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The evaluation of pre and post processing semen analysis parameters at the time of intrauterine insemination in couples diagnosed with male factor infertility and pregnancy rates based on stimulation agent. A retrospective cohort study

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Abstract

Objective—To identify pre or post processing semen analysis parameters that may be predictive of successful pregnancy in couples with male factor infertility undergoing intra uterine insemination (IUI). To evaluate the pregnancy rate based on ovulation inducing agent in couples with male factor infertility per the 2010 world health organization criteria treated with IUI.

Study Design—This retrospective study was performed at Stanford University medical center. All couples with male factor infertility fitting inclusion criteria were included over a 2 year period of time. 147 couples with male factor infertility were included and 356 IUIs were analyzed. All subjects in this study had Kruger strict analysis >4% normal forms. Logistic regression analysis was used to control for confounding effects and multiplicity.

Results—The overall pregnancy rate was 5.3%. No parameter in either the pre or post analysis predicted pregnancy. Furthermore, it was found that natural cycle and letrozole treatment had similar pregnancy rates (3% and 3%) $p=ns$. Similar outcomes were also observed between clomiphene citrate and gonadotropin stimulated cycles (7.5% and 6.0%) $p=ns$.

Conclusions—Total motile sperm count which has been found to be a predictor of pregnancy when evaluated in isolation, may be due to a confounding effect. These low pregnancy rates

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Note: the data was collected at the Stanford University Fertility Center

should be considered when deciding whether to suggest IUI and when selecting a protocol for ovulation induction for couples with male factor infertility.

Keywords

intrauterine insemination; IUI; male factor infertility; stimulation protocol; predictors of pregnancy; semen analysis

Introduction

If indicated couples are encouraged to complete three inseminations before beginning IVF treatment (1). Three inseminations prior to IVF treatments have been usually performed in couples where the cause of infertility was unexplained, due to unilateral tubal factor, mild endometriosis or mild male factor infertility. There are certain variables that are currently known to be predictive of IUI success, most of which relate to the female partner. Tomlison found that four IUI factors were most indicative of success: follicle number, endometrial thickness, duration of infertility, and sperm motility (2). Others found that the number of dominant follicles and endometrial thickness predict successful IUI although, no male factors were found to correlate with the treatment outcome (3). To date, there is only one accepted male semen analysis parameter, either from the pre or post processing analysis, which has been shown to be predictive of IUI outcome in couples with subfertility. That parameter total motile sperm count (TMSC) (4,5,6). However, TMSC has until now only been evaluated in isolation, failing to account for confounding effects.

The aim of this retrospective study is to examine the use of IUI and potentially identify pre or post processing semen analysis parameters that may be predictive of successful pregnancy from intrauterine insemination in couples diagnosed with male factor infertility and Kruger analysis ≥ 4 . Since semen is processed prior to insemination, it would make intuitive sense that post processing parameters may be more predictive of success than pre processed parameters. However, besides total motile sperm count, a PubMed search failed to identify any articles which evaluated any post processing semen analysis parameters. The probability of pregnancy with different stimulation agents in this population was also evaluated.

Material and Methods

All couples undergoing intrauterine insemination for a two year period at the Stanford university fertility center were retrospectively enrolled into our database for evaluation of a diagnosis of male factor infertility. Male factor infertility was diagnosed if the male had at least two pre-treatment semen analyses which were abnormal based on at least one 2010 WHO criteria and had never had a normal semen analysis (7). In this analysis 356 IUI were performed in couples which fit the criteria listed above for male factor infertility. Therefore, this amounted to 356 semen analyses analyzed in this study at the time of IUI on 147 different male patients. Fresh partner's semen specimens were included in the analyses. Donor frozen IUI semen results were not included because only post-processing parameters were available for these samples, and the donor was unlikely infertile.

The couples enrolled in this study had at least one year of primary or secondary infertility with their current partner. All couples underwent an evaluation including medical history and physical exam, documentation of ovulation or an evaluation for the lack of ovulation, and a semen analysis with Kruger strict morphology. If the first semen analysis was normal, the test was not repeated; otherwise, a second semen analysis was performed. If two semen analyses were abnormal, the couple was diagnosed with male factor infertility. If Kruger strict morphology was less than 4% on two samples, the patient was treated with in vitro fertilization and ICSI and, therefore, was not included in this study. All patients had at least one patent fallopian tube, demonstrated by either hysterosalpingogram or laparoscopy with chromopertubation. Ovulation was evaluated with basal body temperature charts, luteal phase progesterone greater than 3 ng/dl, urinary LH kits with regular cycles every 21 to 35 days, or regular cycles every 21 to 35 days where there was a history of premenstrual molimina.

