

High Baseline SHBG, as an Independent Predictor, Was Associated With High Ovulation: a Secondary Analysis of PCOSAct

Hui Chang

heilongjiang university of Chinese medicine <https://orcid.org/0000-0001-6891-1239>

Qi Wu

The Chinese University of Hong Kong

Hang Ge

Heilongjiang University of Chinese Medicine

Jian Li

Heilongjiang University of Chinese Medicine

Meng-Yi Zhu

Heilongjiang University of Chinese Medicine

Xi Luo

Heilongjiang University of Chinese Medicine

Yan-Hua Han

Heilongjiang University of Chinese Medicine

Chi Chiu Wang

The Chinese University of Hong Kong

Xiao-Ke Wu (✉ xiaokewu2002@vip.sina.com)

The First Affiliated Hospital of Heilongjiang University of Chinese Medicine

Research

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Abstract

Background: Sex hormone-binding globulin (SHBG) is related to several human systems such as reproductive system and endocrine system. SHBG binds to testosterone and estradiol, therefore we explored the role of SHBG on reproductive process.

Methods: We carried out secondary analysis of Polycystic Ovary Syndrome and Acupuncture Clinical Trial (PCOSAct) at 21 sites in China, comprising a total of 1000 women with PCOS. A total of 954 women with baseline homocysteine (HCY) were included in the study.

Results: Multivariate analysis of predictors of ovulation showed that age, body mass index (BMI), estradiol (E2), total testosterone (T) and SHBG are predictors of ovulation ($P=0.0211$, 0.0011 , 0.0211 , 0.0029 , 0.0434) whereas luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio is negatively correlated with ovulation ($P=0.0539$). A multivariate logistic regression model (MLRM) of all baseline serum parameters showed the strongest predictive ability for ovulation, followed by MLRM without SHBG. In addition to treatment, baseline SHBG was the strongest single predictor for ovulation. Patients in higher SHBG quartile showed significantly higher ovulation rate ($HR=1.138$; 95%CI $[1.054, 1.229]$, $P=0.0009$). Notably, significance was observed after adjustment for testosterone ($HR=1.139$, 95%CI $[1.055, 1.229]$, $P=0.0009$). However, quartiles of T, free testosterone (FT) and E2 all showed no correlation with ovulation. Kaplan-Meier curves showed that high SHBG is positively correlated with high ovulation, conception and pregnancy rates.

Conclusions: Higher baseline SHBG is associated with higher ovulation rate and is an independent predictive marker.

Trial registration: ClinicalTrials.gov (No. NCT01573858). Registered 10 April 2012, <https://clinicaltrials.gov/ct2/show/NCT01573858?cond=NCT01573858&draw=2&rank=1>.

Background

Polycystic ovary syndrome (PCOS), with a worldwide prevalence ranging from 5–20%¹, is characterized by ovulatory dysfunction, hyperandrogenism and polycystic ovaries², and is considered to induce female infertility in women of reproductive age³.

SHBG is a glycoprotein produced in the liver. It binds to sexual hormones and transports hormones in the bound-state through the circulation as biologically inactive forms. Previous studies report that low level of pre-pregnant SHBG results in metabolic diseases during pregnancy, such as gestational diabetes mellitus^{4,5}, and adverse cardio-metabolic diseases⁶, which may increase risk of negative reproductive outcomes. A study on two randomized clinical trials (RCTs) reports that PCOS women with lower SHBG showed lower conception, pregnancy and live birth rate⁷.

Although previous studies have explored the relationship between reproductive outcomes and SHBG, few clinical studies report on the role of SHBG levels on ovulation. Therefore, the aim of this study was to explore the effects of high SHBG levels on ovulation and other reproductive outcomes in women with PCOS.

