Diminished ovarian reserve is the major cause of infertility among women undergoing their first assisted reproductive technology treatment: a retrospective observational study

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Research

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Abstract

Background

This study aims to investigate the effects of various factors on treatment outcomes in women undergoing *in vitro* fertilization or intracytoplasmic sperm injection (IVF/ICSI) with embryo transfer (ET).

Methods

Of the 8993 eligible women who underwent their first IVF/ICSI–ET cycles, and met our inclusion and exclusion criteria, 2742(30.5%) achieved clinical pregnancy while 6251(69.5%) did not. Multivariable Cox regression analysis, multiple logistic regression analysis, and classification tree analysis were used sequentially to screen key predictors among predictors of various infertility causes and ovarian stimulation protocols through the best subset technique.

Results

Multivariate Cox regression analysis showed that the main factor affecting fertility in first attempts at IVF/ICSI–ET is diminished ovarian reserve (DOR), with a hazard ratio (HR) of 0.406 and 95% confidence interval (CI) of 0.353–0.466. Multiple forward logistic regression with 5-fold cross-validation also found that, with an odds ratio (OR) of 2.522 (95% CI = 2.167–2.937), DOR affects fertility. The classification tree analysis was further used to better visualize the model.

Conclusions

DOR is the major factor affecting success rates in couples undergoing their first attempt at IVF/ICSI-ET. The selection of the most appropriate pairs for IVF/ICSI treatment can not only increase the success rates but also the cumulative cost-effectiveness.

Plain English Summary

Couples visit the reproductive clinics with one purpose which is to have a healthy baby but with different reasons. Factors that influence fertility can be divided into female factors, male factors and other unknown reasons. Female factors include uterine factors, tubal factors, ovarian factors and so on. Among these various factors, which one affects the pregnancy outcome of *in vitro* fertilization (IVF) most? To answer this question, we compared the clinical pregnancy outcome of 8993 women undergoing their first attempt at IVF. The results turned out that women with diminished ovarian reserve (DOR) had the least possibility to be pregnant. Ovarian reserve refers to the quantity and quality of oocytes in ovaries and decreases with age gradually. It can also be impaired by surgery, chemotherapy, infection or some unhealthy living habits. Due to its great impact on pregnancy outcome, women who wish to conceive are

suggested to make their childbearing plans in time before DOR happens and evaluation of ovarian reserve is recommended.

Background

The ability of a couple to establish a clinical pregnancy is known as fertility [1]. Any factor that influences the quantity and quality of oocytes or spermatozoa, as well as the processes of fertilization and embryo implantation, affects human fertility. There is a fixed number of primordial follicles in women, termed ovarian reserve, and female fertility is lost when the ovarian reserve is completely depleted at menopause. For several reasons, an increasing number of women delay their childbearing plans, and this is believed to be the predominant cause of the increased rate of infertility worldwide [2]. However, in 50% of infertility cases, men were also identified to have abnormal semen parameters [3]. It is therefore worth considering if the impact of male infertility is similar to diminished ovarian reserve (DOR) in women.

To the best of our knowledge, no study has established the effects of various causes of infertility on the success rates in couples undergoing assisted reproductive technology (ART) treatments such as *in-vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). To analyze factors associated with infertility, some studies have used logistic regression, using clinical pregnancy rate or live birth rate as outcome variables [4]. Others have used survival analyses to analyze the fertility of reproductive-aged women. The key outcome is the achievement of clinical pregnancy and the time-to-pregnancy (TTP) as the duration to the desired result [5]. We used both the two methods, together with classification tree analysis, to analyze the effects of various infertility factors on success rates in couples undergoing their first ART treatment to make this study more comprehensive.

Methods

From January to December 2018, a total of 16,280 fresh IVF/ICSI cycles were retrospectively exported from the IVF/ICSI registration system in our center. 11,888 first IVF/ICSI cycles were selected, of which 211 cycles using donated spermatozoa were excluded. In addition, cycles not proceeding to embryo transfer (ET) for preimplantation genetic diagnosis/screening (PGD/PGS) or to prevent ovarian hyperstimulation syndrome (OHSS) were removed. A total of 8,993 cycles were finally included for the analysis (Fig. 1). Given that we used de-identified patient data, informed patient consent was waived and approval by the institutional review board was not necessary following the Helsinki declaration.

DOR was defined according to our AAFA model (anti-Müllerian hormone (AMH), antral follicle counts (AFC), basal follicle-stimulating hormone (FSH), and age, in order of their significance) [6]. On menstrual cycle day 2, transvaginal ultrasound scans were used to analyze the AFC of follicles with diameters of 2–9 mm in both ovaries. On the same day, circulating blood samples were collected for FSH, luteinizing hormone (LH), and estradiol (E_2) tests. For AMH assays, samples were collected on any day of the menstrual cycle. A Siemens Immulite 2000 immunoassay system (Siemens Healthcare Diagnostics, Shanghai, P. R. China) was used to measure serum FSH concentrations. Serum AMH concentrations were

measured using an ultrasensitive two-site enzyme-linked immunosorbent assay (Ansh Labs, Webster, TX, USA), following quality controls supplied with the kit.

The data were presented as mean ± SD, number and (percentage) or median and (interquartile range, IQR). A multiple Cox regression analysis was performed using the best subset method among predictors of different causes of infertility and ovarian stimulation protocols to screen for the significant predictors. Parameter estimates were determined at each stage by increasing the number of active effects in the model and the optimal model with the smallest Bayesian Information Criterion (BIC), was selected from all the possible models. The hazard ratio (HR) with a 95% confidence interval (CI) was computed for each selected factor. For the screening process, multiple forward logistic regression with 5-fold cross-validation was also used, and the odds ratio (OR) with 95% CI were computed. The classification tree was used to better visualize the logistic model. A two-sided p-value of < 0.05 was considered statistically significant. All analyses were conducted using SAS JMP Pro (version 14.2; SAS Institute, Cary, NC, USA).

