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Changes and correlations of anti-Müllerian hormone and stem-cell factors in different ovarian reserve patients during GnRH-antagonist protocol and the effects on controlled ovarian hyperstimulation outcomes

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Abstract

Purpose To explore the changes and correlations of anti-Müllerian hormone (AMH) and stem-cell factors (SCF) in different ovarian reserve patients during controlled ovarian hyperstimulation (COH) and the effects on COH outcomes.

Methods Serum at six different timepoints during GnRH-antagonist protocol and follicular fluid (FF) on oocyte retrieval day of 52 patients with polycystic ovary syndrome (PCOS), 61 patients with normal ovarian reserve (NOR) and 42 patients with diminished ovarian reserve (DOR) were collected. AMH and SCF were assessed using enzyme-linked immunosorbent assay.

Results During COH, AMH in the PCOS group was the highest, but SCF did the opposite, and serum AMH gradually decreased, while SCF inversely increased. In the PCOS group, SCF on the first and fourth days of gonadotropin (Gn) administration was negative with Gn dosage ($r=-0.362$, $P<0.05$; $r=-0.344$, $P<0.05$). In the NOR group, the basal AMH was also negative with Gn dosage ($r=-0.297$, $P<0.05$) and positive with COH outcomes (number of retrieved oocytes, MII oocytes, and 2PN fertilization) as well as serum SCF after Gn administration. In the DOR group, both AMH and SCF were significantly associated with COH outcomes. Serum AMH in the DOR group after Gn administration and FF AMH showed a negative correlation with SCF.

Conclusions Serum AMH decreased, while SCF increased during COH. AMH and SCF are effective for Gn time and dosage adjustment and predicting COH outcomes for NOR and DOR patients. In addition, serum AMH in DOR patients after Gn administration and FF AMH has a negative effect on SCF.

Keywords AMH · SCF · Follicular fluid · PCOS · DOR

Introduction

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein hormone secreted by ovarian granulosa cells (GCs) from pre-antral follicles to small antral follicles of size less than 4 mm, and when the size of the antral follicles was greater than 8 mm, the level of AMH decreased sharply [1]. Data from experimental study suggest that the main role of AMH is to inhibit the primordial and cyclic recruitment of ovarian follicles, reduce the sensitivity of growing follicles to follicle-stimulating hormone (FSH), and then protect the follicular pool [2].

Previous studies conducted in the last decades suggested that AMH can directly reflect the size of primordial follicular pool [3], and its value in predicting ovarian reserve and response is better than inhibin B (INHB), FSH, and estradiol (E_2) [4, 5]. Meanwhile, the advantage of AMH in predicting ovarian response is also superior to antral follicle count (AFC), especially in poor ovarian response (POR) [6].

New research confirmed that AMH promotes pre-antral follicle growth, but inhibits antral follicle maturation and

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dominant follicle selection in primates [7]. As one important transcription factor, AMH is instrumental to regulating the transcription of multiple genes and cytokines expression including SCF. Previous researchers have observed that the combination of stem-cell factor (SCF) with c-Kit can activate PI3 K/Akt/Foxo3a signalling pathway, initiate Bcl-2 (an anti-apoptotic gene) expression, promote the survival of germ cells, and prevent oocytes apoptosis in primordial follicles, thereby expanding the primordial follicle pool [8, 9].

Subsequent experiments have found that SCF can not only promote the transformation from primordial follicle to primary follicle, but also stimulate the proliferation of GCs and theca cells as well as steroid hormones production, thus accelerating the meiosis of oocytes [10]. Further studies confirmed that SCF can form a functional feedback loop between oocytes and GCs through growth differentiation factor-9 (GDF-9) or bone morphogenetic protein-15 (BMP-15) secreted by oocytes, and then stimulate steroid hormones and other growth factors' secretion [11]. In addition, SCF-induced vascularization is closely related to the delivery of gonadotrophin (Gn) during controlled ovarian hyperstimulation (COH), and its function in GCs and theca cells differentiation affects the response of infertility patients to Gn during COH, which is dramatically associated with COH outcomes [12].

Polycystic ovary syndrome (PCOS) and diminished ovarian reserve (DOR) are common infertility factors, and often have abnormal response to Gn [13, 14]. Currently, there is no consensus on the relationship of AMH and SCF with ovarian response and COH outcomes in different ovarian reserves [2, 12, 15]. More recently, the changes and correlations of AMH and SCF during COH have been little studied. Hence, this paper aims to study the changes and correlations of AMH and SCF in different ovarian reserve patients undergoing GnRH-antagonist protocol and the effects on COH outcomes, so as to provide theoretical basis for COH treatment and possible pathogenesis for PCOS and DOR.

