

# Role of semen parameters in predicting fertilization / pregnancy outcome in IVF

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## abstract

WHO has established subnormal values for motile density (MD)  $< 10 \times 10^6$ /ml. Another semen parameter, strict sperm morphology did significantly correlate with poor IVF outcome when  $< 4\%$ . However, we presented *in vivo* data in untreated males, that only a MD level of  $< 2.5 \times 10^6$ /ml correlated with subfertility. Similarly, *in vivo*, we could not substantiate that a strict morphology score  $< 4\%$  better predicted male subfertility than normal levels  $> 14\%$ .

The present study was designed to compare fertilization / pregnancy rates from IVF cycles according to motile density / sperm morphology. Also, functional integrity of the sperm membrane, as determined by the hypoosmotic swelling test (HOS), was assessed. A total of 330 first cycles were evaluated and men were divided into groups according to the following parameters:

motile sperm density  
strict sperm morphology  
hypo-osmotic swelling(HOS)

There were no pregnancies (0/10) with MD  $< 2.5 \times 10^6$ /ml. Though only 1/9 group II patients conceived (11%) only 40/239 Group VI patients conceived (17%) with 6 aborting, giving a live birth rate cycle of 14% (p=NS). The highest pregnancy rate was in Group III (19%) with 5 of 26 pregnant but 80% aborted. Thus only 2 of 45 (4.4%) had a live birth with  $< 8 \times 10^6$ /ml compared to 36 of 265 (14%) with  $\geq 8 \times 10^6$ /ml. Yet when all pregnancies were considered 6/45 conceived (13%) with  $< 8 \times 10^6$ /ml vs 42 of 265 (16%).

The only group with a statistically lower poor fertilization was group I. Pregnancy rates with strict morphology was 7 of 62 (11%). Group A patients had a live birth rate of 8.6% compared to 13 of 74 (18%). Group C with 12% live birth. The largest Group (Group B with 268) had pregnancies at 22% with 17% live births. There was only 1 live birth in 26 (3.8%) with HOS  $< 50\%$

compared to 37 out of 284(13%) with HOS > 50%. When  $\geq 60\%$ , the pregnancy rate was still 13%. Thus the  $2.5 \times 10^6/\text{ml}$  level *in vitro*, similar to previous *in vivo* data, seems to be the important cut-off level to predict severe male infertility. Similar to *in vivo* data levels  $< 10 \times 10^6/\text{ml}$  did not clearly identify a subfertile group, but in contrast to *in vivo* data the spontaneous abortion rate increased with levels  $< 8 \times 10^6/\text{ml}$ . Strict morphology  $< 4\%$  did not identify the subfertile group, especially if MD was normal. HOS cores  $< 50\%$  distinguished the subfertile group similar to previous *in vivo* data.

### Introduction

Most centers agree that "male factor" has a lower success rate than other factors as determined by pregnancy rates following IVF/ET. The definition of male factor, however, is at the present time somewhat vague. The World Health Organization (WHO) has established levels below  $20 \times 10^6/\text{mL}$  and 50% motility (or motile density below  $10 \times 10^6/\text{mL}$ ) as subnormal. However, in a previous *in vivo* study evaluation the 6 month pregnant rate according to motile density (MD) in couples where a female factor was identified and corrected, no significant differences were seen until the levels were  $< 2.5 \times 10^6/\text{mL}$  (22%); and MD of 2.5 to  $< 5 \times 10^6/\text{mL}$  resulted in a 60% rate and another subnormal level of 5 to  $10 \times 10^6/\text{mL}$  had the same exact pregnancy rate (81%) as couples where the male partner's MD was  $\geq 15 \times 10^6/\text{mL}$  (Check et al., 1991).

Some researchers believe that morphology is the best parameter to estimate male fertility potential (Rogers et al., 1983). However, using a similar group of infertile couples as the MD study using the WHO cutoff of 50%, there was no difference in rates of pregnancy with 87% in those  $\geq 50\%$  vs 82% in those with  $\geq 50\%$  normal forms (Check, Bollendorf et al., 1992).

Another method for evaluating morphology uses much stricter morphologic criteria (Kruger et al., 1988). Very poor pregnancy rates were found even when males had normal MD where the percentage of strict forms were  $< 4\%$  (Oehninger et al., 1988). However we were not able to confirm these findings *in vivo* when strict morphology was  $< 4\%$  (Check, Adelson et al., 1992). A test of the functional integrity of the sperm membrane known as the hypo-osmotic swelling (HOS) test (Jeyendran et al., 1984) was found to correlate with infertility when  $< 50\%$  swelling was seen (Check et al., 1989).

