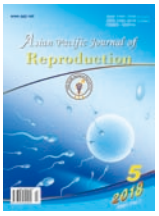

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Live birth rates of assisted reproductive technology treatment and spontaneous conception among subfertile couples in Singapore: A follow-up study

Ho Lee Mee[1], de Souza Nurun Nisa[2,3], Lee Shaw Ni[1], Yu Su Ling[1]✉

[1]*Centre for Assisted Reproduction, Department of Obstetrics and Gynaecology, Singapore General Hospital, Singapore*

[2]*Centre for Quantitative Medicine, Duke-NUS Medical School Singapore, Singapore*

[3]*Epidemiology Department, Singapore Clinical Research Institute, Singapore*

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ABSTRACT

Objective: To explore the potential predictors of a live birth (LB) outcome among subfertile couples of Asian ethnicity undergoing the first fertility treatment cycle; to assess the cumulative live birth rates after successive cycles; and to determine the incidence rate of spontaneous conception (SC). **Methods:** Subfertile couples were grouped according to treatment modalities at the first fertility treatment cycle: intrauterine insemination (IUI), *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI) and no treatment, and were followed-up for duration up to seven years. Multivariable logistic regression analysis was used for statistical analyses. **Results:** Age of female subjects [35-38 years, odds ratio (OR): 0.39; ⩾ 39 years, OR: 0.14], uterine factor subfertility (OR: 5.24), and treatment modalities (ORs: IUI 0.25, IVF 2.33 and ICSI 1.91) significantly predicted a LB outcome (P<0.05). The cumulative live birth rates were 11.7% IUI, 41.5% IVF, 27.5% ICSI and 22.6% from frozen embryo transfer cycles. The cumulative SC rate was 24.6% in the non-treated group and 10.7% in the treated group. All LBs from IVF cycles were delivered by the second cycle and within four years, compared to SC delivery of within five years in the non-treated group and six years in the treated group. **Conclusions:** Age of female subject, uterine factor and modalities of treatment are significant predictors for LB outcome at the first cycle. Higher delivery rates could be achieved following fewer successive IVF cycles and within a shorter duration compared to SC.

1. Introduction

Assisted reproductive technology (ART) has been regarded as an effective treatment for subfertile couples due to the perceived high success rates[1,2]. However, the prediction factors for a successful live birth (LB) outcomes among subfertile couples of Asian ethnicity are limited[3,4].

The primary aim of this study was to explore the potential predictors of a LB outcome among subfertile couples of Asian ethnicity attempting their first treatment cycle (C1). The secondary aims were to assess the cumulative live birth rates (CLBRs) after successive cycles (Cs) and the incidence rate of spontaneous conception (SC) during a follow-up duration up to seven years.

2. Materials and methods

2.1. Subjects

This was a retrospective study of a cohort of subfertile couples of Asian ethnicity who had attended the Fertility Augmentation Clinic (FAC) at Singapore General Hospital, Singapore between December 2003 and up to December 2012. The eligibility criteria were: (i) couples who were unable to conceive after trying for more than one year of unprotected intercourse; (ii) male spouses who had submitted semen samples for seminal analysis and sperm hyaluronan-

First author: Ho Lee Mee, Centre for Assisted Reproduction, Department of Obstetrics and Gynaecology, Singapore General Hospital, Singapore.
E-mail: ho.lee.mee@sgh.com.sg
✉Corresponding author: Yu Su Ling, Department of Obstetrics and Gynaecology, Singapore General Hospital, Singapore.
Tel: + 65 63214673
Fax: + 65 62253464
E-mail: yu.su.ling@sgh.com.sg
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binding assay (HBA) at post-FAC visit for basic male infertility work-up between December 2003 and December 2008; and (iii) couples who had undergone post-FAC fertility treatment, inclusive of intrauterine insemination (IUI) therapy, *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) at Centre for Assisted Reproduction (CARE), Singapore General Hospital.

HBA assessed the ability of the sperm to bind to hyaluronan[5]. Hyaluronan-bound sperm demonstrated fertilizing ability, developmental maturity, reduced frequency of chromosomal aneuploidies and lack of DNA fragmentation[5-7].

The exclusion criteria were: (i) male subjects who were azoospermic or cryptozoospermic (sperm concentration = 0 or <0.2 million/mL, respectively); (ii) female subjects who were recipients of donor embryos; and (iii) couples who went to other institutions for fertility treatment or were lost to follow-up.

