Value of serum and follicular fluid Sirt1,Sirt2 protein levels in predicting the outcome of assisted reproduction

Lingnv Yao
Zhejiang University School of Medicine First Affiliated Hospital
Wenqin Lin
Zhejiang University School of Medicine First Affiliated Hospital
Nan Jiang
Zhejiang University School of Medicine First Affiliated Hospital
Chuyan Li
Zhejiang University School of Medicine First Affiliated Hospital
Haifeng Cao
Zhejiang University School of Medicine First Affiliated Hospital
Hong Li
Zhejiang University School of Medicine First Affiliated Hospital
Jianhua Qian (qianjianhua@zju.edu.cn)
Zhejiang University School of Medicine First Affiliated Hospital https://orcid.org/0000-0003-1320-6860

Research

Keywords: SIRT1, SIRT2, AMH, fertility outcome, assisted reproduction

Posted Date: February 17th, 2020

DOI: https://doi.org/10.21203/rs.2.23749/v1

Abstract

Background: To explore whether serum and follicular fluid Sirt1, Sirt2 can reflect ovarian reserve and predict the outcome of assisted reproduction.

Methods: The study population comprised 125 patients , 39 in OPOI（occult form of premature ovarian insufficiency）group, 49 in advanced age group , and 37 in control group. The levels of serum Sirt1, Sirt2 were measured on the 2nd to 5th day of menstruation (bSirt1,bSirt2) and HCG day. Follicular fluid Sirt1 (FFSirt1) and Sirt2 (FFSirt2), were determined on OPU (oocyte pick up) day.

Results: The level of FFSirt2 in the advanced age group was significantly lower than those in other two groups. FFSirt 2 and Sirt 2 (HCG day) were negatively correlated with age (r=-0.35, r=-0.19), but there were no value of them for assessing DOR (diminished ovarian reserve). The level of bSirt2 in (cumulative) pregnant group was significantly higher (r=0.24, P=0.00).

Conclusions: This was the first study to show that FFSirt2 and Sirt2 (HCG day) might be negatively correlated with age and antral follicle count (AFC). bSirt2 could predict cumulative pregnancy outcome together with anti-Mullerian hormone (AMH), AFC and age.

Background

The assisted reproductive technology (ART) treated patients with diminished ovarian reserve (DOR), whether young or old, are often characterized by low ovarian response, high cycle cancellation rate, reduced number of oocytes, low clinical pregnancy rate and live birth rate, and high abortion rate (1). But some study revealed that DOR patients whose age were less than 37 showed no increase in the proportion of abnormal chromosome karyotypes (2). Thus exploring candidate biomarkers to predict women's fertility potential is helpful for women of childbearing age to make a good fertility plan, especially for DOR and advanced age patients.

At present, serum AMH and AFC (antral follicle count) were considered as the earliest and most accurate diagnostic indices of premature ovarian failure before the symptoms of ovarian reserve decline (3,4). However, the value of AMH and AFC in predicting oocyte and embryo quality is still uncertain (5). There are still no accepted indicators for predicting oocyte quality, embryo development potential and assisted reproductive outcomes.

Sirtuins family is a deacetylase. Its catalysis depends on NAD + and plays different roles in regulating metabolism, cell proliferation and genomic stability. It is related to aging and age-related diseases in mammals, including cancer, metabolic disorders and neurodegenerative diseases. Seven members of this family have been identified so far, named Sirt1-7 (6).

Sirt1 is considered to be the key protein responsible for delaying the aging process of animal models. Up-regulation of Sirt1 protein showed a better resistance to age-related phenotypes, while down-regulation of Sirt1 accelerated aging (7).Ovarian aging is characterized by a gradual decrease in the number of follicles and oocyte quality. Zhang J (8) found that the expression levels of Sirt1, Sirt3 and Sirt6 were significantly decreased in the ovaries of aged mice and mice receiving chemotherapy, and were positively correlated with the number of primary follicles. These results suggested that Sirt1, Sirt3 and Sirt6 are closely related to ovarian reserve.

