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Comparison of Low Hypo-Osmotic Swelling Results to Fertilization Rates in an IVF-ET Program

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Previous studies have shown that in vivo pregnancies correlated with hypo-osmotic swelling (HOS) scores. Men who had subnormal motile densities (MD) but normal HOS scores achieved a high pregnancy rate with female factors corrected. No significant differences were seen in men with normal HOS scores $\geq 60\%$ and grey-zone HOS scores between 50 and 59%. However, there were no pregnancies in the female counterpart when the males HOS score was $< 50\%$ [1–3].

The data presented herein evaluates whether these patients with otherwise normal semen parameters but low HOS scores ($< 50\%$) have reduced fertilization in vitro and subsequent pregnancy rates.

Materials and Methods

Paired semen samples were analyzed at a 2- to 4-week interval prior to the in vitro fertilization (IVF) embryo transfer (ET) cycle. Sperm count, motility, morphology, viability and HOS were performed on both semen samples.

Normals for IVF semen analysis (SA) are: count $\geq 20 \times 10^6$/ml, motility $\geq 30\%$ and morphology $> 60\%$ normal forms [4]. The SA was manually assessed using a phase contrast microscope. The Makler chamber was used for count and motility results. Two stained semen smears were analyzed for sperm morphology. Sperm morphology was evaluated by one of two methods. Earlier the criteria suggested by the World Health Organization (WHO) was used [5]. This criteria was used for approximately one third of the patients. The remaining were evaluated using a more strict criteria report by Kruger et al. [6]. Strict morphology results were considered abnormal if there were $< 5\%$ normal forms.

Sperm viability was assessed using equal parts 0.5% Eosin Y stain and semen. The semen was then placed on a glass slide coverslipped and allowed to sit for 5 min at room temperature. The unstained sperm (viable) were counted microscopically. WHO suggests $> 50\%$ viable as normal [5]. The HOS was performed as previously described [7].

A total of 267 couples with at least one IVF-ET cycle were divided into 4 groups: group 1, normal HOS and normal SA; group 2, normal HOS and abnormal SA; group 3, subnormal HOS and normal SA; group 4, subnormal HOS and abnormal SA.

Follicular Stimulation and Embryo Retrieval

Follicular stimulation was accomplished by first suppressing endogenous gonadotropins with administration of leuprolide acetate (LA), 1 mg s.c. 7 days following ovulation, for a period of 10 days. Human menopausal gonadotropins (hMG) was administered intramuscularly at a dosage of 150 IU twice daily, starting on day 11 of the cycle for 3 days. The hMG was then reduced to 225 IU/day for 1 day, then 150 IU daily thereafter. Patients were monitored daily via vaginal ultrasonography and serum estradiol (E_2) measurement. At the point when a serum E_2 level of at least 600 pg/ml and when at least 3 follicles over 16 mm diameter were observed, hCG (human chorionic gonadotropins) was administered at a dosage of 10,000 IU i.m. Oocyte retrieval was performed via vaginal ultrasound 30–32 h post-hCG.

The retrieved oocytes were classified into four categories: immature, mature, postmature, or atretic. All preovulatory oocytes were incubated in Ham's F-10 medium (Gibco, Grand Island, N.Y.), supplemented with 5% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.), pH 7.4 at 275–280 mosm/kg H_2O. Motile sperm concentrations of $5–10 \times 10^4$/ml were used for inseminations. Higher sperm concentrations of $1–2.5 \times 10^5$/ml were used for oligospermic patients. Semen was centrifuged at 400 *g* for 8 min in a $2\times$ volume of F-10 + BSA and the resulting pellet overlaid with 0.2–0.5 ml modified F-10. Motile sperm were allowed to swim up into the overlying medium for 30–60 min. Following removal of the motile fraction, semen parameters were measured and used for insemination. One to 5 embryos were transferred on the second day after retrieval. When there were more than 5 embryos, the remaining were cryopreserved for subsequent transfer, using a modification of the one-step method. All patients who underwent ET received supplemental progesterone (25 mg in oil i.m.) daily, starting on the day of retrieval and continuing until a negative β-hCG test result was obtained.

Sperm count and motility were evaluated on the day of IVF retrieval. This count and motility was used along with the initial morphology result to place the IVF cycle into its appropriate group.

