MEDICINE & PUBLIC HEALTH
e-ISSN 2956-9443

2025, Vol. 3, No. 1
DOI: 10.48269/MandPH-AFMKU-25-1-004
Received: 2025-05-19
Accepted: 2025-10-06

ORIGINAL RESEARCH PAPER

Very advanced maternal age – chance of pregnancy after ICSI

Jakub Wyroba[1,2,A–B,D,F], *Katarzyna Kostarczyk*[3,C–F], *Joanna Kochan*[1,3,B–D], *Alicja Lachowska*[1,B,E]

[1] Malopolski Institute of Fertility Diagnostics and Treatment – KrakOvi, Krakow, Poland

[2] Fertility Disorders Clinic, Medical College, Andrzej Frycz Modrzewski Krakow University, Poland

[3] Department of Animal Reproduction, Anatomy and Genomics, University of Agriculture in Krakow, Poland

A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Abstract

Background: Postponing procreation becomes an increasing problem in developed countries. Maternal aging contributes to intensive development of assisted reproduction techniques (ART). The aim of the study was to analyze the results of in vitro fertilization in women of very advanced maternal age (AMA, ≥42Y) using autologous oocytes (AO) or donor oocytes (DO) compared to women ≤35Y.

Material and methods: This was a retrospective study of AMA women who underwent intracytoplasmic sperm injection (ICSI) using AO or DO. The control group consisted of young patients with age ≤35Y.

Results: In group of AMA patients, the AMH level was significantly lower (0.56 ng/ml) than in young patients (3.6 ng/ml), which corresponded to obtaining a significantly lower number of oocytes (1.6±0.4 vs 12±4 oocytes/cycle), ($p < 0.001$). In older patients with AO only 0.4±0.1 blastocysts/cycle were obtained compared to 1.9±0.7 when DO were used and 3.8±1.1 from young women. Euploidy rate of blastocysts obtained after fertilization of AO of AMA patients was 26% in compare to 57% in a group of young women and 54% in the group of older patients with DO. The implantation rate was significantly lower in the group of older patients with AO (28%) compared to the other groups (50%, 59%), ($p < 0.001$).

Conclusions: AMA patients must be aware that IVF procedure does not guarantee pregnancy and the birth of a healthy child and they should be offered pre-implantation PGT-A diagnostics due to the high risk of chromosomal defects. These patients should also be informed about the much higher effectiveness of IVF using DO.

Keywords: advanced maternal age, IVF, ART, reproductive aging, oocyte donation

Introduction

Recently, in high-income countries, there has been a tendency to delay childbearing to a later time in a woman's life. The number of births among

✉ Alicja Lachowska, e-mail: ala.lachowska@gmail.com

women of an advanced maternal age is constantly increasing, especially in women over 40 years of age [1,2]. According to US reports from 2006–2007 to 2014–2015, over this timespan there was an 8% increase in the number of births for women aged 40–44 and 26% for women aged 45–54 [3]. It is common knowledge that, as a woman ages, her reproductive potential decreases. An advanced maternal age (AMA), defined as >35 years, is associated with an increased risk of maternal and perinatal complications, especially in women over 40 years of age [4]. For women over 35 years, the fertilized egg will be of lower quality and exhibit reduced developmental competence. Due to decreased tubal function and a delay in oocyte transportation, the risk of ectopic pregnancy is between 4 and 8 times greater [5]. Maternal age is also a risk factor for miscarriages in the first and second trimester of pregnancy. An advanced maternal age increases the risk of chromosomal abnormalities in the foetus, which is a consequence of abnormal separation of chromosomes during cell division [6]. The exponential increase in the risk of chromosomal abnormalities occurs between the ages of 35 and 40 [7]. An advanced maternal age is also associated with an increased risk of pregnancy abnormalities and adverse obstetric outcomes. The newborns of women aged >40 years have a higher risk of heart defects and oesophageal atresia, which is twice as high as in younger mothers [8]. There is an increase in the number of labour dystocia and the rate of intrapartum caesarean sections in AMA women [9].

