AUCTORES
Globalize your Research

Journal of Obstetrics Gynecology and Reproductive Sciences
Lawrence A. Kaplan *

Open Access

Research Article

# The Effect of In-vitro Fertilization Procedures on Alpha-fetoprotein Levels in Second Trimester Screening for Fetal Defects

**Dakai Liu [1], Lawrence A. Kaplan [1]*, Mehdi Tolou [2]**

[1] Co-First Authors Sunrise Medical Laboratories; 250 Miller Place, Hicksville, NY 11801 USA.

[2] UAG School of Medicine; Av. Universidad 700, Lomas del Valle, Zapopan, Jal. 45129 México.

***Corresponding Author:** Lawrence A. Kaplan PhD, Laboratory director of fetal defect screening Sunrise Medical Laboratories 250 Miller Place Hicksville, NY 11801 USA.

**Received date:** April 01, 2024; **Accepted date:** April 09, 2024; **Published date:** April 16, 2024.

**Citation:** Dakai Liu, Lawrence A. Kaplan, Mehdi Tolou, (2024), The Effect of In-vitro Fertilization Procedures on Alpha-fetoprotein Levels in Second Trimester Screening for Fetal Defects, *J. Obstetrics Gynecology and Reproductive Sciences*, 8(3) **DOI:**10.31579/2578-8965/212



## Abstract:

### Objective

Prenatal screening for developmental and chromosomal fetal defects during both the first and second trimesters of pregnancy is considered standard practice. In the second trimester, screening for spinal cord malformations, such as spina bifida and anencephaly (a type of neural tube defect), involves measuring alpha-fetoprotein levels in maternal blood. The cutoffs used to identify increased risks for neural tube defects are typically tailored for factors like race, multiple fetuses, reported smoking, and the presence of maternal insulin-dependent diabetes. What remains unclear is the impact of assisted reproductive technology, commonly known as in vitro fertilization procedures, on alpha-fetoprotein levels and other measured analytes.

### Method

This report presents a retrospective study that examines the impact of reported in vitro fertilization procedures on second-trimester levels of alpha-fetoprotein. The study utilizes consecutive data from March 20, 2019, through March 29, 2023, sourced from laboratory records.

### Result

We illustrate elevated levels of maternal serum alpha-fetoprotein across all racial subgroups undergoing in vitro fertilization procedures. Additionally, we demonstrate that maternal serum alpha-fetoprotein levels rise with maternal age.

### Conclusion

This study underscores the significance of our findings in evaluating the risk of neural tube defects, such as spina bifida, Down syndrome, and other genetic anomalies. It holds considerable value in guiding clinical practices. Furthermore, it highlights the need for further investigation to evaluate how our findings impact the assessment of fetal well-being.

**Keywords:** in vitro fertilization procedures; alpha-fetoprotein; neural tube defect screen

## Introduction

Prenatal screening for spinal cord malformations, typically spina bifida and anencephaly (NTDs), is performed in the second trimester of pregnancy by measuring alpha-fetoprotein (AFP) in maternal blood. The cut-offs used to denote increased risks for NTD are usually modified by the presence of race, multiple fetuses, reported smoking, and the presence of maternal insulin-dependent diabetes [1].

Another factor that has attracted concern is the impact of assisted reproductive technology (ART), also referred to as in vitro fertilization procedures (IVF), on the levels of AFP in pregnant women. The reported effect of IVF treatment on second trimester AFP levels seems to vary. This is partially because previous studies investigated the effect of IVF in general [2-4], or IVF involving transfer of frozen or fresh embryos involving intracytoplasmic sperm injection (ICSI) [5-7].

If second-trimester levels of AFP are truly elevated in IVF-assisted pregnancies, laboratories would need to consider modifying the risk cut-off to account for this bias. This report focuses on the effect of the reported use of all IVF procedures on second-trimester levels of AFP by conducting a non-interventional analysis of retrospective cohort data and a review of the literature.

## Materials and Methods

### 1.Analytical and risk calculation procedures

Serum samples from women in their second trimester of pregnancy are delivered to Sunrise Laboratory. The Beckman Dxi analyzer measures AFP levels in maternal serum.

