Seminal mast cells in infertile asthenozoospermic males

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Introduction

Mast cells are commonly found in connective tissue or in mucosa of many organs including skin, airways, digestive tract, testis, epididymis and the female reproductive tract. They are considered as part of the immune system and are suggested to be generated by different precursor cells in the bone marrow (Metcalfe et al., 1999). They are divided into two subtypes based on differences in their neutral serine protease content; mast cells containing tryptase (MCT) and mast cells containing both tryptase and chymase (Welle, 1997; Yong, 1997). In mammalian testis, MCT is a potent mitogen for fibroblast that can enhance the synthesis of collagen with subsequent fibrosis and thickening of the tubular wall (Guarch et al., 1992).

Several reports have associated increased number of mast cells in the testes and male infertility (Agarwal et al., 1987; Hashimoto et al., 1988; Nagai et al., 1992; Roaiah et al., 2007). Abnormal spermatogenesis is associated with an increased number of testicular macrophages and an increased numbers of B and T lymphocytes; the former is associated with increased production of tryptase enzyme which is a potent mitogen for the fibroblast that can enhance their chemotaxis and activation resulting in tubular fibrosis (Apa et al., 2002). Also, increased number of mast cells is responsible for the marked deposition of collagen and testicular fibers seen in testis with abnormal spermatogenesis (Hussein et al., 2005).

Nagai et al. (1992) found that the number of mast cells was increased and the ratio of mast cell subtypes was changed in idiopathic azoospermia and oligozoospermia. Therefore, mast cell blockers have been empirically used in the treatment of male infertility with some reports of significant improvement in semen quality as well as spontaneous pregnancies (Yamamoto et al., 1995; Matsuki et al., 2000; Hibi et al., 2002). In contrast, other authors showed that mast cell blockers had no benefit in treating infertile men (Cayan et al., 2002).

The aim of this work was to assess seminal mast cell content in asthenozoospermic infertile males.

Materials and methods

One hundred and seventy-six men were selected prospectively after consent from the Andrology Outpatient Clinic of the University Hospital after Institutional Review
Board approval. They were divided into group (Gr)1 ($n = 46$) fertile normozoospermic controls, Gr2 ($n = 62$) idiopathic asthenozoospermia (sperm motility <50%), Gr3 ($n = 32$) asthenozoospermia with scrotal varicocele and Gr4 ($n = 36$) asthenozoospermia with leucocytospermia. A detailed medical history was taken and physical examination with scrotal duplex for varicocele diagnosis was performed for the studied cases. Ejaculates were obtained in the early morning (7:00–9:30 hours) after 4 days of sexual abstinence. The samples were examined immediately conventionally after liquefaction according to the guidelines of WHO (1999) (normally, sperm count >20 $\times 10^6$ sperm ml$^{-1}$, sperm motility >50%, abnormal sperm morphology <70%, vitality >75% and leucocytes <10$^6$ ml$^{-1}$). Semen samples were verified after at least two different analyses.

To evaluate mast cells, four semen slides were prepared for each case. The slides were fixed through air drying and stained with 1% toluidine blue–pyronine (pH 4). Then, they were evaluated under immersion objective. If mast cells were detected in the slides, the cases were considered as mast cells positive (+ve) and if not found, as mast cells negative (−ve) (Fig. 1).

Paired Student’s $t$-test was used to compare parametric groups. Qualitative variable expressed as percentages was compared in different groups using the chi-squared test comparing the frequencies in different groups with theoretical values under the null hypothesis. They were considered statistically significant when $P < 0.05$.

### Results

Mast cells were detected in 100 cases out of 176 (56.4%); 16 in fertile control group (34.8%), 40 in idiopathic asthenozoospermic group (64.5%), 22 in asthenozoospermia with varicocele group (68.75%) and 22 in asthenozoospermia with leucocytospermia group (61.1%). Mast cells (+ve) cases were significantly higher in idiopathic asthenozoospermia and varicocele-associated asthenozoospermia groups in comparison with the controls. Although higher in asthenozoospermia with leucocytospermia group, the difference in comparison with control group was nonsignificant (Table 1). Mast cells (+ve) cases were shown to be significantly higher in those >40 years (32 cases) compared with those <40 years (18 cases). Also, mast cells (+ve) cases were significantly higher in smokers (33 cases) compared with non smokers (17 cases) (Table 2).

