ORIGINAL ARTICLE

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Comparison of Live Birth Rates Between Frozen-Thawed and Fresh Blastocyst Transfer for Male Infertility

Comparación de las tasas de nacidos vivos entre la transferencia de blastocitos congelados y frescos para la infertilidad masculina

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ABSTRACT

Background: In recent years, with the development of assisted reproductive techniques, different in vitro fertilization-embryo transfer protocols have been developed. Embryo cryopreservation has emerged as an alternative to fresh embryo transfer. The superiority of fresh versus frozen-thawed embryo transfer in terms of pregnancy outcome varies among studies. Objective: To compare pregnancy and live birth rates resulting from fresh and frozen-thawed blastocyst transfers in male infertile couples. Methods: In this retrospective study, 803 intracytoplasmic sperm injection-embryo transfer cycles (304 fresh and 499 frozen-thawed) from 2019 to 2022 were reviewed. The results of pregnancy rates were compared as a function of embryo transfer method. In addition, pregnancy rates were evaluated as a function of embryo transfer method and maternal age. Results: The mean age of women in the fresh and frozen-thawed blastocyst transfer groups and the proportion of fresh and frozen-thawed transfers in the age groups were similar. Pregnancy rates of all cases differed between the two groups, with higher rates observed in the group that underwent fresh blastocyst transfer. However, live birth rates were similar. In addition, when pregnancy rates were compared according to maternal age, no significant differences were found. In all cases and fresh blastocyst transfer, pregnancy rates decreased with increasing age. Conclusion: Our results indicate comparable live birth rates with fresh embryo transfer compared to frozen-thawed transfers in male factor infertility.

Key words: Fertilization in vitro, Vitrification, Embryo Transfer, Pregnancy rate, Live birth

RESUMEN

Antecedentes. En los últimos años, con el desarrollo de las técnicas de reproducción asistida se han desarrollado diferentes protocolos de fecundación *in vitro*-transferencia embrionaria. La criopreservación de embriones ha surgido como una alternativa a la transferencia de embriones frescos. La superioridad de la transferencia de embriones frescos frente a los congelados-descongelados en términos de resultados de embarazo varía según los estudios. Objetivo. Comparar las tasas de embarazo y de nacidos vivos resultantes de las transferencias de blastocitos frescos y congelados-descongelados en parejas infértiles masculinas. Métodos. En este estudio retrospectivo se revisaron 803 ciclos de inyección intracitoplasmática de espermatozoides-transferencia de embriones (304 frescos y 499 congelados-descongelados) de 2019 a 2022. Se compararon los resultados de las tasas de embarazo en función del método de transferencia de embriones. Además, se evaluaron las tasas de embarazo en función del método de transferencia de embriones y la edad materna. Resultados. La edad media de las mujeres de los grupos de transferencia de blastocitos frescos y congelados-descongelados y la proporción de transferencias frescas y congeladas-descongeladas en los grupos de edad fueron similares. Las tasas de embarazo de todos los casos difirieron entre los dos grupos, observándose tasas más elevadas en el grupo que se sometió a transferencia de blastocitos en fresco. Sin embargo, las tasas de nacidos vivos fueron similares. Además, cuando se compararon las tasas de embarazo en función de la edad materna, no se encontraron diferencias significativas. En todos los casos y en la transferencia de blastocitos frescos, las tasas de embarazo disminuyeron al aumentar la edad. Conclusión. Nuestros resultados indican tasas de nacidos vivos comparables con la transferencia de embriones frescos en comparación con las transferencias congeladas-descongeladas en la infertilidad por factor masculino.

Palabras clave. Fecundación in vitro, Vitrificación, Transferencia embrionaria, Tasa de embarazo, Nacido vivo

Introduction

Male factors are responsible for 30-40% of infertility cases and affect 7% of the total male population[1]. In recent years, an increasing number of couples diagnosed with infertility have resorted to assisted reproductive techniques (ART), resulting in a higher proportion of ART births, currently reaching 2.4%[2]. Intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of male infertility and currently accounts for 70-80% of the cycles performed. Preserving structural and functional integrity during sperm selection maximizes the success of ICSI[3]. However, other factors, such as advanced maternal age and whether the embryo transfer (ET) is fresh or frozen, are also important to consider when evaluating the results of ICSI. The effect of advanced maternal age on fertility has been extensively investigated. Decreased ovarian reserve and endometrial receptivity particularly reduce the chances of achieving a successful pregnancy[4].

