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ORIGINAL ARTICLE

Intrauterine insemination with donor sperm: only the number of motile spermatozoa inseminated influences both pregnancy and live-birth rates

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Intrauterine insemination with donor sperm (IUI-D) is an assisted reproductive technology (ART) offered to couples with definitive male infertility or risk of genetic disease transmission. Here, we sought to evaluate our practice in IUI-D and identify factors that influenced the success rate. We performed a retrospective, single-center study of all IUI-D procedures performed at Lille University Medical Center (Lille, France) between January 1, 2007, and December 31, 2017. Single and multivariate analyses with a mixed logistic model were used to identify factors associated with clinical pregnancies and live births. We included 322 couples and 1179 IUI-D procedures. The clinical pregnancy rate was 23.5%, and the live birth rate was 18.9% per IUI-D. In a multivariate analysis, the women's age was negatively associated with the live birth rate. The number of motile spermatozoa inseminated was the only factor associated with both clinical pregnancies and live births, with a chosen threshold of 0.75 million. The clinical pregnancy and live birth rates were, respectively, 17.3% and 13.0% below the number of motile spermatozoa inseminated threshold and 25.9% and 21.0% at or above the threshold (all $P = 0.005$). The number of motile spermatozoa inseminated was the only factor that significantly influenced both pregnancies and live-birth rates after IUI-D. Indeed, below a threshold of 0.75 million motile spermatozoa inseminated, those rates were significantly lower. Application of this number of motile spermatozoa inseminated threshold may help centers to allocate donations more effectively while maintaining reasonable waiting times for patients.

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INTRODUCTION

Intrauterine insemination with donor sperm (IUI-D) is the most commonly used assisted reproductive technology (ART) for couples seeking a pregnancy in the context of definitive male infertility. The IUI-D procedure is simple, effective, inexpensive, and noninvasive. In the absence of gynecological problems, IUI-D, therefore, has a very good risk–benefit ratio.

The woman's age is known to be a predictive factor for pregnancy in ART and thus in IUI-D.[1–5] The existence of other factors influencing the outcome of IUI-D is subject to debate; although this topic has been studied intensively, the results are conflicting. Putative factors related to the recipient couple include the ovarian reserve,[2–4] the etiology of male infertility,[3] and the impact of previous pregnancies and deliveries.[1,2,4–6] Donor-related factors include sperm parameters before and after thawing,[4,7–12] impact of male's age,[13,14] and whether pregnancies have already been obtained with donation.[3] Finally, the characteristics and conditions of the attempt itself have been studied, *e.g.*, the use of ovarian stimulation or the need for a two-follicle response to obtain higher pregnancy rates.[2,15–17]

In the current study, we had analyzed procedure-, couple- and donor-related factors for IUI-D carried out at Lille University Medical Center (Lille, France) over the last ten years. The study's primary objective was to identify factors that significantly influenced the clinical pregnancy and/or live birth rates. The study's secondary objective (in the context of a lack of sperm donor and optimization of the cost-effectiveness of IUI-D) was to determine the minimum required number of motile spermatozoa inseminated (NMSI).

PARTICIPANTS AND METHODS

Study population

This study was approved by the Ethics Committee of Lille University Hospital (Lille, France; Reference DEC20150715-0002). All patients had given prior consent for the use of their clinical, hormonal, and ultrasound records. Data were collected retrospectively from medical records at Lille University Medical Center's sperm and oocyte bank, using JFIV software (version 1.8.3, RD Services, Langlade, France). For each couple, data on the reason for requesting donor sperm and the history of intracouple ART were collected, when appropriate. For each recipient woman, the age, body mass index (BMI), and ovarian reserve (assessed in the early follicular phase using the anti-Müllerian hormone [AMH], follicle-stimulating hormone [FSH], and estradiol [E2] levels) were noted. Serum AMH levels were measured using an

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ultrasensitive Enzyme-Linked ImmunoSorbent Assay (ELISA; AMH-EIA, Beckman Coulter, Villepinte, France), according to the supplier's instructions. Levels of FSH were assayed in an immunoassay provided by CISbio International (Saclay, France). E2 concentrations were measured in duplicate using RIA kits (Biomerieux, Marcy l'Etoile, France). We also collected information on previous clinical intrauterine pregnancies (although so-called biochemical and ectopic pregnancies were excluded) and births. For each sperm donor, his age and BMI at the time of donation and the date of the first pregnancy (if any) with his donation were recorded. For each IUI-D attempt, the rank, the use (or not) of ovarian stimulation, the triggering criteria (ovarian follicle ≥15 mm and the serum E2 level), the number of straws used, the NMSI, and the outcome were recorded.