The medical records were retrieved from and did not leave the Health Management Information office, Singapore General Hospital. Each couple was given a coded identifier for which, all statistical analyses were performed. Data for baseline characteristics at FAC (female age, duration of subfertility, obstetrical history and subfertility diagnoses), prior-to-FAC (number of previous treatment cycles) and post-FAC treatment outcome achieved at C1 and after Cs were reviewed. Male factor subfertility was defined as having total motile sperm count (TMSC) of <20 million/ejaculate. TMSC was the product of semen volume, sperm concentration and progressive motility and was divided by 100%. Unexplained subfertility was defined as couples who had normal findings in their fertility work-up. The time-to-live-birth (TTLB) delivery was calculated from the date of fertility work-up to the date of delivery. The duration of follow-up was calculated from the date of fertility work-up to the date of last consultation visit for couples who did not achieve any LB. This study was approved by the Singhealth Centralized Institutional Review Board, Singapore (SHS IRB 2009/068/D). Written informed consent was not obtained from subjects as this was a retrospective study using data retrieved from medical records.

All fresh cycle and frozen embryo transfer (FET) procedures were carried out using couples own gametes. Pregnancy outcome was defined as clinical pregnancy (detection of fetal heart activity at 6-7 weeks of gestation by ultrasonography), LB delivery after SC (SLB), LB (the birth of single, twins or triplets at one delivery) and pregnancy loss (including biochemical, missed abortions; miscarriages; ectopic, voluntary or medical termination of pregnancies).

2.2. Semen samples

Seminal analysis was performed according to the standard laboratory guidelines (World Health Organization,1999)[8]. Progressive motility was the sum of rapid and slow motility (grade A + B). Sperm concentration was assessed using an improved Neubauer haemocytometer. Normal sperm morphology was assessed following Tygerberg strict criteria[9].

HBA was assessed using commercial HBA kits (Biocoat, Fort Washington, USA)[5]. Briefly, an aliquot of the semen was applied on the HBA chamber slide, covered with a Cell-Vu grid coverslip and incubated for 10 min at room temperature. The HBA score (%) was the number of bound motile sperm (no progressive head movement and with rapid beating of the principal piece) divided by the total number of bound and unbound motile sperm (move freely), multiplied by 100%.

2.3. Fertility treatment

2.3.1. IUI

Majority (approximately 90%) of the female subjects received oral supplement of 50 mg or 100 mg of clomiphene citrate (Clomid, Medochemie Ltd, Singapore) on day 2-6 of their menstrual cycle. The rest were either followed through their natural menstrual cycle or received mild ovulation induction with recombinant follicle-stimulating hormone to achieve the growth of 1-2 dominant follicles. When at least one of the follicles was greater than 18 mm in diameter, 5 000 or 10 000 IU of human chorionic gonadotrophin (Pregnyl®, Organon) was administered. On the day of IUI, semen sample was collected and motile sperm was prepared by conventional swim-up method. Single IUI procedure was carried out using a soft catheter (Wallace, Smiths Medical, Australia) to transport 0.25 mL of the prepared sperm through the cervix into the uterine cavity.

2.3.2. IVF / ICSI / FET

The decisions for couples to undergo either the IVF or ICSI treatment at C1 or Cs, the controlled ovarian stimulation regimes, starting and daily doses and the duration of stimulation were made with due consideration and discussion by a team of clinicians at CARE[10]. Fertilization rate (%) was defined as the number of oocytes fertilized, either divided by number of mature oocytes (for IVF) or divided by number of mature oocytes injected (for ICSI), multiplied by 100%. Embryos were not transferred on subjects who were either at risk of having ovarian hyperstimulation syndrome, high progesterone level, fever (no transfer) or occurrence of fertilization failure. The embryos which were not transferred were frozen and subsequently thawed for the FET cycles. All fertility treatment was performed based on the standard operating protocol at CARE.

Post-FAC C1: Couples were categorized according to their C1 treatment, *i.e.* IUI, IVF, ICSI or the non- treatment (NT) group if they did not undergo treatment cycle.

Post-FAC Cs: After the fertility work-up, couples were followed-up for a period up to seven years during which they may have received more than one treatment cycle.

2.4. Statistical analyses

Descriptive analysis was performed; for continuous variables, normality was tested using quantile-quantile plots. The data were summarized as mean and standard deviation (Mean ± SD) for normally distributed data or as median and inter-quartile ranges (IQR) for non-normally distributed data. Categorical data were summarized as frequency and proportion.

