Andrology-Open Access

Branigan et al., Andrology (Los Angel) 2017, 6:2
DOI: 10.4172/2167-0250.1000191

Research Article OMICS International

The Effect of Processed Total Motile Sperm Counts and Twenty Four Hour Sperm Survival on the Efficacy of Intrauterine Insemination in Male Infertility

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Received date: March 2, 2017; **Accepted date:** August 21, 2017; **Published date:** August 28, 2017

Abstract

Objective: To compare the effectiveness of IUI, based on Pre-treatment Semen analysis results, in treating male factor infertility.

Design: A retrospective cohort design of 1,768 infertile couples undergoing 5,219 IUI cycles, who had pre-treatment advanced semen analysis were evaluated. An advanced semen analysis consists of a basic semen analysis and processed total motile sperm counts through a density gradient sperm prep and recording 24 hours sperm survival of these sperm culture media in an incubator. Logistic regression analysis was used to assess the significance of prognostic factors in sperm parameters to predict the pregnancy rates with IUI.

Results: No basic semen analysis parameter accurately predicted IUI success. Clinical pregnancy rate for first cycle of IUI was 15.6% when >10 × 10^6 processed total motile sperm was available and 13.7% in all cycles. This group contained 1264/1768 (71.5%) of couples in the study. The pregnancy rate in the first cycle was 18.2% if their 24 hour survival was >70% and 15.9% in all cycles and 1008/1264 (57%) of couples were in this group. No pregnancy was achieved for processed total motile sperm counts <5 × 10^6 (168/1768) or 9.5% of couples and 3.6% for those with 24 hour survivals <30% in first cycle and 2.0% for all cycles for 601/1768 (34%) of the couples. Strong positive correlations between processed total motile sperm counts and PR (r=0.83; p<0.001) and between 24 hours survival and PR (r=0.79; p<0.001) were seen by linear regression analysis. High correlations were also noted between processed total motile sperm counts (r=0.71; p<0.001) and 24 hours survivals (r=0.76; p<001) in the advanced semen analysis and those in the IUI samples.

Conclusion: Both processed total motile sperm counts and 24 hour survival are useful predictors of whether a couple should be treated with levels below threshold levels have a very poor prognosis with IUIs.

Keywords: Advanced semen analysis; Total motile sperm counts; Sperm survival; Intrauterine insemination; Male factor infertility

Introduction

In Infertile couples, a male factor is identified in almost half of them but the Centres for disease control have shown that a male evaluation is bypassed up to 25% of the time [1]. There are multiple reasons for this including men's reluctance to seek medical care, urologists lack of interest or training in fertility care and the thinking that IVF with ICSI can overcome any potential male factor problem. The other major problem is that the basic semen analysis is a very limited test, except in severe cases, of identifying male factor infertility treatment options.

The basic semen analysis is a static test, which is very operator dependent for quality control of its results and a very poor predictor of IUI success. For insemination success the most important sperm parameters are the number of viable sperm delivered into the uterine cavity and its survival in the reproductive tract for insemination timing. Since these parameters are not usually measured, an empiric trial of 3-6 IUI cycles are often tried before moving on to IVF [2,3]. Studies have tried using total motile count (TMC) which is a calculated value of volume × concentration × motility to predict best treatment options [4-6]. However this has proven to be a very imprecise tool. A large meta-analysis of the use of TMC in the insemination sample itself was unable to predict IUI success because of differences in sperm preps used and a lack of pre-treatment evaluation [7,8].

In 1999, we reported on a simple screening test for IUI, which we called the "Advanced Semen Analysis" [9]. This test includes a basic semen analysis, followed by a density gradient sperm separation to determine the processed total motile sperm count (p-TMC), which is the total sperm available for insemination, and a 24 hour incubation to assess sperm survival. This test measures two important physiologic parameters of sperm necessary for insemination success, the total number of highly motile sperm available for insemination and the survivability of those sperm *in-vitro*. In this initial study we established certain threshold levels of p-TMC and 24 hour sperm survival needed for IUI success. The purpose of this study was to evaluate our over ten year experience using the advanced semen analysis to determine its prognostic value for couples with male factor infertility trying to achieve a pregnancy with IUI. The goal was to establish helpful information for both physicians and couples to select the most efficient and cost effective treatments for the male factor patient.

