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Research article

Elevated baseline LH/FSH ratio is associated with poor ovulatory response but better clinical pregnancy and live birth in Chinese women with PCOS after ovulation induction

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ABSTRACT

Background: What is the association between elevated baseline LH/FSH ratio and reproductive outcomes, especially ovulatory response, among Chinese women with polycystic ovary syndrome (PCOS) after ovulation induction.

Methods: This was a secondary analysis of a multicenter randomized trial in 1000 women with PCOS from 21 sites (27 hospitals) in Mainland China. LH and FSH levels before ovulation induction and the main outcomes including ovulation, biochemical pregnancy, clinical pregnancy, miscarriage, and live birth were measured. A linear regression model, logistic regression models and Cox proportional hazard regression model were used to estimate the association between LH/FSH ratios and reproductive outcomes in PCOS.

Results: LH/FSH ratio was significantly associated with age, body mass index (BMI), total testosterone (TT), estradiol (E2), free testosterone (FT), and antimullerian hormone (AMH). Anovulatory women had significantly higher LH/FSH ratio than ovulatory women ($P = 0.003$), especially in women with young age ($P = 0.023$), high BMI ($P = 0.002$), low E2 ($P = 0.002$), FT ($P = 0.010$), TT ($P < 0.001$) and AMH($P = 0.032$). Women with elevated LH/FSH ratio were associated with lower ovulation (LH/FSH$\geq$1 OR = 0.42, 95% CI, 0.26–0.68; LH/FSH$\geq$2 OR = 0.32, 95% CI, 0.20–0.54; LH/FSH$\geq$3 OR = 0.40, 95% CI 0.21–0.74) when compared with LH/FSH<1. The association was held after adjustment for treatment with or without the confounding factors. Although no association between LH/FSH ratio and biochemical pregnancy, women with $1 \leq$ LH/FSH<2 were associated with higher clinical pregnancy (OR = 1.71; 95% CI, 1.09–2.67) and live birth (OR = 1.73; 95% CI, 1.09–2.75) compared to women with LH/FSH<1. Women with $2 \leq$ LH/FSH<3 were associated with lower miscarriage rate (OR = 0.38; 95% CI, 0.16–0.93).

Conclusions: Elevated baseline LH/FSH ratio in women with PCOS was associated with poor ovulatory response, but women were more likely to achieve clinical pregnancy and live birth than

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women with normal LH/FSH. It suggests LH and FSH in women with PCOS may play a role in successful pregnancy despite of negative impact in ovulation.

1. Introduction

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) released from the pituitary are both complex heterodimeric glycoproteins which consist of common α-subunit and specific β-subunit [1,2]. LH and FSH change periodically and play different roles at different stages of follicle development with the feedback regulation of gonadotropin-releasing hormone (GnRH) and ovarian steroids, which affects oocyte quality, ovulation, and even the overall reproductive outcomes in female reproductive physiology [3,4,5]. While women with polycystic ovary syndrome (PCOS) is a condition often associated with dysregulation of the hypothalamic-pituitary axis [3]. Hyperactivity of GnRH pulse, disruption of feedback mechanism by ovarian estrogen and influence of other endocrine factors are blamed to play role in increased LH release and normal to decreased FSH release, which result in elevated LH/FSH ratios [3,6–8]. Elevated LH/FSH ratios can be observed in approximately 60% of women with PCOS [9].

Reproductive disorder is an important impetus to seek medical treatment in women with PCOS [7,10–12]. Despite spontaneous or assisted ovulation, however, women with PCOS are still at risk of infertility or miscarriage substantially female reproductive health and quality of life [13,14]. Thus, a more thorough understanding of the pathological mechanisms of reproductive disorders in PCOS is needed. Elevated LH/FSH ratio has long been appreciated. Some studies showed PCOS women with higher LH/FSH ratio had decreased pregnancy rate, whereas others had shown no association between higher LH/FSH ratio and pregnancy rate [15,16,17]. Meanwhile, in the PCOS women with elevated LH/FSH ratio, ovulation induction with letrozole was superior to clomiphene citrate in forming a dominant follicle [18]. Whether elevated LH/FSH ratios impact reproductive outcomes in women with PCOS remain a question of considerable debate. The present study aimed to validate the association of baseline LH/FSH ratio and reproductive outcomes in 956 infertile Chinese women with PCOS after ovulation induction.

2. Materials and methods

2.1. Trial design

This was a secondary analysis on LH/FSH ratio in PCOS women utilizing data from the PolyCystic Ovary Syndrome Acupuncture plus Clomiphene Trial (PCOSAct), which was a double-blind (clomiphene), and single blind (participants to type of acupuncture), multicenter, 2 × 2 factorial trial consisting of clomiphene + active acupuncture group, clomiphene + control acupuncture group, clomiphene placebo + active acupuncture group and clomiphene placebo + control acupuncture group from July 2012 to October 2015. This trial was registered at clinicaltrials.gov (NCT01573858) and complied with all regulations, and the protocol was approved by the Ethics Committee at First Affiliated Hospital in Heilongjiang University of Chinese Medicine (2011HZYLL-022). All couples provided written informed consent, and in addition to Ethics Committee this trial was under the supervision of the data and safety monitoring board.