Methods

Patients

This retrospective clinical study was conducted from January 2018 to February 2019 in Shijiazhuang Maternity Hospital. Serum and FF samples were obtained from 52 patients with PCOS, 61 patients with normal ovarian reserve (NOR), and 42 patients with DOR undergoing the GnRH-antagonist protocol. PCOS was diagnosed based on the 2003 Rotterdam criteria [16]. In the NOR group, patients received COH mainly due to oviduct obstruction or male factors, and met the following conditions: (1) menstrual cycle: 26 ~ 35 days; (2) AFC: 8 ~ 20; and (3) normal basic hormone level. Patients with DOR met the criteria of AFC < 5–7 or AMH < 0.5 ~ 1.1 ng/mL [17]. The patients with the following conditions were excluded: ovarian surgery, radiotherapy or chemotherapy, endometriosis, hyperprolactinemia, thyroid dysfunction, hypertension, or ovulation induction within 3 months. Informed consents were obtained from all candidates after the approval of the study by the local Investigation and Ethics Committee.

GnRH-antagonist protocol

The initial doses of 150–450 IU/d recombinant human FSH (r-hFSH, Merck-Serono, Darmstadt, Germany) were used from the second or third days of menstruation depending on the age, body mass index(BMI), basal hormone levels, and antral follicle count (AFC) of the patients. The time and dosage of r-hFSH were adjusted on the basis of ovarian response as monitored by the level of serum E_2 and transvaginal ultrasound. When a follicle was 14 mm in diameter or luteinizing hormone (LH) level ≥ 10 IU/mL, one GnRH-antagonist (0.25 mg, Baxter Oncology GmbH, Germany) was added daily. If the number of follicles with diameter ≥ 18 mm/number of follicles with diameter ≥ 14 mm was equal to or greater than 60% and the average level of serum E_2 of each follicle with diameter ≥ 14 mm was 200–300 pg/mL, the r-hFSH was stopped, and 6000–10,000 IU of recombinant human chorionic gonadotropin (r-hCG, Merck-Serono, Darmstadt, Germany) was administered for triggering. Oocyte retrieval was performed 34–36 h later under transvaginal ultrasound guidance.

Serum and FF collection

The serum samples from 155 patients at six different timepoints during COH were collected. The timepoints are as follows: Gn startup day, the fourth day of Gn administration, GnRH-antagonist initiation day, the day of hCG administration, the next day of hCG administration, and 3 days after oocyte retrieval. The limpid FF was got on oocyte retrieval day. All serum and FF were pooled in sterile tubes and immediately centrifuged at 500*g* for 10 min. Supernatants were aspirated, divided into aliquots, and frozen at − 80 °C for future analysis.

ELISA for AMH and SCF measurements

The level of AMH and SCF in serum and FF was, respectively, assessed by AMH (Anshlabs, USA) and SCF (R and D Systems, MN, USA) enzyme-linked immunosorbent assay (ELISA) kit. All of the procedures were performed according to the protocols of the manufacturers.

Statistical analysis

The software package SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used for all data analysis. The Kolmogorov–Smirnov test was used to evaluate the normality of distribution. Measurement data were expressed by average and standard deviation, and analyzed using One-way ANOVA analysis when appropriate. The correlation analysis was assessed using Pearson correlation coefficient. For all tests, a P value less than 0.05 was considered statistically significant.

Results

General characteristics

155 patients were deemed eligible in this study, including 52 patients with PCOS, 61 patients with NOR, and 42 patients with DOR. The basal level of progesterone (P), prolactin (PRL) and infertility years was similar in the three groups ($P > 0.05$). BMI, AFC, and the basal levels of LH, E_2, testosterone (T) were significantly higher in the PCOS group than in the NOR group ($P < 0.001$), just as the age and the basal levels of FSH, LH, and E_2 in the DOR group, while the basal levels of T and AFC were dramatically lower in the DOR group than in the NOR group ($P < 0.001$) (Table 1).

Laboratory data

The days of stimulation and number of retrieved oocytes in the PCOS group were the highest, but MII oocyte rate was the lowest among the three groups. At the same time, when compared with the NOR group, the Gn dosage, number of retrieved oocytes, MII oocytes, 2PN fertilization, high-quality embryos, MII oocyte rate, 2PN fertilization rate, and pregnancy rate in the DOR group were remarkably reduced. We also found that the daily Gn dosage for per retrieved oocyte was significantly higher in the DOR group than in the PCOS and NOR groups (57.82 ± 6.95 vs. 15.83 ± 1.43 and 19.43 ± 1.11 IU, $P < 0.001$) (Table 2).

Changes of serum and FF AMH and SCF in patients with different ovarian reserves and responses

AMH in the PCOS group were the highest, but SCF did the opposite, and serum AMH gradually decreased, while SCF inversely increased during COH. Furthermore, we found that the reduction rate of AMH on the day of hCG administration (dhCG1) was dramatically higher in the PCOS group than in the NOR and DOR groups ($67.97 \pm 1.76\%$ vs. $49.56 \pm 2.39\%$ and $47.13 \pm 3.33\%$, $P < 0.001$), while the increase rate of SCF on dhCG1 was similar in the three groups ($12.64 \pm 1.14\%$ vs. $11.66 \pm 0.81\%$ vs. $10.33 \pm 1.73\%$, $P > 0.05$) (Table 3 and Fig. 1). In patients with different responses, the level of serum and FF AMH was significantly increased in the high-response group and dramatically decreased in the low-response group ($P < 0.001$). There were no statistical significances of serum and FF SCF in the high- and middle-response groups ($P > 0.05$), but a statistical difference was presented between the high- and low-response groups ($P < 0.001$ or $P < 0.05$). Similarly, the level of AMH visibly decreased, but the level of SCF showed an increased tendency during COH in patients with different ovarian responses (Table 4 and Fig. 2).