Results of the AFP measurements and patient demographics are uploaded to the Benetech software program, which calculates specific risk for an NTD. The AFP results (ng/mL) are adjusted for maternal weight and then normalized to gestational age-specific and race-specific medians to calculate the Multiple of Median (AFP [ng/mL]/weekly median; MoM) for each result. The MoM cut-offs used for a positive screen are: 2.5 for most populations, 3.0 for self-designated Black women, 1.96 if the patient is reported having insulin-dependent diabetes (IDDM), 4.0 for most populations if there are twin gestations, and 5.0 for Black women with twin gestations. The medians of each population are reviewed at least monthly and adjustments to the medians are made to maintain the median of the overall MoM values close to 1.0 with an acceptable range of +/- 0.5 MoM.

### 2.Patient population:

Consecutive data from March 20, 2019 through March 29. 2023, was gathered from the laboratory records.

Exclusion criteria were MoMs greater than 10.0 and results for fetuses whose estimated gestation age was less than 14 weeks or greater than 23 weeks.

The data was sorted by race and the reported use of an in vitro fertilization technique (no IVF or + IVF). No distinction could be made as to the type of IVF procedure used. There was no separation of singleton from multiple gestation pregnancies. Although twin gestations occurred at a rate of 1.4-2.5 % of all serum samples, the prevalence of twin gestations was roughly the same in both IVF pregnancies and non-IVF pregnancies.

### 3.Statistical analysis

The student's t-test was used to compare the means of ages, AFP levels, and AFP MoMs. An Ancova analysis in the R language programming was performed to compare the AFP MoM Mean between IVF and non-IVF groups at the same age, from 30 to 44 years old. The Spearman's Rank Correlation test was used to compare AFP MoMs with maternal age.

## Results

The racial distribution of the patients is listed in Table 1. Notably, the ages of the patients (Table 2) with IVF procedures were significantly higher than the ages of patients without reported use of IVF (t-test, P<0.05). This trend of higher ages with IVF was remined consistent across the various racial groups.

| Race | Total | No IVF | + IVF | % IVF in total |
|---|---|---|---|---|
| Asian | 1574 | 1405 | 169 | 10.7 |
| Black | 1422 | 1371 | 51 | 3.6 |
| Hispanic | 3757 | 3705 | 52 | 1.4 |
| Other | 1677 | 1499 | 178 | 10.6 |
| Unknown | 1156 | 1045 | 111 | 9.6 |
| White | 11998 | 11395 | 603 | 5.0 |
| Total | 21584 | | | |

**Table 1:** Racial distribution of population by presence of IVF

| | Mean age (Years) | | Range (Years) | |
|---|---|---|---|---|
| Race | No IVF | + IVF | No IVF | + IVF |
| Asian | 32.1 | 37.6* | 17-46 | 25-53 |
| Black | 30.6 | 37.6* | 15-49 | 27-55 |
| Hispanic | 29.6 | 36.5* | 14-45 | 20-47 |
| Other | 31.5 | 37* | 15-48 | 25-55 |
| Unknown | 31.3 | 36.2* | 16-43 | 23-48 |
| White | 31.7 | 37.4* | 15-51 | 21-60 |

* mean + IVF and No IVF statistically different by t-test, P< 0.05

**Table 2:** Ages of the various racial populations by presence of IVF

The AFP levels as well as the AFP MoMs for the total patient populations with IVF procedures were significantly higher than the AFP for patients whose pregnancies developed without assisted reproductive technology (Table 3; t-test, P<0.05).

| Mean AFP (ng/mL) | | Median AFP MoMs | |
|---|---|---|---|
| No IVF | + IVF | No IVF | + IVF |
| 47.2 | 57.1* | 1.03 | 1.37* |
| n=27745 | | n=1169 | |

* mean+ IVF vs. no IVF statistically significant by t-test, P< 0.05

**Table 3:** AFP (ng/mL) and AFP MoMs for total population

This pattern of higher AFP and AFP MoMs was also proper for each racial group: both AFP and AFP MoMs were more significant for the patients of each racial group whose pregnancies developed with the aid of assisted reproductive technology (Table 4).

