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Artificial insemination in dromedary camels (*Camelus dromedarius*) using fresh semen extended by physical agitation for achieving liquefaction

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ABSTRACT

Artificial insemination (AI) is widely used in farm animals for efficient utilization of superior males. However, AI is not being used in camels due to various difficulties involved in the process. In an attempt to develop the technology in camels, the present study was carried out to assess the success rate of insemination using fresh semen mixed by physical agitation within the extender media for overcoming difficulties out of gel formation. The study was carried out utilizing 3 males and 10 female Omani camels. Starting from November 2015, 109 semen ejaculates were collected and good quality ejaculates were extended using Triladyl or Tris sucrose based extenders. Ejaculates added with the extender were subjected to physical stirring for 30 sec using plastic straws to achieve mixing of the ejaculate gel. Female camels were scanned regularly for ovarian activity, ovulation was induced and AI done using fresh extended semen doses having at least 100 million motile sperms. Seventeen AI in three rounds resulted in 9 pregnancies giving an overall conception rate of 53%. First insemination conception rate was 50% and 80% of the camels conceived at least by three AI. Various determinants of conception from AI in camels are discussed. It is concluded that insemination using fresh semen extended by physical agitation in Triladyl and Tris Sucrose extenders provided encouraging conception rate for developing AI technology in camels.

Key words: Artificial Insemination, Camel, Conception, Semen, Triladyl

Artificial Insemination (AI) is widely accepted as the breeding technology for faster and extensive dissemination of germplasm from superior males. Even though there are reports of successful AI in camels using freshly extended (Skidmore and Billah 2006) and chilled semen (Bravo *et al.* 2000), AI is not being used as a routine breeding tool in camels because of various practical difficulties of semen collection (Deen and Sahani 2000), semen preservation (Wani *et al.* 2008) and poor conception rate of AI, especially with frozen semen (Skidmore *et al*. 2010). In this context, research trials were initiated with the intention of developing various steps for successful implementation of AI using frozen semen in camels, so that the technology can be used for the augmentation of genetic potential for food production and enhancement of race performances.

Being the initial step detailed studies were carried out in the previous seasons on various aspects of camel semen production such as collection, evaluation, processing and short-term preservation (Kutty and Koroth 2012). Preliminary trials were also conducted on semen freezing, preparation of the female for AI and the insemination technique (Deen *et al.* 2003).

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Unlike other farm animal species, camel ejaculate transforms into thick gel state within few minutes of collection and persists for hours or even days creating difficulties for quality evaluation and processing of the ejaculate (Deen *et al.* 2005). Addition of various chemical substances and enzymes has been tried for overcoming difficulties out of gel formation (Medan *et al.* 2008) and such added chemicals are under suspicion of contributing poor success rate of AI especially with frozen semen. In this context, the major objectives of the present study were to assess the quality of camel ejaculates freshly diluted by physical mixing in two of the common extenders and studying the success rate of insemination using fresh extended semen together with standardization of the technique.

MATERIALS AND METHODS

The study was carried out under Artificial Insemination and Embryo Transfer section of the Authority during November 2015–April 2016. Three adult male camels and 10 females including two heifers, belonging to the Omani breed were utilized for the study. The male camels were managed in individual pens while females were housed in one group. Feeding, general management, and healthcare measures were provided as per standard practices. Starting from November, male camels were trained for semen

collection using modified bovine artificial vagina (AV) (Kutty *et al*. 2016) and two of the female camels as the mount. The routine collection was started from December and was carried out almost regularly at 2–3 days intervals until the end of breeding season.

Ejaculates collected were subjected to quality evaluation and samples of adequate quality were extended using Triladyl extender (El-Hassanein 2017) (Readymade Triladyl media (Minitub) 25 ml, distilled water 75 ml) or Tris sucrose extender (Tris 2.37g, Sucrose 2.22 g, Citric acid 1.30 g, Distilled water 100 ml) added with 20% egg yolk. Dilution rates of 1:1 to 1:5 was used depending upon the sperm concentration and initial oscillatory movement to achieve around 100 million motile sperms/ml. Semen added with the extender was subjected to physical agitation using plastic straw for about 30 sec to enhance mixing within the media and to minimize gel formation. Extended semen was subjected to incubation at 37°C and sperm motility was assessed immediately after extension, at 30 min of incubation and just before insemination. Ejaculates of at least 60% motility were used for AI trials within 2 h of collection to assess the success rate of insemination.

