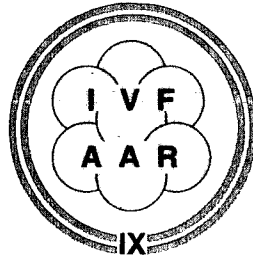


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**IX**

# Cumulus removal (CR), oocyte concentration, and use of follicular fluid (FF) improve pregnancy rate (PRs) following IVF for severe male factor (1)

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## SUMMARY

The study presented herein evaluated the use of non-micromanipulation techniques for severe male factor. Male factor was defined as follows: normal morphology (NM)  $\leq 10\%$  according to strict criteria and motile density  $\leq 10 \times 10^6/\text{mL}$ . Strict morphology (SM) was divided into three groups: Gr I (n=72),  $\leq 2\%$ ; Gr II (n=24), 3-5%; and Gr III (n=29), 6-10%. Modification of standard in vitro fertilization (IVF) techniques included manual cumulus removal, pooling up to 10 oocytes together in 1mL of media, and supplementing media with 20% human follicular fluid. The overall fertilization rate was 57.7% and the pregnancy rate/retrieval cycle was 14.8%. Evaluating patients with  $\leq 5 \times 10^6$  sperm/mL, there were no pregnancies in 5 cycles and 4 transfers following the conventional method, but 2 sets of twins with the modified protocols in 7 cycles.

## INTRODUCTION

One of the indications for IVF-embryo transfer (ET) is male factor. However, a problem in assessing the efficacy of IVF for male factor lies

in identifying the subfertile male based on merely the semen analysis (2,3). Some semen samples with subnormal count, motility, and/or morphology are capable of fertilizing oocytes in vitro (4), while sperm which appears normal may actually be incapable of resulting in pregnancy (5).

Once the problem has been identified as male factor, the difficulty is in selecting an appropriate treatment. When conventional techniques are not effective in achieving fertilization, newer procedures, e.g., micromanipulation and modified insemination techniques are available (6-10). Unfortunately, current treatment methods vary in the degree of reported success, potential damage to gametes and cost.

Cohen et al. (7), advocated micromanipulation for 3 different groups based on NM, motility, and sperm concentration. One of these criteria involves poor SM ( $\leq 2\%$  normal) as suggested by Kruger et al. (11); although some data suggest that this test does not accurately distinguish the subnormal male population, even with an in vivo setting (3).

Other laboratories have reported success with male factor patients without the use of micromanipulation. Some of these programs utilize techniques which bring the gametes in closer proximity, such as combining the sperm and oocytes in microdroplets (12) or in capillary tubes (13,14). Other techniques, such as sperm pre-treatment with follicular fluid (FF) (9) or pentoxifylline (10) may enhance sperm function.

The study presented herein retrospectively evaluated the efficacy of non-micromanipulation for male factor including two modified in vitro insemination techniques, i.e., removal of the cumulus cells from the oocytes with or without the addition of FF, in couples with "male factor" problems undergoing IVF-ET.

## MATERIALS AND METHODS

81 consecutive IVF cycles in which severe male factor was the diagnosis were evaluated. All patients were found to have  $\leq 10\%$  NM according to strict criteria (11), and motile density (MD)  $\leq 10 \times 10^6/\text{mL}$  in the initial semen sample on the day of IVF.

Group I (n=36) received the conventional insemination (protocol A): 1-4 oocytes cultured/1 mL of human tubal fluid (HTF) (Irvine Scientific, Santa Ana, CA) with 0.5% bovine serum albumin (BSA). Group II (n=32) received the cumulus removal (CR) (protocol B): cumulus oophorus was manually dissected away from oocytes and 1-10 oocytes were cultured/mL HTF. Group III (n=13) was treated with (protocol C): the same procedure was followed as protocol B, but HTF was supplemented with 20% FF. Protocol A was used on all patients unless the MD was so poor that the minimum of 10,000 motile sperm with NM/oocyte were not available, or, if there was an adequate number of sperm, but poor progression. Only patients in which the female partner did not have serum antisperm antibodies (ASA) were included in group C.

