Evaluating the Contribution of Oocyte, Sperm, and Uterus in Determining the Outcome of Intracytoplasmic Sperm Injection – A Retrospective Observational Comparative Study on Intracytoplasmic Sperm Injection using Own Oocytes and Donor Oocytes

Deepeka Thangamani Shanthi^{1,2}, Parasuram Gopinath¹, Karthika D. Kumar¹, Ramesh Parameswara Iyer³, Revathi Kesavaramanujam¹, Gopinathan Kannoly Karunakaran¹

¹Department of Reproductive Medicine, Centre for Infertility Management and Assisted Reproduction (A Unit of Edappal Hospitals Private Limited), Kochi, Kerala, ²Guhan Hospital and Infertility Centre, Mayiladuthurai, Tamil Nadu, ³Department of Obstetrics and Gynecology and Reproductive Medicine, IQRAA International Hospital and Research Centre, Kozhikode, Kerala India **Context:** Several studies have assessed the contribution of oocyte, sperm, and endometrium on the outcome of intracytoplasmic sperm injection (ICSI) separately. This study assesses the relative contribution of oocyte, sperm, and uterus in achieving clinical pregnancy (CP) through ICSU. Aim: The aim of the study is to evaluate and compare the strength of contribution of oocyte, sperm, and uterus in achieving CP through ICSI. Settings and Design: This retrospective observational study of ICSI cycles for 20 months including 1000 embryo transfers (ETs). Methodology: Subjects were divided into two groups, Group 1 – ICSI with own oocytes (550 ETs) and Group 2 – ICSI with donor oocytes (450 ETs). Both the groups had 3 subgroups -a (husband sperm, transferred to self), b (donor sperm, transferred to self), c (husband sperm, transferred to a gestational surrogate). CP rate (CPR) as a major outcome was studied in the groups and subgroups. Statistical Analysis: CPR was compared between various subgroups using Z-test and Chi-square of significance of difference between proportions. A P < 0.05 was taken as the level of statistical significance. **Results:** CPR in subgroup 1a < 35 years, $1a \ge 35$ years, and 2a was 42.98%, 26.21%, and 40.92%, respectively (P = 0.001). CPR was compared between 2a and 2c (40.92%, 56.5%, P = 0.044) and between 2a and 2b (40.92%, 42.11%, P = 0.866). Implantation rate was highest in Group 2c (34.88%) compared to other subgroups. Conclusion: The higher CPR in women <35 years undergoing ICSI with own oocytes than older women and a comparable CPR as that of recipients of donor oocytes suggests that age thereby oocyte quality is the strongest determining factor in achieving clinical pregnancy. Among oocyte recipients, higher CPR in surrogate uterus than patient uterus suggests that uterus/endometrium plays a considerable role, and comparable CPR between ICSI using husband sperm and donor sperm indicates that sperm quality might not play a major role in achieving CP.

KEYWORDS: Clinical pregnancy, donor oocytes, donor sperm, gestational surrogate, intracytoplasmic sperm injection, own oocytes

INTRODUCTION

One out of every four women in developing countries of affected by infertility. If difficulty in conception

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Address for correspondence: Dr. Deepeka Thangamani Shanthi, Guhan Hospital and Infertility Centre, Mahadhana Street, Mayiladuthurai - 609 001, Tamil Nadu, India. E-mail: myhforsh@gmail.com

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happens, interventions from simple fertility awareness to complex treatments such as *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are needed. However, for some couples, more advanced procedures such as third-party reproduction are required. This includes treatment with donated oocytes, sperms or embryos, and gestational surrogacy. Third-party outcomes are successful in couples who otherwise may not have achieved parenthood.^[1]

The common indications for which many couples embark onto assisted reproductive technologies (ARTs) include tubal factors, decreased ovarian reserve, uterine factors such as fibroid and adenomyosis, sperm-related factors, and a combination of any of these.

