ORIGINAL

Comparing the ICSI outcome between different causes of subfertility and estimate the role of IL-1β in predicting ICSI outcome.

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Abstract : Background : The physiological regulation of the ovary is a complicated process ; the growth factor, steroid hormones, gonadotropins, and cytokines all take part. Inflammatory cytokines are found in many compartments of the ovary, such as the follicles. The ovarian tissue produces them in addition to the macrophages. In this study, we aimed to compare the level of serum and follicular fluid IL-1Beta (IL-1β) for different groups of infertile patients, comparing the ICSI outcome between sub-fertile couples due to different causes. The results of this study IL-1β was significantly higher in the patients with unexplained subfertility (p-value < 0.05), no significant difference was found in the ICSI outcome between patients with different causes of subfertility. The lowest pregnancy rate was found in patients with unexplained subfertility, but the difference is insignificant. In conclusion, immunity plays a role in unexplained subfertility as patients with unexplained subfertility have a significantly higher serum level IL-1β than the other causes of subfertility. However, IL-1β does not affect ICSI outcome. Therefore, the ICSI outcome is not affected by the cause of subfertility. J. Med. Invest. 69 : 180-184, August, 2022

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INTRODUCTION

Subfertility affects almost 15% of couples (1). It is a reproductive abnormality that leads to the inability to conceive a baby after 12 months of contraception-free regular sexual intercourse (2). Globally, there is an increasing tendency toward assisted reproductive technology (ART) treatment (3, 4). ART remains the best and most effective treatment choice for many indications like bilateral tubal damage, male factor, severe endometriosis, and unexplained subfertility (5). The physiological regulation of the ovary is a complicated process ; the growth factor, steroid hormones, gonadotropins, and cytokines all take part. Immune cells in the ovary cause immune-endocrine interaction ; this modulates the ovarian function through secreting a soluble regulatory factor, especially the cytokines (6). Inflammatory cytokines are found in many compartments of the ovary, such as the follicles. The ovarian tissue produces them in addition to the macrophages (7). Many suggested that inflammatory cytokines directly affect the endocrine cells of the ovary ; on the other side, the production of hormones by the ovary influences the expression of the inflammatory cytokines (8). One of the most prominent mediators of inflammation is the interleukin-1 (IL-1) family, which involves two bioactive ligands : interleukin-1 alpha (IL-1α) and interleukin-1 Beta (IL-1β) (9). The effect of IL-1β on reproduction is still controversial, although several studies support its role. During folliculogenesis, IL-1 promotes proliferation and suppresses differentiation, whereas, during the ovulatory process, IL-1 increases the local production of eicosanoids, steroids, metalloproteases, and vasoactive substances promote ovulation (10). Type I IL-1 receptors are found in the uterine endometrium from day 23 of the menstrual cycle, that how they are associated with implantation (11-13). Following ovulation, the IL-1β increases the granulosa cells' luteal function, leading to the arrest of the proteins and collagenous fibers dissolving in the ovary (14-16). After embryo transfer in ATR, implantation occurs in a narrow window. There will be a feto-maternal cross-talk between invading blastocysts and receptive endometrium for a better outcome. Many identified and unidentified fetal and maternal cytokines and growth factors participate in this dialogue is required for a communication network between the embryo and the mother. Cytokines related to the maternal and fetal membrane have an essential role during normal gestation and successful pregnancy. Among the cytokines are the interleukins. Endometrial epithelium secrets IL-1β during the luteal phase ; it contributes to the embryo apposition, adhesion, attachment, and implantation to the endometrium (17). apart from above, IL-1β had favorable role in reproductive function, but they are not clearly understood.

In this study, we aimed to compare the level of serum and follicular fluid IL-1β for different groups of subfertile patients, comparing the ICSI outcome between sub-fertile couples due to different causes and emphasizing the association of serum and follicular fluid IL-1β with the ICSI outcome, including fertilization rate, cleavage rate, embryo quality, and pregnancy rate.

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MATERIAL AND METHODS

An analytic cross-sectional study was conducted at the fertility center in Al-Sader medical city, in Al-Najaf Governorate, Iraq. The study was done during the period from April 2021 to

December 2021. One hundred eighty couples participated in the study and were enrolled in the controlled ovarian hyper-stimulation program with oocytes pick up and ICSI. The participant had different causes of subfertility (anovulation cause n = 56, tubal factor n = 16, male factor subfertility n = 88, unexplained subfertility n = 20). The ethical committee approved the study of Jabir ibn hayyan medical university, and the whole procedure was clarified for the patients, and informed consent was taken. Age, body mass index (BMI), cause, duration and type of subfertility, parity, abortion if present all this information were taken from the patients. In addition, the female patients' full hormonal profile and complete medical, surgical, and gynecological history were all made.