Effects of AMH and SCF on laboratory data

In the PCOS group, the level of SCF on the first and fourth days of gonadotropin (Gn) administration as well as AMH from hCG administration day was negative with Gn dosage ($r = -0.362$, $p < 0.05$; $r = -0.344$, $p < 0.05$; $r = -0.294, -0.307, -0.322$, $P < 0.05$), and there was no visible correlation of serum AMH and SCF with COH

Table 1 General characteristics in different ovarian reserve patients

Baseline variables	PCOS group ($n = 52$)	NOR group ($n = 61$)	DOR group ($n = 42$)	P
Age (years)	29.71 ± 0.51	30.56 ± 0.41	35.5 ± 0.75^{ab}	< 0.001
Infertility years (years)	3.71 ± 0.24	3.17 ± 0.14	3.81 ± 0.32	0.09
Body mass index (kg/m^2)	27.10 ± 0.55	22.64 ± 0.35^{a}	23.67 ± 0.56^{a}	< 0.001
Basal FSH (IU/L)	4.80 ± 0.16	5.93 ± 0.17^{a}	11.97 ± 0.55^{ab}	< 0.001
Basal LH (IU/L)	9.25 ± 0.41	4.24 ± 0.19^{a}	6.37 ± 0.42^{ab}	< 0.001
Basal E_2 (pg/mL)	68.29 ± 3.12	39.89 ± 1.46^{a}	77.14 ± 6.33^{b}	< 0.001
Basal P (ng/mL)	0.56 ± 0.04	0.54 ± 0.03	0.59 ± 0.04	0.654
Basal T (nmol/L)	1.41 ± 0.06	0.89 ± 0.04^{a}	0.75 ± 0.04^{ab}	< 0.001
Basal PRL (ug/L)	12.13 ± 0.82	13.19 ± 0.71	13.46 ± 1.06	0.513
Antral follicle count (n)	25.69 ± 1.10	11.75 ± 0.37^{a}	4.02 ± 0.24^{ab}	< 0.001

Values are given in mean ± standard deviation

[a]$P < 0.05$ and [b]$P < 0.05$, respectively, represent compared with PCOS group and NOR group

Table 2 Laboratory data in different ovarian reserve patients

Variables	PCOS group ($n=52$)	NOR group ($n=61$)	DOR group ($n=42$)	P
Days of stimulation (days)	10.73 ± 0.2	9.92 ± 0.22[a]	8.55 ± 0.32a[b]	< 0.001
Gn dosage (IU)	2025.8 ± 69.3	2054.7 ± 78.1	1728.7 ± 111.1[ab]	0.019
Number of retrieved oocytes (*n*)	15.46 ± 1.02	11.66 ± 0.39[a]	4.31 ± 0.23[ab]	< 0.001
Number of MII oocytes (*n*)	11.02 ± 0.58	9.89 ± 0.41	2.55 ± 0.25[ab]	< 0.001
Number of 2PN fertilization (*n*)	8.04 ± 0.42	7.64 ± 0.43	1.95 ± 0.23[ab]	< 0.001
Number of high-quality embryos (*n*)	4.85 ± 0.38	4.07 ± 0.36	1.02 ± 0.18[ab]	< 0.001
MII oocyte rate (%)	75.69 ± 2.29	85.02 ± 1.92[a]	58.9 ± 4.97[ab]	< 0.001
2PN fertilization rate (%)	72.44 ± 2.29	77.1 ± 2.4	67.24 ± 5.76[b]	0.049
High-quality embryos rate (%)	51.6 ± 3.12	54.00 ± 3.55	42.07 ± 6.19	0.130
Pregnancy rare (%)	48 (25/52)	58 (39/67)	19 (8/42)[ab]	< 0.001
Daily Gn dosage for per retrieved oocyte (IU)	15.83 ± 1.43	19.43 ± 1.11	57.82 ± 6.95[ab]	< 0.001

Values are given in mean ± standard deviation or *n* (%)

Daily Gn dosage for per retrieved oocyte = Gn dosage/days of stimulation/number of retrieved oocytes

[a]$P<0.05$ and [b]$P<0.05$, respectively, represent compared with PCOS group and NOR group

Table 3 Changes of serum and FF AMH and SCF in patients with different ovarian reserves