Materials And Methods

Design and target population

Metabolic parameters and reproductive outcomes data were retrieved from Acupuncture and Clomiphene in Polycystic Ovary Syndrome Trial (PCOSAct). PCOSAct is a large-sample, multicenter, two-by-two factorial randomized controlled clinical trial conducted from 2012 to 2015 in mainland China. All 1,000 subjects were diagnosed with PCOS following the modified Rotterdam criteria ^{3,8}. Women aged from 20 to 40 years with oligomenorrhea (defined as a menstrual interval ≥ 35 days and/or ≤ 8 menses in the past year) or amenorrhea combined with clinical hyperandrogenism (modified Ferriman-Gallwey score ≥ 5) ^{2,9} and/or polycystic ovaries (defined as the presence of > 12 antral follicles (≤ 9 mm) and ovarian volume > 10 ml by ultrasound) were considered to have PCOS. All participants were randomly grouped into one of four treatment groups including (A) active acupuncture + clomiphene group; (B) control acupuncture + clomiphene group; (C) active acupuncture + clomiphene placebo group; (D) control acupuncture + clomiphene placebo for four menstrual cycles group. Study design, methods, and inclusion and exclusion criteria are described in detail by ¹⁰ and the main results were by ¹¹. A total of 954 women with PCOS who had baseline measurements of SHBG were enrolled in the present study. Reproductive outcomes, including ovulation, conception, pregnancy, pregnancy loss and live birth were recorded at the end of study. Progesterone was measured every week to determine occurrence of ovulation during the trial. Ovulation was defined as elevated progesterone level per cycle. Ovulation rate was calculated as proportion of subjects who had ovulated at least once during all tested cycles in relation to all subjects. Documentation for patency of at least one tube and a normal uterine cavity was obtained through hysterosalpingogram, HyCosy or diagnostic laparoscopy. Male partners of participants were required to have a sperm concentration $\geq 15 \times 10^6/\text{ml}$ and total motility $\geq 40\%$ or viable sperm count ≥ 9 million ^{12,13}. Exclusion criteria included patients with hyperprolactinemia, FSH levels > 15 mIU/mL, uncorrected thyroid dysfunction, poorly controlled Type I or Type II diabetes, patients taking antidiabetes medications and suspected Cushing's syndrome and use of hormones or other medications, pregnancy, abortion and postpartum or breastfeeding within the past 6 weeks.

Data Collection

Baseline laboratory, anthropometric, degree of hirsutism, acne and ultrasonography measurements were performed after an overnight fast, and all biochemical assays were performed in a main laboratory. Fasting blood collected at baseline was used for all hormone assays ¹⁴. Fasting glucose levels were determined using a glucose analyzer following glucose oxidase method, with all intra-assay and inter-assay coefficients of variation less than 1.5% ¹⁴. Total T was measured by radioimmunoassay (Siemens

Diagnostics, Deerfield, Illinois), with intra-assay and inter-assay coefficients of variation less than 7.1%. SHBG levels were available for 954 of the 1,000 women enrolled in the trial. SHBG levels were measured from samples stored at -80 °C using an ELISA assay with no pre-dilution. BMI was calculated as weight in kilograms divided by height in meters squared. Insulin resistance index determined by HOMA-IR was computed using the following formula ¹⁵: $\text{HOMA-IR} = \text{fasting plasma insulin (mIU/L)} \times \text{fasting plasma glucose (mmol/L)} / 22.5$.

Statistical Analysis

Descriptive statistics were used to compare characteristics between different groups. Continuous variables were reported as means \pm standard deviations, whereas categorical variables were summarized as frequencies and percentages. Multiple logistic regression analysis was used to obtain odds ratios (ORs) to assess predictive ability of variables for ovulation. Receiver operator characteristic (ROC) curves were constructed to assess the predictive value of different variables. The area under the curve (AUC) of ROC represents the accuracy with which the test predicts ovulation. Cox proportional hazards regression was used to analyze associations between SHBG, testosterone, free testosterone, estradiol, and ovulation. In predefined analyses, SHBG, testosterone, free testosterone and estradiol were examined as quartiles based on distribution in the entire study population. Based on the observed distribution, SHBG and testosterone were further examined as dichotomous variable comparing quartile 4 with quartiles 1 to 3. Kaplan-Meier survival curves were used to determine association between SHBG levels and several reproductive outcomes over time, including ovulation, conception, pregnancy, miscarriage and live birth. Log-rank test was used to assess statistical significance. All analyses were performed using SAS version 9.3 software (SAS Institute Inc). P-values < 0.05 were considered statistically significant.