In developed countries, where the problem of delayed procreation is more common, women have access to assisted reproductive techniques (ART), which enable them to have children as late as after the age of sixty, by offering oocyte donation programmes [10]. Moreover, following in vitro fertilization (IVF), access to prenatal diagnostics allows foetal chromosomal abnormalities to be detected in women of advanced age. However, access to various ART procedures, as well as the age limit for women, depends on the legal regulations of a given country.

The aim of the study was to analyze the results of in vitro fertilization in women of very advanced maternal age (≥42Y) using autologous oocytes or donor oocytes compared to women ≤35Y.

Material and methods

This was a retrospective study of women of very advanced maternal age (≥42Y), who underwent intracytoplasmic sperm injection (ICSI) in the KrakOvi Clinic in Kraków (Poland) between 2021 and 2024. The control group consisted of patients aged (≤35Y). The research was carried out in accordance with the guidelines of the local bioethics committee.

The patients were treated using either the long agonist protocol (n = 28) or short antagonist protocol (n = 92). The type of protocol used depended on the level of AMH (Anti-Mullerian hormone) and the overall risk of hyperstimulation.

Long agonist protocol started one week before the expected menses (cycle day 18–23), when patients received the GnRH agonist, triptorelin (Decapeptyl, Ferring Pharmaceuticals, 1 mg/d, sc). After successful pituitary downregulation (when the serum estradiol [E2] levels were <40 pg/mL), ovarian stimulation was commenced with a fixed daily dose of 150–300 IU recombinant follitropin alfa (rFSH, sc) with or without an additional 75–150 IU menotropin (hMG).

A GnRH antagonist Cetrorelix (Cetrotide, Merck Europe, 0.25 mg/d, sc or Ganirelix, Gedeon Richter 0.25 mg/d), was administered, commencing when the largest follicle reached a diameter of 14 mm rFSH/hMG was initiated on day 2–4 of the cycle.

The agonist and antagonist protocols were continued up to and including the day of human chorionic gonadotropin (hCG) administration, which was when the leading follicle reached a diameter of 18 mm or more and at least three follicles reached a diameter of 17 mm or more. rFSH was then stopped, and a single sc bolus of 10,000 IU hCG (Eutrig – Samarth Life Sciences) or 6,500 IU rhCG (Ovitrelle – Merck) was administered 36 h before the planned time of oocyte retrieval. When there was a risk of OHSS in an antagonist cycle, the trigger was a single sc bolus of triptorelin 2 mg, and a freeze-all policy was applied. All follicles 12 mm or larger were aspirated.

Oocyte-cumulus complexes (COCs) were identified using a stereoscopic microscope, then washed and after 3 h of incubation (approx. 3 h) the cumulus cells were removed using hyaluronidase (Gynemed, Germany) and mechanical pipetting. Only oocytes in metaphase II with a first polar body were used for further procedures. Intracytoplasmic sperm injection (ICSI) was performed following the standard technique. Embryos were cultured in SAGE® medium (Origio, Denmark) under an atmosphere of 6.0% CO_2, 5.0% O_2 and balance nitrogen at 37°C. Embryo development was assessed every day. The blastocysts were graded according to the Gardner scoring criteria [8]. Blastocysts PGT-A tested were biopsied 120–124 h after ICSI (day 5), using the same micromanipulator and microscope used for ICSI. The zona pellucida was perforated using an Octax® laser (Vitrolife, Sweden) for 250 µsec. The biopsied TE cells were washed with D-PBS and placed in 0.2 mL polymerase chain reaction (PCR) tubes for PGS referral to Igenomix Inc. (Spain) and analyzed using next-generation sequencing (NGS). Following the biopsy, blastocysts were incubated for 1.5 h in Sage®medium and then vitrified.

The blastocysts were vitrified using Kitazato® media and the Cryotop device (Kitazato, Japan) according to the manufacturer's protocol. They were warmed in Kitazato media for a minimum of 1.5 h before transfer and then placed in EmbryoGlue® medium (Vitrolife, Sweden) medium.