The "freeze" strategy of cryopreservation has emerged as a valuable alternative to fresh embryo transfer. Infertility treatment specialists increasingly recommend freezing all high-quality embryos and performing embryo transfer during a natural or modified natural cycle. This approach is especially useful in cases where pathologies prevent immediate embryo transfer or for future use. Although clear benefits have been shown for several patient subgroups, further research is needed before it can be applied to all patients[5,6]. Vitrification is an ultra-rapid cryopreservation method widely used for the cryopreservation of gametes, embryos, and ovarian tissue. The vitrification process involves the use of high-concentration cryoprotectants to solidify to a glass-like state, minimizing the formation of ice crystals[7].

Several studies have shown that cryopreservation improves fertility potential after a single cycle of ovarian stimulation. In addition, embryo cryopreservation significantly reduces the rate of multiple pregnancies and the risk of implantation failure, especially due to ovarian hyperstimulation syndrome (OHSS) and diminished endometrial receptivity[8–11]. Frozen embryos can be thawed and transferred once the deleterious effects of the high doses of hormones administered during controlled ovarian stimulation have been eliminated[12,13]. However, while some studies indicate that embryo cryopreservation may lead to a better prognosis and higher pregnancy rates in women[1], other studies have reported lower pregnancy rates and an increased risk for preterm or low birth weight after transfer of frozen-thawed embryos (FET)[14–16].

The aim of this study was to compare pregnancy outcomes between fresh ET and FET after ICSI treatment, to further investigate the debate between fresh versus frozen embryo transfer in couples with male infertility.

Material and Methods

In this retrospective cross-sectional study, ICSI-ET cycles (304 fresh ET and 499 FET) of 803 cases meeting the study criteria were analyzed. These cases were drawn from a larger pool of 1,163 cases who applied to Acıbadem Altunizade Hospital IVF Center, Istanbul, for ICSI treatment between 2019 and 2022. Inclusion criteria were limited to ICSI treatment for male factor infertility, including oligospermic, cryptozoospermic, azoospermic and asthenozoospermic patients as defined by semen analysis. In addition, all female partners had to be ≤40 years old with anti-Müllerian hormone (AMH) levels 1-3.5 ng/mL, follicle-stimulating hormone (FSH) levels within normal limits on the third day of the menstrual cycle, and endometrial thickness within normal limits. They had no tubal factor and underwent one embryo transfer. Women diagnosed with female factor infertility, endocrine disorders, endometriosis, fibroids, tubal factors, and uterine anomalies were excluded.

The women were divided into three age groups and the study compared the pregnancy outcomes of the different groups according to ET method and age. The study protocol was conducted in accordance with the ethical approval granted by the Ethics Committee of the University of Maltepe (protocol number 2023/18-06).

For follicular development, a GnRH antagonist was used on the third day of the menstrual cycle to obtain a controlled ovarian stimulation. GnRH antagonist Cetrotide (260-270 µg cetrorelix acetate; Merck) and recombinant follicle-stimulating hormone (FSH) (Gonal-F, follitropin alpha, 900IU/1.5mL; Merck Serono, Lyon, France) or follitropin beta (Puregon, 833IU/mL;

MSD, München, Germany) were used. Ultrasound monitoring was performed and when the follicles reached approximately 18-20 mm in diameter, hCG (Ovitrelle 250mg/0.5mL choriogonadotropin-alpha, Merck Serono, Rome, Italy) was administered. A transvaginal USG-guided ovum pick-up (OPU) was performed under general anesthesia 34-36 hours after ovulation was triggered. ICSI was performed on Metaphase II (MII) oocytes immediately after denudation.

Fertilization control was performed 12 to 18 hours following ICSI as evidenced by the presence of two pronuclei and two polar bodies. Embryo development was monitored over a five-day period by a time-lapse system (EmbryoScope™ Time-lapse System; Unisense FertiliTech, Aarhus, Denmark). The blastocysts were classified as poor, fair, good, or excellent in accordance with the grading system established by Gardner and Schoolcraft[17]. All embryo transfers were performed on day 5.

