RESEARCH ARTICLE

Maternal age and intracytoplasmic sperm injection outcome in infertile couples at Khartoum, Sudan [version 1; referees: 1 approved, 2 approved with reservations]

Mohamed Ahmed¹, Osama Shareef², Ishag Adam¹, Duria Rayis¹

¹Department of Obstetrics and Gynecology, Faculty of Medicine, University of Khartoum, Khartoum, 11111, Sudan
²Adam and Hawa Fertility Center, Khartoum, 11111, Sudan

Abstract

Background
Intracytoplasmic sperm injection (ICSI) was considered as the mainstay of treatment for male infertility. Nowadays, the scope of ICSI has been widened to include other causes of infertility. There are few published data on ICSI in countries with low incomes.

Aims
A cross-sectional study was conducted at Saad AbuAlla and Banoun Centers, Khartoum, Sudan to investigate outcomes of ICSI and to determine the parameters that might predict pregnancy success rate following ICSI.

Methods
The study included 191 infertile couples who underwent 296 ICSI cycles between 1st April 2013 and 31 March 2014.

Results
One hundred and ninety one couples (comprising 296 cycles of ICSI) were enrolled to the study. The mean (SD) number of retrieved oocytes was 9.7 (7.5). The mean (SD) number of transferred embryos was 2.9 (1.0). Out of these, 50 (26.2%) and 40 (20.9%) had chemical and clinical pregnancy, respectively. Thirty–six couples (18.8%) and five couples (2.6%) had miscarriage and had ectopic pregnancy, respectively. Under logistic regression, younger age (OR = 0.8, 95% CI= 0.81 ─ 0.96, P = 0.004) and endometrial thickness (OR = 1.3, 95% CI= 1.07─1.60, P = 0.009) were the significant predictors for the success of ICSI in inducing pregnancy.

Conclusion
The rates of successful fertilisation and pregnancy-to-term rates in this setting depend mainly on the maternal age.
Introduction

*In vitro* fertilization (IVF) is recognized as the last treatment option for infertile couples who want biological children, and has been widely accepted as the most important and efficient treatment for infertility (Khalaf *et al.*, 2008). Intracytoplasmic sperm injection (ICSI) is the gold-standard technique for the treatment of male factor infertility (Oehninger *et al.*, 2002). However, ICSI or IVF is also recommended to patients with tubal factor infertility (Staessen *et al.*, 1999), as well as treatment of infertile couples with unexplained infertility and some polycystic ovary syndrome (PCOS) cases (Van der Westerlaken *et al.*, 2005; Youn *et al.*, 2011). Unfortunately due to the high cost, IVF/ICSI services are not widely available at both public and private health institutions in developing countries (ESHRE, 2008). However, in countries with lower incomes, the utility of infertility treatment is not well-established and there are few existing private IVF/ICSI centers, and those that exist are associated with a high cost; beyond the reach of most couples (Giwa-Osagie, 2004; Otuba *et al.*, 2006). Because ICSI has a high cost to both the treatment-seeking couple and the health care system, it is necessary to assess its efficacy in different settings. Research in the IVF/ICSI field is of importance for both the treating physicians and the healthcare policy makers and will yield data necessary for patients’ counseling. Different success rates/outcomes of ICSI have also been observed in different settings. There are few published data on the outcome of ICSI in countries with low income and there is no published data on ICSI in Sudan. The current study was conducted at Khartoum, Sudan to investigate ICSI outcome and to determine the parameters that might predict pregnancy success rate resulting from ICSI. Different causes of infertility, and both male and female infertility were observed in Sudan (Elussein *et al.*, 2008).

Methods

A cross-sectional study was conducted during the period of 1st April 2013 through to 31 March 2014 at Saad AbuAlla and Banoun Centers, Khartoum, Sudan to investigate ICSI outcome and to determine the parameters that might predict pregnancy success rate following ICSI.

After signing an informed consent form, a questionnaire was used to gather information about age, parity, menstrual history, duration of infertility, type of infertility (male infertility, failure of ovulation, tubal infertility, unexplained infertility, endometriosis and PCO), cause of infertility, number of previous cycle, endometrial thickness, number of embryos transferred, and the outcome of ICSI (pregnancy rate, rate of miscarriage and ectopic pregnancy).

