Conservation of camel genetic resources: epididymal sperm recovery

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ABSTRACT: Camels represent part of the Arab heritage. The interest in developing assisted reproductive technologies and cryobanking for the conservation of animal genetic resources has recently increased. However, semen collections in camelids present many problems as sitting position during copulation, slow ejaculation and difficult animal handling. In these cases epididymal sperm from slaughtered or recently died animals will increase the opportunities to create semen storages.

The present work was designed to assess motility of camel epididymal sperm extended in Ovixcell(r) and in Tris-fructose-egg yolk semen extender. Spermatozoa were extracted from 16 epididymides using the retrograde flushing technique, washing sperm cells in a retrograde direction from the ductus deferens through the cauda epididymidis with a syringe loaded with warmed (37°C) extender. Total motility was evaluated after 15 minute of incubation in a water bath at 37°C under phase-contrast microscopy using a pre-warmed (37°C) Makler Chamber. Total motility was similar in Ovixcell(r) or Tris-fructose-egg yolk semen extender (52.8 ± 0.7% vs 41.22 ± 33.56%, respectively). Further studies aiming to the test the fertilizing capacity should be carried out in order to confirm the optimal testicles storage condition for the creation of semen cryo storages in camels.

Introduction

Erosion of animal genetic resources (AnGR) is advancing at high speed. To overcome this phenomenon in last years a major interest in developing assisted reproductive technologies and cryobanking for the conservation of animal genetic resources has observed. In situ conservation of breed diversity, i.e. maintenance of breeds within their production systems, is the preferred strategy to control risks of AnGR losses. However, cryopreservation is an important tool complementary to in situ conservation, as genetic back-up in case of losses of genetic variation, and it is the strategy of choice when in situ strategies are ineffective. Sometimes cryopreservation is not routinely feasible, due to the lack of facilities (Artificial Insemination centres, laboratories) and expertise near the animal farming area.

In camel species, application of assisted reproduction technologies, such as artificial insemination, has been lower in comparison to that in other livestock species (Bravo et al., 2000), probably due to the difficult semen collections that presents many problems as sitting position during copulation, slow ejaculation (Bravo and Johnson, 1994) and problematic animal handling.

When semen collection through standard procedures is difficult or impossible, the post mortem recovery of epididymal sperm from slaughtered or castrated animals can be the only possibility to create semen storages to preserve male gametes from animals of high value or from endangered species (Leibo and Songsasen 2002; Fickel et al. 2007). Viable and functional camel epididymal spermatozoa are also necessary to develop artificial insemination and IVF technique. Moreover optimal diluents and proper storage conditions to maintain the quality and fertilizing ability of the spermatozoa for longer periods, are requested (Waheed et al. 2011).

For this reason we have investigated the effects of two different semen extenders on the quality of camel epididymal sperm in order to support the applicability of this technique in camel species.

Materials and methods

Collection of dromedary camel epididymal spermatozoa

Testicles from 16 mature dromedary were collected post mortem at a local abattoir (El-Basatein) in Cairo, Egypt during the breeding season (November). Both testicles from each animal were removed on average 1.30 h after slaughter and transported to the laboratory in a Styrofoam box at refrigeration temperature with a portable refrigerator. Approximately 180 min after collection, testes and epididymides were removed from the scrotal sac and afterwards caudae epididymides were isolated from testes and from surrounding connective tissue. Epididymal sperm were collected using the retrograde flushing technique as performed by Turri et al. (2012). Briefly the lumen of the ductus deferens was cannulated, before flushing, with a blunted 22-gauge needle to avoid losses of material. Cauda epididymidis and ductus deferens were isolated from the rest of the epididymidis by making a cut with a scalpel near the junction of the corpus and the proximal cauda. Sperm cells were then flushed in a retrograde direction from the ductus deferens through the cauda epididymidis with a syringe loaded with approximately 2 ml of warmed (37°C) extender (Fig. 1A). For each testicles, it was randomly decided which epididymis to process with the following semen extender; (1) The Tris-fructose extender (3.63 g Tris, 0.50 g fructose, 1.99 g citric acid, 20 ml egg yolk, 7% glycerol and double distilled water to 100 ml (Salamon, and Maxwell 2000); 2) Ovixcell(r) extender without animal protein (IMV Technologies, L'Aigle, France).

Quality Assessment

Sperm quality was analyzed immediately after extraction. Collected volume (ml), motility and viability were evaluated. Total sperm motility was evaluated after 15 minute of incubation in a water bath at 37 °C under phase-contrast microscopy using a pre-warmed (37 °C) Makler Chamber. Sperm viability was assessed by Eosin Y-Nigrosin staining according to Emilson et al. (1978).

Results

The effects of Ovixcell(r) and Tris-fructose-egg yolk semen extender on motility and viability of dromedary camel epididymal spermatozoa are presented in Figure 1B. Results indicated that after 15 minutes of incubation at 37°C the percentages of total motility and viability were similar in samples extended with Ovixcell(r) or Tris-fructose-egg yolk semen extender (O = 52.8 ± 40.7%, 83.0 ± 3.4%, respectively; T = 41.22 ± 33.56%, 79.0 ± 2.3%; respectively). However the percentage of motile and viable sperm appeared to be slightly higher when epididymal sperm were recovered and extended in Ovixcell(r).

