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Does age of the sperm donor influence live birth outcome in assisted reproduction?

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Title:

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Running title:

Effect of sperm donor age on live birth outcome.

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Abstract:

STUDY QUESTION: Does age of the sperm donor have an effect on reproductive outcomes (live birth and miscarriage occurrence) of Donor Insemination or In-Vitro Fertilization treatment using donated sperms?

SUMMARY ANSWER: Live birth and miscarriage occurrence in assisted reproduction treatment using donor sperms was not found to be affected by the sperm donor age (recorded up to 45 years of age).

WHAT IS ALREADY KNOWN: Literature on the effect of sperm donor age on outcome of medically assisted reproduction (MAR) is scarce. Most researchers agree that semen parameters deteriorate with increasing paternal age. However, there is no substantial evidence to suggest that this deterioration adversely affects the reproductive outcomes in couples undergoing Medically Assisted Reproduction.

STUDY DESIGN, SIZE, DURATION: A retrospective cohort study analysing 46,078 first fresh In-vitro fertilization (IVF)/Intracytoplasmic sperm injection (ICSI) and Donor insemination (DI) treatment cycles using donated sperms from 1991 to 2012.

PARTICIPANTS/ DURATION/METHODS: The first fresh IVF/ICSI and DI treatment cycles (46,078 treatment cycles) using donated sperms from the UK's regulator, HFEA's long term anonymised data registry from 1991 to 2012 were analysed by the binary logistic modelling technique for association between sperm donor age and reproductive outcomes (live birth occurrence and miscarriage occurrence) .The statistical package SPSS (version 21) was used for analysis and results were considered to be statistically significant if the p value was <0.05.

MAIN RESULTS AND THE ROLE OF CHANCE: In our study, no evidence of declining likelihood of live birth with increasing sperm donor age was found. Results were not suggestive of any unfavourable effect of advancing sperm donor age on the odds of miscarriage occurrence.

LIMITATIONS, REASONS FOR CAUTION: As sperm donors are a select population based on good semen indices, the generalization of results to the paternal population at large may not be possible. Although study sub-groups were controlled for female age, treatment modality and effect of previous treatment cycles, adjustments for certain potential compounding factors like smoking status, BMI of women and stimulation protocol used for stimulation in IVF/ICSI treatment cycles were not possible.

WIDER IMPLICATIONS OF THE FINDINGS: Live birth and miscarriage occurrence following assisted reproduction weren't adversely affected by increasing sperm donor age up to 45 years. In view of the increasing demand for donor sperms, further studies may be required to ascertain the safe upper age limit for sperm donors.

STUDY FUNDING/COMPETING INTEREST(S): No funding was received from any individual or funding agency. NG was on a Commonwealth Scholarship for the duration of conducting the study. Authors do not have any conflicts of interest to declare.

Key words: Sperm donor age/ IVF/ ICSI/ DI/ insemination/live birth /miscarriage.

Introduction:

Infertility affects one in every seven couples in UK and in approximately 25% of these couples is due to factors in the male (National Institute of Clinical Excellence, 2013). Donated sperms may represent the only hope for some of these infertile couples to conceive. With ever-emerging societal changes in lifestyle and personal choices, single women and same sex female couples are also tapping into the resource of donor sperm to form a family. In the UK, 2013 saw a 30% rise of same sex female couples receiving treatment using donated sperm compared to 2012 (Human Fertilisation and Embryology Authority (HFEA), 2014). This increased demand for donated sperms has led to a steady increase in the number of imports of sperm from overseas sperm banks over the years. Overseas sperm donors constituted almost a third of newly registered sperm donors in 2013 (compared to 11% in 2005).

Although, as per current professional guidelines in the UK, men aged 41 years and over should not be accepted as sperm donors, the Human Fertilisation and Embryology Authority's (HFEA) egg and sperm donation statistics report, published in 2014, reveals that the majority of newly registered sperm donors in 2012-2013 were 26 or older with a quarter over 40 years of age (HFEA, 2014). The HFEA long term data registry (1991-2012) reveals that post anonymity removal (2005-2012), 60% of sperm donors were aged between 31-45

94 years whereas this figure was 28% before donor anonymity removal (1991-2004) (HFEA,
95 2013). In our fertility centre, a similar rising trend in sperm donor age was noted with mean
96 age (±SD) of 26.6 years (± 7.38) and 34.72 years (± 7.57) pre and post donor anonymity
97 removal respectively (unpublished data).
98 This raises certain issues in the minds of clinicians and couples alike concerning the effect of
99 sperm donor age on success rates of medically assisted reproduction (MAR) and the upper
100 age cut-off for these donors. There have been numerous studies (Dunson et al., 2002;
101 Noord-Zaadstra et al., 1991; Schwartz and Mayaux, 1982; Scott et al., 1995) in published
102 literature confirming the negative effect of increasing maternal age on female fertility and
103 outcome of MAR, but paternal age influence on these outcomes is less well researched and
104 contentious and still rarer is literature on the effect of sperm donor age on outcome of
105 medically assisted reproduction. Reports on the negative impact of increasing paternal age
106 on fertility outcome and child health (Robertshaw et al., 2013; de la Rochebrochard et al.,
107 2003) may cause anxiety for couples who are faced with limited donor choice and rising age
108 of the sperm donor. The majority of published literature has considered the effect of
109 paternal age in the general population or in IVF cases with ovum donation. Questions
110 concerning the effect of sperm donor age on success rates of assisted reproduction
111 techniques have largely remained unanswered.
112 Hence it is vital that we furnish information to couples based on evidence so as to help
113 them make an informed decision. In the UK, all centers offering donor sperm treatments are
114 registered with HFEA and the HFEA dataset provides a rich mine of raw anonymised data to
115 analyse these treatment outcomes. In this study, using the large, anonymised HFEA national
116 database collected over two decades, we set out to determine the effect of sperm donor
117 age on the chances of live birth occurrence in women undergoing medically assisted

reproduction treatment. Our aim was to answer the question: does the age of sperm donor affect the chances of success in women undergoing medically assisted reproduction?

Material and Methods:

Study population and participants:

In an attempt to find an answer to the above question we looked at the freely available HFEA long term anonymised data registry from 1991 to 2012 (HFEA, 2013).