Couples where males had testicular atrophy, and/or females had uterine anatomical abnormalities, were aged > 44 years, had experience uterine fibroids and/or ICSI failure more than three times were excluded from the study.

In female participants, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured on day 3 of the cycle; preceding ovarian stimulation which was performed followed the short GnRH agonist protocol (Ergenoğlu *et al.*, 2012).

After the workup was done (physical examination, blood group, complete hemogram, viral screening for HIV, HBV and HCV) in the previous cycles, pituitary down-regulation started on the second day of the cycle by daily subcutaneous injection of gonadotrophins and continued until ≥ 3 follicles were present that measured ≥ 17 mm when a 10,000 IU dose of human chorionic gonadotrophin (hCG) was given. Oocyte pickup was scheduled 34–35 hours after the hCG injection. The dose of hCG ranged between 150–450 IU, depending upon the patient’s age, and in response to ovarian stimulation in previous ICSI procedures. Transvaginal ultrasound was done on the day of stimulation to exclude ovarian cysts, and on cycle day seven and every other day to monitor follicle size. E2 (17 beta-estradiol) level was measured on cycle day two and when follicle maturation was achieved. In poor respondents, stimulation was stopped at 20th day of the cycle.

Ovum pickup was done under general anesthesia using a laryngeal mask airway using propofol lipuro 1% (10 mg/ml) 20 ml IV, plus atropine 0.5 mg IV, plus 4 mg dexamethasone as needed to prevent laryngeal spasm, in addition to the anesthetic gas, Nitrous oxide. Fentanyl IV was given as analgesic. Follicles were flushed using flush media from Origio (SynVitro™ Flush, Denmark) using a double lumen needle from (Origio®) if the number of follicles was ≤ 5; otherwise, a single lumen needle from (Wallace®, Wallace Ltd., Colchester, England) was used, without flushing. Embryo transfer was done without anesthesia or sedation using a soft catheter from (Wallace®). Briefly, under sterile condition, vaginal parts were cleaned with saline and draped and a Cusco speculum inserted to expose cervix. Cervical mucus was aspirated. The embryos were deposited in uterine cavity under ultrasound guidance at a position approximately 1 cm shorter than the fundus. The catheter was then checked under a dissecting microscope for retained embryos. If these were found, they were reloaded and transferred again (repeat transfer). The patients were asked to remain in bed for 15–30 min following transfer.

Statistics

The data were entered into computer using SPSS for Windows version 16.0. The mean (SD) of the ICSI variables (age and BMI) were compared between the women who had clinical pregnancy and women who had not using a Student’s t-test. These variables were compared between the different age groups using one–way ANOVA for continuous variables and Pearson’s chi-squared ($X^2$) test for the proportions of the pregnancy rate, ectopic pregnancies and miscarriage. Logistic regression was performed where induction of clinical pregnancy was the dependent variable and the ICSI variables (age, type and duration of infertility, endometrial thickness and the number of oocytes retrieved and their stage of maturation) were the independent variables. A P value < 0.05 was considered significant.

Ethics

The study received ethical clearance from the Research Board at Department of Obstetrics and Gynecology, Faculty of Medicine, University of Khartoum, Sudan.

Results

One-hundred and ninety-one couples were enrolled to the study, comprising 296 total cycles of ICSI. Out of these 191 couples; 82 (42.9%), 48 (25.1%), seven (3.7%) and 54 (28.3%) had male,
female, combined and unexplained infertility, respectively. The vast majority (160; 83.8%) of these 191 couples had primary infertility (failure to achieve pregnancy after one year of unprotected intercourse) and the rest (31; 16.2%) had secondary infertility (failure to achieve pregnancy after one year of unprotected intercourse with previous pregnancy(ies) regardless of its outcome). The mean (SD) duration of infertility was 6.6 (4.4) years. Maternal age range was 18–44 years and the mean (SD) was 32.7 (6.2) years.

The mean (SD) number of retrieved oocytes was 9.7 (7.5). The mean (SD) number of transferred embryos was 2.9 (1.0). The number of retrieved, fertilized oocytes and the transferred embryos was significantly higher in women with age < 30 years (Table 1; Figure 1).

