



Causal Effects of Body Mass Index and Maternal Age on Oocyte Maturation in Assisted Reproductive Technology: Model-Average Causal Effect and Bayesian LASSO Method

Ahad ALIZADEH¹, Reza OMANI-SAMANI², Mohammad Ali MANSOURNIA¹,
Azadeh AKBARI SENE³, *Abbas RAHIMI FOROUSHANI¹

1. Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Medical Ethics and Law, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Shabid Akbarabadi Clinical Research Development Unit, Iran University of Medical Sciences, Tehran, Iran

*Corresponding Author: Email: RahimiFo@tums.ac.ir

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Abstract

Background: Body Mass Index (BMI) and maternal age are related to various disorders of the female reproductive system. This study aimed to estimate the causal effects of BMI and maternal age on the rate of metaphase II oocytes (MII) using a new statistical method based on Bayesian LASSO and model averaging.

Methods: This investigation was a historical cohort study and data were collected from women who underwent assisted reproductive treatments in Tehran, Iran during 2015 to 2018. Exclusion criteria were gestational surrogacy and donor oocyte. We used a new method based on Bayesian LASSO and model average to capture important confounders.

Results: Overall, 536 cycles of 398 women were evaluated. BMI and Age had inverse relationships with the number of MII based on univariate analysis, but after adjusting the effects of other variables, there was just a significant association between age and the number of MII (adjusted incidence rate ratio (aIRR) of age =0.989, 95% CI: [0.979, 0.998], $P=0.02$). The results of causal inference based on the new presented method showed that the overall effects of age and BMI of all patients were significantly and inversely associated with the number of MII (both $P<0.001$). Therefore the expected number of MII decreased by 0.99 for an increase of 1 year (95% CI: [-1.00,-0.97]) and decreased by 0.99 for each 1-unit increase in BMI (95% CI: [-1.01,-0.98]).

Conclusion: Maternal age and BMI have significant adverse causal effects on the rate of MII in patients undergoing ART when the effects of important confounders were adjusted.

Keywords: Infertility; Assisted reproductive technology (ART); Causal effect; Age; Body mass index (BMI); LASSO regression

Introduction

Advanced maternal age and high body mass index (BMI) were recognized as adverse prognostic factors of assisted reproductive technology (ART) cycle (1). These factors are related to various disorders of the female reproductive system,

so it is more complicated to distinguish between causal effects of age and BMI on oocyte outcomes.

Women, ages 20–24 yr old have the best ability to get pregnant; after age 30, a progressive de-



cline in this ability occurs with age (2, 3). Both the gradual depletion of oocytes and the reduction in oocyte quality, at least after the age of 31, are important reasons for decreased fertility capacity (4-6). Female age is the single most important prognostic factor for predicting the outcome of ART cycles (7, 8).

Obese women are three times more likely to develop infertility compared with women with a normal BMI (9). Impaired fertility is occurring both in natural and ART cycles in obese women (10, 11). Furthermore, several studies showed that there was a higher risk of infertility in underweight women (10, 12, 13).

Variations in cycle variables with BMI and age indicated that ART cycles were inversely influenced by increases in both BMI and age (14). In other words, various clinical and demographical risk factors such as polycystic ovary syndrome (PCOS) and primary ovarian insufficiency correlate with BMI and age (1, 12, 14) and affect both the number and quality of oocytes. Therefore, estimating the relatively real contributing effects of BMI and age on oocyte maturation is more complicated.

The associations of age and BMI with oocyte maturation in ART cycles were evaluated by many studies. However, to our knowledge, no study estimated the causal effect of age and BMI on oocyte outcome. In this study, not only adjusted effects of BMI and maternal age were reported, compared with results of previous studies but also causal effects were estimated using a new statistical advanced method that can adjust effects of all-important confounders.