Starting from the middle of November, female camels were examined by palpation and ultrasound scanning to assess the functional status of the reproductive organs. Examinations were carried out mostly at weekly or even shorter intervals as and when required, to assess ovarian follicular dynamics. Three camels without any follicular development even by the middle of December were injected 1500 IU of eCG (Folligon) to initiate follicular growth. Hormonal preparations such as GnRH (Cystorelin 100 µg or Receptal 200 µg) or hCG (Chorulon – 3000 IU) were injected intramuscularly for induction of ovulation in camels possessing at least one mature follicle of 1.2 to 2.0 cm diameter (El-Hassanein *et al.* 2010).

Animals were inseminated following ovulation induction, using at least 2 ml of fresh extended semen loaded in a sterile intrauterine catheter (Bovivet) connected with 5 ml syringe. Semen doses having not less than 100 million motile spermatozoa were deposited into the uterine body or mostly the uterine horn ipsilateral to the ovary possessing mature follicle (Skidmore and Billah 2006). The catheter loaded with semen was covered by a sterile plastic sleeve (Minitub), in order to avoid contamination during the insemination process. Per vaginal insertion of the hand followed by rectovaginal manipulation of the catheter was used for insemination in adult (parous) females while recto vaginal method alone was used in maiden camels (as the hand could not be inserted per vaginum and rectovaginal insertion of catheter was facilitated by narrowness of the vagina and special training for the purpose).

Inseminations were performed either simultaneous with hormonal injections or 24 hours afterward. Inseminated animals were checked with male camels for behavioural signs of pregnancy at 14^{th} and 19^{th} days of insemination (Tibary and Anouassi 1997). Those without any signs were considered not conceived, subjected to ultrasound scanning to rule out pregnancy and included for further trials. Camels that showed behavioural signs or internal changes indicative of conception were checked with the male again for confirmation and scanned for pregnancy at 25 days. Details of semen collection and processing and female side interventions were recorded and analyzed for the description of the findings.

RESULTS AND DISCUSSION

During the study period, a total of 122 semen collection attempts from three camels yielded 109 (89.347%) ejaculates. Short bovine AV without cervix imitations (Deen *et al.* 2003, Kutty and Koroth 2012) was used for 73.95% collections, while remaining collections were taken using AV provided with some type of cervix imitations (Bravo *et al*. 2000, Kutty *et al.* 2016). Ejaculates of sufficient quantity and adequate quality (88) were subjected to extension using Tris sucrose (63.64%) or Triladyl (36.36%) based egg yolk extenders. Immediate addition of the extender together with physical agitation resulted in better mixing of the ejaculate.

The mean value of sperm motility recorded 30 min after the extension was 55%, as against 60% recorded in the previous season, and is attributed to the variations between seasons and different camels being used. Sperm motility proportion showed an increase of 37% between the figures recorded immediately after extension and 30 min afterward, similar to earlier observation (Kutty and Koroth 2012) and is attributed to the time taken for proper mixing and getting the sperms acquainted with new suspending media (Deen *et al.* 2004). Between the two extenders, Triladyl was found to maintain better motility at 30 min of extension concurring the previous findings.

Insemination trials: Preliminary trials consisting of five inseminations did not yield any pregnancy, attributable to the comparatively low initial quality of semen doses during the period of transition from the nonbreeding season (Maiada *et al.* 2013). Average of progressively motile sperm proportion in the ejaculates during the month of preliminary AI was only 56% compared to 76.17% in the subsequent rounds. However, even for the preliminary AI, ejaculates of motility proportion exceeding 60% were selected and the dilution rate of semen was adjusted to ensure deposition of not less than 100 million motile sperms per insemination. Accordingly, the mean count of sperms inseminated in the preliminary round was 188.80±11.24 millions ensuring adequacy of the dose and at the same time including a large proportion of sperms with poor or no motility within the insemination dose.