Sirt2 participates in the mediation of mitosis, oxidative stress, microtubule movement, chromatin agglutination and cell movement (9). Liang Zhang (10) et al. suggested that the level of Sirt2 protein in oocyte of aged mice was significantly decreased, and the age-related meiotic defect could be improved after over expression of Sirt2 in oocyte of aged mice. Sirt2 may be related to the formation or assembly of spindle. Subsequently, Danhong Qiu et al (11) confirmed that the specific deletion of sirt2 could lead to the damage of the kinetochore-microtubule junction of mouse oocyte, thus destroying the maturation process of oocyte and the spindle/chromosome tissue.

The serum Sirt1 concentration had been demonstrated to decreases with age in healthy people (12). This decline was more obvious in patients with AD and MCI, suggesting that Sirt1 may be an early predictor of AD. As the Sirt1 and Sirt2 were proved to be correlated with aging process and age related disease, ovarian reserve was also believed to be correlated with aging process, the present study try to evaluate the clinical value of serum and follicular fluid Sirt1 and Sirt2 proteins in predicting the ovarian reserve and assisted reproductive outcomes in women of different ages and ovarian reserve.

Methods

Cases

The study population comprised 125 patients who underwent their first cycles of in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) treatment from March 2018 to December 2018 at the Reproductive Medicine Center of the First Affiliated Hospital of Zhejiang University School of Medicine. This study was approved by Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (Reference Number:2017-734) and written consent was obtained from all the patients. According to the following criteria, the patients were divided into control group(37 patients), young occult form of premature ovarian insufficiency (OPOI) group(39 patients)and advanced age group(49 patients).

Inclusion criteria:(1) Control group: 20≤ age ≤ 36 years old, normal menstrual cycle, normal basal hormone level (bFSH < 10 IU/L, bE2 < 50 pg/mL), AMH (>1.1 ng/mL), AFC (≥7);(2) OPOI group: 20 ≤ age ≤ 36 years old, bFSH ≥10 IU/L or normal, AMH ≤ 1.1 ng/mL and AFC <7;(3) advanced age group: age (≥37 years old).

Exclusion criteria: (1) PCOS(Polycystic Ovary Syndrome) patients; (2) endometriosis stage II or above, adenomyosis; (3) endocrine diseases such as hyperprolactinemia, thyroid diseases; (4) immune system diseases; (5) repeated implantation failure; (6) patients with uterine cavity adhesion, endometrial

polyps and other factors affecting the history of embryo implantation; (7) surgical history of genital malignant tumors; (8) uterine malformation; (9) contraindication of IVF.

Hormone measurements

The concentration of serum Sirt1, Sirt2(bSirt1,bSirt2), AMH, FSH, LH, and E2 were measured on the 2nd to 5th day of menstruation. Besides, the concentration of serum Sirt1, Sirt2 were measured again on HCG day and follicular fluid Sirt1 and Sirt2 were determined on OPU(oocyte pick up) day. The serum AMH ,Sirt1, Sirt2 and the follicular fluid Sirt1, Sirt2 were measured by enzyme-linked immunosorbent assay (ELISA). The ELISA test kits were from Shanghai Yuduo Company.

Clinical and laboratory data collection

The number of antral follicles with a diameter of 2-8 mm in bilateral ovaries was recorded by transvaginal ultrasonography in our center on the 2nd to 5th day of menstruation. Other data collected included infertility years, age, factors of infertility, body mass index (BMI) , number of eggs, fertilization rate, rate of high-quality embryos, blastocyst formation rate, cumulative clinical pregnancy rate, continued pregnancy and live birth rate etc.

Ovulation stimulation program

Ovulation promotion program was selected according to the individual ovarian function of patients including long-term luteal regimen, short-term regimen, micro-stimulation and natural cycle regimen. Vaginal ultrasound monitored follicular development. If there were more than two dominant follicles with diameter of 18 mm, combined with serum sex hormone level, Gn was discontinued. One HCG 4000-10000 IU or rhHCG (250ug/branch) was injected at night. Oocyte retrieval was performed 34-36 hours after injection.

Definition of Relevant Indicators

Criteria of high-quality embryos: blastomeres are basically uniform in size, regular in shape, homogeneous in cytoplasm or granular cells, with debris less than 20%. Clinical pregnancy: In addition to biochemical indicators, there are clinically visible evidence of pregnancy, including intrauterine pregnancy sac, ectopic pregnancy, abortion or curettage visible chorionic tissue. Cumulative Pregnancy Rate: That is, after transferring all the embryos in an egg retrieval cycle, the percentage of those pregnant women accounted for the total number of egg retrieval cycles in all patients.