### Discussion

In the present study, a significant increase in mast cells was detected among idiopathic asthenozoospermic as well as varicocele-associated asthenozoospermic patients compared with controls. A similar increase was noticed in cases of asthenozoospermia associated with leucocytospermia, though nonsignificant. All these categories of asthenozoospermia were documented to be associated with increased seminal reactive oxygen species (ROS) and decreased seminal antioxidants (Lewis et al. 1995; Mostafa et al. 2001; La Vignera et al. 2006). The relationship between ROS and seminal mast cells was not discussed before, but Santos et al. (2000) suggested that ROS can act as stimulators of mast cell degranulation in burns. Renke et al. (2006) reported that mast cells generate intracellular ROS in response to stimulation with divergent physiologically relevant stimulants.

Few studies have evaluated the relationship between isolated asthenozoospermia and mast cells. Worth mentioning, Weidinger et al. (2003) showed, by immunoelectronmicroscopy, proteinase-activated receptor-2 (PAR-2) in the membranes of the acrosomal region and midpiece of human spermatozoa. These PAR-2 were functional, as exposure of spermatozoa from healthy men with regular standard semen parameters to human recombinant tryptase significantly decreased motility in a dose- and time-dependent fashion. In a study by Cinck & Sezen (2003), semen from 400 randomly selected men was examined for mast cell content in relation to sperm concentration, motility and morphology. 21.5% mast cell-positive cases showed relative decline in sperm parameters, with statistical significance only evident regarding progressive motility, in comparison with mast cell-negative cases.

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**Fig. 1** Mast cell in semen stained by toluidine blue stain.
Nevertheless, medical literature does bear reports on the relationship between other aspects of male infertility and mast cells. Hussein et al. (2005) revealed that increased number of mast cells in testicular tissues was responsible for the marked deposition of collagen in the seminiferous tubules of azoospermic patients with spermatogenic arrest and Sertoli-cell only syndrome. In the same line, Hashimoto et al. (1988) reported an increase in intra-testicular mast cell content in testicular tissue obtained from 16 patients with idiopathic oligozoospermia. Nagai et al. (1992) have also shown that the number of mast cells in testicular tissue was increased and the ratio of mast cell subtypes (chymase and tryptase) was changed in idiopathic azoospermia and oligozoospermia.

We observed that mast cell-positive cases were significantly higher among older subjects, in contrast to mast cell-negative cases. This may point to an ageing process being a possible mechanism by which mast cells contribute to infertility. This finding supports the evidence proposed by Cincik & Sezen (2003) showing that the age of mast cell-positive cases were higher than those of mast cell-negative cases.

Another observation was that seminal mast cells showed higher frequency among smokers compared with nonsmokers, suggesting an aetiological relationship between smoking and mast cell abundance in infertile patients, and therefore an indirect relationship between smoking and infertility. This supports the studies that establish smoking as having an adverse effect on fertility, especially on progressive sperm motility, irrespective of total amount of cigarettes smoked per day (Hassa et al., 2006; Mostafa et al., 2006).

Some of the previous therapeutic trials with mast cell blockers went along with our findings, and some did not. Yamamoto et al. (1995) prescribed randomly mast cell blocker (tranilast) or placebo for 3 months infertile males with severe idiopathic oligozoospermia, showing significant improvement in semen parameters and 28.6% of pregnancy rate in the treated group with no improvement in the placebo group. Matsuki et al. (2000) investigated the effect of ebastine, a mast cell blocker, on semen quality in idiopathic oligozoospermic men, reporting that 66.7% of the patients had improved semen quality and 20% achieved pregnancy with their partners within 6 months of treatment. In contrast to previous studies, Cayan et al. (2002) found that semen parameters of infertile men did not change significantly after mast cell blocker treatment (fexofenadine) and no one achieved spontaneous pregnancy.

It is concluded that mast cells and their products may play a pivotal role in the pathogenesis of asthenozoospermia, possibly proposing a new goal for medical treatment of infertile males to pursue. In addition, this concept may in a way detain smoking, as a cause of male infertility considering the clear abundance of mast cells in semen samples of smokers.

References

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<th>Table 1</th>
<th>Seminal mast cells in different studied groups (n, %)</th>
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<tr>
<td>Group 1</td>
<td>Group 2</td>
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<tr>
<td>Mast cells (+ve)</td>
<td>16 (34.8%)</td>
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<tr>
<td>Chi-square</td>
<td>&lt;0.05*</td>
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<td>*Significant.</td>
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<th>Table 2</th>
<th>Seminal mast cells relationship with age and smoking</th>
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<tr>
<td>&lt;40 years</td>
<td>&gt;40 years</td>
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<tr>
<td>Mast cells (+ve)</td>
<td>18 (43.9%)</td>
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<tr>
<td>Chi-square</td>
<td>&lt;0.05*</td>
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<td>*Significant.</td>
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