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Original Research Article

Efficiency of Intrauterine Insemination with Respect to the Donor Age and Sperm Quality

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Abstract:

Introduction: Artificial insemination was initially used to help male-factor sub fertile couples. In 1978, “in-vitro fertilisation (IVF)” revived interest, leading to safer insemination methods. Implementing sperm preparation processes has improved sperm quality. Modern civilizations have 8%–12% infertility, with male factors playing a major role. The age of the female recipient and proper donor selection may improve donor sperm use.

Aim and objectives: The objective of this study is to evaluate the influence of donor age and sperm quality on the outcomes of “intrauterine insemination (IUI)”.

Method: There were 120 female and 120 male sperm donors in this prospective clinical trial for “intrauterine insemination (IUI)” to treat infertility. From July 2022 to May 2023, researchers looked at patient information, ovarian reserve, sperm donor traits, and IUI techniques. Before IUI treatments, sperm samples were thoroughly tested and cryopreserved, and clinical pregnancies were observed. The purpose of this research was to determine what factors influence clinical pregnancy and live birth outcomes after IUI-D.

Result: The study shows a positive link between pregnancy rates and populations over 0.75 million in numerous areas. The study found that the donor with less than 35 years and NMSI > 0.75 million had the highest pregnancy rate. The donors of more than 35 years old with lower NMSI had the lowest rate. These findings highlight age and NMSI as factors influencing pregnancy rates, though other factors can also affect fertility outcomes in assisted reproductive techniques.

Conclusion: The study indicated that NMSI and mother age significantly affect pregnancy and live births. A 750,000 NMSI threshold increases outcomes without impairing predictions.

Keywords: “Artificial insemination”, “intrauterine insemination (IUI)”, “in vitro fertilization (IVF)”, Donor insemination.

Introduction

Increasing the gamete density at the site of fertilisation was the goal of artificial insemination. Artificial insemination was first created to assist couples who were having trouble becoming pregnant due to severe medical or psychological male factor subfertility.

Nowadays, Male factor subfertility with a modest explanation is the most prevalent indication for artificial insemination using homologous semen. Donor insemination was common in the past century mostly utilised to treat inherited genetic illnesses associated with the Y chromosome in males and to treat azoospermia and an extremely low sperm count in males can prevent conception. Nowadays, women without a male partner are more likely to use donor insemination [1]. Without question, Steptoe and Edwards' invention of “in-vitro fertilisation (IVF)” in 1978 was the primary catalyst for the resurgence of interest in artificial insemination in humans. Early on, the husband's ejaculate was inseminated intrauterinally without preparation, which increased the risk of tubal infections and caused uterine cramping. Techniques for semen preparation were created with the advent of IVF, and IUI once again gained popularity due to it being more painless and safe [2].

Prostaglandins, pathogenic pathogens, and antigenic proteins must all be eliminated by these washing processes. The elimination of leucocytes and immature germ cells, which are nonmotile spermatozoa, is another significant benefit of these methods. Reducing during sperm preparation, the release of cytokines and/or lymphokines as well as the generation of free radicals may have a substantial impact on sperm quality. Techniques for during sperm preparation, it's critical to identify and select sperm cells that have intact genetic & functional features, like little DNA damage, normal cell shape, unharmed cell membranes, and functional binding characteristics [3].

The outcome is an improvement in the capacity of sperm to fertilise both in vitro in addition to in vivo, as well as an increase in the number of motile sperm with proper morphology near the site of fertilisation (Aitken and Clarkson, 1987). The period of conception is shortened by omitting the uterus, which acts as a sperm reservoir. The insemination is even more crucial [4].

The use of Sephadex columns, the mini Percoll, the intermittent Percoll gradient, and the swim-up methods (petite volumes) gradients method, and are the most often used methods. New techniques for sperm selection, such as those based on nonapoptotic sperm selection or sperm surface charging, exhibit encouraging outcomes [5].

A complicated condition with significant effects on psychology, the economy, demographics, and medicine is infertility. In modern civilizations, the prevalence of infertility has been estimated as being between 8% and 12%. Furthermore, it is now widely accepted that while half of the factors contributing to infertility are female (such as oocyte, tubal, uterine, cervical, and endocrine variables), and a further half are caused by male (e.g., poor spermatogenesis, a condition known as low-grade sperm, cancer, and genetic disorders). Considering that around 15% of those infertile males meet the WHO's criteria for normal sperm parameters, more diagnostic tools are required to accurately diagnose male factor infertility and predict the ART's (assisted reproductive technology) success [6].