Statistical analysis

SPSS 19.0 statistical software was used for data analysis. Quantitative data were expressed as a mean±standard deviation. Qualitative data were expressed as counts or percentages. Coparison of quantitative variables between two groups were performed using two-tailed Student's *t*-test. Comparison of qulitative variables was perfomed using chi-square test. Coparison of intergroup among the OPOI group, advanced age group and control group was perfomed using one-way ANOVA followed by the Tukey's or Bonferroni correction. To correlated the level of Sirt1 Sirt2 with other parameters such as age, AMH, AFC, number of eggs and so on, data were analysed with the Person correlation coefficient. Serum and follicular fluid Sirt1 and Sirt2, serum AMH, FSH, AFC and age were used to construct the Receiver Operator Characteristic Curve (ROC Curve) to evaluate the value in predicting cumulative fertility outcomes. p value < 0.05 was considered statistically significant.

Results

Analysis of the differences of laboratories and clinical related indicators among the three groups

The control group mainly used the luteal phase long-term regimen, which was statistically different from the other two groups. The OPOI group and the advanced age group mainly used short-term and micro-stimulation schemes, and there was no statistical difference between the two groups. In the infertility causes, although the proportion of male factor was higher in control group, there was no statistical difference among the three groups. The level of follicular fluid Sirt2 (FFsirt2) in the advanced age group was significantly lower than that in OPOI group and control group. There were no differences between the value of baseline serum Sirt1\Sirt2 and HCG Day serum Sirt1\Sirt2. The high quality embryo formation rate of OPOI group was significantly higher than that of other groups. However, the cumulative pregnancy rate of the control group was 73%, significantly higher than that of the advanced age group and OPOI group, while there was no significant difference between the advanced age group and OPOI group(Table 1).

Table 1

Clinical and laboratory related indicators and pregnant outcomes of OPOI group, advanced age group and normal control group

	OPOI group (n = 39)	Advanced age group (n = 49)	control group (n = 37)	P
FFsirt1	24.9 ± 5.4	23.6 ± 6.7	24.04 ± 5.1	0.58
FFsirt2	23.5 ± 5.4	20.3 ± 5.1#	24.1 ± 5.8	0.00
HCG Day Serum Sirt1	33.6 ± 5.7	35.2 ± 7.5	32.3 ± 7.3	0.15
HCG Day Serum Sirt2	31.4 ± 5.0	30.8 ± 4.8	32.8 ± 4.3	0.12
bsirt1	31.6 ± 6.1	34.4 ± 5.1	32.8 ± 5.0	0.08
bsirt2	31.5 ± 4.7	30.6 ± 5.5	32.1 ± 4.2	0.36
age	30.1 ± 3.8	40.8 ± 2.7#	27.8 ± 4.1	0.00
BMI(kg/m^2)	20.9 ± 2.3	23.7 ± 3.1#	21.2 ± 3.5	0.00
AFC	3.9 ± 2.3	4.9 ± 4.5	13.3 ± 4.5#	0.00
AMH	0.8 ± 0.6*	1.4 ± 1.3*	3.4 ± 1.8*	0.00
bFSH(IU/L)	12.4 ± 4.3*	10.3 ± 4.9	8.5 ± 1.6*	0.06
bE2(pg/ml)	55.4 ± 32.7*	48.8 ± 24.6	40.8 ± 14.6*	0.05
bLH(IU/L)	5.3 ± 2.6	4.6 ± 2.2	5.5 ± 1.9	0.63
FSH/LH	3.1 ± 2.6	2.6 ± 1.5	1.7 ± 0.7#	0.00
The type of infertility				
Primary infertility(%)	64.1(25/39)	10.2(5/49)	35.1(13/37)	0.00
Seconday infertility(%)	35.9(14/39)	89.8(44/49)	64.9(24/37)	0.00
methods of ovulation induction(%)				
long-term luteal regimen	5.1(2/39)	16.4(8/49)	97.3(36/37)	0.00[a] /0.17[b]
short-term regimen	51.3(20/39)	32.6(16/49)	2.7(1/37)	0.00[a] /0.08[b]
micro-stimulation	35.9(14/39)	49.0(24/49)	0(0/37)	0.00[a] /0.28[b]
natural cycle regimen	7.7(3/39)	2(1/49)	0(0/37)	0.13[a]
Infertility factor(%)				
Female factor	82.1(32/39)	71.4(35/49)	64.9(24/37)	0.23[a]
Male factor	5.1(2/39)	4.1(2/49)	16.2(6/37)	0.09[a]
Both factors	10.3(4/39)	22.4(11/49)	10.8(4/37)	0.19[a]
Unknown reason	2.6(1/39)	2.1(1/49)	8.1(3/37)	0.31[a]
Number of eggs retrieved	2.7 ± 2.0	3.7 ± 4.3	11.1 ± 6.0#	0.00
Number of mature eggs	2.3 ± 1.7	3.3 ± 4.0	9.5 ± 5.5#	0.00
Fertilization number	1.7 ± 1.6	2.4 ± 3.2	7.5 ± 5.0#	0.00
Number of high-quality embryos	1.3 ± 1.5	1.6 ± 2.7	4.5 ± 3.7#	0.00
Blastocyst culture number	0.3 ± 1.0	0.8 ± 2.3	3.6 ± 4.2#	0.00