2.2. Participants

The complete list of inclusion and exclusion criteria have been described in detail elsewhere [19]. In brief, 956 Chinese women with PCOS who seek treatments for ovulatory problems and pregnancies were eligible. Participants were between 20 and 40 years, fulfilled the modified Rotterdam diagnostic criteria for PCOS: oligomenorrhea or amenorrhea, together with clinical or biochemical hyperandrogenism and/or polycystic ovaries [9,20]. Women were excluded if they had used any hormone therapy or other medication including Chinese herbal medicinal products in the past 3 months. For the present study, women with FSH levels >15mIU/mL were excluded.

2.3. LH/FSH ratio determination

Baseline LH and FSH were performed in the core laboratory using electro-chemiluminescence immune assay (ECLIA) after an overnight fast (1–5 days after withdrawal bleeding). LH/FSH ratios were calculated according to the formula: baseline LH (mIU/mL)/baseline FSH (mIU/mL). Based on LH/FSH ratios, all participants were divided into four subgroups: LH/FSH<1, 1 ≤ LH/FSH<2, 2 ≤ LH/FSH<3 and LH/FSH≥3 according to the diagnostic criteria concluded by the Japanese Society of Obstetrics and Gynecology [21] and the Group of Endocrinology, Obstetrical and Gynaecological Society, Chinese Medical Association [22,23]. LH/FSH<1 was taken as normal, other subgroups was taken as elevated level.

2.4. Physical measurements

At baseline, participants underwent a physical examination including weight and height measurement, waist, and hip circumferences. BMI (kg/m^2) was calculated using the formulae: weight [kg]/height [m^2]. Waist-to-hip ratio (WHR) was calculated by dividing waist circumference by hip circumference.

2.5. Hormonal and biochemical measurements

Fasting blood collected at baseline was used for assays on sex steroids, gonadotropins, glucose and lipid metabolism measurements, including estradiol (E2), progesterone (P), total testosterone (TT), sex hormone binding globulin (SHBG), free testosterone (FT), free androgen index (FAI, T in nmol/L/SHBG in nmol/L × 100), antimullerian hormone (AMH), fasting glucose, fasting insulin, homeostatic model assessment insulin resistance (HOMA-IR), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Samples were batched and analyzed at the core laboratory in Heilongjiang University of Chinese Medicine [19]. Metabolic syndrome was defined when any 3 of the following 5 criteria presented: waist circumference >88 cm (>35 inch), triglycerides ≥150 mg/dL, HDL <50 mg/dL, systolic blood ≥130 mmHg or diastolic blood pressure ≥85 mmHg and fasting glucose 110–126 mg/dL.

2.6. Clinical phenotypes

Participants underwent a self-reported questionnaire including days between menstruation periods and the number of menstrual cycles per year at baseline. Amenorrhea was defined as an intermenstrual interval >90 days, and oligomenorrhea was defined as an intermenstrual interval >35 days or <8 menstrual bleedings in the past year [24]. Polycystic ovaries were diagnosed when transvaginal scanning present that at least one side of ovary volume >10 mL or antral follicles (2–9 mm) ≥12 [24]. Ferryman–Gallwey (F-G) score and acne score were measured using a standard assessment diagram and definitions [25,26]. Hirsutism was defined as F–G score ≥5 [25,26]. Biochemical hyperandrogenism was defined as total testosterone ≥48.17 ng/dL [27].

2.7. Reproductive outcomes

Reproductive outcomes after ovulation induction were recorded, including live birth ovulation, biochemical pregnancy, clinical pregnancy, and miscarriage. Live birth was defined as the delivery of an infant whose generation above 20 weeks. Ovulation was defined as serum progesterone level >3 ng/mL at local site. Biochemical pregnancy was defined as any positive serum level of hCG at local site. Clinical pregnancy was defined as an intrauterine pregnancy with fetal heart motion and gestational sac detected by ultrasonographic visualization. Miscarriage was defined as a loss of intrauterine pregnancy, including pregnancy loss or fetal demises and stillbirths.

2.8. Statistical analysis

Descriptive statistics were used to compare characteristics between women by different levels of baseline LH/FSH ratios. Baseline characteristics were described using frequencies and percentages for categorical variables and means with standard deviations for continuous variables. Analysis of variance (ANOVA) tests and Chi-square test/Fish's exact tests were used, as appropriate, to determine differences across these groups. Tuckey's test or Chi-square test/Fish's exact tests were used for comparisons between groups. A linear

