Effect of sperm diluent and dose on the pregnancy rate in dromedary camels after artificial insemination with fresh and liquid-stored semen

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Abstract

Considerable scope exists for the use of AI in camel breeding programmes but the technology is not fully developed. The fertility of both fresh and liquid-stored (24 h; 4°C) dromedary camel semen diluted in either Green Buffer (GB) or INRA-96 (INRA) was assessed. Different insemination doses were investigated: 150 and 300 x 10^6 motile sperm for fresh sperm, and 150, 300 and 600 x 10^6 motile sperm for liquid-stored semen. Semen was diluted 1:1 with GB or INRA and asssessed for sperm quality parameters. Diluted semen was inseminated fresh (<2 h of collection) or after liquid-storage (further diluted 1:2; 24 h at 4°C). Dilution of fresh semen in GB resulted in higher motility (67.4±1.7%; mean±sem) than INRA (59.4±2.1%; P<0.05), but sperm membrane integrity was higher for INRA (64.9±2.5%) than GB diluted semen (55.9±5.0%; P<0.05). Sperm viability (GB: 66.7±2.1%; INRA: 66.7±1.9%) and acrosome integrity (GB: 84.9±1.7%; INRA: 88.5±1.4%) were similar (P>0.05). After liquid-storage, sperm motility (GB: 47.6±2.9%; INRA: 48.3±2.5%), membrane integrity (GB: 54.9±3.5%; INRA: 56.7±2.5%) and acrosome integrity (GB: 84.8±1.9%; INRA:84.6±2.1%) were similar between diluents but sperm viability was higher for INRA (62.2±1.5%) than GB (58.9±1.0%; P<0.05). The pregnancy rate following insemination of fresh semen was similar for GB (34%) and INRA (34%) but increased for insemination doses consisting of 300 (41.6%) compared with 150 x 10^6 motile sperm (27.2%; P< 0.05). For liquid-stored semen, the pregnancy rate was higher with INRA (23.5%) than GB (0.0%; P<0.05), and was reduced for 150 (0.0%; P<0.05) compared with 300 (10.5%) and 600 x 10^6 motile sperm (25.0%). INRA is a suitable diluent for the liquid-storage of dromedary semen and insemination doses of 300 and 600 x 10^6 motile sperm are recommended for AI with fresh and liquid-stored sperm respectively.

Keywords: Artificial insemination; Camel; liquid-stored semen; dromedary; INRA; Green Buffer

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Introduction

Artificial insemination (AI) is a powerful reproductive technology and is used worldwide to rapidly disseminate superior genetics in commercially important domestic animal species such as cattle, goats, sheep and pigs. Aspects of the reproductive physiology of the Camelidae family, specifically the difficulties associated with semen collection and the highly viscous nature of the semen (reviewed by Morton et al., 2008) make the application of this technology difficult the methods widely used in domestic ruminants and monograstric species are not directly applicable. Despite these challenges, semen collection in dromedary camels using an artificial vagina mounted inside a dummy, or adjacent to a teaser female is routine and ejaculates are typically obtained (Morton et al., 2008; Skidmore et al., 2013). However, there is still considerable research required as to the most appropriate diluents for semen extension whether that be for fresh, liquid-stored or cryopreserved spermatozoa.

Despite initial success with AI technology in the early 1960s when the birth of a Bactrian camel was reported (Elliot, 1961), there have been only limited reports of offspring born from AI in camels with the majority of offspring having been born after AI with freshly diluted semen (reviewed by Skidmore et al., 2013). Several diluents have been trialled for the dilution of fresh dromedary camel semen including Androhep (Musa et al., 1993), Green buffer (GB; Skidmore and Billah, 2006), Kenney's (skim-milk+glucose; Sieme et al., 1990), Laciphos (Sieme et al., 1990; Musa et al., 1993), and Lactose diluents (Anouassi et al., 1992). Pregnancies have been reported after the AI of fresh semen diluted in Lactose (50%; Anouassi et al., 1992), Laciphos (53%; Musa et al., 1992) and Green Buffer (10-47%; Skidmore and Billah, 2006) but there are fewer reported pregnancies after AI of liquid-stored or frozen-thawed semen. Bravo et al. (2000) stated that the conception rate decreased to 25% with liquid-stored semen but did not provide any other information. Later, Deen et al. (2003) reported a sole pregnancy after the AI of 13 female camels (8%) with frozen-thawed semen but failed to

obtain pregnancies after the AI of liquid-stored semen.

