The Benefit of Semen Cryostorage on Certain Sperm Function Parameters Following in Vitro Activation

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Abstract

Background: Under different circumstances, adequate production of the semen specimen followed by preparation for intrauterine insemination (IUI) is of utmost importance at many centers. Cryostorage for 24 - 48 hours, could solve the problem of semen to the performance of IUI. It is also advantageous if the male partner has difficulty in producing a semen sample or not available at the time of IUI and reducing psychological pressure on the day of his partner's aiming for repeating insemination after 24 hr.

Aim: The objective of this study was to investigate the beneficial effect of semen cryostored at 5 degrees centigrade (5°C) for 24 hr by modifying Tris Solution (MTS) mixed with 30% egg yolk (EY) on maintaining the functional and fecundity sperm parameters of patient`s semen.

Materials & Methods: Thirty eight infertile patient`s semen samples (who was visiting the High Institute of Infertility Diagnosis & Assisted Reproductive Technology (ART) for three weeks) were involved in this study. Semen analysis was done for all samples as recommended by (WHO Laboratory Manual for the examination of human semen and sperm–cervical mucus interaction 1999). The patient`s semen samples were divided into two groups; both groups were activated *in vitro* with FertiCult^{TM} medium using the simple layer method (SLM) for 30 min; a second group treated with (MTS) mixed with 30% (EY) and cryostorage for 24 hr after activation.

Results: The results showed a highly significant ($P<0.001$) improved of all the certain sperm function parameters of group 2 (cryostorage group) compared with group 1. It showed also improvement of all parameters of infertile men semen. There was a highly significant ($P<0.001$) increase in the percentage of grade A and grade B active sperm motility in group 2 compared with group 1.

Conclusions: It is concluded that the use of (MTS) with 30% (EY) maintains adequate sperm viability and fecundity after 24 hr of cryostorage, and it was extremely recommended for patients requiring intrauterine insemination (IUI) in different circumstances.

Key words: Egg yolk, Modified Tris Solution, IUI, Semen Cryostorage.

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Introduction

Male infertility is an expected consequence following treatment of most malignancies with chemotherapy and/or radiotherapy. While infertility may be reversed for some cancer treatment regimens (notably testis cancer) sustained infertility develops in 50–95% of malignancies [1].

The use of preserved human semen for artificial donor insemination (ADI) is steadily increasing, although the cryopreservation techniques presently employed to reduce the fertilization potential of human spermatozoa. The use of preserved spermatozoa provides the advantage of arranging patients and performance of assisted reproductive techniques (ART) such as in vitro fertilization (IVF), gamete intra-fallopian transfer (GIFT), zygote intra-fallopian transfer (ZIFT), etc.) whenever convenient. Injury to spermatozoa is usually attributed to ice or crystal formation during freezing. A newly established technique has been introduced to optimize fertility rates and to decrease difficulty in aligning patients. This technique consists of storing fresh human spermatozoa diluted with Test-Yolk buffer (TYB) at 5°C for approximately 24 to 48 hr. [2]. Meanwhile, Infertility alone often has a profound impact on males ability to produce a seminal specimen on demand. Under these circumstances, adequate production of semen specimens followed by preparation for ART such as intrauterine insemination (IUI) [3]. The cryostorage of semen sample is also more convenient for the male partner, who does not need to attend the clinic at short notice. To reduce the damaging effects of cryostorage on cells it is essential to equilibrate them in the presence of a suitable cryoprotectant [4,5,6,7].

Materials and Method

The men selected to participate in this study were normozoospermia, mild male factor infertility and unexplained infertility. The thirty eight infertile patients (who were visiting the High Institute of Infertility Diagnosis & ART for three weeks) were involved to study the effect of the (MTS) mixed with 30% (EY) on certain sperm function parameters of semen after 24 hr cryostorage.

Semen analysis was done as recommended by WHO (1999) [8,9]. Sperm activation was done by using (SLM) and centrifugation method with a FertiCultTM medium[10,17]. Preparation of culture media and cryostorage of semen patients was made as described by Al-Dujaily and Al-Shammary [11] and Al-Taee [12].