Materials and Methods

This investigation was a historical cohort study and data were collected from women who underwent ARTs in Tehran, Iran from 2015 to 2018. Women were included in the study if they received ART and completely recorded the information of maternal age, BMI, and other important confounders that were reported in Table

1. Exclusion criteria were gestational surrogacy and donor oocyte. BMI is calculated by dividing weight in kilograms by height in meters squared. Unlike most previous studies, BMI and maternal age were evaluated as quantitative variables to save the values of their information. However, the medical information of 536 cycles of 398 women was collected, the oocyte outcome in the last cycle was used to avoid consideration of non-independent and several cycles in the same women. Therefore, this retrospective study was conducted on 398 women. The women underwent controlled ovarian stimulation and received recombinant FSH. Follicle growing was monitored by vaginal ultrasonography. The total number of oocytes and maturity of them were evaluated by expert embryologists and also metaphase II oocytes were considered mature. With at least three or more follicles of 17 mm size, triggering was performed.

The design of the study was approved by the ethics review committee of Tehran University of Medical Science (Ethical No: IR.TUMS.SPH.REC.1396.2421).

Statistical analysis

Data were analyzed using three different statistical tools. First, we used correlation analysis (Spearman or Pearson coefficients) to evaluate primarily the relations between maternal age and BMI with oocyte outcomes. Next, the Poisson regression was performed for comparability of our results with the results of other studies. This method can estimate the crude or adjusted effects of factors on number of MII oocytes. Finally, we used new proposed statistical method based on Bayesian least absolute shrinkage and selection operator (LASSO) and model averaging that can find important confounders and adjust their confounding effects automatically. The main object of various observational studies were estimating the effect of a specific exposure on an outcome while the effects of large number of observed covariates or confounders were controlled (15).

Table 1: The clinical, biochemical, and demographical characteristics of women with infertility

<i>Factors</i>	<i>The patients (n=398)</i>
Baseline characteristics	
BMI, mean±SD	26.18±3.94
Age, mean±SD	31.67±5.39
Infertility history	
secondary infertility, n (%)	36 (9.0)
Infertility Reason, n (%)	
Female	108 (27.2)
Male	136 (34.3)
Both	76 (19.1)
unexplained	77 (19.4)
Polycystic Ovary Syndrome, n (%)	71 (17.8)
Primary Ovarian Insufficiency, n (%)	3 (0.8)
Hypothalamic Amenorrhea, n (%)	5 (1.3)
Poor ovarian response	27 (6.8)
Mullerian anomaly, n (%)	4 (1.0)
Myomatous uterus, n (%)	2 (0.5)
Endometriosis, n (%)	14 (3.5)
Adenomyosis, n (%)	2 (0.5)
OHSS in the previous cycle, n (%)	108 (27.1)
Duration of Infertility, median (IQR)	6.50 (3.00,10.25)
Number of previous ART cycles, median (IQR)	1.00 (1.00,2.00)
Total consumption of gonadotropin (IU), median (IQR)	3350 (1950,5300)
Underlie diseases	
Diabetes, n (%)	2 (0.5)
Thyroid diseases, n (%)	60 (15.1)
Hypertension, n (%)	4 (1.0)
Cardiac disease, n (%)	2 (0.5)
Hematologic disorders, n (%)	6 (1.5)
Renal disease, n (%)	2 (0.5)
Lab results	
AMH, median (IQR)	2.20 (0.90,4.00)
FSH, median (IQR)	6.20 (4.82,9.12)
LH, median (IQR)	5.45 (3.16,7.10)
PRL, median (IQR)	29.75 (17.75,308.30)
Day 3 estradiol, median (IQR)	52.60 (31.9,88.90)

Abbreviation: SD: standard deviation, IQR: interquartile range, AMH: anti-müllerian hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, PRL: prolactin

In such cases, selecting important confounders is an important key to void the bias estimation of effect of exposure. LASSO is an advanced statistical modeling method that performs variable selection in the data sets with large number of confounders (16). The Bayesian form of LASSO was developed (17). Handling the uncertainty in the model selection procedure using model averaging

is one of most important advantages of Bayesian LASSO that helps to estimate the causal effect of exposure (18).