Furthermore, sending couples with poor chances of success to in vitro fertilisation, improved patient selection for intrauterine insemination (IUI), which has benefits that may contribute to improved rates of effectiveness and a shorter time to conception. Conjugal insemination through the uterus is a straightforward and less invasive technique among the several Assisted Reproductive Technologies (ART) that, when properly monitored, may be carried out without the need for expensive infrastructure along with few hazards [7].

The smallest rate of delivery per cycle which may be found in the European register kept the European Society of Human Reproduction & Embryology, or ESHRE, is roughly 8%. Clinical justifications for IUI's use need to be improved, and they must be supported by sufficient stimulation of ovarian function, semen processing, and the integration of ovulation. The sole selection criterion for the male component is typically the amount of motile sperm cells. Although new sperm selection techniques have been created for IUI, the improvement in conception rates is not great. Additionally, several researches on sperm DNA fragmentation has found a link between a high frequency of DNA breakage and couples' poor reproductive outcomes who do IUI [8].

The first-line treatment for certain individuals with ejaculatory problems, minor male aspect, unsettled infertility, immunological infertility, cervical factor, and unresolved infertility is still intrauterine insemination (IUI), which is still affordable, non-invasive, and effective. As a second-line therapy, it is currently advised for some individuals with cervical factors & mild male factors. IUI methodology has largely stayed the same, but various improvements in stimulation regimens and IUI success rates have been improved by gonadotropins, sperm preparation techniques, and ultrasound monitoring that is encouraging [9].

The initial treatment step in assisted reproductive methods is intrauterine insemination (IUI), which is particularly suitable in situations of moderate Anovulation, endometriosis, a minimum of one patent tube, and unexplained infertility are all factors in male factor infertility. Among assisted reproductive technologies, IUI is recognised as the first-line method because of its ease of use, management convenience, affordability, & lack of potentially harmful side effects [10]. Even though semen quality, female cause, age, and body mass index, also known as BMI, are listed as factors impacting the chance of conception following IUI in the literature, there is no agreement on how much of an impact these factors have. In wealthy nations, 8% to 32% of couples (5–10 years old) who are of reproductive age have infertility, according to epidemiological research. Recent statistics for Spain show that 15% of childbearing couples had difficulties getting pregnant [11].

Although many couples benefit from utilising their male partners' sperm for insemination, numerous other groups, including monogamous women, same-sex couples, and marriages with a significant male element infertility, rely on donor sperm. The rate of success of IUI using donor sperm is frequently thought to be higher than IUI using partner sperm. There is research to back this up, however, surprisingly few studies have been done on the subject. In 6360 insemination cycles, according to Dong et al., clinical pregnancy rates with "husband" sperm insemination were 10.8% and 27.5%, respectively. According to some writers, "donor sperm has a higher quality" and "donor sperm quality is expected to be considered excellent, far exceeding the semen grade of the 'general' population," [12].

The reality that many women, who use donor sperm are not infertile themselves, such as those who have no male partner, may help to explain why donor sperm has a higher success rate. In addition, sperm. It is expected that donors will be fertile, as couples typically select donors according to their history of proven fertility. Paternal age has been found to play a factor in reproductive success, and typically, sperm donors for female patients are younger than their husbands who are male [13].

Many writers have stated that women who are forty years of age and older are the most critical age factor for IUI results age and over should start IVF immediately instead of attempting IUI. However, the cost is the first issue that late-reproductive-age women cope with and availability. IUI Age has a significant role in reproductive success, therefore it seems sense that doubts the benefits from donor sperm, as mentioned in the senior population as well as the aforementioned [14].