Sperm cryopreservation

After liquefaction, each sperm sample was prepared and packaged into "high security" straws (CryoBioSystem, L'Aigle, France). Motility was manually assessed by microscopic examination between the slide and the coverslip. The spermatozoa were counted in a counting chamber. Each sample underwent microbiological testing to ensure the absence of infection or contamination. The remainder of the sample was mixed with a cryoprotectant, according to the supplier's recommendations (Sperm Freezing Medium, Medicult, Malov, Denmark; and Sperm Maintenance Medium, Irvine Scientific, Santa Anna, CA, USA), packaged in "high security" 300 µl straws, and identified with the donor code, sample code, current date, and a color. These straws were then thermally welded and frozen by exposure to nitrogen vapor (Freezal, Air Liquide, Paris, France; and Nanodigitcool, CryoBioSystem). At last, the straws were stored at −196°C in liquid nitrogen tanks.

Intrauterine insemination

IUI-D was performed after mono or pauci-follicular ovarian stimulation with gonadotropins. The goal was to develop one or two ovarian follicles. Ovarian stimulation was monitored with endovaginal pelvic ultrasound and serum assays for E2, luteinizing hormone (LH), and progesterone. Once the triggering criteria had been met (between 1 and 3 follicles greater than 15 mm in diameter, concordant estradiolemia [at least 150 pg ml^{-1} per mature follicle], and absence of a spontaneous LH surge), the decision to trigger ovulation was made at the daily staff meeting by gynecologists and biologists from the ART team. The patient self-injected a 250 µg bolus of recombinant human chorionic gonadotropin (hCG; Ovitrelle®, Merck-Serono, Geneva, Switzerland) at a specific time following the medical prescription. The IUI-D procedure took place 36 h to 38 h after the injection.

On the day of the IUI-D procedure, the couple went to collect the sperm straws from the sperm bank and brought them to the ART laboratory. The sperm were prepared in a sterile manner approximately one hour before the IUI-D procedure. After thawing, the spermatozoa were selected by density gradient (two layers; Puresperm®, Nicadon Mölndal, Sweden) and centrifugation. After a washing step, the pellet was concentrated to a volume of 250 µl. Next, the number and motility of the spermatozoa were evaluated to determine the NMSI (% progressive motility × sperm concentration after treatment × 0.25). Next, the sperm preparation was mounted in a flexible, sterile catheter (Elliocath®, Ellios Bio Tek, Paris, France) and transferred to a sterile syringe. After checking the identities of both members of the couple, the gynecologist introduced the catheter into the cervix and gently deposited the sperm preparation at the bottom of the uterine cavity. All women received luteal phase support with vaginal progesterone (200 mg twice daily, morning and evening), which was initiated on the evening of the IUI-D procedure.

Diagnosis of clinical pregnancy

We screened for clinical pregnancies 14 days after IUI-D. A clinical pregnancy was defined as a blood hCG level above 100 IU l^{-1} and was confirmed when a fetal heartbeat was detected on an ultrasound scan 6 to 8 weeks after the IUI-D procedure.

Inclusion

All IUI-D procedures carried out at the Lille University Medical Center between January 1, 2007, and December 31, 2017, were included in the study. The patients' follow-up was censured at delivery (if produced) or at the end of the IUI-D program.

Statistical analyses

Categorical variables were described by the number (percentage), and continuous variables were described by the median (interquartile range). The normality of the distribution was check graphically and using the Shapiro–Wilk test.

A generalized linear mixed model with a random effect for the patient (to take account of the correlation between a patient's IUI-D attempts) was performed to identify associated factors with the occurrence of clinical pregnancy. Variables associated at the level of 0.20 were introduced in a multivariate model. The same methodology was applied to highlight factors associated with the occurrence of live birth.

