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EXPLANATION OF NO OR LOW SPERM MOTILITY IN CAMEL SEMEN

Aminu Deena, Sumant Vyas, Mamta Jain and M. S. Sahani
National Research centre on camel
P. B. No: 07, Bikaner (Rajasthan), India

Abstract

The study on collection and evaluation of camel semen was undertaken with a view to assess possible causes of low sperm motility in this species with an special emphasis on possibility of toxic effect of rubber funnel contact with camel semen. Besides, collection of semen over several years with traditional rubber funnel type of artificial vagina (AV), a separate experiment was conducted on 6 camels which were used for 114 semen collections alternately with AV assembled as traditional rubber funnel (n=63) or with camel collection glass (n=51).

Microscopic examinations of semen revealed that spermatozoa are densely clustered and entrapped. Initially they are not free to move. Sometime later, they can oscillate their tails only. Diluted and chilled semen mixed gently and examined under microscope presents a heterogeneous picture. In certain fields of microscopic glass slide sperms are clustered and entrapped, while at other fields sperms are free and progressively motile. A microscopic picture revealed that the heads of spermatozoa are embedded, tightly secured, appears to have glued together and tails only vibrating strongly. Some process of liquefaction of semen coagulum releases spermatozoa in batches which develop progressive motility.
Semen samples collected either with traditional rubber funnel type AV or camel collection glass did not differ in % motility as revealed by t-test.
It is concluded that low sperm motility is due to coagulation of semen and entrapment of spermatozoa. Rubber funnel contact apparently did not affect motility to any significant extent.

Key words: Camel collection glass, sperm motility

Introduction

Sperm motility is the major criteria used for evaluation of semen for Artificial insemination (AI) in cattle and buffalo. But, scientists working with camel semen have confronted with problems of no (1,2&3) or low sperm motility (4). Brown (2000) reviewed several published reports in American camelids and was of the view that the high viscosity of camelids semen results in oscillatory movement of the spermatozoa and not the progressive sperm motility as occurs in ejaculates from other domestic ruminants. Another commonly

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prevailing belief among the camel reproduction scientist is that contact of camel semen with the rubber funnel of artificial vagina (AV) has a lethal and toxic effect on spermatozoa (4,5,6) which ultimately leads to low motility in camel semen. Some of these workers advised to collect semen directly into glass vial (5,6). Camel collection glass (Catalogue no. ZC008/005463, IMV, France) can be used to replace traditional rubber funnel of AV and as such, the semen is directly ejaculated in glass receptacle, absolutely avoiding any contact between rubber part of AV and ejaculated semen. This study was conducted on collection and evaluation of camel semen with a view to assess possible causes of low sperm motility in camel semen.

Materials and Methods

Experimental Animals

Six adult male camels aged 12-13 years were used in the present experiment, for semen collection in AV alternately with rubber funnel (n=6,1) and camel collection glass (IMV, France) (n=51). These were kept together in a shed but tied individually to a fixture through nose halters. They were maintained on diet of gaar phalgati (Cyamopsis tetragonoloba). The semen collections were attempted during cool winter months of January - March when rutting activity of camels is at peak.

Preparation of Artificial Vagina:

A 12" long AV (I.M.V. France, catalogue no.- 005417) was used in present experiment. The inner chamber was filled with approximately 750ml. water of 45 to 50oC temperature depending upon weather conditions to maintain internal temperature of AV at 41 to 42oC. Air was infiltrated to maintain adequate pressure. In rubber (latex) funnel method, the edges of inner rubber liner are inverted over the edges of hard cylinder in such a way that no loose flap of liner is left behind and a latex cone or funnel with one wider and another narrow end is used to connect AV cylinder with collection tube. In camel collection glass method, one or two inch loose flap of latex liner is left on one side which is used to put over the camel collection glass. The junction between rubber liner and camel collection glass is tightly secured with adhesive tape (Fig.-1). With this modification, the ejaculated semen is directly dropped in collection glass and contact with the rubber funnel is avoided. Insulation bag was applied to cover rubber funnel and collection tube/ camel collection glass in similar way as used in bovine (Fig.-2). Application of lubricant was avoided as camel secretes lot of pre ejaculate prior to intromission of penis into artificial vagina to lubricate it adequately.