Fresh ET on day 5 was performed for patients without contraindications. Fresh ET on day 5 was performed for patients without contraindications. Embryos from patients who developed ovarian hyperstimulation syndrome, those with insufficient endometrial thickness and those with elevated progesterone levels were frozen. Blastocysts from patients without fresh transfers were vitrified using the Kitazato Vitrification Cryotop® kit (Kitazato, Japan) according to the manufacturer's instructions. These patients were treated with oral or transdermal estradiol (E2) on days 1-15 to prepare the endometrium for the planned cycle and a transvaginal ultrasound was performed 10-15 days later. The FET or fresh ET cycle is performed when the endometrial thickness is greater than 8 mm and the progesterone level is below 1.2 ng/mL. The luteal phase was supported by daily oral or injectable progesterone administration for 2 weeks.

Serum β-hCG levels were measured on days 10-12 after embryo transfer. Cases in which a gestational sac was observed were considered positive for pregnancy.

The NCSS (Number Cruncher Statistical System) 2020 statistical software package (NCSS LLC, Kaysville, Utah, USA) was used for statistical analysis. Data are presented as mean ± standard deviation (SD) or number and percentage. Normal distribution of data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, as well as box plots. Categorical data were compared by Pearson chi-square test and Bonferroni post hoc tests. For quantitative assessments of two normally distributed groups, Student's t-test was used. The results were evaluated with a 95% confidence interval and a significance level of $p<0.05$.

Results

A total of 803 infertile couples who met the study criteria were included in the study. Descriptive characteristics, such as age of women (mean ± SD), age group distribution (number [n] and percentage [%]), blastocyst transfer method (n, %), pregnancy (n, %) and live birth (n, %) are shown in Table 1. The cases were divided into two groups according to embryo transfer method, namely fresh blastocyst transfer or frozen-thawed blastocyst transfer, and into three groups according to the age of the women. The study included women aged 21-38, with a mean age of 32.4 ± 3.9. The largest group of women (48.9%) were between 29-34 years old, while 16.9% were between 21-28 years old and 34.1% were over 35 years old. Frozen-thawed blastocyst transfer was performed in 62.1% (n=499) of cases, while fresh blastocyst transfer was performed in 37.9% (n=304). The pregnancy rate was 68.1% and the live birth rate was 50.6%. Blastocyst transfer was performed in all 803 cases.

Table 2 compares the mean age (mean ± SD), age group distribution (n, %), pregnancy rates (n, %), and pregnancy outcomes according to embryo transfer type. Pregnancy rates after fresh blastocyst transfer were higher than after frozen and thawed blastocyst transfer (p=0.039). The

Table 1. Descriptive characteristics of patients. Data are presented as mean ± standard deviation (SD) or number (n) and percentage (%).

		n (%)
Woman age	**Mean±SD**	**32.35±3.90**
Age groups	21-28 years	136 (16.94)
	29-34 years	393 (48.94)
	≥35 years	274 (34.12)
Blastocyst transfer	Frozen-thawed	499 (62.14)
	Fresh	304 (37.86)
Pregnancy	Negative	256 (31.88)
	Positive	547 (68.12)
Live birth		406 (50.56)

Table 2. Comparison of age, age groups, pregnancy rates, and pregnancy outcomes in frozen-thawed blastocyst transfer and fresh blastocyst transfer groups. Data are presented as mean ± standard deviation (SD) or number (n) and percentage (%). *significant difference compared to the fresh blastocyst transfer group ($p<0.05$).

	Frozen-thawed blastocyst transfer group (n=499)	Fresh blastocyst transfer group (n=304)	*p*
Women age (mean±SD)	**32.41±3.90**	**32.25±3.90**	**a0.580**
Age groups n (%)			
21-28 years	83 (16.64)	53 (17.43)	b0.858
29-34 years	247 (49.50)	146 (48.03)	
≥35 years	169 (33.86)	105 (34.54)	
Pregnancy rates n (%)			
All cases	326 (65.33)	221 (72.70)	b0.039*
21-28 years	61 (18.71)	43 (19.46)	b0.306
29-34 years	163 (50.00)	109 (49.32)	b0.055
≥35 years	102 (31.28)	69 (31.22)	b0.636
Pregnancy outcomes (%)			
Abortion	6 (1.83)	4 (1.81)	b0.709
Ectopic pregnancy	2 (0.61)	2 (0.90)	
Clinical pregnancy	34 (10.40)	22 (10.00)	
Chemical pregnancy	31 (9.48)	23 (10.45)	
Live birth	247 (75.54)	159 (72.27)	
Not available	7 (2.14)	10 (4.55)	