Statistical Analysis

Data were expressed as a mean ±standard deviation (SD). Statistical significance was determined by using independent sample student test (T-test). When the probability value reaches ($P<0.05$) the result was considered significant. Probability value reaches ($P<0.001$) the result was considered a highly significant.[13]

Results

The results are presented in Table (1) : These results show a decrease in sperm concentration (m/ml) after 30 min in vitro activation with FertiCultTM medium using (SLM) compared with before activation, the difference was highly significant ($P<0.001$) statistically. There were highly significant ($P<0.001$) improved and increased in the percentage of active sperm motility grade (A & B) and the percentage of normal sperm morphology compared with before activation. The Table (2) shown a highly significant ($P<0.001$) decreased in sperm concentration after sperm activation in vitro of washed semen with 30% (EY) mixed with (MTS) after 24hr cryostorage compared with before activation. Table (2) also shown a highly significant ($P<0.001$) improvement and increased in the percentage of active sperm motility grade (A & B) and the percentage of normal sperm morphology after sperm activation in vitro of washed semen with 30% (EY) mixed with (MTS) after 24hr cryostorage compared with before activation. All sperm functional parameters recorded a highly significant ($P<0.001$)

improvement and increased in the percentage of active sperm motility grade (A & B) and the percentage of normal sperm morphology after sperm activation in vitro of washed semen with 30% (EY) mixed with (MTS) after 24hr cryostorage (except sperm concentration it was decreased) compared with 30 min in vitro activation with FertiCultTM medium using (SLM) in Table (3).

Table (1): Sperm functional parameters before & after 30 (min) *in vitro* activation with FertiCult^{TM} medium.

Sperm parameters		**Before Activation** Mean ± S.D.	**After Activation (30 min)** Mean ± S.D.
Concentration (m/ml)		60.34 ± 24.16	31.31 ± 17.93
Active Motility	**GA (%)**	2.36±7.32	36.05 ± 16.48 €
	GB (%)	36.36±11.65	44.07 ± 11.14 €
Normal Morphology (%)		51.57±16.44	87.44 ± 14.26 €

€: P<0.001

Table (2): Beneficial effect of 30% (EY) mixed with (MTS) after 24hr. cryostorage on sperm *in vitro* activation of semen compared with before activation.

Sperm parameters		**Before Activation** Mean ± S.D.	**After Cryostorage of washed semen for 24hr.** Mean ± S.D.
Concentration (m/ml)		60.34 ± 24.16	22.10 ± 11.21
Active Motility	**GA (%)**	2.36 ± 7.32	52 ± 10.14£
	GB (%)	36.36 ± 11.65	48.60 ± 10.68£
Normal Morphology (%)		51.57 ± 16.44	94.89 ± 8.77£

£: P<0.001

Table (3): Comparison between 30% (EY) mixed with (MTS) after 24hr. cryostorage on sperm *in vitro* activation of semen with 30 (min) after *in vitro* activation using FertiCult^{TM} medium.

Sperm parameters		**After Activation (30 min)** Mean ± S.D.	**After Cryostorage of washed semen for 24hr.** Mean ± S.D.
Concentration(m/ml)		31.31 ± 17.93	22.10 ± 11.21
Active Motility	**GA (%)**	36.05 ± 16.48	52 ± 10.14¥
	GB (%)	44.07 ± 11.14	48.60 ± 10.68¥
Normal Morphology (%)		87.44 ± 14.26	94.89 ± 8.77 ¥

¥: P<0.001

Discussion

In this study, the results showed a highly significant decrease in sperm concentration and highly significantly increased in the percentage of progressive motility (A, B) and sperm morphology after 30 min of activation using FertiCult^{TM} medium and cryostored medium compared with before activation. These findings might be obtained due to the role of sperm preparation techniques and the components of liquid artificial medium which led to separate the largest number of morphologically normal motile spermatozoa in a small volume of culture medium free from seminal plasma, leukocyte, immotile and dead sperms [14,16]. The percentage of progressive motility (A,B) and sperm morphology were increased and improved after 24 hr cryostored with 30% (EY) mixed with (MTS) *in vitro* activation compared with 30 min *in vitro* activation using FertiCult^{TM} medium. The use of egg-yolk buffer has considerable clinical significance in diagnosing and treating male infertility. A short incubation of sperm with egg-yolk buffer before in vitro fertilization can increase the fertilizing pontienal of sperm. The egg-yolk buffer can also be used as a storage medium in the shipment or transporting of specimes to the laboratories. Adding the egg-yolk buffer to the semen with a high viscosity can improve the percentage of sperm motility, viability and morphology.[17]. The data of this study revealed a highly significant improvement of all sperm parameters after 24 hr cryostorage of fertile samples when compared with infertile samples. Moreover, there was a highly significant increased of active sperm grade A and percentage of MNS of washed cryostored semen of fertile patients. These results were similar to other investigator's findings regarding the improvement in sperm characteristics and maintenance of sperm viability of fertile and infertile samples by employing cryostorage technique for 24 hr prior to IUI [11,12].