Results

Baseline biometric features, ultrasonographic findings and fasting serum levels of study participants were determined (Table 1). A multivariate analysis was used to explore the predictive ability of ovulation-related parameters (Table 2). Analysis showed that Age, BMI, LH/FSH, E2, T and SHBG were independent predictors of ovulation. All variables except LH/FSH remained significant after adjusting treatment.

Table 1
Baseline Characteristics of Subjects

Variable	
Biometric features	
Sex hormone-binding globulin, nmol/L	42.51 ± 30.06
Age, yr	27.93 ± 3.31
BMI, kg/m ²	24.18 ± 4.25
Waist to hip ratio (WHR)	0.87 ± 0.07
Modified Ferriman-Gallwey score	3.01 ± 2.76
Acne score	0.44 ± 0.77
Ultrasonographic findings	
Polycystic ovary morphology of any ovary, n (%)	814(88.38)
Fasting serum levels	
LH, mIU/mL	10.57 ± 5.93
FSH, mIU/mL	6.10 ± 1.66
LH to FSH ratio	1.79 ± 1.14
Progesterone, ng/ml	2.58 ± 5.10
Estradiol, pg/mL	260.95 ± 263.66
Total testosterone, ng/dL	1.66 ± 0.64
Free testosterone, pg/ml	2.29 ± 0.84
Fasting glucose, mg/dL	5.03 ± 0.95
Fasting Insulin, µIU/mL	94.43 ± 81.93
HOMA-IR	22.39 ± 23.79

Table 2
Multivariate analysis of predictors of ovulation using multiple logistic regression analysis

Variable	OR	95%CI	P value	P value adjusted treatment
Age, yr	1.063	1.009–1.119	0.0209	0.0211
BMI, kg/m ²	0.922	0.884–0.962	0.0002	0.0011
LH to FSH ratio	0.791	0.654–0.956	0.0153	0.0539
Estradiol, pg/mL	1.002	1.000–1.003	0.0102	0.0211
Total testosterone, ng/dL	0.653	0.494–0.863	0.0027	0.0029
SHBG	1.008	1.000–1.015	0.0364	0.0434

MLRM was constructed based on SHBG, Age, BMI, LH/FSH, E2, T and treatment to explore which parameter was the strongest predictor of ovulation and area under curves for ROC curves determined (Fig. 1). MLRM comprising all variables was effective in prediction of ovulation. MLRM without SHBG showed the second highest predictive ability for ovulation (0.7796, 95%CI [0.7442–0.8149]; 0.7774, 95% [0.7423–0.8126]). Treatment (0.6903, 95%CI [0.6513–0.7293]) showed a higher predictive ability compared with baseline parameters whereas SHBG (0.6102, 95%CI [0.5673–0.6530]) showed the highest predictive ability for ovulation among baseline variables.

HRs for ovulation across quartiles of baseline serum SHBG, T, free T and E2 were calculated to further explore the predictive ability of each baseline sexual hormone (Table 3). Women in the latter quartile of SHBG were at a higher risk of ovulation compared with women in the former quartile of SHBG. Per-quartile increase of SHBG was statistically significant ($P < 0.001$). Testosterone was adjusted due to biological interplay between testosterone and SHBG. After adjusting testosterone, per-quartile increase of SHBG (HR = 1.139; 95%CI [1.055,1.229]; $P = 0.0009$) and Q4 vs. Q1-3 of SHBG were statistically significant (HR = 1.263; 95%CI [1.059,1.507]; $P = 0.0093$). P values of Q2, Q3 and Q4 of free T were all negative. E2 showed no correlation with ovulation.

