

Live Birth Rates After Assisted Reproductive Techniques of Women Aged 42 and Over

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ABSTRACT

Objective: Increased oocyte aneuploidy rates, along with the quantitative decrease of oocytes, are the main consequences of reproductive aging. We aimed to analyze assisted reproductive technique (ART) outcomes of women at extremely advanced age from a single tertiary IVF center.

Material and Methods: This retrospective cohort study included women aged >42 years and underwent oocyte pickup between 2015 and 2020, in a tertiary IVF center. Reproductive outcomes of 178 women who underwent oocyte pickup and subsequent fresh and/or frozen-thawed autologous embryo transfer (ET) were analyzed.

Results: A total of 261 oocyte pickup cycles from 178 women aged >42 years performed during the study period. The mean age of women was 44.5 ± 1.4 . Overall, 118 women (66.3%) underwent either autologous fresh and/or frozen embryo transfer cycles, and 60 women (33.7%) could not reach to embryo transfer. Clinical pregnancy and live birth rates were (5.0%, 1.1% per patient), (5.8%, 1.3% per embryo transfer) and (3.4%, 0.7% per cycle), respectively. Those patients who had live birth were 43 years old at oocyte retrieval, and their pregnancies were obtained from frozen-thawed embryo transfer.

Conclusion: The pregnancy and live birth rates are extremely low for those patients at a very advanced age; however, there might be extreme cases with a successful result. Availability of supernumerary embryos for future frozen-thawed transfers might give a chance these patients to conceive. As with all infertile patients, an individualized approach should be taken into account in advanced age groups.

Keywords: aging, assisted reproductive techniques, pregnancy rate, live birth

INTRODUCTION

The declining number and quality of oocytes by advanced female age is a well-known phenomenon. The success of assisted reproductive techniques (ART) is strictly dependent on the female age. Age is the most established predictor of IVF outcomes [1,2]. Increased oocyte aneuploidy rates, along with the quantitative decrease of oocytes, are the main consequences of reproductive aging. This evidence is also mirrored in the ART outcomes.

Delaying childbirth has been a common problem worldwide. Women's attempts to conceive at advanced reproductive ages are increasing [3]. According to the US national data from 2017, 20.7% of women who underwent an ART cycle were >40 years old,

and 40% of these women was used their oocytes [4]. The reported live birth rates (LBR) were 8.1% for this age group [4]. Fertility treatment for the advanced age group remains difficult and controversial. Some European countries have female age limits for legal access to ART such as 45 years old in Denmark and 51 years old in Bulgaria [5].

ART is believed to offer the best chance for pregnancy in advanced reproductive-aged women [6]. However, ART treatments' success for these poor prognostic women remains constantly low because of their already reduced ovarian reserve and/or high risk of embryonic aneuploidies leading to implantation failure or miscarriage.

Several authors have evaluated the ART

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outcomes of women aged 40 and over [6-10]. Few also analyzed specifically more advanced age groups such cutoff as 43-45 years of age [11-13]. In a study from Korea, including more than 2000 cycles in 1049 women aged 40 and over, the decrease in cumulative LBR's was clearly shown from 12.9% to 0.7% of the women aged 40 to ≥ 45 [7]. The cumulative LBR's were reported as 2.4%, 2.1%, and 0.7% at age of 43, 44, and 45 years old. Similarly, a significant decline in cumulative LBR's has been reported in women ≥ 43 years compared those aged 41 and 42 years in previous studies [8,9]. Gleicher et al. emphasized the age 43 is a breakpoint for IVF outcomes [14]. The chance of the live birth (LB) with an ART treatment is at extremely lower rates, specifically age over 42 years old. On the other hand, there are reports of patients with extremely advanced age and successfully achieved LB after ART using autologous oocytes [15-18]. The decision to proceed or not to proceed to an ART treatment should be based on the patient's findings and their own decision after detailed information on the potential risks and success rates of ART treatment.

Evaluating the chance of LB with their gametes in an ART program is pivotal to counsel advanced aged patients appropriately. We aimed to evaluate the ART outcomes and LB chances of women aged > 42 years who underwent ART treatment in our university-based tertiary IVF center.

