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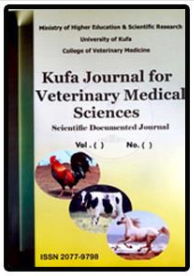

Characterisation of ADAMs (protein)of bovine seminal plasma by Mass Spectrometry

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Abstract

This study has been designed to analyse the secreted and soluble proteins in bovine seminal plasma by mass spectrometry. The majority of these proteins are produced by accessory glands, and partially by testis, epididymis, ductus deferens and vas deferens of male reproductive tract.

Seminal plasma of bovine was collected freshly and isolated after centrifuged and removed the sperm. Non boiled and boiled seminal plasma lysate were run to identify and detect the total proteins, as well as the individual single member of ADAMs protein is determined.

The non-boiled lysate of seminal plasma was displayed diversity of proteins more than boiled sample lysate of seminal plasma.

However, boiled and non-boiled seminal plasma have distinguished several types of ADAMs protein which are included: ADAM10, ADAM9, ADAM7, ADAM15 in non-boiled samples, while boiled lysate of bovine seminal plasma was displayed ADAM10, ADAM9, ADAM28, and ADAM22.

Our finding concluded that bovine seminal plasma is very rich in different types of soluble, cleaved and shed proteins such ADAMs protein which could potentially have a biological and physiological role in protection and interactions of sperm during motility inside female reproductive tract, as it might support it to fertilise the ovum. This protection might be via immunosuppression behaviour or by block specific receptors in female reproductive tract for enhancing sperm motility, and avoid sperm the singling response of immunity.

Keywords **: Bovine seminal plasma, Mass Spectrometry, Proteins, ADAMs, Immunosuppression and Motility.**

وصيف ADAMs (بروتين) في البلازما المنوية البقري بواسطة تقنية المطياف الكتلي للبروتينات

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الخلاصة

صُممت هذه الدراسة لتحليل البروتينات المفرزة والقابلة للذوبان في البلازما المنوية البقريّة بواسطة تقنية المطياف الكتلي للبروتينات ، بصورة رئيسية يتم إنتاج معظم هذه البروتينات عن طريق الغدد الملحقة الذكرية ، وجزئيًا عن طريق الخصية والبربخ والقنوات القاذفة والوعاء الناقل في الجهاز التناسلي الذكري.

في هذة الدراسة تم جمع البلازما المنوية من الثيران بصورة مباشرة وتم عزلها من الحيوانات المنوية بعملية الطرد المركزي. وبعد ذلك عرض قسم منها للغليان والقسم الاخر بدون غليان ، ثم تم حقنها في تقنية المطياف الكتلي للبروتينات للتعرف على البروتينات المكونة للبلازما المنوية ، وكذلك تحديد انواع محددة من بروتينات تدعى (ADAMs) .
اظهرت العينات غير المغلية للبلازما المنوية تنوع كبير في البروتينات مقارنة مع عينات البلازما المنوية المغلية ومع ذلك ، فقد ميزت البلازما المنوية المغلية وغير المغلية عدة أنواع من بروتين ADAMs التي تم تضمينها: (ADAM10، ADAM9، ADAM7، ADAM15) في العينات الغير مغلية ، بينما تم اظهرت العينات المغلية البلازما المنوية البقرية (ADAM10، ADAM9، ADAM28 ، ADAM22) .
خلصت الدراسة الحالية إلى أن البلازما المنوية البقرية غنية جدًا بأنواع مختلفة من البروتينات الذائبة والمجزئة مثل بروتين ADAMs الذي يمكن أن يكون له دور بيولوجي وفسيولوجي في حماية وتفاعل الحيوانات المنوية أثناء الحركة داخل المسالك التناسلية للإناث ، وايضا دعمها لتخصيب البويضة وقد تكون هذه الحماية من خلال سلوك تثبيط المناعة أو عن طريق غلق مستقبلات محددة في الجهاز التناسلي للأنثى لتعزيز حركية الحيوانات المنوية ، بالاضافة الى تجنب الحيوانات المنوية الاستجابة للمناعة اثناء مرورها في الجهاز التناسلي الانثوي.

Introduction

Seminal plasma is secreted with semen after ejaculation of male organ, it is produced by male accessory glands of bull (vesicular (seminal vesicles), prostate, bulbourethral gland (Cowper's glands), ampullary part of the Vas deferens [1, 2].

The biochemical structure of seminal plasma is comprised with different types of sugars and proteins, which consist of main sugars fructose, glucose [3] and three basic proteins which constitute the bovine seminal plasma: BSP-A1/-A2, -A3 and -30 kDa [4]. These biochemical variety compositions of seminal could be impaired fertility and infertility dependent [5].

Many proteomic studies have conducted on seminal plasma and identified very divers of proteins, a few of them is described first time, and some was found in individual seminal plasm of species and other absence in other species [6, 7].

