, UK) for 30 min at 30 °C. The proteomic samples by this step were reduced, alkalyated and digested and ready for fractionation step.

2.8. Strong cation exchange liquid chromatography

Reduced, alkalyated and digested samples were run through strong cation exchange (SCX) liquid chromatography in order to fractionate the samples from major proteins and to allocate them separate tubes. Peptides were separated by the column dimension (first off line dimension). The strong cation exchange process utilised three separation buffers. The injection volume was 200 μL and flow rate was maintained at 0.2 ml/min. Total separation time was 30 min for each sample. The peptide separation was monitored through a UV Detector UV 240U.

2.9. Liquid chromatography–mass spectrometry (LC–MS/MS)

Reverse phase HPLC was performed using CapLC™ system (Waters, UK) to separate the tryptic digests prior to MS analysis. Resolving peptides were first desalted using PepMap18 micro guard column (300 μm internal diameter × 1 mm) (LC Packings, UK). Peptides were transferred and eluted using buffer A (5% (v/v) ACN, 0.1% (v/v) formic acid) and buffer B (95% (v/v) ACN, 0.1% (v/v) formic acid) into. A Q-TOF Micro tandem MS (Micromass-Waters, UK). Two MS ions were selected for collision induced dissociation (CID) fragmentation after an initial precursor MS scan (scan range 400–2000 m/z). Preferential selection was given to +2 and +3 ions with at least 10 ion counts. All spectra acquisitions were performed using Mass Lynx software (Waters) and were searched against the SwissProt database (The Swiss Institute of Bioinformatics and The European Bioinformatics Institute, SwissProt 50.4, on 21st April 2008) using MASCOT 2.0 software (www.matrixscience.com) (Bairoch and Apweiler, 1997, Bairoch and Apweiler, 2000). Search parameters were limited only to mammalian databases; with allowance for 2 miss-cleavages using trypsin as the sole proteolysis agent, and mass tolerances of 0.5 Da MS and 0.3 Da MS/MS. Peptide carbamylation (C) and oxidation (M) were also included as variable modifications. The matched results were filtered using a threshold Mowse score of 30% and only proteins satisfying this criterion were considered for discussions.

2.10. Experimental design

2.10.1. Which sample preparation technique yielded to a higher quantity of proteins?

We compared the protein yield of two different sample preparation techniques, i.e. acetone precipitation and 2D clean up. A set amount of protein samples from uterine horn and oviduct was processed by acetone precipitation and 2D clean up and the resulted samples were compared by using BCA assay and gel electrophoresis images.

2.10.2. Do protein samples depleted have a higher resolution of spots in 2D gels compared to undepleted samples?

The proteomic samples were depleted from the major protein. Depleted and non-depleted samples were run through 2D gel electrophoresis. To understand which technology would produce a higher resolution and an increased number of protein spots detected. The results between the depleted and undepleted sample was assessed by gel spot image analysis.

2.10.3. Is the chance of protein identification by mass spectrometry increased after protein depletion?

To understand if an unknown proteomic sample, after depletion from highly abundant proteins, will have a higher chance to identify a larger set of protein species, we fractionated and examined proteins of depleted and undepleted proteomic samples by mass spectrometry analysis. Both proteomic samples were fractionated through SCX

and separated by LC and the protein peptides were identified by mass spectrometry.

3. Results

3.1. Acetone precipitation a higher quantity of proteins compared with 2D-clean up technique

Acetone precipitation protocol were performed for both samples; the uterine horn and the oviduct. Measurements were carried out in order to assess the protein concentration in each group for pre- and post-clean up techniques. Protein concentration was measured by using BCA assay. All samples were diluted serially as 1:10, 1:20 and 1:60. The experiments were performed in triplicate and the mean value was calculated. The protein concentration was decreased after cleaning step. The total amount of protein lost in uterine horn and oviduct samples were (35.22% ± 1.46) and (47.70% ± 1.75) respectively, as shown in Table 1. The total amount of protein (μg) was also decreased after acetone precipitation. The lost protein was also determined (in percentage).

In addition, the samples prepared from acetone precipitation and 2-D clean up kits were compared by 2-DE (gel image analysis) as depicted in Fig. 1. 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.

3.2. Proteomic samples depleted produce a high resolution of spots in 2D gels compared to undepleted protein samples

The purpose of using albumin and IgG depletion kit was to remove the abundant proteins to allow the mid and low abundant proteins to be detected. 2D-PAGE revealed a significant increase in the number of detected protein spots after depletion of major proteins (Albumin and IG groups). Also, the depleted protein gel is produced with higher resolution and fewer streaks. Fig. 2 illustrated the differences between the two groups.

3.3. The chance of protein identification by mass spectrometry is increased after protein depletion

Protein identification by mass spectrometry revealed a significant detection of low abundant proteins rather than high abundant. Proteins identified by LC–MS/MS from depleted samples (uterine horn and oviduct) are shown in Tables 2 and 3.