Instead of 300-400 sperm reaching an oocyte in the human being following intercourse at mid cycle with a normospermic male, with IVF a minimum of  $50 \times 10^3$  sperm are placed in immediate contact with the oocytes. The expectation would be that a smaller number of quality sperm would be necessary to fertilize by IVF than *in vivo*. The study presented herein evaluated the above semen parameters.

### Materials / Methods

A total of 330 consecutive IVF cycles in which oocytes were successfully retrieved were evaluated. All cycles were performed in one center. IVF outcomes recorded were fertilization rate (percent mature oocytes fertilized and percent all oocytes fertilized), number of embryos

transferred, pregnancy rate, viable birth rate and abortion rate.

Semen parameters evaluated were motile density, normal morphology using strict criteria and hypoosmotic swelling. The motile density (count/mL x % motility) of the specimen obtained on the day of oocyte retrieval was determined using the cell soft computer assisted semen analyzer. The hypo-osmotic swelling (HOS) test and the percentage normal morphology using strict criteria were evaluated on 2 baseline semen specimens obtained 1-3 months prior to the procedure. The average of these two baseline values were used in the analysis. The HOS test was performed as previously described (Jeyendran et al., 1984). Morphology was determined exactly as described by Kruger et al (Kruger et al., 1988).

To assess the relationship between semen parameters and IVF outcome, the fertilization, pregnancy and abortion rates were compared for groups of cycles at the following levels of the semen parameters. For motile density the groups was based on the levels:

- 1)  $< 2.5 \times 10^6$ /mL
- 2)  $\geq 2.5$  to  $< 5.0$
- 3)  $\geq 5$  to  $< 8$
- 4)  $\geq 8$  to  $< 10$
- 5)  $\geq 10$  to  $< 15$
- 6)  $\geq 15 \times 10^6$ /mL

For strict morphology the cycles were grouped for levels:

- A)  $< 4\%$
- B) 4 to  $\leq 14\%$
- C)  $> 14\%$

The levels of HOS test studied were:

- I  $< 40\%$
- II  $\geq 40\%$  to  $< 50\%$
- III  $\geq 50\%$  to  $< 60\%$
- IV  $\geq 60\%$

Chi square analysis was used to test the hypothesis of equal fertilization and pregnancy rates at all levels of the semen parameters. In cases where some levels had small sample sizes, groups were combined for the analysis. A probability level of .05 was used. Mean fertilization rates at various levels of semen parameters were compared using analysis of variance.

## Results

Zero percent fertilization as related to MD was seen in 3 of 10 cycles (30%) in grp 1 patients; 1 of 9 (11.1%) grp 2 couples; 5 of 26 (19.2%) of grp 3 cycles; 2 of 11 (18.2%) of grp 4 cycles; 3 of 15 (20%) grp 5 cycles and 15 of 239 (6.3%) grp 6 cycles. Only grp 1 showed a statistically higher rate of zero fertilization compared other cycles. The percentage of cycles in each group with good fertilization ( $\geq 30\%$ ) is seen in Table 1. The lowest was found in grp 1 (30%) and the highest in grp 6 (84.5%) but there were basically no differences

**Table 1 Pregnancy rates according to motile sperm densities of male partners**

	<u>Tot no.</u>	<u>No. with ≥ 30% fertil</u>	<u>No. preg.</u>	<u>% preg.</u>	<u>Spont AB</u>	<u>% viable</u>
Grp 1	10	3(30%)	0	0	0	0
Grp 2	9	6(66.6%)	1	11.1	0	11.1
Grp 3	26	16(61.5%)	5	19.2	4	3.8
Grp 4	11	6(54.5%)	0	0	0	0
Grp 5	15	10(66.7%)	2	13.2	0	13.3
Grp 6	239	202(84.5%)	40	16.7	6	14.2

**Table 2 Pregnancy rates according to strict morphologic criteria**

	<u>Tot no.</u>	<u>No. with ≥ 30% fertil</u>	<u>No. preg.</u>	<u>% preg.</u>	<u>Spont AB</u>	<u>% viable</u>
Grp A	58	39(67.3%)	6	10.3	2	6.9
Grp B	268	232(83.2%)	43	16.0	9	12.7
Grp C	46	42(91.3%)	5	10.8	2	6.5

**Table 3 Pregnancy rates according to HOS test**

	<u>Tot no.</u>	<u>No. with ≥ 30% fertil</u>	<u>No. preg.</u>	<u>% preg.</u>	<u>Spont AB</u>	<u>% viable</u>
Grp I	9	7(77.7%)	1	11.1	0	11.1
Grp II	17	10(58.8%)	1	5.9	1	0
Grp III	54	40(74.1%)	8	14.8	2	11.1
Grp IV	230	186(80.7%)	38	16.5	7	13.5