Method

Research design

This is a prospective clinical trial, conducted with 120 females and 120 male sperm donors who visited our hospital and underwent "intra-uterine insemination (IUI)" for the management of infertility. This study used demographic data, clinical features, hormonal levels or biochemical investigations and ultrasound characteristics of the female and male patients. The objective of this prospective study is to evaluate the efficacy of the Intra Uterine Insemination (IUI) with respect to the donor's age and quality of sperm in terms of Number of Motile Spermatozoa Inseminated (NMSI). "Intrauterine insemination IUI-D" has been conducted after mono or pauci-follicular ovarian stimulation with gonadotropins. The study encompassed all "intrauterine insemination with donor (IUI-D)" procedures conducted from July 2022 to May 2023.

The follow-up period was terminated either at the time of delivery or upon completion of the IUI-D programme. The research examined multiple variables, encompassing patient demographics, ovarian reserve, sperm donor attributes, and specifics of the IUI-D method. After the IUI-D, this study screened for clinical pregnancies within 14 days. In addition, this clinical pregnancy had described the blood hGG level above IUI-D as well and it had been confirmed that a fetal heartbeat had been seen by ultrasound scan 6 to 8 weeks after the IUI-D process. After mono or pauci-follicular ovarian stimulation with gonadotropins, IUI-D was conducted. The objective was to create one or two ovarian follicles. Ovarian stimulation was measured endovaginally. E2 and LH serum assays and pelvic ultrasound, LH, progesterone. Once triggering parameters were met (which included the presence of 1 to 3 follicles with a diameter exceeding 15 mm, estradiol levels of at least 150 pg ml^{-1} per mature follicle], and [Absence of a naturally occurring LH surge, ovulation was triggered. At the daily team meeting with ART gynaecologists. The patient self-injected 250 µg of recombinant human Chorionic Gonadotropins (rhCG) at a certain time after the medical prescription. The IUI-D occurred 36–38 hours after injection. On IUI-D day, the sperm samples were collected from the bank to the ART laboratory. Approximately sterile sperm were created. IUI-D process one hour before. Thawing spermatozoa were chosen by density gradient and centrifugation. The sperm preparation was then placed in a flexible, sterilised catheter. After verifying the couple's identity, the Gynaecologist gently inserted a catheter into the cervix and placed the sperm preparation at the uterine base. All women received 200 mg vaginal progesterone twice daily, morning and evening, for luteal phase support starting on. The researchers employed a generalised linear mixed model to ascertain the characteristics that are linked to clinical pregnancy and live birth. Through this research, each was prepared and packaged of each sperm same after liquefaction into "high security" straws. The spermatozoa were counted as well as each sample underwent for microbiological testing which provides the absence of infection. In addition, each sample had been combined with cryoprotectants. Each sperm sample was liquefied and then placed in "high security" straws before being packed. A manual microscopic examination of the space between the slide and the coverslip was used to determine motility. A counting chamber was used to tally the spermatozoa. Microbiological analyses confirmed the lack of infection or contamination in every sample. The remaining sample was cryoprotected as per the supplier's instructions, before being placed in "high security" 300 µl straws that were labelled with the donor's information, the sample's unique identification number, the current date, and a colour. After being exposed to nitrogen vapour, these straws were thermally welded and frozen. The straws were then placed in tanks of liquid nitrogen and kept at a temperature of 196°C.

Inclusion and Exclusion criteria

Inclusion criteria

- Patients who underwent IUI-D from July 2022 to the end of 2022 and were followed up within May 2023.
- It was confirmed that the infertility was due to the female factor.

Exclusion criteria

- The study omitted IVF, ICSI, and other ART techniques than IUI-D.
- Missing patient, procedure, or outcome data important to the study's aims.
- Biochemical and ectopic pregnancies were omitted from the analysis.
- The couples who had reproductive disorders, malnutrition, chronic infections or Sexually Transmitted Infections, were not excluded in this study, to prevent bias.

Statistical analysis

Data had been analyzed by employing the SPSS 25.0 software. The charts were created using MS Excel. Descriptive statistics were used for the variables, and then a generalised linear mixed model with random effects at the patient level was used to find causes of clinical pregnancy and live births. This study also used the Chi-square test in this study. Through the Chi-square test, this clinical pregnancy rate and the live birth rate had been described in relation as per the threshold of 0.75 million as well as it had also identified the significance level of P value< 0.05. Statistical testing had been accomplished at the two-tailed α level of 0.05.

Ethical approval

The authors have obtained consent forms from each patient (including male and female) and the study method was approved by the Ethical Committee of the concerned hospital.