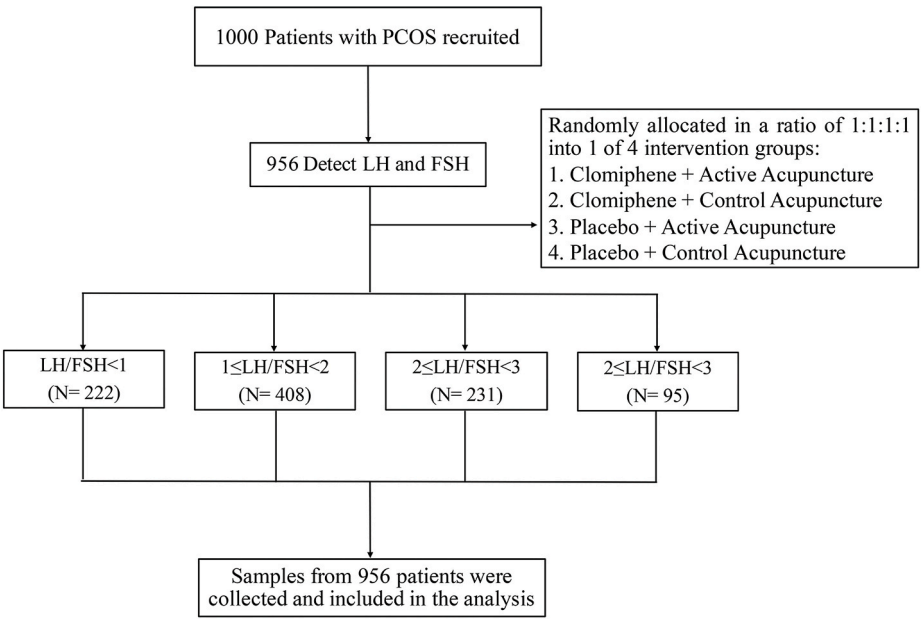

Fig. 1. Flow diagram.

regression model was performed to explore the factor of LH/FSH ratios by backwards method. Logistic regression models were used to estimate odds ratios (OR) and 95% CIs for the association between LH/FSH ratios and clinical reproductive outcomes. Unadjusted models were compared with models that (1) adjusted for treatment alone, (2) adjusted for treatment, age, BMI, T, E2, FT and AMH. The associations of LH/FSH ratios and the cumulative incidence of clinical reproductive outcomes were analyzed by Cox proportional hazard regression model adjusting for treatment, age, BMI, T, E2, FT and AMH. Independent t-tests were used to determine the difference of the baseline LH/FSH ratios between the ovulation and anovulation women in PCOS phenotypes. Analyses were performed in SAS version 9.4 software (SAS Institute, Inc). The difference between the groups was indicated statistical significant if two-side P values were less than 0.05.

3. Results

3.1. Research subject and period

In our analytic sample, the breakdown into PCOS subgroups for the 956 participants was as follows: 222 participants with LH/FSH<1.0 (23.2%), 408 participants with 1 ≤ LH/FSH<2 (42.7%), 231 participants with 2 ≤ LH/FSH<3 (24.2%), 95 participants with LH/FSH≥3 (9.9%) (Fig. 1).

Table 1
Demographic characteristic of women in PCOSAct by baseline LH/FSH ratio.