| | Mean AFP (ng/mL) | | Median of MoMs | |
|---|---|---|---|---|
| **Race** | **No IVF** | **+ IVF** | **No IVF** | **+ IVF** |
| Asian | 49.2 | 67.1* | 1.08 | 1.41* |
| Black | 51.4 | 62.6* | 1.04 | 1.17* |
| Hispanic | 48.8 | 58.5* | 1.02 | 1.31* |
| Other | 45.1 | 54* | 1.01 | 1.23* |
| Unknown | 46.5 | 54.1* | 1.03 | 1.28* |
| White | 45.5 | 54.3* | 1.02 | 1.22* |

* mean + IVF and No IVF statistically significant by t-test, P< 0.05

**Table 4:** AFP (ng/mL) and AFP MoMs by racial group and IVF use

In addition, we compared AFP MoM Mean between IVF and non-IVF groups for ages 30 to 44 years old (Table 5). The cut-off of case number for each age set is 29. Figure 1 visulizes the curves of MoM Mean of each age set for both IVF and non-IVF groups. An Ancova analysis in the R programming language was performed. The P value is 0.01994, indicating a statistically significant difference between IVF; in particular, the non-IVF groups and AFP MoM of IVF group is significantly higher that of non-IVF group.

| | IVF | | Non-IVF | |
|---|---|---|---|---|
| **Age** | **Cases** | **Mean** | **Cases** | **Mean** |
| 30 | 29 | 1.26 | 1079 | 1.1 |
| 31 | 34 | 1.29 | 1180 | 1.11 |
| 32 | 56 | 1.29 | 1175 | 1.14 |
| 33 | 77 | 1.28 | 1173 | 1.12 |
| 34 | 90 | 1.39 | 1142 | 1.13 |
| 35 | 88 | 1.43 | 955 | 1.14 |
| 36 | 103 | 1.32 | 836 | 1.12 |
| 37 | 98 | 1.48 | 647 | 1.12 |
| 38 | 85 | 1.29 | 535 | 1.12 |
| 39 | 84 | 1.36 | 379 | 1.16 |
| 40 | 70 | 1.41 | 293 | 1.13 |
| 41 | 75 | 1.36 | 201 | 1.14 |
| 42 | 53 | 1.37 | 115 | 1.26 |
| 43 | 48 | 1.45 | 60 | 1.15 |
| 44 | 31 | 1.64 | 32 | 1.24 |