MATERIALS AND METHODS

Women aged >42 years and underwent oocyte pickup between 2015 and 2020 in our tertiary IVF center were included in this retrospective cohort study. Based on these criteria, a total of 261 oocyte pickup (opu) cycles of 178 women aged >42 during the procedure were found from the IVF database and included in the study. Subsequent fresh and/or frozen embryo transfer cycle outcomes were evaluated. The variables included clinical and IVF outcomes were retrieved from patient records and the IVF database. The inclusion criteria were; female age >42 years during the oocyte pickup procedure.

Controlled ovarian stimulation was performed using exogen gonadotropins with the antagonist protocol. Human chorionic gonadotropin (hCG) was used for ovulation trigger when the leading follicle reached 18 mm in diameter. Oocyte pickup was performed 34-36 hours after the ovulation trigger. ICSI was used as the fertilization method. A maximum of two embryos was transferred on day 2 to 5. The luteal phase was supported using vaginal micronized progesterone with single dose rechCG on the fifth day of embryo transfer. In case of available surplus embryos after a fresh transfer, vitrified for another thawed cycle. In some cycles, fresh transfer was not performed, and embryos were vitrified, aiming for preimplantation aneuploidy screening or other reasons. Endometrial preparation was performed either with a modified natural cycle or an artificial preparation cycle for thawed embryo transfers.

Table 1. Characteristics of the AMA group

Number of patients	178
No of opu cycles	261
Age ^a	44.5(1.4) (min:43-max:49)
Infertility etiologies (%)	
Unexplained	82.1
Male factor	16.8
Tuboperitoneal factor	1.1
No of Previous IVF cycles ^a	1.0 (1.5) (min:0-max:7)

^a Mean(SD)

Clinical pregnancy was defined as an ultrasonographic visible gestational sac in the uterine cavity, and live birth was defined as the birth of an infant after 24th weeks of gestation. Miscarriage was defined as a clinical pregnancy loss. Ethical approval was obtained from Institutional Ethics Committee.

Statistical Analysis

The main outcome measure was the cumulative live birth rates of the patients. Secondary outcomes were clinical pregnancy rates, the number of total and mature oocytes, fertilization rates, percentages of successful embryo transfer cycles, and miscarriage rates. Continuous variables were given as mean (SD) and ranges, categorical variables were expressed as frequencies and percentages (%). The binary logistic regression model was used to evaluate factors that may associate with the probability of clinical pregnancy and live birth (age, AMH level, the total number of oocytes after the first ovarian stimulation). Statistical significance was considered at p -value < 0.05. Multiple regression analysis was executed if multiple factors were found significant. The IBM SPSS software (ver. 25.0, SPSS Inc., USA) was used for the analyses.

RESULTS

Overall, 261 opu cycles from 178 women aged >42 years performed in our tertiary IVF center between 2015-2020, were included in this retrospective study. Patients' age range was 43 to 49 years old, and the mean age (SD) was 44.5(1.4). In this group of patients, male factor (16.8%) and tuboperitoneal factor (1.1%) were concomitant infertility etiologies. In the study period, a single opu cycle was performed in 138 patients (% 77.5) and 40 patients (%22.5) had recurrent cycles (mean:2.4(1.6), range:1-9) Characteristics of the patients are shown in **Table 1**.

The mean (SD) number of oocytes retrieved was; 2.9 (2.6) (range: 0-13). In total, no oocyte was obtained in 24 cycles and no mature oocytes in 12 cycles. Therefore, there were a total of 225 cycles (86.2%) obtaining at least one oocyte for the ICSI procedure. The mean number of oocytes that underwent ICSI was 2.2 (1.9) (range:1-10) with a fertilization rate of 70.0 \pm 36.5%. Of those 261 cycles; 137 cycles ended up with fresh embryo transfer (ET) whereas in 87 cycles there were no embryos available for transfer, and the embryos of the remaining 37 cycles were cryopreserved. Within overall 16/261 cycles (6.1%), had surplus embryos which were

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Table 2. Cycle outcomes of AMA group (n=178 women)