Characteristic	LH/FSH<1 (N = 222)	1 ≤ LH/FSH<2 (N = 408)	2 ≤ LH/FSH<3 (N = 231)	LH/FSH≥3 (N = 95)	P values
Biometric features					
Age (years)	28.3 ± 3.4	27.9 ± 3.5	27.7 ± 2.9	27.4 ± 3.1	0.079
BMI (kg/m^2)	25.4 ± 4.5^{bc}	24.7 ± 4.4^{de}	23.1 ± 3.7	21.8 ± 3.0	<0.001
WHR	0.9 ± 0.1^{bc}	0.9 ± 0.1^{d}	0.9 ± 0.1	0.8 ± 0.1	<0.001
Endocrine features					
LH (mIU/mL)	4.4 ± 1.8^{abc}	9.2 ± 3.0^{de}	14.8 ± 3.7^{f}	19.8 ± 7.2	<0.001
FSH (mIU/mL)	6.1 ± 1.6^{c}	6.3 ± 1.7^{e}	6.1 ± 1.4^{f}	5.2 ± 1.8	<0.001
Estradiol (pg/mL)	49.8 ± 24.7^{bc}	62.3 ± 46.9^{de}	79.9 ± 65.6^{f}	149.0 ± 199.4	<0.001
Progesterone (ng/ml)	1.8 ± 1.2^{c}	2.3 ± 3.9^{e}	2.9 ± 6.3^{f}	4.7 ± 9.1	<0.001
Total testosterone (ng/dL)	37.3 ± 14.8^{abc}	46.8 ± 16.4^{de}	55.1 ± 17.8	60.3 ± 21.7	<0.001
Sex hormone-binding globulin (nmol/L)	40.8 ± 29.7^{c}	41.6 ± 33.1^{e}	42.4 ± 26.7	51.5 ± 27.1	0.024
FAI	4.8 ± 4.1^{ab}	6.2 ± 4.6	6.4 ± 4.3	5.5 ± 4.2	<0.001
Free testosterone (pg/ml)	2.1 ± 0.8^{abc}	2.4 ± 0.9	2.3 ± 0.8	2.4 ± 0.8	<0.001
AMH (ng/mL)	8.6 ± 5.1^{abc}	11.7 ± 6.0^{de}	14.7 ± 6.1	15.3 ± 7.0	<0.001
Metabolic features					
Glucose (mg/dL)	91.6 ± 18.4	91.4 ± 18.9	91.9 ± 17.0	91.2 ± 13.1	0.932
Insulin (μIU/mL)	15.9 ± 14.8^{bc}	14.4 ± 11.8^{d}	12.4 ± 13.0	10.0 ± 9.5	<0.001
HOMA-IR	3.8 ± 4.2^{c}	3.4 ± 3.2	3.0 ± 4.1	2.4 ± 2.6	0.008
Metabolic syndrome -n. (%)	55/222 [24.8]^{bc}	97/408 [23.8]^{de}	32/231 [13.9]	9/95 [9.5]	<0.001
Triglyceride (mmol/l)	1.6 ± 0.9^{b}	1.7 ± 1.0^{de}	1.5 ± 0.8	1.4 ± 0.7	0.004
Cholesterol (mmol/l)	4.7 ± 1.2	4.8 ± 1.1	4.7 ± 1.0	4.9 ± 1.1	0.292
LDL-C (mmol/l)	2.9 ± 0.9	3.0 ± 0.8	2.9 ± 0.8	3.1 ± 1.0	0.310
HDL-C (mmol/l)	1.2 ± 0.4^{bc}	1.2 ± 0.4^{de}	1.3 ± 0.4	1.4 ± 0.3	<0.001
PCOS clinical phenotypes					
Duration between menstruation periods (days)	59.9 ± 25.2^{ab}	73.8 ± 50.7	73.7 ± 47.0	67.1 ± 32.5	<0.001
No. of menstrual cycles/per year	6.7 ± 2.1^{ab}	6.0 ± 2.1	5.9 ± 2.1	6.2 ± 1.9	<0.001
Amenorrhea -n. (%)	14/222 [6.3]^{ab}	56/407 [13.8]	29/231 [12.6]	11/95 [11.6]	0.031
Oligomenorrhea -n. (%)	219/222 [98.6]	403/408 [98.8]	228/231 [98.7]	94/95 [98.9]	1.000
Polycystic ovaries -n. (%)	184/212 [86.8]^{a}	372/396 [93.9]	204/224 [91.1]	86/91 [94.5]	0.021
Hirsutism -n. (%)	60/169 [35.5]	101/331 [30.5]	62/190 [32.6]	28/77 [36.4]	0.617
Ferriman-Gallwey score	2.8 ± 2.5	2.9 ± 2.6	3.3 ± 3.1	3.3 ± 3.2	0.185
Acne -n. (%)	148/222 [66.7]	284/407 [69.8]	157/231 [68.0]	65/95 [68.4]	0.872
Acne score	0.5 ± 0.9	0.4 ± 0.8	0.4 ± 0.7	0.4 ± 0.6	0.462
Treatment					
Clomiphene + Active Acupuncture	57 [25.68]	96 [23.53]	61 [26.41]	26 [27.37]	0.241
Clomiphene + Control Acupuncture	65 [29.28]	111 [27.21]	41 [17.75]	24 [25.26]	
Placebo + Active Acupuncture	49 [22.07]	105 [25.74]	63 [27.27]	21 [22.11]	
Placebo + Control Acupuncture	51 [22.97]	96 [23.53]	66 [28.57]	24 [25.26]	

Mean ± SD or n/N (%) values are presented. BMI, body mass index; WHR, waist-to-hip ratio; LH, luteinizing hormone; FSH, follicle stimulating hormone; FAI, Free androgen index = T/SHBG × 100; AMH, antimullerian hormone; HOMA-IR, homeostatic model assessment insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. a: P < 0.05 between LH/FSH<1 and 1 ≤ LH/FSH<2; b: P < 0.05 between LH/FSH<1 and 2 ≤ LH/FSH<3; c: P < 0.05 between LH/FSH<1 and LH/FSH≥3; d: P < 0.05 between 1 ≤ LH/FSH<2 and 2 ≤ LH/FSH<3; e: P < 0.05 between 1 ≤ LH/FSH<2 and LH/FSH≥3; f: P < 0.05 between 2 ≤ LH/FSH<3 and LH/FSH≥3.

3.2. Demographic characteristic of PCOS women by baseline LH/FSH ratio

Women in the highest LH/FSH ratios tended to have lower BMI ($P < 0.001$) and smaller WHR ($P < 0.001$), while no differences in age was observed among the four subgroups (Table 1). The level about endocrine, such as estradiol, progesterone, total testosterone, sex hormone-binding globulin, FAI, free testosterone and AMH, all increased with elevated LH/FSH ratios ($P < 0.001$). Although there was no significant difference in the levels of glucose among the four subgroups ($P = 0.93$), the levels of insulin and HOMA-IR decreased with the change in LH/FSH ratios ($P < 0.001$). The data pertaining to lipid Metabolism, including triglyceride, cholesterol, LDL-C and HDL-C did not differ among the four subgroups except for triglyceride ($P = 0.007$) and HDL-C ($P < 0.001$). Furthermore, significant differences in the prevalence of metabolic syndrome ($P < 0.001$) were observed among four groups. Women with lower number of menstrual cycles per year, as well as more days between menstruation periods were observed in women with higher ratios ($P < 0.001$). The proportion of women with amenorrhea and polycystic ovary morphology were significantly different among these four subgroups ($P = 0.031$, $P = 0.021$), while no difference in hirsutism, acne and even their score.

