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Research Article

Documentation of Seminal Plasma Protein Profiles in Dromedary Camels

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Abstract

The purpose of this study was to establish the protein profile of seminal plasma of dromedary camels with reference to their rutting behavior. Semen ejaculates were obtained from seven adult male dromedary Jaisalmeri camels. Five camels were performing copulation successfully and two camels not showing all the signs of rutting. Fresh semen samples were centrifuged at 6000 rpm for 30 min. and clear supernatant of seminal plasma was separated and stored at -20°C until used. Total protein from the seminal plasma was estimated by Lowry Method and the molecular weight was analysed by SDS-PAGE. A total of 10 protein bands were observed in the seminal plasma of rutting camels ranged from 11.7-104.0 kDa, whereas, 9 protein bands were observed in the seminal plasma of non-rutting camels. These 9 protein bands were found to be common in rutting and non-rutting camels. However, additional protein band of 40.0 kDa with very strong intensity was observed only in rutting camels and found to be heat resistant at 38 ºC up to 18 hours. The relevance of 40.0 kDa proteins in the fertility status of dromedary camels deserves further investigation. .

Keywords: Dromedary Camel; Fertility Marker; Seminal Plasma Protein; SDS-PAGE Analysis

Introduction

The semen in mammals including livestock species is the sperm-carrying collective secretions of various accessory glands like the seminal vesicles, the prostate gland and the bulbourethral glands. Seminal plasma proteins are involved in the regulation of osmotic pressure and pH of the seminal plasma, transport of ions, lipids and hormones [1] and play an important role in extending the life of spermatozoa [2].

The camel (*Camelus dromedarius*) has a prostate, two ampullae ductus deferentes and two bulbourethral glands, but no seminal vesicles [3]. Very little is known about the proteins present in camel seminal plasma, and the role of seminal proteins in reproductive physiology of the species. Possibilities for inducing ovulation in females by seminal plasma in bactrian camel were investigated [4] and found that ovulation cannot be induced by manual stimulation of the vagina, and the uterus as is the case in cat and the rabbit, nor by the presence of spermatozoa. The authors showed that the seminal plasma contains an ovulation-inducing factor (OIF) that is very stable for a long time when the semen is stored in the freezer [4]. It has been reported that major OIF in llama (*Llama lama*) seminal plasma is a protein molecule which is resistant to heat and digestion with proteinase K, and has a molecular weight of equal to or higher than 30 kDa [5]. OIF has been identified as β-Nerve growth factor in seminal plasma of llamas [6] and alpacas [7]. Zinc α2-glycoprotein (ZAG), a 40 kDa protein was found in ram, buck, alpaca and camel seminal plasma [8].

The functional significance of seminal plasma is questionable in that pregnancy can be induced in some species by insemination with spermatozoa alone. It appears to be an essential component in natural mating because it serves as a carrier and protector of the spermatozoa [9]. Intramuscular or intrauterine deposition of seminal plasma has been found to induce ovulation in alpacas and llamas [10]. Hence, studies on protein profiles of seminal plasma could provide information on their biological significance in relation to reproductive physiology. Therefore, the objective of the present study was to determine the protein profile of seminal plasma of dromedary camels, with reference to their rutting behavior.

Materials and Methods

Materials

All chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise indicated.

Semen collection, evaluation and seminal plasma separation

109 semen ejaculates were collected after 163 attempts from 7 adult dromedary camels of Jaisalmeri breed at livestock farm of National Research Centre on Camel, Bikaner (India). The animals were apparently healthy and were maintained under uniform standard conditions of feeding and management. The research program was approved by the institute research and ethical committee. Semen samples were collected twice a week during rutting season (December to March) as described previously [11]. A female camel was restrained in the sitting posture and the male was allowed to mount her. Ejaculates were collected with a 12" long artificial vagina (I.M.V. France, catalogue number- 005417), internal temperature of the artificial vagina was maintained at 41 to 42 ºC with hot water during the entire course of copulation. The semen collected was every time subjected to general analysis *viz*., volume, consistency and colour.

Fresh semen samples were centrifuged at 6000 rpm for 30 min. at 4 ºC and sperm-free seminal plasma was separated [12]. Cocktail of protease inhibitors was added in seminal plasma samples to inhibit the proteolytic activity and seminal plasma was stored at -20°C until used (100µl protease inhibitor cocktail used/ml of seminal plasma; Sigma-Aldrich, USA, catalogue number- P 8340). At the time of use, the stored seminal plasma was thawed, and animal wise ejaculates were pooled. A portion of seminal plasma was also kept at 38 ºC for 6-18 hours to see the effect of temperature on seminal plasma protein profiles.

Total protein estimation in seminal plasma

Total protein was estimated in seminal plasma samples so that an equivalent amount of protein could be used for gel preparation. Protein concentrations from each fresh seminal plasma sample were estimated [13].