Out of these 50 (26.2%) and 40 (20.9%) had chemical and clinical pregnancy, respectively. Thirty-six (18.8%) and five (2.6%) had miscarriage and ectopic pregnancy, respectively. The rate of induction of pregnancy was significantly higher in women of < 30 years of age (Table 2; Figure 2).

While the mean (SD) of the age [29.8 (4.7) vs. 33.5 (6.3) years, \( P = 0.001 \)] was significantly higher, the endometrial thickness [11.1 (2.2) vs. 10.2 (1.7) mm, \( P = 0.005 \)] was significantly higher in the women with clinical pregnancy (n=40) than in women who had no pregnancy (n=151). Seventeen (42.05%) out of the 40 couples who experienced successful ICSI had male factor infertility, whereas 65 couples (43.0%; \( P = 0.767 \)) in which ICSI were unsuccessful had male factor infertility (Table 3).

In logistic regression, younger age (OR = 0.8, 95% CI = 0.81 – 0.96, \( P = 0.004 \)) and endometrial thickness (OR = 1.3, 95% CI = 1.07 – 1.60, \( P = 0.009 \)) were the significant predictors for the success of ICSI treatment (Table 4). Raw dataset available in Dataset 1.

### Table 1. Comparison of different parameters between different age groups of ICSI at Khartoum, Sudan.

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;30 year (n=55)</th>
<th>30–34.9 year (n=60)</th>
<th>35–40 year (n=54)</th>
<th>&gt;40 years (n=22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, K/m²</td>
<td>26.9(5.8)</td>
<td>29.0(6.5)</td>
<td>29.5(4.5)</td>
<td>31.3(5.8)</td>
<td>0.016</td>
</tr>
<tr>
<td>Follicle stimulating hormone, IU/L</td>
<td>6.3(2.1)</td>
<td>7.3(3.2)</td>
<td>8.1(3.6)</td>
<td>9.5(3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Luteinizing hormone, IU/L</td>
<td>4.7(2.5)</td>
<td>5.2(24)</td>
<td>4.2(2.6)</td>
<td>4.4(3.6)</td>
<td>0.762</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>11.1(2.1)</td>
<td>10.8(1.8)</td>
<td>11.3(1.6)</td>
<td>11.7(2.9)</td>
<td>0.298</td>
</tr>
<tr>
<td>Endometrial thickness, mm</td>
<td>10.5(1.5)</td>
<td>10.5(1.9)</td>
<td>10.1(1.8)</td>
<td>10.0(1.6)</td>
<td>0.462</td>
</tr>
<tr>
<td>Eggs retrieved</td>
<td>14.6(8.3)</td>
<td>9.7(6.3)</td>
<td>6.8(6.2)</td>
<td>4.5(2.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fertilized ovum</td>
<td>8.4(6.3)</td>
<td>5.7(4.5)</td>
<td>3.9(3.4)</td>
<td>3.0 (1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Egg transfer</td>
<td>3.2(0.8)</td>
<td>3.0(0.9)</td>
<td>2.5(1.0)</td>
<td>2.6(1.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

![Figure 1. The number of the retrieved, fertilized oocytes, the transferred embryos and maternal age.](image-url)
Table 2. Age groups and the outcomes of ICSI among women at Khartoum, Sudan.

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;30 year (n=55)</th>
<th>30–34.9 year (n=60)</th>
<th>35–40 year (n=54)</th>
<th>&gt;40 years (n=22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miscarriage</td>
<td>9(16.4)</td>
<td>11(18.3)</td>
<td>10(18.5)</td>
<td>6(27.3)</td>
<td>0.739</td>
</tr>
<tr>
<td>Ectopic</td>
<td>2(3.6)</td>
<td>3(5.0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0.308</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>18(32.7)</td>
<td>17(28.3)</td>
<td>4(7.4)</td>
<td>1(4.5)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Figure 2. Pregnancy rate and maternal age.