Next, we used a backward deletion procedure to perform a multiple linear regression model (F Value = 53.49, P value < 0.0001; R-Square = 0.2679, Adjusted R-Square = 0.2629) for LH/FSH ratios from the PCOSAct data (Table 2). The model showed age, BMI and free testosterone had a negative correlation with LH/FSH ratios, and total testosterone, estradiol and AMH were positive correlation (Table 2).

3.3. Association between clinical outcomes and baseline LH/FSH ratio after ovulation induction

Totals of 196 (20.5%) women progressed live birth, 749 (78.3%) women became ovulation, 305 (31.9%) women became biochemical pregnancy, 209 (21.9%) women became clinical pregnancy, 104 (10.9%) women became miscarriage after ovulation induction. We observed that participants in $1 \leq$ LH/FSH<2, $2 \leq$ LH/FSH<3 and LH/FSH≥ 3 were associated with lower ovulation compared to participants in LH/FSH <1.0 (OR = 0.42, 95% CI, 0.26–0.68; OR = 0.32, 95% CI, 0.20–0.54; OR = 0.40, 95% CI 0.21–0.74; Table 3). Two multiple logistic regression models were performed for reproductive outcomes. Model 1 entered treatment alone as covariates, as well as treatment, age, BMI, T, E2, FT and AMH were included as covariates in the model2. Participants in $1 \leq$ LH/FSH <2 were associated with higher live birth (OR = 1.73; 95% CI, 1.09–2.75) and clinical pregnancy (OR = 1.71; 95% CI, 1.09–2.67) compared to participants in LH/FSH <1.0 after adjustment for model2. Similar results were also observed for miscarriage, in that participants in the $2 \leq$ LH/FSH<3 as compared to LH/FSH <1.0 were associated with miscarriage after adjustment for model2 (OR = 0.38; 95% CI, 0.16–0.93) (Table 3).

As can be drawn from the diagram (Fig. 2B), participants in LH/FSH <1.0 need shortest duration to achieve the first ovulation ($\chi^2 = 16.10$, $P < 0.001$). While the significant association among the four LH/FSH levels in participants who became live birth ($\chi^2 = 5.74$, $P = 0.12$) (Fig. 2A), biochemical pregnancy ($\chi^2 = 0.29$, $P = 0.96$) (Fig. 2C), clinical pregnancy ($\chi^2 = 0.80$, $P = 0.85$) (Fig. 2D) and miscarriage ($\chi^2 = 0.96$, $P = 0.58$) (Fig. 2E) were not found. As shown in Fig. 3, anovulatory women had significantly higher LH/FSH ratios than ovulatory women in OA + PCO subtype ($P = 0.003$) (Fig. 3A), especially in women with Age<28 years ($P = 0.023$) (Fig. 3B), BMI≥ 23.63 kg/m^2 ($P = 0.002$) (Fig. 3C), E2<54.236 pg/mL ($P = 0.002$) (Fig. 3D), FT < 2.215 ng/dL ($P = 0.010$) (Fig. 3E), TT < 1.59 ng/dL ($P < 0.001$) (Fig. 3F) and AMH<11.549 ng/mL ($P = 0.032$) (Fig. 3G).

4. Discussion

Our study thoroughly investigated the association between elevated baseline LH/FSH ratio and reproductive outcomes in Chinese women with PCOS. We demonstrate that there was significant reducing tendency for ovulation with increasing LH/FSH ratio among women with PCOS. The association was held after adjustment for treatment with or without the confounding factors. Moreover, PCOS women with specific elevated LH/FSH ratio were associated with higher clinical pregnancy and live birth and lower miscarriage.

Elevated LH/FSH ratio appear to have adverse effects on the number of follicles and oocytes, as well as on follicles quality, oocyte maturation and granulosa cell function [28,29]. The lower FSH level in the PCOS women's circulation significantly inhibits the periodic follicular recruitment, there is no dominant follicle formation, and most follicles are in the middle sinus. The higher level of LH in circulation may promote follicular atresia or premature luteinization [30]. Ovulation refers to the complex process in which oocytes and their surrounding cumulus cells are expelled together. Errors in any of these steps will affect the occurrence of ovulation, especially the follicular development and LH/FSH peak-induced ovulation, which may in part explain our finding that elevated baseline LH/FSH

Table 2
Significant variables for baseline LH/FSH ratio.

Label	DF	Parameter Estimate	t Value	Pr > |t|
Age (yr)	**1**	−0.02365	−2.37	0.018
BMI (kg/m^2)	**1**	−0.04314	−5.28	<0.0001
Total testosterone (ng/dL)	**1**	0.01841	7.8	<0.0001
Estradiol (pg/mL)	**1**	0.00426	9.04	<0.0001
Free testosterone (pg/ml)	**1**	−0.1101	−2.2	0.0283
AMH (nmol/L)	**1**	0.02383	4.34	<0.0001

Multiple linear regression model, F Value = 53.49, P value < 0.0001; R-Square = 0.2679, Adjusted R-Square = 0.2629. BMI, body mass index; AMH, antimullerian hormone.