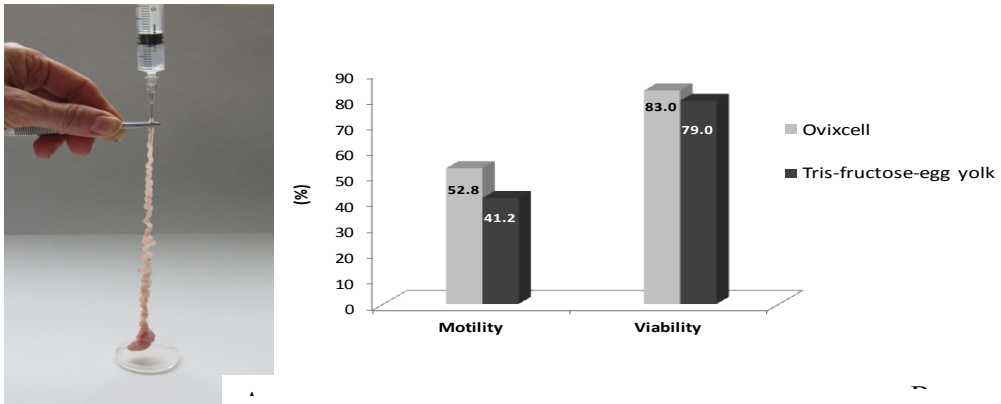

Fig 1. A) Retrograde retrieval method for recovery of spermatozoa from the tail camel cauda epididymidis. B) Sperm motility and viability parameters in fresh camel epididymal sperm samples by extender.

Discussion and Conclusion

Recovery and cryopreservation of viable sperm from the epididymides of slaughtered animals can be an alternative tool to collect male gametes, especially in wild species and in situations where traditional collection is difficult due to the reproductive behavior of the animal and the lack of facilities.

In this work, by using the retrograde flushing technique, never reported in dromedary camel, a good epididymal sperm quality has been achieved.

Sperm motility of camel epididymal sperm seems to be higher in Ovixcell(r) than Tris-fructose semen extender. Moreover, viability for dromedary epididymal spermatozoa was similar in Ovixcell(r) and Tris-fructose semen extenders. Similarly, Khalifa and Lymberopoulos (2013), have founded that Ovixcell(r) is superior to milk-egg yolk extender in preserving ram sperm chromatin stability and motility.

Further studies aiming to the test the fertilizing capacity are carried out in order to test cryopreservation effects and in vitro fertilizing ability of dromedary camel epididymal sperm (Abdoon et al. 2013. Scientific Conference of Camel Research and Production, Khartoum, Sudan. 17-18 April 2013).

In this way epididymal sperm in camel can be considered an alternative source of germoplasm for an efficient management of reproduction and animal genetic resource conservation activities.

REFERENCES

1. Abdoon AS, Kandil OM, Pizzi F, Turri F, El Atrash A, Sabra H (2013). Effect of semen extender on cryopreservation and fertilization rates of dromedary camel epididymal spermatozoa. Scientific Conference of Camel research and Production, Khartoum, Sudan, 17-18/04/2013.

2. Bravo PW, Skidmore JA and Zhao XX (2000). Reproductive aspects and storage of semen in Camelidae, Anim. Reprod. Sci. 62 173-193.

3. Bravo PW and LW Johnson (1994). Reproductive physiology of the male camelid." Veterinary Clinics of North America, Food Animal Practice 10(2): 259-264.

4. Emilson LB, Dougherty KA, Cockett AT and Urry RL (1978). Simultaneous determination of human sperm morphology and viability: simple office technique. Urology 11(5):488-91.

5. Fickel J, Wagener A, Ludwig A (2007). Semen cryopreservation and the conservation of endangered species. Eur J Wildl Res 53, 81-89.

6. Khalifa T and Lymberopoulos A (2013). Changeability of sperm chromatin structure during liquid storage of ovine semen in milk-egg yolk- and soybean lecithin-based extenders and their relationships to field-fertility. Cell Tissue Bank. 2013 Jan 4.

7. Leibo S, Songsasen N (2002). Cryopreservation of gametes and embryos of non-domestic species. Theriogenology 57, 303-326.

8. Salamon S and Maxweel WM (2000). Storage of ram semen. Animal Reproduction Science 474 62, 77-111.

9. Turri F, Madeddu M, Gliozzi TM, Gandini G, Pizzi F (2012). Influence of recovery methods and extenders on bull epididymal spermatozoa quality. Reprod Domest Anim. 2012 Oct; 47(5):712-7.

10. Waheed MM, Al-Eknah MM, El-Bahr SM (2011). Some biochemical characteristics and preservation of epididymal camel spermatozoa (Camelus dromedarius). Theriogenology 76, 1126-1133.

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