Couples were excluded from analysis if they had two blocked fallopian tubes, decreased ovarian reserve, stage 3 or 4 endometriosis, recurrent pregnancy loss (2 or more miscarriages), had two previous ectopic pregnancies or they were anovulatory and folliculogenesis was not successfully induced. All women had serum prolactin levels and serum thyroid stimulating hormone (TSH) levels in the normal range of the assay used prior to starting treatment. Women were included in the analysis if they were anovulatory and ovulation could be induced, had serum follicle stimulating hormone levels less than 12 IU/L on basal and clomiphene citrate challenge testing (if performed), baseline follicle count was greater than 8 on transvaginal ultrasonography or had stage 1 or 2 endometriosis on laparoscopy with at least one patent and undamaged fallopian tube. All women were evaluated with hysterosalpingography or hysteroscopy and any intra-cavitary pathology including polyps, fibroids and synechiae were corrected prior to initiating treatment. Any patients with 4 or more myometrial fibroids of 1 cm or greater in diameter or one leiomyoma of 5 cm or greater in the uterine muscle had surgical resection and appropriate recovery prior to initiating the insemination cycle. Clomiphene citrate (50 or 100 mg daily) and letrozole (5mg daily) were administered orally for 5 days starting on cycle day 2 to 4. Gonadotropin injections were performed daily starting on cycle day 2 or 3 and titrated to develop 2 to 3 mature follicles in patients less than 40-years-old and 2 to 5 follicles in women greater than 40-years-old. Serial ultrasonography was performed to follow folliculogenesis per standard protocols.

For the purpose of semen collection, individuals were asked to refrain from ejaculating for two to four days prior to collection of the specimen. Specimens were produced with masturbation, either in a collection room adjacent to the laboratory or at the patients' homes. To be collected at the patient's home, it was required that the specimen be delivered within 30 minutes of production and that the specimen be kept warm by placement of the receptacle in the patient's axilla.

Freshly ejaculated sperm was allowed to liquefy before initial semen analysis. Liquefied semen was thoroughly mixed before an aliquot was placed on a standard count slide (Leja Products BV, Nieuw-Vennep, the Netherlands) for the pre-processing analysis. The slide

was placed on a 37°C stage of an IVOS computer-assisted semen analyzer (Hamilton Thorn Biosciences, Beverly, MA) and at least three random fields were evaluated for each analysis.

Following the initial semen analysis, the sample was processed by first placing up to 4 ml of raw semen on a differential density gradient column consisting of 1 ml of 40% Pure Sperm and 1 ml of 80% Pure Sperm (Nidacon, Molndol, Sweden). The gradient was centrifuged for 20 minutes at $350 \times g$ and subsequently, the 40% layer and the seminal plasma fraction were removed from the test tube, leaving the 80% layer undisturbed. Approximately 6–8 ml of sperm-washing medium plus 5% HAS (Cooper-Sage, Trumbull, CT) was added to the 80% layer and centrifuged for 10 minutes at $550 \times g$. The sperm pellet was then reconstituted to approximately 0.5 ml. The analysis of an aliquot of the processed sample was performed as previously described using the IVOS computer-assisted semen analyzer.

Intrauterine insemination was performed 24-hours after spontaneous urinary LH surge, or 36-hours after 10,000 IU β -hCG injection (Pregnyl, Merck, West Orange, NJ), (Novarel, Ferring Pharmaceuticals, Inc., Tarrytown, NY) or 250 mcg Ovidrel injection, (Merck-Serono Laboratories, Rockland, MD) and when trans-vaginal ultrasound revealed the largest follicle had a mean diameter of 18 mm. The insemination was performed in a sterile fashion, using a flexible plastic catheter with the patient in the dorsal lithotomy position. The patient did not assume a prone position for at least ten minutes after the end of the insemination.