The predictive value of the NMSI was assessed using the area under the receiver-operating characteristics (ROC) curve (AUC) and the optimal threshold was determined by maximizing the Youden index. The sensitivity, the specificity, the positive predictive value (PPV), and the negative predictive value (NPV) of the thresholds were expressed with their 95% confidence intervals (CIs). The clinical pregnancy rate and the live birth rate were compared according to the threshold of 0.75 million of NMSI using a Chi-square test. A level of $P < 0.05$ was adopted for significance. Statistical testing was done at the two-tailed α level of 0.05. Data were analyzed using the SAS software package, release 9.4 (SAS Institute, Cary, NC, USA).

This retrospective study was registered with the French National Data Protection Commission (Commission nationale de l'informatique et des libertés, Paris, France).

RESULTS

We included 322 couples, of which 240 (74.5%), 60 (18.6%), and 22 (6.8%) had been referred for azoospermia, severe sperm alterations, and transmissible genetic disease, respectively. The 322 couples underwent a total of 1179 IUI-D procedures, of which 277 resulted in a clinical pregnancy (*i.e.*, giving a pregnancy rate per cycle of 23.5%). Two hundred and twenty-three cycles resulted in a live birth (including 13 sets of twins and one set of triplets), giving a live birth rate of 18.9% per cycle and a multiple pregnancy rate of 6.3% among live birth.

Samples from 179 different sperm donors had been used for the 1179 cycles. Eleven of the donors had not fathered any children before their donation. The donors' clinical characteristics are summarized in **Table 1**. In 933 attempts, the donor's straws had already been distributed and had resulted in one or more pregnancies after IUI-D.

The study couple's characteristics are summarized in **Table 1**. In our series, 93 couples had a history of ART (*in vitro* fertilization [IVF] with or without intra-cytoplasmic sperm injection [ICSI]). Each woman's

Table 1: Characteristics of intrauterine insemination attempt

Variable	*n*	*Overall population*
Women characteristic		
Age (year), mean±s.d.[a]	1179	33.1±4.2
BMI (kg m^{-2}), median (IQR)[a]	1006	24 (21–28)
AMH (pmol l^{-1}), median (IQR)[a]	1039	22.9 (13.9–39.1)
FSH on D3 (UI l^{-1}), median (IQR)[a]	1131	5.9 (4.9–7.2)
E2 on D3 (pg ml^{-1}), median (IQR)[a]	1119	36.0 (28.0–45.0)
Previous ART, *n* (%)[b]	322	93 (28.9)
Donor characteristic		
Age (year), mean±s.d.[c]	179	36.1±5.5
BMI (kg m^{-2}), median (IQR)[c]	179	24.7 (22.6–27.0)
Previous pregnancy with donation, *n* (%)[a]	1172	933 (79.6)
Ovarian stimulation and spermatic preparation[a]		
IUI cycles with follicles ≥15 mm on TD, *n*	1140	
IUI cycles with <2 follicles (≥15 mm on TD), *n* (%)		856 (75.1)
IUI cycles with ≥2 follicles (≥15 mm on TD), *n* (%)		284 (24.9)
E2 on TD (pg ml^{-1}), median (IQR)	1127	235 (171–340)
NMSI (million per 250 µl), median (IQR)	1132	1.25 (0.75–2.0)

[a]At IUI attempt level (total=1179); [b]at couple level (total=322); [c]at donor level (total=180). s.d.: standard deviation; BMI: body mass index; IQR: interquartile range; AMH: anti-Müllerian hormone; FSH: follicle-stimulating hormone; D3: day 3 of menstrual cycle; E2: estradiol; TD: triggering day; ART: assisted reproductive technology; NMSI: number of motile spermatozoa inseminated; IUI: intrauterine insemination

previous pregnancies and deliveries (if any) were updated after each attempt: there were 746 IUI-D procedures in women who never had an intrauterine pregnancy and 293 cycles in women who already had given birth to one or more children.

Associated factors with pregnancy

In univariate analyses (**Table 2**), a history of intracouple ART was associated with a poor prognosis for pregnancy rate with an odds ratio (OR) of 0.71 (95% CI: 0.51 to 0.99; $P = 0.044$), while a high NMSI significantly improves the chances of pregnancy (OR: 1.14, 95% CI: 1.04 to 1.25). Donor's characteristics were not significantly associated with pregnancy. In multivariate analysis, only the NMSI stay significantly associated with the occurrence of pregnancy ($P < 0.001$).