No of opu cycles	261
Total gonadotropin used (IU) ^a	2533(903)
Peak E2 level (pg/ml) ^a	646 (472)
No of oocyte retrieved ^a	2.9 (2.6)
No of mature oocytes ^a	2.2 (1.9)
No of fertilized oocytes ^a	1.5 (1.5)
Fertilization rate (%)	70.0(36.7)
No of 2PN's	
Fresh ET cycles (n,%)	137 (52.5%)
Cycles without embryos available for transfer (n,%)	87 (33.3%)
All freeze cycles (n,%)	37 (14.2%)
Clinical pregnancy/per cycle (%)	3.4
Clinical pregnancy/per ET (%)	5.8
Clinical pregnancy/per patient (%)	5.0
Live birth/per cycle (%)	0.7
Live birth/per ET (%)	1.3
Live birth/per patient (%)	1.1

^a Mean(SD)

vitri-fied after fresh embryo transfer. Preimplantation genetic diagnosis for aneuploidy screening (PGD-A) was intended in 10/178 (5.6%) patients in our study population. Of those, no embryo was developed for analysis in a patient. No embryos were found normal in two patients, the remaining

7 had no clinical pregnancy after the transfer of embryos analyzed.

Subsequent autologous fresh (n:137 cycles) and/or frozen-thawed (n:16 cycles) embryo transfer cycles were performed in 118 women (66.3% of the total group). Twelve pregnancies were obtained (biochemical:3, miscarriage:7, and live birth:2). Cumulative reproductive outcomes, including fresh and frozen ET cycles, were analyzed. The cumulative clinical pregnancy rate was 3.4 % and 5.0%, per opu cycle and per patient. The LBR's was, 0.7% and 1.1 %, per opu cycle and per patient, respectively (**Table 2**). Those patients who gave live birth both were 43 years old at oocyte retrieval, and their pregnancies were developed following frozen-thawed ET.

Factors that may predict the cumulative clinical pregnancy and live birth (age, AMH level, total number of oocytes from the first cycle) were evaluated by binary and multiple logistic regression models. The mean AMH levels were 0.68 (0.8) ng/ml (range: 0.01-6.8) and were available in 135 women (75.8% of the total group). Regression analysis showed among the variables, the number of oocytes was independently associated with both clinical pregnancy and LB's (**Tables 3 and 4**). As expected, an increase in oocyte number was significantly associated with likelihood of CP and LB in this group.

DISCUSSION

This study explored the probability of live birth after ART in women aged >42 years with their oocytes. Our results

Table 3. Binary Logistic Regression Models for Single Variables to Predict Cumulative Clinical Pregnancy

Variables	Single Variables		
	p	OR	95% C.I.
Age	0.040 ^a	0.401	0.167-0.959
AMH	0.023 ^a	2.084	1.106-3.928
Number of oocytes	<0.001 ^a	1.606	1.275-2.023
Final Model ^b			
Variables	p	OR	95% C.I.
Age	0.162	0.438	0.137-1.395
AMH	0.693	1.159	0.557-2.410
Number of oocytes	0.027 ^a	1.505	1.048-2.159

^a p<0.05, statistically significant; C.I.: Confidence Interval; OR: Odds ratio

^b Multiple logistic regression final model was executed on all these variables, included together in the model

Table 4. Binary Logistic Regression Models for Single Variables to Predict Cumulative Live Birth

Variables	Binary Logistic Regression Models for Single Variables		
	p	OR	95% C.I.
Age	0.993	-	-
AMH	0.063	2.145	0.960-4.792
Number of oocyte	0.030 ^a	1.696	1.052-2.732

^a p<0.05, statistically significant; C.I.: Confidence Interval; OR: Odds ratio

suggested that live birth rates are extremely low for those patients. The real success of the ART treatments is the birth of a healthy infant. Specific counseling on the ART success rates for advanced reproductive-aged women who have a low chance of live birth is pivotal in clinical practice. Most of those women do not sufficiently know the age-related decline of the pregnancy and live birth rates of ART treatments and also medical risks of delayed childbearing. The center's data results on this specific issue may help patients to realize their expectations.