The sample size was calculated according to the following equation for cross-sectional study with quantitative variables :

Sample size = (Z1-α/2) 2 SD2/d2

Z1-α/2 = is standard normal variant = 1.96

SD = standard deviation of the variable. The value of SD can be taken from a previously done study.

d = absolute error or precision as determined by the researcher.

Sample size = (1.96)2 (0.67)2 / (0.1)2 = (3.84* 0.448)/ 0.01 = 172 patients.

So the sample size is 172 patients, our study involved 180 patients.

The study included patients with a regular cycle, female age (18-40) years old, BMI = 18-35 kg/m^2, cycles reached to the oocytes retrieval and transfer of embryos. We excluded the poor responders and severe male factor. Male partner evaluated by doing seminal fluid analysis.

After a complete evaluation, the female patients underwent controlled ovarian hyper-stimulation (COH) programs with a short agonist protocol. The patients received a GnRH-a a 0.1 mg per day from the second day of the cycle until the ovulation triggered. Recombinant FSH in a dose determined according to the patients' age, BMI, and cause of subfertility. The dose was starting from 150 IU to 450 IU injected subcutaneously. The patients were followed up by serial ultrasound examination and serial measurement of the serum estrogen (E2) level to assess their response to the treatment. Ovulation triggered by human chorionic gonadotropin 10000 IU is given to the patients with good response to COH (> 3 follicles of diameter > 17 mm and the serum E2 level > 400 pg\ml). Thirty-six hours after the ovulation trigger, oocytes pick up was achieved. ICSI was done to the suitable retrieved oocytes. After ICSI, the oocytes would be examined for fertilization success after 16-18 hr. that is confirmed by the presence of two pronuclei (2PN). On the second-day post-injection, and under U\S guidance, the best resulting embryos would be transferred, maximally three embryos. The patients were given progesterone supplements for luteal phase support (Duphaston tablet 10 mg daily), and two weeks later pregnancy test was done.

The number of follicles and oocytes quality and number were assessed. The ICSI outcome represented by the number of 2PN, fertilization rate, the total number of embryos, embryo quality, cleavage rate, no. of transferred embryos, pregnancy rate were all compared between different causes of subfertility. Results were analyzed and statically explained.

SAMPLE COLLECTION AND ANALYSIS

Follicular fluid (FF) : The follicular fluid was collected from the mature ovarian follicles during the oocytes pick up under ultrasound guidance. The sample was centrifuged, and we stored the supernatant at -20 ºC.

Serum sample : a blood sample was drowned from the patients on the exact day of oocytes pick up. It is left standing for at least 15 mint, then we centrifuged and stored it at -20 ºC.

ELISA kits (Elabscience Biotechnology Inc.), which are commercially available, were used to quantitatively measure the levels of IL-1β in the follicular fluid and serum.

The serum and follicular fluid IL-1β were measured and compared between patients with different causes of subfertility.

STATISTICAL ANALYSIS

The data analysis was achieved by the Microsoft Office Excel 2016 and the SPSS 26 programs (Statistical Package for social sciences). Categorical data were plotted as numbers, and percentages and the numerical data were plotted as mean ±SE (stander error). One Way ANOVA test was used to interpret the difference between the numerical variables. The significant P-value is < 0.05.

RESULTS

Table 1 compares the serum and follicular IL-1β on the day of oocyte retrieval between the four studied groups. Using the One Way ANOVA test, the data are expressed in mean ± SE. There is a statistically significant difference in the serum level of IL-1β among the different causes of subfertility (P-value < 0.05).

Table 2 compares the embryological data of the four groups expressed in mean ± Standard error using One Way ANOVA. Though there is a lower number of follicles, retrieved oocytes, injected MII, no. of 2PN, and no. of embryos ; however, there was no statistically significant difference in embryological data among the different causes of subfertility (P-value > 0.05). Likewise, the pregnancy rate was lower in patients with unexplained subfertility ; still, the difference is insignificant.