The baseline characteristics at C1 were stratified to several categories. The number of couples who had LBs was compared between the stratification categories and the modalities of treatment using *Chi*-squared test. The semen parameters, age of female subjects (at FAC,

first cycle, and first LB delivery), TTLB delivery or duration of follow-up, the number of mature oocytes, fertilized oocytes, embryos transferred, cryopreserved and fertilization rates among couples who had LBs were compared to those who did not have LB using Mann-Whitney *U* test, for each modalities of treatment. The clinical pregnancy/cycle, pregnancy loss/cycle, LB/cycle rates and the number of childbirths (singletons, twins and triplets) by the different modalities of treatment were compared using *Chi*-squared test. The relationship between two continuous variables were evaluated using non-parametric Spearman's correlation coefficient, *r*.

Multivariable logistic regression analysis was performed to explore the potential predictors of LB outcomes at C1. $P<0.05$ was considered as statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences (version 21; Chicago, IL, USA). Graphs were used to present the CLBRs for the various modalities of conception against the number of Cs, as well as against the TTLB deliveries. The graphs were generated using the Excel 2010 (Microsoft Office).

3. Results

A total of 983 couples had attended the FAC during the study period and their medical records were retrieved. One hundered and seventeen couples were excluded from the study due to: spouses who were azoospermic or cryptozoospermic (*n*=8); had repeated seminal analyses and HBA (*n*=27); couples who were recipients of donor embryos (*n*=7) and couples who went to other institutions for fertility treatment or were lost to follow-up (*n*=75). Therefore, 866 couples were eligible and were included in the final data analyses. The mean age of the female subjects at FAC visit was (32.6 ± 4.5) years and the median duration of subfertility was 3.0 years (IQR: 1.5-5.0 years).

There were 259 couples who received IUI, 47 IVF, 193 ICSI and 367 did not undergo any treatment at C1. Female age at FAC was stratified to ⩽ 30 years (*n*=284), 31-34 years (*n*=307), 35-38 years (*n*=178) and ⩾ 39 years (*n*=97). Duration of subfertility were stratified to ⩽2 years (*n*=359), >2-5 years (*n*=318), >5-7 years (*n*=83), >7 years (*n*=83) and unknown (*n*=23). Obstetrical history were categorized as primary (*n*=615) and secondary subfertility (*n*=251). Subfertility diagnoses were tubal factor (*n*=37), endometriosis (*n*=47), ovulatory disorders (*n*=108), uterine factor (*n*=87), mixed female factor (*n*=172), male factor (*n*=58), mixed female and male factor (*n*=130) and unexplained subfertility (*n*=227). Previous treatment cycles were stratified to 0 (*n*=722), 1-3 (*n*=124), 4-6 (*n*=17) and ⩾7 (*n*=3) (Table 1).

3.1. C1

In this cohort study, the overall LB/cycle rate achieved at C1 was 16.6% (144/866). The clinical pregnancy/cycle rates achieved by the various modalities of treatment were significantly different: 8.5%

Table 1

Baseline characteristics of couples who have had LBs, grouped according to the modalities of treatment at C1 [%].