Serum β -human chorionic gonadotropin (β -hCG) levels were analyzed 15 to 17 days after IUI to determine pregnancy status. A level greater than 5 mIU/ml was considered positive for pregnancy. However, most normal singleton pregnancies have levels in the range of 50 to 100 mIU/ml at this gestation. To conduct the analysis, blood samples were assayed on the Immulite 2500 (Diagnostic Products Corporation, Los Angeles, CA) for a quantitative measurement of β -hCG. The Immulite uses a solid-phase two-site chemoluminescent immunometric assay with a sensitivity of 1mIU/ml and a calibrated range to 5000mIU/ml. Intra- and inter-assay coefficients of variation were each less than 7%.

All statistical analyses were done using the statistical package for social sciences 16.0 (SPSS, Inc., Chicago, IL). Continuous variables were evaluated for normal distribution using the Kolmogorov-Smirnov test. Any variables which were not normally distributed were logarithmically transformed for the sake of analysis. Results are reported as mean value $\pm$ standard deviation (SD). Discriminators of pregnancy vs. no pregnancy among the pre and post processing semen analysis parameters were evaluated using logistic regression analysis, which controlled for confounding variables and multiplicity. Statistical significance was accepted as a two-sided $P = 0.05$. The Stanford University Committee for the Protection of Human Research Subjects approved this study. The authors declare that they have no conflict of interest.

Results

From 356 inseminations performed on 147 couples, the overall pregnancy rate was 5.3% (19 out of 356) of inseminations and 12.9% (19 out of 147) of couples. Three out of 94 times,

couples conceived with natural cycle insemination for a per cycle pregnancy rate of 3.2%. One out of 39 time couples conceived with letrozole for a per cycle pregnancy rate of 2.6%. Eight out of 107 times couples conceived with clomiphene citrate for a per cycle pregnancy rate of 7.5%. Seven out of 116 times couples conceived with gonadotropins for a per cycle pregnancy rate of 6.0%. As expected, when compared pregnancy rates did not differ for natural cycle and letrozole or gonadotropins and clomiphene citrate, $p=NS$ in both cases.

A comparison of female characteristics of subjects who conceived as compared to those who did not is presented in table I. It can be noted that those who conceived, did so after fewer insemination cycles than those who did not get pregnant, 2.0 ± 1.1 compared to 3.7 ± 4.0 respectively. Individuals who did not get pregnant also had thinner uterine linings, 8.3 ± 2.2 mm, at the time of hCG triggering as compared to those who conceived, 9.6 ± 3.4 mm. Otherwise the two groups did not significantly differ.

Using logistic regression analysis and controlling for confounding effects, the pre and post processing semen analysis parameters were compared in the pregnant and not pregnant groups (Table II). When comparing the semen analysis parameters between the two outcome groups, there was no significant difference with any parameter. Thus none of the pre or post processing semen analysis parameters considered in this study were found to be predictors of pregnancy in couples with identified male factor infertility undergoing intrauterine insemination.

Comments

The total pregnancy rate observed in this study, 5.3% cycles, is considerably lower than the traditionally quoted pregnancy rate for IUI of up to 20% (8,9,10). Couples should be made aware of their low chances when considering IUI as a treatment option for male factor infertility, even when the Kruger analysis is $>4\%$. The outcome of this study also reflected similar pregnancy rates for different types of stimulation during IUI, with comparable success rates between natural cycle (3%), and letrozole (3%), or clomiphene citrate (8%) and gonadotropin stimulation (6%). In this study, it has been demonstrated that letrozole was equivalent to not using an ovulation inducing agent for this patient population. However, this finding makes intuitive sense since letrozole often results in monofolliculogenesis reflected in extremely low multiple pregnancy rates (11). Therefore, letrozole should not be used in patients with male factor infertility. Moreover, the more costly gonadotropin stimulation led to a statistically similar, although lower pregnancy rate per treatment than the inexpensive clomiphene citrate, in patients with male-factor infertility. It should be noted that some of the patients who did not conceive with clomiphene would then have received treatment with gonadotropins, perhaps leading to a selection bias. However, the pregnancy rate of 6% with gonadotropins was very low and given the multiple gestation risk and significant cost with this medication (10,1,13), gonadotropin use for male factor infertility does not seem indicated. Therefore, if an ovulation inducing agent is to be used with insemination, clomiphene citrate is likely the medication to select.