	PCOS group ($n=52$)	NOR group ($n=61$)	DOR group ($n=42$)	P
AMH (ng/mL)				
dGn1	9.91 ± 0.52	3.21 ± 0.20[a]	0.76 ± 0.07[ab]	< 0.001
dGn4	7.20 ± 0.39	2.60 ± 0.11[a]	0.62 ± 0.06[ab]	< 0.001
dGnRH-A1	4.79 ± 0.33	2.16 ± 0.10[a]	0.51 ± 0.05[ab]	< 0.001
dhCG1	2.97 ± 0.17	1.55 ± 0.74[a]	0.38 ± 0.04[ab]	< 0.001
dhCG2	2.61 ± 0.15	1.34 ± 0.60[a]	0.33 ± 0.03[ab]	< 0.001
dOPU3	1.79 ± 0.10	0.94 ± 0.05[a]	0.25 ± 0.02[ab]	< 0.001
FF	8.80 ± 0.44	2.98 ± 0.09[a]	1.57 ± 0.13[ab]	< 0.001
Reduction rate of AMH on dhCG1 (%)	67.97 ± 1.76	49.56 ± 2.39[a]	47.13 ± 3.33[a]	< 0.001
SCF (pg/mL)				
dGn1	636.04 ± 11.98	734.84 ± 11.08[a]	753.22 ± 13.33[a]	< 0.001
dGn4	656.13 ± 12.49	765.98 ± 11.38[a]	796.22 ± 15.79[a]	< 0.001
dGnRH-A1	678.51 ± 11.98	791.36 ± 11.98[a]	808.12 ± 17.09[a]	< 0.001
dhCG1	714.65 ± 13.70	818.95 ± 12.26[a]	830.32 ± 19.44[a]	< 0.001
dhCG2	729.02 ± 14.23	845.54 ± 11.6[a]	846.80 ± 19.56[a]	< 0.001
dOPU3	642.02 ± 11.74	775.65 ± 11.63[a]	778.47 ± 18.31[a]	< 0.001
FF	646.29 ± 11.78	724.49 ± 15.67[a]	730.56 ± 16.96[a]	< 0.001
Increase rate of SCF on dhCG1 (%)	12.64 ± 1.14	11.66 ± 0.81	10.33 ± 1.73	> 0.05

Values are given in mean ± standard deviation or *n* (%)

Reduction rate of AMH on dhCG1 (%) = (AMH on dhCG1 − on dGn1)/AMH on dGn1 × 100%

Increase rate of SCF on dhCG1 (%) = (SCF on dhCG1 − on dGn1)/SCF on dGn1 × 100%

dGn1 Gn startup day, *dGn4* the 4th day of Gn administration, *dGnRH*-A1 GnRH-antagonist initiation day, *dhCG1* day on hCG administration, *dhCG2* the next day of hCG administration, *dOPU3* 3 days after oocyte retrieval, *FF* Follicular fluid

[a]$P<0.05$ and [b]$P<0.05$, respectively, represent compared with PCOS group and NOR group

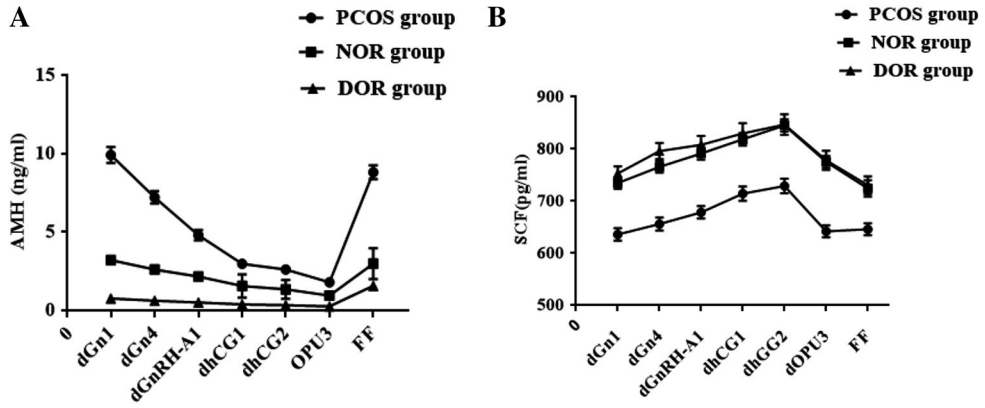

Fig. 1 Changes of serum and FF AMH and SCF in patients with different ovarian reserves. **a** During COH, AMH were the highest in the PCOS group and lowest in the DOR group. In addition, serum AMH gradually decreased and reached to the lowest level on 3 days after oocyte retrieval ($P < 0.001$). **b** SCF showed an increasing trend after Gn startup, peaked after trigger, and decreased significantly 3 days after oocyte retrieval in different ovarian reserve patients. The level of SCF was dramatically lower in the PCOS group than in the NOR and DOR groups ($p < 0.001$)

Table 4 Changes of serum and FF AMH and SCF in patients with different ovarian responses

