Separating of bovine seminal plasma proteins by using 2D-PAGE revealed the different profile of PDC-109

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Abstract: Several lines of evidence showed that some proteins in seminal plasma were correlated to bull fertility and can be used as the potential protein markers for identifying the fertile bulls. This preliminary study aimed to find out any different expression proteins in seminal plasma of the bulls by using proteomic approach. Seminal plasma proteinsfrom 4 Brahman bulls, 3-5 years of age, were separated using 2D-PAGE followed by staining with Coomassie blue. At least a triplicate gels was performed for each sample. Each gel was scanned with an ImageScanner System and analyzed for spots by ImageMasterTM 2D Platinum software. The different expression protein spot(s) was cut from the gel and identified by LC MS/MS. The results showed that at least 200 protein spots were detected in the seminal plasma of the Brahman bulls. Of all these spots, the relative protein content of2 spots at 16.5 kDa, pI 5.3 and 5.5 expressed differently among 4 bulls. Both spots were identified as PDC-109. It was concluded that by using 2D-PAGE, PDC-109 was a different expression protein in seminal plasma of the Brahman bulls. Further study of this protein in relation to bull fertility may lead to one promising marker for selecting the fertile bull.

Introduction

Bull fertility is an important factor affecting the bovine reproduction. Breeding with high fertility bulls increases cattle production and gains more profitability. The classical evaluation of bull fertility bases on breeding soundness examination (BSE) which consists of physical examination, reproductive tract examination, measurement of scrotal circumference and semen quality examination are routinely used (Chenoweth et al., 1993). However, there were reports on the range beef bulls and the bulls at some AI centers that differently showed as much as 7 to 40% non-return rates, including bulls that have passed routine BSE test (Bellin et al., 1998; Killian et al., 1993). These evidences indicated that the using of BSE, though providing useful information but some limitations was existed. Recently, with the advance of proteomic analysis, it revealed the seminal plasma origin-proteins as the potential fertility markers and can co-consider used with BSE to evaluating bull fertility. Osteopontin (OPN) and lipocalin-type prostaglandin D syntase (PGDS) were good examples of such seminal plasma proteins as they were found predominated in higher-fertility bulls (Cancel et al., 1997; Gerena et al., 1998; Killian et al., 1993). Moreover, the bulls with higher fertility possessed seminal plasma origin-proteins such as acidic seminal fluid protein (sSFP) and fertility associated antigen (FAA) in spermatozoa membrane (Roncolleta et al., 2006; Sprott et al., 2000).

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As mention above, seminal plasma which is a complex mixture fluid secreted from testis, epididymis and accessory sex glands contains the potential proteins that modulate bull fertility and can be used as the protein markers. This preliminary study aimed to investigate the seminal plasma proteins of the bulls that have normal fertility (passed BSE test) whether there is any proteins that express differently.If so, it may be a promising fertility marker.

Materials and Methods

Experimental animals

Four Brahman bulls—*maintained at the NorthEasternBull Center, Thailand*—were used. The bulls were between 3 to 5 years of age with normal fertility, proved by BSE test according to minimum requirements of the Society for Theriogenology{Chenoweth, 1993, Guideline for Using the Bull Breeding Soundness Evaluation Form}(Chenoweth et al., 1993). The criteria are thattheir semen quality in term of progressive motility and normal morphology greater than 70%. The animals were fed concentrated feed and roughage. Only healthy bulls on the day of semen collection were included in this experiment.

Semen and seminal plasma collection

Semen samples were collected by using an artificial vagina. The first ejaculated semen was used in this study. Seminal plasma was obtained from the semen by centrifuging at 800× *g* for 5 min. The supernatant seminal plasma was then transferred to 1.5 ml tubes and centrifuged at 10,000× *g* for 60 min at 4ºC. The supernatant of each sample was divided into 1.5 ml aliquots and stored at -70ºC.

Seminal plasma proteins separation by 2D-PAGE

Before performing 2D-PAGE, the seminal plasma was assayed for total protein concentration (Bradford, 1976) using bovine serum albumin as the standard. The 2D-PAGE was performed according to O'Farrell (1975). Briefly, the isoelectric focusing (IEF) was performed in an EttanIPGphor II Isoelectric Focusing System in 7 cm IPG DryStrip with a pH range 3-10. Prior to the IEF, the seminal plasma sample was diluted to 60 μg of protein in 125 μl of re-hydration solution. This mixing solution was loaded to strip holder, placed IPG dry strip face down and covered with mineral oil. The strip holder was then placed on the plate of Electro-focusing chamber and re-hydrated for 12 h. A low voltage gradient was applied (0-3500 V) for 90 min and then 3500 V for 7 h. The focused strip was immediately equilibrated in a buffer containing 1% w/v DTT for 30 min then changed to equilibrate in a buffer containing 2.5% w/v IAA for 30 min. After being equilibrated, the strip was done in a second dimension of 13% SDS-polyacrylamide gel. The low molecular weight standard—range, 14.4-97 kDa —was loaded to the gel. The vertical setup was used for 1 gel, using 10 mA for 15 min and then changed to 20 mA at constant voltage of 150 V for 105 min. The gel was fixed for 1 h and stained in colloidal Coomassie Brilliant Blue G-250 overnight. The gel was de-stained with de-ionized water until the background had cleared. At least triplicate gels were performed for each sample. Each gel was scanned with an ImageScanner System and analyzed spots by ImageMaster™ 2D Platinum software.

Protein identification by LC MS/MS

The different expression protein spot(s) among experimental bulls was cut from gel and sent for identification at the GENOME Institute, Thailand, using the LC MS/MS technique. The spot samples were digested with trypsin enzyme; then the digested peptides were analyzed by LCMS/MS mass spectrometry. The molecular weight values of the trypsinized peptides—obtained by LC MS/MS—were then used to identify the predicted proteins using MASCOT web-base search engines.