Results

Of the 8993 eligible women who underwent their first ART cycle and met our inclusion criteria, 2,742 (30.5%) achieved clinical pregnancy while 6251 (69.5%) did not. The TTP and numbers of different causes of infertility related to the clinical pregnancy rates are indicated in Table 1.

Figure 2A shows the selection of variables based on the best subset using multivariate Cox regression analysis. In Fig. 2B, the horizontal axis shows the numbers of active effects (predictors) included in this model, and the vertical axis shows the BIC. The optimal model with the smallest BIC is indicated by the red line. The order of variables included in our best subset was: DOR, the ovarian stimulation protocol, and uterine associated factors. The major factors affecting the clinical pregnancy rates of these women undergoing their first ART treatment were DOR and uterine-related factors after adjusting for ovarian stimulation protocols. The parameter estimates and HR of each predictor are shown in Table 2. The HR for DOR versus non-DOR was 0.406 (95%CI 0.353–0.466), and that for uterine associated factors was 0.764 (95%CI 0.660–0.885). Further, Kaplan-Meier survival analyses of clinical pregnancy rates over TTP revealed that DOR was statistically significant for this outcome, with both log-rank *p*- and Wilcoxon *p*-values of < 0.0001, as shown in Fig. 2C. Intrauterine factors were also significant, with log-rank *p*-value of 0.0029 and Wilcoxon *p*-value of 0.0266 (Fig. 2D).

Figure 3A shows the selection of variables based on the best subset using multiple forward logistic regression analysis with 5-fold cross-validation. In Fig. 3B, the horizontal axis shows the numbers of active effects (predictors) included in this model, and the vertical axis shows the BIC, similar to the Cox regression model. The optimal model is indicated by the red line. The first and second variables included in this best subset were DOR and the ovarian stimulation protocol, respectively. The third variable was TTP. The parameter estimates and OR of each predictor are outlined in Table 3. DOR was the most significant, with an OR of 2.522 (95% CI = 2.167–2.937), consistent with the Cox regression analysis results.

Classification tree analysis with 5-fold cross-validation was conducted to demonstrate the outcome more clearly. In Fig. 4A, the blue line represents the training set and the red line represents the validation set. The optimal model is indicated by the vertical line when there are four splits. As shown in Fig. 4B, the first split is DOR, with LogWorth of 33.72 ($p < 0.0001$). The clinical pregnancy rate of the group with DOR is 15.78%, while that of non-DOR is 33.54%. The following splits are different ovarian stimulation protocols and infertile years.

Discussion

Estimating the influence of various factors on the evaluation of fertility is of great importance in counseling couples. Choosing the most appropriate couples for ART treatment can not only increase success rates but is also cost-effective. In this retrospective cohort study using three different analysis methods, we showed that DOR is the most important factor affecting outcome success in couples undergoing their first attempt at ART.

The ovarian reserve decreases with age and is commonly known to be a major factor affecting human fertility [7]. Decreased fecundity begins 10 years before menopause, reflecting the DOR [8–9]. Although DOR has long been regarded as an important factor affecting fertility, little research has been conducted to support this concept for three main reasons. First is the discrepancies in the definition of DOR. According to the Bologna criteria, DOR is defined as an AMH level of < 0.5–1.1 ng/ml and/or AFC < 5–7 [10], which causes confusion in clinical practice. For instance, a patient with an AFC of 6, whose age, AMH and FSH are satisfactory, according to our AAFA model [6], has a good ovarian reserve. However, the same patient is considered to have DOR according to the Bologna criteria. Secondly, in-depth research about human fertility is extremely difficult because of the worldwide use of contraceptives [11]. Lastly, there is no acknowledged method for fertility assessment during ART for infertile couples. Therefore, analysis of the fertility of couples undergoing ART has always been difficult and controversial.

The duration of infertility is crucial in fertility since infertility is defined as 12 months of unsuccessful, and unprotected intercourse. We, therefore, used multiple Cox regression analysis. In women who naturally conceive, survival analysis is commonly used to determine their fertility, using TTP as the duration of the event, and clinical pregnancy as the outcome event. However, it is worth investigating whether TTP represents the potential to develop a clinical pregnancy in ART populations since the duration of the outcome is an unfixed duration on how long the infertile couple has sought treatment from specialist clinics. We, therefore, tried to use other indicators as the duration of the event for survival analysis. For example, we used the number of ART attempts as the event duration variable for survival analysis. However, the number of such attempts is not a time-related variable and does not consider the time before couples went for treatment and the variations of the time interval between each ART attempt in different couples. It is therefore not suitable as a variable of the duration of outcome for survival analysis. Therefore, using TTP before the first ART attempt is more appropriate in evaluating the fertility of infertile couples than the number of such attempts.

Some statisticians state that survival analysis cannot determine outcomes in IVF/ICSI populations because pregnancies do not occur spontaneously. Logistic regression analysis has been used in some studies that use clinical pregnancy rate or live birth rate as the outcome [12–13]. We also conducted logistic regression analysis using clinical pregnancy as the outcome variable. Results showed that DOR was the most significant factor affecting clinical pregnancy, consistent with the Cox regression analysis results. This verifies our assertion that DOR is a major factor affecting outcomes for couples undergoing their first ART cycle.

Classification tree analysis has not been widely used before on infertility research. Ahlstrom et al. used classification-and-regression-tree analysis to conduct prediction of live birth through the embryo's morphology [14]. We also conducted the classification tree analysis to illustrate with simplicity and adaptability the best splits. The results showed that DOR was the first split to differentiate between the chances of clinical pregnancy, in line with the results of two previous statistical approaches.

