Assessment of epididymal sperm obtained from dromedary camel

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Summary

Testicles were isolated from dromedary camels in a local slaughterhouse at breeding and non-breeding seasons. Sperms were recovered from different parts of the epididymis (caput, corpus and cauda) and stained separately on slide glasses by eosin nigrosin staining method and dried by a hair dryer and carried to the laboratory. In the lab, slides were observed for evaluation of the proportion of live sperms and the proportion of sperms with cytoplasmic droplets under a light microscope. The proportions of live sperm cells were 76.8, 86.9 and 88.8% for caput, corpus and cauda epididymis, respectively. In the left testicle these values were 85.3, 83.1 and 88.4 for caput, corpus and cauda epididymis, respectively. No significant difference was also observed in the live sperm cells obtained from right and left testicles. The proportions of live sperm cells were 83, 90 and 86% in breeding and 80, 82 and 90.5% in non-breeding seasons for caput, corpus and cauda epididymis, respectively, which were not significantly different. The proportions of live sperms with protoplasmic droplets were 66, 70 and 74% in breeding and 73, 70 and 82% in non-breeding seasons for caput, corpus and cauda epididymis, respectively, which were not significantly different. The proportions of live sperms with protoplasmic droplets were significantly different neither among right and left testicles nor in different parts of epididymis. We concluded that sperm cells could be obtained from every part of the epididymis.

Key words: Camel, Epididymal sperm, Protoplasmic droplets

Introduction

Epididymal sperm has been used in many laboratories because it is easier to get in some especial species. Cryopreserved epididymal sperm is now used for intracytoplasmic sperm injection (ICSI) in human insemination (Jansen *et al*., 2000; Patrizio, 2000). Epididymal sperms have been obtained and individual variations in cryoprotectant toxicities have been studied from African antelope (Loskutoff *et al*., 1996). Epididymal sperms have successfully been obtained at necropsy from goats and used for *in vitro* fertilization (IVF) (Blash *et al*., 2000). One year later, goat epididymal sperm was cryopreserved using a chemically defined model system (Kundu *et al*., 2001). Yu and Leibo (2002) have successfully recovered motile and membrane-intact spermatozoa from canine epididymis stored for 8 days at 4ºC. James *et al*. (2002) have stored equine sperm in the epididymis at 4ºC for 24, 48, 72 and 96 hrs. Some experiments have also been done by Kaabi *et al*. (2003) on the quality of cauda epididymal ram spermatozoa. On the other hand, artificial insemination and embryo transfer as well as IVF have been used for camelids (Anouassi *et al*., 1992; Musa *et al*., 1992; McKinnon *et al*., 1994; Tinson and Singh, 1998; Bravo *et al*., 2000). Surprisingly some researchers have used epididymal sperm from South American camelids but no offspring was resulted from their works (Del Campo *et al*., 1994). The present study was conducted to assess the proportion of dromedary camel live sperm in different parts of the epididymis and also to compare left and right testicles in breeding and non-breeding season for this aspect, for further use of camel epididymal sperm in AI and IVF.

Materials and Methods

Sperm cells preparation

Testicles from 50 slaughtered dromedary camels (100 testicles) were isolated in a local slaughterhouse at breeding and non-breeding seasons. Selected animals were between 5 to 12 years old. Sperm cells were recovered from different parts of the epididymis (caput, corpus and cauda) and stained separately on slide glasses by eosin nigrosin staining method according to our previous study (Tajik *et al*., 2003) and dried by a hair dryer. For each camel 6 slides were prepared. Slides from caput, corpus and cauda epididymis were marked LH, LB and LT, respectively for the left testicle and marked RH, RB and RT from the same pain in the right testicles.

Slide preparation was completed in slaughterhouse and then carried to the laboratory. In the lab, slides were observed for evaluation of the proportion of live sperms and the proportion of sperm cells with cytoplasmic droplets in different parts of the epididymis under a light microscope. For each slide 200 sperm cells were observed and the mean ± SE were calculated for 50 testicles in breeding and non-breeding seasons as well as for the right and left testicles.