The previously known correlation between endometrial thickness at time of insemination with a significantly higher pregnancy rate, was confirmed by these results (3). It should be

stated that the mean endometrial thickness was 8.3 ± 2.2 mm compared to 9.6 ± 3.4 mm when the groups that did not and did conceive were compared. This finding suggests that endometrial thickness remains important and that an 8 mm lining may impair likelihood of pregnancy not just a lining under 6 mm. However, it should be noted that at least one prospective performed on 168 couples undergoing ovarian stimulation with clomiphene citrate and IUI failed to find an association between ongoing pregnancy rate and endometrial thickness (14). In this study the authors concluded that No discriminative ability of endometrial thickness on the achievement of ongoing pregnancy could be shown by receiver operating characteristic curve analysis (14). It is possible that the discrepancy in this study with the one listed previously is due to the use of other ovulation inducing agents and natural cycle IUI in this analysis. More studies will be needed to further explain this inconsistency.

Patients did not have testing for sperm DNA fragmentation, given the current American society for reproductive medicine guidelines against ordering this test. These guidelines are based on the fact that the DNA fragmentation tests lack data from fertile populations and that the different assays give discordant results when used on the same specimen. The average age of the women analyzed were 38-years-old. This is consistent with the population treated in American fertility centers where more that 50% of couples are seen because the women has delayed child bearing until an older age. This may seem old for insemination since in-vitro fertilization may be offered based this maternal age. However, most patients are self pay and many cannot afford in-vitro fertilization. This remains the situation in most countries therefore, this data remains important. It should be noted that female age was not the factor which determined pregnancy because the mean age of the women who conceived and those who did not was exactly the same, and two samples did not differ statistically. In addition women treated were as young as 24-years-old.

No parameter from the pre or post processing semen analyses was found to be predictive of treatment outcome in the current study. In the context of male factor infertility, this could have been a powerful tool for the identification of couples who could be strong candidates for insemination and to allow couples with less favourable chances to move directly to IVF. However, the findings remain important that post processing semen analysis parameters are not more predictive of pregnancy than are pre processing semen analysis results in couples with male factor infertility. Total motile sperm count was not found to be a predictor of pregnancy. This is the first study to control for confounding effects which may explain why total motile sperm count was not found to be a predictor of pregnancy. Most importantly, irrelevant of parameters in these patients with an acceptable Kruger morphology, pregnancy rates remain low with insemination irrelevant of whether ovarian stimulation occurs or not, when male factor infertility is present. Although 357 inseminations cycles were evaluated, the pregnancy rate with male factor infertility was low in this study. It is possible that the sample size may not be enough to detect statistical significance for some parameters with small differences expected in the groups. Further studies will help elucidate the literature.