The three different methods used in this study identified DOR as the major factor affecting clinical pregnancy in couples undergoing their first ART, in line with other studies that found a correlation between DOR and fertility [15–16]. However, controversy has been also reported in several publications. Steiner et al. [17] analyzed the early follicular phase serum level of AMH and FSH of 750 women aged between 30 and 44 without a history of infertility. They recorded their cumulative probability of conception by 6 and 12 cycles of attempt and the probability of conception in a given menstrual cycle. Women with low AMH or high FSH values did not have a significantly different predicted probability of conceiving compared to women with normal hormonal values after adjusting for age, body mass index, ethnicity, current smoking status, and recent hormonal contraceptive usage. Analysis by Hagen et al [18] showed similar results. Low AMH did not predict decreased fecundity in healthy women in their mid-20 s when assessed by the monthly probability of conceiving. The key explanation for these controversial findings could be the inaccurate selection of one single marker to represent ovarian reserve. As earlier mentioned, according to our AAFA model, a patient with low AMH or high FSH but satisfactory age and AFC is considered to have moderate ovarian reserve [6]. However, the previous studies classified such patients in the DOR group, leading to false-negative results. Secondly, these studies used healthy women who were trying to conceive naturally as participants, which has more confounding variables such as menstrual cycles, tubal functions, and male factors. Infertility patients undergoing ART were included in our population, which is more instructive for clinical practice. We also had a bigger sample size compared with the previous studies.

Although it is acknowledged that female fertility decreases with age, age was not included in our analysis as an independent factor. This is because DOR and age are well correlated from the statistical perspective, which can cause collinearity, and therefore they should not be included simultaneously. Furthermore, the included predictors are the different causes of infertility, which excludes age as it is not a type of infertility. The age of the male partner was also not included in the model for the same reason.

While follicle quantity and quality change substantially with age, there is still little attention on the high heterogeneity in the ovarian reserve among women undergoing ART treatments [9, 11]. DOR is only reported when signs of irregular cycles and menopause appear, leaving limited clinical options [19]. Thus, a woman of reproductive age needs to assess her ovarian reserve in time. We previously published our AAFA model for ovarian reserve analysis using AMH, AFC, FSH, and age [6]. We have developed an algorithm based on this model to predict the starting age of DOR and menopause, which would help women of reproductive age to plan their childbearing according to their ovarian reserve status, thereby reducing the global needs for infertility treatment.

The male factor accounts for 50% of all infertile cases [20–21]. Azoospermia, including obstructive azoospermia (OA) and nonobstructive azoospermia (NOA), is a major cause of male infertility, accounting for 10–15% of male factors [20, 22]. Azoospermia used to be considered an untreatable condition, with sperm donation the only remedy for conception. The introduction of ICSI has revolutionized the treatment of such patients [23]. A live birth rate of up to 28% has been recorded even in patients with NOA [24]. Using ICSI during ART cycles can be one of the reasons why azoospermia does not affect overall outcomes in couples receiving ART treatment. However, we analyzed ART cycles in which the spermatozoa and oocytes of the couples were used. Sperm donation cycles were excluded for males suffering from severe spermatogenesis defects. This is another likely reason why azoospermia as a factor did not affect outcomes in couples receiving their first IVF/ICSI attempts.

One of the limitations of our study is that clinical pregnancy or not was used as the outcome event in all three methods. Future studies should hence focus on other outcome variables such as retrieved oocyte numbers, transferable embryo numbers, miscarriage rate, live birth rate and even obstetric outcomes. Besides, as a retrospective study, some confounding variables, such as body mass index, the selection of treatment and the dosage of medication may have existed in the analysis. Also, the study was performed in a single reproductive center in China. Therefore, a multi-center study is needed to represent the whole country or even all Asian countries.

Conclusions

In summary, the selection of the most appropriate pairs for IVF/ICSI treatment can not only increase the success rates but also the cumulative cost-effectiveness. On their first attempt at IVF/ICSI-ET, DOR is the major factor affecting success rates in couples.

Abbreviations

AFC, antral follicle counts; AMH, anti-Müllerian hormone; ART, assisted reproductive technology; BIC, Bayesian Information Criterion; DOR, diminished ovarian reserve; E_2, estradiol; ET, embryo transfer; FSH, follicle-stimulating hormone; ICSI, intracytoplasmic sperm injection; IVF,in-vitro fertilization; LH, luteinizing hormone; NOA, nonobstructive azoospermia; OA, obstructive azoospermia; OHSS, ovarian

hyperstimulation syndrome; PGD/PGS, preimplantation genetic diagnosis/screening; TTP, time-to-pregnancy.

Declarations

Ethics approval and consent to participate: Given that we used de-identified patient data, informed patient consent was waived and approval by the institutional review board was not necessary following the Helsinki declaration.

Consent for publication: Not applicable.

Availability of data and materials: The data that support the findings of this study are available from Center for Reproductive Medicine, Peking University Third Hospital, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Peking University Third Hospital.

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: Mengqian Zhang contributed to manuscript drafting and revising. Hongxian Zhang and Rui Yang contributed to design of the work and manuscript drafting. Guoshuang Feng contributed to data analysis and interpretation. Huiyu Xu contributed to the conception of the study and manuscript revising. Jie Qiao contributed to the conception of the study and final approval.

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Tables

Due to technical limitations, table 1, 2, 3 is only available as a download in the Supplemental Files section.