Statistical analysis

The proportion of live and dead sperm in different parts of the epididymis in breeding and non-breeding seasons and the proportion of sperm cells containing protoplasmic droplets were subjected to an arc-sine transformation; the transformed values were assigned for one-way ANOVA. When ANOVA revealed a significant effect, values were compared by Duncan's multiple range test.

Results

Fig. 1, shows the mean values ± SE of live sperm cells in the right and left testicles. In the right testicle these values were 76.8, 86.9 and 88.8% for caput, corpus and cauda epididymis, respectively and were not significantly different. In the left testicle these values were 85.3, 83.1 and 88.4 for caput, corpus and cauda epididymis, respectively (Fig. 1). Fig. 2 shows the mean values ± SE of live sperms in breeding and non-breeding seasons. As shown in Fig. 2, the proportion of live sperms (Mean ± SE) in breeding and non-breeding seasons was not significantly different for, caput (83 ± 4.4% vs. 80 ± 10.4%), corpus (90 ± 3.3% vs. 82 ± 7%) and/or cauda epididymis (86 ± 3.6% vs. 90.5 ± 4.5%) for breeding and non-breeding seasons, respectively. No significant difference was observed in the proportions of live sperms obtained from right and left testicles during breeding and non-breeding seasons (data were not shown here).

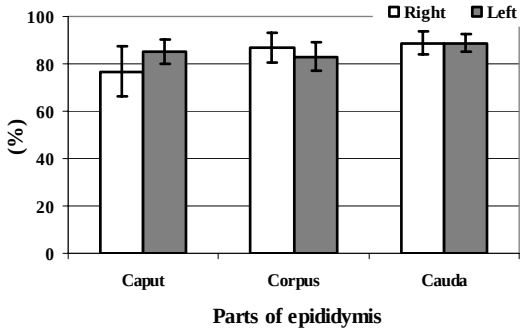

Fig. 1: Proportion of live sperms in the right and left testicles

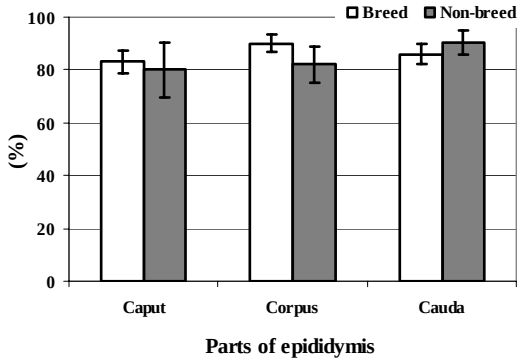

Fig. 2: Live sperms in different parts of the epididymis in breeding and non-breeding seasons

The proportions of live sperms with protoplasmic droplets were not significant with the sperms obtained from caput (67.8% and 74.5%), corpus (70.9% and 68.8) and cauda epididymis (81.1% and 74.4%) for the right and left testicles, respectively (Fig. 3). No significant difference was observed between the right and left testicles and among different parts of the epididymis.

These values (Mean ± SE) were 66 ± 6.2% vs. 73 ± 5.5% for caput, 70 ± 5.2% vs. 70 ± 9.2% for corpus and 74 ± 9.5% vs. 82 ± 7.1% for cauda epididymis in breeding and non-breeding seasons, respectively (Fig. 4). No significant difference was observed between breeding and non-breeding and among different parts of the epididymis. Fig. 5 shows live and dead sperm stained by eosin nigrosin staining method.

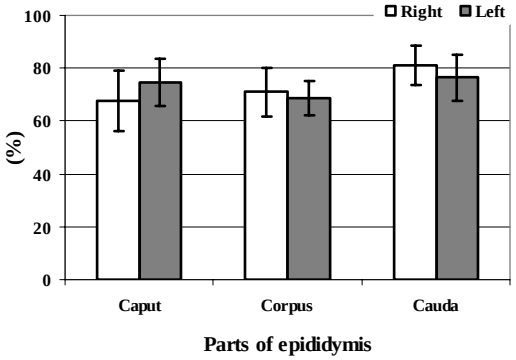

Fig. 3: Proportion of live sperms with protoplasmic droplets in the right and left testicles