Table 3. We have divided the patients into two age groups ; those with ≤ 35 years old and those with > 35 years old. compared the serum and follicular IL-1β concentration on the day of oocytes retrieval between patients according to their age. The data are expressed in mean ± SE using an independent sample t-test. There was no significant difference in the serum and follicular IL-1β concentration between the patients ≤ 35 years and those > 35 years old (P-value > 0.05).

Table 1. Serum and follicular IL-1β in different causes of subfertility

Variables	male factor (n = 88)	unexplained (n = 20)	Anovulation (n = 56)	Tubal factor (n = 16)	P value
Serum IL-1β (pg/ml)	5.20 ± 1.70	26.80 ± 17.80	3.10 ± 0.67	3.20 ± 1.10	0.014
Follicular IL-1β (pg/ml)	2.90 ± 0.82	1.20 ± 0.20	2.10 ± 0.38	1.00 ± 0.00	0.41

Table 2. Embryological data in the four groups of subfertility

Variables	Male factor (n = 88)	unexplained (n = 20)	anovulation (n = 56)	tubal factor (n = 16)	P value
Follicles number	12.77 ± 0.99	12.20 ± 3.02	13.86 ± 1.96	6.25 ± 2.86	0.18
Retrieved oocytes	9.95 ± 1.12	10.40 ± 3.32	11.86 ± 2.15	4.00 ± 1.47	0.21
Injected MII oocytes	8.59 ± 1.07	9.80 ± 3.56	10.7 ± 1.93	3.25 ± 1.31	0.26
2 PN	6.41 ± 0.81	7.40 ± 2.65	6.07 ± 0.9	2.75 ± 1.18	0.3
Fertilization rate	75.99 ± 4.69	63.4 ± 37.44	66.5 ± 6.8	90.00 ± 10.00	0.28
Embryos number	5.82 ± 0.69	7.4 ± 2.65	5.71 ± 0.91	2.75 ± 1.18	0.3
Cleavage rate	94.32 ± 2.22	80.00 ± 20.00	94.07 ± 3.18	100.00 ± 0.00	0.31
Transferred embryos	2.45 ± 0.15	2.40 ± 0.6	2.86 ± 0.09	1.75 ± 0.479	0.07
Grade I embryos	1.32 ± 0.28	0.20 ± 0.200	2.07 ± 0.54	1.00 ± 0.57	0.12
Grade II embryos	3.68 ± 0.57	5.40 ± 3.02	3.00 ± 0.54	1.25 ± 0.25	0.23
Endometrial thickness	9.68 ± 0.43	8.40 ± 0.51	10.08 ± 0.48	8.83 ± 0.946	0.39
Pregnancy rate	40.9%	20%	35.7%	50%	0.79

MII : metaphase two oocytes, 2PN : two pronuclei, GI : grade one, GII : grade two, GIII : grade 3

Table 3. Relation of IL-1β and patient's age. Data expressed by mean ± SE

IL-1β	≤ 35 years (n = 124)	> 35 years (n = 56)	P value
Serum IL-1β (pg/ml)	12.20 ± 1.90	5.20 ± 1.30	0.2
Follicular IL-1β (pg/ml)	3.60 ± 1.10	1.90 ± 0.30	0.1

DISCUSSION

Our study analyzes the association between the intracytoplasmic sperm injection outcome and the concentration of serum and follicular IL-1β at the day of oocyte collection to evaluate the local and systemic role of the pro-inflammatory cytokine in reproductive physiology. Our study measured the serum and follicular fluid IL-1β at the day of oocyte retrieval and detected the concentration of IL-1β in all patients (100%). A study detected it in serum and follicular fluid of all patients (18)