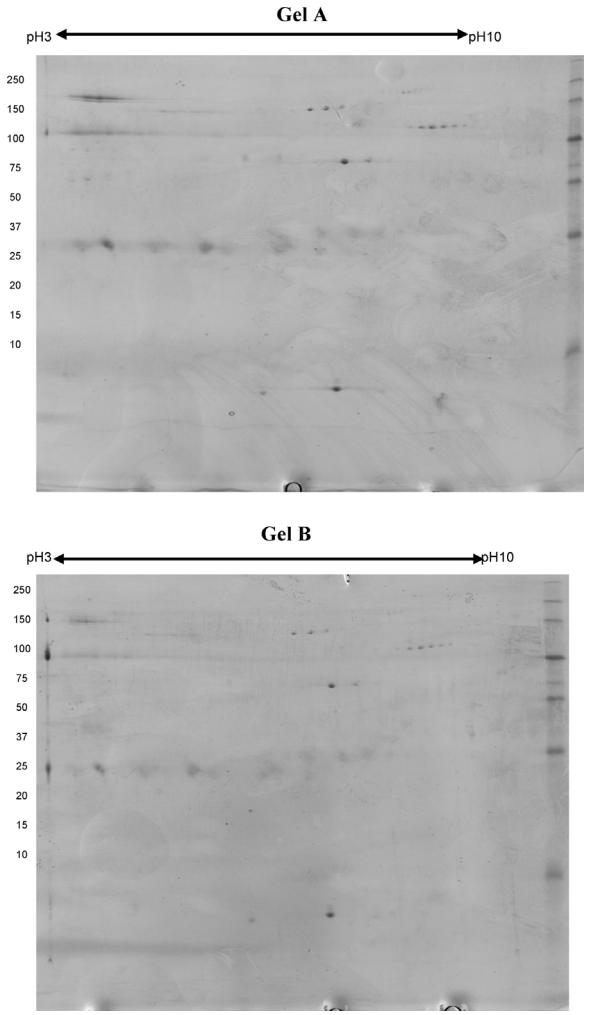

Fig. 1. Two different gels for oviduct with detectable spots of protein. Gel (A) was cleaned by the commercial kit 2-plus clean up while gel (B) was cleaned by acetone precipitation.

4. Discussion

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. 2DE is a powerful technique and allows for simultaneous separation of thousands of proteins. However, 2DE is a labour-intensive technology and has a relatively low throughput. The treatment of 2DE samples involves several steps of sample cleaning, cell lysis and protein solubilisation. Indeed, 2DE has limitations and

Table 1
Data from uterine horn and oviduct samples cleaned with acetone precipitation technique. The values are the mean value of triple measurements. Protein concentration was measured by BCA assay and mean ± SEM was calculated. AP: acetone precipitation.

	Uterine horn		Oviduct	
	Pre AP	Post AP	Pre AP	Post AP
Concentration (μg/μl)	12.42	4.53	8.95	2.40
Protein amount (μg)	298.08	226.75	304.3	143.7
Protein lost (%) after acetone precipitation	36.50% (±1.46)		47.22% (±1.83)	

Table 2
Identified protein from depleted oviductal sample.

Symbol	Acc. number	Protein name	Species	Score
TRFE	P09571	Serotransferrin	Sus Scrofa	44
HEMO	P50828	Heamopexine precursor	Sus Scrofa	47
HBA	P01965	Hemoglobin subunit alpha	Sus Scrofa	67
HPT	Q8SPS7	Haptoglobin precursor	Sus Scrofa	113
HBB	P02067	Hemoglobin subunit bet	Sus Scrofa	158

Table 3
Identified proteins from depleted uterine horn sample.

Symbol	Accession number	Name	Species	Score
HBB	P02067	Hemoglobin subunit beta	Sus Scrofa	85
HBA	P01965	Hemoglobin subunit alpha	Sus Scrofa	68

