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Physical and Biochemical Studies in Jack's Semen

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Abstract: Semen samples of five French donkeys (Poitu breed) maintained at cryopreservation laboratory were evaluated at different intervals during both breeding and non-breeding seasons, for their physical and biochemical parameters, to assess their individual potential and further use for cryopreservation. Samples were collected using artificial vagina at weekly interval throughout the year. Semen was creamy to 'off white' in color and of thin to thick consistency in all the jacks during period of the study. Semen volume was significantly higher during non-breeding season as compared to breeding season, while reverse was true for live sperm per cent, initial motility, progressive motility and sperm concentrations. A significant positive correlation was observed among spermatozoa concentration, live sperm per cent, initial motility and progressive motility and all these were negatively correlated with semen volume. Among biochemical parameters including different enzymes, metabolites and ions, significant differences in their values during breeding and non-breeding seasons, as well as among individuals were observed. Activity of SDH enzyme remained almost constant throughout the study indicating that fructose may not be the direct source of energy for spermatozoa. Among all the jacks, the quality of two jacks was appreciably better than the rest. Overall semen quality was better during breeding season than non-breeding season.

Key words: Jacks, seminal plasma, semen, seasons, physical, biochemical.

In India, there are more than one million donkeys constituting a part of draught animal power mainly as pack and cart animals. Because of their low price, little maintenance cost and casual attention, donkeys are well suited to the meager resources of poverty stricken people as a source of their income and livelihood (Birthal *et al.*, 1999). Except a small number of large sized white donkeys, most of the donkeys available in India are gray colored, small in size and measure an average height of 0.9 m with low fecundity and productivity. Probably these gray donkeys are the smallest in the world (Varshney and Gupta, 1994). Because of their small size and lesser draught power than big size white donkeys, ponies and mules, the gray donkeys are not the animals of first choice. The equine owners prefer large sized donkeys and mules for their use as pack or cart animals. Large size exotic donkeys (Italian, French, etc.) had previously been imported for upgradation of indigenous donkeys and production of superior quality mules. In order to adopt a donkey improvement and mule production program using a superior exotic germplasm, it is mandatory to evaluate the breeding potential of these exotic donkeys individually for their further use in artificial insemination by semen extension and cryopreservation. The present study was undertaken to evaluate the quality of semen of individual exotic jack and the effect of non-breeding and breeding seasons on the semen quality

Materials and Methods

Five healthy adult male, French (Poitu) donkeys (*E. asinus*) of Martina franca breed maintained at an organized farm and well adapted under hot and dry climatic conditions, were used for this study. All jacks were kept individually under same management conditions and were trained to donate semen with artificial vagina (AV). The semen was collected from jacks between 7.00 to 8.00 AM using an oestrus or induced oestrus donkey mare as a dummy. Semen was collected using standard AV containing inner polyethene liner to avoid the toxic effect of rubber latex liner. Semen samples were collected at weekly interval from all the jacks through out the year. Semen was immediately used for its physical and biochemical evaluations. The gel portion of the ejaculate/semen was separated by passing the semen through a sterile cotton gauge to record the gel free volume. Gel free semen was kept at 37°C in an incubator. Semen was processed for seminal plasma collection by centrifuging the samples at 3,000 rpm for 15 minutes at 4°C and used for various biochemical evaluations. The color and consistency of the semen were evaluated by direct visual observation. Semen volume was measured in a graduated sterile cylinder after removing the gel, if any. Spermatozoa concentration was estimated in gel free semen using a haemocytometer. For this, semen was diluted 1:100 with diluent (10 ml of 35% formalin v/v with 50 g sodium bicarbonate in 1 L distilled water). The number of spermatozoa counted in five small squares were multiplied with the dilution factor to calculate the total number of spermatozoa present per ml. The pH of the semen was measured with a pH paper strip within five minutes after semen collection. At the time of each collection, live and dead spermatozoa were counted using eosin-nigrosin staining dye technique. The initial and progressive motility of spermatozoa were determined using a light microscope and pre heated glass slides and were expressed in per cent.

