DETRIMENTAL EFFECTS OF POLYMER STYRENE MALEIC ANHYDRIDE (SMA) ON CANINE SPERMATOZOA: A POTENTIAL FOR CONTRACEPTION IN MALE DOGS

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ABSTRACT

The *in vitro* Interaction of positively charged polymer styrene maleic anhydride (SMA) with the negatively charged canine epididymal spermatozoa resulted in damage to acrosome and leaching out of enzymes. Moreover, the polymer reduced the pH of the environment to a level which was spermicidal. The damage to acrosome, mid piece and tail of canine spermatozoa brought about by addition of polymer SMA was evident by the Scanning Electron Microscopy and Atomic Force Microscopy studies. This study provides scope for development of a new contraceptive for male dogs.

Keywords: Atomic Force Microscopy, Canine contraception, Polymer, Scanning Electron Microscopy, SMA

INTRODUCTION

Sterilization is the most effective way of controlling pet overpopulation. Yet with the huge number of pet and stray dogs in India, the available sterilization programmes are not enough. Therefore, the search for a safe and effective mean to permanently eliminate fertility remains a long-standing goal in addressing dog overpopulation. At the forefront of non-hormonal contraceptive research in humans lies RISUG®, which involves the injection of an active compound (Styrene maleic anhydride, SMA) combined with DMSO into the vas deferens. Its success offers a sign of hope for male fertility control in other species. The present study aims to investigate the detrimental effects of polymer SMA on canine spermatozoa.

MATERIALS AND METHODS

The testis of healthy adult male dogs (age, 1-6 yr) were subjected for orchiectomy as per standard technique and the testis epididymal complexes were surgically removed and washed with normal saline solution to remove any vestiges of blood and contaminants followed by dissecting away the epididymis from the testis. The blood vessels on the surface of the epididymis were removed. To collect the transmigrated sperm, the cauda epididymis was isolated and minced with a scalpel in a petri dish containing 1 ml of Tris buffer. All the procedures from excision of epididymis to sperm collection were carried out at room temperature and completed within 1 h. The sperms recovered from cauda epididymis using the mincing method were microscopically examined and subsequently graded for their motility on a scale of 0-4 (0, absent; 1, weak or sluggish; 2, definite; 3, good; 4, vigorous; Mortimer, 1994). Sperm samples with a motility grade of 3 or more were utilized for the study.

For assessing the kinematic properties of spermatozoa and the phenomenon of attachment of the positively charged polymers with the negatively charged plasma membrane, both undiluted epididymal sperm solution in normal saline and polymer SMA in DMSO (1.0 mg/100 µl DMSO) were subjected to zeta potential analysis using zeta analyser (HORIBA - SZ100, Japan). Since the sperm cells become less

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negatively charged with the onset of capacitation, the zeta potential had to be measured immediately after collection.

To assess whether the polymer had any effect on pH, the polymer was suspended in normal saline and the saline was changed daily while retaining the original polymer. The pH was measured every 24 h prior to change of saline.

The spermicidal property of polymer was evaluated *in vitro* using epididymal as well as ejaculated sperm cells. The epididymal sperm cells collected as per the procedure described above and the ejaculated sperm cells, the sperm rich fraction of the semen collected by digital manipulation from adult male dogs, were microscopically evaluated and graded for motility. The sperms with motility grade of 3 or more were considered for further study. Each of the epididymal and ejaculated sperm suspension was divided into fractions A and B. To fraction A, small pieces of the hydrated polymer were added, while fraction B served as untreated control. Thereafter, microscopic analysis of sperms was carried out at 5 minute interval until all the sperms were dead.

RESULTS AND DISCUSSION

In the present study, the zeta potential of normal saline alone was -0.2 mV, whereas, the zeta potential of sperm suspension in normal saline was -8.6 to -33.11 mV, thus indicating the development of negative charge in the suspension due to sperm cells. Furthermore, the zeta potential of DMSO alone ranged from -1.5 to -15.6 mV, while addition of SMA to DMSO resulted in a zeta potential of -1.0 to 0.7 mV suggesting that polymer SMA was positively charged.