Result

The features of intrauterine insemination attempts in the whole population of the study are presented in Table 1. The data represents essential features of female and donor participants in an ART or IVF trial. The female group has an average age of 31.52 years, a typical BMI of 23.55 kg/m², and varying AMH and FSH levels, as well as significant characteristics such as past ART history and pregnancy history. On the donor side, donors average 38.22 years old, have a similar BMI to women, and many have had previous pregnancies with donated sperm.

The data also covers ovarian stimulation and sperm preparation, including the percentage of IUI cycles with different follicle counts and E2 levels on the triggering day and the number of motile spermatozoa inseminated. This data snapshot may help analyse fertility treatment and ART outcomes.

Table 1: Characteristics of the patients in this study

Variable	Value
Female characteristic	
Age (year)	31.52 ± 5.69
BMI (kg m−2)	23.55 ± 3.15
AMH <8 pmol l−1	61 (50.83%)
AMH ≥8 pmol l−1	59 (49.16%)
FSH on D3 <10 UI l−1	66 (55%)
FSH on D3 ≥10 UI l−1	54 (45%)
E2 on D3 (pg/ml)	58.69 ± 26.39
History of Previous ART	12 (10.00%)
History of pregnancy	25 (20.83%)
Donor characteristic	
Age (year)	38.22 ± 3.74
BMI (kg m−2)	23.55 ± 3.15
Previous pregnancy with donated sperms	45 (37.50%)
Ovarian stimulation and spermatic preparation	
IUI cycles with <2 follicles (≥15 mm on Triggering day)	95 (%)
IUI cycles with ≥2 follicles (≥15 mm on Triggering day)	25 (%)
E2 on Triggering day (pg ml−1)	32 (%)
Number of Motile Spermatozoa Inseminated (million per 250 µl)	1.28

N/A: Not Apllicable; BMI (Body Mass Index in kg/m²), AMH (Anti-Müllerian Hormone) <8 pmol/l, AMH (Anti-Müllerian Hormone) ≥8 pmol/l, FSH (Follicle-Stimulating Hormone) on D3, FSH (Follicle-Stimulating Hormone) on D3 <10 UI/l, FSH (Follicle-Stimulating Hormone) on D3 ≥10 UI/l, E2 (Estradiol) on D3 (pg/ml), Previous ART (Assisted Reproductive Technology); IUI (Intra Uterine Insemination) Table 2 presents the results of an analysis aimed at identifying factors associated with clinical pregnancy in a univariate and multivariate context. Clinical pregnancy is a significant outcome in fertility and assisted reproductive contexts. In the univariate analysis, various factors related to female characteristics, donor characteristics, and aspects of ovarian stimulation and spermatic preparation were examined. The p-values indicate the statistical significance of each factor's association with clinical pregnancy. Some notable findings from the univariate analysis include, age of the female participant (p=0.063) and E2 levels on D3 (p=0.052) show a borderline significance in their association with clinical pregnancy. The number of motile spermatozoa inseminated (p=0.005) appears to be significantly associated with clinical pregnancy.

In the multivariate analysis, the factors with statistically significant associations with clinical pregnancy are determined after accounting for potential confounding variables. Overall, these results suggest that age, E2 levels on D3, and the number of motile spermatozoa inseminated are potential predictors of clinical pregnancy in this context.

Additional research with a larger sample size and more comprehensive analysis may be needed to confirm these findings and explore the impact of other variables not considered in the multivariate analysis.

Table 2: Analysis of the associated factors with clinical pregnancy in univariate and multivariate analyses

Variable	**p-value**	
	Univariate analysis	**Multivariate analysis**
Female characteristic		
Age (year)	0.063	0.091
BMI (kg m−2)	0.33	N/A
AMH <8 pmol l−1	0.57	N/A
AMH ≥8 pmol l−1		N/A
FSH on D3	0.99	N/A
FSH on D3 <10 UI l−1		N/A
FSH on D3 ≥10 UI l−1	N/A	N/A
E2 on D3 (pg ml−1)	0.052	0.052
Previous ART, yes versus no	0.044	0.096
Previous pregnancy, yes versus no	0.73	N/A
Previous live birth, yes versus no	0.62	N/A
Donor characteristic		
Age (year)	0.27	N/A
BMI (kg m−2)	0.85	N/A
Previous pregnancy with donationa	0.53	N/A
Ovarian stimulation and spermatic preparation		
IUI cycles with follicles ≥15 mm on TD	0.082	0.067
IUI cycles with <2 follicles (≥15 mm on TD)		
IUI cycles with ≥2 follicles (≥15 mm on TD)		
E2 on TD (pg ml−1)	0.27	N/A
Number of Motile Spermatozoa Inseminated (million per 250 µl)	0.005	<0.001