Figure 1

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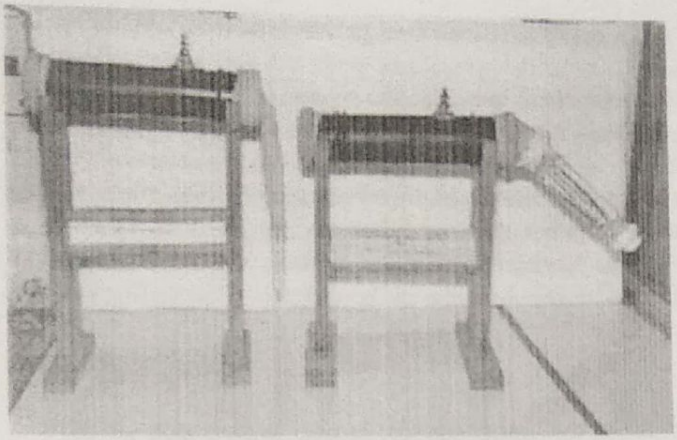
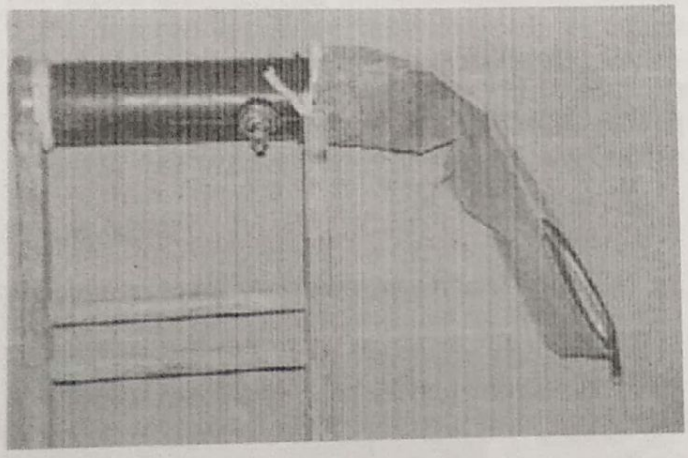


Figure 2:



Semen Collection:

A female camel was restrained in the sitting posture and the male was allowed to mount her. Operator approached the male from left side as camel usually falls on right side after copulation. The AV was held firmly in the left hand of operator and erected penis was directed into AV with the right hand holding prepucial sheath. The sheath was supported throughout the course of copulation to prevent extrusion of penis from AV. During the entire course of copulation, at least two assistants were required to control camel and avoid accidental falling on operator side. Copulation time was recorded with a timer watch. Semen was collected at an interval of 5 to 7 days from individual males using AV with rubber funnel and camel collection glass alternately for each camel.

Semen extension and evaluation of sperm motility:

A small bunch of whole semen was spread over a pre warmed glass slide and examined under microscope for mass motility. Remaining amount of collected semen

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sample is diluted with Tris egg yolk extender (7) at room temperature and transferred into refrigeration unit for slow cooling to 4oC over 2 hours. Semen was examined using a drop of diluted semen over pre-warmed slide covered by a cover glass using 400X magnification of the microscope by same technician throughout the period of study. Extended camel semen usually presented heterogeneous picture. At certain areas sperms use to be clustered while at others these were individually scattered and free. Sperm motility was recorded for each semen sample from an area where spermatozoa are individually scattered. Means of sperm motility with rubber funnel method and camel collection glass were compared by paired "t" test (8).

Results

Freshly ejaculated camel semen does not exhibit mass motility apparently because sperms are densely clustered and entrapped. A dark central spot in Fig. 3 shows densely packed camel spermatozoa. Video pictures of microscopic examination of camel semen showed densely clustered and entrapped spermatozoa, some of which can oscillate their tails only, otherwise tightly secured to prevent free movement. Video pictures also revealed that heads of spermatozoa are embedded while tails strongly vibrate (Fig. 4). Even in small clumps of 3-10 spermatozoa, the heads of the spermatozoa appear to be glued together. Video pictures also revealed that spermatozoa which are rendered free from entrapment develop progressive motility.