As women's age increases, oocyte production and fertilization rate decreases. Furthermore, couples tend to postpone pregnancy owing to professional ambitions and social issues. The quality of the uterus also declines with age.^[2] Endometrial receptivity plays a vital role in the establishment of healthy pregnancy both in natural conception and ICSI. Although aging clearly affects female fertility, some studies have shown that increasing male age affects semen parameters and hence male fertility.^[3,4]

Data collected by the Human Fertilisation and Embryology Authority, UK, has shown that the percentage of IVF cycles resulting in live birth decreases significantly as women's age increases. According to the National Institute for Health and Care Excellence (NICE), the success rate of 20%–35% per cycle but the likelihood of getting pregnant decreases with each successive round, which increases not only the cost but also the frustration to the couples. Some women fail to conceive even after three or more cycles of ICSI in whom using donor gametes and gestational surrogacy could be viable options according to their cause of infertility.

A prospective study was published by Banker *et al.* in 2016 to study the outcomes of ICSI in terms of positive pregnancy rates, clinical pregnancy rate (CPR) from ICSI with own oocytes and donor oocytes. CPR in women undergoing ICSI with own oocyte was 40.8% and donor oocytes was 50.2%.^[4] Many studies have assessed the effect of oocyte quality, sperm quality, and the endometrial factor on the outcome of ICSI cycles. However, studies which compare relative contribution of all the factors toward ICSI outcomes are lacking.

Our study aimed at evaluating the effect of quality of oocyte, sperm, and uterine factor individually and their contribution toward achieving success in ICSI cycles.

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We analyzed the CPRs in cycles of ICSI-ET with own gametes, donor gametes, and gestational surrogacy.

Methodology

This was a retrospective observational comparative study carried out at the department of Reproductive Medicine in a tertiary level fertility center. We included patients who underwent ICSI-ET cycles from September 2018. Couples who underwent ICSI with own oocytes were put together as Group 1 and who underwent ICSI with donated oocytes were grouped into Group 2. The sample size was calculated from the parent study^[5] as 437 for each group with expected prevalence of 40.8% in Group 1, 50.2% in Group 2, and 95% confidence interval. Group 1 (own oocytes) and Group 2 (donor oocytes) were divided into three subgroups:

- a. Husband sperm and embryos transferred to self
- b. Donor sperm and embryos transferred to self and
- c. Husband sperm and embryos transferred to gestational surrogate.

Both fresh and frozen embryo transfers (ETs) during the study period were included. Couples who had at least three failed attempts in the particular type of ART treatment were excluded from the same subgroup. Data including women age, duration of infertility, ovarian reserve test results, semen parameters, age of oocyte donor and surrogate mother, protocol used for ART, number of ETs, number of embryos transferred, type of ET (fresh or frozen), endometrial thickness before ET, and the outcome were collected from patient files and entered into MS office excel sheet.

The patients of own oocyte group or the oocyte donors had undergone ART using standard long protocol (downregulation with injection Leuprolide acetate 3.75 mg Depot (Lupride Depot, Sun Pharma, India), followed by injection Menotropin (IVF-MTM, LG Lifesciences, India) for ovarian stimulation. The monitoring and final oocyte triggering and ICSI were as per standard practice. For fresh transfer, patients/recipients received intramuscular or vaginal progesterone (injection HALD 100 mg. Intas Pharmaceuticals Ltd, India, Naturogest 8% gel, Zydus Healthcare Ltd, India). For frozen transfer of embryos with own oocytes, oocyte recipient cycles and surrogate cycles, oral estradiol valerate (Progynova, Bayer Zydus Pharma Pvt., Ltd, India) was used to prepare endometrium and once endometrial thickness was at least 8 mm and good morphology, progesterone was started. ET was done on D2/D3 as per the institute's protocol. Luteal phase support was given and serum β-human chorionic gonadotropin (HCG) was checked 14 days after the day of ET.

Outcomes assessed were positive pregnancy (positive β -HCG of at least 50 μ IU/ml or more), CP, defined as finding an intrauterine pregnancy with fetal heartbeat at 6 weeks of pregnancy by transvaginal ultrasonography, ongoing pregnancy, defined as the pregnancies which cross beyond 12 weeks of gestation, chemical pregnancy (positive pregnancy, but failed to reach the level of CP, clinical miscarriage (including anembryonic pregnancy) and ectopic pregnancy. Implantation rate (IR) was defined as the number of gestational sacs observed divided by the number of embryos transferred. Perinatal outcomes and live birth rates were assessed for 82.8% of the samples but due to missing data caused by patients moving to the referral hospitals for delivery and not traceable through phone and mail, statistics was not applied to the same.