Items	IUI (*n* = 17)	IVF (*n* = 16)	ICSI (*n* = 44)	NT (*n* = 67)	*P* value
Female age (years):					
⩽ 30	8 (47.1)	5 (31.2)	15 (34.1)	32 (47.8)	0.260
31 to 34	7 (41.1)	7 (43.8)	20 (45.4)	25 (37.3)	
35 to 38	2 (11.8)	2 (12.5)	5 (11.4)	10 (14.9)	
⩾ 39	0 (0)	2 (12.5)	4 (9.1)	0 (0)	
Duration of subfertility (years):					
⩽ 2	9 (52.9)	4 (25.0)	12 (27.3)	33 (49.2)	0.218
> 2 to 5	7 (41.2)	9 (56.3)	19 (43.2)	27 (40.3)	
> 5 to 7	1 (5.9)	1 (6.2)	6 (13.6)	5 (7.5)	
> 7	0 (0)	1 (6.2)	5 (11.4)	1 (1.5)	
Unknown	0 (0)	1 (6.2)	2 (4.5)	1 (1.5)	
Obstetrical history:					
Primary	14 (82.4)	12 (75.0)	32 (72.7)	48 (71.6)	0.841
Secondary	3 (17.6)	4 (25.0)	12 (27.3)	19 (28.4)	
Subfertility diagnosis:					
Tubal factor	0 (0)	1 (6.2)	1 (2.3)	2 (3.0)	< 0.001*
Endometriosis	1 (5.9)	2 (12.5)	2 (4.5)	4 (6.0)	
Ovulatory disorders	5 (29.4)	1 (6.2)	1 (2.3)	14 (20.9)	
Uterine factor	3 (17.6)	5 (31.3)	3 (6.8)	8 (11.9)	
Mixed female factor	3 (17.6)	2 (12.5)	5 (11.4)	12 (17.9)	
Male factor	1 (5.9)	0 (0)	8 (18.2)	0 (0)	
Mixed female+male factor	3 (17.6)	0 (0)	14 (31.8)	5 (7.5)	
Unexplained subfertility	1 (5.9)	5 (31.3)	10 (22.7)	22 (32.8)	
Previous treatment cycles:					
0	16 (94.1)	10 (62.5)	32 (72.7)	63 (94.0)	0.009*
1 to 3	1 (5.9)	5 (31.3)	9 (20.5)	4 (6.0)	
4 to 6	0 (0)	1 (6.2)	3 (6.8)	0 (0)	
⩾ 7	0 (0)	-	-	0 (0)	

LB: live birth; C1: the first treatment cycle; IUI: intrauterine insemination; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection; NT: no treatment.

*Statistically significant $P<0.05$.

(22/259) for IUI, 38.3% (18/47) IVF, 29.0% (56/193) ICSI and 22.1% (81/367) after SC in the NT group, $P<0.001$. The pregnancy loss/cycle rates were: 1.9%, 4.3%, 6.2% and 3.8%, respectively, $P>0.05$. Thus, the corresponding LB/cycle rates were 6.6% IUI, 34.0% IVF, 22.8% ICSI and 18.3% SC in the NT group. The number of live births achieved at C1 varied across the baseline characteristic stratifications and treatment modalities (Table 1). In the univariate analysis, subfertility diagnosis and the number of previous treatment cycles were significantly associated with LB outcomes, $P<0.05$. The number of childbirths were significantly different for the various treatment modalities; *i.e.* 17 singletons were born after IUI, 8 after IVF, 32 after ICSI and 67 in the NT group; 7 pairs of twins were born after IVF and 10 pairs after ICSI; one set of triplets was born after IVF and 2 sets were born after ICSI, $P<0.001$.

The semen parameters and HBA scores were compared by whether or not a LB was achieved for the various modalities of treatment (Table 2). The parameters were significantly different: progressive motility ($P=0.047$) for IUI group; sperm concentration ($P=0.027$) for ICSI group; and sperm concentration ($P=0.001$), normal morphology ($P=0.033$), TMSC ($P=0.004$) and HBA scores ($P=0.020$) for the NT group.

Progressive motility, sperm concentration, morphology and TMSC were significantly correlated to HBA score, $r = 0.419$, 0.322, 0.553 and 0.344, respectively, $P<0.001$. HBA scores were not correlated

Table 2

Comparison on the descriptive parameters and the outcome of C1 between couples with and without LBs.