aStudent-t Test bPearson Chi-Square Test *p<0.05

mean age, age group distribution and pregnancy rates of age groups 21-28, 29-34 and ≥35 did not differ between women undergoing fresh blastocyst transfer and frozen-thawed blastocyst transfer (p=0.580; p=0.858; p=0.306; p=0.055; p=0.636, respectively). Pregnancy outcomes were compared between fresh and frozen blastocyst transfer and no statistically significant differences were found (p=0.709). Live birth rates were similar in both groups.

Table 3 compares pregnancy rates in all cases, in the fresh blastocyst transfer group and in the frozen-thawed blastocyst transfer group as a function of maternal age. Pregnancy rates decreased significantly over the age of 35 years when all cases were evaluated (p=0.013). Although there was no significant difference in pregnancy rates between the 21-28 and 29-34 age groups (76.5% vs. 69.2%, p=0.108), there was a statistically significant difference between the 21-28 and over 35 age groups (76.5% vs. 62.4%, p=0.004). A similar decline was observed in the fresh blastocyst transfer group when fresh and frozen-thawed blastocyst groups were evaluated separately (p=0.045). Although pregnancy rates were not different between the 21-28 and 29-34 age groups (81.1% vs. 74.7%, p=0.381), a statistically significant decrease was observed

Table 3. Comparison of pregnancy rates in all cases, in the fresh blastocyst transfer group and in the frozen-thawed blastocyst transfer group by woman age. Data are presented as n (%). Significant difference compared to the 21-28 years group (#$p<0.05$, ##$p<0.01$). Significant difference compared to the age group (^$p<0.05$).

	Pregnancy positive (%)			
	21-28 years	**29-34 years**	**≥35 years**	***p***
All cases n (%)	104 (76.47)	(272) 69.21	(171) 62.41##	b0.013^
Frozen-thawed Blastocyst Transfer n (%)	61 (75.31)	163 (66.00)	102 (60.36)	b0.160
Fresh Blastocyst Transfer n (%)	43 (81.13)	109 (74.66)	69 (65.71)#	b0.045^

bPearson Chi-Square Test #p<0.05 ##p<0.01 ^p<0.05

above age 35 compared to the 21-28 age group (65.7% vs. 81.1%, p=0.028). However, in the case of frozen-thawed blastocyst transfer, there was no difference (p=0.16) in pregnancy rates between different age groups.

Discussion

Cryopreservation of human gametes and embryos have revolutionized the treatment of infertility and is increasingly used to preserve fertility[18–21]. Recently, the Practice Committee of the American Society for Reproductive Medicine has issued a guideline stating that oocyte cryopreservation is no longer an experimental treatment, paving the way for its use as a treatment modality[22]. Moreover, in recent years, infertility treatment specialists are increasingly recommending the freezing of good-quality embryos and planning patients for deferred embryo transfer in more controlled cycles[6]. Vitrification is becoming an important option for fertility preservation by freezing embryos for later use, especially in patients with OHSS and germ cells in patients with gonadal failure. However, some studies have reported that cryopreservation negatively affects embryo quality, decreases pregnancy rates, and increases premature births[14–16].

Implantation potential and pregnancy rates are key outcomes evaluated to compare embryo transfer methods. To our knowledge, no study has been performed on the effect of fresh and frozen blastocyst transfer on pregnancy outcomes in male factor infertile couples stratified by maternal age groups.

In this study, the mean age of women in the fresh and frozen-thawed blastocyst transfer groups was similar with a comparable distribution of age groups. This may prevent the debate that the difference in pregnancy rates may be due to differences in average age. Similarly, Weiss et al. compared fresh and frozen-thawed blastocyst transfer groups in their study. Similar to our findings, the mean maternal age was similar in the fresh ET and FET groups[23].