Statistical analysis

A11 data were analyzed using RStudio version 3.6 (RStudio team 2020, PBC, Boston). Categorical variables were summarized using percentages. Chi-square test and Z test of significance of difference between proportions were used to assess the significance of difference between categorical variables. P < 0.05 was considered statistically significant.

Ethics

The study protocol was approved by the Institutional Ethics Committee (approval number-05/20). Consent was obtained from all the patients for the use of anonymized data for research purposes during recruitment for ICSI.

Results

As this was a retrospective study, data collection was carried out along with the inclusion of subjects for the study during the study period. In Group 1 (own oocyte group), we included 550 ETs of 413 couples who underwent 422 cycles of ICSI. In Group 2 (donor oocyte group), we included 450 embryo transfers of 377 couples who underwent 406 ICSI cycles. Overall, 1000 ETs and their outcomes were studied. Figure 1 depicts the distribution of ETs among groups and subgroups.



Figure 1: Distribution of number of embryo transfers in groups and subgroups

The mean age in Group 1 was 31.96 ± 4.67 years and in Group 2, 38.17 ± 5.49 years. The mean age of oocyte donors was 24.67 ± 2.68 years. The mean number of embryos transferred was (Group $1 = 2.37 \pm 0.77$, Group $2 = 3.14 \pm 0.69$). Figure 2 depicts the comparison of outcomes between Group 1 and Group 2.

Group 1 had a pregnancy rate of 48.36% compared to 59.11% in Group 2 (P = 0.001); however, there was no statistically significant difference in the CPRs between the groups (Group 1 = 38.36%, Group 2 = 42.65%, P = 0.18). The ongoing pregnancy rates (OPR) and chemical pregnancy rates also did not differ significantly between the groups (P = 0.26, P = 0.18, respectively). However, Group 2 had a high proportion of clinical miscarriage compared to the Group 1 (18.56% vs. 8.72%, P = 0.09). The IRs were not significantly different between the groups; Group 1 (24.77%) and Group 2 (24.07%), P = 0.06.

Comparison of outcomes among all the subgroups

Among subgroups, there was no significant difference in the pregnancy rates, CPR, and OPR between subgroups 1a, 1b, and 1c [Table 1]. The clinical miscarriage rates in subgroups 1a and 1b were 8.72% and 21.53%, respectively, and the difference



Figure 2: Comparison of outcomes in Group 1 and Group 2

was statistically significant, P = 0.07. In Group 2, there was a significantly higher pregnancy rate in subgroup 2c (78.2%) compared to 2a (56.77%) and 2b (57.89%), P = 0.02. However, there was no significant difference in CPR and OPR between subgroups 2a, 2b, and 2c.

Table 2 enlists the pregnancy rate, CPRs, and OPR among subgroups 1a <35 years, 1a \geq 35 years, and 2a. Subgroup 1a <35 years had higher CPR (42.98%) compared to those in 2a (40.92%). 1a \geq 35 years had the lowest CPR (26.21%), *P* = 0.001. Similar trend was observed in the OPR as well, *P* = 0.005. Figure 3 indicates that highest CPR (62.81%) was achieved in age <30 years, followed by those aged 30–34 years (50.96%), *P* < 0.001 in own oocytes group (Group 1). The CPRs in Group 2 <35 years was



Figure 3: Clinical pregnancy rate among different age groups in Group 1 (own oocytes)

50%, whereas in Group $2 \ge 35$ years, it was 42.57% and was not statistically significant, P = 0.192.

The CPR in donor oocyte recipients with husband sperm (2a) and donor sperm (2b) was not different; 40.92% and 42.1%, P = 0.866 [Table 3]. Among recipients of donor oocyte, a significantly higher CPR was found when transferred to surrogate uterus (subgroup 2c, 56.5%) compared to the own uterus (subgroup 2a, 40.92%), P = 0.044 [Table 4].