The seminal plasma was used immediately for estimation of enzymes, metabolites and ions, namely alkaline phosphatase (ALP), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), glutamic aspartic transaminase (GOT), glutamic alanine transaminase (GPT), albumin, protein, urea nitrogen, cholesterol, glucose and chloride using single step reagent kit for each parameter in Auto chemistry analyzers (Beckman DU Clinical System, USA and Polimak M/10, Italy). Sodium and potassium were analyzed using a flame photometer (Mediflame, India).

The data were statistically analyzed using SPSS 7.5 GLM multivariate program to find out the significant differences among individuals. "T" test was used to assess the effect of season on both physical and biochemical indices. Correlation coefficients were calculated among different physical characteristics along with effect of seasons on them.

Results and Discussion

Physical characteristics

The color of semen was 'off white' to 'creamy' in appearance. In general, creamy appearance was observed very

Table 1. Physical indices of semen during non-breeding and breeding seasons (Mean±SEm)

Parameters	Season	
	Non-breeding (n=110)	Breeding (n=150)
Semen volume (ml)	48.05*±1.24	43.71±0.94
pH	7.15±0.02	7.31**±0.02
Sperm concentration (x 10^6)	282.78±7.52	331.56**±5.49
Live spermatozoa (%)	81.93±0.34	84.25*±0.29
Initial motility (%)	79.15±1.02	84.97**±0.52
Progressive motility (%)	70.27±0.81	75.13**±0.56

* Significant P<0.05, ** Significant P<0.01.

frequently irrespective of the season as well as jacks. In horse semen, milky white appearance of the semen is considered as good quality semen, however this criteria may not hold good for donkey jacks. The consistency of the semen differed among jacks. The semen was of thin consistency, i.e., 58%, 62% and 64% times in jack no. 1, 2 and 5, respectively, while in jack no. 3 and 4, thin consistency was only 48% and 46% times, respectively. However, this variation in consistency was irrespective of the season.

The semen volume (gel free) as observed at different intervals ranged from 20 to 100 ml per collection irrespective of a particular Jack and season. The semen volume was significantly higher (P<0.05) during non-breeding season (September to January) than breeding season (Table 1). These results are in agreement with the observations of Pickett and Voss (1972) and Squires *et al.* (1979). Further, among the individual Jacks, overall semen volume, irrespective of season, was significantly higher (50.29 ml) in Jack no. 5, while it was the minimum (40.29 ml) in Jack no. 4 (Table 2).

In general, pH of the semen was neutral to slightly alkaline as it ranged from 7.0 to 7.5 throughout the year at different sampling intervals in both the seasons. During breeding season, it was significantly (P<0.05) higher (7.31±0.019) than that in non-breeding season (7.15±0.024). Among Jacks, the overall pH was significantly (P<0.05) lower in Jack no. 3 and 4 than Jack nos. 2 and 5 (Table 2).

All the jacks were more potent and productive during breeding season as mean concentration of spermatozoa was significantly higher (P<0.01) during this season than the non-breeding season (Table 1). These observations are in agreement with the earlier findings in horse stallions (Costa *et al.*, 1991). Significant variations were also observed in spermatozoa concentration in individual jack as the mean values ranged between 226.56 to 375.39 x 10^6 sperms per ml of semen (Table 2). Spermatozoa concentration was maximum in jack no. 4 followed by jack no. 3 and their values were significantly higher than the rest of the jacks. In general, Jack nos. 3 and 4 were found to be more useful than other jacks in terms of higher expected production. Though semen volume was significantly lower during breeding season, yet the total spermatozoa concentration was

Table 2. Physical parameters of individual Jack's semen during non-breeding and breeding season (Mean±SEm)