Methods

Patient population

Patient who was candidates for IUI for male factor infertility at Bellingham IVF and Infertility Centre from November 2002 through November 2012 were included in the study. Male factor infertility in this study was defined by World Health Organization (WHO) [10] standards of either a sperm concentration of $<20 \times 10^6$ ml, overall motility of <50%, sperm morphology of <30% or the combination of these abnormalities. Inclusion criteria included the following:

- Female age 36 years or younger
- Documented patent tubes by either hysterosalpingogram or laparoscopy,
- Documented ovulatory cycles with adequate luteal phases either naturally or with clomiphene citrate (gonadotrophin cycles were excluded);
- Men had to have a Minimum of 1×10^6 processed TMC. Exclusion criteria included women>age 36 because of age related fertility declines, gonadotropin stimulated cycles because of the larger number of oocytes available in those patients and women with ovulatory dysfunction or tubal diseases. Informed consent was obtained for the study protocol.

Advanced semen analysis

An advanced semen analysis was obtained prior to beginning IUI from all couples. The men had a 2-5 day abstinence period before collecting the sperm sample. Samples were obtained by masturbation in a sterile specimen container. A standard basic semen analysis by WHO criteria was performed after liquification for semen volume, sperm concentration, motility, sperm morphology and seminal leucocytes. A discontinuous two layered density gradient isolate (Irvine scientific, santa Ana, CA) was set up in two blue-capped conical falcon tubes. The samples were centrifuged at 350 xg for 20 minutes. The supernatant were removed from each tube and pellet were combined with 2 ml of sperm wash (Irvine scientific, santa Ana, CA) and centrifuged at 250 xg for 8 minutes. The supernatant was removed and another wash with 2 ml of sperm wash medium at 250 xg for 5 minutes were performed. The final pellets were suspended in 2 ml of sperm wash media. The sperm count and assessment of sperm motility was then performed on the final sample. The specimen concentration was adjusted to a concentration of 2×10^6 motile sperm per millimetre of media incubated for 24 hours at 37C in 5% CO_2. The percentage of sperm motility was re-evaluated 24 hours later and all findings were recorded.

Intrauterine insemination cycle

All insemination cycles were monitored in the follicular phase with the use of trans-vaginal ultrasound (siemens sonoline, siemens,USA) beginning of the cycle day 10-12 and repeated as necessary. When the mean diameter of the lead follicle was ≥20mm, 10,000 IUI hCG was given IM and a single IUI was performed 22-30 hours later. The same sperm preparation as described in the advanced semen analysis was used. The final sperm pellet was re-suspended in 0.3 ml of sperm wash medium and drawn into an insemination catheter (Tefcat, cook OB/GYN, IN) and then deposited high in the fundus of the uterus. The patient remained supine for 15 minutes after the IUI. All ultrasounds and inseminations were performed by the same physician and all lab work performed in the same lab by three lab technicians. Ovulations were confirmed by mid-luteal progesterone values. Sperm hCG levels were drawn to confirm pregnancies and 7 week gestation ultrasounds confirmed clinical pregnancies. Couple who failed to conceive with IUI and moved on to IVF had their IVF results recorded and analysed. IVF was performed in a standardized fashion and by the same physician and the lab used for the IUI. Decisions to use ICSI were based in past pregnancy history, semen analysis and strict morphology results. Embryo transfers were performed on either day 3 or 5 post oocytes retrieval based on patient age and number of embryos available. Chi square analysis was used for categorical variables to compare various basic semen analysis parameters and advanced semen analysis parameters to clinical pregnancy rates. Linear regression analysis was used for continuous variables to correlate processed TMC and 24 hours survival with clinical pregnancy rates. Data for the first IUI cycles were recorded separately. Receiver operating curve analysis (ROC) was used to generate cut off values for processed TMC and 24 hours survival and to determine the predictive power of various sperm parameters.

Results

One thousand seven hundred sixty eight patients met the inclusion criteria and completed 5,219 IUI cycles. The range was 1-12 cycles with a mean of 2.9 cycles. Mean female age was 30.6+2.7 years and 54% were null gravid with a mean duration of infertility of 2.1 ± 1.1 years. Seven hundred fifty nine of the 1786 patients achieved a pregnancy using IUI for an overall cumulative PR of 42.9%. 19% of these couples conceived on their first IUI cycle, 53% of the pregnancies occurred by the third IUI cycle and 96% were achieved by the sixth cycle. Of the 1109 patients who failed to achieve pregnancy, three hundred eighty four had an IVF cycle at our centre for analysis. The distribution of processed TMC and 24 hour sperm survival from the advanced semen analysis and the first IUI cycle and the total IUI cycles associated PRs are shown in Table 1.