Hormonal injections for induction of follicular growth was carried out in three of the five camels inseminated in the preliminary round owing to the lack of follicular growth even by December, deviating from the normal expectations (Tibary and Anouassy 1992) and the same might have contributed to the low conception.

Excluding the preliminary trials, three rounds of inseminations (based on the periodicity of AI) were carried out in 10 camels using fresh extended semen. Details of

camels inseminated and corresponding figures for conception are shown in Table 1. Seventeen inseminations in 10 camels resulted in 9 conceptions (including one pregnancy loss after 45 days of conception) giving a conception rate of 52.94% and was in agreement with the report of Morton *et al.* (2013) and Skidmore *et al.* (2013).

Irrespective of the rounds of AI, conception from first and second repeat inseminations after the trial round was 50% and 60% respectively. The better success rate for the second insemination can be attributed to coincidence with the most favourable period of the breeding season (El-Hassanein 2017).

Conception rates of first (9), second (5) and third (3) rounds of insemination were 55.56%, 60.00% and 33.33% respectively. The low success rate of the third round was attributable to more problem animals passed on from the previous rounds of inseminations. After the three rounds of AI, eight camels conceived yielding herd pregnancy rate of 80% and remaining two were considered as repeat breeders. Both these non conceived camels had the history of treatment for reproductive tract infection (RTI) prior to inseminations indicating the probable contribution of RTI to conception failure (Tibary *et al.* 2006).

Overall conception of AI in the season with extended fresh semen including five preliminary inseminations and loss of one pregnancy was 40.91% (9 pregnancies out of 22 AI). Further, excluding the last three inseminations among those camels failed to conceive from the previous rounds, success rate of AI was 57.14%, which is better than reported conception rate for AI using fresh extended semen (Deen *et al.* 2003 and Skidmore and Billah 2006), and lower than the success rate reported by Morton *et al.* (2011).

Among the male camels, Soghan was used for 13 AI as against Mayyaz used for 4 AI. Even though semen of all the three males were used for preliminary AI, none of them conceived. Better quality of semen obtained on the days of AI was the major criteria for selecting the male. In the first round, semen of Soghan resulted in 4 conceptions out of 6 AI (66.67%) as against 33.33% for Mayyaz (only 3 AI). In the second round, Soghan was used for 4 out of 5 AI and resulted in three conceptions (75%) while in the third round yielded 33.33% conceptions from 3 AI. The overall conception rate for the semen of Soghan was 69.23% as against 25% for Mayyaz. Even though there is marked difference in the total number of AI between the two males used (13 versus 4), it can be inferred that chances of getting conception were better with the semen of Soghan (OR = 4.89966).

Determinants of AI success: Quality attributes of the semen that was used in conceived camels are given in Table 2. The major semen quality parameter affecting conception of AI is the count of actively motile sperms inseminated since other quality parameters were adjusted through the selection and processing of the ejaculates. Motile sperms deposited as low as 100 million yielded pregnancies in present study, even though conception is reported with semen doses as low as 80 million by Skidmore *et al.* (2013). Hence, the possibility of reducing the semen dose further need to be tried for increasing the number of inseminations from each ejaculate.

Another factor expected to influence the success rate of AI was the extension media used for the semen. Out of 17 AI, Tris sucrose and Triladyl extended semen was used in 13 (76.57%) and 4 (23.53%) inseminations respectively and resulted in 7 (53.85%) and 2 (50%) conceptions showing not much difference between the two media. Even though Triladyl having glycerol among the components is intended for cryopreservation, earlier studies have shown that Triladyl extended semen maintained better motility during room temperature as well as chilled storage compared to constituted Tris extenders without glycerol (Kutty and Koroth 2012). However, Triladyl extended semen was used only in 4 inseminations, as the preliminary round of 5 AI using the extender failed to achieve any conception.