The inherent negative charge of the spermatozoa particularly at the acrosome head gets disturbed by the positive electric charge generated by SMA interaction. This electrostatic interaction was probably responsible for the damage to the spermatozoa which was evident by Scanning Electron Microscopy (SEM) and Atomic Fluorescent Microscopy (AFM) studies. The findings of the present study were in accordance with previous reports (Guha, 1996 and 2007; Lohiya *et al*., 1998 and Kumar *et al*., 2006). Due to such an imbalance caused by the electrostatic interaction, it was believed that chloride ions were no longer kept out and the flow of extra amount of such ions with water through the membrane caused the sperm head to swell up leading to membrane rupture and leaching out of acrosin and hyaluronidase. Thus, the sperm becomes deficient of such enzymes required to fertilize the ovum (Guha, 1996 and 2007; Lohiya *et al*., 1988). Such damage to acrosome and leaching out of enzymes were also evident by SEM and AFM studies (Fig. 1).

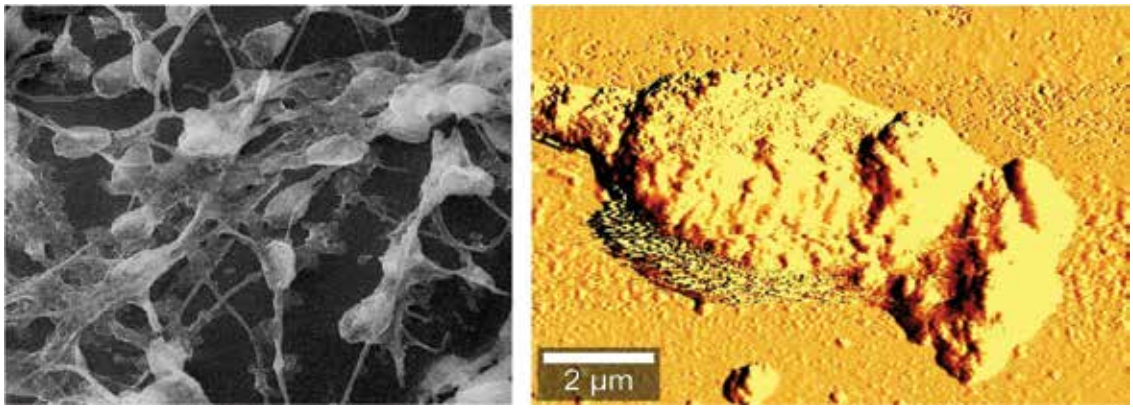

Figure 1: Polymer SMA treated canine spermatozoa. Left - Scanning Electron Microscopy showing damage to acrosome, mid-piece and tail of spermatozoa; Right - Atomic Fluorescent Microscopy showing rupture of acrosome and leaching of enzymes

Table 1: Viability percentage (Mean±SE) of untreated (control) and polymer SMA treated canine epididymal spermatozoa at different time intervals

Sample	Pre-treatment viability, %	Post-treatment viability, %			
		5 min		10 min	
		Control	SMA	Control	SMA
Epididymal	75.0±2.6	70.0±1.8[a]	25.0±1.8[a]	60.0±2.2[a]	0.33±0.21[a]
Ejaculated	80.8±2.4	80.8±2.4[b]	20.0±2.2[a]	70.0±1.8[b]	0.17±0.17[a]

p<0.05, Means bearing different superscripts within a row differ significantly

In vitro studies have also shown that polymer SMA altered the pH of the saline to as low as 4.5 which concurred with the previous findings (Misro *et al*., 1979; Guha *et al.,* 1985 and Sethi *et al*., 1992). Since, the spermatozoa were very sensitive to pH of medium, a pH <5.5 and >8.5 was fatal to spermatozoa. The acidic pH was probably responsible for death of all the spermatozoa within 10 min in the present study. Hence, we conclude that the destruction of spermatozoa occurred by the virtue of lowering of pH.

When the spermicidal action of the polymer SMA was tested *in vitro*, using both canine epididymal and ejaculated sperm cells, it was found that the addition of SMA to both canine epididymal and ejaculated spermatozoa declined (p<0.05) viability percent at 5 min with 100 per cent spermicidal effect on both canine epididymal and ejaculated sperm cells within 10 min (Table 1).The spermicidal activity of the polymer was probably due to the presence of carboxylic group. It might be possible that low pH environment due to ionization of carboxyl group fixed in the polymer matrix was responsible for killing the spermatozoa (Singh *et al*., 1984). Hydrogen ion concentration was undoubtfully one of the most important factors which influenced the motility, viability and metabolism of spermatozoa in species extending from sea urchin to man.

The results of the present study clearly indicated that polymer SMA has a pH lowering action that is detrimental to canine spermatozoa. In addition, the positive charge generated by the polymer disturbs negative charge of sperm membrane, ultimately resulting in loss of functional competence, thus providing scope for development of a new contraceptive for male dogs.

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