Processed Total Motile Sperm			
Number of sperm recovered (× 10^6)	**Numbers of men with indicated sperm count/Total number of men (%)**	**Pregnancy rate 1st cycle**	**Pregnancy rate all cycle**
<5	168/1768 (9.5%)	0	0
5-10	336/1768 (19.0%)	3.1	7.2
>10	1264/1768 (71.5%)	15.9*	13.7*
24 hours Sperm Survival			
Motility (%)	**Numbers of men with indicated sperm count/Total number of men (%)**	**Pregnancy rate 1st cycle**	**Pregnancy rate all cycle**
<30	601/1768 (34%)	3.6	2.1
31-70	159/1768 (9%)	0.6	4.4
>70	1008/1768 (57%)	18.2	15.9
*P<0.001 versus other groups by chi square analysis			

Table 1: The distribution of processed TMC and 24 hour sperm survival rate.

Clinical pregnancy rate for first cycle of IUI was 15.6% when >10 × 10^6 processed total motile sperm was available and 13.7% in all cycles. This group contained 1264/1768 (71.5%) of couples in the study. The pregnancy rate in the first cycle was 18.2% if their 24 hour survival was >70% and 15.9% in all cycles and 1008/1264 (57%) of couples were in this group. No pregnancy was achieved for processed total motile sperm counts<5 × 10^6 (168/1768) or 9.5% of couples and 3.6% for those with 24 hour survival<30% in first cycle and 2.0% in all cycles 601/1768 (34%) of couples. Strong positive correlations between processed TMC and PR (r=0.83; p<0.001) and between 24 hour survival of sperm and PR (r=0.79; p<0.01) were noted by linear regression analysis. Receiver operating curve analysis (ROC) suggested cut off values for processed TMC of 10 × 10^6 with a slight but non-significant increase up to counts of 30 × 10^6 with no further increase over these counts. The cut off for 24 hour sperm survival was at 70% or more with significant differences the survival was less than 30%.

However using only the processed TMC of 10 × 10^6 alone to predict IUI success was not helpful because 81% of the men in the study were above this level. The lower limit of 5 × 10^6 for processed TMC was very predictive of a negative result as no men in this group produced a pregnancy. The sperm survival test was more predictive when used alone with men with >70% survival having an 18.2% per cycle PR in their first cycle and very good negative predictive value if <30% survival with only a 3.6% per cycle rate for these men. Men with processed TMC of >10 × 10^6 with >70% survival accounted for 89% of all the pregnancies in the study. However, if the men with >10 × 10^6 had survival of <30%, only 11% produced a pregnancy.

High correlations were also noted between advanced semen analysis pre-treatment processed TMC (r=0.71; p<0.001) and 24 hour survivals (r=0.76; p<0.001) and the TMC and survivals in the IUI samples.
There were no significant relationships found between PRs and any of the basic semen analysis parameters of concentration, motility or morphology using WHO standards for normal by chi square analysis. Individual semen parameters were also unable to predict IUI success using ROC analysis. Basic semen parameters had no predictive value for sperm survival in the advanced semen analysis. Good correlations (r=0.86; p<0.001) were noted with calculated TMC from the basic semen analysis when this value was >30 × 10^6 with having a processed TMC of 10 × 10^6 after the density gradient prep. However calculated TMC with values between 10 and 30 × 10^6 had poor correlations with the threshold level.

IVF results for those who to conceive with IUI were compared primarily by fertilization rates and secondarily by PR because most had at least some oocytes treated with ICSI which overcame their sperm problems. Unlike IUI procedures men with >10 × 10^6 processed TMC but <30% survival had normal fertilization rates in their IVF cycles. In men with above threshold TMC and survivals who failed to conceive with IUI 32% had poor or no zona binding in their IVF cycle but normal fertilization with ICSI oocytes. 19% of these same men had poor progressive motility with good zone binding but poor to no fertilization with their inseminated sperm. This group of men with good processed TMC and good 24 hour survival but no pregnancy from their IUI cycles are at high risk for a sperm function problem and should have ICSI when they move to IVF. ROC analysis suggested a cut off of processed TMC of 3 × 10^6 before decreased fertilization rates were observed with IVF. All of these sperm problems were alleviated if ICSI was used in the IVF cycle Pregnancy rates for the IVF cycles were not significantly affected by any of the sperm problems noted because ICSI was performed on at least some oocytes in most cycles and all cycles had appropriate numbers of embryos available for transfer.