The HFEA pipe delimited dataset was converted to an Excel spreadsheet format and was imported to MS Excel package 2007. Of the total 1,048,575 treatment cycles, the number of cycles using donated sperms was 237,852. Figure 1 depicts the breakdown of the number of the donor sperm treatments analysed following exclusions. Restricting the analysis to first cycles enabled us to alleviate the compounding effect of previous cycles and to report results as per individual woman rather than per cycle. To adjust for women's age, two groups were selected and separately analysed - women between 18-34 years with optimum reproductive potential and women > 37 years (i.e. 38-50 years) which is taken as the conventional cut off for decline of female fertility. An additional analysis was conducted to determine the influence of women at the farthest end of the age spectrum (45-50 years) whereby results for women in the 38-50 year and 38-44 year age brackets were compared (data provided upon request). As the results were no different, the whole group (38-50 year old women) was included in the final analysis. For entirety, women aged 35-37 years were also studied as a third group. Within these three groups, donor insemination (DI) treatment cycles and IVF/ICSI treatment cycles were analysed separately. In all the six groups, donor age categories were compared for live birth occurrence rates and occurrence of miscarriage. The categories chosen for sperm donor age (≤20, 21-25, 26-30, 31-35, 36-40 and 41-45)

were similar to those used by the HFEA for data collection. Live birth occurrence (one live birth occurrence is one birth event in which at least one baby is born alive) rather than actual numbers of live births was used as the output measure. Likewise miscarriage occurrence rather than actual number of miscarriages was analysed. HFEA data was also searched for congenital abnormalities at birth in all the study groups.

Exclusions:

The cycles with missing data for the woman or sperm donor age were excluded from the analysis. Treatment cycles involving gamete or zygote intra-fallopian transfer (GIFT, ZIFT), cycles, using egg donation, frozen embryos or surrogacy were excluded from the analysis. Out of 49,242 women undergoing first sperm donation treatment cycles 46,078 women were finally included in the live birth analysis (the remaining 3,164 women were excluded due to reasons explained in Figure 1). For the miscarriage analysis, a further 2,990 treatment cycles were excluded due to unrecorded data on early outcome (Supplementary data-Figure 1).

Statistical Analyses:

Live birth occurrence was taken as the dependent variable and donor age, in categorical fashion, was used as a covariate. As the outcome could take one of two qualitative categories (e.g. live birth occurrence or no live birth occurrence), the binary logistic modelling technique was employed to establish the strength and pattern of association between outcome and sperm donor age (Lewis-Beck, 1980). This modelling technique requires few distributional assumptions and is applicable with either continuous or discrete explanatory variables, or both. The statistical package SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) was used for analysis and results were considered to be statistically significant if the p value was <0.05. In an effort to

improve the transparency of the results, the odds ratio (OR) and 95% CI (confidence intervals) were also calculated. In this study, the binary logistic model expresses the odds of live birth occurrence as:

$$\left(\frac{p}{1-p}\right) = \exp(\theta + \beta_1 x_1 + \beta_2 x_2 + \cdots \beta_5 x_5)$$

p is the probability of a live birth occurrence.

$\beta_1, \beta_2, \ldots \beta_5$ and θ are unknown parameters that are estimated from the data.

For the categorical covariate, one category acts as the reference category – all other categories are compared to the reference category. In this study, donor age 41-45 years was taken as the reference level. As older sperm donors act as the reference category – the model enabled us to estimate the effect (on live birth occurrence) of being in a younger age group rather than the oldest age group. We hypothesise that a younger sperm donor would have an increased odds of live birth compared to an older donor assuming that increasing age has a similar effect on male fertility as on female fertility. Therefore, we decided to take older sperm donors as the reference category.

As the number of embryos transferred in each cycle may be an independent compounding factor, a one way ANOVA test was used to compare mean embryos transferred and embryos created per cycle in each donor age category. This test was conducted in all three female age subgroups undergoing IVF/ICSI treatment. If the overall ANOVA test proved significant, it was decided to use the multiple comparisons Dunnett's test to establish if each of the younger sperm donor age categories had a higher mean number compared to oldest (41-45 year old) category.

Similarly the relationship between donor age and the binary response "occurrence of miscarriage" was investigated. We investigated overall pregnancy loss combining biochemical pregnancy loss (after positive pregnancy test) and clinical pregnancy loss (after gestational sac has been demonstrated by ultrasound scan). Ectopic pregnancies, pregnancy terminations and heterotropic pregnancies were excluded from the analysis. The binary logistic modelling technique was again used. A wealth of medical literature proves that the likelihood of miscarriage increases if the woman is aged ≥35 years (Nybo Andersen et al. 2000; Heffner, 2004; Cleary-Goldman et al, 2005). Also there are studies in published literature (de La Rochebrochard and Thonneau, 2002; Kleinhaus et al., 2006) which indicate that increased paternal age is a risk factor for miscarriage therefore, as a corollary, we hypothesised that an older sperm donor would have an increased odds of miscarriage compared to a younger donor. Hence, here too the 41-45 years sperm donor age group was taken as the reference category for analysis.

HFEA data was also consulted for congenital abnormalities at birth in all six study groups.

Ethical Approval:

Formal ethical approval was not indicated as the freely available HFEA anonymised dataset was used for data procurement.

Results:

Of 46,078 women, 84.58% (N=38,974) underwent donor insemination (DI) treatment and the rest 15.42% (N=7,104) had IVF/ICSI treatment with donor sperms. In the donor insemination group, 66.51% (N=25,925) women were aged 18-34 years, 16.82% (N=6,559) were aged 35-37 years whilst the remaining 16.65% (N=6,490) were between 38-50 years of age. Similarly in the IVF/ICSI treatment group, 51.01% (N=3,624) of the women were in the

18-34 years age category, 19.10% (N=1,359) were aged 35-37 years and 29.85% (N=2,121) of the women belonged to the 38-50 years age category. The numbers of women in each sperm donor age category in all six study groups have been tabulated in supplementary table-1.

Mean embryos transferred per cycle in IVF/ICSI treatment group:

The mean number of embryos transferred per women was 1.86, 1.88 and 1.81 for women aged 18-34, 35-37 and 38-50 years respectively. Single embryo transfer was recorded in 17.85%, 17.44% and 16.78% women in these age brackets respectively. It was observed that the mean embryos transferred per cycle were higher in younger sperm donor categories than in the 41-45 years sperm donors (Supplementary Table 2). To assess whether number of embryos transferred were linked to number of embryos generated, we analysed the number of embryos created per cycle using a 1-way ANOVA. For all three women's age subgroups undergoing IVF/ICSI treatment there was no evidence of a difference between the sperm donor age categories as regards the mean number of embryos created (Supplementary Table 3).

