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Bovine Epididymal Sperm Morphology Obtained from Caput, Corpus and Cauda Epididymides

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Abstract: To investigate the proportion of normal sperm cells in bovine epididymis, bovine testicles (n = 50), obtained from a local slaughterhouses, epididymides were incised and sperm cells were transferred into slide glasses where eosin nigrosin stain was applied either in the place or in laboratory. When sperm were stained in slaughterhouse, 88% of caput epididymal sperm were alive; 9% without protoplasmic droplet (NPD), 10.2 and 68.8% had distal (DD) and Proximal Droplets (PD), respectively. Of dead sperm, 10.4% were NPD and 0.33 and 1.27% had DD and PD, respectively. Of corpus epididymal sperm, 77.2% were alive of which 14.7% were NPD, 58.3 and 4.2% had DD and PD, respectively. Of dead sperm, 20.4% were NPD and 2.2 and 0.2% had DD and PD, respectively. When spermatozoa were stained in laboratory, 71.7% were alive of which 17.4% were NPD, 49.7 and 4.6% had DD and PD, respectively. Of dead sperm, 23.1% had no droplet and 4.21 and 0.99% were DD and PD, respectively. The proportion of live spermatozoa from caudal epididymis was 86.1%, of which 9.9% were NPD, 68.3 and 7.9% had DD and PD, respectively. Of dead spermatozoa, 10.1% had no droplet and 3.3 and 0.5% had DD and PD, respectively. No significant difference observed between different parts of epididymis and also between slaughter house staining and laboratory staining of sperm cells. Data showed that approximately all parts of epididymis contained similar status of live sperm cells and the sperm cells containing protoplasmic droplets.

Key words: Bovine, epididymal sperm, morphology

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 for African antelope (Loskutoff *et al*., 1996). Epididymal sperms have successfully been obtained at necropsy from goats and used it 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 4°C (Yu and Leibo, 2002). James *et al*. (2002) have stored equine sperm in the epididymis at 4 °C for 24, 48, 72 and 96 hours (James *et al*., 2002). Some experiments have also been done by Kabbi *et al*. (2003) on the quality of cauda epididymal ram spermatozoa (Kaabi *et al*., 2003). On the other hands artificial Insemination and embryo transfer as well as IVF has been used for camelids (Anouassi *et al*., 1992; Bravo *et al*., 2000; McKinnon *et al*., 1994; Musa *et al*., 1992 and Roberets, 1991). Surprisingly some researchers have used epididymal sperm from South American camelids but no offspring was resulted from their works (Del Campo, 1994). There are few studies concerning morphological study of bovine epididymal sperm. The present study was carried to study the proportion of bovine sperm cells containing proximal or distal protoplasmic droplets in different parts (caput, corpus and caudae) epididymides.

MATERIALS AND METHODS

Sperm cells preparation: Testicles from 50 slaughtered bulls (100 testicles) were isolated in a local slaughterhouse in Isfahan suburb. Selected animals were

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between 18-24 months 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 bull 6 slides were prepared. Slides from caput, corpus and cauda epididymis were marked LH, LB and LT, respectively for left testicle and marked RH, RB and RT from the same pain in 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 protoplasmic 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 for right and left testicles.

Experiment was carried during one year (12 month) started in June 2005 and finished the next year. Similar Results were observed during the experiment time, so the data were pooled.

Statistical analysis: The proportions of sperm cells containing proximal or distal protoplasmic droplets in different parts of epididymis were analyzed by squrae test.

RESULTS

When sperm cells were stained in slaughter house, 88% of sperm cells from caput epididymis were alive of which 9% did not have protoplasmic droplet (NPD), 10.2 and 68.8% had distal (DD) and proximal droplets (PD), respectively. Of dead sperm cells, 10.4% were NPD and 0.33 and 1.27% had DD and PD, respectively (Fig. 1).

Lab staining (2 h after slaughter), revealed that 87.2% of caput epididymal sperm were alive of which 7% were NPD, 6.4 and 73.8% had DD and PD, respectively.

The results of corpus epididymal sperm staining showed that 77.2% of them were alive of which 14.7% were NPD, 58.3 and 4.2% had DD and PD, respectively. Of dead sperm cells, 20.4% were NPD and 2.2 and 0.2% had DD and PD, respectively (Fig. 2)

The same results were obtained when sperm cell were stained in laboratory (71.7% alive of which 17.4% were NPD, 49.7 and 4.6% had DD and PD, respectively). Of dead sperm cells, 23.1% had no droplet and 4.21 and 0.99% were DD and PD, respectively.

Sperm cells from caudal epididymis were 86.1% alive of which 9.9% were NPD, 68.3 and 7.9% had DD and PD, respectively. Of dead sperm cells, 10.1% had no droplet and 3.3 and 0.5% had DD and PD, respectively (Fig. 3).