Disintegrin and metalloproteinase (DAMs) are a family of single-pass transmembrane and classified as sheddase proteins because is secreted metalloendopeptidases. ADAMs have externally particular domains presenting a pro-domain, a disintegrin, a metalloprotease, a cysteine-rich, an epidermal-growth factor like and a transmembrane domain, and internally C-terminal cytoplasmic tail [8, 9].

There are different types of ADAMs proteins are distinguished in human and these proteins were expressed in different locations of the body, this is indicated that ADAMs might be had main biological functions in human body. Also, they have metalloprotease and integrin receptor-binding activities, and a cytoplasmic domain and this specify award them establishes for various signal transducing proteins [10, 11].

Many researchers have characterised several types of ADAMS family of human and detected ADAMs (1,2,7,8,9,10,11,12,15,17,17,18,19,20,21,22, 23,28,29,30, and 33)[12-20].

Furthermore, ADAMs have basic functions in the body: cell-cell reactions, regulations of cells, motifs, signals, presence of different of domains, C-terminus and a few have peptidase, all these different jobs make them impact on development, homeostasis, and diseases where they are expression in the body [6].

Eventually, 34 members of ADAMs family has been identified up to date, and most of the ADAMs contain a metalloprotease-like domain [10]. Moreover, a few of ADAMs are shown that have a main role in spermatogenesis and sperm functions, possibly by influencing on maturation of sperm and their adhesion and motility of sperm in the uterus and uterine tube [16] .

ADAMs are considered a proteolysis and adhesion proteins, these particular structure and contents of them they might be affected on sperm-egg fusion, ecto-domain shedding, somatic cell-cell adhesion, myoblast fusion and development [21, 22].

This study is directed to determine total proteins of bovine seminal plasma, and to characterise the presence of ADAMs proteins of seminal plasma.

Materials and Methods

Samples

Fresh bovine sperm was collected and placed in Eppendorf and centrifuged for 5 minutes at 4^{o}C and 18400 g rpm. The supernatant was removed, and diluted with HEPES and frozen (-80^{o}C) for three hours, then thawed and store again for next day. The pellet was neglected. Next day, samples was thawed and added 10%Tissue cell lysis buffer, some samples were boiled and other were not boiled. After that, boiled and non-boiled samples were treated as the same.

Digestion of Protein and Mass Spectrometer

The lysate of both samples were dissolved by the addition of 600 µl of fresh 8M urea and mixed thoroughly. After that, 50 µl of the suspension was relocated into a 0.5 ml Eppendorf, and 1µl of DTT (10 mM final concentration) was added, vortex, and incubated for 10 minutes at room temperature. Also, 2.5 µl of 10mM iodoacetamide was added, and followed by 150 µl of 200mM ammonium bicarbonate buffer to adjust the pH to neutral. Added 245 µl of reaction buffer plus 5 µl (µg) trypsin, and the digestion was incubated overnight at 37^{o}C. Next day, the lysate was run and analysed by Mass Spec machine (FT-ICR/Orbitrap) in the Conway Institute. University College Dublin.

Lysis buffer

Tissue cell lysis buffer was used according to manufacturer instructions, and before using the tissue buffer and thawed it, one tablet of protease inhibitor (Roche cat no. 11836170001) was added.

Analysis of data

The files were collected from Mass Spec machine (FT-ICR/Orbitrap) in the Conway Institute and analysed by Peak7 studio software which exhibited the sequence of amino acids and similarity of protein. The parameters which were followed in Peak7 studio software showed in the table (1).

1	Peptide -10lgP	≥15
2	Protein -10lgP	≥15
3	Proteins unique peptides	≥0
4	De novo ALC Score	≥50%

Result

The boiled and non-boiled lysate were run in Mass Spec machine (FT-ICR/Orbitrap) and analysed to classify different types of proteins. The two groups are presented variety of proteins. However, the non-boiled lysate was showed the highest level and abundances of proteins (1970 different types of proteins) figure (1, and 2) and supplementary (1) compare to the boiled lysate which is displayed (1033 different types of proteins) figure (3, and 4) supplementary (2).

Figure (1) False discovery rate (FDR) curve for Non boiled Seminal plasma. X axis is the number of peptide-spectrum matches (PSM) being kept. Y axis is the corresponding FDR.