Sequeira *et al*. measured the serum IL1-β before HCG injection, and they found that the level was higher in women who achieved pregnancy (19). Another study measured it in serum and follicular fluid, and its level in the follicular fluid was higher than in the serum and detected in all patients (20). In contrast, a study for serum IL-1β on the day of oocytes retrieval detected it in 8.3% of the overall patients and 11.5% of patients with female factors subfertility (21). This variation may be due to different profiles of the studied patients (idiosyncratic variation between the patients) or the techniques used for cytokines measurement, which have been excluded in this study by performing all measurements by the same professional scientist. A remarkable variation in cytokines level is observed in many relevant studies (18, 22). In our study, there is a statistically significant difference in the serum level of IL-1β between the different causes of subfertility (P-value < 0.05). In addition, the patients with unexplained subfertility have shown a statistically significant higher level of serum IL-1β compared with the patients with other causes of subfertility. This may be due to an immunological factor affecting the fertility in patients with unexplained subfertility by a negative effect on the no. of oocytes and no. of 2PN (18, 20). The research found a lower concentration of serum IL-1β in patients with tubal factor subfertility compared with other subfertility causes and undetected in patients with unexplained subfertility (23). We found no significant difference in the level of follicular fluid IL-1β among the different causes of subfertility (P-value > 0.05) ; this may be because we studied the IL-1β in pooled follicular fluid rather than in the follicular fluid of individual follicles. The ICSI outcome is compared according to the cause of subfertility. There is no significant difference in ICSI outcome in various causes of subfertility. This can be attributed to the role of ICSI in overcoming all causes of subfertility ; the same study reported that the ICSI improves the outcome in IVF for treating the male factor subfertility (24). Many previous studies show that fertilization and pregnancy outcomes are not affected mainly by the parameters of male seminal fluid like motility, morphology, concentration, and DNA fragmentation (25, 26). It is thought that the ICSI outcome critically depends on the quality of the oocytes, which is affected by cytokines (27, 28). Therefore, we have studied the cytokine levels in the follicular fluid and compared them according to the ICSI outcome.

There is no statistically significant difference between the groups on comparing the pregnancy rate between the different causes of subfertility. A lower pregnancy rate is found in patients with unexplained subfertility. This is concomitant with the significantly higher serum interleukins IL-1β level in the group of patients with unexplained subfertility so that it may be a negative predictor for ICSI outcome, and this was agreed with a study done by Rihab *et al*., which showed that there is a negative correlation between the serum IL-1β and ICSI outcome (6). Our finding can be explained that ICSI itself leads to no significant association between the subfertility cause and the clinical outcome as (25) had stated. Some studies found similar observations in their research (18, 29, 30). Bhattacharya *et al*. disagreed with this finding and mentioned that the male factor subfertility was less likely to have treatment failure (31). On the other hand,

Hunault *et al*. stated a lower pregnancy rate in male factor or unexplained subfertility compared to tubal factor subfertility (32). Cai *et al*. mentioned that the pregnancy rate varied significantly with various causes of subfertility (33).

Even though the difference is not statistically significant, the serum and follicular fluid IL-1β was higher in older, which negatively affects the pregnancy rate. Similar findings are present in Nikolettos *et al*. and Mukheef *et al*. (18, 34). Asimakopoulos *et al*. (35) reported a negative association between age and follicular fluid IL-1β

CONCLUSION

immunity plays a role in unexplained subfertility as patients with unexplained subfertility have a significantly higher level of serum IL-1β than the other causes of subfertility. IL-1β has no effect on ICSI outcome. The ICSI outcome is not affected by the cause of subfertility.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states no conflict of interest.