	High-response group ($n = 28$)	Middle-response group ($n = 95$)	Low-response group ($n = 32$)	*P*
AMH (ng/mL)				
dGn1	9.51 ± 0.91	4.56 ± 0.36[a]	1.34 ± 0.44[ab]	< 0.001
dGn4	6.97 ± 0.68	3.51 ± 0.25[a]	0.94 ± 0.28[ab]	< 0.001
dGnRH-A1	4.55 ± 0.45	2.70 ± 0.21[a]	0.57 ± 0.09[ab]	< 0.001
dhCG1	2.99 ± 0.26	1.77 ± 0.10[a]	0.41 ± 0.07[ab]	< 0.001
dhCG2	2.61 ± 0.26	1.53 ± 0.09[a]	0.38 ± 0.07[ab]	< 0.001
dOPU3	1.75 ± 0.14	1.09 ± 0.07[a]	0.27 ± 0.04[ab]	< 0.001
FF	7.65 ± 0.68	4.52 ± 0.34[a]	1.94 ± 0.42[ab]	< 0.001
SCF (pg/mL)				
dGn1	681.64 ± 17.54	703.02 ± 10.10	739.42 ± 18.50[a]	0.025
dGn4	704.02 ± 19.50	732.17 ± 10.51	781.76 ± 22.10[a]	0.006
dGnRH-A1	725.98 ± 18.76	754.24 ± 10.73	797.39 ± 23.27[a]	0.013
dhCG1	754.16 ± 20.46	780.77 ± 11.05	834.41 ± 24.58[ab]	< 0.05
dhCG2	771.52 ± 21.47	802.71 ± 11.02	849.77 ± 25.27[a]	0.010
dOPU3	680.09 ± 21.67	728.49 ± 10.69[a]	785.84 ± 22.94[ab]	< 0.05
FF	687.56 ± 16.94	629.17 ± 11.85	733.64 ± 21.74	> 0.05

Values are given in mean ± standard deviation or *n* (%)

dGn1 Gn startup day, *dGn4* the 4th day of Gn administration, *dGnRH*-A1 GnRH-antagonist initiation day, *dhCG1* day on hCG administration, *dhCG2* the next day of hCG administration, *dOPU3* 3 days after oocyte retrieval, *FF* Follicular fluid

[a]$P < 0.05$ and [b]$P < 0.05$, respectively, represent compared with high- and low-response groups

outcomes (number of retrieved oocytes, MII oocytes, 2PN fertilization) (Table 5).

In the NOR group, the basal level of AMH was negative with Gn dosage ($r = -0.297$, $P < 0.05$) and positive with the number of oocytes retrieved ($r = 0.313$, $P < 0.05$) and COH outcomes. Meanwhile, serum SCF after Gn administration was also positively related to COH outcomes (Table 6).

In the DOR group, both AMH and SCF have good association with the number of oocytes retrieved and COH outcomes ($P < 0.05$) (Table 7).

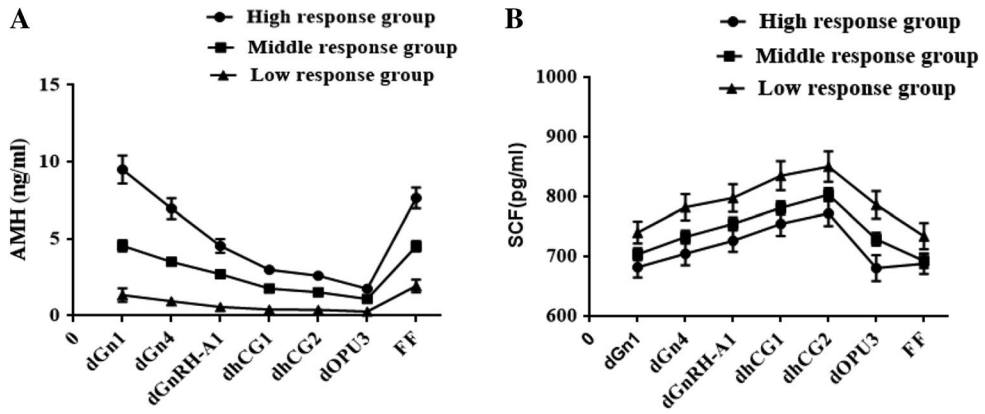

Fig. 2 Changes of serum and FF AMH and SCF in patients with different ovarian response. **a** Level of serum and FF AMH was significantly increased in the high-response group and dramatically decreased in the low-response group ($P<0.001$). During COH, the level of AMH also tends to be decreased. **b** There were no statistical significance of serum and FF SCF in the high- and middle-response groups ($P>0.05$), but a statistical difference was presented between the high- and low-response groups ($P<0.001$ or $P<0.05$). The level of SCF increased after Gn administration

Table 5 Correlations of AMH and SCF with COH outcomes in the PCOS group

	AFC	No.RO	Gn.D	No.MII	No.2PN	No.HQE
AMH						
dGn1	0.492#	0.177	0.019	0.111	− 0.006	− 0.049
dGn4	0.398#	0.191	0.000	0.075	− 0.028	− 0.063
dGnRH-A1	0.355*	0.143	− 0.224	0.149	0.060	0.074
dhCG1	0.433#	0.206	− 0.294*	0.263	0.170	− 0.033
dhCG2	0.438#	0.123	− 0.307*	0.159	0.143	− 0.021
dOPU3	0.407#	0.141	− 0.322*	0.161	0.103	− 0.043
FF	0.359#	− 0.086	− 0.040	− 0.041	− 0.094	− 0.132
SCF						
dGn1	0.309*	0.289*	− 0.362*	0.238	0.185	0.143
dGn4	0.314*	0.204	− 0.344*	0.212	0.155	0.077
dGnRH-A1	0.203	0.210	0.011	0.175	0.159	0.109
dhCG1	0.133	0.172	− 0.313*	0.086	0.022	0.074
dhCG2	0.153	0.180	− 0.287*	0.070	0.019	0.032
dOPU3	0.336*	0.154	− 0.451#	0.127	0.061	− 0.011
FF	0.118	0.323*	− 0.314*	0.300*	0.239	0.288*

