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The impact of semen processing on sperm parameters and pregnancy rates with intrauterine inseminations.

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Abstract

Objective: Semen processing is routinely performed prior to intrauterine insemination (IUI). The expectation is that semen processing improves semen parameters. However, no studies have evaluated the impact of semen processing for IUI on those parameters. This study aims to evaluate the impact of semen processing on quantitative measures of sperm function and the resultant changes in semen parameters on pregnancy rates.

Methods: A retrospective study was performed on the fresh non-donor semen used for infertile couples undergoing IUI during a two-year period. A total of 981 couples undergoing 2231 IUI cycles were evaluated and the freshly collected semen was analyzed before and after density gradient centrifugation.

Results: Most sperm parameters improved after laboratory processing, including significant increases in sperm concentration by 66 ± 74 mil/ml ($p=0.0001$), percentage motile by 24 ± 22 % ($p=0.0001$), concentration motile by 27 ± 58 mil/ml ($p=0.0001$), sperm progression by 18 ± 14 u/sec ($p=0.0001$). However, in 95% of cases, there was a decrease in total motile sperm counts (TMSC), with an average decrease of $50 \pm 124\%$ of pre processing results ($p=0.0001$). This decrease was as much as 100% of baseline. Importantly, the decrease in TMSC did not negatively affect pregnancy rates ($p=0.45$).

Conclusions: Semen processing led to significant increases in most sperm parameters, with the exception of total motile sperm count. Remarkably, the decrease in TMSC did not affect the pregnancy rate. This should reassure clinicians when the TMSC is negatively affected by processing.

Keywords

semen processing; density gradient centrifugation; intrauterine insemination; pregnancy rate

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Introduction:

Intrauterine insemination (IUI) is a minimally invasive, common strategy for the treatment of infertile couples (Ombelet, Puttemans et al. 1995, Montanaro Gauci, Kruger et al. 2001, Cohlen 2005, Ombelet 2005, Akanji Tijani and Bhattacharya 2010, Ombelet, Dhont et al. 2014). Several factors have been found to predict success at IUI including patient age, use of controlled ovarian hyper-stimulation, follicle number, endometrial thickness, and duration of infertility (Hendin, Falcone et al. 2000, Montanaro Gauci, Kruger et al. 2001, Van Voorhis, Barnett et al. 2001, van Rumste, Custers et al. 2008). Among semen specific characteristics forward progression, percentage motile, and total motile sperm count (TMSC) have been found to be important predictors of pregnancy (Hendin, Falcone et al. 2000, Montanaro Gauci, Kruger et al. 2001, Van Voorhis, Barnett et al. 2001, van Rumste, Custers et al. 2008). It should be noted that of semen parameters TMSC has consistently been found to be the best and most reliable predictor of pregnancy rate with IUI (Van Voorhis, Barnett et al. 2001, Akanji Tijani and Bhattacharya 2010, Kleppe, van Hooff et al. 2014, Ombelet, Dhont et al. 2014).

Prior to its use in IUI, semen must be processed to separate spermatozoa from seminal plasma and to remove unfavourable debris, prostaglandins, non-sperm cells, and dead spermatozoa (Ombelet, Dhont et al. 2014). The expectation is that processing improves semen by yielding a final preparation containing a high concentration of morphologically normal and motile cells. However, to date no studies have evaluated the impact of semen processing for IUI on sperm analysis measures. This study aims to evaluate the impact of semen processing on quantitative measures of sperm function and the resultant changes in semen parameters on pregnancy rates.

Materials and Methods:

A retrospective cohort study was performed on the semen analyses used in a total of 981 couples undergoing 2231 IUI cycles at the Stanford University Fertility Center over a two-year period. Freshly collected semen was analyzed, using a computer-assisted semen analyzer before and after density gradient centrifugation processing. The effect of processing on sperm concentration, percentage motile, concentration motile, total motile sperm count (TMSC), sperm progression, sperm linearity, lateral head displacement and average sperm velocity was analyzed. Data on volume was not compared since the post-processing volume is fixed to 0.5 ml and is not dependent on the processing procedure. Donor and frozen semen results were excluded because only post-processing parameters were available for these samples, and the donor was unlikely infertile.

