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A low total motile sperm count in donor sperm obtained from commercial banks does not affect pregnancy rates from intrauterine insemination

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ABSTRACT

Objective: Women are often concerned about the absolute quantity and quality of sperm in a thawed donor sample at the time of intrauterine insemination (IUI). The aim of this study was to determine how the total motile sperm count (TMSC) of donor sperm obtained from commercial sperm banks affects the pregnancy rate after IUI.
Study design: We performed a retrospective cohort study including single women and women in same-sex relationships undergoing IUI at a single academic fertility center between January 2011 and March 2018. Our primary outcome was pregnancy rates per IUI cycle, stratified by post-washed TMSC. The data was analyzed according to TMSC and included three different groups: samples with a TMSC less than 5 million; TMSC of 5–10 million; and a TMSC greater than 10 million. Pregnancies were defined by a serum Beta-human chorionic gonadotropin (Beta-HCG) of greater than 5 mIU/mL. Chi-squared analyses and correlation coefficients were performed.
Results: Overall, 9341 IUIs were conducted during the study period. Of these, 1080 (11.56%) were performed for single women and women in a same-sex relationship using commercially available donor sperm. We found that there were no differences in the pregnancy rates per insemination based on TMSC. The pregnancy rates per cycle were 15/114 (13.3%) for the group with a TMSC of less than 5 million; 34/351(9.5%) with a TMSC of 5–10 million; and 61/609 (10.0%) for samples with a TMSC greater than 10 million ($p = 0.52$). We found an insignificant correlation ($r = -0.072$) between donor sperm TMSC and pregnancy after IUI ($p = 0.46$). Furthermore, a reassuring beta-HCG level (>100IU/L) drawn 16 days after IUI was unrelated to TMSC ($r = 0.0071$, $p = 0.94$).
Conclusion: The pregnancy rate following IUI is unaffected by the TMSC of commercially available donor sperm. This result is useful in reassuring patients when freshly thawed donor sperm is found to have a lower TMSC. Frozen sperm samples from commercial banks typically represent just a portion of an ejaculate produced by a donor who meets the banks' standards for age, health and sperm quality. As such, exaggerated sperm death caused by freezing does not result in worse outcomes with donor sperm.

1. Introduction

The successful use of cryopreserved sperm for artificial insemination was first demonstrated in 1953 [1]. Since then, intra-uterine insemination (IUI) with the use of frozen anonymous donor sperm has become a common mode of assisted reproduction for couples with various needs, such as same-sex couples, single parents or those with severe male factor infertility [2].

The use of frozen donor sperm is highly regulated in most jurisdictions. The selection and screening process for donors is designed to ensure high quality samples and prevent transmission of disease. Studies have shown that frozen donor sperm may be associated with decreased pregnancy rates compared to fresh donor samples [3]. Reasons behind this phenomenon include the use of cryoprotectants during the freezing process, which may reduce motility and viability of sperm, ultimately affecting pregnancy rates [4]. Another possible explanation includes the division of the specimen into multiple aliquots suitable for repetitive insemination, although this theory is mostly untested.

Multiple sperm parameters and their effect on pregnancy rates have been studied in the literature [5,6]. There is no consensus on an optimal

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total motile sperm count (TMSC) for IUI with cryopreserved sperm, as there have been multiple studies published with varying outcomes [5]. Donor sperm samples sold by commercial banks typically represent just a portion of an ejaculate produced by the donor who meets the bank's standards for age, health and minimum sperm quality, of which different sperm banks may still provide varying quantities and quality [7]. A study from Denmark showed that increasing the donor TMSC to greater than 19 million increased the likelihood of achieving a pregnancy [8]. Another study found that frozen donor sperm ejaculates containing at least 20 million sperm yielded pregnancy rates similar to fresh samples that contained higher concentrations of sperm [9]. However, these levels of TMSC are high compared to the standard 10 million upheld by many studies as required for best pregnancy rates at IUI with partner sperm and as such are suspect [10,11].

It is unclear if the TMSC in frozen donor sperm samples plays an independent role in influencing pregnancy rates. Women using donor sperm are often concerned about the absolute quantity and quality of sperm in a thawed donor sample at the time of IUI. Therefore, our aim was to determine if the TMSC of donor sperm obtained from commercial sperm banks affects the pregnancy rate after IUI and whether there is an optimal minimal threshold for TMSC in these specimens.