Results and Discussions

When 60 µg of total protein from bovine seminal plasma was assayed by 2D-PAGE and stained with colloidal Coomassie Brilliant Blue, more than 200 protein spots with a pI of 3-10, and M_r of 10-97 kDa could be detected (Figure 1). From all of these spots, there were two spots of 16.5 kDa, pI 5.3 (X_1) and 16.5 kDa at pI 5.6 (X_2) which clearly showed different expression among the 4 Brahman bulls (**Figure 1**). Of 4 bulls (a) one had the high relative protein content (volume), (b) two of medium amount and (c) one of low amount of spot X_1. For spot X_2, 2 bulls had a large amount and the other 2 bulls had a few amount. Interestingly, identification of spot X_1 and X_2 by LC MS/MS found that both spots matched to the bull seminal plasma, named PDC-109 with a respective score of 413 and 562.

PDC-109 was the member of BSP protein family which was largely secreted from the seminal vesicles (Nauc and Manjunath, 2000). This protein binds to spermatozoa membrane at ejaculation (Manjunath et al., 1993). It was referred to the identical of BSP-A1 and BSP-A2 in their primary structure of 109 amino acid residue and differed only in the degree of glycosylation (Manjunath and Sairam, 1987). According to the report of Zaia (2004) the glycosylation proteins increases the complexity of protein molecules and causes them to migrate as diffuse spots on SDS-PAGE gels, it make room to believe that the various M_r/pI values of PDC-109 which found in this study occurred from glycosylation.

The function of PDC-109 involved in sperm capacitation by anchoring heparin to the sperm surface and by stimulating sperm cholesterol efflux (Moreau et al., 1998). In addition, PDC-109 also plays a role in forming an oviductal spermatozoa reservoir by enabling sperm to bind to oviductal epithelium (Gwathmey et al., 2003). Furthermore, in vitro study showed the functions of PDC-109 as a molecular chaperone, suggesting that it may assist the proper folding of proteins involved in the bovine spermatozoa capacitation pathway (Sankhala and Swamy, 2010).

These evidences indicated that PDC-109 was the seminal plasma originated-protein and binds to spermatozoa membrane at ejaculation. Since PDC-109 plays a crucial role on fertilization, especially the acrosome reaction and capacitation process, result of this studythat there were different profile of PDC-109, both of level and its various M_r/pI values,may imply the different ability of spermatozoa to fertilization and subsequently bull fertility. This should be elucidated, so that PDC-109 could be the potential fertility marker for fertile bulls and have valuable applications in bovine reproduction.

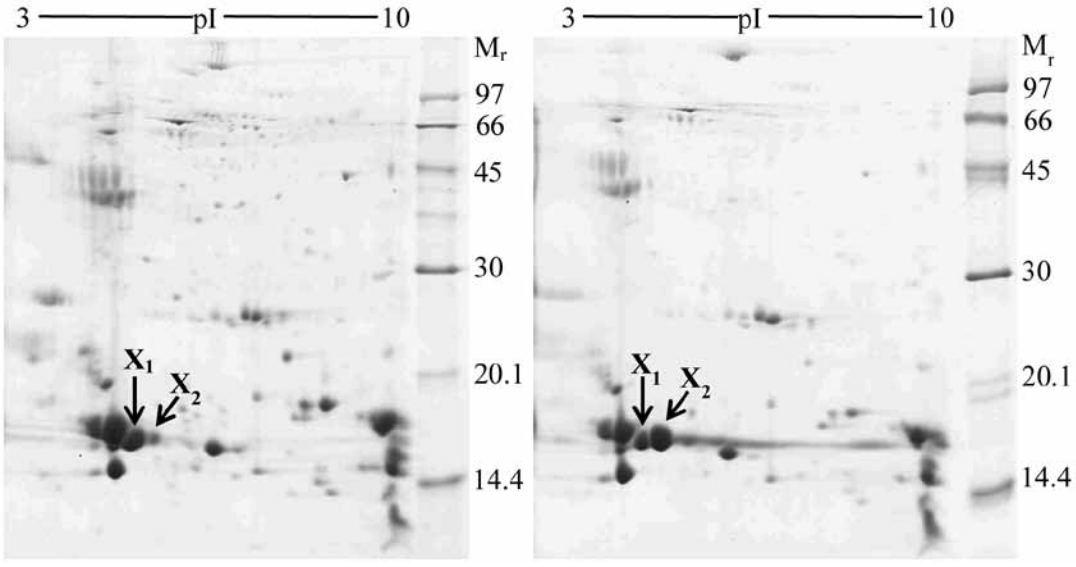

Figure 1 2D-gel of seminal plasma proteins from bulls with different expression of spot X1 and X2. Location of spot X1 (16.5 kDa, pI 5.3) and X2 (16.5 kDa, pI 5.6) are indicated with an arrow. 60 µg of protein was electro-focused in 7 cm DryStrip gel (pH range, 3-10). SDS-PAGE was conducted in a 13% acrylamide gel plate. The Mr standard used ranged between 14.4-97 kDa. In this figure, Mr is on the Y-axis and pI (3-10) on the X-axis. Colloidal Comassie Brilliant Blue G-250 was used for protein staining.

Conclusions

This study found PDC-109, the seminal plasma protein shown at 16.5 kDa, pI 5.3, and 16.5 kDa, pI 5.6 on 2D-gels. The relative protein content of PDC-109 at various M_r/pI was clearly differed in 4 bulls. It provided promising fertility marker in which further study required to elucidate.

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