Items	IUI (n = 259)		IVF (n = 47)		ICSI (n = 193)		NT (n = 367)	
	LB (n = 17)	No LB (n = 242)	LB (n = 16)	No LB (n = 31)	LB (n = 44)	No LB (n = 149)	LB (n = 67)	No LB (n = 300)
Semen parameters of spouses:								
Semen volume (mL)	2.4 (1.5-2.8)	2.5 (1.9-3.4)	2.6 (2.0-3.8)	2.8 (1.8-3.8)	2.5 (1.7-3.6)	2.5 (1.8-3.5)	2.7 (1.8-3.7)	2.5 (1.8-3.6)
Progressive motility (%)	**40.0 (29.0–47.5)**	**46.0*** **(38.0–53.0)**	53.0 (46.0-61.8)	52.0 (45.0-61.0)	37.5 (22.3-46.0)	36.0 (21.5-46.0)	46.0 (35.0-56.0)	43.5 (34.0-53.0)
Sperm concentration (million/mL)	49.0 (20.6-133.5)	68.9 (32.2-135.3)	99.0 (73.6-123.8)	126.0 (63.5-147.0)	**29.8 (14.8–62.9)**	**45.0*** **(21.7–85.0)**	**101.0 (42.0–148.0)**	**54.5*** **(24.3–109.0)**
Normal morphology (%)	5.0 (2.5-8.0)	5.0 (3.0-8.0)	7.5 (5.3-11.5)	7.0 (6.0-11.0)	3.0 (2.0-4.8)	3.0 (1.0-5.0)	**5.0 (3.0–8.0)**	**4.0*** **(2.0–7.0)**
TMSC (million)	58.7 (13.4-111.3)	69.9 (33.2-156.6)	165.2 (82.7-250.2)	137.0 (77.0-256.4)	21.5 (8.9-63.8)	35.6 (10.4-84.5)	**89.6 (43.3–190.4)**	**62.3*** **(21.3–138.0)**
HBA score (%)	72.0 (59.0-81.5)	76.0 (59.8-87.0)	83.5 (76.5-90.0)	85.0 (77.0-88.0)	53.0 (33.3-71.0)	55.0 (32.0-75.0)	**79.0 (58.0–88.0)**	**70.0*** **(46.3–82.0)**
Characteristic of female subjects:								
Age at FAC (years)	31.0 (28.0-33.5)	32.0 (29.0-35.0)	32.0 (28.0-34.8)	32.0 (30.0-35.0)	**32.0 (29.0–34.0)**	**34.0*** **(31.0–38.0)**	**31.0 (27.0–33.0)**	**32.0*** **(29.0–36.0)**
Age at C1 (years)	31.7 (29.4-34.4)	32.5 (30.3-36.2)	33.5 (29.3-36.6)	34.1 (30.8-37.3)	**33.4 (30.3–35.5)**	**35.0*** **(32.2–39.2)**	-	-
Age at first LB delivery (years)	32.4 (30.1-35.0)	34.2 (31.2-37.2)	34.1 (30.0-37.3)	34.9 (32.1-38.4)	**34.0 (30.9–36.2)**	**36.7*** **(33.4–39.6)**	32.9 (29.3-34.9)	33.4 (30.2-36.9)
Time-to-live-birth delivery *vs* duration of follow-up (months)	**10.0 (8.7–11.7)**	**47.4*** **(35.0–62.5)**	**10.6 (9.6–12.1)**	**41.7*** **(32.1–58.8)**	**11.5 (10.4–13.2)**	**38.5*** **(26.8–51.4)**	**10.4 (9.6–13.7)**	**40.7*** **(26.6–56.9)**
Outcome of C1:								
Mature oocytes	-	-	8.5 (6.0-12.8)	10.0 (5.0-12.0)	9.0 (5.0-12.0)	8.0 (4.0-12.0)	-	-
Oocytes fertilized	-	-	6.0 (4.3-10.8)	6.0 (3.0-8.0)	6.0 (4.0-9.0)	5.0 (2.0-10.0)	-	-
Fertilization rate (%)	-	-	84.5 (66.7-92.7)	66.7 (50.0-85.7)	80.0 (72.7-94.8)	75.0 (61.0-100.0)	-	-
Embryos transferred	-	-	**3 (2–3)**	**2*** **(1–3)**	2 (2-3)	2 (2-3)	-	-
Cryopreserved embryos	-	-	1 (0-5)	3 (0-4)	2 (0-5)	0 (0-4)	-	-

Parameters in median (inter-quartile range).

C1: the first treatment cycle; LB: live birth; IUI: intrauterine insemination; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection; NT: no treatment; TMSC: total motile sperm concentration; HBA: sperm hyaluronan-binding assay; FAC: Fertility Augmentation Clinic visit.

Comparison of LB versus no LB: *$P<0.05$.

Data are bold if significant.

to the rates of oocyte fertilization among the IVF or the ICSI group. The number of mature oocytes retrieved, fertilized and cryopreserved embryos were negatively correlated to female age among the ICSI group, r = -0.268, -0.261 and -0.294, P<0.001, respectively. These correlations were not observed among the IVF group.

The study also investigated factors that could potentially influence on LB outcome at C1 (Table 3). Older women were less likely to have a succcessful delivery compared to women aged <30 years. Women with uterine factor subfertility were more likely to succeed compared to those with tubal factor. There was a higher likelihood of achieving a LB among women who had undergone IVF or ICSI compared to the NT, while those who had undergone IUI were 75% less likely to achieve a LB.

Table 3
Multivariable associations of potential predictors for LB outcome following C1.