In all 3 groups, oocytes were cultured in organ culture dishes (Falcon 3037) in a humidified incubator at 37°C with 5% CO<sub>2</sub> in air. Culture medium was overlaid with mineral oil (Squibb). The FF used in this study was pooled from two or more mature follicles during the patient's retrieval, then centrifuged at 795g (Clay-Adams Dynac II table top centrifuge) for ten minutes. Oocytes in all three groups were inseminated with  $\leq 10 \times 10^3$  motile sperm with normal SM/oocyte. Sperm was processed using Percoll density gradient.

The pregnancy rate (PR) and fertilization rate (FR) for mature oocytes were evaluated according to the insemination protocol A, B, or C. SM was divided into three groups: Gr I (n=27),  $\leq 2\%$ ; Gr II (n=24), 3-5%; and Gr III (n=30), 6-10%. Pregnancies from subsequent frozen ET were included in the PRs.

## RESULTS AND CONCLUSIONS

The FR for patients in the combined groups was 57.7%. The PR/retrieval cycle was 14.8% for the combined groups and 18.2% when cycles without fertilization were excluded. There were no differences noted even if % normal forms by SM was  $\leq 2\%$  vs  $> 5\%$ .

While the FR seemed to improve when protocols B and C were used in Gr I, the difference was not significant. In all three groups the sample sizes were too small to make any inferences.

Evaluating patients with  $\leq 5 \times 10^6$  sperm/mL, there were no pregnancies in 5 cycles and 4 transfers following protocol A, but one set of twins each with protocol B and C in 7 cycles.

It is not yet clear what factors should be included in the definition of severe male factor. While various semen parameters can indicate a reduced chance of success with fertilization, they cannot accurately predict the outcome following IVF (15). Our results confirmed this since average fertilization and PRs were demonstrated following IVF-ET for male factor patients. Perhaps other factors such as hypo-osmotic swelling scores, or, previous failure to fertilize any oocytes may better define the male factor patient, but even these have been shown to be inconsistent (16).

Appropriate treatment for male factor patients should be based on the severity of the problem and high cost treatments, such as micromanipulation, should be reserved for the most severe cases. In our program, we attempt to enhance the cell culture environment to improve the conditions necessary for IVF for male factor patients. This is done by removing the cumulus cells from around the oocytes, pooling more oocytes together in a small volume, and adding FF to the culture medium. The removal of cumulus cells from around the oocyte may improve the chances of sperm with low or abnormal motility to come in contact with the zona-pellucida. Cumulus removal also allows pooling more oocytes together in the culture dishes to increase the proximity of sperm and oocytes. Human FF has been found to contain factors which improve the rate of fertilization of human oocytes in vitro (9), possibly by enhancing sperm motility (17), or by providing factors necessary for the completion of the acrosome reaction in vitro (18). The study presented herein was not designed to compare the efficacy of standard and modified techniques. We were able, however, to demonstrate a clinical PR in patients with male factor that compares favorably with PRs in non-male factor cases without the use of micromanipulation. In general, protocol A was used for better quality sperm and protocol C for the poorest. The highest PRs were seen following addition of FF (protocol C) at least suggesting that CR and FF may improve PRs. However, a prospective randomized study is needed to determine whether CR, adding FF, or pooling more oocytes improves the PRs and FRs compared to standard protocol.

We have shown that pregnancies can be achieved without the use of micromanipulation in patients with male factor. As micromanipulation procedures are often expensive, they should not be used indiscriminately. Furthermore, the implications of bypassing the natural sperm selection

process and the risk of selecting abnormal sperm for micromanipulation have not been fully investigated. Only a few IVF centers have the means to perform micromanipulation. If non-micromanipulation enhancing procedures are found to help a segment of the infertile population, then assisted reproductive technology may become available to a larger group of patients who fail to benefit from standard techniques.