Association of live birth occurrence and sperm donor age:

The overall live birth occurrence was 9.54% in the DI treatment group and 22.76% in women undergoing IVF/ICSI treatments. In the DI treatment group, the live birth occurrence was 11.07% in 18-34 year old women, 8.30% in 35-37 year old women and 4.70% in 38-50 year old women. The corresponding figures in the IVF/ICSI treatment group were 28.89%, 22.00% and 12.91% respectively. Figures 2 and 3 compare odds of having a live birth occurrence in the different sperm donor age categories for DI and IVF/ICSI treatment cycles for different women's age brackets.

When applying binary logistic regression modelling, we hypothesised that the odds of having a live birth would increase as the sperm donor age decreased (compared to the oldest donor age group) – however this pattern was not apparent. It was also observed that the difference in odds of live birth between each age category and the oldest donor age group was generally not statistically significant. Tables 1, 2 show the odds ratio for live birth occurrence among all the donor age categories in the DI and IVF/ICSI treatment groups respectively taking the odds of having a live birth occurrence in the reference sperm donor category (41-45 years) as one. By including "number of embryos transferred per cycle" as a covariate in the binary logistic regression model, we obtained adjusted estimates for the age categories: younger sperm donors were observed to have a lower odds ratio of live birth occurrence when compared to the oldest sperm donor age group and this was generally statistically significant in 18-34 year old women. The difference, generally, did not show statistical significance in 38-50 year old women (Supplementary data-Table 4).

Association of miscarriage occurrence and sperm donor age:

Overall, 1.50% women miscarried during their first donor insemination treatment cycle. When adjusted for female age, the miscarriage occurrence (i.e. number of miscarriages per 100 women commencing treatment) was 1.31% in 18-34 year old women, 1.86% in 35-37 year old women and 1.89% in 38-50 year old women undergoing donor insemination treatment. In the sperm donation IVF/ICSI treatment group, these figures were 6.58% (overall), 5.74%, 8.44% and 6.83% respectively. Miscarriage rate (i.e. number of miscarriages per 100 positive pregnancy tests) for 18-34 year old women was 10.55% in the DI group and 8.70% in IVF/ICSI treatment group. Corresponding figures in 35-37 year old women were 15.35% and 14.94% and in older women (38-50 years) were 23.24% and 18.50%.

The association between miscarriage occurrence and the age of sperm donor was explored using binary logistic regression. We hypothesised that the odds of having a miscarriage would be lower in the younger sperm donor age categories (vis-a-vis the oldest donor age group). For the donor insemination group, no significant difference was observed between each sperm donor age category and the oldest group as regards odds of miscarriage (Table 3). In the IVF/ICSI treatment group, following adjustment for "number of embryos transferred", the odds of miscarriage occurrence, was generally not significantly different for the various sperm donor age categories when compared to the oldest group (Table 4).

Association of congenital abnormalities at birth occurrence and sperm donor age:

Upon examination of the HFEA long term data registry, the congenital abnormalities if mentioned, were recorded in extremely low numbers, hence, a meaningful analysis was not feasible.

Discussion*:*

There is no consensus amongst the key professional bodies about the upper sperm donor age limit, with HFEA (UK) recommending <41 years (HFEA code of practice: guidance note 11), ASRM (USA) 40 years (Practice Committee of the American Society for Reproductive Medicine, and Practice Committee of the Society for Assisted Reproductive Technology., 2013), Human Reproduction Act (Australia), 45 years (Reproductive Technology Accreditation Committee., 2010) and ESHRE (Europe) recommending <50 years (ESHRE task force on ethics and law., 2002). This lack of consensus demonstrates the paucity of evidence to show linkage of increasing male age and reproductive outcomes. In our study, we report that there is no evidence to showcase any decline in the likelihood of live birth with

increasing sperm donor age. Adjusting for female age, treatment modality and alleviating the compounding effect of previous cycles it was found that each donor age category was not significantly different when compared to the oldest donor age group (up to 45 years of age) with respect to live birth and miscarriage occurrence. Interestingly, we found a higher number of embryos transferred per cycle in younger sperm donor categories when compared to older sperm donor categories (generally statistically significant), even though the number of embryos created per cycle was comparable in all the donor age categories. After adjusting for number of embryos transferred per cycle, the odds of live birth occurrence was in general, lower for younger sperm donors compared to older sperm donor age category in 18-34 year old women. A conceivable explanation for this observation (young sperm donors requiring more embryos to be transferred and lower live births compared to older sperm donors), may be attributed to the embryo quality or other unknown compounding factor unrecorded in the HFEA data. As data on embryo quality was not recorded in HFEA dataset, further exploration into this subject was not feasible and hence we refrain from making concrete conclusions on this issue and suggest the need for further studies.

Most researchers agree that semen parameters especially semen volume (de La Rochebrochard and Thonneau , 2005; Neme et al., 2007) and often sperm motility (Brian et al., 2011) deteriorate with increasing paternal age while sperm morphology and concentration largely remain unaffected (Frattarelli et al., 2008). However published literature is not in consensus about whether this deterioration in semen parameters translates into decreased clinical pregnancy rate in couples undergoing MAR (Brian et al., 2011; Duran et al., 2010).

Though there is paucity of published work evaluating association of sperm donor age and live birth rate, our study agrees with Paulson and co-workers (Paulson et al., 2001), Whitcomb and colleagues (Whitcomb et al., 2011) and Begueria and colleagues (Begueria et al., 2014) who observed no correlation between paternal age and pregnancy outcomes in ovum donation models. Similar to our study, all the three studies have examined live birth rate in proportion to all treatment cycles. The results of the present study are also in consensus with a study by Luna and co-workers (Luna et al., 2009) who observed no significant decrease in clinical pregnancy rates in couples with paternal age below 60 years using ovum donation models. Frattarelli and coworkers (Frattarelli et al., 2008) observed a decrease in live birth rate (among known pregnancies) after 50 years of paternal age in their study on donor oocyte treatment cycles although this study was not adjusted for recipient female age. Our study findings were different from the results of a study undertaken by Robertshaw and colleagues (Robertshaw et al., 2013) which demonstrates 26% lower odds of live birth rate with each 5-year increase in paternal age from 25 years of age, however small sample size was their major limitation. Another key feature that we need to take into account is that the studies listed above look at effect of paternal age and live birth outcome but do not specifically address the effect of sperm donors' age as sperm donors are likely to be a selective population based on their optimum sperm quality. Unpublished data from our centre shows only 1 in 5 enquiries progress on to become sperm donors.