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REFERENCES

1. Azuonwu G, Nduka CT : Technology Alternatives to Challenging Infertility Issues in Sub-Saharan Africa. Int J of Res and Reports in Gyn 4 : 1-9, 2021
2. Sahakian JP K K, El Helou E, Azoury J : Infertility within the Lebanese population : beliefs and realities. Middle East Fertil Soc J 25 : 1-8, 2020. https://doi.org/10.1186/s43043-020-00037-0
3. Adamson GD, de Mouzon J, Chambers GM, Zegers-Hochschild F, Mansour R, Ishihara O, Banker M, Dyer S : International Committee for Monitoring Assisted Reproductive Technology : world report on assisted reproductive technology 2011. Fertil Steril 110 : 1067-1080, 2018. https://doi.org/10.1016/j.fertnstert.2018.06.039
4. Ali RA, Mukheef MA, Majeed HH, Hadi BAA : Ovarian hyper-stimulation protocols in good prognosis patients : Agonist versus Antagonist protocol, an Iraqi view. J Pak Med Assoc 71 : 550-555, 2021
5. Ombelet W, van Eekelen R, McNally A, Ledger W, Doody K, Farquhar C : Should couples with unexplained infertility have three to six cycles of intrauterine insemination with ovarian stimulation or in vitro fertilization as first-line treatment? Fertil Steril 114 : 1141-1148, 2020. https://doi.org/10.1016/j.fertnstert.2020.10.029
6. Ali RA, Al-Murshidi SY, Al-jarah DM : The Role of Serum and Follicular IL-1Beta in Predicting the ICSI Outcome in Infertile Women. Indian J Public Health 10 : 511-521, 2019
7. Hammadeh ME, Fischer-Hammadeh C, Amer AS, Rosenbaum P, Schmidt W : Relationship between cytokine concentration in serum and preovulatory follicular fluid and in vitro fertilization/intracytoplasmic sperm injection outcome. Chem Immunol Allergy 88 : 80-97, 2005. https://doi.org/10.1159/000087822
8. Miceli F, Tropea A, Minici F, Navarra P, Lanzone A, Apa R : Interleukin-1 beta stimulates progesterone production by in vitro human luteal cells : evidence of a mediatory role of prostaglandins. J Clin Endocrinol Metab 88 : 2690-2694, 2003. https://doi.org/10.1210/jc.2002-020819
9. Huang HY : The cytokine network during embryo implantation. Chang Gung Med J 29 : 25-36, 2006
10. Stassi AF, Baravalle ME, Belotti EM : Altered expression of cytokines IL-1α, IL-6, IL-8 and TNF-α in bovine follicular persistence. Theriogenology 97 : 104-112, 2017
11. Uri-Belapolsky S, Shaish A, Eliyahu E, Grossman H, Levi M, Chuderland D, Ninio-Many L, Hasky N, Shashar D, Almog T, Kandel-Kfir M, Harats D, Shalgi R, Kamari Y : Interleukin-1 deficiency prolongs ovarian lifespan in mice. Proc Natl Acad Sci U S A 111 : 12492-12497, 2014. https://doi.org/10.1073/pnas.1323955111
12. Pretto CM, Gaide Chevronnay HP, Cornet PB, Galant C, Delvaux D, Courtoy PJ, Marbaix E, Henriet P : Production of interleukin-1alpha by human endometrial stromal cells is triggered during menses and dysfunctional bleeding and is induced in culture by epithelial interleukin-1alpha released upon ovarian steroids withdrawal. J Clin Endocrinol Metab 93 : 4126-4134, 2008. https://doi.org/10.1210/jc.2007-2636
13. Alizzi FJ, Abbas WAJA , Fawzi HA : Assessment of the role of cholecystokinin in hyperemesis gravidarum and correlation with its severity. J of Pharm Sciences and Research 10 : 272-275, 2018
14. Lee MY, Kim SH, Ihm HJ, Chae HD, Kim CH, Kang BM : Up-regulation of p21-activated kinase 1 by in vitro treatment with interleukin 1-beta and its increased expression in ovarian endometriotic cysts. Fertil Steril 96 : 508-511, 2011. https://doi.org/10.1016/j.fertnstert.2011.05.082
15. Woolery KT, Kruk PA : Ovarian epithelial-stromal interactions : role of interleukins 1 and 6. Obstet Gynecol Int. 358493, 2011. https://doi.org/10.1155/2011/358493
16. Mohammed MA, Kadhim KA, Jasim GA , Fawzi HA : Metformin compared to insulin for the management of gestational diabetic. Int J Res Pharm Sci 9 : 1063-1070, 2018
17. Rehman R, Jawed S, Zaidi SF, Baig M, Ahmeds K : Role of interleukin-l 3 in conception after intracytoplasmic sperm injection. J Pak Med Assoc 65 : 49-53, 2015
18. Nikolettos N, Asimakopoulos B, Nicolettos N, Efthimiadou A, Mourvati E, Demirel C : Evaluation of leptin, interleukin-1beta, tumor necrosis factor-alpha and vascular endothelial growth factor in serum and follicular fluids of women undergoing controlled ovarian hyperstimulation as prognostic markers of ICSI outcome. In Vivo. 18 : 667-673, 2004
19. Sequeira K, Espejel-Núñez A, Vega-Hernández E, Molina-Hernández A, Grether-González P : An increase in IL-1β concentrations in embryo culture-conditioned media obtained by in vitro fertilization on day 3 is related to successful implantation. J Assist Reprod Genet 32 : 1623-1627, 2015. https://doi.org/10.1007/s10815-015-0573-4
20. Yang H, Pang H, Miao C : Ovarian IL-1α and IL-1β levels are associated with primary ovarian insufficiency. Int J Clin Exp Pathol 11 : 4711-4717, 2018
21. Bonetti TC, Salomao R, Brunialti M, Braga DP, Borges E Jr, Silva ID : Cytokine and hormonal profile in serum samples of patients undergoing controlled ovarian stimulation : interleukin-1beta predicts ongoing pregnancy. Hum Reprod 25 : 2101-2106, 2010. https://doi.org/10.1093/humrep/deq171
22. Tsai EM, Yang CH, Chen SC, Liu YH, Chen HS, Hsu SC, Lee JN : Leptin affects pregnancy outcome of in vitro