Figure 3:

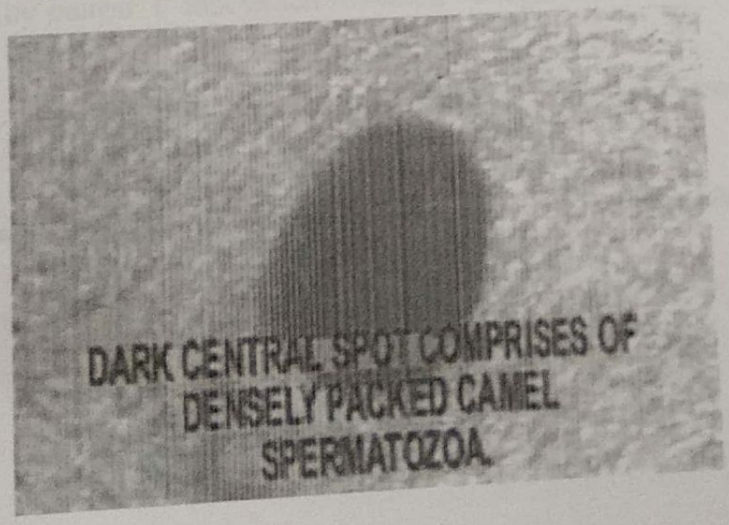

Figure 4:

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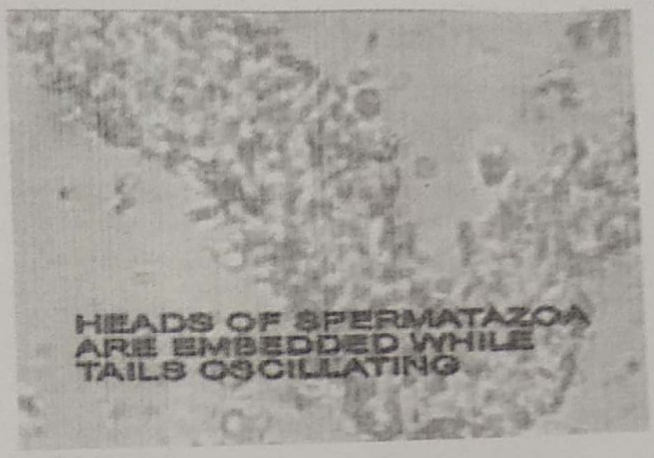

Most puzzling aspects of camel semen evaluation is that a large proportion of ejaculates collected artificially do not develop motility. As for example, 439 ejaculates collected over 3 years with rubber funnel type of AV, 31.89% failed to develop sperm motility.

Data on sperm motility of a total of 114 semen samples harvested from 6 camels with camel collection glass and rubber funnel have been presented in Table-1. The motility varied from 0 to 90% in different semen samples. Semen samples showing 0 to < 20% were 17/63 (26.9%) with rubber funnel method as against 11/51 (21.5%) with camel collection glass. Means were compared by paired "t" test which revealed no significant difference between the 2 groups.

Table-1 Comparison of sperm motility of camel semen collected by Rubber funnel and camel collection glass method

		Camel Rubber funnel method			Camel collection glass method		
No.	No.	No. of observations	Actual motility (%)	Average ± S. E.	No. of observations	Actual motility (%)	Average ± S. E
1	A-273	7	20, 70, 80, 50, 70, 10, 60	51.42 ± 10.95	5	70, 60, 70, 80, 40	64 ± 7.58
2	J-322	14	50, 5, 2, 60, 55, 70, 70, 70, 90, 80, 90, 80, 20, 20	54.42 ± 8.5	12	50, 10, 10, 60, 10, 70, 30, 90, 70, 70, 0, 40	42.5 ± 9.12
3	B-346	11	5, 2, 40, 30, 70, 50, 70, 40, 50, 50, 90	45.18± 8.41	11	5, 20, 20, 50, 40, 70, 50, 80, 40, 70, 104	41.36± 8.07
4	J-321	10	50, 60, 70, 60, 70, 70, 50, 20, 0, 60	51± 7.76	5	50, 60, 80, 30, 30	50± 10.6