References

[1] S.Kelleher, S. M. Wishart, P. Y. Liu, L. Turner, I. DI Pierro, A. J. Conway & D.J. Handelsman. Long-term outcomes of elective human sperm cryostorage. Human Reproduction 2001;16.(12):2632–2639.

[2] Zavos, P., Correa, J., Sofikitis, K., Kofinas, G., & Zarmakoup, P. A Method of Short-Term Cryostorage and Selection of Viable Sperm for Use in the Various Assisted Reproductive Techniques. Tohoku J. Exp. Med. 1995; 176: 75-81.

[3] Shanner, L. & Nisker, J. Bioethesis for clinicians: assisted reproductive technologies. C. M .J. 2001;164:11-14.

[4] Kofinas, G.D.& Zavos, P.M. Selection of viable spermatozoa via sperm filtration following 24 hr of cryostorage at 5ºC in test –yolk buffer. Mol. Androl.1992;4:113-119.

[5] Mazur, P., Kemp, J.A., & Miller, R.H. Survival of fetal rat pancreases frozen to -78 and -196 degrees. Proc. Natl. Acad. Sci. 1976;73:4105-4109.

[6] Crister, J.K., & Russell, R.J. Genome resource banking of laboratory animal models. Institute of Laboratory Animal Research J. 2000; 41:183-186.

[7] Nalesnik, J.G., Sabanegh, E.S., Jr., Eng, T.Y. & Buchholz, T.A. Fertility in men after treatment for stage 1 and 2A seminoma. American J. of Clinical Oncology. 2004; 27: 584-588.

[8] World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 1999; 4th ed:6-22. London: Cambridge University Press.

[9] World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus

Interaction. 2010; 5th ed:13-32. Switzerland Press.

[10] Al-Azzawi, K. S. Activation of epididymal sperms *in vitro* in mice. Msc. thesis, college of Science, Al-Mustansiriya University.1997.

[11] Al-Dujaily, S.S., and Al-Shammary, R.N. Effect of certain additive to Tris solution for maintenance of human semen during cryostorage. J. University of Karbala. 2008, 13:106-112.

[12] Al-Taee, A.M. Impact of Semen Cryostorage on Intrauterine Insemination Outcome. Diploma thesis, Institute of Embryo Researches and Infertility Treatment, Al-Nahrain University. 2011.

[13] Sorlie, D.E.,: In: Medical Biostatistics and Epidemiology. Examination and Board Review.1st ed. Appleton and Lange, Norwalk, Connection. 1995:47-88.

[14] Pardo, M., and Bancells, N. Artificial insemination with husband's sperm (AIH). Techniques for sperm selection. Arch. Androl. 1989, 22 :15-27.

[15] Centola, G.M. Sperm preparation for insemination. In: Office Andrology. Patton, P.E., Battaglia, D.E., eds. Totowa, NJ: Humana Press. 2005: 39-52.

[16] Al-Ghazi, M.K., Al-Azzawi, K.S., Al-Saadi, R.A., & Flayeh, N.K. Effect of incubation time on certain sperm function parameters following *in vitro* activation test FertiCult™ medium. Iraqi Journal of Embryos & infertility Researches 2012; 2. (3): 31-34.

[17] Hallak, J., Sharma, R.K., Wellstead, C. & Agarwal, A. Cryopressravtion of human spermatozoa: Comparison of Test-Yolk Buffer and Glycerol. Int. J. Fertil. 2000; 45(1):38-42.