In the evaluation of all our cases, we observed that fresh embryo transfer resulted in higher pregnancy rates. However, live birth rates, which are the primary indicator of IVF success, were similar between women who underwent fresh blastocyst transfer and those who underwent frozen-thawed blastocyst transfer. Similarly, Zaat et al. reported that there was no statistically significant difference between live birth rates after fresh and frozen embryo transfer[24]. Although Venetis reported that the freezing strategy resulted in a higher live birth rate in the OHSS group, no differences were obtained in patients with normal responders[25]. While Weiss et al. found higher pregnancy and live birth rates with fresh embryo transfers, after adjustment by log-linear models and propensity score analysis, no statistically significant difference in singleton live birth rates was demonstrated[23]. Furthermore, Gullo et al. reported that there was no difference in pregnancy outcomes, including cumulative live birth rates, between fresh ET and FET, but preterm birth was more frequent in those who underwent FET[16].

Contrary to the above results, other studies have reported higher live birth rates in FET cycles than in fresh ET cycles[26,27], which may be explained by the absence of ovarian hyperstimulation. Other studies have also reported higher pregnancy rates with FET compared to fresh ET, but also rates of placenta previa, placenta abruption, preterm birth, large baby syndrome, and perinatal mortality were observed more frequently in women who underwent FET[14,28,29]. Although the reasons for these adverse effects are not fully understood, they are thought to be due to possible genomic, transcriptomic, and epigenetic changes that cryopreservation may induce. However, only a few studies have reported the effects of cryopreservation at the molecular level[19,30,31]. The potential cellular damage caused using high concentrations of cryoprotectants is thought to cause severe stress to the blastocysts, leading to a low ART success rate and potential adverse effects[8,32].

It is a well-known fact that as the age of the woman increases, the pregnancy rate decreases. In this study, while pregnancy rates decreased with increasing maternal age in fresh blastocyst transfer, age did not influence pregnancy rates in frozen-thawed blastocyst transfer. The difference in the number of cases is thought to account for this. Adebayo et al. also reported a significant reduction in pregnancy rates after IVF-ET in women older than 34 years, like the results of our study[33]. Vitagliano et al. also published that increasing maternal age was associated with a decreased ART success rates after embryo transfer[34]. In addition, Bagheri et al. included 223 infertile women with various causes of infertility and examined pregnancy rates according to embryo transfer method and age[35]. Pregnancy rates were higher in the 25-30-year-old age group undergoing FET than in those undergoing fresh ET, but no difference was observed in those older than 35 years. A recent study showed that decrease in pregnancy rates with advancing maternal age is due to the gradual decline in ovarian reserve and oocyte/embryo quality, which is related to telomere shortening and impaired mitochondrial metabolic activity[36].

Conclusions

In the present study, only couples with male infertility who underwent fresh or frozen-thawed blastocyst transfer were selected. A limitation of the present study was the relatively small sample size. Our results indicate similar live birth rates between the use of fresh ET and FET. The different results described in the literature may be due to methodological differences and different characteristics of the study populations. ASRM guidelines published in 2021 advise clinicians to implement an individualized protocol by evaluating patient characteristics and clinical outcomes. Cryopreservation of high-quality embryos is recommended for those who cannot undergo a fresh embryo transfer due to complications or for use in subsequent cycles, even if a fresh transfer is performed.