**Table 5:** MoM Mean and case number of each age

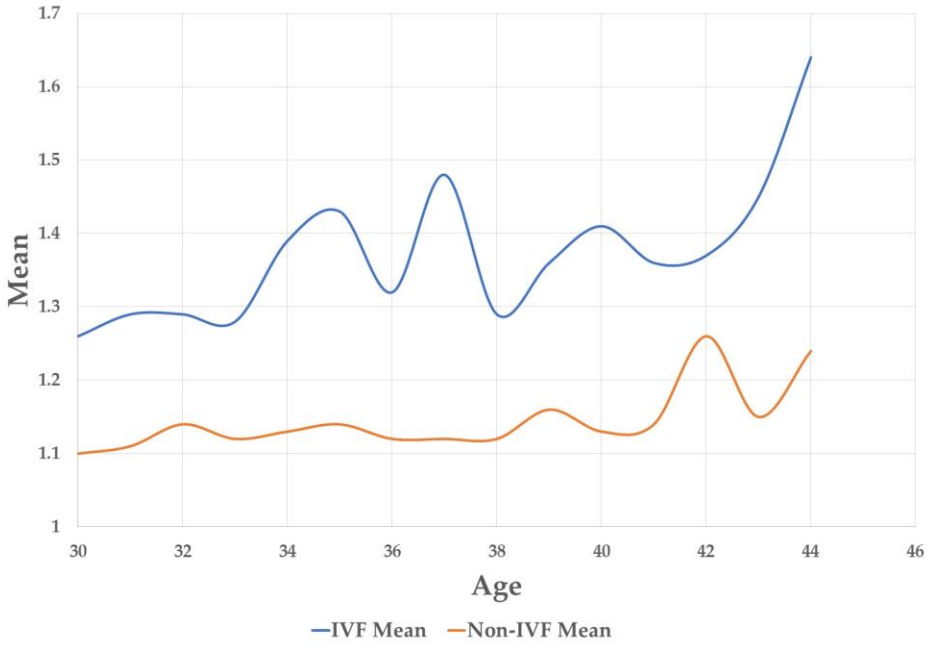


**Figure 1:** Comparison AFP MoM between IVF and non-IVF groups at various age groups

Finally, we considered the relationship between patient age and the AFP MoMs. The AFP MoMs for patients with no IVF trended higher with increased maternal age (Table 6A). The Spearman's Rank Correlation Coefficient Rs value is more significant than 0.78 with Critical Value $\alpha = 0.01$, suggesting that there is a statistically significant correlation between higher MoM of no IVF individuals and older ages.

| Age | Case number | no IVF AFP MoM mean |
|---|---|---|
| 14 | 1 | 0.75 |
| 15 | 12 | 1.31 |
| 16 | 19 | 1.07 |
| 17 | 47 | 1.06 |
| 18 | 77 | 1.05 |
| 19 | 137 | 1.09 |
| 20 | 183 | 1.06 |
| 21 | 210 | 1.10 |
| 22 | 283 | 1.09 |
| 23 | 360 | 1.04 |
| 24 | 408 | 1.06 |
| 25 | 527 | 1.09 |
| 26 | 502 | 1.13 |
| 27 | 666 | 1.10 |
| 28 | 762 | 1.09 |
| 29 | 939 | 1.09 |
| 30 | 1079 | 1.10 |
| 31 | 1180 | 1.11 |
| 32 | 1175 | 1.14 |
| 33 | 1173 | 1.12 |
| 34 | 1142 | 1.13 |
| 35 | 955 | 1.14 |
| 36 | 836 | 1.12 |
| 37 | 647 | 1.12 |
| 38 | 535 | 1.12 |
| 39 | 379 | 1.16 |
| 40 | 293 | 1.13 |
| 41 | 201 | 1.14 |
| 42 | 115 | 1.26 |
| 43 | 60 | 1.15 |
| 44 | 32 | 1.24 |
| 45 | 19 | 1.37 |
| 46 | 4 | 1.30 |
| 47 | 5 | 1.18 |
| 48 | 3 | 1.19 |

**Table 6A:** Relationship between maternal age and AFP MoM

The Spearman's Rank Correlation Coefficient test is not conducted on the IVF data because, in some IVF age groups, there are no cases or only one case (Table 6B).

| Age | IVF Case number | AFP MoM mean |
|---|---|---|
| 20 | 1 | 0.85 |
| 21 | 1 | 1.02 |
| 22 | 1 | 1.05 |
| 23 | 1 | 1.28 |
| 24 | 5 | 1.18 |
| 25 | 10 | 1.31 |
| 26 | 4 | 1.58 |
| 27 | 9 | 1.31 |
| 28 | 11 | 1.60 |
| 29 | 17 | 1.40 |
| 30 | 29 | 1.26 |
| 31 | 34 | 1.29 |
| 32 | 56 | 1.29 |
| 33 | 77 | 1.28 |
| 34 | 90 | 1.39 |
| 35 | 88 | 1.43 |
| 36 | 103 | 1.32 |
| 37 | 98 | 1.48 |

| | | |
|---|---|---|
| 38 | 85 | 1.29 |
| 39 | 84 | 1.36 |
| 40 | 70 | 1.41 |
| 41 | 75 | 1.36 |
| 42 | 53 | 1.37 |
| 43 | 48 | 1.45 |
| 44 | 31 | 1.64 |
| 45 | 26 | 1.53 |
| 46 | 15 | 1.27 |
| 47 | 21 | 1.30 |
| 48 | 8 | 1.50 |
| 49 | 2 | 1.70 |
| 50 | 3 | 1.98 |
| 51 | 3 | 1.31 |
| 52 | 0 | |
| 53 | 2 | 1.00 |
| 54 | 1 | 0.81 |
| 55 | 3 | 2.06 |
| 56 | 1 | 1.48 |
| 57 | 1 | 1.99 |
| 58 | 0 | |
| 59 | 0 | |
| 60 | 1 | 1.10 |