Associated factors with live birth

The woman's age was significantly negatively associated with the occurrence of a live birth. Indeed, the chances of having a live birth in our series fell by 40.0% every 10 years (OR: 0.63, 95% CI: 0.43 to 0.94; $P = 0.022$). Like for the occurrence of pregnancy, a history of intracouple ART and NMSI were significantly associated with live birth (OR: 0.61, 95% CI: 0.41 to 0.90; $P = 0.014$; and OR: 1.15, 95% CI: 1.04 to 1.26; $P = 0.007$, respectively). In multivariate analysis, the woman's age and the NMSI stay significantly associated with live rate (**Table 3**).

Threshold of NMSI

The predictive value of the NMSI was low for both outcomes, with an AUC of 0.55 (95% CI: 0.52 to 0.59) for the clinical pregnancies and 0.55 (95% CI: 0.51 to 0.60) for the live births (**Figure 1**). The threshold of NMSI appointed by the Youden index was 1 million for both clinical outcomes, with a sensitivity of 71.2% (95% CI: 65.4% to 76.5%), a specificity of 37.7% (95% CI: 34.5% to 41.1%), a PPV of 26.5% (95% CI: 23.3% to 29.8%), and an NPV of 80.6% (95% CI: 76.4% to 84.4%) for clinical pregnancies.

Using a lower threshold value of 0.75 million, the diagnostic values of NMSI for the clinical pregnancy were 83.8% (95% CI: 78.8% to 88.0%) for the sensitivity, 24.4% (95% CI: 21.6% to 27.4%) for the specificity, 25.9% (95% CI: 23.0% to 28.9%) for the PPV, and 82.7% (95% CI: 77.5% to 87.1%) for the NPV. Similar diagnostic values were found for the live births (data not shown).

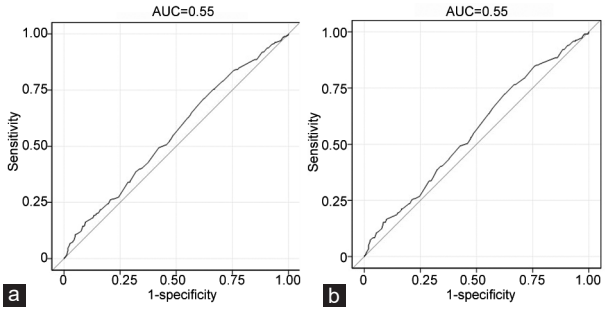

Figure 1: ROC curves and AUC for diagnostic values of the NMSI in (**a**) pregnancy rate and (**b**) live birth rate. ROC: receiver-operating characteristics; AUC: area under curve; NMSI: number of motile spermatozoa inseminated.

In our center, a couple could make up to six IUI-D attempts. We obtained a cumulative pregnancy rate of 64.7% for rank 4 and 79.4% for rank 6. The cumulative live birth rates were 56.3% for rank 4 and 69.4% for rank 6. At last, the cumulative clinical pregnancy and live birth rates according to NMSI <0.75 million versus NMSI ≥0.75 million are represented on the **Figure 2**. The cumulative pregnancy rate for rank 4 was 45.9% for procedures below the NMSI threshold and 70.2% for procedures above or at the NMSI threshold ($P = 0.0004$); for rank 6, the values were 69.2% and 82.1%, respectively ($P = 0.033$). The cumulative live birth rate for rank 4 was 36.7% below the NMSI threshold and 63.3% above or at the NMSI threshold ($P = 0.002$); for rank 6, the values were 56.9% and 73.8%, respectively ($P = 0.32$).

By regarding both women's age and NMSI threshold, we observed that cumulative pregnancy and live birth rates were higher when more than 0.75 million motile spermatozoa were inseminated despite of women's age (**Figure 3**).

DISCUSSION

The primary objective of the present study was to identify factors that influenced the outcome of IUI-D. The study's secondary objective was to optimize the distribution of donor sperm donation by setting an NMSI threshold.