Table 3
HRs for Ovulation Across quartiles of serum SHBG, testosterone, free testosterone, and estradiol

	No. of Ovulation / No. at Risk	HR (95% CI)	P value
SHBG, µg/mL			
Q1 (≤ 2.5)	160/238	1.00 (referent)	
Q2 (2.5–3.8)	180/239	1.278 (1.023,1.597)	0.0307
Q3 (3.8–6.2)	191/240	1.282 (1.016,1.618)	0.0362
Q4 (≥ 6.2)	208/237	1.538 (1.211,1.953)	0.0004
Per-quartile increase		1.138 (1.054,1.229)	0.0009
Per-quartile increase, adjusted for testosterone†		1.139 (1.055,1.229)	0.0009
Q4 vs. Q1-3		1.267 (1.062,1.511)	0.0085
Q4 vs. Q1-3, adjusted for Testosterone*		1.263 (1.059,1.507)	0.0093
Testosterone, ng/dl			
Q1 (≤ 34.6)	197/234	1.00 (referent)	
Q2 (34.6–45.8)	192/240	0.919 (0.746,1.132)	0.4266
Q3 (45.8–58.8)	167/239	0.755 (0.607,0.938)	0.0112
Q4 (≥ 58.8)	178/236	0.856 (0.682,1.073)	0.1780
Per-quartile increase		0.933 (0.868,1.004)	0.0622
Q4 vs. Q1-3		0.983 (0.821,1.176)	0.8486
Q4 vs. Q1-3, adjusted for SHBG*		0.975 (0.814,1.168)	0.7820
Free testosterone, pg/ml			
Q1 (≤ 1.67)	193/234	1.00 (referent)	

	No. of Ovulation / No. at Risk	HR (95% CI)	P value
Q2 (1.67–2.22)	197/234	1.145 (0.931,1.407)	0.1995
Q3 (2.22–2.83)	172/234	0.890 (0.719,1.102)	0.2857
Q4 (≥ 2.83)	162/234	0.812 (0.652,1.012)	0.0636
Per-quartile increase		0.917 (0.857,0.981)	0.0120
Estradiol, pg/ml			
Q1	198/237	1.00 (referent)	
Q2	168/237	0.807 (0.654,0.996)	0.0453
Q3	167/238	0.838 (0.676,1.039)	0.1075
Q4	201/237	1.259 (1.011,1.568)	0.0397
Per-quartile increase		1.072 (0.996,1.154)	0.0624

Kaplan-Meier curves were constructed using survival data of SHBG for reproductive outcomes (Fig. 2). Q4 of SHBG showed significantly higher ovulation rate, conception rate and pregnancy rate compared with Q1-Q3 of SHBG over time ($P < 0.0001$; $P = 0.0260$; $P = 0.0246$). High SHBG was not correlated with miscarriage ($P = 0.2414$). Notably, high SHBG had no link with miscarriage ($P = 0.2414$). And high SHBG quartile, Q4, had comparatively higher live birth rate and a critical P value ($P = 0.056$).

Discussion

SHBG showed the strongest prediction for ovulation. Women with high SHBG, independent of testosterone, had higher ovulation rate. In addition, women in high SHBG quartile showed significantly higher conception rate and high pregnancy rate compared with the other quartiles. These findings imply that baseline SHBG level is an independent prediction marker for ovulation in women with PCOS, which further affects conception, pregnancy and even live birth.