N/A: Not Apllicable; BMI (Body Mass Index in kg/m²), AMH (Anti-Müllerian Hormone) <8 pmol/l, AMH (Anti-Müllerian Hormone) ≥8 pmol/l, FSH (Follicle-Stimulating Hormone) on D3, FSH (Follicle-Stimulating Hormone) on D3 <10 UI/l, FSH (Follicle-Stimulating Hormone) on D3 ≥10 UI/l, E2 (Estradiol) on D3 (pg/ml), Previous ART (Assisted Reproductive Technology). The prevalence of pregnancy is shown to be higher in regions with a population of at least 0.75 million people in Figure 1. With a pregnancy rate of 19% for the region that came in first place and 70% for the region that came in sixth place. When compared to regions with a population of less than 0.75 million people. With a pregnancy rate ranging from 29% for the top-ranked region to 81% for the sixth-ranked region), regions with larger populations had higher birth rates.

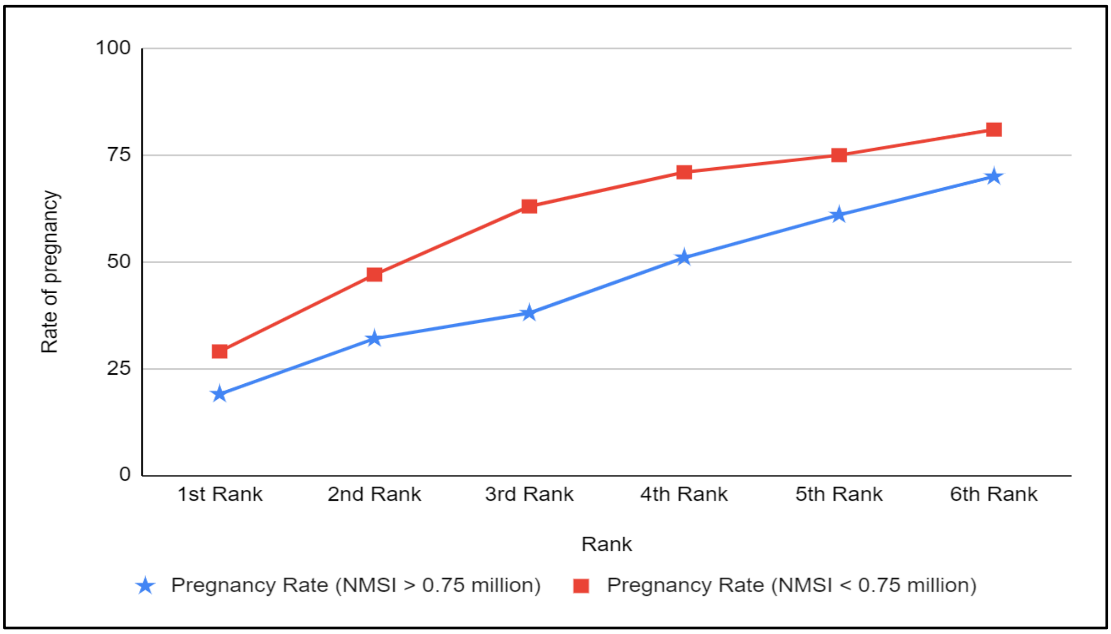

Figure 1: Pregnancy Rate obtained among patients with NMSI more than 0.75 mn and less than 0.75 mn