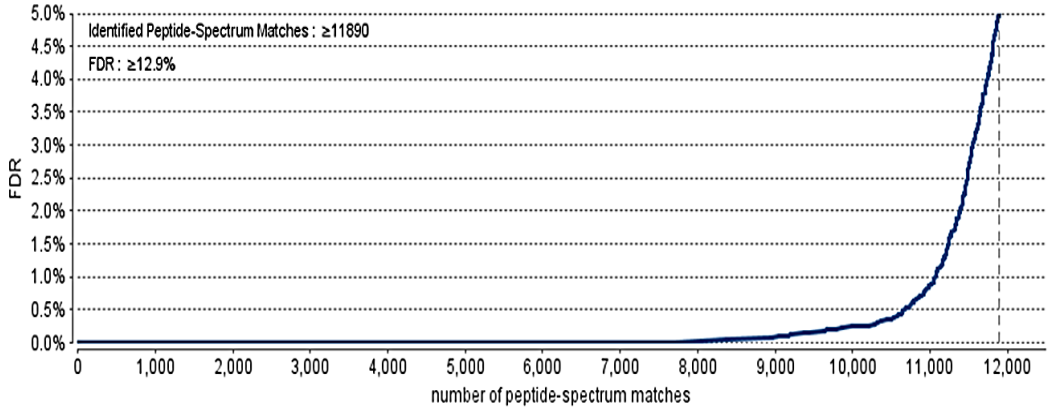


Figure (2) PSM score distribution. **(A)** Distribution of PEAKS peptide score; **(B)** Scatterplot of PEAKS peptide score versus precursor mass error. (Non-boiled Seminal plasma).

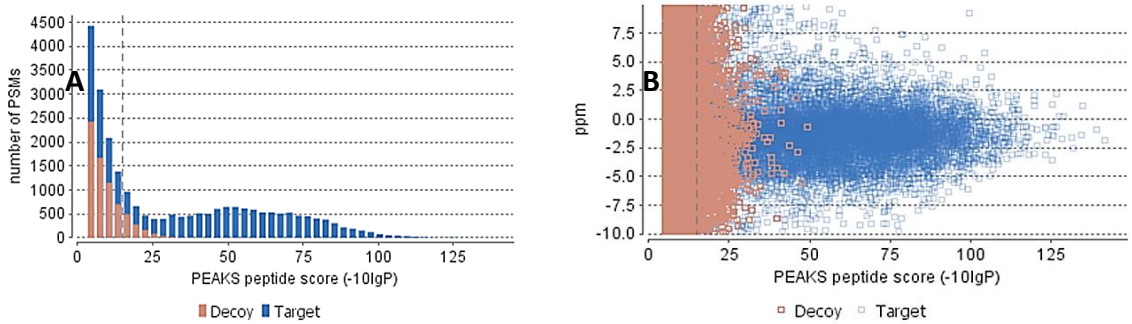


Figure (3) False discovery rate (FDR) curve for Boiled Seminal plasma. X axis is the number of peptide-spectrum matches (PSM) being kept. Y axis is the corresponding FDR.

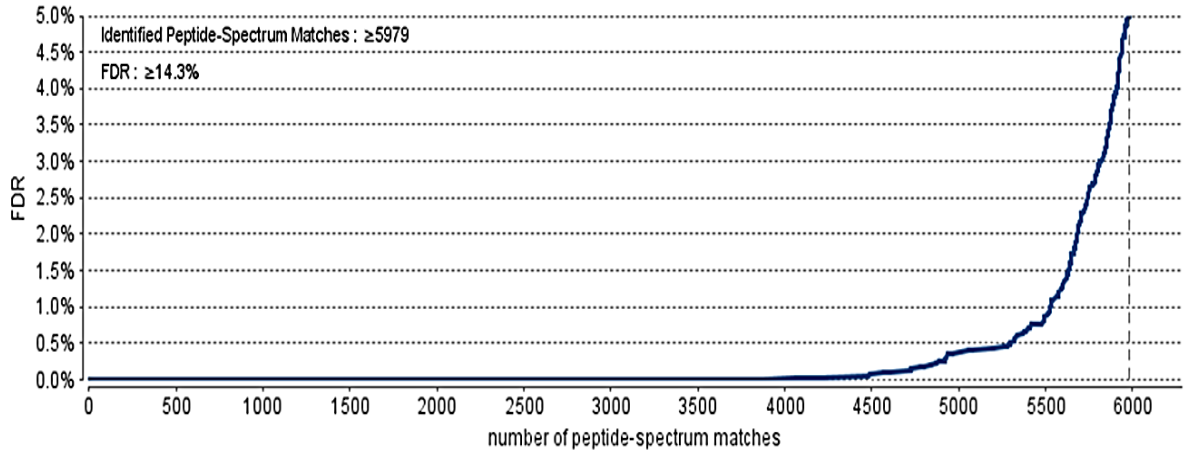
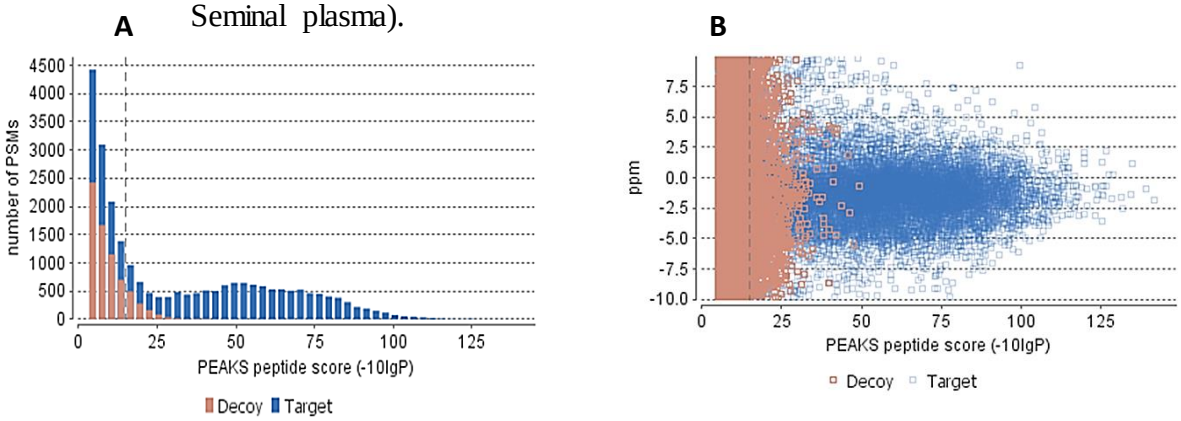