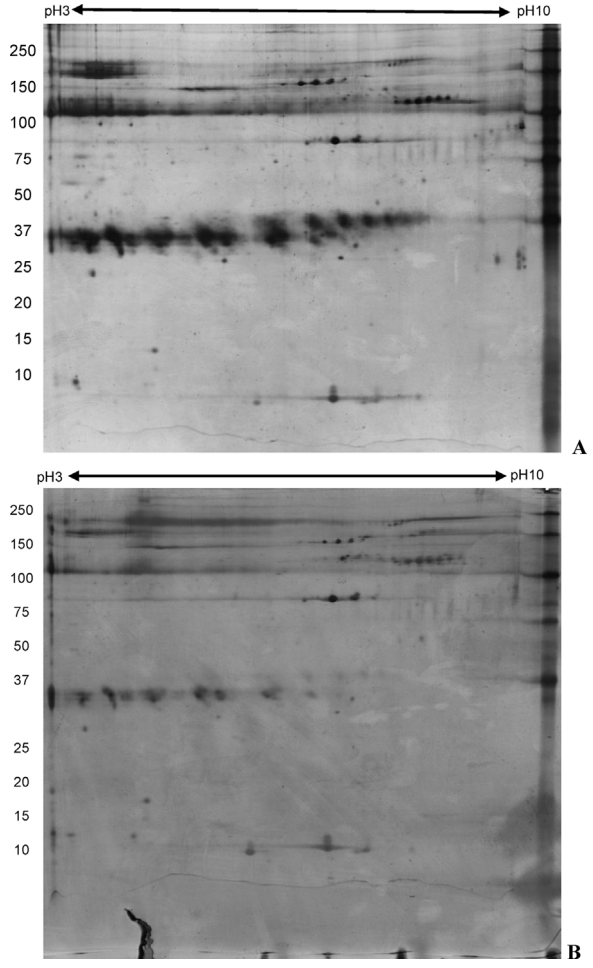

Fig. 2. Two different gels for the oviduct. (A) shows the undepleted proteins, (B) shows the depleted sample protein. The depletion step is crucial to eliminate the major abundant protein such as albumin and immunoglobulin.

many experimental drawbacks may happen during sample perpetration and protein separation. Extensive efforts have been made in order to overcome these limitations.

In the current study, we compared two different techniques for sample cleaning preparation. One technique was the acetone precipitation and the other was 2D commercial clean up kit. The total amount of lost proteins from both techniques was significant due to the cleaning processes. This observations were expected because of the chemical properties and desalting process. We confirmed these observations further by performing 2DE gel electrophoresis from both samples. Interestingly, no significant differences were observed between the two gels produced by the two techniques (Fig. 1). All our further experiments were based on an acetone precipitation clean up technique. Acetone precipitation showed to be easy-to-use and cost effective when compared to the 2D clean up commercial kit. In addition, this technique can be repeated twice or more for further purification of the proteomic samples.

We also attempted to deplete the samples from the major protein species. In the depleted samples it was possible to detect a significant number of spots, which represent mainly the mid and low abundant proteins. Thus, more spots are related to mid and low abundant proteins can be detected in depleted samples when compared to undepleted samples. In addition, in the depleted 2D-gels less spot streaking was visible. A number of gel spots that were dominant were excised from the gels and used for identification by mass spectrometry. Albumin and immunoglobulin were the major proteins identified by mass spectrometry in undepleted samples. Depletion process will increase the chance of identification of protein species other than highly abundant proteins such as albumin and immunoglobulins. Our data also revealed that the most dominant proteins such as albumin and IgG proteins were no longer detected in uterine horn and oviduct samples after depletion step. As a result of depletion and removing the major proteins, only mid and low abundant proteins were detected. In contrast, data from undepleted samples demonstrated that the albumin and IgG proteins were the most dominant detected protein species. Hence, a further step is required to remove the other dominant proteins such as transferrin and haemoglobin in order to detect further the low abundant proteins. Our current data clearly recommend using a depletion step strategy prior to determination of the proteome profile of a given proteomic sample.

In fact, the protein spots presented in 2DE gel represent only a portion of all the proteins that are present in a sample [24]. Generally, the proteins are visualised in 2D gels

by Coomassie staining methods are high-abundance proteins. On the other hand, low-abundance proteins, which are not detected by the staining, include regulatory proteins, receptors, and other proteins that play key roles in cellular processes. In addition, the other particular proteins class that is not detectable by 2DE are membrane proteins. The main factors for 2DE analysis of membrane proteins are the poor solubility of the samples in standard protein extraction solutions and low abundance [25].

In conclusion, we demonstrated different techniques to purify the proteomic samples. We identified acetone precipitation as a more efficient and cost effect technique for cleaning up of proteomic samples prior to 2D gel analysis. Depletion of given proteomic samples from the most abundant protein species is strongly recommended to allow the mid and low abundant protein species to be detected. In addition, a better resolution of the gels will be achieved by removing the major proteins such as albumin and immunoglobulin.

Although a proteomic approach to establish and study maternal interaction with spermatozoa or embryo looks interesting and attractive, there are several hurdles and shortcomings in application of proteomic analysis to defined sperm-oviduct experimental systems. For example the amount of the lost proteins during the sample preparation process is significant. Also, the subculture of oviductal cells and the number could have an effect [26]. An alternative method should be found to replace this process. The quantity of samples needed for analysis, poor resolution of analysis, aggregation of proteins and streaking, the semi quantitative nature of proteomic analysis and the labour and time utilised to obtain data, encouraged us to look for other technologies for studying transcription activities in oviductal cells as a result of interaction with spermatozoa. Thus, an established system and well characterised *in vitro* system based on genomic-technologies [27] is suitable to test our hypothesis in future. Such system will allow us to maintain and handle the shortcoming of using primary cell culture system.

Conflict of interest

The authors declare that no conflict of interests.

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