The pregnancy rates per IUI-D cycle reported in the literature range from 6.1% to 28.1%, and the birth rates range from 5% to 22%.[1–15,18–28] In our study, the pregnancy rate per cycle was 23.5%, and the birth rate per cycle was 18.9%. Our rates are similar to the average values for France published in the French Federation of Sperm and Oocyte Banks' annual report for 2015 (24% for pregnancies and 18.6% for live births).

As mentioned above, our study included all IUI-D procedures performed at Lille University Medical Center between January 1, 2007, and December 31, 2017. All patients were stimulated with gonadotropins. Some researchers have demonstrated the superiority of ovarian stimulation with gonadotropins with regard to the IUI-D outcomes,[1,15,18] whereas others have not.[19,20] Stimulation should be moderate and well controlled, so as to avoid the risk of multiple pregnancies. In the present study, we considered that ovarian follicles with a diameter greater than 15 mm were mature. When two or more mature follicles were present, the pregnancy rate (27.5%) was not significantly higher than that for single follicles (22.4%; $P = 0.1$). This moderate, nonsignificant elevation in pregnancy rates during a two-follicle stimulation contrasts with the literature data.[4,7] This disparity might be due to the size above which the follicle is considered to be mature (from 16 mm to 20 mm in the literature).

Table 2: Associated factors with clinical pregnancy in univariate and multivariate analyses

Variable	*n*	*Univariate analysis*		*Multivariate analysis*	
		OR (95% CI)	*P*	*OR (95% CI)*	*P*
Women characteristic					
Age (year)	1179	0.73 (0.52–1.02)[c]	0.063	0.73 (0.51–1.05)	0.091
BMI (kg m^{-2})	1006	1.08 (0.93–1.25)[b]	0.33	–	
AMH	1039				
AMH <8 pmol l^{-1}		Ref			
AMH ≥8 pmol l^{-1}		1.22 (0.62–2.40)	0.57	–	
FSH on D3	1131				
FSH on D3 <10 UI l^{-1}		1.00 (0.55–1.79)	0.99	–	
FSH on D3 ≥10 UI l^{-1}		Ref			
E2 on D3 (pg ml^{-1})	1119	0.91 (0.83–1.00)[c]	0.052	0.91 (0.83–1.00)	0.052
Previous ART, yes versus no	1179	0.71 (0.51–0.99)	0.044	0.71 (0.51–0.99)	0.096
Previous pregnancy, yes versus no	1179	1.05 (0.79–1.40)	0.73	–	
Previous live birth, yes versus no	1179	1.08 (0.79–1.49)	0.62	–	
Donor characteristic					
Age (year)	1174	1.15 (0.90–1.47)[c]	0.27	–	
BMI (kg m^{-2})	1174	1.02 (0.83–1.26)[b]	0.85	–	
Previous pregnancy with donation[a]	1172	0.99 (0.64–1.26)	0.53	–	
Ovarian stimulation and spermatic preparation					
IUI cycles with follicles ≥15 mm on TD	1140				
IUI cycles with <2 follicles (≥15 mm on TD)		Ref		Ref	
IUI cycles with ≥2 follicles (≥15 mm on TD)		1.32 (0.97–1.80)	0.082	1.36 (0.98–1.89)	0.067
E2 on TD (pg ml^{-1})	1127	1.04 (0.97–1.13)[d]	0.27	–	
NMSI (million per 250 µl)	1132	1.14 (1.04–1.25)[a]	0.005	1.18 (1.07–1.29)	<0.001

[a]OR calculated for 1-unit increase; [b]OR calculated for 5-unit increase; [c]calculated for 10-unit increase; [d]calculated for 100-unit increase. Ref: reference; OR: odds ratio; CI: confidence interval; BMI: body mass index; AMH: anti-Müllerian hormone; FSH: follicle-stimulating hormone; D3: day 3 of menstrual cycle; E2: estradiol; TD: triggering day; ART: assisted reproductive technology; -: multivariate analysis not performed; IUI: intrauterine insemination; NMSI: number of motile spermatozoa inseminated