The couples had at least one year of either primary or secondary infertility with their current partner. All couples underwent a comprehensive evaluation including medical history and physical examination, documentation of ovulation or an evaluation for the lack thereof, as well as a semen analysis using Kruger strict morphology. If Kruger strict morphology was less than 4% on two samples, the patient was excluded, since these patients were offered IVF. All patients had at least one patent fallopian tube on either hysterosalpingogram or laparoscopy with chromopertubation. Ovulation was evaluated with a luteal phase

progesterone > 3 ng/dl, basal body temperature charts, urinary LH kits with regular cycles every 21 to 35 days, or regular cycles every 21 to 35 days with a clear history of premenstrual molimina. 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.

Couples were excluded from analysis if they had stage 3 or 4 endometriosis, recurrent pregnancy loss (2 or more miscarriages), or two previous ectopic pregnancies. Women were included if they were anovulatory with inducible ovulation, if they had serum follicle stimulating hormone (FSH) levels < 12 IU/L on basal and clomiphene citrate challenge testing (if performed), a baseline follicle count of greater than 8 on endovaginal ultrasonography or stage 1-2 endometriosis on laparoscopy. 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 four or more intra-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.

Semen collection, analysis and processing:

Individuals were asked to refrain from ejaculation for two to four days prior to the collection of the specimen. Specimens were produced with masturbation, either in a collection room at the fertility clinic or at the patient's home. To be collected at home, the specimen had to be delivered within thirty minutes of production while being kept warm (e.g. by placement under the axilla).

Freshly ejaculated sperm was allowed to liquefy before 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). At least three random fields were evaluated for each analysis. Inter and intra assay coefficients of variability were under 10% for the parameters evaluated.

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 and 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 above using the IVOS computer-assisted semen analyzer.

Intrauterine insemination and beta-HCG assay

Intrauterine insemination was carried out 24 hours after spontaneous urinary LH surge, or 36 hours after 10,000 IU beta-HCG injection (Pregnyl, Merck, West Orange, NJ), (Novarel, Ferring Pharmaceuticals, Inc., Tarrytown, NY) or 250 mcg Ovidrel injection (Merck-Serono

Laboratories, Rockland, MD), administered when trans-vaginal ultrasound revealed the largest follicle had a mean diameter of at least 18 mm. A sterile flexible plastic catheter was used to conduct the insemination, with the patient in the dorsal lithotomy position. The patient remained supine for at least 10 min after the end of the insemination.

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

Statistical Analysis:

All statistical analyses were performed using the statistical package for social sciences 11.0 (SPSS, Inc., Chicago, IL). The pre-processing value, post-processing value, absolute change and percentage change in each sperm variable was calculated and presented as a mean $\pm$ standard deviation. Pearson correlation coefficients were performed to evaluate the impact of absolute change and percent change in each variable on the resultant biochemical pregnancy rate. Comparison of within patient means was performed by paired sample t-t tests. Data is presented as mean plus or minus standard deviations. Two sided p values are presented. The absolute change in semen parameters was calculated as the post processing value minus the pre processing value. The percent change was calculated as the post processing value minus the pre processing value. The result was then divided by the pre processing value and the sum of which was then multiplied by 100.

Ethical Approval:

The Stanford University Committee for the Protection of Human Research Subjects approved this study.

Results:

A total of 981 women underwent 2231 IUI cycles over a two-year period. The average number of cycles per patient was 2.3. The total number of pregnancies was 453, resulting in cycle pregnancy rate of 20%, and a couple pregnancy rate of 46%. The demographics of the group are presented in table 1.

There was a significant change in all sperm parameters after density gradient centrifugation ($p=0.0001$) (Table 2). Significant increases were seen in the average sperm concentration by 66 ± 74 mil/ml, percentage motile by 24 ± 22 %, concentration motile by 27 ± 58 mil/ml, sperm progression by 18 ± 14 u/sec, lateral head displacement by 1.4 ± 4.6 u, and average sperm velocity by 20 ± 17 u/sec. In 95% of cases, there was a decrease in TMSC after semen processing, average 50 ± 124 %. This decrease was as much as 100% of baseline (Table 3).

The correlation between the change in each parameter and the likelihood of pregnancy is presented a correlation coefficient in Table 4. The decrease in TMSC did not correlate with a change in the pregnancy rates ($p=0.45$). The absolute change in sperm concentration post-processing significantly correlated with the likelihood of pregnancy ($p=0.04$). The absolute change in linearity correlated with pregnancy ($p=0.006$) with an average decrease of 2 ± 16 ($P=0.0001$), with processing. The percentage change in path speed correlated with pregnancy ($P=0.022$).