High SHBG contributed to high ovulation, conception and pregnancy rates. More subjects should be enrolled to further explore the effect of high SHBG on live birth rate and there may be a probably statistical correlation between live birth rate and SHBG. Previous studies report similar findings on the effect of SHBG. Women with regular ovulation showed statistically higher serum SHBG compared with

PCOS women with anovulation¹⁶. A secondary analysis of two trials conducted among PCOS women showed that SHBG is positively correlated with conception, pregnancy and live birth which is consistent with our results⁷. However, another prospective study comprising 251 infertile women with clomiphene citrate resistant polycystic ovary disease, reports that SHBG is not predictor of spontaneous ovulation within eight weeks after laparoscopic ovarian drilling¹⁷. More studies should be conducted to explore relationships between SHBG and reproductive outcomes of PCOS women.

In addition, SHBG is implicated in several pregnant complications. Firstly, SHBG is a marker in predicting GDM in PCOS subjects¹⁸. Moreover, a previous prospective study reports that SHBG is an independent risk factor for PCOS women who had preeclampsia (PE)¹⁹. However, a previous case-control study report that PCOS women with a preeclamptic history have no significant difference compared with normal subjects²⁰. Additionally, SHBG can be used as an integrative biomarker in prediction for adverse cardio-metabolic profile in pregnant women with pregestational plus gestational obesity. SHBG levels, independent of BMI and other metabolic and endocrine variables, are associated with c-reactive protein and systolic blood pressure⁶. These findings imply that low SHBG level of PCOS women is correlated with low pregnancy rate, live birth rate and a high incidence of several pregnant complications which have adverse effects on both maternal and child health. PCOS women have significantly lower serum SHBG level compared with normal women²¹. Therefore, if PCOS women with lower SHBG desire to have children, they should take measures to elevate SHBG levels thus increasing ovulation rate. Increase in ovulation rate will affect conception, pregnancy and delivery.

In addition to treatment, baseline SHBG, showed high predictive ability for ovulation compared with other baseline serum parameters (Fig. 1). SHBG level in higher quartile showed higher ovulation rate after adjustment for testosterone. Testosterone and estradiol had no effect on ovulation rate. Therefore, prediction of ovulation by SHBG is independent of testosterone and estradiol. Most previous studies report on relationships between other sexual hormones and ovulation. Patients with high FSH/LH ratio showed fewer mature oocytes aspirated, indicating that high FSH/LH ratio might has negative effects on follicular development²². Urinary FSH peak²³ and kisspeptin surge in serum and urine²⁴ are useful biomarkers for predicting the day of ovulation. Premature ovulation rate is positively correlated with preovulatory E2/-1E2 ratio and premature LH surge²⁵. A previous prospective study reports that the surge in serum and urinary LH are associated with ovulation²⁶. Moreover, LH level between 25–30 MIU/ml tested before day 7 of the menstrual cycle showed the strongest prediction for ovulation²⁷. A prospective trial reports that ovulatory women with PCOS, showed significantly lower anti-mullerian hormone (AMH) and antral follicle count after using clomiphene citrate and highly purified FSH, compared with anovulatory women with PCOS after undergoing the same intervention²⁸. PCOS women with reduced response to ovulation showed higher AMH²⁹. Oocyte in-vitro maturation treatments showed that AMH can be used to identify women candidate with suitable number of oocytes³⁰. However, our findings show that SHBG has higher predictive ability for ovulation compared with other parameters.

Quartiles of SHBG, T and E2 were further calculated and adjusted analyses were performed due to the interaction between SHBG and T and E2. Higher SHBG was positively correlated with higher ovulation rate. Notably, this correlation was statistically significant after adjustment for T, whereas this trend was not observed for T and E2. Therefore, the prediction of ovulation using SHBG levels is independent of testosterone and estradiol. This is consistent with previous findings that SHBG has a stronger prediction value compared with T and E2. A previous polymorphism study conducted on PCOS patients who underwent IVF-ET (in vitro fertilization-embryo transfer) reports that SHBG rs6259 GA + AA genotype carriers show decreased number of retrieved oocytes and embryo, and fertility rate [= (number of fertilized eggs/number of retrieved oocytes) × 100%]³¹. Further, high-molecular-weight adiponectin level is positively correlated with SHBG³². Adiponectin modulates follicular growth and maturation³³, therefore, correlation between adiponectin and follicle showed be further investigated. A previous study reports that granulosa cell proliferation is increased in anovulatory PCO compared with both normal and ovulatory PCO³⁴. In addition, AQP-9 mRNA levels in granulosa cells of patients with PCOS were significantly correlated with SHBG levels in follicular fluid³⁵. Therefore, further studies on the correlation between granulosa cell and SHBG should be conducted.