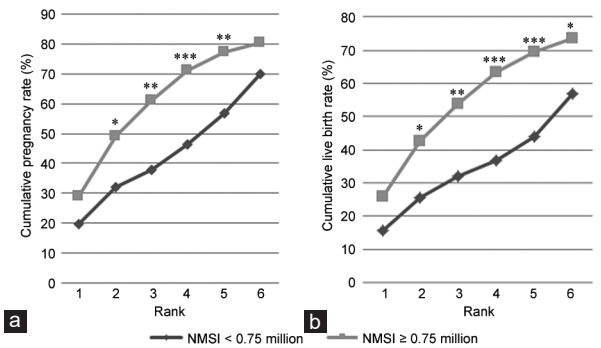

Figure 2 : Cumulative (**a**) pregnancy and (**b**) live birth rates in function of NMSI. The two curves of cumulative (**a**) pregnancy and (**b**) live birth rates are shown in order to compare IUI-D carried out above and under our NMSI's threshold. χ^2 test, $^{*}P < 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. NMSI: number of motile spermatozoa inseminated; IUI-D: intrauterine insemination with donor sperm.

In the present study, we recorded 13 twin pregnancies and 1 triplet pregnancy. Eight of the twin pregnancies occurred during a single-follicle cycle. The multiple pregnancy rate was 4.2% overall and 7.7% for multifollicular procedures. The relative increase in the twin pregnancy rate was not statistically significant and thus contrasts with literature reports.[4,7] The only triple pregnancy came from a cycle with three mature follicles. In our center, we rarely apply two-follicle stimulation for IUI-D procedures. Although the objective is to obtain a pregnancy, physicians and patients must be aware of the elevated risk of multiple pregnancy.

The women's age is a critical factor in ART. In the present study, the women's age was found to be negatively associated with live birth in both univariate and multivariate analyses; there was a 40.0% decrease

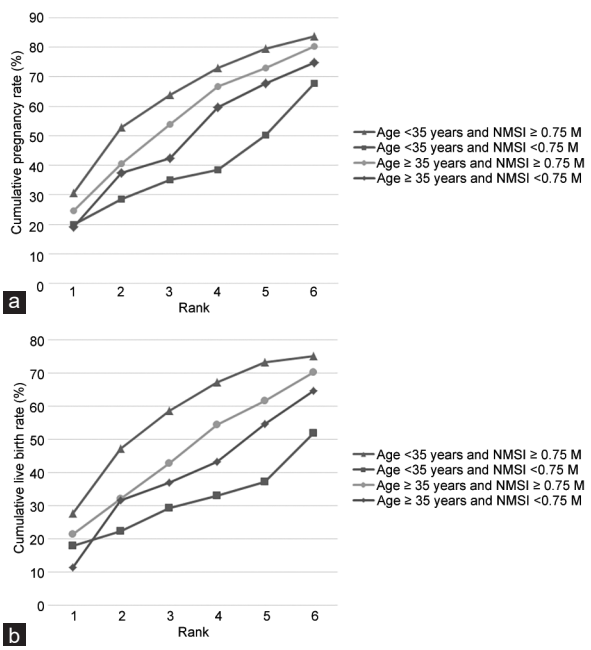

Figure 3: Cumulative (**a**) pregnancy and (**b**) live birth rates depending on women's age and NMSI. Four curves represents cumulative (**a**) pregnancy and (**b**) live birth rates in function of women's ages: younger than 35 years old or older; and also in function of NMSI: lower than 0.75 million or with at least 0.75 million motile spermatozoa inseminated. NMSI: number of motile spermatozoa inseminated; M: million.

in the likelihood of a live birth per 10-year age increment. It should be noted that our study included a small number of cycles (n = 44)

Table 3: Results of univariate and multivariate statistical analyses about IUI-D characteristics' effect on live-birth outcomes