Parameters	Season	Jack 1	Jack 2	Jack 3	Jack 4	Jack 5
Semen volume (ml)	Non-breeding	50.91±2.56	46.59±2.31	49.77±3.623	42.73±2.41	50.23±2.66
	Breeding	43.17±1.72	41.67±1.60	44.90±2.17	38.50±1.95	50.33±2.41
	Mean	46.44^{b}±1.55	43.75^{c}±1.37	46.96^{b}±1.99	40.29^{a}±1.53	$50.29^{b,d}$±1.77
pH	Non-breeding	7.23±0.05	7.16±0.05	7.07±0.05	7.16±0.05	7.14±0.06
	Breeding	7.28±0.05	7.42±0.04	7.28±0.046	7.17±0.044	7.41±0.03
	Mean	7.26±0.04	7.31^{b}±0.03	7.19^{a}±0.04	7.16^{a}±0.03	7.29^{b}±0.04
Sperm conc. (x 10^{6})	Non-breeding	265.27±9.62	288.41±6.44	328±5.76	355.86±13.40	176.36±14.56
	Breeding	305.57±10.5	328.70±3.99	370.47±11.77	389.70±9.13	263.37±8.66
	Mean	$288.52^{b,c}$±7.7	$311.65^{b,d.e}$±4.5	$352.50^{b.d,f,g}$±7.7	$375.39^{b,d,f.h}$±8.0	226.56^{a}±9.89
Live sperm (%)	Non-breeding	78.68±0.79	82.41±0.75	83.77±0.59	84.27±0.47	80.85±0.52
	Breeding	80.03±0.46	85.03±0.62	85.88±0.45	86.70±0.39	83.70±0.57
	Mean	79.46^{a}±0.44	$83.92^{b,d,e}$±0.51	$84.92^{b,d}$±0.38	$85.67^{b,d,f}$±0.34	$82.35^{b,c}$±0.45
Initial mass motility (%)	Non-breeding	73.6±1.54	80.00±1.85	76.91±3.77	86.59±1.50	78.64±0.91
	Breeding	78.67±1.31	88.33±0.91	85.83±0.96	86.90±0.088	85.13±0.90
	Mean	76.54^{a}±1.05	84.81^{b}±1.10	$82.06^{b,c}$±1.79	$86.77^{b,d}$±0.68	$82.38^{b,c}$±0.80
Prog. motility (%)	Non-breeding	63.64±1.55	70.68±1.87	70.68±1.98	77.37±1.29	68.64±1.00
	Breeding	68.83±1.33	79.00±1.11	76.17±1.06	76.67±1.00	75.00±0.93
	Mean	66.64^{a}±1.06	$75.48^{b,d}$±1.16	$73.85^{b,e}$±1.10	$77.12^{b,d,f}$±0.77	$72.31^{b,c}$±0.81

Superscripts (a&b) indicate significant differences ($P<0.05$) among their mean values. Smilarly c&d; E&f and g&h indicate signficant differences in their values.

higher during this season, which could possibly be due to increased rate of spermatogenesis (Johnson and Thompson, 1983; Johnson and Tatum, 1988).

Beside spermatozoa concentration and semen volume, per cent live spermatozoa, initial motility and progressive motility are other important indices for assessing the quality of semen. Live spermatozoa were observed to be 81.93 and 84.25% during non-breeding and breeding season, respectively, and these values differed significantly ($P<0.05$) from each other. Similarly, significant differences were observed in live sperm counts among different jacks with maximum sperm counts in jack no. 4 followed by jack nos. 3 and 2.

The average initial motility and progressive motility of spermatozoa were significantly ($P<0.01$) higher during breeding than during non-breeding season (Table 1). Similarly, among individual jacks, both initial spermatozoa motility and progressive motility was maximum and significantly ($P<0.05$) higher in jack no. 4 than jack no. 1 (Table 2). Progressive motile spermatozoa are good for fertilization as compared to weakly motile ones. Both mean initial and progressive motility of spermatozoa were significantly higher

Table 3. Biochemical indices of semen during non-breeding and breeding seasons (Mean±SEm)