Items	OR (95% CI)	P value
Female age (years):		
⩽30	Reference	
31 to 34	0.81 (0.52-1.26)	0.355
35 to 38	**0.39 (0.21–0.72)**	**0.003**
⩾39	**0.14 (0.05–0.38)**	**< 0.001**
Duration of subfertility (years):		
⩽2	Reference	
>2 to 5	1.43 (0.92-2.22)	0.117
>5 to 7	1.01 (0.49-2.07)	0.985
> 7	0.74 (0.30-1.85)	0.517
Unknown	0.96 (0.29-3.20)	0.957
Obstetrical history:		
Primary	Reference	
Secondary	0.75 (0.37-1.50)	0.409
Subfertility Diagnosis:		
Tubal factor	Reference	
Endometriosis	2.23 (0.58-8.64)	0.247
Ovulatory disorders	2.89 (0.84-9.96)	0.094
Uterine factor	**5.24 (1.49–18.37)**	**0.010**
Mixed female factor	1.42 (0.42-4.72)	0.572
Male factor	1.72 (0.43-6.81)	0.441
Mixed female + male factor	2.32 (0.66-8.11)	0.188
Unexplained subfertility	2.58 (0.80-8.35)	0.114
Modality of treatment at first cycle:		
No Treatment	Reference	
IUI	**0.25 (0.14–0.45)**	**< 0.001**
IVF	**2.33 (1.11–4.92)**	**0.026**
ICSI	**1.91 (1.16–3.16)**	**0.011**

LB: live birth; C1: first treatment cycle; OR: odds ratio; CI: confidence interval; IUI: intrauterine insemination; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection.
Data are bold if significant.

3.2. Cs

The median duration of follow-up in this study was 3.6 years (IQR: 2.5-4.8 years). 58% (504/866) of the couples had attempted a total Cs of 1 017, median of two cycles (IQR: 1-3 cycles). The maximum number of cycles attempted was: IUI=7, IVF=3, ICSI= 4, and FET=4. In total, 266 couples had undergone 491 successive IUI cycles, 65 couples had 77 IVF cycles, 247 couples had 312 ICSI cycles and 106 couples had 137 FET cycles (Table 4).

Table 4
Couples' attempts at Cs and the treatment outcomes.

Outcome after successive treatment cycles	IUI	IVF	ICSI	FET	NT
Total OHSS [n (%/cycle)]	-	5 (6.5)	13 (4.2)	-	-
Total fertilization failure [n (%/cycle)]	-	2 (2.6)	10 (3.2)	-	-
Total no transfer [n (%/cycle)]	-	2 (2.6)	4 (1.3)	1 (0.7)	-
Total pregnancy loss [n (%/cycle)]	10 (2.0)	3 (3.9)	18 (5.8)	12 (8.8)	19 (5.2)
Total subjects with LBs (n)	31	27	68	24	89
Total LB/cycle rate (%)	6.3 (31/491)	35.1 (27/77)	21.8 (68/312)	17.5 (24/137)	-
CLBR (%)	11.7 (31/266)	41.5 (27/65)	27.5 (68/247)	22.6 (24/106)	24.6 (89/362)
Total infants delivered					
- singleton	29	13	46	18	89
- twins	2	13	20	6	-
- triplets	-	1	2	-	-

Cs: successive cycles; IUI: intrauterine insemination; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection; FET: frozen embryo transfer; NT: no treatment; OHSS: ovarian hyper stimulation syndrome; LB: live birth; CLBR: Cumulative live birth rate.

The corresponding total LB/cycle rates achieved were: 6.3% IUI, 35.1% IVF, 21.8% ICSI and 17.5% FET. The CLBRs were: 11.7% after IUI, 41.5% IVF, 27.5% ICSI and 22.6% FET. The CLBRs peaked at the seventh cycle after IUI, at the second cycle after IVF and ICSI and at the third cycle after FET (Figure 1A). Among these 504 couples who had successful or unsuccessful Cs: 24 couples who had undergone one treated cycle, subsequently conceived spontaneously and had SLBs (refered as the second cycle); 18 couples who had 2 treated cycles, conceived spontaneously and had SLBs (the third cycle), 11 couples who had 3 treated cycles, had SLBs (fourth cycles) and one couple who had 4 treated cycles, had SLB (fifth cycle); thus, a total of 54 couples had treated cyles had SLBs (CLBR 10.7%), (Figure 1A).