Electrophoretic analysis of seminal proteins

Standard SDS-polyacrylamide-gel electrophoresis of seminal plasma proteins was carried out under reducing conditions at 30 mA constant current [14] using 15% polyacrylamide gel. The gel containing electrophoresed proteins was stained with Commassie brilliant blue R-250. The gel was destained with 10% (v/v) acetic acid solution until the gel was free of excessive stain and the protein bands were clearly visible. Gel images were scanned with Chemi Doc XRS System (Bio-Rad) and analyzed by using Quantity One Software (Bio-Rad). The molecular weight of the proteins was estimated by using reference protein molecular weight markers run simultaneously (Bangalore Genei, India catalogue number- RPMW-M) ranging from 14.3-97.4 kDa: Phosphorylase b (97.4 kDa), bovine serum albumin (66.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (29.0 kDa), Soyabean trypsin inhibitor (20.1 kDa), and lysozyme (14.3 kDa).

Results

Semen collection and its evaluation

The volume, colour and consistency of semen varied from animal to animal. Seminal volume ranged from 1.0 to 9.8 ml with a mean ejaculate volume of 3.09 ± 0.19 ml. In our study, camel semen was observed to be white or yellowish white in colour and had thick to thin viscosity. 46.49% semen samples were highly thick, 32.46% thick and 21.05% thin water-like consistency. Semen was successfully collected from all camels and semen collection frequency was found to be 66.87% (Table 1). The incidence of aspermic ejaculates varied from 6.25 to 18.75% in five rutting camels and 66.67 to 71.43% in two non-rutting camels (Table 1). Thick to thin consistency was also observed in a spermic ejaculate.

Total protein estimation in seminal plasma

The total seminal plasma proteins were estimated in seminal plasma samples and presented in table 1.

Electrophoretic analysis of seminal proteins

Ten protein bands of different intensities were observed and found to be in range of 11.7-104.0 kDa (i.e., 11.7, 17.4, 23.0, 28.0, 40.0, 65.1, 70.8, 81.6, 88.1 and 104.0 kDa) in the seminal plasma of animal numbered as J-228, J-230, J-218, J-242 (Figure 1 lanes 2-5) and J-112 (Figure 2 lanes 2-5) respectively. A total of nine protein bands were observed in seminal plasma of J-120 (Figure 1 lane 6) and J-224 (Figure 2 lanes 7-10). A protein band of around 40.0 kDa with a strong intensity was not observed in J-120 (Figure 1) and J-224 (Figure 2).

Camel No. (Rutting status)	No. of attempts	No. of times ejaculated semen	No. of times a zoo spermic ejaculate	Total protein (g/dl) Mean ± S.E (Range)
J-112 (In rut)	23	15	2	3.55 ± 0.10 (2.67-4.39)
J-224 (Not in rut)	26	14	10	3.69 ± 0.12 (2.50-3.97)
J-228 (In rut)	18	12	1	3.06 ± 0.09 (2.90-3.67)
J-230 (In rut)	20	16	3	2.46 ± 0.07 (2.50-3.43)
J-218 (In rut)	24	18	2	2.63 ± 0.11(2.20-3.56)
J-242 (In rut)	20	16	1	2.88 ± 0.14 (2.50-3.28)
J-120 (Not in rut)	32	18	12	2.95 ± 0.12 (2.65-3.78)

Table 1: Number of attempts, successful semen ejaculations, a zoo spermic ejaculates and total proteins in seminal plasma (g/dl) of 7 camels.

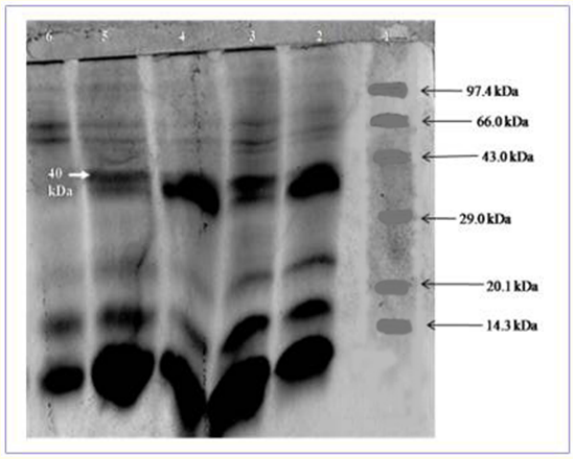

Figure 1: Fresh seminal plasma protein profiles of Jaisalmeri (J) camels. Lane 1: Marker; Lane 2: J-228; Lane 3: J-230; Lane 4: J-218; Lane 5: J-242; Lane 6: J-120. 40 kDa protein band expressed in 4 camels (Lanes 2-5) and not in 1 camel (Lane 6).