Figures

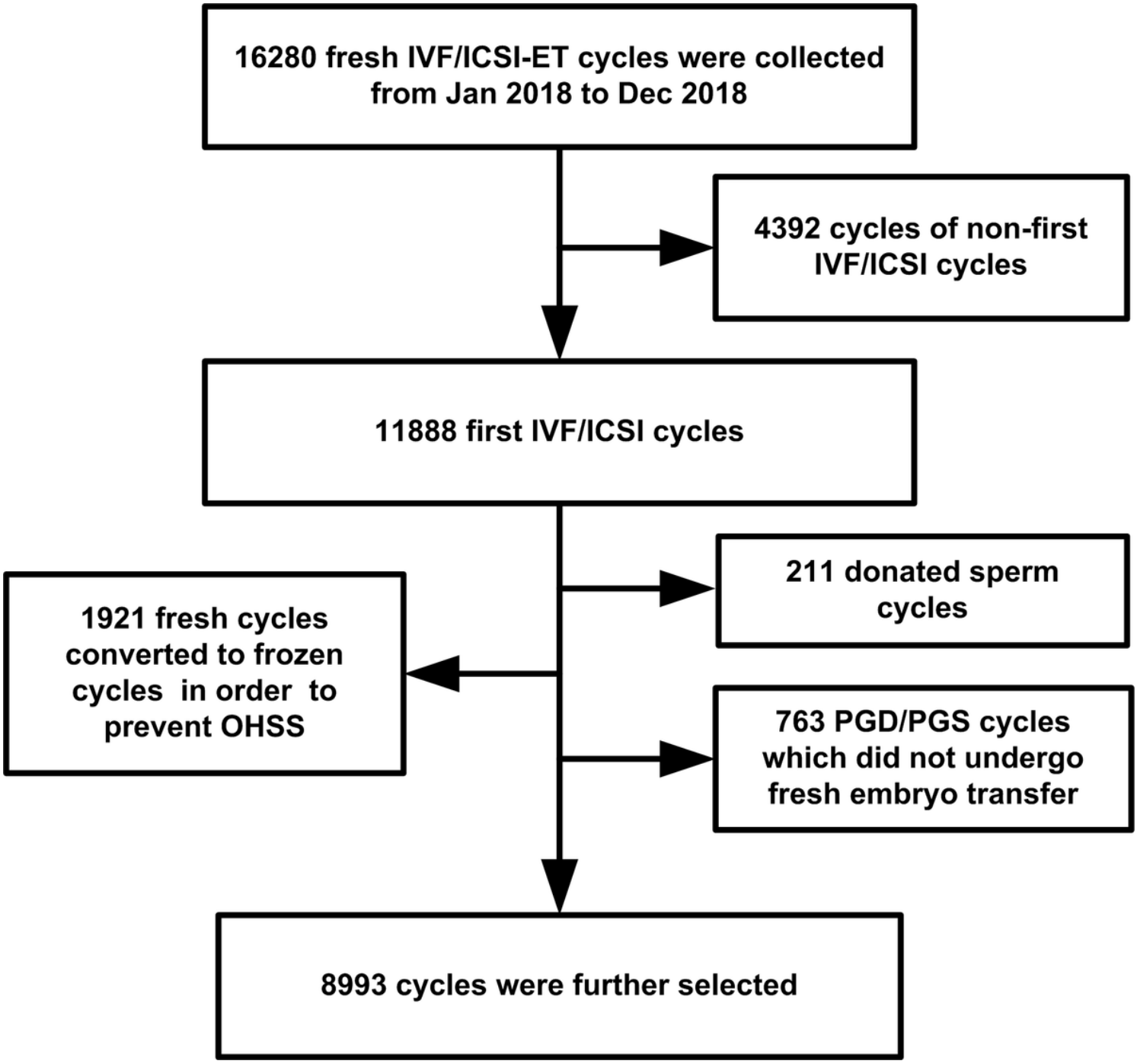
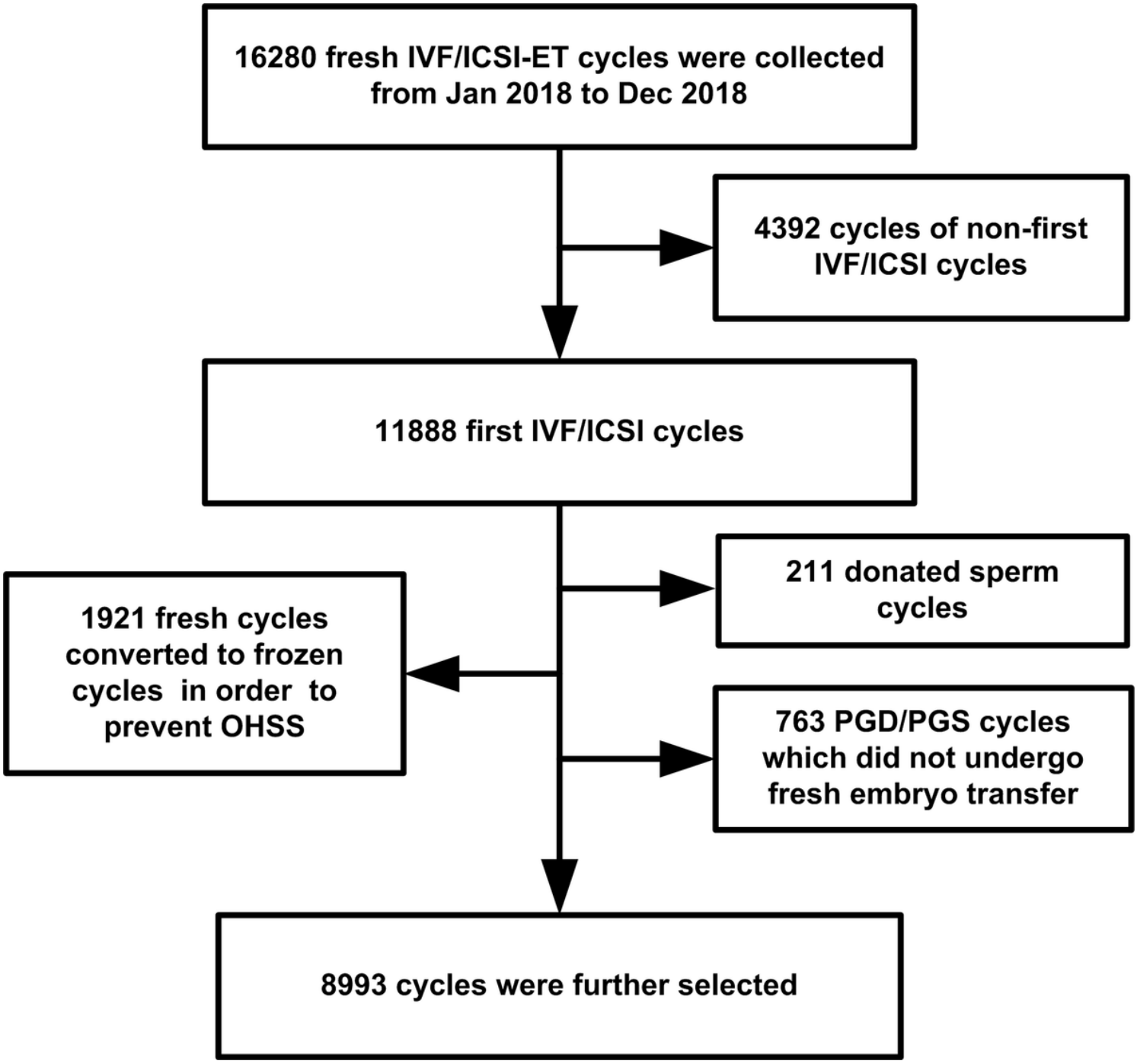