fertilization and steroidogenesis of human granulosa cells. J Assist Reprod Genet 19 : 169-176, 2002. https://doi.org/10.1023/a:1014889928191
23. Sarapik A, Velthut A, Haller-Kikkatalo K, Faure GC, Béné MC, de Carvalho Bittencourt M, Massin F, Uibo R, Salumets A : Follicular proinflammatory cytokines and chemokines as markers of IVF success. Clin Dev Immunol 60 : 59-64, 2012. https://doi.org/10.1155/2012/606459
24. Ashrafi M, Jahanian Sadatmahalleh S, Akhoond MR, Ghaffari F, Zolfaghari Z : ICSI Outcome in Infertile Couples with Different Causes of Infertility : A Cross-Sectional Study. Int J Fertil Steril 7 : 88-95, 2013
25. Abu-Hassan D, Koester F, Shoepper B, Schultze-Mosgau A, Asimakopoulos B, Diedrich K, Al-Hasani S : Comet assay of cumulus cells and spermatozoa DNA status, and the relationship to oocyte fertilization and embryo quality following ICSI. Reprod Biomed Online 12 : 447-452, 2006. https://doi.org/10.1016/s1472-6483(10)61997-9
26. Karydis S, Asimakopoulos B, Papadopoulos N, Vakalopoulos I, Al-Hasani S, Nikolettos N : ICSI outcome is not associated with the incidence of spermatozoa with abnormal chromatin condensation. In Vivo. 19 : 921-925, 2005
27. Nori W, Ali AI : Maternal alpha-1-antitrypsin as a noval marker for growth restriction in pre-eclampsia. J Obstet Gynaecol Res 47 : 4250-4255, 2021. https://doi.org/10.1111/jog.15043
28. Nori W, Hamed RM, Roomi AB, Akram W : Alpha-1antitrypsin in pre-eclampsia ; from a clinical perspective. J Pak Med Assoc. 71 : 553-556, 2021. https://jpma.org.pk/supplement-article-details/670
29. Lintsen AM, Eijkemans MJ, Hunault CC, Bouwmans CA, Hakkaart L, Habbema JD, Braat DD : Predicting ongoing pregnancy chances after IVF and ICSI : a national prospective study. Hum Reprod 22 : 2455-2462, 2007
30. Al-Ghamdi A, Coskun S, Al-Hassan S, Al-Rejjal R, Awartani K : The correlation between endometrial thickness and outcome of in vitro fertilization and embryo transfer (IVF-ET) outcome. Reprod Biol Endocrinol 6 : 37-44, 2008. https://doi.org/10.1186/1477-7827-6-37
31. Bhattacharya S, Maheshwari A, Mollison J : Factors associated with failed treatment : an analysis of 121, 744 women embarking on their first IVF cycles. PLoS One. 8 : e82249, 2013. https://doi.org/10.1371/journal.pone.0082249
32. Hunault CC, Eijkemans MJ, Pieters MH, te Velde ER, Habbema JD, Fauser BC, Macklon NS : A prediction model for selecting patients undergoing in vitro fertilization for elective single embryo transfer. Fertil Steril 77 : 725-732, 2002. https://doi.org/10.1016/s0015-0282(01)03243-5
33. Cai QF, Wan F, Huang R, Zhang HW : Factors predicting the cumulative outcome of IVF/ICSI treatment : a multivariable analysis of 2450 patients. Hum Reprod 26 : 2532-2540, 2011. https://doi.org/10.1093/humrep/der228
34. Mukheef MA, Ali RA, Majeed HH : How Can the Maternal Age and the Basal Level of Serum FSH Predict the ICSI Outcome?. Indian J Forensic Med Toxicol 15 : 1695-1702, 2021
35. Asimakopoulos B, Abu-Hassan D, Metzen E, Al-Hasani S, Diedrich K, Nikolettos N : The levels of steroid hormones and cytokines in individual follicles are not associated with the fertilization outcome after intracytoplasmic sperm injection. Fertil Steril 90 : 60-64, 2008