The IR in Group 1 was 24.77% and in Group 2, it was 24.07%. The highest IR was in subgroup 2c (donor oocyte, husband sperm, and embryo transferred to surrogate), i.e. 34.88%, however, there was no statistically significant difference in IR among the groups and subgroups, P = 0.06.

After leaving the missing data, the live birth rate for subgroup are as follows: 1a - 29.74%, 1b - 29.41%, 1c - 33.33%, 2a - 21.47%, 2b - 23.21%, 2c - 25%, and the missing data in subgroup are as follows: 1a - 23.22%, 1b - 26%, 1c - 21.05%, 2a - 6.05%, 2b - 24.56%, 2c - 60.86%, hence test of statistical significance was not applied.

DISCUSSION

Our study was a retrospective observational study analyzing the outcomes of ICSI in couple using own gametes, donor gametes, and gestational surrogacy.

In an overview, in our study, the CPR in donor oocyte group was only marginally higher than own oocyte

Table 1: Outcomes of intracytoplasmic sperm injection-embryo transfers in all subgroups								
Outcomes	Group 1			Group 2				
	1a (<i>n</i> =508), <i>n</i> (%)	1b (<i>n</i> =23), <i>n</i> (%)	1c (<i>n</i> =19), <i>n</i> (%)	Р	2a (<i>n</i> =347), <i>n</i> (%)	2b (<i>n</i> =57), <i>n</i> (%)	2c (<i>n</i> =46), <i>n</i> (%)	Р
Pregnancy	243 (47.83)	15 (65.21)	8 (42.11)	0.223	197 (56.77)	33 (57.89)	36 (78.2)	0.02*
СР	194 (38.18)	10 (43.47)	7 (36.84)	0.87	142 (40.92)	24 (42.1)	26 (56.52)	0.13
Ongoing pregnancy	183 (36.02)	10 (43.47)	7 (36.84)	0.767	135 (38.9)	21 (36.84)	24 (52.17)	0.197
Chemical pregnancy	12 (2.36)	0	0	-	12 (3.45)	2 (3.5)	5 (10.86)	0.06
Clinical miscarriage	42 (8.72)	5 (21.53)	1	0.07	44 (12.68)	11 (19.29)	6 (13.04)	0.398
Ectopic pregnancy	1 (0.197)	0	0	-	5 (0.98)	0	0	-

1a=Own oocyte, husband sperm, transferred to self, 1b=Own oocyte donor sperm, transferred to self, 1c=Own oocyte, husband sperm, transferred to self, 1b=Donor oocyte, donor sperm, transferred to self, 1c=Donor oocyte, husband sperm, transferred to self, 1b=Donor oocyte, donor sperm, transferred to self, 1c=Donor oocyte, husband sperm, transferred to surrogate, CP=Clinical pregnancy

Table 2: Outcomes in subgroups own oocyte, husband sperm, transferred to self <35 years, own oocyte, husband sperm, transferred to self ≥35 years and donor oocyte, husband sperm, transferred to self (*z*-test of significance of difference between proportions)

unterence between proportions)				
Outcomes	1a <35 years (<i>n</i> =363), <i>n</i> (%)	1a ≥35 years (<i>n</i> =145), <i>n</i> (%)	2a (<i>n</i> =347), <i>n</i> (%)	Р
Pregnancy	179 (49.31)	48 (33.1)	197 (56.77)	0.001
СР	156 (42.98)	38 (26.21)	142 (40.92)	0.001
Ongoing pregnancy	146 (40.22)	37 (25.52)	135 (38.9)	0.005

1a=Own oocyte, husband sperm, transferred to self, 2a=Donor oocyte, husband sperm, transferred to self

Table 3: Clinical pregnancies in donor oocyte, husband
sperm, transferred to self and donor oocyte, donor
sperm, transferred to surrogate (Chi-square test)

СР	2a (<i>n</i> =347), <i>n</i> (%)	2b (<i>n</i> =57), <i>n</i> (%)	Р
Positive	142 (40.92)	24 (42.1)	0.866
2a=Donor	oocyte, husband sperm,	, transferred to self,	2b=Donor
oocyte, dor	nor sperm, transferred to sperm	urrogate, CP=Clinical	pregnancy