No evidence of an increase in odds of miscarriage among the older sperm donors was suggested by our study. In our study we investigated miscarriage occurrence (miscarriages among all women undergoing treatment) rather than calculating miscarriage rate based on total number of pregnancies, as this approach is less prone to theoretical risk of over-estimation of miscarriage risk due to decrease in the probability of a pregnancy by

increasing age. Our study findings are in agreement with Begueria and colleagues (Begueria et al., 2014) who observed no difference in miscarriage occurrence among different male age groups using an ovum donation model. Ferreira and colleagues (Ferreira et al., 2010) also observed no influence of paternal age on miscarriage outcome, after making adjustments for maternal age, in couples undergoing ICSI treatment. However, the majority of published medical literature examines miscarriage rate in proportion to all known pregnancies. Our findings are in consensus with Luna and co-workers (Luna et al., 2009) who, in an ovum donation model, reported no statistically significant correlation between clinical pregnancy loss and increasing paternal age, whereby estimating pregnancy loss in proportion to number of clinical pregnancies. Similar results were observed by Andersen and colleagues in a Danish population based cohort study of spontaneous pregnancies (Nybo Andersen et al., 2004). The study results differ from work by Rochebrochard and Thonneau who, in a population based study examining spontaneous conceptions, have shown 6.73 (95% CI, 3.50–12.95) OR of miscarriage in couples where females were aged ≥35 years and male partners were aged 40-64 years, with couples having partners aged 20-29 years being used as the reference category (Rochebrochard et al., 2002). This study was not adjusted for the compounding effect of female age on miscarriage rate as couple age instead of male age was analysed. Likewise Slama et al. have shown a 1.26 times higher risk of spontaneous miscarriages (6-20 week pregnancy loss) if the paternal age was 35 years or above as compared to fathers aged less than 35 years (Slama et al., 2005). However, our findings suggest that a sperm donor aged 41-45 years does not have higher odds of miscarriage when compared to a younger sperm donor.

Zhu Jin Laing and co-workers (Zhu et al., 2005) in a population based cohort study on 71,937 couples found no association between paternal age and congenital malformations at birth.

These findings are also supported by other studies (Polednak., 1976 and Kazaura et al., 2004). Any positive relationship of trisomy 21 and advancing paternal age is conflict-ridden as it is observed by some (Stene et al., 1981) and refuted by others (Carothers et al., 1984; Martin and Rademaker., 1987; Hook and Regal., 1984). Martin and Rademaker demonstrated significant higher frequency of hyper haploid sperm complement in younger men as compared to older men thus indirectly showing negative evidence for a relationship between paternal age and numerical chromosomal abnormalities (Martin and Rademaker., 1987). Autosomal dominant disorders like anchondroplasia, Apert syndrome, Marfan syndrome etc. have been observed to be associated with increasing paternal age (Jones et al., 1975). Published literature has also linked increasing paternal age to neurocognitive disorders like autism, schizophrenia and bipolar disorders (Wiener-Megnazi et al., 2012). Plas et al., in a review study has recommended that the sperm donor age should be less than 50 years in consideration of increased risk of structural chromosomal abnormalities with advancing paternal age (Plas et al., 2000).In the present study, as very few congenital abnormalities recorded were in the HFEA data (the number was nil in many subgroups), an evocative analysis in this regard was not possible. Exploration of association of autosomal dominant diseases and neurocognitive disorders is beyond the scope of this study.

Alio and co-workers (Alio et al., 2012) have observed increased risk of stillbirth, preterm births and low birth weights in infants born to fathers greater than 45 years of age and elevated likelihood of small for gestational age, prematurity and low weight births in those born to fathers less than 24 years in a population based study. The study does not look into these outcomes and concentrates on live birth as the key parameter of success of assisted reproduction and the outcome expected by couples seeking this treatment. Moreover, our study does not address reproductive outcomes for sperm donors more than 45 years and is

based on age of selected population of sperm donors rather than paternal age of men undergoing fertility treatment.

One of the major strengths of this study lies in the fact that it analyses one of the largest and comprehensive databases available on fertility and medically assisted reproduction outcomes. This greatly increases the power of the study and its internal validity. In an effort to reduce confounding variables, different study sub-groups were controlled for female age, treatment modality and effect of previous treatment cycles. Sub-groups were not adjusted for potential compounding factors like smoking status, BMI of women and stimulation protocol used for stimulation in IVF/ICSI treatment cycles as no data was available on these factors in the anonymised HFEA data registry. However we presume that, given the large data, any unknown compounding factors (and their confounding effect on results) will be randomly distributed among groups. Sperm donor age was analyzed in categorical fashion as the format of HFEA anonymised data precludes exploration of sperm donor age as a continuous variable. We were unable to analyze sperm donors beyond 45 years of age as sperm donor age is recorded only up to 45 years in the anonymised data. As sperm donors are a select population based on good semen indices, the generalization of results to the paternal population at large may not be possible. In addition, HFEA doesn't collect or record paternal age in its database, precluding future studies using its database to assess the impact of paternal age rather than just a select population of sperm donor age on live birth outcome.

Conclusions:

Presence of limited and inconclusive medical literature on the effect of paternal age on success of medically assisted reproduction has precluded a firm ceiling on male or sperm donor age in relation to paternal reproductive potential. The study suggests that there is a

lack of evidence for any adverse effect of advancing sperm donor age up to 45 on live birth and miscarriage occurrence, which arguably are the most important measures of success of MAR. We postulate that, perhaps, moderation is required as regards the conservative limitation of the upper limit of age for semen donors, although this would require further studies to decide on an appropriate cut-off. In addition, we hope this study would provide reassurance to women limited by choice of available sperm donors regarding the impact of age of sperm donor on achieving a live birth.

Authors' roles:

N.G. contributed to the conception of the study and was involved in data collection, statistical analysis, manuscript preparation and revision, construction of figures and tables and submission of manuscript. E.C. contributed to understanding of the sperm donor selection process, provision of local sperm donor data and critical appraisal of the manuscript. K.P. helped with statistical analyses, data interpretation and revising the manuscript. M.C. conceived the idea for the study, supervised the project and contributed to data collection, interpretation, critical appraisal of the manuscript including amendments and final approval.