The present study is based on a large multi-center, randomized, double-blind, placebo-controlled design with a representative sample of PCOS population to explore and demonstrate the correlation between SHBG level with reproductive outcomes, including ovulation, conception, pregnancy and live birth. Meanwhile, higher baseline SHBG is associated with higher ovulation rate and is an independent predictive marker.

There are limitations of this study. First, this study is a secondary analysis of PCOSAct. The study does not include certain data, such as data of normal controls. Second, the relationship between SHBG and ovulation for each menstrual cycle was not determined. Besides, larger samples are needed to certify that high SHBG statistically associated with high live birth.

Conclusions

Higher baseline SHBG level is associated with higher ovulation rate and is an independent predictive marker.

Abbreviations

AUC: area under the curve

BMI: body mass index

FSH: follicle-stimulating hormone ()

HCY: homocysteine

LH: luteinizing hormone

MLRM: multivariate logistic regression model

OR: odds ratio

PCOS: polycystic ovary syndrome

PCOSAct: Polycystic Ovary Syndrome and Acupuncture Clinical Trail

PE: preeclampsia

RCT: randomized clinical trial

ROC: receiver operator characteristic

SHBG: Sex hormone-binding globulin

Declarations

Ethics approval and consent to participate

The study was approved by the regional ethics committee at The First Affiliated Hospital of Heilongjiang University of Traditional Chinese Medicine, Harbin, China. All participants and their partners provided written informed consent before participation in the study.

Consent for publication

All authors critically reviewed the article and approved it for submission.

Availability of data and materials

Dr. Xiao-Ke Wu had full access to all of the data in the study (including statistical reports and tables) and thus takes responsibility for the integrity of the data and accuracy of the data analysis.

Competing interests

All authors (Hui Chang, Qi Wu, Hang Ge, Jian Li, Meng-Yi Zhu, Xi Luo, Yan-Hua Han, Chi Chiu Wang, Xiao-Ke Wu) declare that they have no conflict of interest or financial conflicts to disclose.

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Authors' contributions

Study concept and design: Dr. Xiao-Ke Wu and Chi Chiu Wang. Drafting of the manuscript: Hui Chang and Hang Ge. Data collection and data interpretation: Hui Chang, Meng-Yi Zhu, Xi Luo, and Yan-Hua Han. Statistical analysis: Qi Wu, Jian Li, and Dr Chi Chiu Wang. Supervision: Dr. Xiao-Ke Wu.