Table 3. Comparison of various variables between pregnant and non-pregnant women following ICSI at Khartoum, Sudan.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pregnancy (n=40)</th>
<th>No pregnancy (n=151)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>29.8(4.7)</td>
<td>33.5(6.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Body mass index, kg/cm²</td>
<td>28.6(6.6)</td>
<td>28.8(5.7)</td>
<td>0.825</td>
</tr>
<tr>
<td>Duration of infertility, years</td>
<td>5.8(3.1)</td>
<td>6.9(4.7)</td>
<td>0.174</td>
</tr>
<tr>
<td>Follicle stimulating hormone, IU/l</td>
<td>7.2(3.0)</td>
<td>7.6(3.3)</td>
<td>0.501</td>
</tr>
<tr>
<td>Luteinizing hormone, IU/l</td>
<td>5.8(9.1)</td>
<td>4.4(2.7)</td>
<td>0.109</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>11.4(2.4)</td>
<td>11.1(1.9)</td>
<td>0.354</td>
</tr>
<tr>
<td>Endometrial thickness, mm</td>
<td>11.5(2.2)</td>
<td>10.2(1.7)</td>
<td>0.005</td>
</tr>
<tr>
<td>17 beta-estradiol at triggering, Pg/ml</td>
<td>3326(2710)</td>
<td>2878(2379)</td>
<td>0.305</td>
</tr>
<tr>
<td>Eggs collected</td>
<td>11.6(8.0)</td>
<td>9.3(7.3)</td>
<td>0.082</td>
</tr>
<tr>
<td>Fertilized ovum</td>
<td>7.2(5.6)</td>
<td>5.3(4.7)</td>
<td>0.028</td>
</tr>
<tr>
<td>Egg transfer</td>
<td>3.2(0.7)</td>
<td>2.8(1.0)</td>
<td>0.016</td>
</tr>
<tr>
<td>Days of transfer</td>
<td>3.9(0.8)</td>
<td>3.7(0.8)</td>
<td>0.139</td>
</tr>
</tbody>
</table>
In the current study, ICSI outcomes such as eggs retrieved, fertilized ovums, embryos transferred and the rate of successful induction of pregnancy depend primarily on age of the woman, where the optimal outcomes were observed in women < 30 years of age. This is consistent with Tan et al.’s (2014) findings where optimal IVF outcomes (the number of oocytes retrieved) was highest among women aged < 30 years, with a reduced number of oocytes retrieved per cycle, lower pregnancy and live birth rates seen among women of older age groups. Likewise, Nouri et al. (2015) observed that age was an independent factor for pregnancy rate following IVF/ICSI. The decreasing ovarian reserve (Speroff, 1994), poor oocyte quality (Simpson et al., 2000), higher embryo implantation failure (Navot et al., 1991), ovulatory dysfunction due to poor hormonal environment (Hull et al., 1996; Sherman et al., 1976) and uterine problems (Faddy et al., 1992; Schwartz & Mayaux, 1982) were the postulated effects of the aging process that could have a detrimental effect on the efficacy of IVF/ICSI.

In the current study the pregnancy rate was associated with endometrial thickness. This agrees with the several previous studies which have shown a significant correlation between pregnancy rate and endometrial thickness (Al-Ghamdi et al., 2008; Kasius et al., 2014; Okohue et al., 2009). Endometrial thickness < 7 mm was reported to have a significant reduction in the implantation rate and pregnancy rate. It has recently been shown that (systematic review and meta-analysis) probability of clinical pregnancy for an endometrial thickness ≤7 mm was significantly lower compared with cases with endometrial thickness >7 mm which investigated for pregnancy outcomes after IVF (Kasius et al., 2014).

We conclude that the fertilization and pregnancy rates in this setting depend mainly on maternal age.

Data availability

Discussion and conclusions
The main findings of the current study were that the number of eggs retrieved, fertilized ovum, the number of embryos successfully transferred and the rate of successful induction of pregnancy depend on age of the woman and endometrial thickness. The pregnancy rate (20.9%) in this study was lower than the rates recently reported in Nigeria (30%; Athue et al., 2012); Tunisia (32.4%; Fourati et al., 2009); Vienna, Austria (27.3%; Nouri et al., 2015) and in Singapore (Tan et al., 2014). It is worth mentioning, however, that all of these studies (with exception of Fourati et al.) report the pregnancy rate following IVF/ICSI and not the rate following ICSI alone, as in our study.