We used a new method based on Bayesian LASSO and model average to capture important confounders using a specific prior distribution. The method is based on two models, which connected and estimate spontaneously the coefficients of

covariates over the iterations of unique Monte Carlo Markov Chain (MCMC). The prior links the two models of exposure and outcome in each MCMC iteration. Therefore, this prior distribution gives a higher weight to the covariates that relate to both exposure and outcome and forced them to include in the outcome model in the each iteration.

Some other classical methods can estimate the causal effect of an exposure using a two separated step approach (19, 20) but this approach has some disadvantages (21). To fix the disadvantages of the methods based on this two-step approach, we proposed our method that can use with small number of samples, large number of confounders, and handles the uncertainty of both parameter and model selection procedure. The Bayesian inferences are performed using MCMC chains run for 40,000 iterations and the first 2000 iterations discarded as burn-in for posterior inference. In the present study, a value of P less than 0.05 was accepted as a statistical significance and a value more than 0.05 and less than 0.10 was set as a borderline significance. The data were analyzed using R software (ver. 3.4.1) (22).

Overall, 398 women were evaluated. The total mean BMI and maternal age were 26.12 kg/m² (95% CI: [25.71, 26.53]) and 31.67 yr (95% CI: [31.13, 32.2]), respectively. The clinical, biochemical, and demographical characteristics of women are reported in Table 1. Overall, 398 women, 108 (27.2%) of which were from patients with female infertility factor, 136 (34.3%), 76 (19.1%), and 77 (19.4%) were male factor, both female and male, and unexplained, respectively. The prevalence of PCOS was 27.2% (95% CI: [22.1, 32.8]), and thyroid disorder was the most common underlying disease in women who underwent assisted reproductive technology treatment (prevalence= 15.08% [11.82, 18.84]). Generally, about 39.2% of women did not have any previous ART cycles, 24.4%, 18.6%, and 18.8% had one, two, and more than two cycles, respectively. The medians of retrieved oocytes and number of MII were both equal to 7 (IQR for the total oocytes: [4, 7] and for the number of MII: [3, 10]). Correlation analysis was showed that the number of MII had significant negative correlations with both age and BMI (Fig. 1).