2. Materials and methods

We performed a retrospective cohort study of women undergoing IUI using donor sperm at a single academic fertility center between January 2011 and March 2018. This group included single women, women in same-sex relationships, and married women with azoospermic partners. Our primary outcome was pregnancy rate per IUI, stratified by post-washed TMSC. Commercially available donor sperm insemination cycles were included in the study. The data was analyzed according to three groups: samples with less than 5 million TMSC; 5–10 million TMSC; and greater than 10 million TMSC. All IUI cycles were analyzed regardless of prior fertility treatments. We included IUIs completed in unstimulated natural cycles as well as those using oral stimulation medications, which included clomiphene or letrozole, and gonadotropin-stimulated cycles. Doses used were clomiphene citrate 50 or 100 mg, letrozole 5 mg, and injectable FSH 50–75 IU daily.

This was a retrospective study, and as such patient consent was not required. The Institutional Review Board of McGill University Health Centre approved this study.

All patients had at least one patent fallopian tube. None of the subjects had stage 3 or 4 endometriosis, untreated uterine polyps or submucosal fibroids, or a male partner with sperm.

Exclusion criteria included women with cervical blockage or severely abnormal cervical physiology precluding IUI, women with Asherman's syndrome, women with severe maternal diseases (such as bleeding syndromes and severe autoimmune disease), and alcohol or drug addiction.

The protocol for IUI was previously reported in the study by Ruiter-Ligeti et al.[12]. In natural cycles, patients were screened for ovulation with a luteinizing hormone (LH) level and an ultrasound. If the serum LH was positive ($\geq$ 20 IU/L), the IUI was scheduled 24 h later. If a follicle greater than 17 mm in diameter was present and serum LH was negative (<20IU/L), the patient would receive a recombinant–HCG 250 mcg subcutaneous injection (Merk Serono, Canada) and the IUI would be scheduled 24–36 h later. Patients using clomiphene citrate or letrozole took their medication on days 2–6 of the cycle and had an ultrasound on day 10 to monitor follicular growth. Patients planning to use gonadotropins would have a baseline ultrasound on days 2 or 3 of the cycle. If the ultrasound was normal (endometrial thickness less than 5 mm and no ovarian cysts), the patient would begin gonadotropin stimulation and return for ultrasound monitoring one week later. If the leading follicle was at least 17 mm in diameter, the patient would receive hCG and the IUI would be scheduled 24–36 h later. Luteal support with vaginal progesterone was started the day after insemination for all gonadotropin-stimulated cycles (vaginal micronized progesterone 200 mg daily, various makers).

Cycles were canceled if: (a) an ovarian cyst or endometrial polyp were present at baseline scan, (b) if no dominant follicle was recruited, (c) if there was no sperm sample for insemination, (d) if the ovulation was missed, (e) if the patient requested cancelation for personal reasons, or (f) if the risk of multiple pregnancy was deemed to be too high based on the ASRM recommendations for ovulation induction [13]. Cancelled cycles were excluded from our analysis. Cycles were also excluded if they were converted to IVF.

Frozen donor sperm samples were received from three sperm banks: Repromed; Can-Am (Fairfax, Seattle Sperm Bank; Xytex); and Canada Cryobank. Samples were obtained with pre-freezing concentration and motility. All samples were thawed at room temperature for 15–20 min and then examined by the Makler microscope for concentration and motility post-thawing. Unwashed samples were additionally treated with a basic wash and density gradient 80% centrifugation (Origio Gradient 100 diluted to 80%: 40 mL of Origio Gradient 100 + 10 mL of Origio Sperm wash). Sperm was then layered on top of 1 mL of 80% gradient with a spin for 20 mins at 1600 rpm. The pellet was then transferred to another tube containing 2 mL of Origio Sperm Wash solution with a spin for 10 min at 900 rpm. The concentrated pellet was reconstituted to a volume of 0.5 mL with tubal media (Ferticult, Beemm, Belgium). Thereafter, concentration & motility were checked again on the mackler. All samples were inseminated into the uterus using a Cook catheter (Indiana, USA). Patients were instructed to ambulate immediately post-insemination or to remain recumbent if experiencing pain or at patient request. Pregnancy was defined as a positive serum beta-HCG test (> 5 mmol/L) taken 16 days after the IUI.

Table 1 -
Pregnancy rate per cycle in the three groups of total motile sperm count (TMSC).