Figure 2 shows an overall comparison of pregnancy rates across four distinct groups of sperm donors, categorized by their age and the number of motile spermatozoa inseminated (NMSI). Firstly, it's noteworthy that the group with the highest pregnancy rate is the one consisting of sperm donors under the age of 35 years with an NMSI greater than 0.75 million (81 pregnancies). This group showcases the highest likelihood of achieving a successful pregnancy within the dataset. Next, the group of younger sperm donors (age <35 years) with an NMSI lower than 0.75 million still demonstrates a respectable pregnancy rate of 68 pregnancies. While this rate is slightly lower than the first group, it suggests that even with lower NMSI, younger age may positively impact pregnancy success. Conversely, when we examine the groups involving sperm donors aged 35 years or older, we see a difference in pregnancy rates based on NMSI. The group of older donors (age ≥ 35 years) with a higher NMSI of over 0.75 million has a pregnancy rate of 75. This indicates that while age may be a factor, a higher NMSI can mitigate some of the age-related impact on pregnancy rates. Finally, the group of older donors with an NMSI lower than 0.75 million has the lowest pregnancy rate among the four groups, with 60 pregnancies. This suggests that when both age and NMSI are less favorable, there is a reduced likelihood of achieving a successful pregnancy. In summary, this analysis reveals that younger sperm donors tend to have higher pregnancy rates, and a higher NMSI can help mitigate the age-related decline in pregnancy rates. However, it's essential to remember that pregnancy outcomes can be influenced by numerous factors, including female fertility and the specific assisted reproductive techniques used, so these findings should be considered within the broader context of fertility treatments.

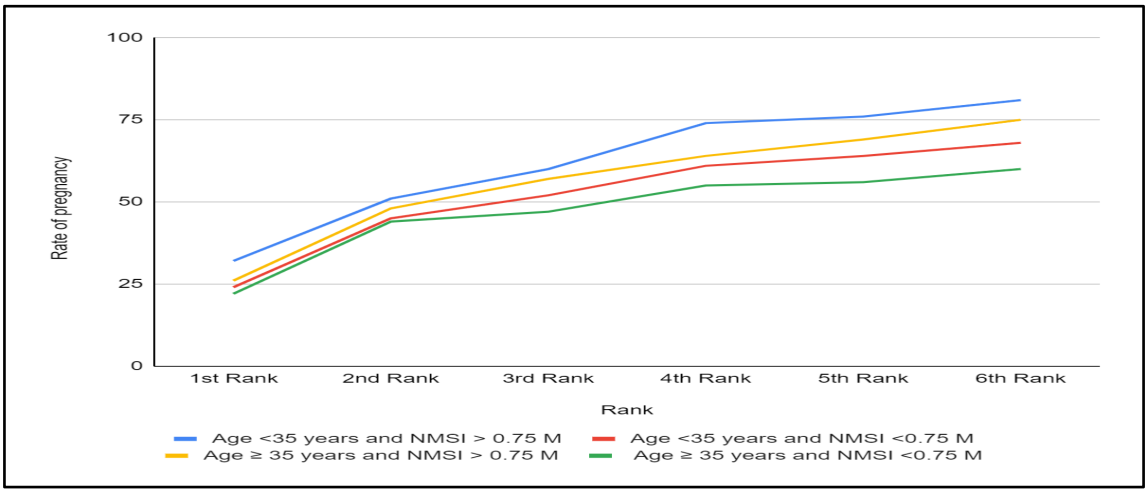

Figure 2: Pregnancy Rate obtained among patients with respect to the donor's age and NMSI

Discussion

The study sought to understand how a woman's age towards the end of her reproductive life affected the efficacy of IUI in combination with ovarian stimulation and IUI during natural cycles. Between January 2011 through March 2018, we conducted a retrospective cohort analysis of women they, during the time of, were no less than 43 years old IUI at a single academic infertility centre [15]. The main results were the each IUI cycle, a total of live births & pregnancies. Data is shown as a percentage or as the mean SD. Analyses using Fisher exact & chi-squared were carried out. Women 43 years of age & older should not utilise intrauterine insemination as a therapeutic option. This holds true whether donor sperm or ovarian stimulation is used. When compared with natural cycle IUIs, both expensive gonadotropin injections and oral medications did not enhance the likelihood of conception [16].

In intrauterine insemination (IUI) cycles, to assess the birth rates of partner & donor sperm in 38-year-old women and beyond. The results of another educational fertility clinic near Montreal, Canada, treated 944 women, ages 38 to 43, with 1596 IUI cycles between February 2009 and April 2018 Are shown were used in a retrospective cohort analysis. When compared to partner sperm, utilising donor sperm during IUI with regulated ovarian stimulation did not increase conception rates in women between the ages of 38 and 43 [17].