Results

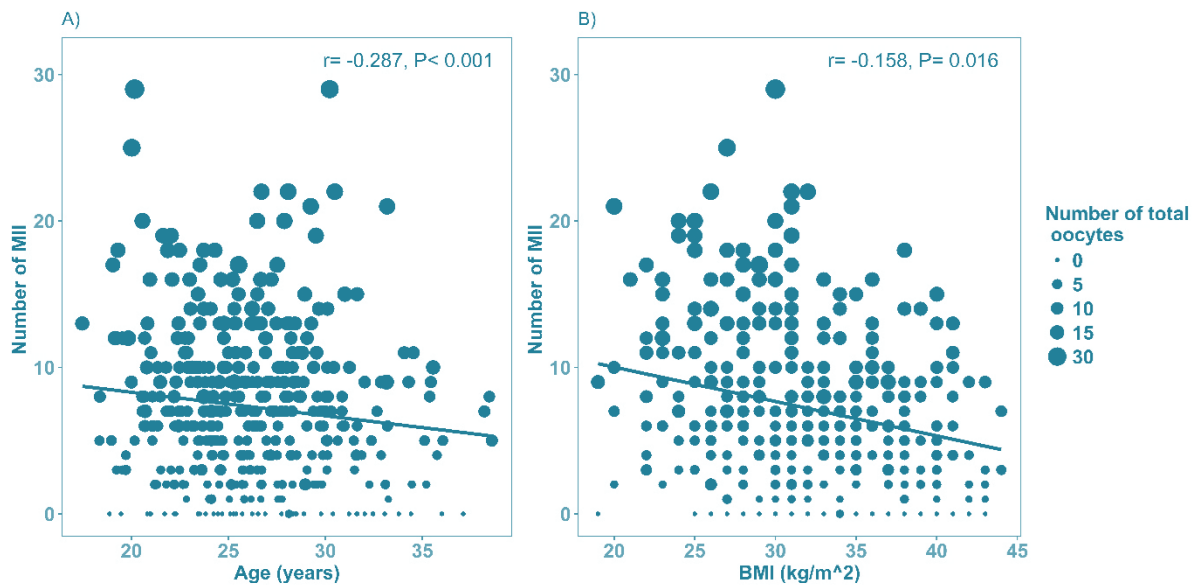


Fig. 1: Correlation analysis of age (A) and BMI (B) with the number of MII. The size of bullets are showed the total number of retrieved oocytes

According to the aim of this study, BMI and age as primary exposers had inverse relationships with the number of MII based on univariate analysis (IRR of BMI =0.98, 95%CI: [0.97, 0.99], $P < 0.001$; IRR of Age=0.97, 95% CI: [0.96, 0.98], $P < 0.001$), but after adjusting the effects of other variables, there was just a significant association

between age and the number of MII (aIRR of age=0.989, 95% CI: [0.979, 0.998], $P=0.02$).

Based on multivariate analysis of Poisson regression, the expected numbers of MII in women with female infertility factor, unexplained, and both male and female infertility were 0.79 and 0.84, and 0.8 times less than women with male factor infertility, respectively (Table 2).

Table 2: Univariate and multivariate analysis of several factors on number of MII to estimate their effects

Factors	Univariate Analysis		Multivariate Analysis	
	IRR (95% CI)	P value	aIRR (95% CI)	P value
BMI	0.98(0.97,0.99)	<0.001	0.99(0.98,1.001)	0.066
Age	0.97(0.96,0.98)	<0.001	0.99(0.98,0.99)	0.020
Infertility Reason				
Male*	1	-	1	-
Female	0.7 (0.63,0.77)	<0.001	0.73 (0.63,0.85)	0.002
Both	0.81 (0.73,0.9)	<0.001	0.80 (0.68,0.94)	0.007
unexplained	0.81 (0.73,0.89)	<0.001	0.81 (0.72,0.91)	0.007
Secondary infertility*	1.21 (1.06,1.40)	0.005	1.09 (0.93, 1.29)	0.270
Polycystic Ovary Syndrome	1.19 (1.08,1.30)	<0.001	1.16 (0.99,1.36)	0.067
Hypothalamic Amenorrhea	0.71 (0.47,1.02)	0.085	0.76 (0.49,1.17)	0.214
Poor ovarian response	0.43 (0.34,0.52)	<0.001	0.71 (0.55,0.92)	0.011
Thyroid diseases	1.08 (0.98,1.19)	0.114	0.88 (0.79,1.003)	0.055
Hematologic disorders	0.85 (0.60,1.15)	0.310	1.14 (0.79,1.64)	0.486
OHSS in the previous cycle	1.91 (1.78,2.06)	<0.001	1.90 (1.72,2.10)	<0.001
Total consumption of gonadotropin	1.52 (1.41,1.63)	<0.001	1.53 (1.42,1.65)	<0.001
Duration of Infertility	0.99 (0.99,1)	0.174	1.01 (1.001,1.02)	0.024
Number of previous cycles	1.03 (1.01,1.05)	0.005	1.04 (1.01,1.07)	0.004
AMH	1.06 (1.04,1.07)	<0.001	-	-
FSH	0.97(0.96,0.98)	<0.001	0.99 (0.96,0.999)	0.033
LH	1(0.99,1.00)	0.060	1.01 (1.002,1.03)	0.019
PRL	1.00(1,1.00)	0.378	1.00 (1,1.00)	0.934
Day 3 estradiol	1(0.99,1.00)	0.005	-	-

Abbreviation: CI: confidence interval, AMH: anti-müllerian hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, PRL: prolactin, IRR: incidence rate ratio, aIRR: adjusted incidence rate ratio.
*Male factor and Primary type of infertility were reference levels in their own categories.