Clinical pregnancy was identified by the ultrasound confirmation of an intrauterine gestational sac after 8 weeks of gestation with visible foetal cardiac activity. Ongoing pregnancy was confirmed when over 12 weeks of gestation had passed with visible foetal cardiac activity.

Statistical analysis

Non-parametric data, such as differences in the percentage values between groups, were assessed using the chi-squared test. Parametric data were expressed as means±SD and compared by two-way ANOVA. Differences were considered significant when the P-value was ≤0.05. The statistical analysis was performed using PQStat 1.6.2 (PQStat Soft, Poznan, Poland).

Results

The study analyzed the results of in vitro fertilization in 75 patients with advanced maternal age (≥42Y) and 45 patients aged <35Y. In the group consisting of patients ≥ 42Y, 45 of them used autologous oocytes and 30 took advantage of the oocyte donation programme. The results of IVF in the study groups are presented in Table 1 and Figure 1. In the group consisting of AMA patients, the AMH level was significantly lower (0.56 ng/ml) than in younger patients (3.6 ng/ml), ($p < 0.001$), which corresponded to a significantly lower number of oocytes (1.6±0.4 vs. 12±4 oocytes/cycle), ($p < 0.001$). In patients using donor oocytes, a standard package of six oocytes was used for one cycle. In older patients after fertilization with autologous oocytes only 0.4±0.1 blastocysts/cycle were obtained compared to 1.9±0.7 when donor oocytes were used and 3.8±1.1 from younger women. Due to the risk of genetic defects associated with AMA in the study group with autologous oocytes, as many as 83% of blastocysts were PGT-A tested with PGT-A and only 26% of euploid blastocysts were obtained. For comparison, in the group consisting of younger patients, only 51% of blastocysts were PGT-A tested, and the euploid rate was 57%. A similar euploidy rate (54%) was obtained in the group of older patients using donor oocytes. Only seven cycles (15%) ended in embryo transfer in the AMA group with autologous oocytes compared to 70% in the AMA group with donor oocytes and 75% in the younger group. Furthermore, the implantation rate was significantly lower in the group consisting of older patients with autologous oocytes (28%) compared to the other groups (50%, 59%), ($p < 0.001$).

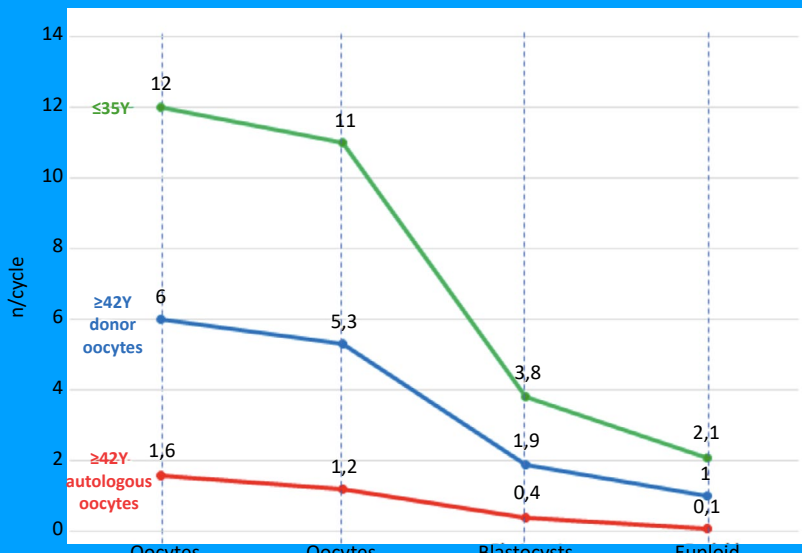
However, the number of ET (embryo transfer) in this group was so small that a large statistical error must be taken into account.