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Figures

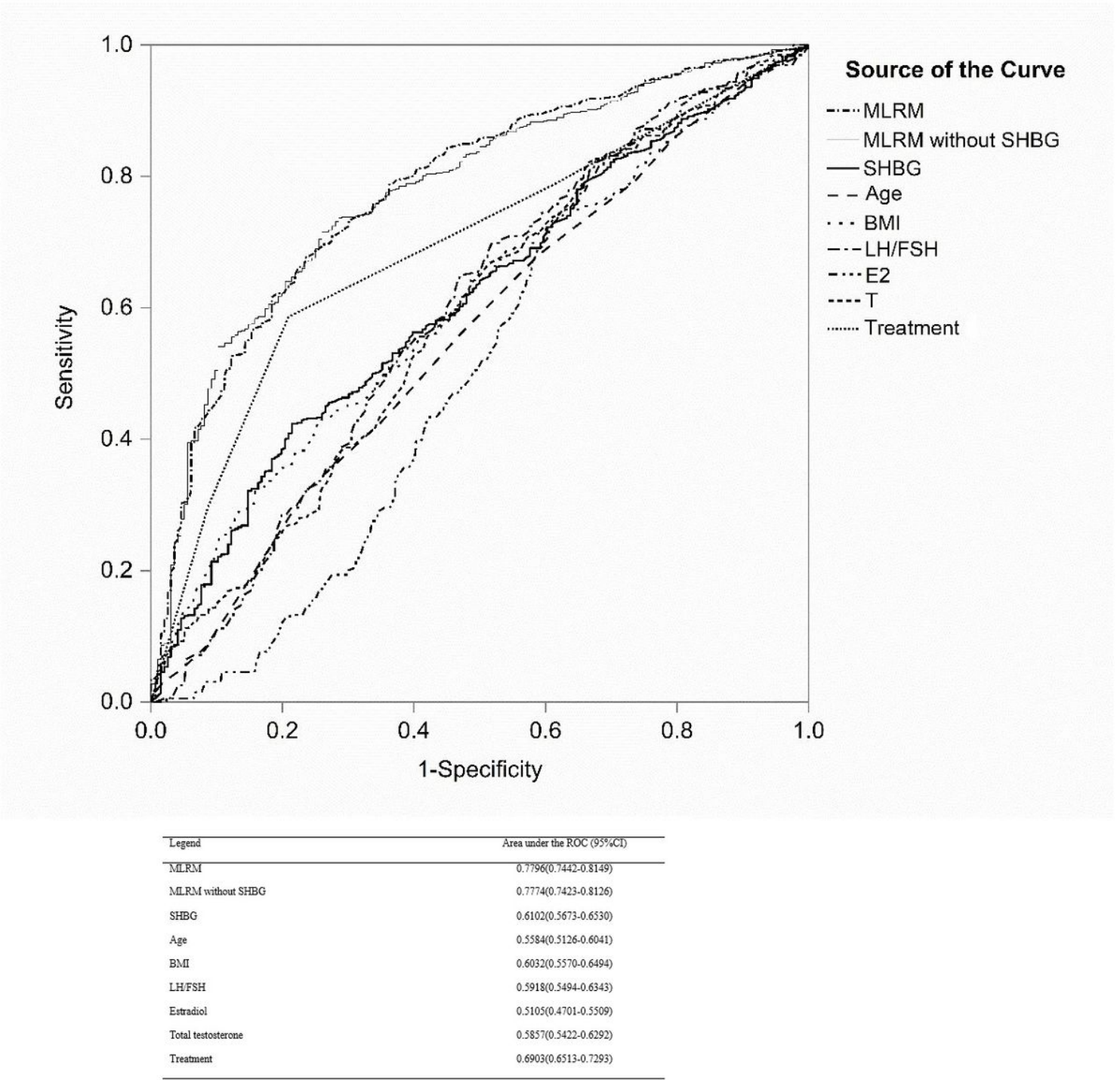


Figure 1

Receiver operating characteristics (ROC) curves of a multivariate logistic regression model (MLRM), MLRM without SHBG, and other individual variables included in MLRM for predicting ovulation of women

with PCOS. Area under curves for receiver operator characteristics (ROC) curves shown in Fig 1 constructed based on multivariate logistic regression model (MLRM), MLRM without SHBG, SHBG, Age, BMI, LH/FSH, E2, T