The expected number of MII sharply increased by PCOS in the crude analysis (IRR=1.49, 95% CI: [1.36, 1.64], $P < 0.001$), but it was borderline in

the multivariate analysis where the effect of other confounders was adjusted (aIRR=1.16, 95% CI: [0.99, 1.36], $P=0.067$). Poor ovarian response

significantly decreased the incidence risk ratio of MII oocytes by a factor of 0.71 (95% CI: [0.55, 0.93], $P=0.011$).

The expected number of MII in the women who had ovarian hyper-stimulation syndrome (OHSS) in the last previous cycle had an incidence risk ratio of 1.9 (95% CI: [1.72, 2.10], $P<0.001$).

Unexpectedly, an increase in the duration of infertility and the number of previous cycles had a weak positive association with the number of MII using multivariate analysis (aIRR of duration of infertility=1.01, 95% CI: [1.002, 1.02], $P=0.024$; aIRR of the number of previous cycles=1.04, 95% CI: [1.01, 1.07], $P=0.004$). Additionally, the effects of some important hormones, including anti-Müllerian hormone (AMH), follicle-stimulating hormone, luteinizing hormone, prolactin (PRL), and estradiol on day three are reported in Table 2.

In the current study, the causal effects of age and BMI were estimated using the newly presented method. The overall effects of age and BMI of all patients were significantly and inversely associated with the number of MII (both $P<0.001$) (Table 3). The expected number of MII decreased by 0.99 for an increase of 1 year (95% CI: [-1.00, -0.97]) and decreased by 0.99 for each 1-unit increase in BMI (95% CI: [-1.01, -0.98]). The posterior distributions and their 95% Bayesian credible intervals of age and BMI coefficients are shown in Fig. 2. Evaluating the causal effect of age and BMI among women with male factor infertility showed that, in line with results of all women, increasing maternal age and BMI had significant negative effects on the expected number of MII in women with a normal female reproductive system (Table 3).

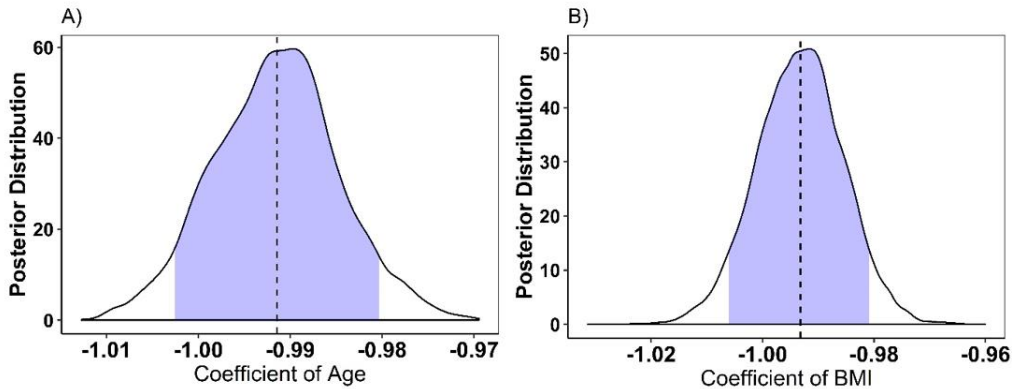


Fig. 2: The posterior distributions of coefficients of age (A) and BMI (B). The vertical dashed lines show the mean of distributions and the blue regions refer to 95% Bayesian credible intervals

Table 3: The causal effects of Age and BMI on number of MII oocytes

Factor	All women		Women with male factor infertility	
	Coefficient (95% CI)	P value	Coefficient (95% CI)	P value
Age (years)	- 0.99(-1.00,-0.97)	<0.001	- 0.99(-1.07, -0.92)	<0.001
BMI (kg/m ²)	- 0.99(-1.01,-0.98)	<0.001	- 1.05(-1.16, -0.96)	<0.001

CI: Bayesian credible interval.