Figure (4) PSM score distribution. **(a)** Distribution of PEAKS peptide score; **(b)** Scatterplot of PEAKS peptide score versus precursor mass error. (Boiled Seminal plasma).



The existing study is focused on specific interesting proteins which called ADAMs protein for first time in bovine species, for that reason the analysis of data was revealed two types of ADAMs proteins: characterised and uncharacterised ADAMs proteins, in the lysate of bovine seminal (non-boiled Lysate) is revealed:

1. Disintegrin and metalloproteinase domain-containing protein 10 (ADAM10)
2. Uncharacterized protein (ADAM9)
3. Uncharacterized protein (Fragment) (ADAM7)
4. Uncharacterized protein (ADAM15)
5. Uncharacterized protein (ADAM32)
6. Uncharacterized protein (Fragment) (ADAM1B)

While boiled lysate of bovine seminal plasma presented:

1. Disintegrin and metalloproteinase domain-containing protein 10 (ADAM10)
2. Uncharacterized protein (ADAM9)
3. Uncharacterized protein (Fragment) (ADAM28)
4. Uncharacterized protein (Fragment) (ADAM22).

Boiled and non-boiled lysate of seminal plasma is shown same ADAMs members (9 and 10), but also they are revealed other different members of ADAMs, this might be caused by influence of temperature on contents of seminal plasma.

Discussion and Conclusion

In this study, the expression of several soluble and cleaved proteins were detected on bovine seminal fluid by mass spectrometry and some of these proteins are described first time in bovine seminal plasma. However, non-boiling seminal plasma was revealed more abundance of proteins compare to boiling seminal plasma, this confirmed that boiling temperature can be changed proteins structure and effect on particular proteins dependent [23]

Many studies have applied on seminal plasma and identified various proteins in seminal plasma, these proteins have variable biological kinds which can play a major role sperm migrations and fertilisation [6, 24].

Interestingly, result is firstly discovered new ADAMs proteins in bovine seminal plasma, some characterised ADAMs and other not. Additionally, non-boiled lysate samples is shown (ADAM9, ADAM7, ADAM15, ADAM32 and ADAM1B) and characterised protein ADAM10, while boiled lysate samples revealed characterised (ADAM10), and uncharacterized protein (ADAM9, ADAM28and ADAM22) some of these

proteins have been reported in bovine seminal fluid[6]

Common expression patterns of ADAMs on seminal plasma lysate suggests an extent biological functions may be mediated sperm dependent interactions within female reproductive tract [10, 25], this evident that ADAMs could be involved in several regions including such as: regulation of sperm migration, transcervical mucus progression, as well as the interaction of sperm with the oviductal epithelium and reservoir before interaction with oocyte.

These ADAMs also may have intracellular signalling domains and may intermediate specific sperm functions including motility, egg-sperm fusion and energy metabolism [26, 27].

Overall, the result is shown that components of bovine seminal plasma has variety of several types of soluble proteins, as boiled lysate may be degenerated or destructed the amino acid sequences of some of protein due boiling temperature. On the other hand, mass spectrometry is determined different members of ADAMs family in boiled and non-boiled lysate of seminal plasma, which could internally impact on sperm development and maturation process and externally during migration of sperm in external environment such as regulation, signal transduction, plasma membrane cell interaction, immunosuppression, motility and oocyte-sperm reaction.

This research has been detected specific sheddase and cleaved proteins which are described first time in bovine seminal plasma, subsequently they could be a good indicator for researchers to bring the clear understanding for migration of sperm and fertilisation.

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