Note:one-way ANOVA followed by the Tukey's or Bonferroni correction was perfomed to detect the differecnce among the 3 gourps repeatly. Statistically siginificant difference were marked in bold.

#: statistically significant difference in the intergroup comparision with the other two groups.

*: statistically significant difference in the intergroup comparision between the two groups.

[a] Chi-square test among three groups. [b] chi-square test between OPOI group and advanced age group

	OPOI group (n = 39)	Advanced age group (n = 49)	control group (n = 37)	P
Number of blastocyst formation	0.3 ± 0.9	0.4 ± 1.2	2.0 ± 2.1#	0.00
High quality blastocyst number	0.2 ± 0.9	0.2 ± 0.8	1.2 ± 1.3#	0.00
Transplanting high quality embryos	0.5 ± 0.5	0.41 ± 0.5	0.8 ± 0.4#	0.00
Normal fertilization rate	64.7%(68/105)	66.1(119/180)	67.5(278/412)	0.85
High quality embryo formation rate	77.9%(53/68)*	68.3(80/117)	61.4(167/272)*	0.03
No egg obtained cycle rate	5.1%(2/39)	13.8%(6/49)	0	0.06
Untransplanted cycle rate	23%(9/39)	28.5%(14/49)	21.6%(8/37)	0.75
Blastocyst formation rate	84.6%(11/13)	48.8(21/43)	57.6(76/132)	0.07
Clinical pregnancy rate(%)	46.6(14/30)	28.5(10/35)	51.7(15/29)	0.13
Biochemical pregnancy rate(%)	0(0/30)	2.8(1/35)	10.3(3/29)	0.12
implantation rate(%)	45(18/40)	21.7(10/46)	28.0(16/58)	0.07
Spontaneous abortion rate(%)	0	8.7(3/35)	0(0/29)	0.07
Ectopic pregnancy rate(%)	0(0/30)	0(0/35)	3.4(1/29)	0.32
Live Birth and Continued Pregnancy Rate(%)	46.6(14/30)	20(7/35)*	48.3(14/29)*	0.03
Cumulative pregnancy rate(%)	41.0(16/39)	30.6(15/49)	73.0(27/37)#	0.00
Note:one-way ANOVA followed by the Tukey's or Bonferroni correction was perfomed to detect the differecnce among the 3 gourps repeatly. Statistically siginificant difference were marked in bold.				
#: statistically significant difference in the intergroup comparision with the other two groups.				
*: statistically significant difference in the intergroup comparision between the two groups.				
[a] Chi-square test among three groups. [b] chi-square test between OPOI group and advanced age group				

Analysis of the differences of laboratories and clinical related indicators between pregnancy group and non-pregnancy group

According to the cumulative pregnancy outcome, all patients were divided into pregnancy group (56 cases) and non-pregnancy group (69 cases). The level of bsirt2, AMH, age, AFC, egg number, fertilization number, number of high-quality embryos, blastocyst culture number, blastocyst formation number and number of transplanted high-quality embryos in pregnant group were significantly higher than those in the non-pregnant group(Table 2).