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References

1. Basirat Z, Adib Rad H, Esmailzadeh S, Jorsaraei SGA, Hajian- Tilaki K, Pasha H, et al. Comparison of pregnancy rate between fresh embryo transfers and frozen-thawed embryo transfers following ICSI treatment. Int J Reprod Biomed. 2016;14(1):39–46. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4837924/pdf/ijrb-14-039.pdf
2. Molgora S, Fenaroli V, Acquati C, De Donno A, Baldini MP, Saita E. Examining the Role of Dyadic Coping on the Marital Adjustment of Couples Undergoing Assisted Reproductive Technology (ART). Front Psychol. 2019;10:415. doi: 10.3389/fpsyg.2019.00415
3. Simon L, Shamsi MB, Carrell DT. Sperm Selection Techniques and their Relevance to ART. In: Schatten H, editor. Human Reproduction [Internet]. 1st ed. Wiley; 2017 [cited 2024 Mar 15]. p. 1–43. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9781118849613.ch1
4. Choi HW, Park YS, Lee SH, Lim CK, Seo JT, Yang KM. Effects of maternal age on embryo quality and pregnancy outcomes using testicular sperm with intracytoplasmic sperm injection. Clin Exp Reprod Med. 2016;43(4):221. doi: 10.5653/cerm.2016.43.4.221
5. Bosch E, De Vos M, Humaidan P. The Future of Cryopreservation in Assisted Reproductive Technologies. Frontiers Endocrinol. 2020;11:67. doi: 10.3389/fendo.2020.00067
6. Coutifaris C. Freeze-only in vitro fertilization cycles for all? Fertil Steril. 2017;108(2):233–4. doi: 10.1016/j.fertnstert.2017.06.028
7. Tavukcuoglu S, Al-Azawi T, Khaki AA, Al-Hasani S. Is vitrification standard method of cryopreservation. Middle East Fertil Soc J. 2012;17(3):152–6. doi: 10.1016/j.mefs.2012.07.007
8. Chen H, Zhang L, Meng L, Liang L, Zhang C. Advantages of vitrification preservation in assisted reproduction and potential influences on imprinted genes. Clin Epigenet. 2022;14(1):141. doi: 10.1186/s13148-022-01355-y
9. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update. 2017;23(2):139–55. doi: 10.1093/humupd/dmw038
10. Veleva Z, Karinen P, Tomás C, Tapanainen JS, Martikainen H. Elective single embryo transfer with cryopreservation improves the outcome and diminishes the costs of IVF/ICSI. Hum Reprod. 2009 Jul 1;24(7):1632–9. doi: 10.1093/humrep/dep042
11. Zhu D, Zhang J, Cao S, Zhang J, Heng BC, Huang M, et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles—time for a new embryo transfer strategy? Fertil Steril. 2011 Apr;95(5):1691–5. doi: 10.1016/j.fertnstert.2011.01.022
12. Belva F, Henriet S, Van Den Abbeel E, Camus M, Devroey P, Van Der Elst J, et al. Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. Hum Reprod. 2008 Jul 8;23(10):2227–38. doi: 10.1093/humrep/den254
13. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. Fertil Steril. 2013 Jan;99(1):156–62. doi: 10.1016/j.fertnstert.2012.09.003
14. Alviggi C, Conforti A, Carbone IF, Borrelli R, De Placido G, Guerriero S. Influence of cryopreservation on perinatal outcome after blastocyst- vs cleavage-stage embryo transfer: systematic review and meta-analysis. Ultrasound in Obstet & Gyne. 2018;51(1):54–63. doi: 10.1002/uog.18942
15. Braga DPAF, Setti AS, Figueira RCS, Azevedo MDC, Iaconelli A, Lo Turco EG, et al. Freeze-all, oocyte vitrification, or fresh embryo transfer? Lessons from an egg-sharing donation program. Fertil Steril. 2016 Sep;106(3):615–22. doi: 10.1016/j.fertnstert.2016.05.004
16. Gullo G, Giuseppe B, Cucinella G, Greco M, Perino A, Chiantera V, et al. Fresh vs. frozen embryo transfer in assisted reproductive techniques: a single center retrospective cohort study and ethical-legal implications. Eur Rev Med Pharmacol Sci. 2023;27(14):6809-6823. doi: 10.26355/eurrev_202307_33152
17. Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts: Curr Op Obstet Gynaecol. 1999 Jun;11(3):307–11. doi: 10.1097/00001703-199906000-00013
18. Arav A. Cryopreservation of oocytes and embryos. Theriogenology. 2014;81(1):96–102. doi: 10.1016/j.theriogenology.2013.09.011
19. Casciani V, Monseur B, Cimadomo D, Alvero R, Rienzi L. Oocyte and embryo cryopreservation in assisted reproductive technology: past achievements and current challenges. Fertil Steril. 2023;120(3):506–20. doi: 10.1016/j.fertnstert.2023.06.005
20. Estudillo E, Jiménez A, Bustamante-Nieves PE, Palacios-Reyes C, Velasco I, López-Ornelas A. Cryopreservation of Gametes and Embryos and Their Molecular Changes. Intern J Mol Sci. 2021;22(19):10864. doi: 10.3390/ijms221910864