Discussion

This study showed that both processed TMC and 24 hour sperm survivals are useful predictors of whether a couple should be treated with IUI. Those with processed TMC below 5 × 10^6 and those with 24 hour sperm survivals <30% are very unlikely to conceive with IUI Couples with processed TMC >10 × 10^6 and 24 hour sperm survivals >70% are excellent candidates for IUI. These measurements can be easily made by performing a pre-treatment advanced semen analysis which correlates well with samples used during the IUI. This could be an effective screening test for couples considering IUI and could help those with a poor prognosis move to IVF without doing multiple IUIs with little chance of success.

The strength of this study lies first in its large numbers. All of the couples had pre-treatment advanced semen analyses with determinations of processed TMC and 24 hour sperm survivals using the same sperm preparation used in the inseminations. The same lab and same physician performed all the analyses, sperm preps and inseminations every cycle used the same trans-vaginal ultrasound follicular monitonng protocol, hCG for insemination timing and the same sperm preparation. No women over age 36 were included in the study because female age would be a confounding variable [11]. Likewise no gonadotropin stimulated cycles were used as this has been shown to affect pregnancy rates. We also reported both first cycle and the multiple cycle pregnancy rates. Most studies only report multiple cycle pregnancy rates but this could bias the results because the less fertile couples have more cycles in the analysis.

Men who have severe sperm problems identified on their basic semen analysis are not good candidates for IUI. However we found that none of the basic semen parameters of concentration, motility or morphology was predictive of IUI success. We also found that calculated TMC made horn the basic semen analysis was not well correlated with having a processed TMC of >10 × 10^6 unless it was >30 × 10^6. Basic semen parameters had no predictive value for poor sperm survival in the advanced semen analysis.

The total number of sperm available for insemination and the survival of these sperm physiologically should be important variables for IUI success and much less of a factor for successful IVF. In IVF the sperm are placed directly on the oocytes and can be removed in 1-2 hours without affecting the fertilization rates. Smaller amounts of sperm are needed in IVF because the sperm can be concentrated around the oocyte. Both of these findings were confirmed in this study. In IUI the timing between the insemination and the oocyte release is highly variable so the number of functional sperm over time is important. The advanced semen analysis can provide the clinician with these important numbers.

There are several studies that have concluded that IUI is not an effective treatment in male factor infertility [12,13]. We used the standard definition of male factor infertility using the WHO criteria for normal semen parameters in this study. This is a very non-discriminating male factor definition and the majority of the couples in the study had processed TMC above the 10 × 10^6 threshold with this definition of male factor infertility. However the true value of the processed TMC lies in its ability to identify those unlikely to conceive with IUI. Poor 24 hour sperm survival identifies another group of men with poor conception chances with IUI. The identification of these two

groups could greatly help counselling these couples toward IVF. In IVF these couples would have normal conception rates.

One major limitation of this study is that we used only one sperm separation technique for both the pre-treatment sperm analysis and the insemination procedure. Different numbers for the processed TMC would be generated if a different sperm preparation was done. Two studies have looked at swim-up for their sperm separation technique and found that a lower cutoff for threshold TMC than found in our study [14,15].

Another study showed that a simple sperm wash produced similar IUI pregnancy rates when compared to discontinuous density gradient technique [16]. Since it is unclear if one sperm preparation technique is superior to another for IUI, if a clinic is using a different sperm preparation they would need to establish their own threshold values for TMC and establish the 24 hour sperm survival test for that preparation in their laboratory.

In conclusion, the value of the advanced semen analysis lies in its ability to discriminate between couples who are candidates for a trial of IUI and more importantly identifying those unlikely to benefit from this procedure. The two most important criteria for IUI success are the processed total motile sperm for the IUI and its 24 hour survival for timing the insemination. These are easily performed tests for most reproductive medicine laboratories.

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