Table 1. Comparison of semen results of the four patient groups

	Group 1	Group 2	Group 3	Group 4
Patients	221	44	10	5
Mean count, $\times 10^6$/ml	98 ± 98.2	64.2 ± 62.2	112.6 ± 62.13	39.77 ± 33.29
Mean motility, %	64.5 ± 13.35	53.1 ± 21.85	58.3 ± 12.58	53.18 ± 25.99
Mean WHO criteria morphology % normal forms	65.5 ± 11.7	43.4 ± 15.3	69.5 ± 1.5	35.0 ± 4.1
Mean strict morphology % normal forms	13.9 ± 16.1	4.9 ± 5.6	14.3 ± 4.2	02.5 ± 0.5
Mean HOS, %	74.2 ± 8.9	70.0 ± 9.8	41.2 ± 5.9	40.9 ± 16.6

Table 2. Comparison of IVF results of the four patient groups

	Group 1	Group 2	Group 3	Group 4
Patients	221	44	10	5
Retrievals	377	60	13	11
Mean number of eggs inseminated	9.05 ± 6.4	8.46 ± 5.56	9.3 ± 3.72	8.6 ± 5.3
Mean number of embryos transferred	3.26 ± 4.76	2.08 ± 2.19	2.92 ± 1.6	2.09 ± 2.38
Mean number of embryos frozen	1.32 ± 2.57	0.76 ± 1.7	1.38 ± 2.2	1.18 ± 1.78
Pregnancies	59	2	2	0
Pregnancy rate/retrieval, %	15.7	3.3	15.4	0
Abortion rate, %	19	100	50	
Mean fertilization rate, %	52.65 ± 31.37	33.3 ± 34.0	36.3 ± 31.8	33.63 ± 42.9

Results

In the control group (group 1) the count, motility and percent swelling (HOS) are higher than in groups 2, 3 and 4 (table 1).

In table 2 the number of eggs inseminated, number of embryos transferred and number of embryos frozen are essentially equal in all groups. Also, in groups 2, 3 and 4 the percent fertilization in vitro was essentially

the same. Percent fertilization in group 1 (control group) was twice as high as in groups 2, 3 and 4. The pregnancy rate in groups 1 and 3 per retrieval is also much greater than that in groups 2 and 4. Differences between groups 1 and 3 are due to differences in HOS parameters only, i.e. fertilization rate. But at the same time, pregnancy rate per retrieval does not follow this trend.

Discussion

It has been reported by various IVF centers that an abnormal semen analysis is associated with low fertilization rates in vitro [4]. In our study presented herein, groups 1 and 3, both having a normal semen analysis, should have similar results. Interestingly, group 3 (normal SA but subnormal HOS scores) had lower fertilization rate than group 1. Group 3 also had similar fertilization rate as groups 2 and 4 (both containing men with subnormal semen analysis).

These data suggest that a subnormal HOS score does suggest reduced fertilization rates in vitro but 0% pregnancy rate in vivo. It appears to be an important semen parameter that can indicate a male factor contribution to the low fertilization rates in vitro which could have been classified as unexplained infertility.

Studies are currently being performed to evaluate a larger group of patients with subnormal HOS scores in vitro. These data suggest that in vitro fertilization may be an alternate approach to the treatment of men with subnormal HOS scores.

References

1 Check, J.H.; Epstein, R.; Nowroozi, K.; Shanis, B.S.; Wu, C.H.; Bollendorf, A.: The hypo-osmotic swelling test as a useful adjunct to the semen analysis to predict fertility potential. Fertil. Steril. *52:* 159–161 (1989).

2 Check, J.H.; Nowroozi, K.; Wu, C.H.; Bollendorf, A.: Correlation of the semen analysis and the hypo-osmotic swelling test with subsequent pregnancies: A preliminary report. Arch. Androl. *20:* 256–260 (1988).

3 Check, J.H.; Nowroozi, K.; Wu, C.H.; Bollendorf, A.: The Clinical Significance of the Hypo-Osmotic Swelling (HOS) Test. Meeting of the Pacific Coast Fertility Society, Palm Springs, Calif., April 1989.

4 Yates, C.A.; De Kretser, D.M.: Male factor infertility and in vitro fertilization. J. In Vitro Fertil. Embryo Transfer *4:* 141–147 (1987).

5 World Health Organization Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction (Cambridge University Press, Cambridge 1987).

6 Kruger, T.F.; Acosta, A.A.; Simmons, K.F.; Swanson, R.J.; Matta, J.F.; Oehninger, S.: Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil. Steril. *49:* 112–117 (1988).

7 Jeyendran, R.S.; Van der Ven, H.H.; Perez-Palaez, M.; Crabo, B.G.; Zaneveld, L.J.D.: Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. J. Reprod. Fertil. *70:* 219–228 (1984).

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