A number of different diluents (reviewed by Morton et al., 2008) have been trialled for short-term liquid-storage of camel semen and many studies have reported success (as measured by *in vitro* characteristics such as motility) when semen has been diluted in Lactose (55% at 10 h; Hassan et al., 1995), Green Buffer (50% at 24 h; Morton et al., 2010), INRA-96 (50% at 24 h; Morton et al., 2010), or Tris (40% at 24 h; Deen et al., 2004). More recently Wani et al. (2008) examined the liquid storage of dromedary camel semen in either Tris-tes, Tris-lactose, Citrate, Sucrose or Tris-fructose diluents. While a high proportion of sperm displayed motility (40-50%) after 24 h storage at 4°C, subsequent insemination (150 x 10^6 motile, deep intra-uterine) failed to result in any pregnancies (Wani et al., unpublished data), suggesting that either the diluents were unsuitable, or that the insemination procedure for liquid-stored semen may be sub-optimal.

Previous studies have reported high levels of fertility after the AI (deep-intra uterine) of fresh camel semen diluted in GB. Semen was inseminated 24 h after the induction of ovulation at doses of 150 x 10^6 motile sperm and yielded pregnancy rates of 53% (Skidmore and Billah, 2006) and 72.7 % (Morton et al., 2011), suggesting that the timing of insemination relative to ovulation is unlikely to be the cause of the low pregnancy rates after AI with liquid-stored semen. Rather, the poor results after AI with liquid-stored semen are more likely to be attributable to a reduction in fertility of the sperm owing to sub-optimal liquid storage (diluent) or insufficient numbers of sperm inseminated into the reproductive tract.

The effect of insemination dose of motile sperm on the resultant pregnancy rate in camels after the AI of liquid-stored semen has yet to be investigated, although previous studies using fresh semen have reported 150 or 300 x 10^6 motile sperm as fertile doses. The choice of diluent for the liquid-storage of camel semen also requires further research, and given the success of previous studies with GB and INRA, a study was designed to compare the *in vivo* fertility of fresh and liquid-stored dromedary camel semen diluted in GB or INRA at a dose of 150, 300 or 600 (liquid-stored only) x 10^6 motile sperm.

Materials and Methods

Animals

Male (n=4, aged 10 - 20 years) and female (n=79) camels were housed singly (males) or in groups of 20 - 25 (females) in sand pens at The Camel Reproduction

Centre in Dubai, UAE. All camels were exposed to natural day length and ambient temperatures, had *ad libitum* access to water and were maintained on a ration of commercially formulated camel feed (Za'abeel Feed Mills, Dubai, UAE) and grass-hay.

Collection of semen

Semen was collected from four adult male camels (n=5 ejaculates per male; 20 ejaculates total) as previously described by Morton et al. (2011) using a modified bull artificial vagina (AV; IMV Technologies) with a silicone liner (Morton et al., 2008) and a camel collection glass (IMV Technologies). Collecting glasses containing semen were stored in a 35°C waterbath until dilution and processing (which occurred within 10 min of collection). During the experiment, semen from individual males was kept separate throughout all procedures.

Semen dilution and preservation

Each ejaculate was divided into two aliquots of equal volume and diluted 1:1 with either warmed (35°C) GB + 20% hens egg yolk or INRA and placed in a waterbath (35°C) for 30 min to partially liquefy. Diluted semen was then assessed for quality parameters (as described below) and prepared for insemination. For liquid-storage, ejaculates were further diluted 1:2 with the same diluent as used previously and placed in an Equitainer (Hamilton-Thorne, USA) and stored at 4°C for 24 h prior to insemination (liquid-stored).

Semen assessment

Sperm concentration, motility, viability, membrane integrity and acrosome integrity were assessed immediately after collection (neat), after dilution, and following 24 h of storage at 4°C. Briefly, sperm concentration was determined using a haemocytometer after 1:10 dilution of semen in a 3% (W/V) solution containing 1% collagenase (Sigma). The methods described by Evans and Maxwell (1987) and Morton et al. (2008) were used to subjectively estimate sperm motility to the nearest 5%. Motility of neat semen was assessed as ocillatory while motility of diluted semen was assessed on forward progressive motion (for further explanation see Morton et al., 2008). Sperm viability was determined using the Eosin-Nigrosin stain (Evans and Maxwell, 1987). Equal volumes of semen and staining solution (10 μL each) were placed on pre-warmed glass slides (at 37°C), mixed and smeared into thin films. Smears were then dried at 37°C and examined using phase contrast microscopy (magnification x400) or oil immersion (magnification x1000). At least 200 sperm were counted per

