Imprisonment-associated Sperm Clumping and Male Infertility

FS AL-JOUDI1,a AND JAK JAMIL2,b

1Infertility Clinic, Maternity and Children's Hospital, and 2Department of Urology, Ramadi Teaching Hospital, Ramadi, Iraq

OBJECTIVE: This observational, retrospective study investigated infertility among Iraqi men who had been long-term prisoners of war. METHODS: Routine seminal fluid analyses were undertaken in former prisoners with suspected infertility attending the Ramadi Infertility Clinic, Ramadi, Iraq; semen samples were also microscopically evaluated in these patients. RESULTS: In 16 out of 38 patients (42%), male infertility was found to be associated with the formation of unusually large clumps of sperm. These sperm clumps contained large numbers of sperm; clumping led to sperm immobilization and infertility. CONCLUSIONS: The causes of sperm clumping are unclear. It is possible that the sperm clumping observed in the present study might be a result of developmental defects, possibly related to stress or other undiscovered factors, although this requires further investigation.

KEY WORDS: MALE INFERTILITY; IMPRISONMENT; SPERM CLUMPING; SPERM MOTILITY; PSYCHOLOGICAL STRESS

Introduction

The effect of stress in causing male infertility is probably a slow process that can take years to develop, and may be influenced by hormonal, physical and psychological factors.1–9 The present study retrospectively evaluated a previously unreported phenomenon: large clumps of sperm were observed during the analysis of seminal fluid in several infertile male patients who were former prisoners of war. The research stemmed from observations in routine seminal fluid analyses, which form the preliminary investigations for each male partner of a couple with suspected infertility.

Patients and methods

PATIENTS

This observational, retrospective study included Iraqi men who were former prisoners of war; the men had been released from prison some time after the end of the Iran–Iraq war in August 1988 and were attending the Ramadi Infertility Clinic, Ramadi, Al-Anbar, Iraq, between 1994 and 1996, for treatment for infertility. After observing sperm clumping in some men who had been prisoners of war, the decision was taken to study this phenomenon further in the clinic's patient population.

Immediately after their prison discharge, the Iraqi health authorities would have ensured that these men underwent comprehensive medical and psychological...
examinations. They were then allowed to resume their normal lives. The medical examinations did not, however, include investigations of fertility. Over the intervening years, some former prisoners who failed to have children started seeking medical advice and attended the Ramadi Infertility Clinic, Ramadi, Al-Anbar, Iraq, for treatment. Only patients with evidence of sperm clumps, who had been prisoners of war during the Iran–Iraq war and who were attending the clinic, were included in the study. There were no other specific inclusion criteria. Patients with other medical illnesses or systemic diseases, those who had testicular or scrotal disorders, and those who consumed alcohol or who were heavy smokers were excluded.

For political reasons, verbal approval of the study protocol was sought from the Al-Anbar Health Authority, Iraq; approval was granted in 1994. Although the examinations included in the study were routine and no treatment was included (therefore, no formal consent was required from patients), oral approval was obtained from all patients to include their data.

INITIAL INVESTIGATIONS OF INFERTILITY

The main investigations performed in the men included in the study were tests for serum levels of reproductive hormones (including follicle stimulating hormone [FSH], luteinizing hormone [LH], prolactin and testosterone). These investigations were performed at Gedeon Laboratory (Baghdad, Iraq), which is a private medical laboratory. Standard laboratory enzyme immunoassays were used: as a guideline, standard reference ranges for these reproductive hormones are: FSH 1.3 – 8.4 IU/l; LH 1.6 – 8 IU/l; prolactin 50 – 400 mIU/l; testosterone: 9 – 30 nmol/l).10

The detection of antisperm antibodies in blood and seminal plasma samples was carried out by an indirect immunobead assay (Immunobead® Solid Phase Reagents, Irvine Scientific, Santa Ana, CA, USA) as described previously.11 Approximately 1 ml of blood was collected from each patient and allowed to clot at room temperature before serum was prepared by centrifugation at 1500 g for 10 min. Additionally, 1 ml of plasma was prepared from freshly obtained liquefied seminal samples by centrifugation at 1500 g for 10 min. The sera and seminal plasma samples were stored at −20°C until further use. To perform the antisperm antibody assay, fresh sperm from a normal donor was prepared by washing with saline, containing 1% bovine serum albumin (BSA), before adding 100 µl of the patient’s serum or seminal plasma and incubating the sample at room temperature for 1 h. After washing with saline containing 1% BSA, the immunobead-conjugated antihuman immunoglobulin rabbit antibodies (100 µl of a 1:100 dilution in saline containing 1% BSA) were added and incubated for 30 min at 37°C. Samples and assay controls that were positive for antisperm antibodies would result in cross-linking of the immunobeads.

Further analyses of the seminal fluid samples were performed at the laboratory of the Ramadi Infertility Clinic. Manual manipulations of the large sperm clumps were performed, by using pointed glass rods or squeezing the clumps between two glass slides, in an attempt to separate bound sperm. Immediately after ejaculation and collection, each seminal sample was placed in a 37°C incubator until liquefaction became evident. Next, the sample was shaken gently. A drop of it was then placed on a slide with a cover slip for light microscope examination using both low- (×100) and high-power (×400) magnification (Olympus Corporation, Tokyo, Japan).
microscopy included visual evaluation of the general sperm concentration, distribution, morphology, movement type and relative speed. Samples were also examined for the presence of any pus cells, epithelial cells or red blood cells.