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References

1. The ESHRE Capri Workshop Group. Intrauterine Insemination. *Human Reprod Update*. 2009; 15(3):265–277.
2. Tomlison MJ, Amissah-Arthur JB, Thompson KA, Kasraie JL, Bentick B. Prognostic indicators for intrauterine insemination (IUI): statistical model for IUI success. *Hum Reprod*. 1996; 11(9):1892–1896. [PubMed: 8921060]
3. Farimani M, Amiri I. Analysis of prognostic factors for successful outcome in patients undergoing intrauterine insemination. *Acta Medica Iranica*. 2012; 45(2):101–106.
4. Guven S, Gunalp GS, Tekin Y. Factors influencing pregnancy rates in intrauterine insemination cycles. *J Reprod Med*. 2008; 53(4):257–265. [PubMed: 18472648]
5. Merviel P, Heraud MH, Grenier N, Lourdel E, Sanguinet P, Copin H. Predictive factors for pregnancy after intrauterine insemination (IUI): an analysis of 1038 cycles and a review of the literature. *Fertil Steril*. 2010; 93(1):79–88. [PubMed: 18996517]
6. Van Voorhis BJ, Barnett M, Saprks AE, Syrop CH, Rosenthal G, Dawson J. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and in vitro fertilization. *Fertil Steril*. 2001; 75(4):661–668. [PubMed: 11287015]
7. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th edition. Cambridge: Cambridge University Press; 2010.
8. Deaton JL, Gibson M, Blackmer KM, Nakajima ST, Badger GJ, Brumsted JR. A randomized, controlled trial of clomiphene citrate and intrauterine insemination in couples with unexplained infertility or surgically corrected endometriosis. *Fertil Steril*. 1990; 54(6):1083–1088. [PubMed: 2245833]
9. Demir B, Dilbaz B, Cinar O, Karadag B, Tasci Y, Kocak M, Dilbaz S, Goktolga U. Factors affecting pregnancy outcome of intrauterine insemination cycles in couples with favourable female characteristics. *J Obstet Gynaecol*. 2011; 31(5):420–423. [PubMed: 21627427]
10. Nyboe AA, Goossens V, Bhattacharya S, Ferraretti AP, Kupka MS, de Mouzon J, Nugren KG. European IVF-monitoring (EIM) Consortium for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology and intrauterine inseminations in Europe, 2005: results generated from European registers by ESHRE: ESHRE. The European IVF Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). *Hum Reprod*. 2009; 24(6):1267–1287. [PubMed: 19225009]
11. Mitwally MF, Biljan MM, Casper RF. Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation. *Am J Obstet Gynecol*. 2005; 192(2):381–386. [PubMed: 15695975]
12. Derom C, Derom R, Vlietinck R, Maes H, Van den Berghe H. Iatrogenic multiple pregnancies in East Flanders, Belgium. *Fertil Steril*. 1993; 60(3):493–496. [PubMed: 8375532]
13. Dodson WC, Hughes CL, Haney AF. Multiple pregnancies conceived with intrauterine insemination during superovulation: an evaluation of clinical characteristics and monitored parameters of conception cycles. *Am J Obstet Gynecol*. 1988; 159(2):382–385. [PubMed: 3136651]
14. Kolibianakis EM, Zikopoulos KA, Fatemi HM, Osmanagaoglu K, Evenpoel J, Van Steirteghem A, Devroey P. Endometrial thickness cannot predict ongoing pregnancy achievement in cycles stimulated with clomiphene citrate for intrauterine insemination. *Reprod Biomed Online*. 2004 Jan; 8(1):115–111. [PubMed: 14759299]

Table I

Comparison of the baseline characteristics of subjects who did or did not conceive a pregnancy with insemination, when male factor infertility was present.

	Pregnant N= 19	Not Pregnant N=128	p=
Maternal Age (years)	38±4	38±4	0.59
Duration of infertility (months)	25±19	28±23	0.58
Previous Pregnancies	1.2±1.4	0.8±1.2	0.18
Number of mature follicles	2.5±1.7	2.4±1.7	0.86
Lead follicle mean diameter at Triggering (mm)	17±4	18±3	0.17
Endometrial Thickness (mm)	9.6±3.4	8.3±2.2	0.03*
Mean Number of IUI cycles	2.0 ± 1.1	3.7±4.0	0.0001*
Max Day 3 FSH (mIU/ml)	7.6±2.8	8.4±5.2	0.65

Mean±standard deviation

*
p < 0.05.

Table 2

Comparison of the pre and post processing semen analysis parameters of subjects who did or did not conceive a pregnancy

	Pregnant (N=19)	Not pregnant (N=337)	p =
Initial Volume (ml)	3.4±1.9	3.1±1.8	0.74
Initial Concentration (M/ml)	46±35	44±35	0.33
Initial Percent Motile (%)	48±24	43±22	0.45
Initial Concentration Motile (M/ml)	27±30	24±28	0.17
Initial Total Motile Sperm Count (M)	75±34	58±29	0.43
Initial Progression (u/sec)	46±8	42±9	0.29
Initial Path Speed (u/sec)	76±14	73±18	0.65
Initial Linearity (0–100)	60±6	59±9	0.5
Initial Lateral Head Displacement (u)	3.2±0.6	3.2±2.6	0.2
Initial Velocity Average Path (u/sec)	54±9	50±11	0.62
Post Volume (ml)	0.5±0.00	0.5±0.05	0.41
Post Concentration (M/ml)	68±53	53±64	0.49
Post Percent Motile (%)	74±18	71±22	0.39
Post Concentration Motile (M/ml)	58±53	45±61	0.12
Post Total Motile Sperm Count (M)	25±48	19±54	0.33
Post Progression (u/sec)	67±11	61±14	0.62
Post Path Speed (u/sec)	113±16	109±28	0.21
Post Linearity (0–100)	59±6	56±8	0.9
Post Lateral Head Displacement (u)	4.5±0.8	4.5±1.2	0.22
Post Velocity Average Path (u/sec)	75±12	71±17	0.33

M=Million

Mean±standard deviation

*
p < 0.05