**Table 4 Percentage of oocytes fertilized according to group**

	<u>% fertil. all oocytes</u>	<u>% fertil. mature oocytes</u>
Grp 1	29.5±29.1	36.4±29.1
Grp 2	47.2±33.8	45.6±33.3
Grp 3	41.7±32.0	55.2±24.7
Grp 4	39.2±29.1	47.6±32.4
Grp 5	49.5±33.2	78.5±10.3
Grp 6	62.6±28.2	71.8±16.4
Grp A	45.8±29.6	53.6±27.9
Grp B	60.5±29.1	68.8±18.7
Grp C	63.9±23.0	71.1±15.5
Grp I	63.7±32.7	66.2±31.5
Grp II	46.8±31.5	48.7±29.7
Grp III	51.7±26.5	62.3±19.0
Grp IV	60.0±30.6	70.0±18.1

**Table 5 Mean percent normal strict morphology and HOS scores according to motile density**

<u>Mean motile density</u>	<u>Mean % normal morph</u>	<u>Mean HOS %</u>
Grp 1 (0.67)	3.58	49.90
Grp 2 (3.37)	6.29	56.67
Grp 3 (6.49)	5.87	59.23
Grp 4 (8.90)	3.90	64.55
Grp 5 (12.31)	6.20	62.40
Grp 6 (64.09)	10.18	71.42

between grps 2-5. The pregnancy rates are seen in Table 1 also. No pregnancies were seen in grp 1 but there were only 10 cases. No pregnancies were found in the 11 grp 4 cases either. Using the WHO cutoff of  $\geq 10 \times 10^6/\text{mL}$  as normal, there were 42 pregnancies (including aborters) in 254 first cycles (16.5% per cycle) compared to 6 of 56 (10.7%) with MD's  $< 10 \times 10^6/\text{mL}$ . Chi square analysis showed  $p = \text{NS}$ .

This would be the appropriate group to compare if one assumes that spontaneous abortions are not related to male factor. However some data indicate that male factor may allow fertilization but increase abortion rate comparing viable pregnancies as determined by pelvic sonography at 6 weeks from retrieval. The grp  $\geq 10 \times 10^6/\text{mL}$  resulted in 36 viable pregnancies (14.2%) vs only 2 pregnancies (3.6%) in those with MD's  $< 10 \times 10^6/\text{mL}$  (Fisher's exact test  $p = < 0.02$ ).

The pregnancy rates according to strict morphologic criteria is seen in Table 2. Statistical analysis comparing all pregnancies in grp A (6/58, 10.3%) to grps B and C combined (48/314, 15.3%) using chi-square analysis found  $p = 0.3$ . Comparing viable pregnancies (4/58, 6.9% vs 37/314, 11.8%) using Fisher's exact test found  $p = 0.3$ . However comparing grp C (the best morphologic group) to grp A and B combined (5/46, 10.8% vs 49/326, 16.0%) ( $p = \text{NS}$  by chi-square analysis) and comparing live birth rates (3/46, 6.5% vs 38/326, 11.6%, ( $p = \text{NS}$  Fisher's exact test). Zero fertilization was seen in 8 of 58 (13.8%) of grp A cycles 21 of 268 (7.8%) grp B cycles and 2 of 46 (4.3%) of grp C cycles.

The pregnancy rates according to HOS scores are seen in Table 3. Using the 50% cutoff that we found previously to distinguish fertile from subfertile males, there were 2 pregnancies in 26 (7.7%) vs 46 in 284 (16.2%) ( $p = \text{NS}$ , Fisher's exact test) and comparing live births 1/26 (3.8%) vs 37/284 (13.0%) ( $p = \text{NS}$ , Fisher's exact test).

When the HOS test was  $< 40\%$  there were no cycles with zero percent fertilization and in group 2 of 26 (7.7%) vs 27 of 284 (9.5%) with levels  $\geq 50\%$  ( $p = \text{NS}$  Fisher's exact test). Mean fertilization rates were significantly lower for group 1 than group 6, but the same for MD levels above  $2.5 \times 10^6$  ( $p < .05$ , ANOVA and Tukey post hoc tests). The mean % normal strict morphology and HOS scores according to MD is seen in Table 5. The mean MD and HOS scores according to strict morphology is seen in Table 6. The mean number of embryos transferred is seen in Table 7 as well as the MD following swim-up or Percoll separation on the day of retrieval.