In the NT group, 5 couples had attempted a total of 8 successive cycles of IUI without success (Table 4). The remainder 362 couples did not undergo any fertility treatment throughout the follow-up period. 73 of these couples conceived spontaneously and had one LB, 14 subsequently had a second LB and two couples had a

third LB, thus, a total of 89 SLBs (CLBR 24.6%) were delivered (Figure 1A).

All LBs were delivered within TTLB of four years after IVF, compared to SLBs deliveries within five years among the non-treated couples and six years among couples who had treatment (Figure 1B). In all, 33.8% (293/866) of the couples achieved at least one LB at the end of the follow-up period.

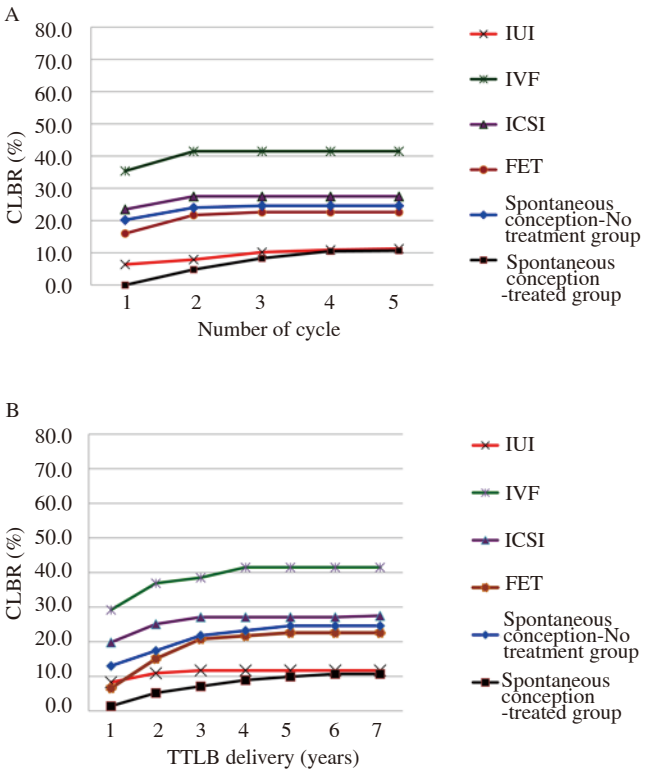

Figure 1. CLBRs for the total cohort of 866 couples undergoing successive treatment cycles during the duration up to 7 years of follow-up period.

A: Comparison of the CLBRs according to the modalities of conception. X-axis is the number of treatment cycles, truncated to 5 cycles.

B: Comparison of the CLBRs according to the modalities of conception. X-axis is the TTLB delivery (years).

CLBR: Cumulative live birth rate; TTLB: Time- to-live-birth delivery; IUI: intrauterine insemination; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection; FET: frozen embryo transfer.

In tracking the actual treatment attempts during the followed-up period, couples who had started with IUI at C1 subsequently underwent a total of 481 successive IUI cycles, 18 of these couples proceeded with 21 IVF cycles, 49 couples attempted 58 ICSI cycles, 19 couples had 27 FET cycles and 34 couples had SLBs: the CLBR achieved was 33.6% (87/259) and 106 LBs (inclusive of singleton, twins and triplets) were delivered in all. Likewise, couples who had started with IVF treatment subsequently underwent a total of two IUI cycles, 56 successive IVF, 5 ICSI, 22 FET cycles and had 8 SLBs: CLBR was 57.4% (27/47) and a total of 42 LBs delivered. The ICSI group did not attempt any IUI or IVF cycles, had 249 successive ICSI cycles, 88 FET cycles and had 12 SLBs: CLBR was 37.3% (72/193) and a total of 103 LBs deliveries.

4. Discussion

In this retrospective cohort study on subfertile couples attempting fertility treatment at our Centre, we sought to determine the prognostic factors to predict LB outcome at the first fertility treatment. Our study demonstrated women younger than 35 years had higher odds of achieving LBs and the odds greatly reduced as age increased. This trend could be due to the decline in the number of retrievable mature and fertilizable oocytes as women get older. Our findings are in line with previous studies on the adverse effect of increasing age on the success rates of fertility treatment[10–12].

Our study has shown that women with uterine factor subfertility were more likely to achieve a LB than tubal factor. In the review by Zarinara *et al*, demographic and clinical factors influence treatment success, *e.g.* duration of infertility, body mass index, obstetrical history, ovarian size, ovulation disorders, tubal factors, unexplained infertility, male factor or previous treatment cycles, *etc*[13].