Table 1. In vitro fertilization results depending on women's age and type of oocytes (autologous or donor)

	Advanced maternal age		**Control**
	≥42Y autologous oocytes	**≥42Y donor oocytes**	**≤35Y autologous oocytes**
No. of patients (cycle), *n*	45	30	45
Age (years), range, mean±SD	42–50 43.5±1.5	42–49 43.7±1.6	28–35 32±2.8
BMI, mean±SD	23±2	23±3	22±2
AMH (ng/ml), mean±SD	0.56±0.3[a]	0.51±0.2[a]	3.6±0.8[c]
Oocytes recovery, *n* mean±SD	72 1.6±0.4[a]	-	540 12±4[c]
Oocytes M II, *n* mean±SD	54 1.2±0.1[a]	159 5.3±0.5[b]	495 11±2[c]
Blastocysts, *n* mean±SD	18 0.4±0.1[a]	57 1.9±0.7[b]	171 3.8±1.1[c]
PGT-A tested blastocysts, *n* (%)	15/18 (83%)[a]	24/57 (53%)[c]	88/171 (57%)[c]
Euploidy blastocysts, *n* (%)	4/15 (26%)[a]	13/24 (54%)[c]	50/88 (57%)[c]
ET (day 5) *n* (%)	3/45 (6%)[a]	10/30 (30%)[c]	16/45 (35%)[c]
FET, *n* (%)	4/45 (8%)[a]	11/30 (36%)[c]	18/45 (40%)[c]
Implantation rate, *n* (%)	2/7 (28%)[a]	11/22 (50%)[c]	20/34 (59%)[c]
Cycle cancelled due to lack of oocytes or blastocysts	21/45 (46%)[a]	7/30 (23%)[c]	6/45 (13%)[c]

BMI – the body-mass index is the weight in kilograms divided by the square of the height in metres, AMH – Anti-Mullerian hormone, ET – embryo transfer, FET – frozen embryo transfer,
a:b – values with different superscripts within the same rows differ significantly ($p < 0.05$)
a:c, b:c – values with different superscripts within the same rows differ highly significantly ($p < 0.001$)

Figure 1. Efficiency of IVF depending on women's age and type of oocytes (autologous or donor)



Discussion

Postponing procreation is becoming an increasing problem in developed countries. The issue of maternal aging is contributing to the intensive development of ART, preimplantation genetic diagnosis (PGD) and oocyte donation programmes (ODP) [11].

A marker of ovarian reserve and thus the chances of natural conception is AMH, which decreases with age, and a low or extremely low ovarian reserve makes natural conception impossible [12]. In our study, in the group of women of advanced maternal age we observed drastically lower AMH levels compared to women ≤35Y. In AMA patients we obtained only an average of 1.6±0.4 oocytes after hormonal stimulation, compared 12±1.1 oocytes from younger patients. Therefore, it can be assumed that for these women IVF is the only chance of achieving pregnancy. In one in four AMA patients we failed to obtain an oocyte capable of fertilization. With such a small number of oocytes, a correspondingly small number of embryos at the blastocyst stage are obtained.

In AMA patients, the problem is not only the quantity but also the quality of oocytes, which age together with the patient, and the chromosomal

disorders that occur in them lead to genetic defects in embryos [13,14]. There is no doubt that maternal aging contributes to the increased rates of aneuploidy in embryos. Therefore, for AMA patients, preimplantation genetic testing (PGT-A) is recommended. Over the last decade, preimplantation genetic testing (PGT-A) has become a very important tool for the selection of healthy blastocysts for transfer [15]. In the group of older patients, the embryo transfer at day 5 is most often abandoned because the patients decide to undergo PGT-A, but the problem is the small number of blastocysts that can be tested for PGT-A. Another key problem is the euploidy rate, which decreases dramatically with age. The euploidy rate of blastocysts obtained after fertilization of the autologous oocytes of AMA patients was 26%, compared to 57% in the group consisting of young women.