Table 3
Likelihood of clinical outcomes by baseline LH/FSH ratio.

Model	LH/FSH<1 (n = 222)	1 ≤ LH/FSH<2 (n = 408)	2 ≤ LH/FSH<3 (n = 231)	LH/FSH≥3 (n = 95)
Live birth n(%)	38/222 (17.12%)	90/408 (22.06%)	47/231 (20.35%)	21/95 (22.11%)
Unadjusted	Reference	1.37 (0.90, 2.09)	1.24 (0.77, 1.99)	1.37 (0.76, 2.50)
Adjusted model 1	Reference	1.44 (0.94, 2.20)	1.36 (0.84, 2.20)	1.41 (0.77, 2.58)
Adjusted model 2	Reference	**1.73 (1.09, 2.75)**	1.73 (0.98, 3.04)	1.49 (0.71, 3.13)
Ovulation n(%)	197/222 (88.74%)	314/408 (76.96%)	166/231 (71.86%)	72/95 (75.79%)
Unadjusted	Reference	**0.42 (0.26, 0.68)**	**0.32 (0.20, 0.54)**	**0.40 (0.21, 0.74)**
Adjusted model 1	Reference	**0.42 (0.26, 0.69)**	**0.34 (0.20, 0.58)**	**0.38 (0.20, 0.73)**
Adjusted model 2	Reference	**0.47 (0.27, 0.80)**	**0.31 (0.17, 0.57)**	**0.27 (0.12, 0.61)**
Biochemical pregnancy n(%)	70/222 (31.53%)	135/408 (33.09%)	68/231 (29.44%)	32/95 (33.68%)
Unadjusted	Reference	1.07 (0.76, 1.52)	0.91 (0.61, 1.35)	1.10 (0.66, 1.84)
Adjusted model 1	Reference	1.13 (0.79, 1.62)	1.00 (0.66, 1.52)	1.13 (0.67, 1.91)
Adjusted model 2	Reference	1.25 (0.85, 1.85)	1.10 (0.68, 1.79)	1.01 (0.53, 1.91)
Clinical pregnancy n(%)	42/222 (18.92%)	97/408 (23.77%)	48/231 (20.78%)	22/95 (23.16%)
Unadjusted	Reference	1.34 (0.89, 2.01)	1.12 (0.71, 1.78)	1.29 (0.72, 2.31)
Adjusted model 1	Reference	1.40 (0.93, 2.12)	1.23 (0.77, 1.98)	1.32 (0.73, 2.39)
Adjusted model 2	Reference	**1.71 (1.09, 2.67)**	1.60 (0.92, 2.78)	1.34 (0.64, 2.78)
Miscarriage n(%)	29/222 (13.06%)	43/408 (10.54%)	21/231 (9.09%)	11/95 (11.58%)
Unadjusted	Reference	0.63 (0.34, 1.15)	0.59 (0.29, 1.19)	0.69 (0.29, 1.65)
Adjusted model 1	Reference	0.63 (0.34, 1.15)	0.59 (0.29, 1.21)	0.70 (0.29, 1.68)
Adjusted model 2	Reference	0.51 (0.26, 1.00)	**0.38 (0.16, 0.93)**	0.45 (0.15, 1.36)

Odds ratios (95% CI) are presented. Bolds: $p < 0.05$ by logistic regression models. Adjusted model 1: adjusted for treatment only. Adjusted model 2: adjusted for treatment, age, body mass index, total testosterone, estradiol 2, free testosterone and antimullerian hormone.

ratio in women with PCOS was associated with poor ovulatory response. Moreover, we observed that the women with elevated LH/FSH had higher level of testosterone than women with normal LH/FSH, which are consistent with prior studies [13]. Higher LH level in women with PCOS promotes the production of androstenedione and testosterone in theca cells and ovarian stromal cells [31,32,33]. At the same time, LH can also increase the activity of insulin-like growth factor I (IGF-I) in the ovary, and its binding to the IGF-I receptor on follicular theca cells is considered to be another way to promote androgen production, while another research questioned this finding [34,35]. The results showed that although high level of androgen in PCOS women can accelerate spontaneous development of primordial follicles, it can increase the expression of Bcl-2 in follicles, reduce the expression of Bax and p53, and reduce the level of growth differentiation factor-9 (GDF-9), thereby inhibiting the growth of follicles to a certain extent, inhibiting the apoptosis of follicles, increasing the number of small follicles, and ultimately affecting ovulation [36,37]. More interestingly, we observed that the women with elevated baseline LH/FSH had higher level of progesterone than women with normal LH/FSH. This result is consistent with prior studies that higher LH can promote follicular atresia or premature luteinization, resulting in higher progestin [30]. Higher level progesterone may provide a good growth environment for oosperm, which may in part explain that women with elevated LH/FSH are more prone to pregnancy and live births, and more difficult to have miscarriages, even after adjustment for treatment with or without the confounding factors. Because of the large number of participants and the independence explore about association between LH/FSH and reproductive outcomes, our study got findings differ from the observations of prior studies that women with higher LH/FSH ratios have an adverse effect on pregnancy rates [23,38] and seems to predict a greater possibility for miscarriage [36,39,40], while some studies are in agreement with our findings [3,27].