Retrospective details of the semen doses used for AI that did not conceive are given in Table 3. Attributes of semen used in unsuccessful AI did not reveal striking differences from that of successful inseminations, except minor variations of sperm count and motility proportions. The number of motile sperms deposited was more than 100 million in all the inseminations except one (75 million), while the mean figure of motility proportions was 5% less in case of semen doses that failed to conceive (73.75% vs 78.33%) compared to others resulted in conception.

Table 1. Details of animals inseminated and conceived in three rounds of AI done

Female camel #	First round		Second round		Third round		Total	
	Male	Result	Male	Result	Male	Result	AI	Result
34			Mayyaz	NP	Soghan	NP	2	NP
35	Soghan	Pregnant					1	Pregnant
36	Soghan	NP	Soghan	Pregnant			2	Pregnant
37	Soghan	Pregnant					1	Pregnant
38	Soghan	Pregnant					1	Pregnant
39	Mayyaz	Pregnant					1	Pregnant
40	Soghan	NP	Soghan	Pregnant			2	Pregnant
41	Mayyaz	NP	Soghan	NP	Soghan	NP	3	NP
43	Mayyaz	NP	Soghan	Pregnant			2	Pregnant
44	Soghan	Pregnant		Pregnancy lost	Soghan	Pregnant	2	Pregnant

Table 2. Attributes of semen samples used for the inseminations that turned successful

Camel No	Male used	Semen volume (ml)	Sperm. Conc. (millions/ml)	Progressive motility (%)	Media used	Dilution rate	Motile sperms deposited (millions)
35	Soghan	6	200	75	Tris Suc	1:2	100
36	Soghan	3	270	85	TrisSuc	1:3	115
37	Soghan	2	1120	75	Triladyl	1:4	336
38	Soghan	6	200	75	Tris Suc	1:2	100
39	Mayyaz	2	800	70	Triladyl	1:3	280
40	Soghan	3	270	85	TrisSuc	1:3	115
43	Soghan	3.5	670	80	TrisSuc	1:2	357
44–1	Soghan	3	280	80	TrisSuc	1:2	149
44–2	Soghan	2.5	300	80	TrisSuc	1:3	160

Table 3. Quality of semen samples used for AI turned unsuccessful in the three rounds

Camel No	Male used	Semen volume (ml)	Sperm Conc. (millons/ml)	Progressive motility (%)	Media used	Dilution rate	Motile sperms deposited (millions)
34–1	Mayyaz	3	160	70	Tris Suc	1:2	75
34–2	Soghan	2.5	300	80	Tris Suc	1:3	160
36	Soghan	3	280	80	Tris Suc	1:2	149
40	Soghan	6	200	75	Tris Suc	1:2	100
41–1	Mayyaz	1	500	75	Triladyl	1:3	188
41–2	Soghan	3.5	670	80	Tris Suc	1:2	357
41–3	Soghan	2	200	60	Tris Suc	1:1	120
43	Mayyaz	2	800	70	Triladyl	1:3	280

Besides semen characteristics, functional characteristics of the reproductive tract and regulation of ovarian activity are important factors affecting conception. In this respect, striking differences noticed between successful and unsuccessful AI was the time of giving the hormonal injection for induction of ovulation. In 66.66% of the successful AI, either GnRH / hCG was injected on the same day along with insemination, as against 75% of the camels injected with the hormones on the previous day failed to conceive. This is in contrary to the observations made by Skidmore *et al.* (2013) that maximum conception was obtained for AI 24 h after GnRH injection, even though the dose of GnRH used was same in both the studies.

It can be concluded that 8 out of 10 camels used for insemination trials conceived by 1 to 3 AI using fresh semen extended by physical agitation for achieving liquefaction of the gel. First service conception rate of 50% and overall success rate of 53% was obtained from the 17 AI performed in three rounds. Besides quality attributes of ejaculates collected, semen donor, extension media used and time of ovulation induction appears to influence the success rate of AI in camels. However, the number of inseminations done being very less, there is a need to perform more studies to arrive at valid conclusions on determinants of conception rate.

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