slide. Sperm membrane integrity was assessed using the hypoosmotic-swelling test (HOS; Morton et al., 2008). Briefly, 50 μL semen were transferred to a micro-centrifuge tube, diluted 1:4 with fructose solution (60 mOsm) and incubated at 37°C for 35 min. Semen samples (10 μL) were then placed on a pre-warmed slide, covered with a coverslip and examined using phase contrast microscopy (magnification x400). At least 200 sperm per slide were examined. Sperm with coiled tails were considered to have intact membranes whilst those with straight tails were considered non-intact.

Sperm acrosomal status was assessed by fluorescent isothiocyanate-conjugated peanut agglutin (FITC-PNA; Sigma) as described by Morton et al. (2007). Briefly, an aliquot of semen was smeared on a slide and air-dried. Slides were then stored at 4°C for less than two weeks. Immediately before assessment, slides were stained with 100 μg mL^{-1} FITC-PNA in PBS and incubated for 30 min in a humid 37°C atmosphere. Slides were then rinsed with PBS to remove excess stain and placed in the dark at 37°C to dry. Fade retardant, consisting of 90% (v/v) glycerol, 10% (v/v) PBS and 0.1% (w/v) *p*-phenylenediamine, was then placed on the stained area of the slide and covered with a coverslip. Spermatozoa with acrosomes stained green and were considered intact, while those with no staining or a single band of staining at the equatorial segment were considered as having non-intact acrosomes.

Preparation of females and artificial insemination

Transrectal ultrasonography was used to monitor ovarian follicular activity, and measure ovarian follicles in female camels according to the methods described by Morton et al. (2011). When the dominant follicle was observed to measured 1.3 – 1.8 cm in diameter, ovulation was induced by i.v. administration of 20 μg buserelin (Receptal®, Hoechst Animal Health, Bedfordshire, UK). Female camels were then artificially inseminated 24 - 28 h following the induction of ovulation with either fresh (150 or 300 x 10^6 motile sperm) or liquid-stored (150, 300 or 600 x10^6 motile) sperm using the technique described by Morton et al. (2011). Briefly, extended semen was deposited in the uterine body using a standard bovine AI pipette (IMV Technologies) which was manually guided through the cervix per rectum. Ovulation was detected and confirmed (collapsed dominant follicle) using ultrasound examination of the ovaries 48 h after buserlin administration. Pregnancy (presence of conceptus) was also confirmed using ultrasound 18 - 25 days post-insemination (Morton et al. 2011)

Experimental design

The ejaculate was split into two aliquots and immediately diluted 1:1 (v:v) with Green Buffer (IMV Technologies, USA) containing 20% (V/V) hen egg yolk or INRA-96 (INRA; IMV, France). Semen was then assessed for sperm motility, sperm concentration, sperm membrane integrity, viability and acrosome integrity, and then either used for insemination (within 2 h of collection; fresh), or further diluted (1:2 with respective diluent) and stored for 24 h at 4°C prior to re-assessment and insemination (liquid-stored). Artificial inseminations were performed 24-28 h post induction of ovulation and only females who ovulated were inseminated. For fresh semen sperm was inseminated at either 150 or 300 x 10^6 motile sperm, while liquid-stored semen was inseminated at doses of 150, 300 or 600 x 10^6 motile sperm. Pregnancy was then determined by ultrasound 18-25 days post-insemination.

Statistical analysis

Statistical differences in the sperm parameter data were determined by ANOVA after arcsine transformation of the data and means were compared by least significant differences. Statistical differences among pregnancy rates were determined using a Chi-square test using Genstat (Release 7.2; Ceanet, Brisbane, Qld, Australia) with $P<0.05$ considered significant.

Results

Semen quality parameters

Sperm quality parameters for neat and semen diluted in GB and INRA are described in Table 1. Neat semen volume was 2.2 ± 0.5 mL (range: 0.5 – 6.0 mL), and sperm concentration was 237.4 ± 47.3 x 10^6 sperm mL^{-1}.