Discussion

The current study tried to evaluate whether the rate of MII oocytes was affected by maternal age and BMI. The structure of the result was de-

signed hierarchically in three steps. At the first step, as a primary view, correlations of age and BMI with the rate of MII oocytes were reported (Fig. 1). After that, the crude and adjusted effects of these two risk factors on the number of MII

oocytes were estimated using Poisson regression are summarized in Table 2. Finally, at the last step, the causal effects of age and BMI were estimated by the new proposed statistical method and presented in Table 3.

The correlation analysis of age and BMI with the number of MII showed that there were significant correlations between these two factors and the number of MII. Results of the correlation analysis provide a primary view to suggest possible effects of age and BMI on oocyte outcome and are not valid to estimate adjusted or causal effects because of several existing important confounders.

According to the results of the second step of the analysis, increasing maternal age and BMI had significant offensive effects on the number of MII in univariate models. However, after adjusting the effects of other confounders, there was just a significant association between age and the number of MII. Therefore, the adjusted effect of BMI was not statistically significant based on multivariate analysis.

Until now, to our knowledge, there are no prior studies about the causal effects of maternal age and BMI on oocyte outcome in assisted reproductive technology and some have only used some classical statistical methods. There was an interaction between maternal age and BMI on mature oocytes. Therefore, fertility was altered by BMI in relation to maternal age. Some studies were contradictory and reported a decline in the number of retrieved oocytes because of an increase in BMI (23-25). Women with a BMI greater than 24 kg/m² were related to a significant rise in the number of follicles observed on ultrasound (26). A recently published study has some aspects that are similar to our study. As the BMI of patients increased, the number of oocytes retrieved significantly decreased. In agreement with this result, the current study found that there was an inverse association between the number of collected oocytes and BMI. However, Kudesia's study had a very large sample size; no advanced statistical causal method was applied to estimate the causal effect of BMI on in vitro fertilization (IVF) outcomes. In contrast, our study used a

new statistical causal model to estimate causal effects of maternal age and BMI on oocytes response. Based on these results, we found that generally, the expected of MII decreased by 0.99 for an increase of 1 year and decreased by 0.99 for each 1-unit increase in BMI.

Fertility was reduced by both rising BMI and maternal age in the normal population (27). In the current study, evaluating the causal effect of age and BMI among women with male factor infertility can be valuable because of their normal sexual system. In agreement with previous studies, there were invasive effects of both age and BMI in women with normal reproductive systems.

The strength of this study included the new statistical method developed by the authors and applied to the data set. Unlike previous studies, BMI and age were evaluated as quantitative variables to save the values of their information so the interpretation of results may be a bit more complex. However, we tried to collect all-important confounders; the current study is limited by its retrospective design.

Conclusion

Maternal age and BMI have significant adverse casual effects on the number of MII in patients undergoing ART when the effects of important confounders were controlled. Increasing maternal age and BMI have the same inverse effects on fertility in women with a normal reproductive system (woman with male factor).

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors have no conflict of interest to report.