Author contributions
MAA - data collection, laboratory work, manuscript preparation. OS - study design, data analysis, and manuscript preparation. IA - data collection, data analysis. DAR - data collection, manuscript preparation. All authors have read and approved the final content of the manuscript.

Competing interests
The authors declare that they have no competing interests.

Grant information
The author(s) declared that no funding was involved in supporting this work.

Table 4. Logistic regression of the predictors of pregnancy following ICSI at Khartoum, Sudan.

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.8</td>
<td>0.81–0.96</td>
<td>0.004</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1.0</td>
<td>0.97–1.11</td>
<td>0.264</td>
</tr>
<tr>
<td>Male infertility</td>
<td>0.6</td>
<td>0.23–1.58</td>
<td>0.306</td>
</tr>
<tr>
<td>Follicle stimulating hormone</td>
<td>0.9</td>
<td>0.83–1.13</td>
<td>0.762</td>
</tr>
<tr>
<td>Luteinizing hormone</td>
<td>1.0</td>
<td>0.96–1.16</td>
<td>0.205</td>
</tr>
<tr>
<td>Estradiol levels</td>
<td>1.0</td>
<td>1.00–1.00</td>
<td>0.864</td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>1.3</td>
<td>1.07–1.60</td>
<td>0.009</td>
</tr>
<tr>
<td>Type of the catheter</td>
<td>2.532</td>
<td>0.195–2.888</td>
<td>0.478</td>
</tr>
</tbody>
</table>

Dataset 1. Raw data for Ahmed et al., 2015 ‘Maternal age and intracytoplasmic sperm injection outcome in infertile couples at Khartoum, Sudan’

http://dx.doi.org/10.5256/f1000research.7386.d107727

Age= maternal age (years). BMI= Body mass index (maternal, Kg/m²). FSH= Follicle stimulating hormone, IU/l. LH= Luteinizing hormone, IU/l. Factor= infertility factor: 1= male, 2= tubal, 3= ovarian, 4= combined, 5= unexplained. Protocol= Protocol used (1= long, 2= short, 3= antagonist, 4= agonist –antagonist, 5= short soft). Days= days of stimulation. Endometrium= Endometrial thickness (mm). E2= 17 beta-estradiol at triggering, (Pg/ml). EC= eggs collected. M2= eggs at metaphase 2. Fertilized= no. of fertilized eggs. DOT= day of transfer. ET= eggs transferred. Chempreg= chemical pregnancy (0= no, 1= Yes). Clinpreg= clinical pregnancy (0= no, 1= yes). Catheter= catheter type. Duration of infertility= duration of infertility (years). Primsec= type of infertility (0= primary infertility, 1= secondary infertility). Miscarriages= miscarriage (0= no, 1= yes). Ectopic pregnancies= ectopic pregnancy (0= no, 1= yes). Age group (0= less than thirty years, 1= thirty-thirty five years, 2= thirty five – forty years, 3= More than forty years) (Ahmed et al., 2015).
Supplementary materials

Questionnaire for collection of ICSI data.

After signing an informed consent form, the questionnaire was used to gather information about age, parity, menstrual history, duration of infertility, type of infertility (male infertility, failure of ovulation, tubal infertility, unexplained infertility, endometriosis and PCO), cause of infertility, number of previous cycle, endometrial thickness, number of embryos transferred, and the outcome of ICSI (pregnancy rate, rate of miscarriage and ectopic pregnancy).

Click here to access the data.

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Version 1

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doi:10.5256/f1000research.7960.r14119

Silke J Dyer, Paversan Archary
Department of Obstetrics & Gynaecology, University of Cape Town, Cape Town, South Africa

Thank you for giving us the opportunity to review this article. The authors report on female factors impacting outcome and pregnancy success rate following ICSI for treatment of infertility in a low income setting.

We congratulate and thank the authors on publishing findings on the effectiveness of ART in their country, as data pertaining to the availability, utilization, effectiveness and safety of ART in sub-Saharan Africa are overall scant and much needed.

The paper is generally well written. Data are presented clearly, and the tables and illustrations are relevant. The study design is appropriate, and the analysis and interpretation of data are overall sound. The conclusions derived are balanced and justified. Although the main finding, especially in relation to age is not novel, the paper contributes relevant information on the status of ART in Sudan.