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J-58	10	0, 30, 50, 60, 70, 70, 20, 50, 40, 40	43± 7.37	8	10, 50, 60, 40, 70, 40, 15, 50	41.87± 7.83
J-56	11	40, 0, 5, 70, 20, 60, 0, 50, 70, 70, 5	35.45± 9.50	10	50, 50, 40, 60, 0, 60, 70, 70, 50, 50	50± 6.66
Overall	63		46.80± 3.40	51		46.47± 3.35

Discussion

Microscopic pictures illustrate that there is no mass motility in camel semen, comparable to that of cattle and buffalo, which exhibit waves and swirls. The reason is quite obvious, contrary to the free spermatozoa in semen ejaculate of cattle and buffalo, freshly ejaculated camel spermatozoa are entrapped in a sort of fibrinous network and do not find space to move. This resembles to those described for human semen ejaculate (12). This provision of entrapment of spermatozoa in semen may act as a type of sperm reservoir, fulfilling a similar physiological function to the so called sperm cervical reservoir in other domestic ruminants. Further more, the high viscosity of the semen may be important in maintaining the viability of sperm within the uterus (13). It is speculated that coagulated semen undergo liquefaction slowly, releasing spermatozoa over a prolonged period of time. As reported by Brown (2000), time taken for liquefaction of alpaca semen averaged 23 h. This sort of sperm reservoir appears to be essential as ovulation in this species is induced type and might require 36-48 hrs period after mating.

Puzzling aspect in camel semen is that a large proportion of semen samples do not exhibit motility under laboratory handling conditions. The reported literature though vary but supports the findings of poor motility in camel semen. As for example, some of the previous workers observed no sperm motility at all in camel semen either fresh or diluted semen upto 12 hours of collection (1,2 & 3). Some other workers reported that progressive sperm motility in raw semen examined 15 minutes after collection ranged from 30-50% (9). Yet other reported very low initial motility (5%) at the time of collection, which improved as ejaculate becomes more liquid (4, 11). These workers have observed sperm motility ranging from 0-85% in 125 semen samples from 5 males of proven fertility. As per the annual progress report of National Research Centre on camel, Bikaner, 10/24 (41.66%), 14/40 (35%) and 7/20 (35%) semen samples collected during three consecutive years 1996-98 were only found to exhibit sperm motility, which, means as many as 60-65% semen samples exhibited no motility. Initial motility of +4 or +5 grade has been reported by some workers (10) from the same group who previously reported no motility. Procedural faults in form of pressure and temperature of AV were suspected by them to be responsible for no sperm motility. But, this

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plea does not seem to be convincing. Some workers are of the opinion that conditions of semen collection and state of liquefaction affect sperm motility. Type of rubber in AV and length of time semen stays in contact with the rubber liner is said to have adverse effect on motility of spermatozoa (4). Dilutor containing caffeine has been shown to have beneficial effect on regaining motility in non motile samples, which substantiated the hypothesis that rubber tended to paralyze the spermatozoa rather than killing them. The adverse effect of rubber on semen was suspected by other workers as well (5,6) and it was advised to collect semen directly into a glass vial. Results of present study in regards to motility of semen samples collected with rubber funnel and camel collection glass indicated no difference. It can be concluded that the rubber funnel contact of camel semen apparently had no adverse effect on motility of spermatozoa. Beneficial effects of caffeine observed by some workers may be due to potentiating effect of this chemical on motility. More rational explanation of motility status in camel semen can be derived from microscopic pictures of semen in which spermatozoa are entrapped in coagulum with no space to move. In this way it resembles with human semen in which a freshly formed semen coagulum presents a dense network of long fibers approximately 0.15 micromillimeter in diameters, separated by spaces too narrow to allow free movement of the enmeshed spermatozoa. As liquefaction gets under way amorphous material consisting of small globules appear on the fiber surface until the fibers disappear and the globules take over (12). Sperm can develop motility only after liquefaction of coagulum. It appears that many of the previous workers were unaware of this sort of status of camel semen and were unable to undertake suitable form of evaluation. It is still felt that a significant proportion of camel semen samples fail to exhibit motility and one need to examine the effects of current handling practices, mechanisms of gelification and subsequent liquefaction.

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