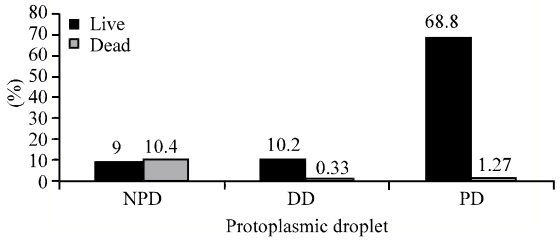

Fig. 1: Protoplasmic droplet in caput epididymal sperm cell just slaughte NDP = No protoplasmic droplets; DD = distal droplet and PD = Proximal droplet

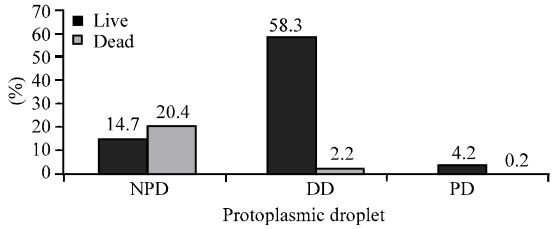

Fig. 2: Protoplasmic droplet in corpus epididymal sperm cell just slaughter NDP = No protoplasmic droplets; DD = distal droplet and PD = Proximal droplet

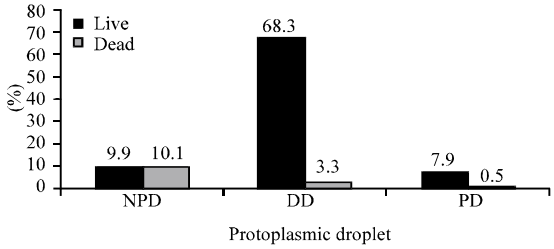

Fig. 3: Protoplasmic droplet in cauda epididymal sperm cell just after slaughter NDP = No protoplasmic droplets; DD = distal droplet and PD = Proximal droplet

In caudal epididymis sperm cells 86.2% were alive in laboratory staining of which 6.6% were NPD, 69.2 and 10.4% had DD and PD, respectively. Of dead sperm cells, 11.6% had no droplet and 1.8 and 0.4% had DD and PD, respectively. No significant differences were observed between different parts of epididymis and also between slaughter house staining and laboratory staining of sperm cells.

Figure 4 shows the protoplasmic proportion compared between bulls of 12-18 month of age with dose of 18-24 months. The proportion of sperm cells with no protoplasmic droplets were 11 and 6.75% for bulls under 18 and over 18 moths of age, respectively and were not

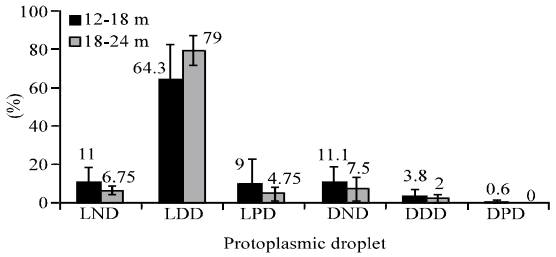

Fig. 4: Protoplasmic droplets in epididymis according to LND = live no droplet; LDD = Live distal droplets; LPD = Live proximal and D** = Dead

significantly different. These values were neither significantly different for live sperm cells with distal droplets (64.3 and 79% for live sperm with distal droplets for the same groups of age, respectively).

No significant difference was observed between sperm containing proximal or distal protoplasmic droplets of right and left epididymides.

DISCUSSION

It is believed that sperm cells acquire their maturity during passing the epididymis. However, there is few data informing status of sperm cells in different parts of epididymis (Roberets, 1991). Tajik *et al*., assessed the morphology of Zell ram (Tajik *et al*., 2003) and dromedary camel (Tajik and Hasan-Nejad, 2002) and postulated that there is no significant different in live sperm in different parts of epididymis them. In the present study the same results were observed.

There is no data suggesting the proportion of sperm containing protoplasmic droplets in different parts of epididymis. The results of the present study showed that there is no significant different in sperms with protoplasmic droplets in different parts. However, proximal and distal droplets containing sperm were significantly different which are in agreement with Roberets, 1991, but not with the results of our previous study on Zell ram (Tajik *et al*., 2003) and or dromedary camel (Tajik and Hasan-Nejad, 2002) sperm cells. Goovaerts *et al*. (2006) have studied bovine caudae epididymal sperm by assessing the morphology as well as motility. In their study the proportion sperm containing of proximal and distal protoplasmic droplets are 11.7% and 47.3%, respectively. They did not clarify the reason for the large variation between the proportion recorded (0-77% for proximal droplets and 10-84% for distal droplets). However, those values are different from the present study. The other different between their studies and the present one is the different between proximal and distal protoplasmic droplets of right and left testicles in the experiment of Goovaerts *et al*. (2006). In the present study, no significant difference was observed between sperm containing proximal or distal protoplasmic droplets of right and left epididymides.

We know that the protoplasmic droplets in ejaculatory sperm are very rare. It is not really obvious that what will isolate the droplet of sperm cells during ejaculation. In the present study, other fertility indicators of bovine epididymal sperm were not studied. Other studies should be carried to help us in better understanding of this phenomenon.

CONCLUSIONS

Data showed that approximately all parts of epididymis contained similar status of sperm cells regarding the proportion of live sperm cells and the sperm cells containing protoplasmic droplets.

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