For semen diluted in both GB and INRA, forward progressive motility was increased by dilution compared with neat semen (P<0.05; Table 1). Immediately after dilution sperm motility was higher (P<0.05) for semen diluted with GB (67.4 ± 1.7%) than INRA (59.4 ± 2.1%) but by 24 h storage at 4°C, there were no difference (P>0.05) in sperm motility between these diluents. However, sperm motility was lower (P<0.05) for both diluents after 24 h storage at 4°C than immediately after dilution. Despite this reduction, a lower proportion of the intitial motility was retained for semen diluted in both the GB (47.6/67.4; 70.6%) or INRA dilutes (48.3/59.4; 81.3%). Compared with neat semen, sperm viability was reduced by dilution (P<0.05; Table 1) but there was no difference (P>0.05) between sperm diluted in GB and INRA (Table 1). Sperm viability remained similar during storage (ie 24 hr storage

compared with diluted samples) for sperm diluted in INRA ($P>0.05$) but was reduced during storage in GB diluted sperm ($P<0.05$; Table 1).Sperm membrane integrity was reduced by dilution in GB but not INRA diluent ($P<0.05$) but was similar for diluents after 24 h storage at 4°C (Table 1). During storage for 24 h at 4°C, sperm membrane integrity did not change for GB diluted sperm but was reduced for sperm stored in the INRA diluent (Table 1). Sperm acrosome integrity was unaffected by dilution and 24 h storage at 4°C ($P>0.05$) and did not differ between diluents ($P>0.05$).

Table 1. Sperm quality parameters for dromedary camel neat semen, fresh diluted (immediately after collection) and liquid-stored (24 h storage at 4°C).

Sperm Parameter	Diluent	Neat Semen	Fresh diluted	Liquid-stored (24 h; 4°C)
Sperm Motility (%)	GB	45.5 ± 5.5^{A}	$67.4 \pm 1.7^{a,B}$	$47.6 \pm 2.9^{a,A}$
	INRA		$59.4 \pm 2.1^{b,B}$	$48.3 \pm 2.5^{a,A}$
Sperm Viability (%)	GB	73.2 ± 4.6^{A}	$66.7 \pm 2.1^{a,B}$	$58.9 \pm 1.0^{b,C}$
	INRA		$66.7 \pm 1.9^{a,B}$	$62.2 \pm 1.5^{a,B}$
Sperm Membrane Integrity (%)	GB	68.4 ± 6.3^{A}	$55.9 \pm 5.0^{a,B}$	$54.9 \pm 3.5^{a,B}$
	INRA		$64.9 \pm 2.5^{b,A}$	$57.6 \pm 2.5^{a,B}$
Acrosome integrity (%)	GB	89.4 ± 5.2^{A}	$84.9 \pm 1.7^{a,A}$	$84.8 \pm 1.9^{a,A}$
	INRA		$88.5 \pm 1.4^{a,A}$	$84.6 \pm 2.1^{a,A}$

Data are mean ± s.e.m. and values within a row (A,B,C) or column (a,b,c) with a different superscript letter are significantly different ($P<0.05$).

In vivo fertility

For fresh semen there was no difference in pregnancy rate between females inseminated with sperm diluted in GB or INRA (P>0.05; Table 2). However, pregnancy rate was increased by insemination of 300 (41.6%) compared with 150 x 10^6 motile sperm (27.2%; P<0.05; Table 2). Pregnancy rate for liquid-stored semen (Table 2) was increased for semen liquid-stored in INRA compared with GB (23.5 vs 0.0%), and was increased by AI with 600 (25.0%) compared with 300 (10.5%) and 150 x 10^6 motile sperm (0.0%).

Table 2. In vivo fertility of freshly diluted and liquid-stored dromedary camel semen.

Sperm Type	Diluent / Motile Sperm Dose	No. Inseminated	No. Pregnant	Pregnancy Rate (%)
Freshly diluted	Green Buffer	23	8	34.0[a]
	INRA	23	8	34.0[a]
Liquid-stored (24 h, 4°C)	Green Buffer	16	0	0.0[b]
	INRA	17	4	23.5[c]
Freshly diluted	150 x 10^6	22	6	27.2[a]
	300 x 10^6	24	10	41.6[b]
Liquid-stored (24 h, 4°C)	150 x 10^6	10	0	0.0[c]
	300 x 10^6	19	2	10.5[d]
	600 x 10^6	8	2	25.0[a]

Data are mean ± s.e.m. and values with a different superscript letter are significantly different (a,b) (P<0.05).