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Conflicts of interest:

Authors have no conflict of interests.

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Figure Legends:

Figure 1: Flow chart depicting the number of cases at each stage of selection.

Exclusions in dashed line boxes.

Inclusions in solid line boxes.

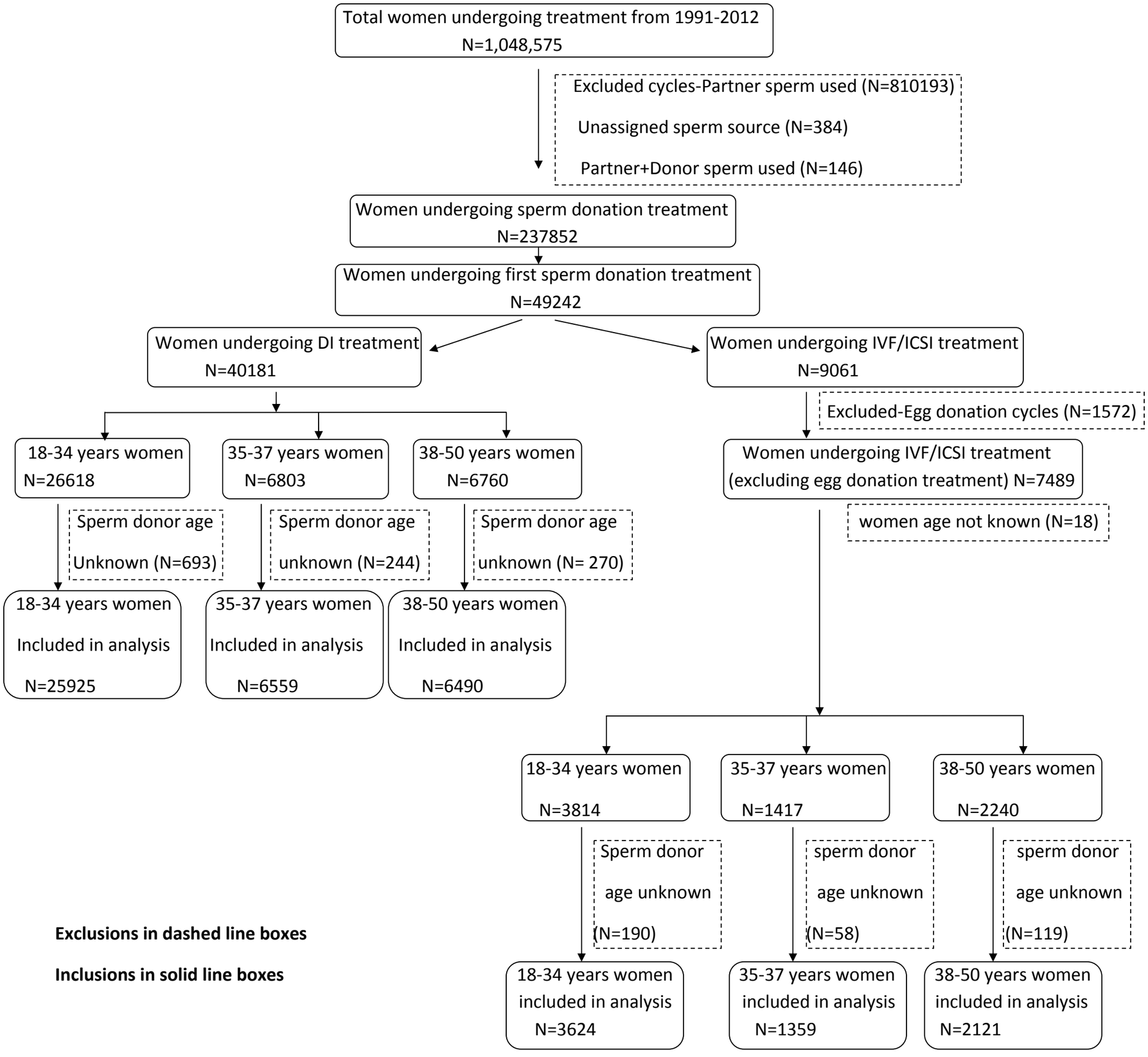
Figure 2: Odds of having a live birth occurrence in different sperm donor age categories in DI treatment cycles

Figure 3: Odds of having a live birth occurrence in different sperm donor age categories in IVF/ICSI treatment group.

Supplementary data- Figure 1: Flow chart depicting the number of cases included in miscarriage analysis.

Exclusions in dashed line boxes.

Inclusions in solid line boxes.



Exclusions in dashed line boxes

Inclusions in solid line boxes

Figure 1: Flow chart depicting the number of cases at each stage of selection.