Variable	*n*	*Univariate analysis*		*Multivariate analysis*	
		OR (95% CI)	*P*	*OR (95% CI)*	*P*
Women characteristic					
Age (year)	1179	0.63 (0.43–0.94)[c]	0.022	0.64 (0.41–0.99)	0.045
BMI (kg m^{-2})	1006	1.07 (0.90–1.29)[b]	0.44	–	
AMH	1039				
AMH <8 pmol l^{-1}		Ref			
AMH ≥8 pmol l^{-1}		1.19 (0.54–2.59)	0.67	–	
FSH on D3	1131				
FSH on D3 <10 UI l^{-1}		1.19 (0.58–2.45)	0.64	–	
FSH on D3 ≥10 UI l^{-1}		Ref			
E2 on D3 (pg ml^{-1})	1119	0.91 (0.81–1.02)[c]	0.097	0.90 (0.80–1.01)	0.072
Previous ART, yes versus no	1179	0.61 (0.41–0.90)	0.014	0.61 (0.41–0.90)	0.061
Previous pregnancy, yes versus no	1179	0.97 (0.70–1.35)	0.85	–	
Previous live birth, yes versus no	1179	1.17 (0.82–1.67)	0.39	–	
Donor characteristic					
Age (year)	1174	1.22 (0.93–1.61)[c]	0.16	1.23 (0.91–1.66)	0.18
BMI (kg m^{-2})	1174	0.98 (0.78–1.24)[b]	0.88	–	
Previous pregnancy with donation[a]	1172	0.83 (0.57–1.20)	0.32	–	
Ovarian stimulation and spermatic preparation					
IUI cycles with follicles ≥15 mm on TD	1140				
IUI cycles with <2 follicles (≥15 mm on TD)		Ref		Ref	
IUI cycles with ≥2 follicles (≥15 mm on TD)		1.30 (0.92–1.83)	0.14	1.26 (0.85–1.87)	0.25
E2 on TD (pg ml^{-1})	1127	1.08 (0.99–1.17)[d]	0.090	1.08 (0.98–1.19)	0.13
NMSI (million per 250 µl)	1132	1.15 (1.04–1.26)[a]	0.007	1.19 (1.07–1.32)	0.001

[a]OR calculated for 1-unit increase; [b]OR calculated for 5-unit increase; [c]calculated for 10-unit increase; [d]calculated for 100-unit increase. Ref: reference; OR: odds ratio; CI: confidence interval; BMI: body mass index; AMH: anti-Müllerian hormones; FSH: follicle-stimulating hormone; D3: day 3 of menstrual cycle; E2: estradiol; TD: triggering day; ART: assisted reproductive technology; –: multivariate analysis not performed; IUI: intrauterine insemination; IUI-D: intrauterine insemination with donor sperm; NMSI: number of motile spermatozoa inseminated

in women over the age of 40. In this small group, the pregnancy and birth rates were 18.2% and 13.6%, respectively, *i.e.*, 8 pregnancies and 6 births, two of which were twin births. Other researchers have reported on the decline in female fertility from the age of 38 onward.[4,7,15] The mean age was higher in women with a history of autologous ART (34.3 years) than that in those without (32.5 years). However, this difference was not statistically significant ($P = 0.37$). The lower pregnancy and birth rates for these couples cannot therefore solely be explained by the women's older age. The indication of IUI-D after the failure of IVF-ICSI is widely accepted in practice and in the literature.[3,7,21,22] Our present results and a number of other studies have shown that pregnancy and birth rates for IUI-D are lower after the failure of IVF-ICSI.[4,23] Hence, one can reasonably hypothesize that another factor for female infertility remains to be discovered in the partners of men with oligozoospermia – preventing them from compensating for sperm anomalies.[24] Nevertheless, IUI-D remains an option when IVF/ICSI with the male spouse's sperm has failed and the woman's fertility is not objectively impaired.

In the present study, the woman's characteristics (such as ovarian status [AMH, FSH, and E2 levels] and a history of pregnancies and deliveries) were not significantly related to the chances of pregnancy and birth after IUI-D. This could be explained by the fact that the women admitted to donor sperm programs also have etiologic infertility (endometriosis, tubal infertility and/or pelvic adhesion) and are directly steered toward IVF-ICSI with sperm donor. A recent study found that the AMH level does not predict the success of IUI-D.[25] Like our present results, these findings emphasize that AMH is irrelevant outside the context of controlled ovarian hyperstimulation. In our study, previous pregnancy or childbirth was not predictive of successful IUI-D. The literature data on this topic are contradictory.[1,4,6]

In line with most of the literature data, we found that the woman's BMI did not influence pregnancy or birth rates in IUI-D.[2,4] We also observed that donor age was not associated with the pregnancy and birth rates. This was probably due to the donor age limit of 45 imposed by the French legislation. A retrospective study of 2142 IUI-D cycles in women under 40 found that donor age of 45 was associated with lower pregnancy rates.[14] Other researchers have reported that along with a lower likelihood of pregnancy, advanced donor age is associated with an elevated risk of psychiatric diseases, polymalformative syndromes, and Down syndrome (trisomy 21) in the children.[5,13,14,26] We consider that these risks justify the age limit imposed on sperm donors in France. Moreover, in our study, the sperm donors' screening reveals the importance of sperm quality in achieving pregnancy in women over 35 years of age (**Figure 3**).