Results

Data were available for 38 former male prisoners of war who sought infertility testing. The age range of included patients was 27 – 41 years (median age 33 years), and the patients had been imprisoned for periods ranging between 2 and 8 years (median imprisonment time 4.5 years). The results of the reproductive hormone profile tests fell within the reference ranges\(^{10}\) and did not suggest abnormalities at the time of testing (data not shown).

The presence of unusually large clumps of sperm (Table 1; Fig. 1A, 1B) was identified in 16 out of the 38 patients (42%) included in the study. The clumps were not of immunological aetiology since, morphologically, they did not appear to show any particular antibody binding pattern\(^{12}\) and they were large in size. Seminal fluid and serum samples from all patients were negative for antisperm antibodies. A normal semen sample is shown in Fig. 1C for comparison.

Manual manipulation of the large sperm clumps, to try and separate the bound sperm, failed. Once the pressure was released, the bound sperm clumps would resume their earlier shape, behaving very much like fibrous tissue. Only small numbers of free-moving sperm were observed in patient samples. Pus cells, epithelial cells and red blood cells were not observed.

Discussion

The present observational, retrospective study – which was undertaken in former prisoners of war who underwent investigation for male infertility – showed the presence of large clumps of sperm in several patients. These clumps showed strong linkages that are likely to have formed prior to ejaculation. Similar smaller sperm clumps are occasionally detectable in samples of semen taken from men with proven fertility; however, despite such clumps, such samples are normal because they also harbour a high proportion of free-moving sperm. In comparison, only small numbers of free-moving sperm were observed in infertile patients in the present study. The sperm in the subfertile seminal samples in the present study may have been essentially normal, although the functionality of individual sperm was not tested. These sperm were, however, bound together in clumps (possibly before ejaculation), thus reducing the chance of normal movement and function; the consequent loss of sperm motility led to infertility. Other causes of male infertility observed in the same clinic, among patients who had not been previous prisoners of war, mainly included oligosperma or azoospermia and low sperm activity; a small percentage of cases were of immunological aetiology.\(^{13}\)

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<tr>
<th>TABLE 1: Patients included in a study investigating male infertility in former Iraqi prisoners of war from the Iran–Iraq war</th>
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<tbody>
<tr>
<td>Previously imprisoned</td>
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<tr>
<td>With sperm clumps</td>
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<tr>
<td>With clumps and primary infertility(^a)</td>
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<tr>
<td>With clumps and secondary infertility(^b)</td>
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<tr>
<td>(^a) Couples who had never conceived.</td>
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<tr>
<td>(^b) Couples who had difficulty conceiving, but who had conceived previously.</td>
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The formation of sperm clumps may have followed systemic and local changes over long periods of stress in the former prisoners of war, which may have had a detrimental effect on the testes and/or epididymis, leading to alterations in sperm development. This view may be supported by the fact that five of the 16 patients with sperm clumps had secondary infertility; i.e. they had been married and had children before their imprisonment (Table 1). In addition to hormonal influences, such pathological changes may be associated with (or enhanced by) high oxidative stress that develops over time.14 – 16 The possible use of medications such as long-term inhibitors of libido while the patients were imprisoned cannot be excluded, although such use has never been disclosed.

There were several limitations to the present study. The small sample size, the lack of a control group and the unrandomized, observational nature of the study limits the conclusions one is able to draw regarding the links between major stress and the development of infertility. In addition, certain technical difficulties meant that tests could not be carried out to try to understand the underlying pathology behind the sperm clumping observed in these patients. Furthermore, if stress due to imprisonment was a major cause of infertility in these men, it might be expected that these problems would resolve over time, and that such cases would not be detectable. Finally, the lack of any similar reports appearing in the

FIGURE 1: (A and B) Large sperm clumps, observed in seminal samples obtained from two infertile Iraqi males who had previously been prisoners of war during the Iran–Iraq war. The clumps were deemed not to be immunological in aetiology as, morphologically, they did not show any particular antibody binding pattern and were large in size. (C) Small sperm clumps can be observed in this sample of seminal fluid taken from a fertile Iraqi male during routine testing for secondary infertility; this sample was deemed normal because, despite the small clumps, there was a high proportion of free-moving sperm.
previously published literature on infertility is also a major limitation.

In conclusion, high rates of infertility – caused in several cases by the presence of unusually large clumps of sperm – were observed retrospectively in men who were former prisoners of war. In these patients, infertility may have been related to severe psychological stresses resulting from long-term imprisonment during the 1980s, although evidence to support this is limited and further detailed investigations are required.

**Conflicts of interest**
The authors had no conflicts of interest to declare in relation to this article.

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**References**

**Author’s address for correspondence**

Dr Fawwaz Shakir Al-Joudi
Department of Microbiology, Royal College of Medicine Perak, No. 3 Jalan Greenwood, 30450 Ipoh, Perak, Malaysia.
E-mail: fajoudi@yahoo.com