No.RO: number of retrieved oocytes, *Gn.D* Gn dosage, *No.MII* number of MII oocytes, *No.2PN* number of 2PN fertilization, *No.HQE* number of high-quality embryos

*$p<0.05$, #$p<0.001$

Correlations of serum and FF AMH with SCF in different ovarian reserve patients

In our study, no significant correlation was found between serum AMH and SCF during GnRH-antagonist protocol in the PCOS and NOR groups ($P>0.05$), but there was a distinct negative effect of serum AMH in the DOR group after Gn startup ($r=-0.339, -0.392, -0.363, -0.407, -0.423$; $P<0.05$ or $P<0.001$) and FF AMH ($r=-0.323, -0.323, -0.323$; $P<0.05$) on SCF (Table 8).

Discussion

In addition to controlling follicular recruitment and maturation, AMH can inhibit steroid hormone production by restraining CYP17A1 transcription. A signaling cascade of AMH plays an essential role in the etiology of PCOS and DOR, and when AMH or AMHRII gene is abnormal, it may significantly reduce the signal activity of AMH cascade [18, 19].

Table 6 Correlations of AMH and SCF with COH outcomes in the NOR group

	AFC	No.RO	DGU	No.MII	No.2PN	No.HQE
AMH						
dGn1	0.261*	0.313*	− 0.297*	0.371#	0.468#	0.203
dGn4	0.342#	0.128	− 0.037	0.255*	0.442#	0.268*
dGnRH-A1	0.286*	0.102	− 0.018	0.218	0.402#	0.126
dhCG1	0.230	0.005	0.088	0.047	0.212	0.109
dhCG2	0.131	− 0.121	0.141	− 0.066	0.094	0.018
dOPU3	0.038	0.048	0.088	0.071	0.141	0.071
FF	0.238	0.047	0.038	0.037	0.095	− 0.100
SCF						
dGn1	0.127	0.038	− 0.157	0.089	0.242	0.104
dGn4	0.174	0.202	− 0.232	0.308*	0.298*	0.109
dGnRH-A1	0.194	0.234	− 0.294*	0.324*	0.315*	0.099
dhCG1	0.188	0.178	− 0.251	0.284*	0.294*	0.078
dhCG2	0.166	0.159	− 0.266*	0.258*	0.280*	0.104
dOPU3	0.114	0.130	− 0.299*	0.228	0.238	0.006
FF	− 0.050	− 0.022	− 0.123	0.032	− 0.002	− 0.139

No.RO number of retrieved oocytes, *Gn.D* Gn dosage, *No.MII* number of MII oocytes, *No.2PN* number of 2PN fertilization, *No.HQE* number of high-quality embryos

*$p < 0.05$, #$p < 0.001$

Table 7 Correlations of AMH and SCF with COH outcomes in the DOR group

	AFC	No.RO	DGU	No.MII	No.2PN	No.HQE
AMH						
dGn1	0.433#	0.530#	− 0.067	0.558#	0.448#	0.185
dGn4	0.436#	0.562#	− 0.134	0.565#	0.510#	0.239
dGnRH-A1	0.405#	0.556#	− 0.192	0.604#	0.565#	0.234
dhCG1	0.367*	0.554#	− 0.228	0.518#	0.532#	0.238
dhCG2	0.262	0.468#	− 0.334*	0.471#	0.401#	0.214
dOPU3	0.377*	0.484#	− 0.297	0.454#	0.368*	0.279
FF	0.359*	0.468#	− 0.215	0.500#	0.505#	0.419#
SCF						
dGn1	− 0.162	− 0.293	0.264	− 0.413#	− 0.378*	− 0.033
dGn4	− 0.230	− 0.285	0.243	0.366*	− 0.307*	− 0.055
dGnRH-A1	− 0.269	− 0.357*	0.241	− 0.401#	− 0.337*	− 0.049
dhCG1	− 0.326*	− 0.371*	0.227	− 0.318*	− 0.274	− 0.071
dhCG2	− 0.313*	− 0.408#	0.249	− 0.358*	− 0.323*	− 0.063
dOPU3	− 0.311*	− 0.411#	0.285	− 0.349*	− 0.323*	− 0.043
FF	− 0.107	− 0.150	0.334*	− 0.143	− 0.182	0.201

No.RO number of retrieved oocytes, *Gn.D* Gn dosage, *No.MII* number of MII oocytes, *No.2PN* number of 2PN fertilization, *No.HQE* number of high-quality embryos

*$p < 0.05$, #$p < 0.001$

PCOS was often accompanied with an aberrant increase in AMH, and it was completely opposite in DOR, which were also confirmed in the present study. The possible reasons were as follows: the abnormal LH/FSH ratio of PCOS and the increased activity of aromatase p450-17-α will lead to the increase of endogenous androgen, and then significantly heighten follicular recruitment and add the sensitivity of GCs to FSH, thus intensify AMH secretion [20].