#### REFERENCES

1. Hourani CL, Check JH, Baker AF, Hoover LM, Summers DC, Benfer KM. Cumulus removal and addition of follicular fluid possibly improves pregnancy rates with in vitro fertilization for male factor. *Arch Androl* 34:47-52;1995.
2. Check JH, Nowroozi K, Bollendorf A. Correlation of motile sperm density and subsequent pregnancy rates in infertile couples. *Arch Androl* 27:113-115;1991.
3. Check JH, Adelson HG, Schubert BR, Bollendorf A. Evaluation of sperm morphology using Kruger's strict criteria. *Arch Androl* 28:15-17;1992.
4. Coates TE, Check JH, Choe J, Nowroozi K, Lurie D, Callan C. An evaluation of couples with failure of fertilization in vitro. *Hum Reprod* 7:978-981;1992.
5. Check JH, Framroze A, Liss JR, Bollendorf A. Therapeutic insemination by donor (TDI) achieves high pregnancy rates in infertile couples regardless of male motile sperm density. *Arch Androl* 25:169-171;1990.
6. Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smitz J, Wisanto A, Devroey P. High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 8:1061-1066;1993.
7. Cohen J, Alikani M, Malter HE, Adler A, Talansky BE, Rosenwaks Z. Partial zona dissection or subzonal sperm insertion: microsurgical fertilization alternatives based on evaluation of sperm and embryo morphology. *Fertil Steril* 56:696-706;1991.
8. Ord T, Patrizio P, Balmaceda JP, Asch RH. Can severe male factor infertility be treated without micromanipulation? *Fertil Steril* 60:110-115;1993.
9. Ghetler Y, Ben-Nun I, Kaneti H, Jaffe R, Gruber A, Fejgin M. Effect of sperm preincubation with follicular fluid on the fertilization rate in human in vitro fertilization. *Fertil Steril* 54:944-946;1990.
10. Tesarik J, Mendoza C. Sperm treatment with pentoxifylline improves the fertilizing ability in patients with acrosome reaction insufficiency. *Fertil Steril* 60:141-148;1993.
11. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil Steril* 49:112-117;1988.
12. Tournaye H, Devroey P, Camus M, Staessen C, Bollen N, Smitz J, Van Steirteghem AC. Comparison of in-vitro fertilization in male and tubal infertility: a 3 year survey. *Hum Reprod* 7:218-222;1992.
13. Al Hasani S, Van Der Ven K, Diedrich K, Krebs D. In vitro fertilization in capillary tubes for male factor infertility. *Ann Acad Med Singapore* 21:489-491;1992.
14. Hammitt DC, Walker DL, Syrop CH, Miller TM, Bennett MR. Treatment of severe male-factor infertility with high concentrations of motile sperm by microinsemination in cryopreservation straws. *J In Vitro*

Fert Embryo Transfer 8:101-110;1991.

15. Check JH, Bollendorf A, Lee MA, Nazari A, Nowroozi K. Correlation of computerized semen analysis with successful fertilization of oocytes in an in vitro fertilization program. Arch Androl 24:229-234;1990.

16. Check JH, Epstein R, Nowroozi K, Shanis BS, Wu CH, Bollendorf A. The hypoosmotic swelling test as a useful adjunct to the semen analysis to predict fertility potential. Fertil Steril 52:159-161;1989.

17. Chao HT, Ng HT, Kao SH, Wei YH, Hong CY. Human follicular fluid stimulates the motility of washed human sperm. Arch Androl 26:61-65;1991.

18. Siegel MS, Paulson RJ, Graczykowski JW. The influence of human follicular fluid on the acrosome reaction, fertilizing capacity and proteinase activity of human spermatozoa. Hum Reprod 5:975-980;1990.

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