**Table 6B:** Relationship between maternal age and AFP MoM

## Discussion

Screening for neural tube defects (NTDs) by measuring alpha-fetoprotein in serum from women in their second trimester of pregnancy is a routine part of prenatal care. However, there is an inconsistency with reports regarding the effect of ART (IVF) on the levels of AFP and their normalized values (the Multiple of the Mean, MoM). AFP values are often reported to be raised, lowered, or have no change [1, 5-7]. The discrepancies result partially from relatively small numbers in these clinical studies and partially from the investigation of multiple variations of ART procedures, such as transfer of frozen or fresh embryos or IVF involving intracytoplasmic sperm injection (ICSI). Each of these sub-variants of IVF may have different associated clinical risks and issues, complicating the simple question of whether or not the reported use of ART is associated with changes in second-trimester AFP levels.

In this report, based on an extensive and ethnically diverse database, we can confirm that the answer is affirmative. AFP levels and the AFP MoMs are raised in the IVF-reported group as compared to the pregnancies reporting no ART to assist in conception (Table 2). We do not consider the type of IVF procedure employed; we only consider the global effect of all IVF procedures. The elevated AFP levels in IVF-assisted pregnancies occur for each of the racial groups investigated: Caucasians, Blacks, Asians, Hispanics, and the two sub-groupings for which no race had been assigned: Other and Unknown (Table 3). The MoMs, which are used to assign risk for NTDs, are raised by about 20% for both overall and each racial group in IVF-assisted pregnancies. These results compare well with the recent report by Lanes A et al. [4], in which higher AFP values are also reported in second-trimester serum. Any bias from twins in the current populations studied will be small slight, with twin gestations reported at about 1.4-2.5% of totals. Since twin gestations were rarely associated with IVF, any bias of twin gestation would tend to increase the AFP MoMs of the non-IVF population, making the actual bias we see even more real.

We do see a significant association of AFP MoMs with the age of non-IVF patients (Spearman's Rank Correlation Coefficient Rs value is more significant than 0.78 with Critical Value $\alpha = 0.01$); this is similar to what was previously observed by others [8, 9]. When we compared the mean AFP levels (ng/mL) in various maternal ages for both the IVF and non-IVF groups, the AFP levels were consistently higher for the IVF group for each age. Thus, the observation that AFP levels are elevated in the IVF cohort is still tenable despite the overall difference in age between the IVF group and the non-IVF group.

Maternal serum AFP is significantly increased in IVF-assisted pregnancies for every racial cohort examined. This finding needs to be addressed in particular in terms of its clinical application.

Fetal defects associated with IVF-assisted pregnancies range from physical defects [10-13], or increased risk for cancer [14], to chromosomal changes [15]. However, one report concludes there are no significant risk of fetal defects [16]. There is no clear explanation as to the biological or physiological cause for any higher risk associated with IVF. The higher risk for birth defects may stem from either the IVF procedures themselves, the manipulation of individual gametes or embryos, or from the underlying need for IVF, namely infertility.

Increased risk for fetal risk with IVF may be due to delayed conception (older, more damaged oocytes [11], and other underlying causes of infertility [17]. Drugs used in IVF for older women may increase their risk of having a baby with Down's syndrome (DS). Some suggest that the increased DS incidence may be attributable to the underlying cause of patient infertility or its determinants, since couples who take longer than a year to conceive have a similar increased risk of having babies that exhibit birth defects [16, 17]. Stated, women undergoing IVF tend to be older (see Tables 2 and 5) and/or have other independent factors that might by themselves increase DS or NTD risk; therefore, it is difficult to correct for IVF by itself reliably. In this study the ages of all the populations reporting the use of IVF were significantly higher (Table 1) than the populations with commonly conceived fetuses.