There are several limitations to our study. First, this study is limited by the relatively small sample size, although we have included 1000 women. Larger trials are required to elucidate the association between elevated LH/FSH ratios, not in specific ratios, and higher clinical pregnancy, live birth, as well as lower miscarriage. Second, we did not include normal women in study, and cannot identify the LH/FSH cut-off point for reproductive outcomes. Additional research is required to further clarify the diagnostic vale of baseline LH/FSH ratios for reproductive outcomes in Chinese women with PCOS compared with HA, PCO or OA.

5. Conclusion

In this study, elevated baseline LH/FSH ratio is associated with poor ovulatory response but better clinical pregnancy and live birth in Chinese women with PCOS after ovulation induction. It suggests LH and FSH in Chinese women with PCOS may play a role in successful pregnancy despite of negative impact in ovulation.

Author contribution statement

Xiaoke Wu: Conceived and designed the experiments.
Qing Xia and Liangzhen Xie: Analyzed and interpreted the data; Wrote the paper.
Qi Wu, Jing Cong and Hongli Ma: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
Jian Li and Wangyu Cai: Contributed reagents, materials, analysis tools or data.

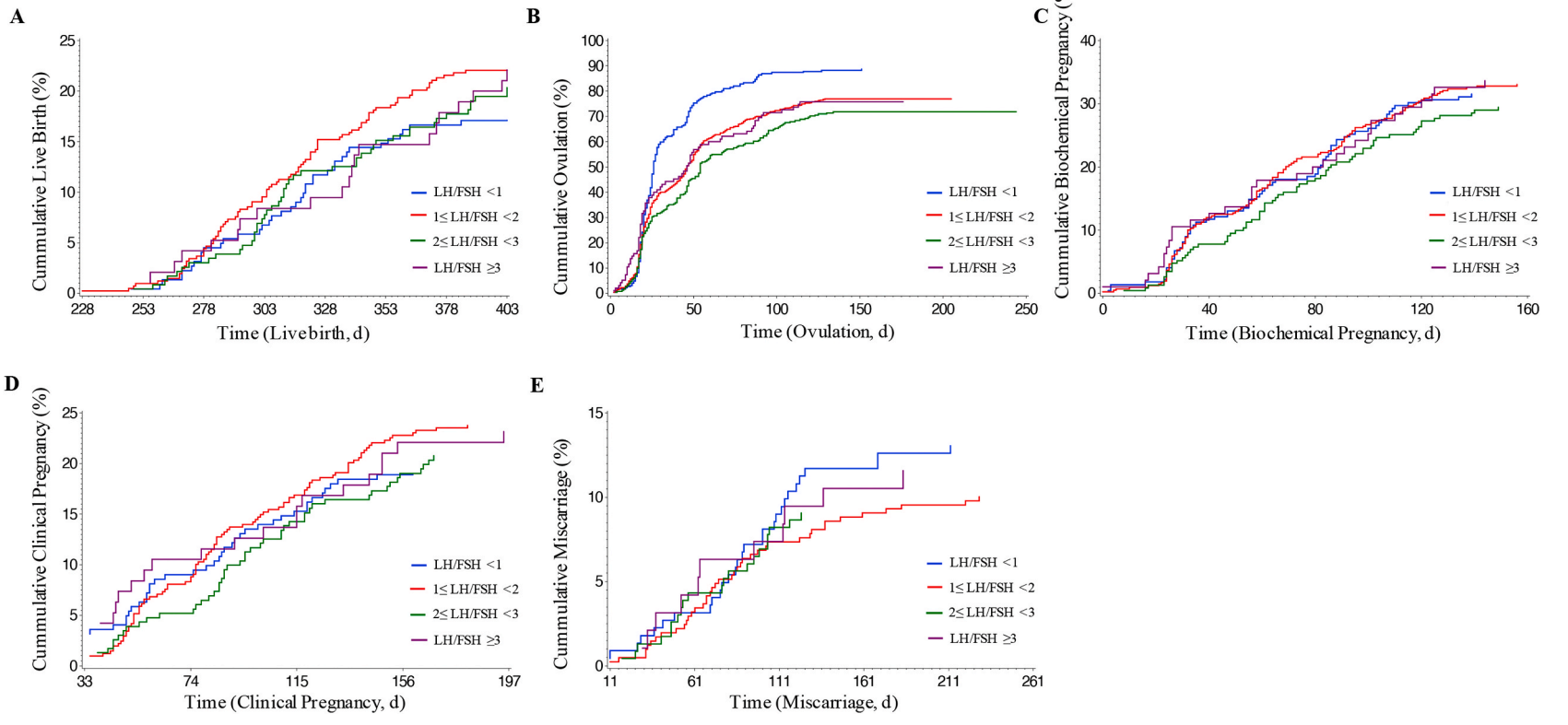

Fig. 2. Cumulative clinical outcomes by baseline LH/FSH ratio. Cox regression models for (A) live birth, (B) ovulation, (C) biochemical pregnancy, (D) clinical pregnancy, and (E) miscarriage after randomization.