Discussion

In the present study, the liquid storage of domedary camel semen in Green Buffer (GB) and INRA diluents, and the resultant *in vivo* fertility of various insemination doses of fresh and liquid-stored semen were examined. The results indicated that dromedary camel semen can be successfully liquid-stored (24 h, 4°C) in both Green

Buffer and INRA diluents, as confirmed by *in vitro* sperm quality parameters. Pregnancies were also achieved with fresh semen diluted in GB and INRA diluents and liquid-stored (24 h, 4°C) semen diluted in INRA but not GB.

Neat semen characteristics were similar to those previously described for this species (Skidmore and Billah, 2006; Wani et al., 2008; Morton et al., 2011 and Wani et al., 2011). The initial motility was high for semen diluted in both GB and INRA, and was similar to that previously reported for the GB diluent (70.4%; Morton et al., 2011) and other diluents (reviewed by Morton et al., 2008). Immediately after dilution, sperm motility was higher for semen diluted in GB compared to that in INRA but acrosome integrity was similar for both diluents, demonstrating their respective suitabilities for use with fresh camel semen.

Comparisons between studies examinig the liquid storage of dromedary camel semen are difficult as many have not provided sufficient details to enable a direct comparision. For example, generally a dilution rate of 1:3 (semen: diluent) is used (Deen et al., 2004; Wani et al., 2008) but a number of studies have failed to specify the dilution rate (Vyas et al., 1998; Morfeld et al., 2003). Initial studies reported that lactose based extender was superior to Androhep, sodium citrate, Laiciphos and Green Buffer diluents (Sieme et al., 1990). Subsequently, Vyas et al. (1998) concluded that Tris based diluent was superior to Lactose based diluent but sperm motility was not disclosed. Morfeld et al. (2003) also examined the liquid storage of camel semen in 11% fructose, 11% glucose or 11% lactose-based diluents and observed similar motility (near 30%) after 48 hours of storage between the diluents used. In a later comparison of Tris and Biociphos diluents, Deen et al. (2004) concluded that Tris diluent was superior as none of the samples diluted Biociphos were motile after 24 h liquid-storage. Further to this, Wani et al. (2008) compared different types of Tris based diluents (Tris-fructose, Tris-lactose, Tris-tes, citrate), concluding that whilst all diluents are suitable, Tris-lactose diluent provides superior motility. In a more recent study, Morton et al. (2010) demonstrated the suitability of INRA diluent for the liquid-storage of dromedary camel semen.

The results of the present study show that a high proportion (70-80%) of the initial motility and other sperm quality parameters (viability, membrane integrity and acrosome integrity) were retained after 24 h of storage at 4°C. This suggests that the procedure for the liquid-storage of camel spermatozoa was effective. Despite this, a higher

proportion of motility was retained for semen diluted and stored at 4°C in INRA than GB diluents (81.3 vs 70.6%). This coupled with the higher viability of semen diluted at stored in INRA compared with GB suggests that INRA is more suitable diluent for the liquid-storage of camel semen at 4°C than GB.

Despite a difference in the sperm membrane integrity immeditely after dilution, there was no difference observed in the pregnancy rates after AI with semen freshly diluted in either GB or INRA. The overall pregnancy rate after AI with freshly diluted semen was 34.0% which is similar to previous studies (Musa et al., 1993; Skidmore and Billah, 2001). However, the pregnancy rate in dromedary camels after AI, reported in the literature, has varied consiberably (ranging from 0 - 10%: Deen et al., 2003; 20 - 30%: Bravo et al., 2000; 40 - 50%: Skidmore and Billah, 2006; > 50%: Anouassi et al., 1992 and Morton et al., 2011). Comparisons between studies are often difficult as a number of studies have failed to provide adequate information regarding the specifics of the AI procedure, for example omitting the number of spermatozoa inseminated (Deen et al., 2003) or site of insemination (Bravo et al., 2000).

In dromedary camels, AI is routinely performed 24 h after the induction of ovulation (generally using a GnRH injection) and sperm is deposited either in the uterine body or uterine horn (reviewed by Skidmore et al., 2013). However, the optimal dose of motile sperm for AI with fresh and liquid-stored semen has yet to be determined. In the present study, increasing the dose of sperm used for AI with freshly diluted semen from 150 to 300 x 10^6 motile sperm, increased the pregnancy rate increased from 27.2% to 41.6% respectively. Previously, Skidmore and Billah (2001) reported increased pregnancy rates with fresh semen from 10 to 47% when the sperm dose was increased from 100 to 300 x 10^6 motile sperm. This is similar to 47% reported by Musa et al. (1993) when 300 x 10^6 motile sperm were used for AI. These results would suggest that 300, rather than 150 x 10^6 motile sperm would be the preferred AI dose for freshly diluted semen in the dromedary camel. However, considerably higher pregnancy rates of 42% (Niasari-Naslaji et al., 2001), 43-53% (Skidmore and Billah, 2006) and 72.7% (Morton et al., 2011), have been reported after the AI of 150 x 10^6 motile fresh sperm.