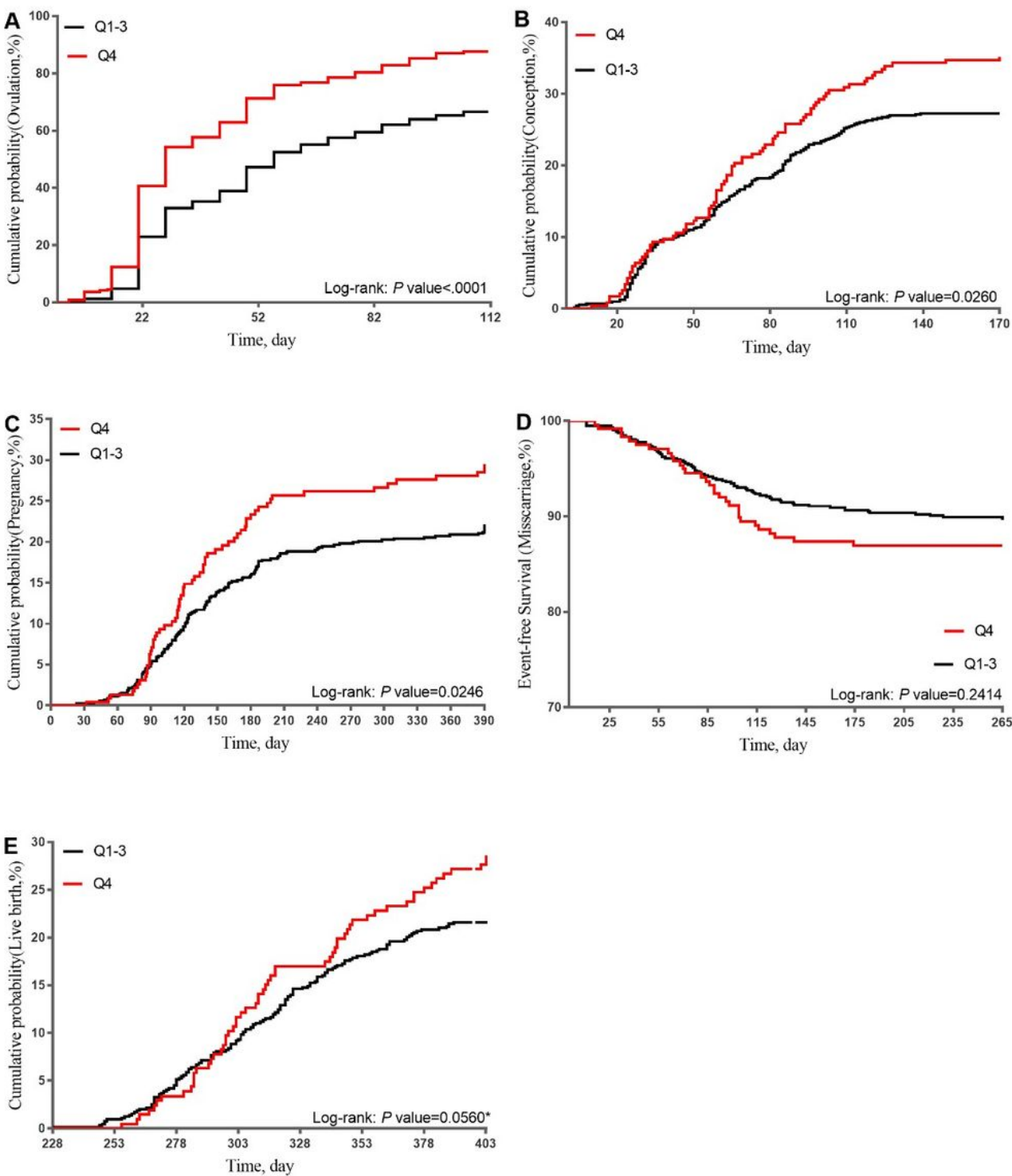


Figure 2

Kaplan-Meier Plots based on SHBG levels. Kaplan-Meier curves of event-free survival by SHBG for Ovulation (A), conception(B), pregnancy (C), miscarriage (D), live birth (E). In quartile 4 of SHBG (red lines)

or quartiles 1 to 3 (black lines). P value assessed by log-rank test.