There have been suggestions that the levels of specific second-trimester analytes should be adjusted to offset the changes associated with IVF-assisted pregnancies [18, 19]. However, reducing AFP MoMs for the presence of IVF would decrease the sensitivity for detecting NTDs and other possible adverse fetal outcomes while, in combination with other second-trimester tests for DS screening (Human chorionic gonadotropin, unconjugated Estriol, Inhibin), the artificially lowered AFP MoMs might decrease the specificity of DS screening and increase the already high rate of false positives (~3-5%). In addition, elevated serum AFP levels (MoMs >1.00) below the cut-off for increased NTD risk levels may well reflect other adverse fetal outcomes [8, 20, 21]. This lack of knowledge should preclude artificial modifications of the data until the clinical significance of the higher AFP MoMs is better known.

## Conclusion

We illustrate elevated levels of maternal serum alpha-fetoprotein across all racial subgroups undergoing in vitro fertilization procedures and demonstrate that these levels rise with maternal age. This study underscores the significance of our findings in evaluating the risk of neural tube defects, such as spina bifida, Down syndrome, and other genetic anomalies. It holds considerable value in guiding clinical practices. Furthermore, it highlights the need for further investigation to evaluate how our findings impact the assessment of fetal well-being.

## Acknowledgments

We would like to thank Peter M. Colaninno; Kristina M. Brewi; Jennifer M. Welsch; David A. Barnard; Laura M. Chavez; Mary Joy for their technical assistance.

## Conflicts of Interest

The authors declare no conflict of interest.

## Author Contributions

Conceptualization: D.L., K.A.L.; Data curation: D.L., K.A.L.; Investigation: D.L., K.A.L.; Supervision: K.A.L.; Statistics analysis: D.L., K.A.L., M.T.; Writing, Reviewing & Editing: D.L., K.A.L. M.T.; All authors have read and agreed to the published version of the manuscript.

## Funding

This research received no external funding.

## Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Western Institutional Review Board (WIRB) Copernicus Group (WCG) IRB (Study Number: 1-1711850-1 and date of approval: Nov. 6, 2023.

Informed Consent Statement: Patient consent was waived by WCG IRB as the Board found that this research meets the requirements for a waiver of consent under 45 CFR 46 116(f) [2018 Requirements] 45 CFR 46.116(d) [Pre-2018 Requirements]), since the study will use existing da-ta/sample collection.

## Data Availability Statement

The data presented in this study are available upon request from the corresponding authors.