21. Konc J, Kanyó K, Kriston R, Somoskői B, Cseh S. Cryopreservation of Embryos and Oocytes in Human Assisted Reproduction. BioMed Res Internat. 2014;2014:e307268. doi: 10.1155/2014/307268

22. The Practice Committee of the American Society for Reproductive Medicine. Evidence-based outcomes after oocyte cryopreservation for donor oocyte in vitro fertilization and planned oocyte cryopreservation: a guideline. Fertil Steril. 2021;116(1):36–47. doi: 10.1016/j.fertnstert.2021.02.024

23. Weiss MS, Luo C, Zhang Y, Chen Y, Kissin DM, Satten GA, et al. Fresh vs. frozen embryo transfer: new approach to minimize the limitations of using national surveillance data for clinical research. Fertil Steril. 2023;119(2):186–94. doi: 10.1016/j.fertnstert.2022.10.021

24. Zaat T, Zagers M, Mol F, Goddijn M, Van Wely M, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database of Systematic Reviews. 2021(2). doi: 10.1002/14651858.CD011184.pub3

25. Venetis CA. Pro: Fresh versus frozen embryo transfer. Is frozen embryo transfer the future? Hum Reprod. 2022 Jun 30;37(7):1379–87. doi: 10.1093/humrep/deac126

26. Sik A, Oral S, Aba YA, Ozolcay O, Koc M, Sismanoglu A. Pregnancy results after fresh embryo transfer and selective frozen-thawed embryo transfer: Single-center experience. J Gynecol Obstet Hum Reprod. 2020;49(4): 101707. doi: 10.1016/j.jogoh.2020.101707

27. Wang SF, Seifer DB. Age-related increase in live-birth rates of first frozen thaw embryo versus first fresh transfer in initial assisted reproductive technology cycles without PGT. Reprod Biol Endocrinol. 2024 Apr 13;22(1):42. doi: 10.1186/s12958-024-01210-0

28. Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? Hum Reprod. 2014;29(3):618–27. doi: 10.1093/humrep/det440

29. Sha T, Yin X, Cheng W, Massey IY. Pregnancy-related complications and perinatal outcomes resulting from transfer of cryopreserved versus fresh embryos in vitro fertilization: a meta-analysis. Fertil Steril. 2018;109(2):330-342.e9. doi: 10.1016/j.fertnstert.2017.10.019

30. Edgar DH, Gook DA. A critical appraisal of cryopreservation (slow cooling versus vitrification) of human oocytes and embryos. Hum Reprod Update. 2012;18(5):536–54. doi: 10.1093/humupd/dms016

31. Wong KM, Mastenbroek S, Repping S. Cryopreservation of human embryos and its contribution to in vitro fertilization success rates. Fertil Steril. 2014;102(1):19–26. doi: 10.1016/j.fertnstert.2014.05.027

32. Choi HW, Jang H. Application of Nanoparticles and Melatonin for Cryopreservation of Gametes and Embryos. CIMB. 2022;44(9):4028–44. doi: 10.3390/cimb44090276

33. Adebayo FO, Ameh N, Adesiyun AG, Ekele BA, Wada I. Correlation of female age with outcome of IVF in a low-resource setting. Intern J Gynecol Obstet. 2023;161(1):283–8. doi: 10.1002/ijgo.14545

34. Vitagliano A, Paffoni A, Viganò P. Does maternal age affect assisted reproduction technology success rates after euploid embryo transfer? A systematic review and meta-analysis. Fertil Steril. 2023 Aug 1;120(2):251–65. doi: 10.1016/j.fertnstert.2023.02.036

35. Bagheri RB, Bazrafkan M, Sabour A, Ataei M, Badehnoosh B, Mashak B, et al. The comparison of pregnancy outcomes in fresh and frozen embryo transfer: A cross- sectional study. IJRM [Internet]. 2023 Aug 19 [cited 2024 Feb 19]; https://knepublishing.com/index.php/ijrm/article/view/13891

36. Ubaldi FM, Cimadomo D, Vaiarelli A, Fabozzi G, Venturella R, Maggiulli R, et al. Advanced Maternal Age in IVF: Still a Challenge? The Present and the Future of Its Treatment. Front Endocrinol (Lausanne). 2019;10:94. doi: 10.3389/fendo.2019.00094