To further evaluate the effect of processing in patients with male factor infertility we performed a subgroup analysis on subjects with an initial pre-processed total motile sperm count of less than 10 million. This was an analysis of 355 cycles. The results are presented in Table 5. Again, the change in TMSC did not correlate with pregnancy rates. Both the absolute and percentage change in linearity correlated with pregnancy, ($p=0.04$ and $p=0.02$, respectively).

Discussion:

To the best of our knowledge, this is the first study to evaluate the clinical significance of semen processing on sperm analysis measures as a predictor of pregnancy at IUI. By comparing semen analysis before and after processing we were able to quantify the change in each semen parameter. Indeed, processing significantly changed all sperm parameters studied. Improvements were seen in sperm concentration, percentage motile, concentration motile, sperm progression, lateral head displacement and average sperm. Conversely, we found semen processing resulted in significant decreases in TMSC. This decrease did not negatively effect pregnancy rates.

Semen processing techniques have long been an important part of semen preparation in both IUI and IVF (Goldenberg, Rabinovici et al. 1992). Semen processing is designed to yield the highest concentration of morphologically and functionally normal sperm. By filtering out white blood cells, bacteria and dead spermatozoa that produce oxygen radicals. Semen processing is designed to improve the ability of normal spermatozoa to fertilize the egg (Aitken and Fisher 1994, Parinaud, Le Lannou et al. 1997, De Jonge 2002).

Furthermore, unprepared semen risks causing pelvic inflammatory disease, endometritis, cervicitis and vaginitis, by introducing bacteris (Busolo, Zanchetta et al. 1984, Leiva, Peterson et al. 1985, Boomsma, Heineman et al. 2007) and likely creates a milieu which favors against pregnancy. These conditions can lead to an increased likelihood of miscarriage, and premature delivery (Busolo, Zanchetta et al. 1984, Leiva, Peterson et al. 1985, Boomsma, Heineman et al. 2007). When comparing the effectiveness of a different processing techniques a Cochrane meta-analysis of randomized controlled trials found no difference in pregnancy rates between swim-up versus density gradient centrifugation (DGC) or a basic wash (Boomsma, Heineman et al. 2007). However, Karamahmutoglu et al. found that in the “unexplained” infertility subgroup the DGC produced significantly higher pregnancy rates compared to a swim up technique (Karamahmutoglu, Erdem et al. 2014). They hypothesize that DGC resulted in a higher fertilization rate because it selects sperm with better DNA and chromatin structures (Karamahmutoglu, Erdem et al. 2014).

In this study, DGC was used on all samples. DGC involves overlaying the liquefied ejaculate on a column of layered density media to create a gradient, followed by low speed centrifugation. The most highly motile sperm traverse the gradient more rapidly and are recovered for use in IUI. However, the improvements in percentage motile, concentration motile, sperm progression, lateral head displacement, and average sperm velocity did not correlate with increased pregnancy rates at IUI. This is consistent with published literature that shows few semen variables consistently predict success at IUI (Ombelet, Dhont et al. 2014).

Of the semen parameters analyzed to date, TMSC has been among the most studied: likely because it is a composite measure representing the product of sperm motility and its concentration. Many published studies have been able to establish an association between TMSC and successful pregnancy with IUI (Van Voorhis, Barnett et al. 2001, Akanji Tijani and Bhattacharya 2010, Kleppe, van Hooff et al. 2014, Ombelet, Dhont et al. 2014). However, determining the optimal threshold of TMSC that is predictive of success in IUI has been challenging because isolating male factor infertility is nearly impossible (Duran, Morshedi et al. 2002). Further, the lack of standardization in interpreting semen analysis results, along with confounding factors such as the duration of infertility, female age, and methods of sperm preparation impede our ability to draw conclusions. (van der Westerlaken, Naaktgeboren et al. 1998, Duran, Morshedi et al. 2002, Ombelet, Dhont et al. 2014). Some studies suggest a minimum TMSC of 5 million in the post-washed ejaculate for IUI to be offered (Francavilla, Romano et al. 1990, Martinez, Bernardus et al. 1993, Huang, Lee et al. 1996, Dickey, Pyrzak et al. 1999, Khalil, Rasmussen et al. 2001, Badawy, Elnashar et al. 2009, Ombelet, Dhont et al. 2014), whereas others insist on at least 10 million (Van Voorhis, Barnett et al. 2001, Duran, Morshedi et al. 2002, Miller, Hollenbeck et al. 2002, Dorjpurev, Kuwahara et al. 2011). The systematic review by Ombelet et al. concludes that a post wash TMSC >1 million with IUI “is probably the best cost-effective treatment before starting IVF, irrespective of sperm morphology” (Ombelet, Dhont et al. 2014). However, a recent study by Kleppe et al. found the cumulative pregnancy rate after four cycles of IUI was not clinically different when comparing couple with at least one washed TMSC less than one million to couples consistently over one million (Kleppe, van Hooff et al. 2014).