Differences between the present study and that of Niasari-Naslaji et al. (2001) could be attributable to differences in semen collection and handling techniques, insemination procedure and techniques, camel management or

inherent fertility of the populations of camels used. However, the results of the present study are considerably lower than the 53% and 72.7% reported by Skidmore and Billah (2006) and Morton et al. (2011), which is surprising given the same insemination technique, number of motile sperm (150 x 10^6) and population of experimental animals were used. Variation between successive annual breeding seasons may explain this result, and has been previously reported for other species (sheep; Evans and Maxwell, 1987).

There have few studies examining the fertility of liquid-stored dromedary camel sperm after AI with Bravo et al (2000) first mentioning the use of liquid-stored semen in the literature. The authors merely stated that the conception rate decreased to 25% with cooled semen diluted in Green Buffer with 20% egg yolk although no further details were provided, such as the number of animals inseminated, motility of sperm or the number of motile sperm inseminated. In the present study, despite achieving pregnancies after the AI with liquid-stored sperm, the overall fertility of liquid-stored sperm (4/37; 10.8%) was reduced compared with fresh sperm (16/46; 34.0%; $P<0.05$). Moreover, this reduction in fertility was observed for both the 150 (fresh: 27.2%; liquid-stored: 0%) and 300 x 10^6 (fresh: 41.6%; liquid-stored: 10.5%) sperm doses, clearly demonstrating that liquid-storage affects the fertility of dromedary camel semen. Both Bravo et al. (2000) and Deen et al. (2003) have also reported a reduction in the fertility of liquid-stored dromedary camel semen, concurring with the results of the present study and other domestic species (boar: Johnson et al., 2000; bull: Vishwanath and Shannon, 2000; ram: Salamon and Maxwell, 2000).

During liquid-storage, the main changes occuring to sperm are a reduction in motility and morphological integrity, which result from the accumultion of toxic metabolic products, mainly in the form of reactive oxygen species resulting from the lipid peroxidation of spermatozoa membranes (Salamon and Maxwell, 2000). This was reflected in a decline in transport and survival of sperm in the female reproductive tract and reduction in fertility (Salamon and Maxwell, 2000). The reduction in sperm motility, membrane integrity and viability observed in the present study after storage for 24 h at 4°C supports this process as being the mechanism behind the reduction in fertility of liquid-stored dromedary camel sperm. Interestingly, in the present study sperm liquid-stored in Green Buffer failed to yield any pregnancies despite previous success with dromedary camel semen stored in Green Buffer (Bravo et al., 2000).

Given that the studies were performed in the same laboratory, it was quite surprising.

The results of the present study demonstrate that increasing the number of liquid-stored motile sperm inseminated from 150 to 600 x 10^6 increases the pregnancy rate from 0 to 25% suggesting that increasing the AI dose with liquid-stored sperm in the dromedary camel is warranted, as is routine for other species when liquid-stored sperm is used (Evans and Maxwell, 1987; Salamon and Maxwell, 2000). Despite the success at achieving pregnancies with liquid-stored dromedary camel sperm, further research is required to elucidate the optimal sperm storage diluent and AI dose.

In summary, the present study demonstrates prengancies can be achieved in dromedary camels after AI of semen liquid-stored (24 h, 4°C). In addition, INRA is a more suitable diluent for liquid-storage than GB. However, both GB and INRA are suitable diluents for semen being inseminated immediately after dilution. For freshly diluted semen, insemination with 300 x 10^6 motile sperm results in a higher pregnancy rate than 150 x 10^6 motile sperm; whilst AI with a minimum of 600 x 10^6 motile sperm is recommended for liquid-stored semen.

Acknowledgements

This research was kindly supported by His Highness General Shaik Mohammed bin Rashid Al Maktoum, Vice President and Prime Minister of the UAE and Ruler of Dubai. The authors wish to thank Aijaz Hussain and Abdul Rahman for their expert technical assistance during the conducting of the experiment.

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