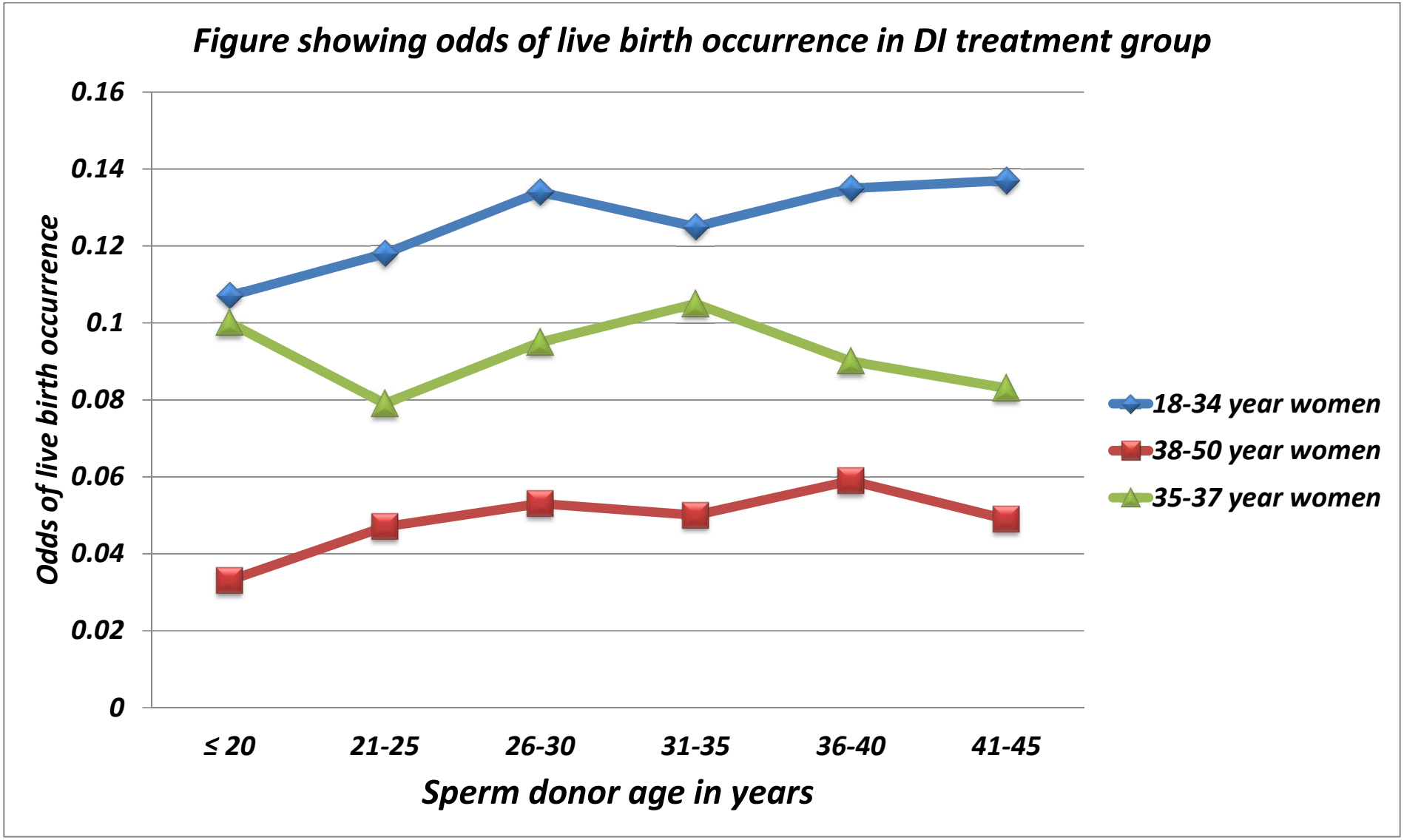


Figure showing odds of live birth occurrence in DI treatment group
Odds of live birth occurrence
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
≤ 20
21-25
26-30
31-35
36-40
41-45
Sperm donor age in years
18-34 year women
38-50 year women
35-37 year women

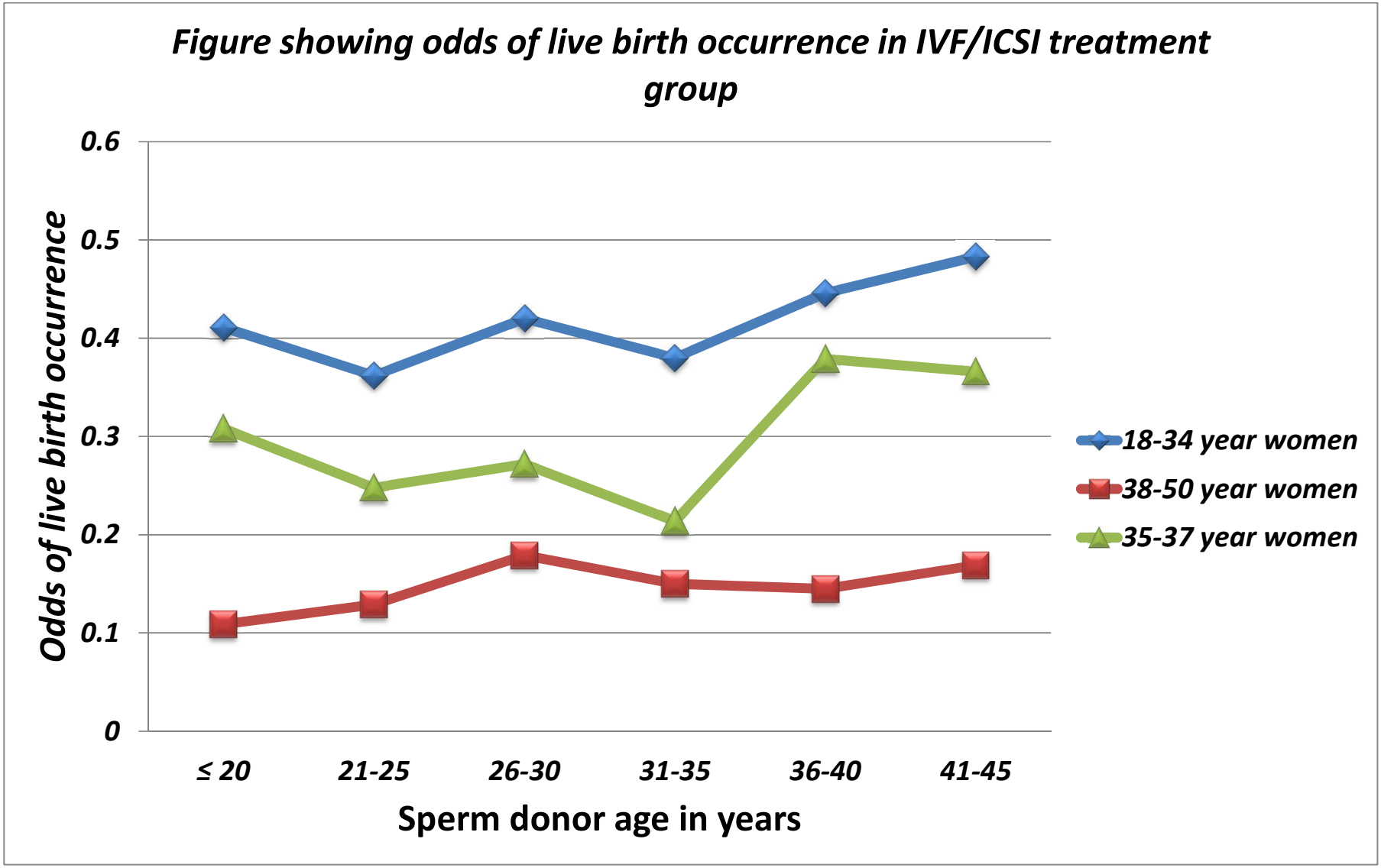


Figure showing odds of live birth occurrence in IVF/ICSI treatment group
Odds of live birth occurrence
0.6
0.5
0.4
0.3
0.2
0.1
0
≤ 20
21-25
26-30
31-35
36-40
41-45
Sperm donor age in years
18-34 year women
38-50 year women
35-37 year women