Our study adds the understanding that in the majority of cases semen processing by DGC results in significant decreases in TMSC (average $50\% \pm 124\%$ decrease). Similar results, though not specifically addressed, can also be found in other papers which report on both pre and post processed semen samples (Zhao, Vlahos et al. 2004, Tan, Ha et al. 2014). For example, Tan et al. looked at total progressively motile sperm count (TPMSC) post DCG. They found TPMSC decreased by 56% after processing in both those who achieved pregnancy and those that did not. Our findings confirm that processing significantly decreases TMSC but that this decrease does not correlate with a decrease in pregnancy rates. Importantly, this result holds true in the male factor infertility subgroup.

The change in linearity was found to correlate with pregnancy rates in both the entire cohort and the male factor infertility subgroup. This was an unexpected result. The absolute decrease in linearity on average appeared to be minimal (-2 ± 16). In fact, linearity improved in only 30% of cases and decreased in the remaining 70% of cases. Future study

of linearity normograms and pregnancy rates pre and post processing could help to elucidate this relationship. Other studies should be performed to evaluate the value of linearity on pregnancy outcomes at IUI. It makes intuitive sense that sperm which loses its ability to travel in a linear manner would have difficulty to swim to the oocyte and fertilize.

A major strength of this study is the novelty of its design. No other project has specifically compared CASA semen parameters before and after processing. Further, we show for the first time that the significant decreases in TMSC post processing does not negatively correlate with pregnancy outcomes. Another key asset to this study is the fact that all semen samples were analyzed with computer assistance in the same laboratory over a relatively short period of time, ensuring standardization and reducing variation in the interpretation of results.

However, our study is not without limitations. Other than the drawbacks of its retrospective design, the DGC processing method is not universal. Outcomes seen here may not be useful when different methods of semen processing and sperm analysis are used. Another drawback to our study was the pregnancy rates per IUI cycle were slightly higher than expected based on the existing literature (Allen, Herbert et al. 1985, Ombelet, Puttemans et al. 1995). The high cycle pregnancy rates of 20%, is likely due to multiple factors such as the above average use of ovulation induction (93% of patients received ovulation induction) and the maintenance of the sperm specimen in a warming bath, up to the time of insemination. Further, by excluding semen with a Kruger strict morphology of less than 4% may have favored couples with greater potential for success at IUI. Unfortunately, the study design precludes the ability to analyze post-processing morphology at the time of IUI because evaluation of strict morphology kills the sperm and it is not commonly performed on IUI specimens.

Whether biochemical pregnancy or clinical pregnancy should be used to measure semen parameter related success is debatable. In our study, pregnancy rates were determined using serum B-hCG results, rather than with evidence of clinical pregnancy or live birth. However, it can be countered that semen capability is best measured in fertilization and biochemical pregnancy, while clinical pregnancy or live birth depends more on maternal age, uterine environment, and embryo developmental capacity. All these factors are sperm independent.

To conclude, semen processing prior to IUI leads to significant increases in most sperm measures, with the exception of total motile sperm count, which decreases in most cases. Remarkably, the decrease in TMSC was not associated with a decreased pregnancy rate. Therefore, our findings should reassure clinicians when the TMSC is negatively affected by semen processing.

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Table 1:

Patient Demographics

Demographic	Maternal Age (years)	Duration of infertility (months)	Previous Pregnancies	Number of mature follicles	Max Day 3 FSH (mIU/ml)
Mean $\pm$ SD	37 $\pm$ 6	24 $\pm$ 15	1.2 $\pm$ 1.1	2.4 $\pm$ 1.3	8.0 $\pm$ 2.5

Table 2:

Mean Absolute Change in Sperm Analysis Measures After Processing.