Table 2
Comparison of clinical and laboratory indices and clinical outcomes between pregnant and non-pregnant groups

	Pregnancy group(n = 56)	Non-pregnant group(n = 69)	P
FFsirt1	24.4 ± 5.9	23.9 ± 5.6	0.687
FFsirt2	22.6 ± 5.4	22.3 ± 5.9	0.726
HCG day serum sirt1	33.8 ± 6.0	33.9 ± 7.7	0.948
HCG day serum sirt2	32.3 ± 5.3	30.6 ± 4.6	0.057
bsirt1	32.7 ± 5.3	33.3 ± 5.6	0.531
bsirt2	32.6 ± 4.6	30.2 ± 4.9	0.006
Age	32.4 ± 5.3	35.4 ± 6.9	0.008
BMI(kg/m^2)	21.5 ± 3.4	22.5 ± 3.1	0.097
AFC	8.8 ± 5.8	5.6 ± 5.0	0.001
AMH	2.4 ± 2.0	1.3 ± 1.2	0.000
bFSH(IU/L)	9.4 ± 2.9	11.3 ± 9.2	0.143
bE2(pg/ml)	43.8 ± 28.9	52.2 ± 23.7	0.075
bLH(IU/L)	4.7 ± 2.1	5.4 ± 6.1	0.438
FSH/LH	2.5 ± 2.4	2.4 ± 1.3	0.943
Number of eggs obtained	8.1 ± 6.7	3.5 ± 3.8	0.000
Mature eggs	7.0 ± 5.8	2.9 ± 3.1	0.000
Fertilization number	5.7 ± 5.0	2.1 ± 2.7	0.000
Number of high quality embryos	4.0 ± 3.5	1.0 ± 1.7	0.000
Blastocyst culture number	2.8 ± 3.9	0.4 ± 1.4	0.000
Number of blastocyst formation	1.6 ± 2.0	0.2 ± 0.9	0.000
High quality blastocyst number	1.0 ± 1.3	0.1 ± 0.6	0.000
Transplanting high quality embryos	0.7 ± 0.4	0.4 ± 0.5	0.000

Statistical significance was evaluated using Student T tes. Statistically significant differences($P < 0.05$) were marked in bold.

Table 3 Analysis of the correlation between serum and follicular fluid Sirt1 and Sirt2 protein levels and some laboratory and clinical related indicators

	FFsirt1		FFsirt2		HCG day serum sirt1		HCG day serum sirt2		bsirt1		bsirt2	
	r	p	r	p	r	p	r	p	r	p	r	p
Age	-0.78	0.38	-0.35	0.00	0.15	0.08	-0.19	0.03	0.13	0.14	-0.08	0.34
BMI (kg/m2)	-0.23	0.15	-0.1	0.54	-0.09	0.56	-0.12	0.54	-0.00	0.99	-0.03	0.84
AFC	0.01	0.84	0.20	0.02	-0.16	0.07	0.18	0.03	0.00	0.9	0.06	0.51
AMH	-0.12	0.16	0.04	0.63	-0.04	0.63	0.12	0.16	0.00	0.91	0.14	0.10
bFSH(IU/L)	-0.05	0.57	0.02	0.85	0.07	0.42	0.00	0.96	-0.03	0.71	-0.05	0.56
bE2(pg/ml)	0.172	0.05	0.03	0.70	-0.01	0.87	-0.18	0.04	-0.07	0.39	-0.03	0.67
bLH(IU/L)	-0.11	0.22	0.11	0.19	0.09	0.31	0.11	0.19	-0.00	0.92	-0.03	0.73
FSH/LH	0.03	0.67	-0.11	0.19	-0.02	0.79	-0.01	0.84	-0.03	0.7	0.06	0.45
Number of eggs obtained	-0.06	0.49	0.14	0.1	-0.11	0.21	0.22	0.01	-0.05	0.57	0.14	0.12

Person correlation coefficient was used. Statistically significant differences($P < 0.05$) were marked in bold.