Table 1: Odds ratio for live birth occurrence among all the donor age categories in DI treatment group

SPERM DONOR AGE CATEGORIES	LIVE BIRTH IN DI TREATMENT GROUP					
	18-34 YEARS OLD WOMEN		35-37 YEARS OLD WOMEN		38-50 YEARS OLD WOMEN	
	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value
≤ 20 years	0.78 (0.64-0.95)	0.015	1.20 (0.78-1.85)	0.397	0.67 (0.36-1.24)	0.202
21-25 years	0.86 (0.73-1.02)	0.079	0.95 (0.66-1.35)	0.765	0.97 (0.63-1.48)	0.882
26-30 years	0.98 (0.82-1.16)	0.795	1.14 (0.79-1.65)	0.472	1.08 (0.68-1.67)	0.736
31-35 years	0.91 (0.76-1.10)	0.321	1.26 (0.86-1.84)	0.234	1.02 (0.64-1.61)	0.940
36-40 years	0.98 (0.81-1.19)	0.866	1.08 (0.72-1.61)	0.713	1.14 (0.71-1.82)	0.589
41-45 years	1		1		1	

Table 2: Odds ratio for live birth occurrence among all the donor age categories in IVF/ICSI treatment groups

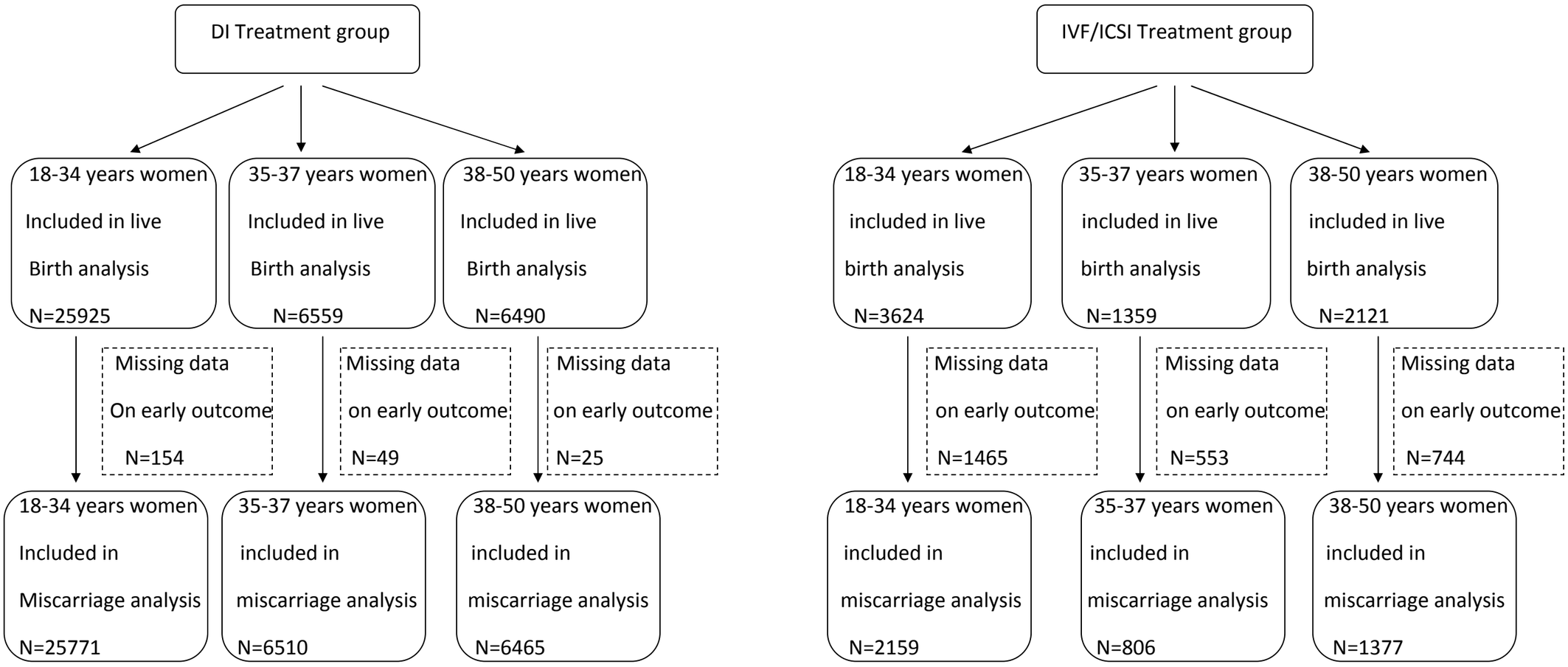
SPERM DONOR AGE CATEGORIES	LIVE BIRTH IN IVF/ICSI TREATMENT GROUP					
	18-34 YEARS OLD WOMEN		35-37 YEARS OLD WOMEN		38-50 YEARS OLD WOMEN	
	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value
≤ 20 years	0.85 (0.61-1.19)	0.340	0.84 (0.46-1.52)	0.564	0.64 (0.34-1.22)	0.179
21-25 years	0.76 (0.59-0.99)	0.042	0.68 (0.43-1.06)	0.089	0.76 (0.49-1.18)	0.227
26-30 years	0.87 (0.66-1.14)	0.306	0.74 (0.47-1.18)	0.210	1.06 (0.68-1.65)	0.797
31-35 years	0.79 (0.59-1.06)	0.110	0.59 (0.35-0.98)	0.044	0.89 (0.55-1.45)	0.639
36-40 years	0.92 (0.69-1.24)	0.598	1.035 (0.64-1.69)	0.889	0.86 (0.53-1.39)	0.532
41-45 years	1		1		1	

Table 3: Odds ratio for miscarriage occurrence among all the donor age categories in DI treatment group

SPERM DONOR AGE CATEGORIES	MISCARRIAGE IN DI TREATMENT GROUP					
	18-34 YEARS OLD WOMEN		35-37 YEARS OLD WOMEN		38-50 YEARS OLD WOMEN	
	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value
≤20 years	1.15 (0.62-2.12)	0.665	1.86 (0.78-4.42)	0.161	0.92 (0.45-1.89)	0.813
21-25 years	1.36 (0.79-2.33)	0.267	1.11 (0.51-2.41)	0.789	0.60 (0.34-1.08)	0.089
26-30 years	1.24 (0.71-2.17)	0.453	1.24 (0.56-2.75)	0.592	0.56 (0.30-1.04)	0.066
31-35 years	1.48 (0.84-2.62)	0.180	1.14 (0.49-2.64)	0.761	0.65 (0.34-1.23)	0.185
36-40 years	1.49 (0.82-2.72)	0.187	1.43 (0.62-3.30)	0.409	0.62 (0.31-1.23)	0.170
41-45 years	1		1		1	

Table 4: Odds ratio for miscarriage occurrence among all the donor age categories in IVF/ICSI treatment groups including embryos transferred per cycle as covariate.