Parameter	Mean pre value $\pm$ SD	Mean post value $\pm$ SD	Change (Post-Pre)	Minimum Value	Maximum Value	Percentage with a positive change	P value of Change
Concentration (M/ml)	53 $\pm$ 43	69 $\pm$ 74	16 $\pm$ 55	–22	553	90%	0.0001
Percent Motile (%)	48 $\pm$ 22	72 $\pm$ 24	24 $\pm$ 22	–61	91	80%	0.0001
Concentration Motile (M/ml)	32 $\pm$ 40	59 $\pm$ 72	27 $\pm$ 58	–975	410	70%	0.0001
Total Motile Sperm Count (M)	85 $\pm$ 102	30 $\pm$ 39	–55 $\pm$ 81	–1244	153	<5%	0.0001
Progression (u/sec)	44 $\pm$ 9.5	63 $\pm$ 16	18 $\pm$ 14	–46	81	80%	0.0001
Path Speed (u/sec)	78 $\pm$ 33	113 $\pm$ 30	35 $\pm$ 37	–1141	186	95%	0.0001
Linearity (0-100)	58 $\pm$ 9	56 $\pm$ 15	–2 $\pm$ 16	–67	561	30%	0.0001
Lateral Head Displacement (u)	3.4 $\pm$ 2.5	4.8 $\pm$ 3.9	1.4 $\pm$ 4.6	–54	97	90%	0.0001
Velocity Average Path (u/sec)	53 $\pm$ 12	73 $\pm$ 19	20 $\pm$ 17	–54	108	80%	0.0001

Table 3:

Percentage Change in Sperm Analysis Measures.

Parameter	% change (post-pre/pre $\times$ 100)	Minimum Value	Maximum Value	Percentage with a positive change
Concentration (M/ml)	37 $\pm$ 119	-98	2081	95%
Percent Motile (%)	96 $\pm$ 206	-100	3700	80%
Concentration Motile (M/ml)	149 $\pm$ 310	-100	4770	70%
Total Motile Sperm Count (M)	-50 $\pm$ 124	-100	4766	5%
Progression (u/sec)	45 $\pm$ 42	-100	511	80%
Path Speed (u/sec)	55 $\pm$ 96	-100	2083	95%
Linearity (0-100)	3 $\pm$ 100	-100	2700	30%
Lateral Head Displacement (u)	52 $\pm$ 132	-100	3034	90%
Velocity Average Path (u/sec)	64 $\pm$ 557	-100	16250	80%

Table 4:

Relationship Between the Absolute and Percentage Change in Sperm Parameters and Pregnancy Rates as Measured by Correlations.

Parameter	Change (Post-Pre) r value	p value	% change ((post-pre)/pre) × 100) r value	p value
Concentration (M/ml)	0.043	0.04*	0.027	0.20
Percent Motile (%)	-0.012	0.56	-0.006	0.77
Concentration Motile (M/ml)	0.038	0.08	0.008	0.70
Total Motile Sperm Count (M)	-0.002	0.97	0.016	0.45
Progression (u/sec)	0.037	0.08	0.039	0.072
Path Speed (u/sec)	0.019	0.38	0.05	0.022*
Linearity (0-100)	0.06	0.006*	0.014	0.51
Lateral Head Displacement (u)	0.016	0.46	0.014	0.51
Velocity Average Path (u/sec)	0.19	0.38	0.017	0.44

Table 5:

Subgroup analysis in couples with initial TMSC less than 10 million. Relationship Between the Absolute and Percentage Change in Sperm Parameters and Pregnancy Rates as Measured by Correlations.

Parameter	Change (Post-Pre) r value	p value	% change ((post-pre)/pre) × 100) r value	p value
Concentration (M/ml)	0.006	0.91	0.064	0.23
Percent Motile (%)	-0.08	0.15	-0.08	0.88
Concentration Motile (M/ml)	-0.04	0.52	-0.006	0.91
Total Motile Sperm Count (M)	-0.003	0.95	-0.052	0.33
Progression (u/sec)	0.002	0.97	0.014	0.79
Path Speed (u/sec)	-0.09	0.11	-0.07	0.21
Linearity (0-100)	0.11	0.04*	0.13	0.02*
Lateral Head Displacement (u)	-0.03	0.65	-0.02	0.68
Velocity Average Path (u/sec)	-0.05	0.35	-0.07	0.22