With regard to sperm parameters, our results differ markedly from the literature data.[9,11,27,28] In the present study, the most important factor influencing pregnancy and birth rates was the NMSI. This topic is subject to debate. Some researchers have found that pregnancy did not depend on the NMSI. However, the average NMSI in these studies was >10 million, >2 million and >1 million, respectively.[3,12,15] One study reported a significantly higher pregnancy rate for an NMSI >20 million.[16] An optimal NMSI of 1.5 million to 2 million was suggested in two retrospective studies of IUI-D cycles.[7,17] In the present study, the mean NMSI was 1.7 million, and 403 IUI-D procedures (34.2%) were performed with an NMSI below 1 million. Hence, the present study is one of the first to have evaluated results obtained with such a low NMSI.

In France, sperm donation is unpaid and voluntary; hence, donations must be optimized to meet the demand from infertile

patients. An optimal threshold of NMSI of 1 million was found in our study as in previous studies[27–29] and as recommended by the French National Guidelines on ART for IUI with couple sperm (IUI-C). However, the predictive value of the NMSI is low (AUC = 0.56, 95% CI: 0.52 to 0.59), so no threshold seems to be obvious. A threshold of 0.75 million of NMSI had a PPV and an NPV not significantly different from the threshold of 1 million (PPV of 0.75 million *vs* 1 million: 25.9% [95% CI: 23.0% to 28.9%] *vs* 26.5% [95% CI: 23.3% to 29.8%]; NPV of 0.75 million *vs* 1 million: 82.7% [95% CI: 77.5% to 87.1%] *vs* 80.6% [95% CI: 76.4% to 84.4%]). A study conducted in seven ART centers in France[29] showed that beyond 1 million, the NMSI had no influence on the pregnancy and birth rates following IUI-C.

Our results indicate that IUI-D and IUI-C differ with regard to the NMSI and the number of attempts before referral for IVF. In fact, women of infertile men in donor sperm programs have not usually ever been pregnant - with the exception of women with a history of intracouple ART attempts, which might explain the negative association for our IUI-D results. However, the gynecologists at our center tend to change their technical strategy rapidly and (in line with the French National Guidelines on ART) offer IVF after of 3 or 4 intracouple IUI procedures have failed. The gynecologists often apply the same pattern to IUI-D, although our results emphasize the clear benefit of carrying out a fifth and even a sixth attempt before turning to IVF-ICSI. This result is not in line with the literature data.[4,7,15] Given the difficulty, risks and cost of IVF-ICSI treatment and the absence of significantly higher associated pregnancy and birth rates, it makes sense to carry out all six IUI-D attempts.

Our study could comfort ART centers in optimizing each valuable sperm donation by lowering the esteemed number of mobile spermatozoa per straw. And, in countries where ART's cost is supported by patients, our results could help to reduce the cost of procedure using sperm donor by decreasing the number of straws needed to reach fair chances of success.

CONCLUSION

The NMSI was the only associated factor with both pregnancy and live birth after multivariate analysis. The woman's age was the second factor highlighted for live birth only. A threshold of 0.75 million of NMSI was found as efficient to provide good chances of pregnancy and live birth to patients. The use of this lower NMSI threshold than the one usually employed does not reduce the analytical performances of it to predict IUI-D success. That is why its application is relevant in the current context (a shortage of donations, and ever-increasing demand for IUI-D programs). And, our results may help ART centers to allocate donations more effectively while maintaining reasonable waiting times for patients.

AUTHOR CONTRIBUTIONS

MCL collected data and drafted the manuscript. BD conceived the study design and participated in its coordination. AU helped to collect data. HB performed the statistical analysis and helped to draft the manuscript. ALB participated in the study design and helped to draft the manuscript. GR conceived the study, and participated in its design and helped to draft the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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