According to Tvrdá et al., 11 found that donor sperm has more stable chromatin and less DNA damage than control sperm. According to their research, donor sperm samples exhibit much-reduced DNA fragmentation than normozoospermic patient samples. While A study by Robinson et al.12 revealed that damaged sperm may effectively fertilise oocytes, increasing sperm DNA damage may increase the risk of miscarriage in the early stages of pregnancy. The conception rate with donor sperm was noticeably greater than "husband" sperm in the above-mentioned research by Dong et al.2 (27.5% vs 10.8%) [18]. in the study they conducted, women were on average 27.5 years old, whereas in the current inquiry, it was 27.5 years. Nevertheless, this was also the case with a much younger sample that we evaluated. the partner & donor groups' ages were 39.8 and 40.2 years, respectively. Given that age affects reproductive success, the current study concentrates on a completely diverse group [19]. Definitions of the For women, the Clinical pregnancy rate (CPR) and live birth rate (LBR) under the age of 40 having an induction of ovulation (OI)/intrauterine insemination (IUI) are the main goals of this research. The best chance of having a live delivery while utilising homologous sperm for women under 40 is through IVF [20]. OI/IUI may be examined for a maximum of four cycles using partner materials or for 6 cycles utilising donor sperm if IVF is not a possibility. OUI is probably ineffective for women over 45, however, a little trial may be thought of for psychological reasons while promoting adoption or donor oocyte IVF. In this age bracket, using gonadotropins does not seem to be any more beneficial than using oral medications [21].

In order to define the optimal conditions for achieving the greatest outcomes and to properly educate the individuals undergoing these operations about the possibility of becoming pregnant. Set out to determine the effect of all the variables regulating "artificial insemination (AI)" results. We highly advise ovarian stimulation instead of AI in elderly individuals. Nevertheless, in wanting to inseminate with the highest likelihood of success, we must get an acceptable number of sperm that have high motility [22].

The study sought to understand how a woman's age towards the end of her reproductive life affected the effectiveness of full ovarian stimulation in addition to "intrauterine insemination (IUI)" throughout a cycle. Women who are 43 years old or older should not utilise intrauterine insemination as a therapeutic option. This holds true whether donor sperm or ovarian stimulation is used. Compared to typical IUIs, both expensive gonadotropin injections and oral medications did not enhance the likelihood of conception [23].

To separate the motile, typical spermatozoa the development of Techniques for semen preparation for IUI and other assisted reproductive technologies. Oxygen radicals produced by leucocytes, bacteria, and decomposing spermatozoa impair egg fertilisation. The production of many motile, morphologically healthy spermatozoa may impact treatment options and consequently results [24]. To evaluate the effects of clinical results and semen parameters following intrauterine insemination (IUI) in subfertile couples using gradient, swim-up, wash, & centrifugation. The data is insufficient to endorse any particular preparation method. It is difficult to examine, in sizable, excellent randomised controlled studies, the impact on the clinical result resulting from the gradient, swim-up, rinse, & centrifugation operation. Additional randomised trials are necessary. Results of research assessing semen parameters may point to a predilection for the gradient method, but it is impossible to make definite conclusions without taking the constraints into account [25].

Conclusion

This study has concluded that the Number of Motile Spermatozoa Inseminated was the major factor related to both pregnancy and live birth, as evidence shown by the multivariate study. The age of the female (mother) was identified as the second significant factor exclusively associated with live birth. A threshold of 750,000 NMSI (Non-Motile Spermatozoa Index) was determined to be effective in increasing the likelihood of successful pregnancy and live births among patients. The utilisation of a lower NMSI threshold, as opposed to the typically employed threshold, does not compromise the analytical capabilities of predicting the effectiveness of IUI-D. Hence, the implementation of this technology holds significance within the present context, characterised by a scarcity of donors and a growing demand for IUI-D programmes. Furthermore, the outcomes of our study have the potential to assist “assisted reproductive technology (ART)”centres in optimising the allocation of donations in a manner that maximises effectiveness, all the while ensuring that patients experience reasonable waiting times. Overall, the findings of this study can be clinically important in the management of IUI-D.

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