Variable	< 5 million TMSC N = 114 (%)	5–10 million TMSC N = 351 (%)	> 10 million TMSC N = 609 (%)
Pregnancy rate per cycle	15 (13.3)	34 (9.5)	61 (10)

3. Calculation

Statistical analysis was performed with SPSS 23.0 (IBM corporation, Chicago, Il, USA). Data are presented as numbers and percentages or mean ± SD as appropriate. Chi-squared analyses and correlation coefficients were performed. P values < 0.05 were accepted as significant.

4. Results

There were 9341 IUIs conducted during the study period. Of these, 1080 were performed for women using commercially available donor sperm. Female age did not differ between the three groups of TMSC ($p > 0.05$). We found that there were no differences in the pregnancy rates per insemination based on TMSC. The pregnancy rates per cycle were 15/114 (13.3%) in the group with less than 5 million TMSC, 34/351 (9.5%) in the group with 5–10 million TMSC, and 61/609 (10.0%) in the group with greater than 10 million TMSC ($p = 0.52$) **(**Table 1**)**. We found an insignificant correlation ($r = -0.072$) between donor sperm TMSC and pregnancy after IUI ($p = 0.46$). Furthermore, a reassuring beta-HCG level (>100IU/L) drawn 16 days after IUI was unrelated to TMSC ($r = 0.0071$, $p = 0.94$).

5. Discussion

The use of donor sperm for IUI has become widely used in medical practice, as it is a simple yet effective method for achieving a pregnancy [2,14]. It is minimally invasive and remains an affordable first line treatment. Alongside an increasing prevalence of infertility and

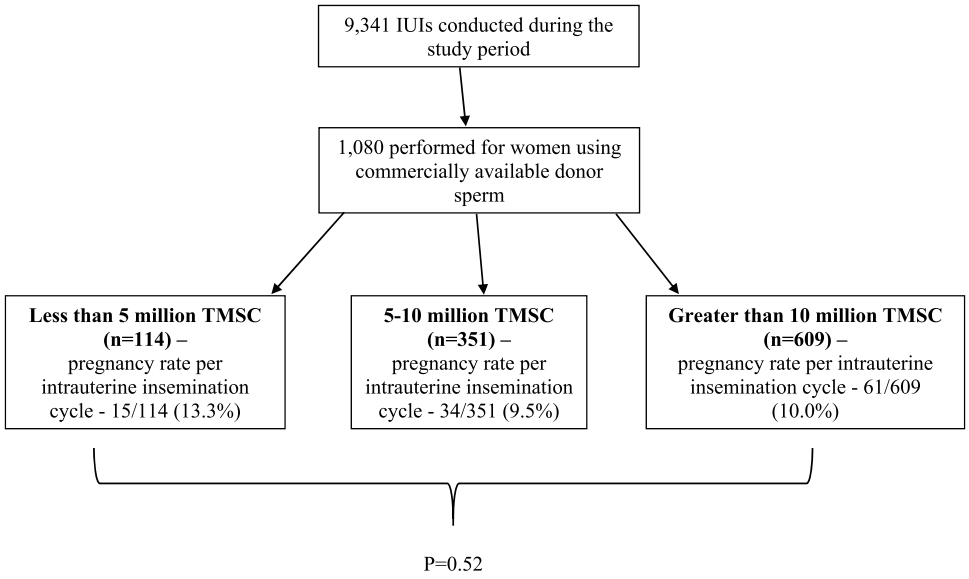

Fig. 1. Study population flowchart.

same-sex couples seeking parenthood, the demand for donor sperm continues to grow [15]. Not only are sperm donor banks closely regulated with numerous policies and standards of care in place, but it is also in their best interest to provide sperm of high quality to ensure an ongoing profit. As most sperm banks provide a guaranteed level of sperm quality with each donor sample, this has brought into question whether certain parameters of cryopreserved sperm may be more likely to produce higher pregnancy rates. As there is no consensus on an optimal TMSC for IUI with cryopreserved sperm, we aimed to address this issue.

In our study, the absolute TMSC in a thawed anonymous donor sample did not affect pregnancy rates. The success rate of donor insemination is reliant on numerous factors, such as maternal age, use of stimulated vs. unstimulated protocols, tubal patency and sperm quality [5]. Given these factors, the pregnancy rate per IUI cycle with donor sperm in published literature varies from 6.4% to 18% [4,16]. Despite the differences in TMSC, all three groups in our study had pregnancy rates within this range, suggesting that varying TMSC may not have a clinical effect on pregnancy rates in these patients. Similar findings have been seen in other studies demonstrating no differences in pregnancy rates per IUI cycle with donor sperm related to TMSC [5,17–19].