## References

1. American College of Obstetricians and Gynecologists (ACOG) (2001). Practice Bulletin No. 27: Clinical Management Guidelines for Obstetrician-Gynecologists Prenatal diagnosis of fetal chromosomal abnormalities. *Obstet Gynecol.* 97(5 Pt 1): suppl 1-12. PMID: 11501567.
2. Räty R, Virtanen A, Koskinen P, Anttila L, Forsström J, et al. (2002). Serum free beta-HCG and alpha-fetoprotein levels in IVF, ICSI and frozen embryo transfer pregnancies in maternal mid-trimester serum screening for Down's syndrome. *Hum Reprod*. 17(2):481-484
3. Barkai G, Goldman B, Ries L, Chaki R, Dor J, et al. (1996). Down's syndrome screening marker levels following assisted reproduction *Prenat Diagn.* 16(12):1111-1114
4. Lanes A, Dougan S, Fell DB, Huang T, Sprague A, et al. (2018). Comparing Maternal Serum Screening Markers Among IVF and Spontaneous Conceptions in Ontario Through Registry Data. *J Obstet Gynaecol* Can. 40(12):1608-1617. Epub PMID: 30539731.
5. Perheentupa A, Ruokonen A, Tuomivaara L, Ryynänen M, Martikainen H (2002). Maternal serum beta-HCG and alpha-fetoprotein concentrations in singleton pregnancies following assisted reproduction. *Hum Reprod*. 17(3):794-797.
6. Hui PW, Tang MH, Lam YH, Ng EH, Yeung WS, et al. (2003). Maternal serum hCG and alpha-fetoprotein levels in pregnancies conceived after IVF or ICSI with fresh and frozen-thawed embryos. *Hum Reprod.* 18(3):572-575.
7. Lam YH, Yeung WS, Tang MH, Ng EH, So WW, et al. (1999). Maternal serum alpha-fetoprotein and human chorionic gonadotrophin in pregnancies conceived after intracytoplasmic sperm injection and conventional in-vitro fertilization. *Hum Reprod.* 14(8):2120-2123.
8. Dai X, Zhang H, Wu B, Ning W, Chen Y, et al. (2023). Correlation between elevated maternal serum alpha-fetoprotein and ischemic placental disease: a retrospective cohort study. *Clin Exp Hypertens.* ;45(1):2175848.
9. Szabó M, Veress L, Münnich A, Papp Z (1995). Maternal age-dependent and sex-related changes of gestational serum alpha-fetoprotein. *Fetal Diagn Ther*. 10(6):368-372.
10. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, et al. (2012). Reproductive technologies and the risk of birth defects. *N Engl J Med.* 366(19):1803-1813.
11. Liberman RF, Getz KD, Heinke D, Luke B, Stern JE, et al. (2017). Assisted Reproductive Technology and Birth Defects: Effects of Subfertility and Multiple Births. Birth Defects Res. 109(14):1144-1153.
12. Luke B, Gopal D, Cabral H, Stern JE, Diop H (2017). Pregnancy, birth, and infant outcomes by maternal fertility status: the Massachusetts Outcomes Study of Assisted Reproductive Technology. *Am J Obstet Gynecol.* 217(3): 327.e1-327.e14.
13. Boulet SL, Kirby RS, Reefhuis J, Zhang Y, Sunderam S, et al. (2016). States Monitoring Assisted Reproductive Technology (SMART) Collaborative. Assisted Reproductive Technology and Birth Defects Among Liveborn Infants in Florida, Massachusetts, and Michigan, 2000-2010. *JAMA Pediatr*. 170(6): e154934.
14. Luke B, Brown MB, Nichols HB, Schymura MJ, Browne ML, et al. (2020). Assessment of Birth Defects and Cancer Risk in Children Conceived via In Vitro Fertilization in the US. *JAMA Netwo*rk Open. 3(10): e2022927.
15. Serafin D, Grabarek BO, Boroń D, Madej A, Cnota W, et al. (2022). Evaluation of the Risk of Birth Defects Related to the Use of Assisted Reproductive Technology: An Updated Systematic Review. Int *J Environ Res Public Health*. 19(8):4914
16. Mills M, Rindfuss RR, McDonald P, te Velde E (2011). ESHRE Reproduction and Society Task Force. Why do people postpone parenthood? Reasons and social policy incentives. *Hum Reprod* Update. 17(6):848-860.
17. Ubaldi FM, Cimadomo D, Capalbo A, Vaiarelli A, Buffo L, et al. (2017). Preimplantation genetic diagnosis for aneuploidy testing in women older than 44 years: a multicenter experience. *Fertil Steril.* 107(5):1173-1180.
18. Wald NJ, Watt HC (1996). Serum markers for Down's syndrome in relation to number of previous births and maternal age. *Prenat Diagn.* 16(8):699-703.
19. Wald NJ, White N, Morris JK, Huttly WJ, Canick JA (1999). Serum markers for Down's syndrome in women who have had in vitro fertilisation: implications for antenatal screening. *BJOG*. 106(12):1304-1306.

20. Konchak PS, Bernstein IM, Capeless EL (1995). Uterine artery Doppler velocimetry in the detection of adverse obstetric outcomes in women with unexplained elevated maternal serum alpha-fetoprotein levels. Am *J Obstet Gynecol.* 173(4):1115-1119.

21. Aboughalia H, Bastawrous S, Revzin MV, Delaney SS, Katz DS, et al. (2020). Imaging findings in association with altered maternal alpha-fetoprotein levels during pregnancy. Abdom Radiol (NY). 45(10):3239-3257.





**DOI:10.31579/2578-8965/212**