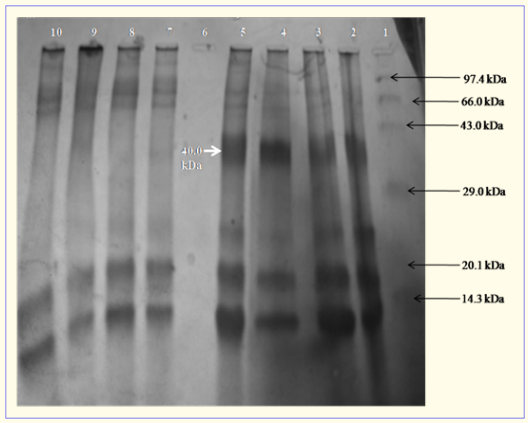

Figure 2: Seminal plasma protein profiles of Jaisalmeri (J) camels. Lane 1: Marker; Lanes 2-5 and 7-10 having samples of J-112 and J-224, respectively. Lane 6: negative control (No seminal plasma). Lanes 2 and 7: fresh seminal plasma; lanes 3 and 8: seminal plasma kept at 38ºC for 6 hours; lanes 4 and 9: seminal plasma kept at 38ºC for 12 hours; lanes 5 and 10: seminal plasma kept at 38ºC for 18 hours. 40 kDa protein band expressed in lanes 2-5 (J-112) and not in lanes 7-10 (J-224).

Discussion

Present study was undertaken to compare seminal protein profile of single humped male dromedary camels of Jaisalmeri breed and seminal fluid analysis of the animals. The study was motivated by earlier findings [15] who reported four fertility-associated proteins in bovine seminal plasma that were of value in predicting significant differences in relative fertility among the bulls studied. The fertility was found to fluctuate in and among stallion, and fertility-associated proteins of seminal plasma of stallions could be correlated with their fertility [16].

Camels are induced ovulators where seminal plasma proteins play a crucial role in induction of ovulation. Recent studies show that role of seminal plasma includes effects via multiple mechanisms on ovarian function and induction of ovulation in the inseminated females including camelids such as llama and alpaca (10, 5).

The average volume of semen (3.09 ± 0.19 ml) obtained in the present study agrees with earlier studies, which have reported an average volume of 3 ml [17], 4.3 ml [18], but are lower than 5.1-11.0 ml [19] reported in other studies. There could be many reasons for the reduced semen volume including age of the animals, agroclimatic conditions, semen collection frequency, and training of animals. Additionally, male camel can mate at least two to three times in a lone day with several females [20]. Rutting season has also been seen to influence the quality and quantity of semen in dromedary camels [21]. Camel semen reported to be grey to milky white in colour with thick viscous consistency immediately after collection [22]. The highly viscous nature of semen contributes to the oscillatory movement of camelid spermatozoa [23,24] as compared to progressive motility of spermatozoa in other animals and may be important in maintaining the viability of spermatozoa in uterus [25]. Short breeding spell, little libido and high ferociousness are still some of the foremost bases of monetary loss, underprivileged reproductive enactment and injuries, for camel breeding and productiveness. Strong information of animal erudition and precise procedure could be beneficial for camel breeders, experts , and scientists [26].

As shown in Table 1, highest protein concentration found was 3.69 ± 0.12 g/dl in animal J-224, whereas lowest concentration was 2.46 ± 0.07 g/dl, in animal J-230. The average protein concentration was 2.74 g/dl making it a rich and important source for studying proteins and their role in fertility (12). The highest protein concentration being found in one of the non-rutting camels might be due to higher percentage of aspermic ejaculates (Table 1) as destruction of immature cells may contribute to increased protein content in these specimens [27].

It is evident that seminal plasma contains a large protein component which has been implicated in the function, transit and survival of spermatozoa within the female reproduction tract. In addition, majority of these proteins are unknown and a direct comparison between the domestic mammalian species has yet to be made [8].

Our study shows 9-10 protein fractions with 2-4 major components in addition to the minor fractions in the seminal plasma of Indian dromedary camels (Jaisalmeri breed). 10-14 protein fractions were reported [2], with varying percentages along with 3-4 major components in the camel seminal plasma of Egyptian dromedary camels (Saidi breed). The variations in total number of protein fractions in the present study and with that of El-Naggar and Abdel-Raouf, 1977 may be due to difference in camel breeds.

One of the possible reasons for differential expression of protein profiles in our study may be due to variable levels of fertility, as reported in human and other animal species that protein expressions could be correlated to fertility indices [15,16]. 40 kDa protein band was expressed in the seminal plasma of only in those camels performing copulation successfully and 40 kDa protein was found to be heat resistant at 38 ºC up to 18 hours. It will be really interesting to characterize and purify 40 kDa protein band to further investigate its possible role in rutting camels. A 40 kDa zinc α2-glycoprotein (ZAG) was reported in ram, buck, alpaca and camel seminal plasma [8]. The role of ZAG is not well established but may be involved in the regulation of sperm motility via the cAMP pathway [28]. β-NGFs was purified from dromedary camel seminal plasma [29]. During liquefaction, proteins with molecular masses of 24.55 kDa and 22.07 kDa seemed in conjunction with the disappearance of intact 26.00 kDa protein after 18-24 hours. These proteins were identified as β-nerve growth factors (β-NGFs) in liquefied camel semen [30]. Our study possibly will provide the firsthand evidence that some proteins are unique and expressed precisely in some camels. The significance of 40 kDa protein in the seminal plasma of rutting dromedary camels is certainly worthy of further investigation and these proteins must be characterized. Further research work could be undertaken to establish the rationale for low or no expression of 40 kDa protein in the dromedary camel semen plasma [31]. Characterization of these proteins will be needed to improve understanding of mechanisms involved in male reproduction in camels.

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