Parameters	Season	
	Non-breeding (n=110)	Breeding (n=150)
Lactate dehydrogenase (IU L^{-1})	447.90±11.78	538.12**±18.80
Alkaline Phosphatase (IU L^{-1})	40.07±1.30	54.79**±0.91
SDH (IU L^{-1})	18.24±0.73	20.24±0.91
GOT (IU L^{-1})	339.59±10.16	383.19**±12.17
GPT (IU L^{-1})	18.06±0.51	19.82*±0.66
Albumin (g dl^{-1})	2.56**±0.07	2.23±0.032
Urea nitrogen (mg dl^{-1})	11.27±0.28	17.27**±0.95
Cholesterol (mg dl^{-1})	16.94±0.54	16.26±0.46
Glucose (mg dl^{-1})	9.46*±0.32	8.35±0.32
Protein (g dl^{-1})	4.28±0.08	5.04**±0.05
Sodium (mEq L^{-1})	104.36±1.14	110.82**±0.48
Chloride (mEq L^{-1})	119.35**±1.11	115.20±0.46
Potassium (mEq L^{-1})	21.54**±0.33	19.69±0.08

during breeding seasons, which further support that quality of semen was better during breeding season than non-breeding season. Picket *et al.* (1970) had also reported higher motility in stallion semen during breeding season.

On the basis of micro and macroscopic indices, jack nos. 3 and 4 were observed to be better in their quality than other jacks as total number of spermatozoa per ejaculate were observed to be higher in jack nos. 3 and 4 along with higher per cent of live spermatozoa and their greater initial and progressive motility. These micro and macroscopic indices clearly revealed that among different jacks, quality of semen varies so selection of a more potent and useful jack is very important for any breeding program. Secondly season has also significant effect on the quality of semen.

Correlation studies carried among different physical parameters of semen indicated a positive and significant correlation between initial motility, progressive motility, live sperms and spermatozoa concentration while pH had non-significant positive correlation with them (Table 5). Overall season had a positive and significant correlation with all the above indices while semen volume was observed to have a negative and significant correlation ($P<0.01$) with initial motility, progressive motility and sperm concentration only. Similar significant positive correlation between sperm concentration and motility has also been observed by Singler and Kiracofe (1988).

Biochemical parameters

Activities of all the enzymes except SDH were significantly higher in seminal plasma during breeding season than non-breeding seasons (Table 3). SDH activity was at par during both the seasons. Among different jacks, overall activities of LDH

Table 4. Mean values of different biochemical parameters in individual Jack's seminal plasma during non-breeding and breeding seasons