There have been studies showing differences in pregnancy rates when comparing lower ranges of total motile sperm count at < 5 million. A Chinese study performed by Dong et al. assessed pregnancy rates in women undergoing donor insemination with cryopreserved sperm and found that samples with a progressive motile sperm count (PMSC) ≤ 2 million had a significantly lower rate of pregnancies compared to groups of 2–4 million, 4–6 million, 6–8 million, 8–10 million and > 10 million [14]. There were no significant differences between any other groups of TMSC. In addition, Achard et al. reported that the TMSC after thawing donor sperm played an independent factor in increasing pregnancy rates when TMSC was > 1.5 million compared to < 1.5 million [20]. Given the groups in our study were stratified differently, our study possibly overlooked any significant differences that may be present at a lower TMSC, as seen in other studies. Therefore, in keeping with our conclusion, there are unlikely any major differences between sperm at TMSCs that are > 5 million. However, at lower concentrations this may not be the case. Of note, there remains contradictory evidence regarding the effect of donor TMSC on pregnancy rates following IUI, with several studies using donor sperm for IUI showing an improved pregnancy rate with a TMSC of over 10–20 million compared to lower TMSCs [8,9,21–23].

A major limitation of our study design is that variables such as medical history, BMI, and conception history were unavailable, thus our study represents all patients in an unselected population using cryopreserved donor sperm. Additionally, due to our study's retrospective design, we lacked data regarding female factors that may have contributed to infertility. Having said that, our cohort of patients included single women, women in same-sex relationships, and married women with azoospermic partners, making infertility due solely to female factors unlikely. Moreover, if female factors contributing to infertility did exist, one would expect these to be relatively similarly distributed between groups, and as such, to have little impact on results. We also did not have data regarding maternal race, maternal tobacco smoking, the rates of different stimulation drugs used in each group, nor the number of dominant follicles before HCG administration. These potential confounders were not controlled for in the comparison between the three groups and may have influenced the results. Given this heterogeneity within the study population, other factors may be responsible for differences seen in pregnancy rates. Another limitation is that our laboratory does not examine parameters such as rapidly progressive (after 30 min) or slow progressive sperm (after 1 h) post-thawing of frozen sperm, variables indicative of sperm quality that may affect pregnancy rates. Additionally, commercial banks do not report on sperm morphology of frozen donor sperm, although they do assess strict morphology pre-freeze on the donors and do not accept donors with male factor infertility. Furthermore, our outcome measure was biochemical evidence of pregnancy rather than live births or clinical pregnancy. However, pregnancy is more likely a good measure of sperm potential based on TMSC than are these other parameters, which are altered by multiple other factors such as uterine milieu and maternal age. Additionally, because we had data on pregnancy and not clinical pregnancy rates, multiple pregnancy data is missing.

A strength of this study is its sample size, which is moderately large.

6. Conclusions

In conclusion, our data demonstrates that the absolute total motile sperm count found in thawed donor sperm on the day of insemination does not affect pregnancy rates after IUI. This result is useful in reassuring patients when freshly thawed donor sperm may be found to have a lower TMSC. An exaggerated gamete mortality caused by freezing does not appear to result in worse outcomes with donor sperm. However, given the conflicting evidence currently available, more studies are needed to confirm these findings and further elucidate whether TMSC

influences pregnancy rates and outcomes..

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Declaration of Competing Interest