In a study by Devesa et al. (2018), cumulative LBR's including fresh and frozen ET's after single stimulation cycles of 4570 infertile women aged ≥ 38 years were evaluated according to the age and number of oocyte yields [19]. In their study, the most prominent decrease in LBR's was shown at 42-43 years, and the cumulative LBR was the only 1.2% in women aged ≥ 44 years. Authors also reported that a higher number of oocytes related with higher cumulative LBR's more evidently up to 41 years old, but without clear benefit beyond 44 years of age [19]. Substantial contribution of cryopreserved embryos to cumulative LBR's was shown until women aged 42 years. The observed benefit was limited to 3% and 0% for 42-43 years and aged beyond 44 years, respectively. The majority of the cryopreservation method in that study was slow freezing; thus, the authors emphasized that the results need to be interpreted with caution. The authors also concluded that IVF with own oocytes should be discouraged in women aged over 43 years based on their results.

Spandorfer et al. evaluated the IVF outcomes of women age between 45-49. The authors reported that, of the 231 initiated IVF cycle, 2.2% ended with delivery, and only patients at 45 years of age and with good response have been achieved this result [11]. A decade later, in another retrospective study from the same center, Gunnala et al. analyzed 1078 fresh autologous IVF cycles of women aged 45 and over, without preimplantation genetic diagnosis (PGD), including a 20-year period. [12]. The LBR per cycle was 2.9% at 45 years and 0.5% at 46 years group and the oldest age who delivered was 46 in this group. The authors noticed high pregnancy loss rates (82.3%). They concluded that live birth with autologous IVF in women aged over 46 is unlikely.

The increased embryo aneuploidy rates by advanced women age are well known to cause the age-related decline in pregnancy rates [20]. Mechanisms of age-related oocyte aneuploidies and impairment of oocyte competence have been discussed in detail [21-23]. Preimplantation genetic screening has been suggested for advanced age women as a logical approach for improving ART success by selecting euploid embryos [24,25]. Ubaldi et al. (2017) evaluated results of PGD-A of 137 women aged ≥ 44 years with clinical outcomes [25]. No euploid blastocysts for patients aged over 45 were reported. The delivery rate of 10.0% per cycle for the age of 44 and 2.6% for the age of 45 was shown after PGD-A

in their study group. The authors suggested the use of PGD-A for these women with good ovarian reserve [25].

However, low oocyte yield is a limiting factor for PGD in advanced age women. To overcome this situation, embryo or oocyte pooling with several consecutive conventional stimulation cycles or both follicular and luteal phase stimulation in the same cycle has been proposed [26-28]. Nevertheless, limited data on this approach's efficiency and cost-effectiveness has been noticed [19,25,29]. In our study population, PGD-A was performed only in 5.6% of the patients, possibly due to the procedure's cost and time need.

In a recent study, Warshaviak et al. compared morphokinetic parameters of embryos from women aged >42 years (51 women, 496 embryos) and <38 years (71 women, 653 embryos), during the first 3 days of development, by time-lapse microscopy [30]. No significant differences in the evaluated parameters except tendency toward developmental arrest until the 8-cell stage in the advanced age group were reported. The authors suggested, a possible significant delay could be seen in later morphokinetic parameters if the culture period extended to the blastocyst stage. In their group, the LBR's were 2% for women aged >42 years old, with significantly lower than the younger group (27%). Gleicher et al. specifically reported the pregnancy outcomes of the extremely advanced age women (43 to 51 years) following IVF with autologous oocytes during the 2014-2016 periods [13]. The LBR's were 1.1%, 0.43%, and 1.3% per cycle for the yearly bases, respectively. They have reported two pregnancies with autologous IVF in the two oldest women aged 47 in the presented group. One of them ended with live birth and other loss of pregnancy with complex aneuploidy. They concluded that in selected women at very advanced age with a good ovarian reserve and with individualized managing, IVF with own oocytes can be an option.

Our study presents, pregnancy outcomes of women aged >42 years following ART from a single tertiary IVF unit. This study's strength is evaluating cumulative pregnancy outcomes of a significant number of advanced age women from a single IVF unit including the last five-year period. Although its retrospective nature is a limitation, the study results are expressing the daily clinical practice.

In conclusion, our study results showed extremely low live birth rates in women aged >42 years. However, there might be cases with a successful result. Good ovarian response and availability of supernumerary embryos for future frozen-thawed transfers might give a chance for these patients to conceive. As with all infertile patients, an individualized approach should be taken into account in advanced age groups.

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