Parameter	Seasons	Jack 1	Jack 2	Jack 3	Jack 4	Jack 5
LDH	Non-breeding	426.59	460.61	467.41	437.98	446.93
($IU\ L^{-1}$)	Breeding	566.61	512.27	417.01	588.39	606.31
	Mean	507.37	490.41	438.33^{a}	524.76^{b}	538.88^{b}
ALP	Non-breeding	42.47	38.30	37.22	39.20	43.18
($IU\ L^{-1}$)	Breeding	58.24	55.00	51.93	54.62	54.17
	Mean	51.57^{b}	47.94	45.71^{a}	48.10	49.52
SDH	Non-breeding	17.50	18.89	18.39	16.82	19.59
($IU\ L^{-1}$)	Breeding	19.57	19.67	24.17	16.54	21.26
	Mean	18.69	19.34	21.73^{b}	16.66^{a}	20.55
GOT	Non-breeding	250.42	385.04	382.68	412.04	267.78
($IU\ L^{-1}$)	Breeding	252.49	442.46	506.23	451.07	263.68
	Mean	251.61^{a}	418.17^{b}	453.96^{b}	434.56^{b}	265.42^{a}
GPT	Non-breeding	16.45	17.46	22.62	17.80	15.99
($IU\ L^{-1}$)	Breeding	13.48	19.90	27.07	23.14	15.50
	Mean	14.74^{a}	$18.87^{b,c}$	$25.19^{b,d}$	$20.89^{b,c}$	15.71^{a}
Albumin	Non-breeding	2.01	2.80	2.74	2.66	2.60
($g\ dl^{-1}$)	Breeding	2.17	2.19	2.30	2.27	2.20
	Mean	2.10^{a}	2.45^{b}	2.49^{b}	2.44^{b}	2.37^{b}
Urea nitrogen	Non-breeding	14.16	11.57	11.53	10.03	9.07
($mg\ dl^{-1}$)	Breeding	17.41	18.02	19.33	18.04	13.55
	Mean	16.03^{b}	15.29^{b}	16.03^{b}	14.65^{b}	11.66^{a}
Cholesterol	Non-breeding	14.77	17.55	17.53	18.42	16.46
($mg\ dl^{-1}$)	Breeding	13.05	14.72	19.99	17.06	16.46
	Mean	13.78^{a}	$15.92^{b,c}$	$18.95^{b,d}$	17.63^{b}	16.46^{b}
Glucose	Non-breeding	8.63	10.09	9.48	8.76	10.36
($mg\ dl^{-1}$)	Breeding	6.56	7.97	9.26	7.99	9.99
	Mean	7.43^{a}	$8.87^{b,c}$	9.35^{b}	$8.31^{a,c}$	$10.15^{b,d}$
Protein	Non-breeding	3.74	4.31	4.70	4.07	4.57
($g\ dl^{-1}$)	Breeding	5.16	4.79	5.14	5.01	5.08
	Mean	4.56^{a}	4.59^{c}	$4.95^{b.d.f}$	4.61^{e}	$4.86^{b,d}$
Chloride	Non-breeding	126.55	116.41	114.23	119.73	119.82
($MEq\ L^{-1}$)	Breeding	115.47	114.80	115.47	115.47	115.27
	Mean	119.88^{b}	115.87^{a}	114.56^{a}	117.27	117.19
Potassium	Non-breeding	19.76	21.29	23.28	21.06	22.33
($MEq\ L^{-1}$)	Breeding	19.21	19.63	21.36	19.58	19.68
	Mean	19.44^{a}	20.33^{c}	$21.60^{b,d}$	20.21^{c}	20.80^{b}
Sodium	Non-breeding	113.59	103.36	97.23	103.96	103.59
($MEq\ L^{-1}$)	Breeding	110.70	110.50	110.80	110.47	111.63
	Mean	111.92^{b}	107.48^{a}	105.10^{a}	107.71^{a}	108.23^{a}

Superscripts (a&b) indicates significant difference (P<005) among their mean values. Similarly c&d and e&f indicate significant differences in their values.

Table 5. Correlation coefficients among different physical indices and season

Parameters	Live sperm	Motility	pH	Progressive motility	Sperm conc.	Semen volume
pH	0.009	0.018				
Progressive motility	0.335**	0.877**	0.065			
Sperm conc.	0.347**	0.189**	0.054	0.216**		
Semen volume	0.060	-0.169**	0.089	-0.187**	-0.234**	
Season	0.305**	0.323**	0.312**	0.302**	0.317**	-00174**

** Indicates significant (P<0.01) correlation between them.

and ALP were significantly lower (P<0.05) in jack no. 3 while activities of SDH, GOT and GPT were significantly higher in this jack than jack nos. 1 and 5 (Table 4). Further, activities of LDH and GOT varied to a greater extent among different jacks that is evident from higher standard error of mean (SEm) values.

In the present study, significantly higher activity of enzymes namely GOT, GPT, LDH and ALP during breeding season seems to be due to higher spermatozoa concentration and their progressive motility during this season. GOT and GPT provides free amino acids in seminal plasma which have greater physiological significance as chelating agents as well as oxidizable substrates for spermatozoa (Mann and Mann, 1981). Biochemical indices like enzymes, metabolites and ions have already been observed to have significant effect on sperm motility, sperm concentration and sperm damage during cryopreservation, conception rate, etc. (Mann and Mann, 1981; Hafez, 1987). Further higher activities of enzymes during breeding season is also in agreement with the similar observations in stallions (Herak *et al.*, 1978, 1979).