SPERM DONOR AGE CATEGORIES	MISCARRIAGE IN IVF/ICSI TREATMENT GROUP					
	18-34 YEARS OLD WOMEN		35-37 YEARS OLD WOMEN		38-50 YEARS OLD WOMEN	
	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value
≤ 20 years	2.46 (0.77-7.82)	0.128	0.93 (0.29-2.94)	0.902	0.63 (0.22-1.77)	0.379
21-25 years	3.14 (1.20-8.23)	0.020	0.70 (0.30-1.64)	0.416	0.63 (0.32-1.25)	0.187
26-30 years	2.31 (0.86-6.22)	0.099	0.42 (0.17-1.06)	0.066	0.48 (0.23-1.02)	0.055
31-35 years	1.55 (0.52-4.59)	0.433	0.67 (0.27-1.65)	0.384	0.55 (0.25-1.17)	0.121
36-40 years	3.37 (1.26-9.02)	0.016	0.61 (0.25-1.46)	0.268	0.81 (0.41-1.61)	0.554
41-45 years	1		1		1	



Exclusions in dashed line boxes

Inclusions in solid line boxes

Supplementary data-Figure 1: Flow chart depicting the number of cases included in miscarriage analysis.

Table 1: Numbers of women in different donor age categories.

Donor age	DI TREATMENT GROUP			IVF/ICSI TREATMENT GROUP		
	18-34 WOMEN	35-37 WOMEN	38-50 WOMEN	18-34 WOMEN	35-37 WOMEN	38-50 WOMEN
≤ 20 YEARS	2823	548	505	309	102	153
21-25 YEARS	9012	2106	1879	1106	393	613
26-30 YEARS	5880	1459	1457	832	322	467
31-35 YEARS	3990	1064	1096	544	204	321
35-40 YEARS	2705	849	910	483	200	332
41-45 YEARS	1515	533	643	350	138	235
TOTAL	25925	6559	6490	3624	1359	2121

Table 2: Mean number of embryos transferred per cycle in IVF/ICSI treatment group. Dunnett t (>control)

SPERM DONOR AGE CATEGORIES (I)	18-34 YEARS WOMEN SUB-GROUP			35-37 YEARS WOMEN SUB-GROUP			38-50 YEARS WOMEN SUB-GROUP		
	Mean embryo transferred per cycle	Difference of Means when compared to Reference category (95% CI- lower bound)	p-value	Mean embryo transferred per cycle	Difference of Means when compared to Reference category (95% CI-lower bound)	p-value	Mean embryo transferred per cycle	Difference of Means when compared to Reference category (95% CI-lower bound)	p-value
<= 20 YEARS	2.05	0.49 (0.34)	0.000	2.14	0.49 (0.23)	0.000	1.99	0.34 (0.12)	0.001
21-25 YEARS	1.99	0.44 (0.32)	0.000	2.04	0.39 (0.20)	0.000	1.89	0.24 (0.07)	0.003
26-30 YEARS	1.94	0.39 (0.27)	0.000	1.89	0.24 (0.04)	0.016	1.86	0.22 (0.05)	0.010
31-35 YEARS	1.71	0.16 (0.03)	0.017	1.80	0.15 (-0.06)	0.175	1.81	0.17 (-0.01)	0.071
36-40 YEARS	1.66	0.10 (-0.03)	0.146	1.64	-0.005 (-0.22)	0.812	1.66	0.02 (-0.17)	0.732
41-45 YEARS	1.55			1.65			1.64		

Table 3: Mean number of embryos created per cycle in IVF/ICSI treatment group. One way ANOVA was used to compare means.

SPERM DONOR AGE CATEGORIES	18-34 YEARS WOMEN SUB-GROUP		35-37 YEARS WOMEN SUB-GROUP		38-50 YEARS WOMEN SUB-GROUP	
	Mean embryo created per cycle	One way ANOVA p-value	Mean embryo created per cycle	One way ANOVA p-value	Mean embryo created per cycle	One way ANOVA p-value
<= 20 YEARS	6.51	0.895	5.23	0.561	4.73	0.534
21-25 YEARS	6.31		5.14		4.42	
26-30 YEARS	6.21		4.88		4.57	
31-35 YEARS	6.30		5.34		4.82	
36-40 YEARS	6.42		5.59		4.28	
41-45 YEARS	6.53		5.31		4.38	

Table 4: Odds ratio for live birth occurrence among all the donor age categories in IVF/ICSI treatment groups with number of embryos transferred as covariate.

SPERM DONOR AGE CATEGORIES	LIVE BIRTH IN IVF/ICSI TREATMENT GROUP WITH NUMBER OF EMBRYOS TRANSFERRED AS COVARIATE					
	18-34 YEARS OLD WOMEN		35-37 YEARS OLD WOMEN		38-50 YEARS OLD WOMEN	
	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value	ODDS RATIO (95% CI)	p-value
≤ 20 years	0.64 (0.45-0.90)	0.010	0.62 (0.34-1.14)	0.125	0.52 (0.27-0.99)	0.048
21-25 years	0.59 (0.45-0.77)	0.000	0.52 (0.33-0.84)	0.007	0.65 (0.41-1.01)	0.058
26-30 years	0.70 (0.53-0.92)	0.010	0.63 (0.39-1.01)	0.057	0.92 (0.59-1.45)	0.722
31-35 years	0.71 (0.52-0.95)	0.022	0.52 (0.31-0.89)	0.017	0.80 (0.50-1.32)	0.385
36-40 years	0.86 (0.64-1.17)	0.342	1.04 (0.64-1.71)	0.870	0.84 (0.51-1.37)	0.473
41-45 years	1		1		1	