Table 4: Clinical pregnancies in donor oocyte, husbandsperm, transferred to self and donor oocyte, husbandsperm, transferred to surrogate (Chi square test)

СР	2a (<i>n</i> =347)	2c (<i>n</i> =46)	Р		
Positive	142 (40.92)	26 (56.5)	0.044		
2a=Donor oocyte, husband sperm, transferred to self, 2c=Donor					

oocyte, husband sperm, transferred to surrogate, CP=Clinical pregnancy

Group 1 (42.65% and 38.36%, respectively). Banker *et al.* reported a CPR of 40.8% in ICSI with own oocytes and 50.2% in ICSI with donor oocytes.^[5]

As per the society for ART (SART) registry in 2013 across 467 clinics in the USA, a total of 135,423 fresh ETs using own oocytes, CPR from 6.9% to 54.3% was reported among different age groups.^[6] In our study, among own oocytes group (Group 1), the CPR is highest in age group <30 years, i.e. 62.81% and lowest CPR in age group 40 years and above, i.e. 17.32% [Figure 2].

A study published in 2014 by Jason *et al.*^[7] by comparing the pregnancy rate between autologous oocyte and donor oocytes by analyzing 26,457 cycles reported to SART between 2008 and 2010 showed a CPR of 59% and 67%, respectively, however, the mean age of women using own oocytes in their study was 28.0 ± 2.0 , whereas in our study, it is 31.96 ± 4.67 years. The mean age of oocyte donors is 25.4 ± 2.6 years^[7] while it is 24.67 ± 2.68 years in our study. The mean age of recipients is comparable to our study (40.2 ± 4.0 vs. 38.17 ± 5.49 years in our study).

Among women who had ICSI with own oocyte (Group 1), there was no significant difference between subgroups 1a, 1b, and 1c in the pregnancy rates, CPR and OPR as seen from Table 1. However, the number of individuals in Groups 1b and 1c was less as opposed to Group 1a and therefore, the finding might be statistically underpowered.

Among donor oocytes group (Group 2), subgroup 2c has the highest pregnancy rates of followed by subgroup 2b and then by subgroup 2a. The CPR and OPR were highest in subgroup 2c however not statistically significant.

Our CPR outcome in surrogate carrier agrees with Perkins *et al.*^[8] that the gestational carrier cycle had

higher ART success than nongestational carrier cycles; however, in our study, donor oocyte in gestational carrier has the highest CPR, while according to Perkins *et al.*, nondonor gestational carrier cycles has the highest CPR and IR.

On segregating women in subgroup 1a into women age <35 and ≥35 years and comparing the CPR between them and with all in donor oocyte group, i.e. 2a, there was a significant difference observed. 1a <35 years achieved a CPR of 42.98%, whereas 1a ≥35 years had only 26.21% CPR. Various studies have suggested that reproductive outcome such as CP and ongoing pregnancy decreases with increasing female age.^[9-12]

Furthermore, CPR in 1a <35 years and 2a is not significantly different or comparable (42.98% and 40.92%, respectively). The mean age of the donors of oocyte was 24.67 ± 2.68 years. Age is a significant factor which differentiates between the groups. Aging affects oocyte quality significantly according to the observation by SART. We can also infer that age is a significant factor in deciding the success in ICSI from Figure 3, where in own oocvte Group 1, highest CPR is achieved in women <35 years, and as age increases, the chance of achieving CPR reduces. As suggested by Dai et al.,^[13] it is a widely accepted fact that age plays a crucial role in determining oocyte quality. Our study also agrees with Speroff that decreased reproductive potential has been associated with increasing age and the quality of oocyte.^[14]

On the other hand, among the recipients of donor oocytes (Group 2), CPR between women in <35 and ≥ 35 years was not different. This implies that age of recipients of donor oocytes did not affect the outcome in our study. This observation in our study is in accordance with Wang *et al.*^[15] that recipient's age does not affect the outcome in donor oocyte recipient ICSI cycles. However, Gupta *et al.*^[16] observed higher OPR and IRs in younger women.

