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Determination of osteopontin and antioxidant concentrations from epididymal fluids in mongrel dogs

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Abstract

The extracellular matrix phosphoprotein, Osteopontin (OPN) has been found in tissue fluids and organs, including the male reproductive tract. The vesicular gland and the ampullary region in the male reproductive system have been documented to secrete OPN, a 55 kDa fertility associated protein. The objective of this study was to quantify OPN and antioxidant concentrations in different epididymal sections in mongrel dogs. Current results showed a significantly ($p<0.01$) higher concentration of OPN in cauda epididymal fluid than in the caput. Glutathione peroxidase (GPx) and Catalalse (CAT) concentrations were also higher in the cauda epididymal segment than in the caput. To the author's best knowledge, this is the first report on quantification of OPN levels in the epididymal fluid harvested from the different sections of epididymis. Further studies should focus on correlation and association of this protein from the different epididymal segments with seminal attributes.

Keywords: *Osteopontin (OPN), cauda epididymis, antioxidants, caput epididymis*

Osteopontin (OPN) is a secreted phosphoprotein that is found in several tissues and fluids, including those of the female and male reproductive tracts. It has been found predominantly in bull's seminal vesicular fluid, ampullary fluid and accessory sex gland fluid (Cancel *et al*., 1999), the epithelium of the male reproductive tract in humans (Brown *et al*., 1992), epididymis, testis, and sperm surface in rats. Presence of OPN mRNA in the testis and epididymis was identified by reverse transcriptase polymerase chain reaction and its presence in tail region of sperm was confirmed by immunofluorescence studies (Siiteri *et al*., 1995).

Souza *et al*. (2009) reported that the seminal plasma (SP) and sperm membrane of dogs contain several isoforms of OPN, though its exact function was not elucidated in this species. Osteopontin has been identified as a fertility associated protein in Holstein bulls (Cancel *et al*., 1997), Arabian Horses (Waheed *et al*., 2013), dromedary camels (Waheed *et al*., 2015) and dogs

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(Abedin *et al*., 2020). Several other fertility related proteins such as bovine seminal plasma (BSP) has been identified in crossbred bull semen (Adarsh, 2015) and its binding pattern with OPN on ejaculated sperm before and after incubation with isthmic and ampullary oviductal fluid has been documented (Souza *et al*., 2008).

Until now there are no published reports of OPN concentration (ng/mL) in different epididymal sections in mongrel dogs. In this study, testes along with epididymis were collected after orchiectomy from 6 male dogs that were brought to the hospital for birth control. The collected testes and epididymis were transported on ice to the laboratory. Sperm and fluid from the cauda and caput regions of each epididymis were recovered by back-flushing the epididymis through the vas deferens with sterile PBS (Killian and Amann, 1972) separately. For the present study, 1000 microlitres (1 mL) of sterile PBS was used for both the epididymal segments separately and the resultant concentrations by ELISA were arrived as per that dilution. Flushes from each epididymis were centrifuged (600 *g*) for 10 min at room temperature. Cauda and caput epididymal fluids was aspirated from the sperm pellet and centrifuged (10,000 *g*) for 60 min at 4-8ºC to remove remaining sperm. From the resultant supernatant, the OPN and antioxidant enzymes concentration were determined by species specific ELISA Kits (Sincere Biotech, Beijing-101300, China) as per manufacturer's protocol. Statistical analyses were performed via t test using IBM SPSS Statistics 23. P values <0.01 were considered significant unless otherwise specified. The Mean values with standard error are depicted in Table 1.

The present study showed a higher ($p<0.01$) OPN concentration (ng/mL) in cauda epididymal fluid (14.75±0.96) than in the caput (7.03±0.15). Moura *et al*. (2006) studied the proteins of cauda epididymal fluid associated with fertility index in mature Holstein bulls and concluded that multiple proteins such as α-L-fucosidase, cathepsin-D and prostaglandin-D-synthase present in the cauda epididymal fluid are potential markers of fertility in mature Holstein bulls. Osteopontin being a fertility associated protein should also be present in higher concentrations in the cauda epididymal fluid but there is no literature regarding the comparison of OPN concentration in cauda and caput epididymal fluids in any species.

Angrimani*etal*.(2014)studiedtheenzymatic activity of CAT, GPx and SOD in epididymal corpus, cauda and caput in the epididymal spermatozoa of dogs and observed that GPx activity was higher in the epididymal cauda when compared to the other regions of the epididymis or ejaculated samples. Jervis and Robaire (2001) also found similar results in rat epididymis and stated that superior GPx activity in the cauda occurred due to enhanced capacity of protein synthesis in the final stage of the sperm maturation cycle.

Angrimani *et al*. (2014) reported that no detectable levels of this enzyme were found in samples collected from epididymal segments. This may suggest a primary contribution of male accessory glands on seminal catalase content in dogs. However, in the present study significantly higher ($p<0.05$) CAT concentration was observed in the cauda when compared against the caput. There is no available literature that could be reviewed to substantiate our finding in this regard.

Summary

To the authors best knowledge, this is the first report on quantification of OPN levels in epididymal fluid harvested from different sections of epididymis. Further studies are warranted to focus on correlation and association of this protein from the different epididymal segments with seminal attributes.

Conflict of interest: The authors declare no conflict of interest.

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Table 1. Comparison of OPN, GPx, CAT and SOD concentrations (ng/mL) in cauda and caput epididymis (Mean±SE).

Sl. No.	Parameters	Cauda (ng/mL) Mean±SE	Caput (ng/mL) Mean±SE	t value	p value
1.	OPN	14.75±0.96	7.03±0.15	7.680	0.001**
2.	GPx	21.18±0.57	5.17±0.69	21.010	0.000**
3.	CAT	19.42±0.64	13.74±1.03	3.678	0.014*
4.	SOD	8.87±0.33	9.26±1.30	-0.322	0.761NS

***- Highly significant (p<0.01) *- Significant (p<0.05) NS- Non significant*

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