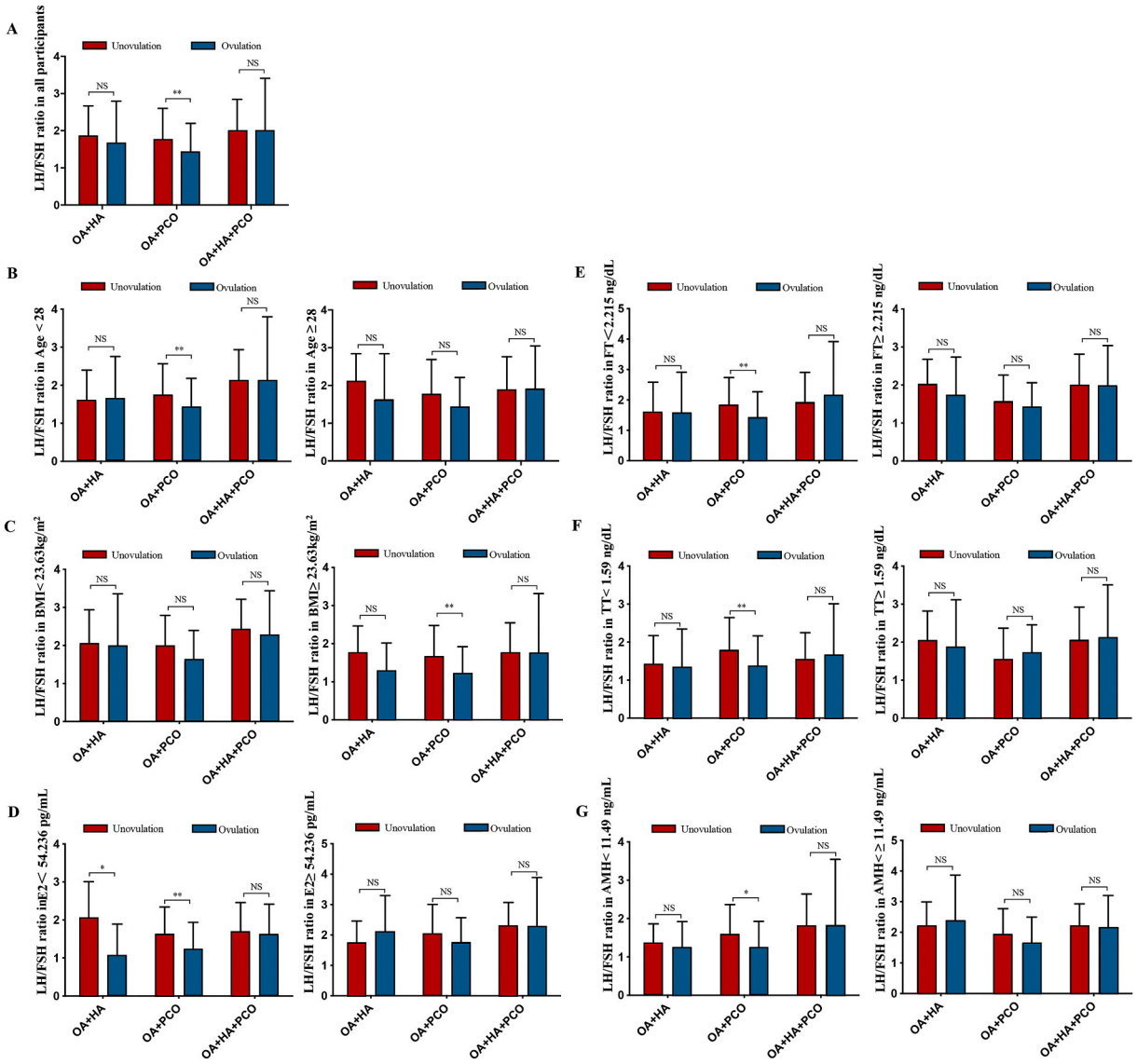

Fig. 3. Comparison of baseline LH/FSH ratio by ovulatory status. Differences of LH/FSH ratios according to different phenotypes of PCOS in (A) overall women, (B) Age≥28 years and Age<28 years, (C) BMI≥23.63 kg/m^2 and BMI<23.63 kg/m^2, (D) E2≥54.236 pg/mL and E2<54.236 pg/mL, (E) FT ≥ 2.215 ng/dL and FT < 2.215 ng/dL, (F) TT ≥ 1.59 ng/dL and TT ≥ 1.59 ng/dL, and (G) AMH≥11.549 ng/mL and AMH<11.549 ng/mL. OA, anovulation; HA, hyperandrogenism; PCO, polycystic ovaries. NS, no significant, *P < 0.05, **P < 0.01 ovulated versus unovulated.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Additional information

No additional information is available for this paper.

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