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References

[1] Bunge RG, Sherman JK. Fertilizing capacity of frozen human spermatozoa (In eng) Nature 1953;172(4382):767–8. https://doi.org/10.1038/172767b0.
[2] Zuzuarregui JL, Meseguer M, Garrido N, Simón C, Pellicer A, Remohí J. Parameters affecting the results in a program of artificial insemination with donor sperm. A 12-year retrospective review of more than 1800 cycles (In eng) J Assist Reprod Genet 2004;21(4):109–18. https://doi.org/10.1023/b:jarg.0000029494.55273.a2.
[3] Steinberger E, Smith KD. Artificial insemination with fresh or frozen semen. A comparative study. Jama 1973;223(7):778–83 (In eng).
[4] Botchan A, Hauser R, Gamzu R, Yogev L, Paz G, Yavetz H. Results of 6139 artificial insemination cycles with donor spermatozoa (In eng) Hum Reprod 2001;16(11):2298–304. https://doi.org/10.1093/humrep/16.11.2298.
[5] Williams RS, Alderman J. Predictors of success with the use of donor sperm (In eng) Am J Obstet Gynecol 2001;185(2):332–7. https://doi.org/10.1067/mob.2001.116733.
[6] Thijssen A, Creemers A, Van der Elst W, et al. Predictive factors influencing pregnancy rates after intrauterine insemination with frozen donor semen: a prospective cohort study (In eng) Reprod Biomed Online 2017;34(6):590–7. https://doi.org/10.1016/j.rbmo.2017.03.012.
[7] Allamaneni SS, Bandaranayake I, Agarwal A. Use of semen quality scores to predict pregnancy rates in couples undergoing intrauterine insemination with donor sperm (In eng) Fertil Steril 2004;82(3):606–11. https://doi.org/10.1016/j.fertnstert.2004.02.113.
[8] Carrell DT, Cartmill D, Jones KP, Hatasaka HH, Peterson CM. Prospective, randomized, blinded evaluation of donor semen quality provided by seven commercial sperm banks (In eng) Fertil Steril 2002;78(1):16–21. https://doi.org/10.1016/s0015-0282(02)03179-5.
[9] Keel BA, Webster BW. Semen analysis data from fresh and cryopreserved donor ejaculates: comparison of cryoprotectants and pregnancy rates (In eng) Fertil Steril 1989;52(1):100–5. https://doi.org/10.1016/s0015-0282(16)60797-5.
[10] Nielsen NC, Risum J, Bogaard Hansen K, Nissen U. Obtained pregnancies by AID using frozen semen in relation to specific qualities of the semen (In eng) Gynecol Obstet Invest 1984;18(3):147–51. https://doi.org/10.1159/000299070.
[11] Ruiter-Ligeti J, Dahan MH, Steiner N, Volodarsky-Perel A, Buckett W. Is intrauterine insemination a viable treatment option for women over 43 years old? An analysis by ovarian stimulation protocol and sperm source (In eng) J Assist Reprod Genet 2020. https://doi.org/10.1007/s10815-020-01976-3.
[12] Dong F, Sun Y, Su Y, Guo Y, Hu L, Wang F. Relationship between processed total motile sperm count of husband or donor semen and pregnancy outcome following intrauterine insemination (In eng) Syst Biol Reprod Med 2011;57(5):251–5. https://doi.org/10.3109/19396368.2011.603792.
[13] Van den Broeck U, Vandermeeren M, Vanderschueren D, Enzlin P, Demyttenaere K, D'Hooghe T. A systematic review of sperm donors: demographic characteristics, attitudes, motives and experiences of the process of sperm donation (In eng) Hum Reprod Update 2013;19(1):37–51. https://doi.org/10.1093/humupd/dms039.
[14] Sidhu RS, Sharma RK, Agarwal A. Effects of cryopreserved semen quality and timed intrauterine insemination on pregnancy rate and gender of offspring in a donor insemination program (In eng) J Assist Reprod Genet 1997;14(9):531–7. https://doi.org/10.1023/a:1021131511209.
[15] Cressman BE, Pace-Owens S, Pliego JF, Wincek TJ, Kuehl TJ. Effect of sperm dose on pregnancy rate from intrauterine insemination: a retrospective analysis. Tex Med 1996;92(12):74–9.
[16] Pittrof RU, Shaker A, Dean N, Bekir JS, Campbell S, Tan SL. Success of intrauterine insemination using cryopreserved donor sperm is related to the age of the woman and the number of preovulatory follicles (In eng) J Assist Reprod Genet 1996;13(4):310–4. https://doi.org/10.1007/bf02070144.
[17] Juan Liu JW, Wang BS, Liu Y, Li Q, Yu HZ. Effects of sperm-treated methods and total motile sperm count on pregnancy outcome. Chinese J Birth Health Hered 2006;33:877–83.
[18] Byrd W, Bradshaw K, Carr B, Edman C, Odom J, Ackerman G. A prospective randomized study of pregnancy rates following intrauterine and intracervical insemination using frozen donor sperm (In eng) Fertil Steril 1990;53(3):521–7. https://doi.org/10.1016/s0015-0282(16)53351-2.
[19] Kang BM, Wu TC. Effect of age on intrauterine insemination with frozen donor sperm (In eng) Obstet Gynecol 1996;88(1):93–8. https://doi.org/10.1016/0029-7844(96)00074-9.