The paper could be further strengthened if (1) information on biochemical pregnancies was excluded and simply considered under “not pregnant”; (2) data on live births and on complications of ART especially multiple pregnancies were included or, if not available, acknowledged as a study limitation; (3) more information on semen quality and prevalence of severe male factor infertility in the study group were presented; (4) a section on study strengths and limitations was included.

We have the following additional major and minor observations/ suggestions in order of appearance in the manuscript:

Major:
1. According to current evidence ICSI should be considered first line intervention for severe male factor infertility. The use for other indications is of no proven benefit according to our understanding of current evidence, although we acknowledge that it is used for other indications as indeed in the index study (see ICMART World report 2008-2009-2010 and accompanying Editorial). The authors may wish to stay away from a discussion on the role and indications of ICSI in the introduction as in our opinion it weakens the paper; a comment under study limitations may suffice. Instead, it would be of interest to briefly expand on the current situation of ART in Sudan in the introduction.
In table 3, number of eggs fertilized and numbers of eggs transferred also differed between the two groups (p<0.05). It is not clear if these variables were included in the regression analysis- and if not, why. Either way it justifies some attention in both the result and discussion section.

Minor:
1. The title is somewhat misleading and could be rephrased. Perhaps “Factors predicting pregnancy following ART with ICSI in Sudan” or similar would capture the study better?

2. The conclusion in the abstract is not strictly based on the results as no information pertaining to fertilisation are presented in the abstract.

3. Page 3 column 2; line 1 and 2 should be revised. At present the reader gets the impression that gonadotrophins rather than GnRH agonists are used for pituitary down-regulation. In ‘The dose of hCG ranged between 150 – 450IU’ presumably FSH and not hCG is meant. In addition, the entire section on methods could perhaps be shortened, at least if we assume that readers are familiar with the basic principles of ART.

4. Presumably “pregnancy” in table 2 and figure 2 refers to “clinical pregnancies’ only. If the authors follow our recommendation to include biochemical pregnancies under “not pregnant” no changes are required; otherwise an asterix in both the table and figure is needed to specify clinical versus biochemical pregnancy.

5. The choice of age groups is not in keeping with age categories used by regional/international data registries. This limits valuable comparisons. It would be advantageous to consider these age categories in future work.

References

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Referee Report 26 May 2016
doi:10.5256/f1000research.7960.r13995

Akmal El-Mazny
Department of Obstetrics and Gynecology, Cairo University, Cairo, Egypt

The title is appropriate for the content of the article, and the abstract represents a suitable summary of the work. The design, methods, and analysis of the results from the study have been explained and are
appropriate for the study. The conclusions are sensible, balanced, and justified on the basis of the results, and enough information has been provided in a usable format to be able to replicate the study.

Recommendations:

Methods:
- Adequately describe inclusion and exclusion criteria.
- Mention appropriate details of ovarian stimulation and ICSI technique.

Results:
- The text should complement the tables and figures (do not duplicate).
- Clarify the effect size for the main outcome.

Discussion:
- Mention the differences from other studies.
- Mention the limitations of the study.
- Suggest clinical implications.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Frank H de Jong
Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, 3000 CA, Netherlands

In this manuscript, results of an ICSI programme in Khartoum, Sudan are reported. The amount of new information is limited: as indicated in the discussion, a host of published papers already concluded that maternal age is an important factor determining the success of ICSI or IVF procedures. The manuscript might be improved on the following aspects:

1. There is hardly information on the male partners. Data on hormone levels and sperm quality might be as important as the data on hormone levels, endometrial thickness and age in the female partners.

2. There is no information on sperm retrieval, ICSI procedure or embryo culture.

3. On page 3, right-hand column, line 6 "HCG" should probably read "FSH".

4. What happens when pregnancy results are normalized for numbers of embryos transferred? Can the difference in numbers of pregnancies in the different age groups be explained on basis of this number?

5. It might be better to replace "ovum" (line 2 under Discussion and Conclusions) and "ovums" (line 2 in right-hand column on page 6) with "ova".

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I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Competing Interests:** No competing interests were disclosed.