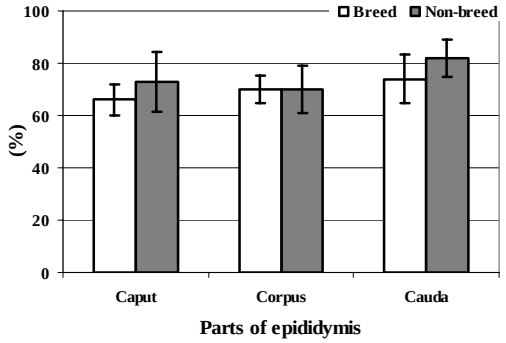

Fig. 4: Live sperms with protoplasmic droplets in different parts of the epididymis in breeding and non-breeding seasons

Discussion

As a report by Merkt *et al.* (1990) the average percentage of live sperm from dromedary camel was 55%. Zhao (2000) has reported that 5% of Bactrian camel ejaculatory sperms are dead and 4.9% are abnormal. El-Hassanein (2003) has reported the proportion of 25, 21 and 12% for dead sperm in dromedary camel by electroejaculation, artificial vagina (AV) and teaser and camel dummy, respectively. He has also reported the proportion of 22, 19 and 11% for abnormal sperm by the 3 mentioned methods. Tingari *et al.* (1986) have reported a mean value of 43% (25-66%) live sperm cells when they used electroejaculation. Musa *et al.* (1992) have reported that 81 and 82% sperm cells were alive when electroejaculation or AV was used for semen collection of dromedary camel, respectively. Deen *et al.* (2003) have reported that 73.3% of ejaculatory sperms from dromedary camel are live. In the present study 86-90.5% of sperm cells were alive in cauda epididymis during breeding and non-breeding seasons which is in agreement with Musa *et al.* (1992). In our study more sperm cells were alive in cauda epididymis than in caput. However, this difference was not statistically significant.

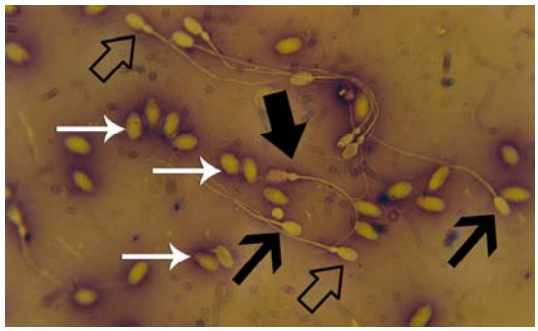

Fig. 5: Bold arrow shows a dead sperm with proximal protoplasmic droplet. Transparent arrows show live sperm with proximal protoplasmic droplets. Narrow arrows show live sperm without droplet. White arrows show camel's ovoid red blood cells

Some workers have mentioned that cytoplasmic droplets in sperm cells may be considered as an abnormality but some others do not agree with them. Flores *et al.* (2002) who worked on alpaca semen, have reported 9.3% sperm cells with cytoplasmic droplet and counted them as abnormality. Tingari *et al.* (1986) have reported that 84% of dromedary camel sperm cells were normal when they used electroejaculation. They also noted that cytoplasmic droplet was commonly present in sperm cells, but they did not mention the proportion of sperm cells with cytoplasmic droplets. It may suggest that they did not count cytoplasmic droplet as an abnormality. However, Bravo *et al.* (1997) counted cytoplasmic droplet as an abnormality and reported that only 3.8%

of ejaculatory alpaca sperms have cytoplasmic droplets. In the present study 74-82% cauda epididymal sperms of dromedary camel had cytoplasmic droplets during breeding and non-breeding seasons which are in agree with Tingari *et al*. (1986). The percentage of spermatozoa with cytoplasmic droplets has been reported to be significantly higher in the caput than in the corpus or cauda epididymis (McKinnon *et al*., 1994). However, in the present study, there was no significant difference between proportions of spermatozoa with protoplasmic droplets in different parts of the epididymis.

The results of the present study indicates that epididymis of dromedary camels have reasonable proportion of alive sperm cells and may have the potential uses in laboratory studies in IVF as well as in the means of AI as a useful tool in animal breeding programs. The results also show that almost three quarters of live sperms in epididymis have protoplasmic droplets. Some reports indicated that around 73% ejaculatory sperms of on humbled camel are normal. It is not really known whether sperms loose their droplets soon before ejaculation or the workers had not categorized cytoplasmic droplets as abnormality.

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