## Discussion

Similar to the *in vivo* study, the lowest pregnancy rate was seen in grp 1 where the MD was  $< 2.5 \times 10^6/\text{mL}$ . However grp 4 also had zero pregnancies. If the data from grp 1 is eliminated there would have been 6 pregnancies in 46 couples (13.0%), which is comparable to the 16.5% for those with MD's  $\geq 10 \times 10^6/\text{mL}$ .

The low viable pregnancy rates with MD  $< 10 \times 10^6/\text{mL}$  may be interpreted as being indicative of this cutoff as an appropriate level to distinguish a fertile from subfertile male. This would contrast with the previous *in vivo* data where only until the MD dropped to  $< 2.5 \times 10^6/\text{mL}$  was

**Table 6 Mean motile density and HOS scores according to strict morphology**

<u>Mean Morphology</u>	<u>Mean motile density (x10<sup>6</sup>/mL)</u>	<u>Mean HOS %</u>
Grp A (1.82)	19.67	58.94
Grp B (8.26)	52.55	69.63
Grp C (18.46)	81.11	72.22

**Table 7 Mean numbers of embryos transferred and mean swim-up or percoll prepared MD**

<u>Groups</u>	<u>Swim-up or Percoll prepared MDx10<sup>6</sup>/mL</u>	<u>No. embryos transferred</u>
1	69.5±7.0	1.9±2.1
2	20.8±18.0	3.2±2.3
3	17.5±20.3	2.5±2.1
4	24.1±37.8	3.1±1.9
5	32.3±33.0	3.3±2.0
6	77.7±108.8	3.5±1.8
A	26.1±24.0	3.1±1.9
B	65.5±56.5	3.6±1.8
C	81.5±59.2	3.5±1.9
I	33.6±60.1	2.8±1.6
II	41.1±56.1	3.2±1.9
III	51.5±65.6	2.7±2.0
IV	79.8±110.9	3.5±1.9

a difference in pregnancy rates seen (Check et al., 1991). A selection bias may explain these differences. The *in vivo* study evaluated the 6 month pregnancy rates in newly registered patients where a female factor was identified and corrected. The type of problem requiring IVF with the highest success rate is tubal occlusion. The majority of couples with female partner tubal occlusion have male partners with normal MD's. This is seen in our data where the majority of indications for IVF was tubal factor.

Previously we demonstrated that in couples who failed to conceive after 8 cycles of all female factors corrected, there was a very high pregnancy rate in the next 8 cycles following therapeutic donor insemination (TDI) regardless of whether MD was low or excellent (Check, Framroze et al., 1990).

Because we have seen so many pregnancies *in vivo* with low MD's or poor Kruger scores these patients would have been encouraged to try many *in vivo* cycles before attempting IVF. Some of these patients not conceiving may have had defective oocytes or male or female genetic factors leading to infertility rather than subnormal sperm; they may have had physiological abnormalities with the sperm not reflected in the MD or morphology. We have previously published that MD was not predictive of failed fertilization using a shared oocytes program where we in fact demonstrated, quite frequently, good fertilization in the couple with the inferior MD (Check, Coates et al., 1992; check, 1991).

The conclusions of the importance of strict morphology as a determinant of IVF success is similar to the previous *in vivo* data (Check, Adelson et al., 1992), ie. we cannot support this test as being helpful in predicting the subfertile male. Small numbers in Oehninger's study as well as this study can lead to wrong conclusions. In our study both the worst and best morphology groups had the worst pregnancy rates. In fact making the "Kruger" test even less significant is the fact that the MD's were significantly lower in grp A than grp C and yet the severe asthenospermia still allowed the same pregnancy rate as the superior group.

IVF data did not show any inferiority of semen specimens with HOS scores <50% normal as far as fertilization of oocytes or in number of embryos transferred. However, as we previously demonstrated *in vivo* there were considerably fewer clinical pregnancies with HOS <50%, thus, the possibility exists that problems with the functional integrity of the sperm membrane lead to impairment of progressing from embryos to blastocysts or may result in very early spontaneous abortions.

Though the live birth rate of 3.8% with HOS <50% seems much lower than 13.0% for those  $\geq 50\%$ , there were insufficient numbers to gain statistical significance. Thus, these data suggest, similar to our previous *in vivo* data, the two best parameters to detect subfertile sperm are MD's  $< 2.5 \times 10^6/\text{mL}$  or an HOS score <50%. Once again, we did not find severe asthenospermia as determined by <4% normal morphology using strict criteria to be very useful in identifying the subfertile male. Perhaps a confirmation of these factors, similar to previous IVF data using the computerized assisted semen analyzer, may be useful in cases that are not severely poor.

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