Treatment modalities varied in their effect on LB outcomes in our cohort. The LB/cycle rate and the pregnancy loss rate were considerably low with IUI therapy, probably due the stimulation regime with clomiphene citrate. In contrast, other studies reported higher pregnancy/cycle rate of 12.6% to 19.6% with stimulation regime of gonadotrophin, albeit high rates of pregnancy loss, cycle cancellation and multiple pregnancy[11,14]. We propose IUI therapy should not be recommended at C1 to the following groups of female subjects due to substantially low success rate; *i.e.* age $\geqslant$ 35 years, duration of subfertility of >5 years, tubal factor, endometriosis, unexplained subfertility, spouse having male factor and those who had previous treatment cycles, similarly reported by Nuojua-Huttunen *et al*[11].

In our study, IVF could be offered as the treatment of choice as the likelihood of achieving a LB was significantly higher. When considering IVF and ICSI as related treatment in our cohort, the overall LB/cycle rate at C1 was 25% (60/240), similar to 26.5% in the study by Witsenburg *et al*[15]. Purcell *et al* has reported Asian women achieved a LB rate of 26.9% compared to Caucasians (34.9%)[3]. Other studies have shown lower clinical pregnancy or LB rates in Asian women compared to other ethnicities, which could explain the overall low IVF/ICSI success rate in our study cohort of Asian subjects[4,16,17].

To our knowledge, our study is the first to show sperm concentration, morphology, TMSC and HBA were significantly higher in the non-treated couples who had SLBs compared to those who did not have SLB. However, these parameters lack significance for prediction of LB outcome. Other studies reported SC rates of 34% and 38% in infertile couples and semen parameters were significantly associated

with the occurrence of SC[18,19]. Our findings also showed no associations between HBA scores and fertility treatment outcome, which were consistent with several other studies[20,21]. In contrast, HBA scores have been shown to significantly correlate with embryo quality, cleavage rates, fertilization rates, implantation, clinical pregnancy, miscarriage and pregnancy loss rates[22,23].

The strength of our study lies in our approach into investigating the prognostic factors contributing to the success rate at C1. Nevertheless, we acknowledge the limitation of using these factors; they were assessed prior to starting a fertility treatment, applicable only for the prediction of success rate at C1 and for subjects of Asian ethnicity. In contrast, success of fertility treatment is also dependent on other post-treatment factors, *i.e.* the numbers and quality of fertilized oocytes or transferred embryos which we did not include in the prediction analysis[16,17,22].

Our study has shown that the total LB/cycle rates achieved after Cs for the individual treatment modalities were rather similar to the rates at C1, indicating that increases in the number of treatment cycles do not result in additional increase to the LB outcome. The CLBRs showed a trend across the seven years. We propose four cycles of IUI therapy to be sufficient as 94% of the LBs were delivered within TTLB of two years, similar to other reports of 97% to 100% pregnancies within the first three or four cycles[11,14]. The CLBRs for IVF treatment in our cohort reached the peak at the second cycle and within TTLB delivery of four years. In contrast, other studies reported high ART success rates; CLBRs of 59% to 69.4% after six or seven cycles, or between five to seven years of follow-up duration[15,24,25].

The 3-year CLBR of SC in our cohort was considerably low, 21.8% among couples who remained untreated and 9.1% among the total cohort, compared to higher CLBRs found in other studies[26,27]. We observed CLBR of 10.7% of SLB among the treated couples. Other follow-up studies have reported cumulative delivery rates after SC of 4.6% to 22.2% in couples who had finished or discontinued unsuccessful or successful IVF/ ICSI treatment[24,28-30].

One final strength of our study was that we had followed the actual treatment attempts of couples over a long duration of up to seven years. Our findings were that IVF was the most effective modality of treatment at the first cycle as well as after successive cycles; couples who had started with IUI cycles and proceeded with ART treatment achieved higher success rate and SC occurred among the subfertile couples, albeit lower incidence rate and longer time to achieve a LB compared to attempting fertility treatment.

To the best of our knowledge, this is the first follow-up study on subfertile couples of Asian ethnicity undergoing successive treatment cycles. Our study suggests female age, subfertility diagnosis and modalities of treatment are key factors when deciding on fertility program. Future research could explore semen analysis and sperm functional assessment as a concerted effort to improve fertility outcomes.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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