Among the individual jacks, activities of GOT and GPT were significantly high in jack nos. 3 and 4 than jack nos. 1 and 5, while activities of LDH and ALP were in reverse order. ALP is another important enzyme which can be used as diagnostic tool in stallion with azoospermia and oligospermia. In present study, its activity in jacks was quite low as compared to that observed in horse stallions (Turner and Sertich, 2001). Such a difference in ALP activity could be due to different species as well as environmental factors. Enzyme sorbitol dehydrogenase which is responsible for maintaining equilibrium between fructose and sorbitol concentration in plasma remained almost constant during both the seasons indicating that fructose may not be the direct source of energy for spermatozoa. This is in agreement with the earlier findings that stallion spermatozoa readily metabolize monosaccharide namely glucose, but have limited ability to utilize fructose (Mann, 1964). Activities of most of the enzymes in individual jack seminal plasma were quite low than the values reported for horse stallions (Herak *et al.*, 1979; Castro *et al.*, 1991), which could be due to species difference.

Levels of various metabolites in seminal plasma differed significantly during both the seasons as well as among different jacks. Albumin and glucose contents were observed to be significantly higher during non-breeding than breeding season while reverse was observed for urea nitrogen and protein levels (Table 3). Cholesterol contents were at par during both these seasons. For the viability and motility of spermatozoa, a large proportion of metabolic energy is required which must come from the glycolysable sugar moieties present in the seminal plasma.

Glucose content as estimated in present study, was significantly lower during breeding season than the non-breeding season which could possibly be due to higher number of spermatozoa concentration present during that season. Beside, glucose, sorbitol and fructose have also been observed as sugars present in very small amounts in seminal plasma (Mann, 1975). The level of cholesterol, which is generally present as free and esterified form, remained constant during both the seasons.

In jacks, significantly higher protein contents along with lower albumin contents were observed during breeding season than non-breeding season. Levels of protein content in jacks were comparatively higher than those reported for stallion seminal plasma (Mann, 1964). Among jacks, overall levels of albumin, urea nitrogen, cholesterol and glucose contents were significantly higher in jack no. 3 than either jack no. 1 or 5 (Table 4). Cholesterol and protein contents in jack no. 3 were also significantly higher than jack no 2. Albumin:globulin (A:G) ratio was 0.793 and 1.488 during breeding and non-breeding seasons, respectively. Among individual jacks also A:G ratio was also quite low during breeding season than non-breeding season and it ranged from 0.726 to 0.842 and 1.161 to 1.887 in these seasons, respectively.

These basic values of metabolites are of importance during preparation of diluter/extenders for cryopreservation of jack semen as cholesterol which is present in semen is considered to stabilize the sperm plasma membrane during freezing and post-thawing. Selection of an individual jack for cryopreservation of its semen is quite important for success of any breeding program.

Significant variations were also observed in all the ions during both the seasons as well as among different jacks. Chloride and potassium contents were significantly higher ($P<0.05$) in seminal plasma during non-breeding season than breeding season, while sodium contents were higher during breeding season. In general, significant differences in overall ion contents, irrespective of season, were also observed in seminal plasma of different jacks. Sodium and chloride contents were significantly lower with higher levels of potassium in jack no. 3 as compared to jack no. 1 (Table 4). Levels of sodium and potassium in jacks seminal plasma were appreciably lower than those reported in Pega donkey and stallion seminal plasma (O'Reilly *et al.*, 1979; Morais *et al.*, 1994) suggesting that procedures used for *in vitro* cryopreservation of horse semen cannot be used in totality.

This study, in general, indicated that among the five exotic jacks included in this study, the quality of Jack nos. 3 and 4 was better than others as these had maximum spermatozoa concentration per ejaculate along with higher live sperm per cent, initial and progressive sperm motility. The quality of the semen was better during breeding season than non-breeding season.

The base line data generated on various biochemical indices in jack semen can be utilized for the cryopreservation of jack semen and post-thaw studies.

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