Furthermore, the latest investigation demonstrated that insulin resistance will give rise to compensatory increase of endogenous insulin, and the incremental insulin can directly promote AMH expression, but in turn, the excessive AMH will inhibit the promoting effect of insulin on aromatase

Table 8 Correlations between AMH and SCF in different ovarian reserve patients

	PCOS group	NOR group	DOR group
dGn1	0.048	− 0.049	− 0.302
dGn4	0.016	0.166	− 0.339*
dGnRH-A1	− 0.042	0.160	− 0.392*
dhCG1	0.053	0.130	− 0.363*
dhCG2	0.010	0.064	− 0.407$^{\#}$
dOPU3	0.247	− 0.057	− 0.423$^{\#}$
FF	− 0.323*	− 0.255*	− 0.375*

*$p < 0.05$, $^{\#}p < 0.001$

expression in GCs, further bringing about the increased level of AMH [21]. The elevated level of AMH in PCOS patients reflects the opposite effect of reproductive stimulation and metabolic inhibition [22]. The decreased sensitivity of FSH to GCs caused by the high level of FSH, LH, androgen, and the sharply reduced size of primordial follicle pool may be the main reasons for the declined expression of AMH in DOR patients.

SCF/c-Kit system plays an important role in primordial follicular initiation and oocyte maturation and the abnormal expression of SCF/c-Kit will affect follicular recruitment, growth, and ovulation [10, 15]. Our results showed that the level of SCF was remarkably lower in the PCOS group than in the NOR and DOR groups. The low expression of SCF is closely related to follicular dysplasia in PCOS patients that may provide a new breakthrough for PCOS treatment.

Apart from that, we found that there was no obvious difference of SCF in DOR and NOR groups. For this phenomenon, we consider that although the number of AFC in DOR patients was markedly reduced, which would lead to SCF reducing, but DOR was often associated with early follicular recruitment and development, which was partly responsible for the raised level of SCF. Moreover, the increased positive regulation by high FSH and the decreased inhibition by low AMH might result in further increase of SCF. Therefore, the level of SCF in DOR patients was the same as in NOR patients.

Presently, there were few studies on the changes of AMH and SCF in different ovarian reserve patients during GnRH-antagonist protocol. Only in 2011, Saldrawings et al. [23] made a pioneering study of the dynamic changes of SCF in NOR patients undergoing GnRH-agonist long protocol. Li et al. [24] also only studied the change of AMH during COH in DOR patients. In our paper, the data showed that the level of serum AMH in different ovarian reserve and response patients during GnRH- antagonist protocol gradually decreased after Gn startup, and reached to the lowest on 3 days after oocyte retrieval, but SCF presented an opposite change.

What are the possible causes? First, the decreased AMH was strongly correlated with declined pre-antral follicles and small antral follicles during COH. Second, studies confirmed over physiological E_2 could significantly inhibit AMH and AMHRII expression in GCs, and there was a significant negative correlation between AMH and E_2, indicating that the declined AMH was related to the inhibitory effect of E_2 on AMH [25]. Besides, exogenous FSH can notably reduce AMH by inhibiting the activity of AMH promoter, manifesting that FSH itself has a negative effect on AMH [26, 27]. As a result of increased ovarian blood flow, the level of vascular endothelial growth factor (VEGF) radically elevated during COH that might increase AMH binding by inducing the expression of AMHR II on the surface of GCs, thus attenuate the level of AMH [28, 29]. Ulteriorly, some GCs were discharged and some were left to form corpus luteum after oocyte retrieval, then the level of AMH on 3 days after oocyte retrieval showed a further decline.

SCF made crucial functions in follicular recruitment and growth by increasing ovarian vascularization, stromal cells' proliferation, and reducing cells' apoptosis. The secretion of SCF was regulated by FSH [23], SCF gradually increased with exogenous FSH stimulation in the present study, demonstrating that SCF may have an important effect on human follicular development and selection. Previous studies have concluded that hypoxia inducible factor-1 alpha (HIF-1α) and its target gene products VEGF and erythmpoietin (EPO) can promote the secretion of SCF [30], indicating that the increased level of HIF-1α induced by hypoxia may be related to the increased level of SCF during COH. Beyond that, our results showed that although SCF in the PCOS group was significant lower, the increase rate of SCF on hCG administration day in the three groups was similar ($12.64 \pm 1.14\%$ vs. $11.66 \pm 0.81\%$ vs. $10.33 \pm 1.73\%$, $P > 0.05$), which may contribute to triggering.

On 3 days after oocyte retrieval, SCF reduced because of the disappearance of exogenous FSH. Moreover, the high level of E_2, P, and inhibin B (INHB) in late follicular stage had negative effects on endogenous FSH secretion [31, 32] that would lead to further decline of SCF. In addition, the decrease or damage of GCs caused by oocyte retrieval would deeply result in decreased SCF. On account of the limitations of clinical work, serum samples on the day of oocyte retrieval and after embryo transplantation were not collected in the present study, which need to be further investigated.