Figure 1

Flowchart of the study population selection strategy.



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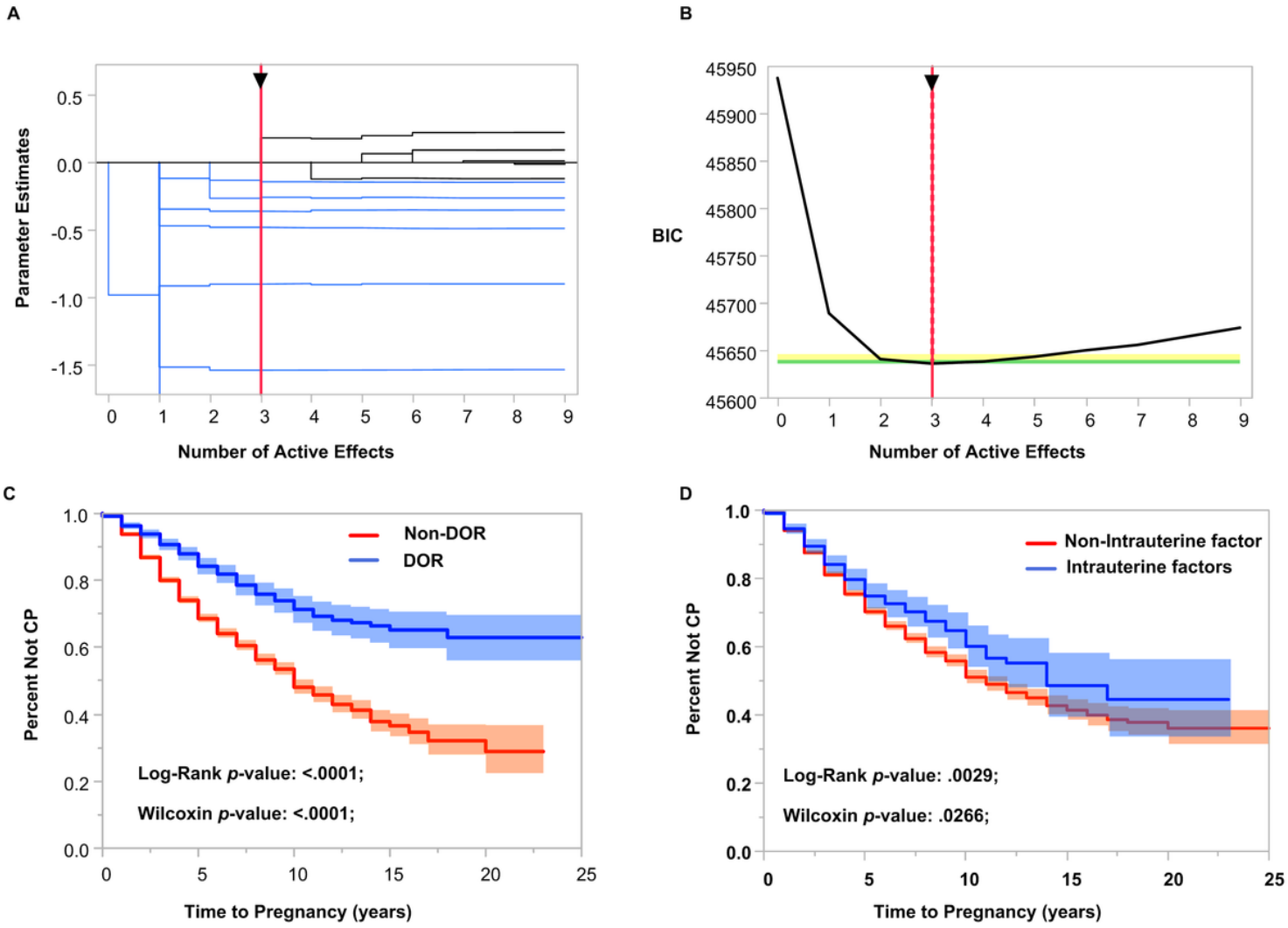
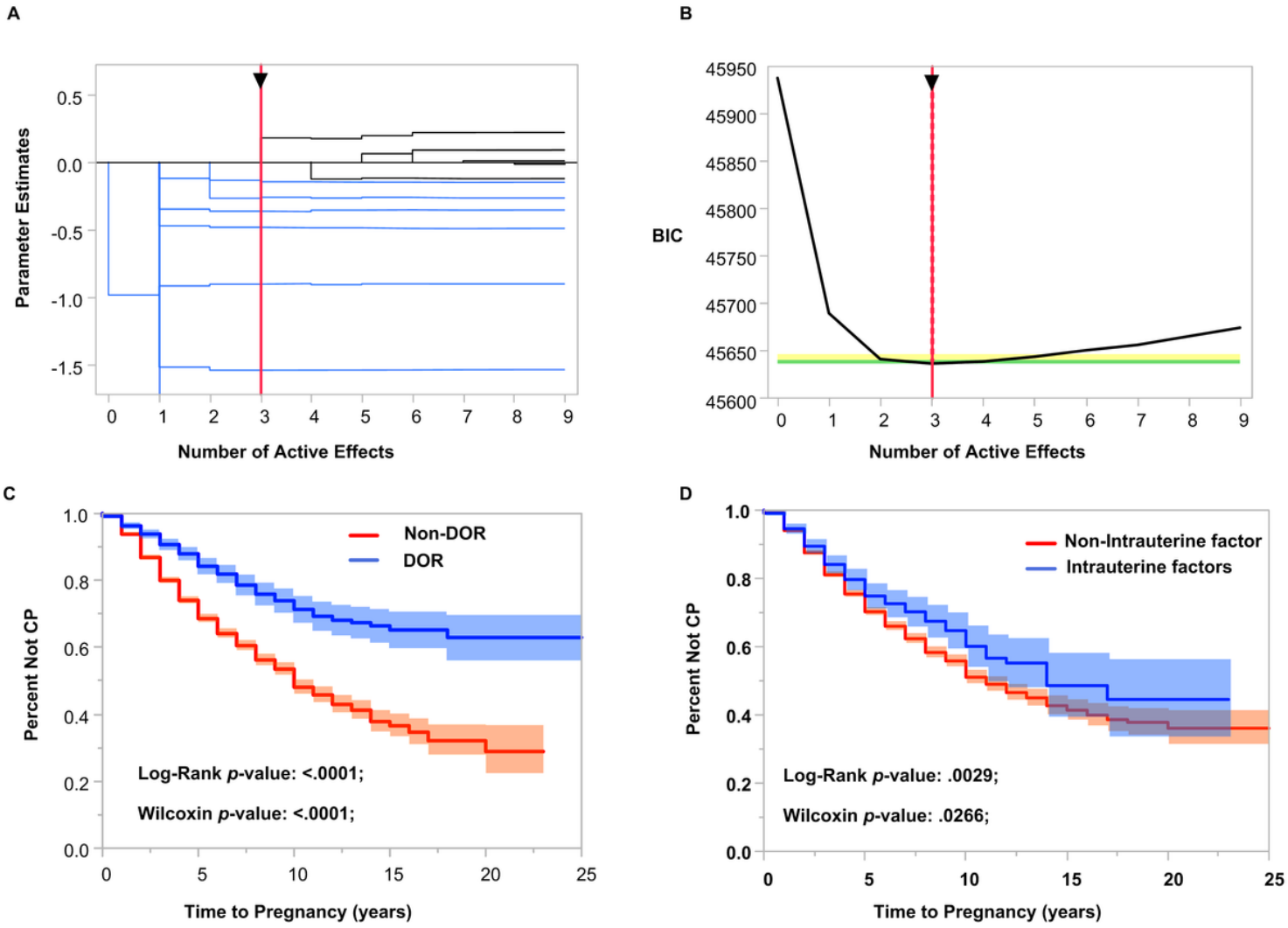