On comparing 2a and 2b, i.e. (donor oocyte, husband sperm vs. donor oocyte donor sperm), CPRs are almost similar, i.e. 40.92% and 42.1%, respectively. This finding implies that by assuming donor oocyte of a fertile donor as a matching factor, sperms of infertile men who have come for ART and sperms of sperm donors are not differently affecting the CPRs. This is complying with the many studies which suggest that the severe sperm motility problems are overcome by ICSI, and outcome of ICSI in severe sperm abnormality is similar to those with normal sperm parameters.^[17-20]

The CPR of 2a (donor oocyte, husband sperm, transfer to self) is 40.92% and that of 2c (donor oocyte,

husband sperm, transfer to surrogate mother) is 56.5%. Since donor oocyte was used in both the groups, the use of surrogate mother's uterus had higher CPR than using the uterus of the patient herself possibly because patients in 2a had previous implantation failures too apart from poor quality ovum. The major indications for surrogate uterus were MRKH syndrome, medical illness contraindicating pregnancy followed by recurrent implantation failure.

It may be summarized that younger women (<35 years) with own oocytes had as higher CPR than older women (\geq 35 years) (P = 0.001) and similar CPR as that of recipient of donor oocytes. Since age is a strong oocyte quality determining factor, oocyte quality is the strongest determining factor in achieving CP. Surrogate uterus has better CPR than an infertile patient uterus with donor egg (P = 0.044). Hence, uterus contributes considerably to CP. Since there is no difference in the CPR of donor oocyte with donor sperm and subfertile patient's sperm, quality of the sperm did not affect the chance of achieving CP.

The clinical miscarriage rate (CMR) was statistically higher in Group 2, i.e., than Group 1, i.e., 18.56% versus 8.72% [Figure 2]. In the study by Banker et al. too, CMR was higher with donor oocytes than own oocytes (21.8% vs. 15.9%).^[5] The CMR in subgroups 2b (donor ooycte, donor sperm) is 19.29% followed by 2c (donor oocyte, surrogate) with 13.04%, followed by 2a with 12.68% [Table 1]. Speroff suggests that the abortion rate increases with the age of the oocyte recipient from 14.0% among those aged 20-24 years to 44.5% among those aged 35 years and older.^[14] Since the mean age of donor oocyte recipients is 38.17 ± 5.49 years in our study, higher miscarriage rate in recipients of donor gametes could be coincidental or due to advanced recipient age or due to impaired immune tolerance. Larger studies are needed to study this aspect.

IR is an important indicator of the overall performance of the laboratory. The IR in own oocyte group (Group 1) is 24.77% and in donor oocyte group (Group 2) is 24.07% [Figure 1], which are both near the competence level of 25% as indicated by the Vienna consensus report analysis of the key performance indicators of IVF laboratories.^[21] The IRs are similar in all the groups and subgroups, except 2c where it is substantially high (34.88%) which is near the benchmark level of 35%.^[21] This indicates that the laboratory conditions have almost similar effects on the oocytes and embryos in all the groups and subgroups in our study, and there were no confounding effects arising out of the culture conditions.

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Limitations

In our study, perinatal outcome was studied only for 82.8% of study population, as our center is a referral center and as most of the patients went back to other cities and nearby hospitals after becoming pregnant. However, conclusions could not be drawn due to missing data and also live birth was affected by pregnancy and maternal factors such as preeclampsia and diabetes.

The difference in the outcome between fresh and frozen ET was not studied as it was not a part of our objective. Furthermore, some of the outcomes of subgroup analysis are statistically underpowered.

CONCLUSION

Age thereby the oocyte quality is the strongest determining factor in achieving CP in ICSI. Surrogate uterus has higher CPR in donor oocyte recipient cycles, and hence, uterus also contributes considerably to achieving CP in ICSI. The quality of sperm did not affect the chance of achieving CP in our study. Finding sperms for ICSI are sufficient to achieve CP. Future studies with larger sample size are required to understand the difference in the outcomes among the subgroups described in our study.

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Data availability

Data of the study are available with the authors and the authors are willing to provide the dataset upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

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