In our results, AMH during COH in different ovarian reserve patients were observably positive with AFC, indicating that AMH can sensitively reflect ovarian reserve. Vembu et al. [33] found that the cut-off value of basal serum AMH in predicting high response in PCOS group was 6.85 ng/ml, while that in non-PCOS group was 4.85 ng/ml. Individualized FSH administration scheme based on AMH level can significantly reduce

the occurrence of ovarian hyperstimulation syndrome (OHSS) [34, 35]. Delayed OHSS was strongly related to hCG administration [33], our results showed the daily Gn dosage for per retrieved oocyte was distinctly lower in the PCOS group than in the NOR and DOR groups (15.83 ± 1.43 vs. 19.43 ± 1.11 and 57.82 ± 6.95 IU, $P < 0.05$), and the serum AMH after hCG administration in the PCOS group was arresting negative with Gn dosage, suggesting that AMH might be an effective biological indicator to predict the risk of OHSS in PCOS patients, especially for delayed OHSS. Meanwhile, SCF in the PCOS group on the first and fourth days of Gn administration were negative with Gn dosage ($r = -0.362$, $P < 0.05$; $r = -0.344$, $P < 0.05$), which would be conducive to the early adjustment of Gn dosage and reducing the high risk of OHSS for PCOS patients during COH.

AMH and SCF secreted by GCs played an essential role in regulating follicular growth and the development of oocyte and GCs [2, 5, 36, 37]. Our study showed that there was no visible correlation of serum AMH and SCF with COH outcomes (number of retrieved oocytes, MII oocytes, and 2PN fertilization) in the PCOS group. That might because of the complex pathogenesis and unclear mechanisms of PCOS. At the same time, both AMH and SCF could exert biological effects through autocrine or paracrine pathways, and it was still not clear which pathway was dominant [2]. This needs to be confirmed by the relationship of AMH and SCF in GCs and FF from a single follicle with COH outcomes. In addition, Sneed et al. [38] found that increased BMI has a negative impact on clinical outcomes, but diminished with the growth of age. Although the age structure of the PCOS group in our study was the same as the NOR group, the BMI in the PCOS group was significantly increased, which may influence the evaluation value of AMH and SCF in PCOS patients' clinical outcomes. In addition, considering that AMH and SCF serve as reactivity indicators, their effects may be weakened in high responders, and on the contrary, amplified in some low responders. It was still meaningful to further expand samples for these problems.

In the NOR group, the level of AMH on the first and fourth days of Gn administration was observably positive with COH outcomes, and negative with Gn dosage, suggesting that AMH had a certain value in guiding the time and dosage of Gn startup for NOR patients. Simultaneously, SCF after Gn administration had a good advantage in predicting ovarian response and COH outcomes, which appeared a value in Gn dosage adjustment during COH. Follicular growth depended on the stimulation of exogenous Gn during COH, and the low responders were not completely DOR patients, partly because of the amount of exogenous Gn not reaching to the follicular development threshold. Similarly, high responders are not completely PCOS patients, partly due to overstimulation of exogenous Gn, so the combined detection of AMH and SCF was more advantaged for NOR patients during COH.

Taking 0.93 ng/ml as the boundary, the specificity of AMH in predicting POR was 90%, the sensitivity was 74.1%, the area under ROC curve (AUC) was 0.929, which was notably higher than 0.615 of FSH, and AMH was an effective biomarker for predicting POR during COH [39]. POR was often seen in DOR patients during COH. Our study demonstrated that serum and FF AMH in DOR patients were very effective biological indicators to evaluate ovarian response and COH outcomes, and SCF was also one of the good indicators. On account of some DOR patients might have low or undetectable AMH level, the evaluation of serum SCF during COH in these patients might be conducive to a better assessment of their COH outcomes, and thus solve the problem of whether the cycle would be canceled or continued.

Early research verified AMH can reduce SCF expression at cellular level by inhibiting the cAMP/PKA-signaling pathway in NOR patients [40]. In PCOS patients, AMH had a negative role in SCF secretion of GCs, but the specific mechanism was still unclear [41]. Studies on whether AMH involved in the regulation of SCF secretion in DOR patients were rarely reported. Our results indicated that serum AMH in DOR patients after Gn startup and FF AMH in different ovarian reserves was negative with SCF.

As the conclusion, serum AMH in different ovarian reserve patients during GnRH-antagonist protocol gradually decreased, while SCF did the opposite, suggesting that AMH and SCF participate in the pathophysiological processes of PCOS and DOR, and also have critical role in folliculogenesis and dominant follicle selection. Meanwhile, the combined detection of serum AMH and SCF is more beneficial to the adjustment of Gn time and dosage as well as the choice of triggering time, then to assist clinicians in determining whether to interrupt the cycle or proceed and obtain a better clinical outcome. In addition, AMH in serum of DOR patients after Gn administration and FF have a significant negative effect on SCF.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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