In the group of AMA patients using donor oocytes, significantly better results were achieved than with autologous oocytes, but worse than in younger patients, even though the oocyte donors are of a similar age to the control group. The poorer results may be due to the fact that the donor oocytes were vitrified, which could have impaired their developmental competence. In addition, the donation programme used packages of six oocytes/cycle and in the group of young patients, an average of 11 oocytes were fertilized. However, the euploidy rate of blastocysts was similar after the donor oocytes and oocytes from the group of young patients had been fertilized

To sum up, patients of advanced age must be made aware that using the IVF procedure does not guarantee pregnancy and the birth of a healthy child. Moreover, AMA patients should be offered pre-implantation PGT-A diagnostics due to the high risk of chromosomal defects. These patients should also be informed about the much higher effectiveness of IVF using donor oocytes.

References

1. Tough SC, Newburn-Cook C, Johnston DW, Svenson LW, Rose S, et al. Delayed childbearing and its impact on population rate changes in lower birth weight, multiple birth, and preterm delivery. *Pediatrics*. 2002;109(3):399–403.
2. Martin JA, Hamilton BE, Osterman MJK. Births in the United States, 2017. *NCHS Data Brief*. 2018;(318):1–8.
3. Sheen J-J, Wright JD, Goffman D, Kern-Goldberger AR, Booker W, et al. Maternal age and risk for adverse outcomes. *Am J Obstet Gynecol*. 2018;219(4):390.e1–390.e15.

4. Zipori Y, Linder R, Khatib N, Weiner Z, Barzilay E. Advanced maternal age and perinatal outcome in twin pregnancies: a meta-analysis. *J Matern Fetal Neonatal Med*. 2020;33(18):3193–3199.
5. Farr SL, Schieve LA, Jamieson DJ. Pregnancy loss among pregnancies conceived through assisted reproductive technology, United States, 1999–2002. *Am J Epidemiol*. 2007;165(12):1380–1388.
6. Kolevzon A, Gross R, Reichenberg A. Prenatal and perinatal risk factors for autism: A review and integration of findings. *Arch Pediatr Adolesc Med*. 2007;161(4):326–333.
7. Savva GM, Walker K, Morris JK. The maternal age-specific live birth prevalence of trisomies 13 and 18 compared to trisomy 21 (Down syndrome). *Prenat Diagn*. 2010;30(1):57–64.
8. Gill SK, Broussard C, Devine O, Green RF, Rasmussen SA, et al. Association between maternal age and birth defects of unknown etiology: United States, 1997–2007. *Birt Defects Res A Clin Mol Teratol*. 2012;94(12):1010–1018.
9. Martin JA, Hamilton BE, Osterman MJK, Driscoll AK, Mathews TJ. Births: final data for 2015. *Natl Vital Stat Rep*. 2017;66(1):1.
10. Laskov I, Michaan N, Cohen A, Tsafrir Z, Maslovitz S, et al. Outcome of twin pregnancy in women ≥45 years old: a retrospective cohort study. *J Matern Fetal Neonatal Med*. 2013;26(7):669–672.
11. Mastenbroek S, de Wert G, Adashi EY. The Imperative of Responsible Innovation in Reproductive Medicine. *N Engl J Med*. 2021;385(22):2096–2100.
12. Russell N, Gilmore A, Roudebush WE. Clinical Utilities of Anti-Müllerian Hormone. *J Clin Med*. 2022;11(23):7209.
13. Wasielak-Politowska M, Kordowitzki P. Chromosome Segregation in the Oocyte: What Goes Wrong during Aging. *Int J Mol Sci*. 2022;23(5):2880, https://doi.org/10.3390/ijms23052880
14. Van der Reest J, Nardini Cecchino G, Haigis MC, Kordowitzki P. Mitochondria: Their relevance during oocyte ageing. *Ageing Res Rev*. 2021;70:101378.
15. Yang H, DeWan AT, Desai MM, Vermount SH. Preimplantation genetic testing for aneuploidy: challenges in clinical practice. *Human Genomics*. 2022;16(1):69.