Figure 2

Multiple Cox regression analysis and Kaplan–Meier survival analysis of ART outcomes. A, shows the process of selecting variables based on the best subset of multivariate Cox regression. In B, the horizontal axis shows the number of active effects (predictors) included in this model, and the vertical axis shows the Bayesian Information Criterion (BIC). The optimal model is indicated by the red line, with the smallest BIC. C and D show the major differences between curves using the log-rank and Wilcoxon tests.



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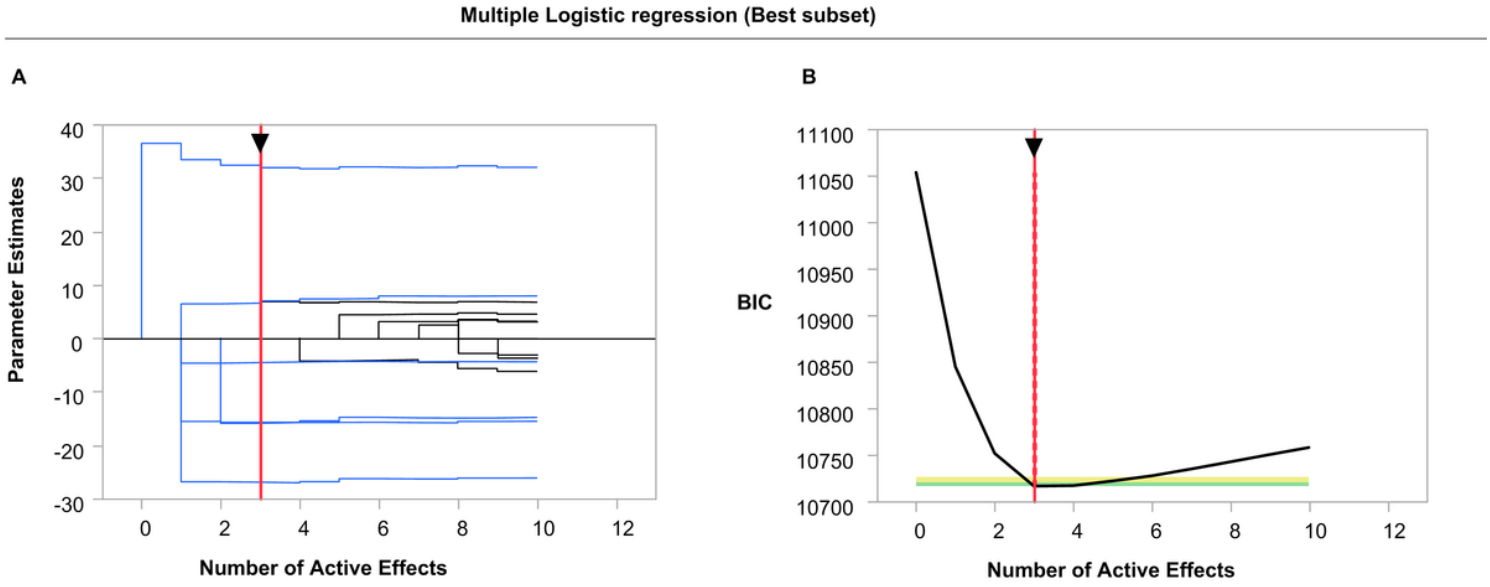
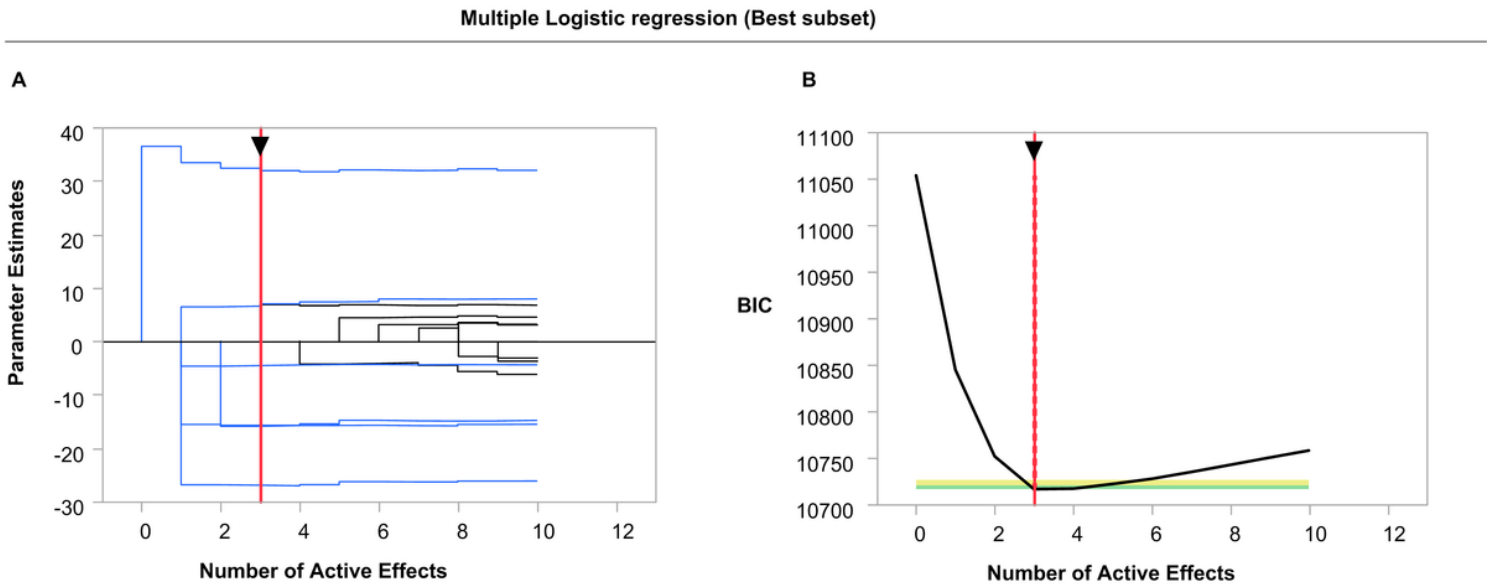


Figure 3

Multiple logistic regression analysis. A, shows the process of selecting variables based on the best subset of multiple logistic regression. In B, the horizontal axis shows the number of active effects (predictors) included in this model, and the vertical axis shows the Bayesian Information Criterion (BIC). The optimal model is indicated by the red line, with the smallest BIC.