References

- Lashen H, Ledger W, Bernal AL, et al (1999). Extremes of body mass do not adversely affect the outcome of superovulation and in vitro fertilization. *Hum Reprod*, 14(3): 712-5.
- Al-Obaidi MT, Mahdi HB, Alwasiti E (2018). The impact of age and BMI on oocyte maturation and embryo development. *Biomedical Research*, 29(9): 1920-1924.
- Fritz MA, Speroff L. (2012). *Clinical gynecologic endocrinology and infertility*. Lippincott Williams & Wilkins.
- Grøndahl ML, Christiansen SL, Kesmodel US, et al (2017). Effect of women's age on embryo morphology, cleavage rate and competence—A multicenter cohort study. *PLoS One*, 12(4): e0172456.
- Tatone C, Amicarelli F, Carbone MC, et al (2008). Cellular and molecular aspects of ovarian follicle ageing. *Hum Reprod Update*, 14(2): 131-42.
- Broekmans FJ, Knauff EA, te Velde ER, et al (2007). Female reproductive ageing: current knowledge and future trends. *Trends Endocrinol Metab*, 18(2): 58-65.
- Hourvitz A, Machtinger R, Maman E, et al (2009). Assisted reproduction in women over 40 years of age: how old is too old? *Reprod Biomed Online*, 19(4): 599-603.
- Van Loendersloot L, Van Wely M, Limpens J, et al (2010). Predictive factors in in vitro fertilization (IVF): a systematic review and meta-analysis. *Hum Reprod Update*, 16(6): 577-89.
- Rich-Edwards JW, Goldman MB, Willett WC, et al (1994). Adolescent body mass index and infertility caused by ovulatory disorder. *Am J Obstet Gynecol*, 171(1): 171-7.
- Zaadstra BM, Seidell JC, Van Noord P, et al (1993). Fat and female fecundity: prospective study of effect of body fat distribution on conception rates. *BMJ*, 306(6876): 484-487.
- Crosignani PG, Colombo M, Vegetti W, et al (2003). Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. *Hum Reprod*, 18(9): 1928-32.
- Green BB, Weiss NS, Daling JR (1988). Risk of ovulatory infertility in relation to body weight. *Fertil Steril*, 50(5): 721-6.
- Grodstein F, Goldman MB, Cramer DW (1994). Body mass index and ovulatory infertility. *Epidemiology*, 5(2): 247-50.
- Kalem MN, Kalem Z, Sari T, et al (2016). Effect of body mass index and age on in vitro fertilization in polycystic ovary syndrome. *J Turk Ger Gynecol Assoc*, 17(2): 83-90.
- Wilson A, Reich BJ (2014). Confounder selection via penalized credible regions. *Biometrics*, 70(4): 852-61.
- Tibshirani R (1996). Regression shrinkage and selection via the lasso. *J R Stat Soc Series B Stat Methodol*, 58(1): 267-288.
- Park T, Casella G (2008). The Bayesian lasso. *J Am Stat Assoc*, 103(482): 681-6.
- Hans C (2010). Model uncertainty and variable selection in Bayesian lasso regression. *Statistics and Computing*, 20(2): 221-229.
- Rosenbaum PR, Rubin DB (1983). The central role of the propensity score in observational studies for causal effects. *Biometrika*, 70(1): 41-55.
- Garrido MM, Kelley AS, Paris J, et al (2014). Methods for constructing and assessing propensity scores. *Health Serv Res*, 49(5): 1701-1720.
- McCandless LC, Gustafson P, Austin PC (2009). Bayesian propensity score analysis for observational data. *Stat Med*, 28(1): 94-112.
- Team RC (2017). R: A language and environment for statistical computing. Vienna, Austria. R Foundation for Statistical Computing. Available from: <https://www.R-project.org/>
- Spandorfer SD, Kump L, Goldschlag D, et al (2004). Obesity and in vitro fertilization: negative influences on outcome. *J Reprod Med*, 49(12): 973-7.
- van Swieten EC, van der Leeuw-Harmsen L, Badings EA, et al (2005). Obesity and Clomiphene Challenge Test as predictors of outcome of in vitro fertilization and intracyto-

- plasmic sperm injection. *Gynecol Obstet Invest*, 59(4): 220-4.
25. Maged AM, Fahmy RM, Rashwan H, et al (2019). Effect of body mass index on the outcome of IVF cycles among patients with poor ovarian response. *Int J Gynaecol Obstet*, 144(2):161-166.
26. Frattarelli JL, Kodama CL (2004). Impact of body mass index on in vitro fertilization outcomes. *J Assist Reprod Genet*, 21(6): 211-5.
27. Pasquali R, Pelusi C, Genghini S, et al (2003). Obesity and reproductive disorders in women. *Hum Reprod Update*, 9(4): 359-72.