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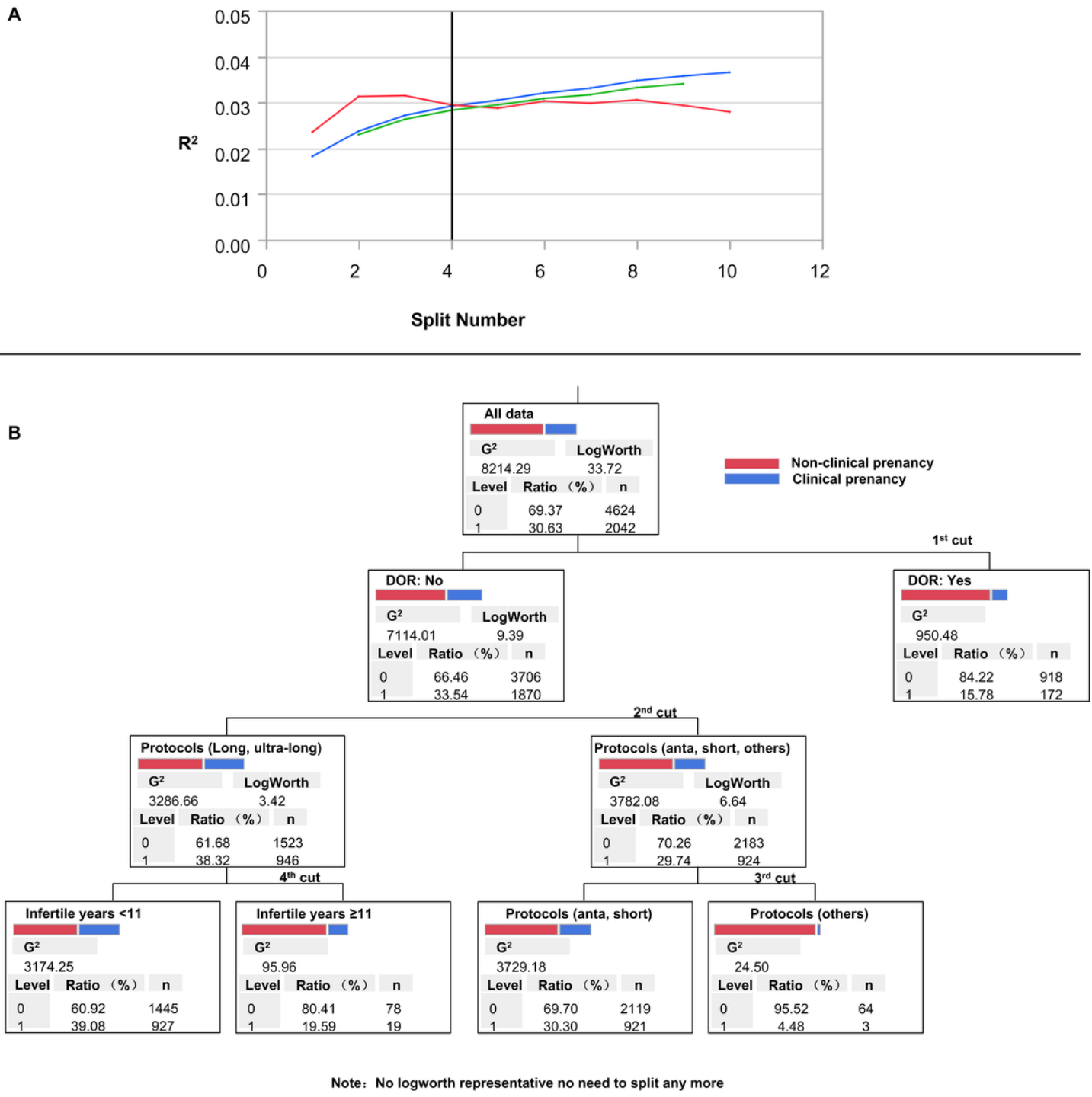
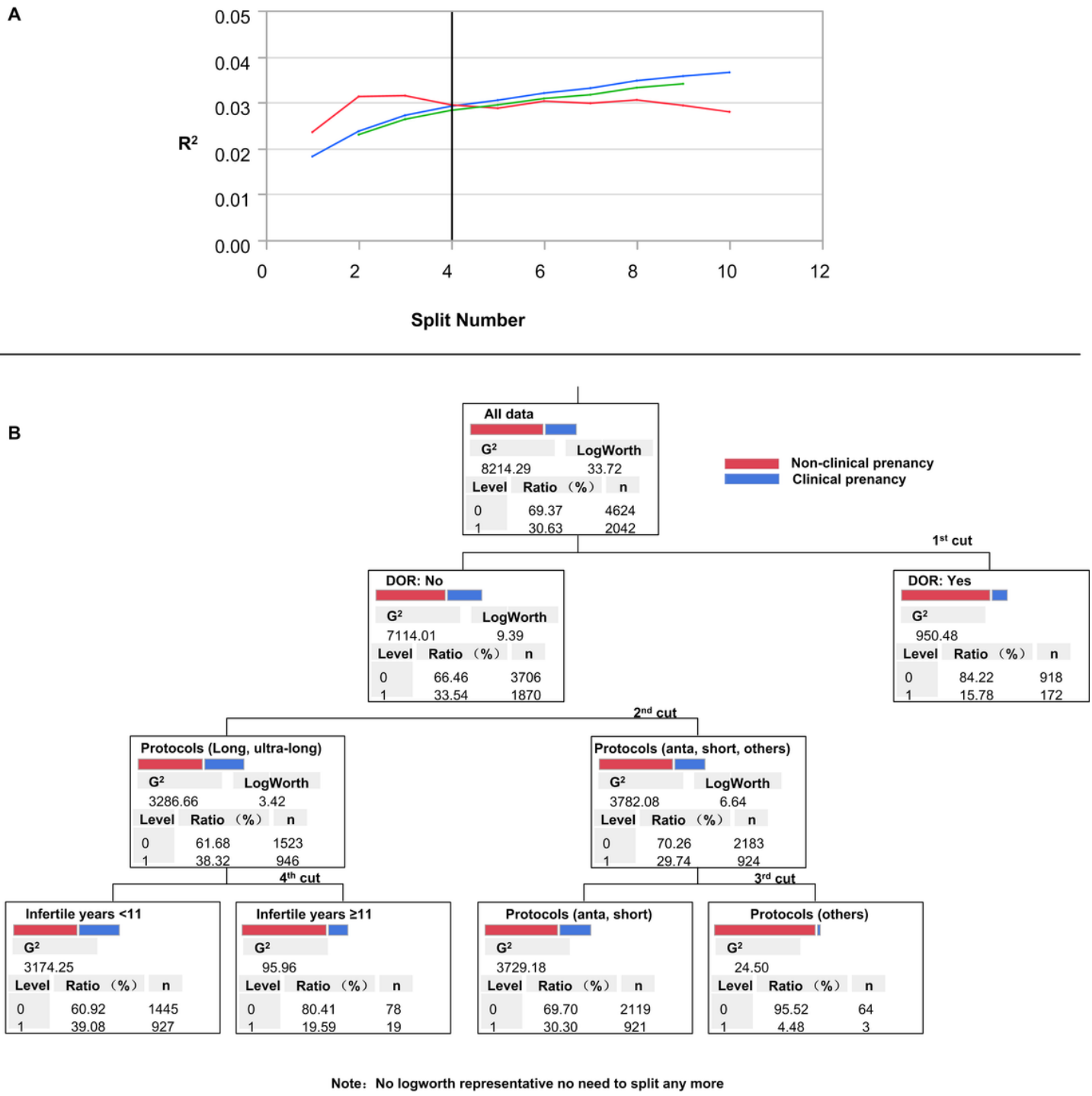


Figure 4

Classification tree analysis. In A, the blue, green and red lines represent the training set, the inner validation set, and the outer validation set, respectively. The optimal model is indicated by the vertical line when there are four splits. B shows the splits of the classification tree.



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Supplementary Files

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