	Pregnancy group(n = 56)				Non-pregnant group(n = 69)				P			
Mature eggs	-0.11	0.21	0.04	0.59	-0.11	0.19	0.22	0.01	-0.05	0.57	0.12	0.16
Fertilization number	-0.09	0.31	0.01	0.90	-0.13	0.13	0.22	0.01	-0.05	0.53	0.10	0.24
Number of high quality embryos	-0.07	0.45	0.02	0.81	− .012	0.17	0.17	0.05	-0.05	0.55	0.05	0.19
Blastocyst culture number	-0.15	0.07	0.00	0.96	-0.10	0.23	0.16	0.06	-0.03	0.72	0.14	0.10
Number of blastocyst formation	-0.12	0.16	-0.02	0.81	-0.18	0.04	0.21	0.01	-0.07	0.39	0.14	0.12
High quality blastocyst number	-0.09	0.30	0.04	0.59	-0.11	0.21	0.17	0.05	-0.02	0.75	0.17	0.05
Number of frozen embryos	-0.12	0.16	-0.11	0.88	-0.21	0.01	0.20	0.02	-0.10	0.23	0.05	0.56
Abortion number	-0.00	0.93	-0.08	0.35	0.19	0.03	0.05	0.55	-0.03	0.70	0.13	0.14
Cumulative pregnancy	0.03	0.68	0.03	0.72	-0.00	0.95	0.17	0.05	-0.05	0.53	0.24	0.00

Table 4. Comparison of sensitivity and specificity of related sensitive parameters in predicting cumulative pregnancy outcomes of the three groups of patents

Parameters	Area under ROC curve(AUC)	sensitivity	specificity	critical point	P Value
bsirt2	0.636	0.75	0.53	29.95	0.010
AMH	0.683	0.36	0.94	3.31	0.000
AFC	0.662	0.55	0.76	6.5	0.002
Age	0.632	0.85	0.45	38.5	0.012

Table 5. Comparison of sensitivity and specificity of related sensitive parameters in predicting cumulative pregnancy outcomes in three groups

Parameters	OPOI group(P > 0.05)				Advanced age group(p > 0.05)				Control group(p > 0.05)			
	AUC	sensitivity	specificity	critical point	AUC	sensitivity	specificity	critical point	AUC	sensitivity	specificity	critical point
bsirt2	0.611	0.875	0.391	28.05	0.592	0.733	0.515	29.98	0.687	0.88	0.5	28.21
AMH	0.639	0.813	0.565	0.57	0.627	0.333	0.970	3.35	0.602	0.6	0.833	3.63
AFC	0.530	0.125	0.957	6	0.618	0.867	0.424	2.5	0.577	0.6	0.833	15
Age	0.457	0.813	0.348	33.5	0.813	0.533	0.912	38.5	0.618	0.68	0.667	28.5

Person correlation coefficient was used. Statistically significant differences(P < 0.05) were marked in bold.

Correlation analysis between serum and follicular fluid Sirt1 and Sirt2 protein levels and some laboratory and clinical related indicators

Table 3 showed that FFsirt 2 and Sirt 2(HCG day) were negatively correlated with age (r=-0.35, r=-0.19), FFSirt2 was positively correlated with AFC (r = 0.2). Serum Sirt1 on HCG day was negatively correlated with blastocyst formation and frozen embryos (r=-0.18, r=-0.21), but positively correlated with abortion (r = 0.19); serum Sirt2 on HCG day was positively correlated with AFC,number of eggs obtained, number of mature eggs, number of fertilization, number of blastocyst formation, number of frozen embryos(r = 0.18, r = 0.22, r = 0.22, r = 0.22, r = 0.21, r = 0.20), and negatively correlated with basic E2 (r=-0.18). bSirt2 was positively correlated with cumulative pregnancy (r = 0.24, p = 0.00).

ROC curve analysis of serum and follicular fluid Sirt1 and Sirt2 protein levels in predicting DOR and cumulative pregnancy outcomes

The diagnostic criteria of DOR were AMH less than 1.1 ng/ml. Serum and follicular fluid Sirt1 and Sirt2 protein levels did not show statistically predictive meaning(P > 0.05)(Fig. 1). Figure 2 showed that the AUC values of bsirt2, AFC, AMH and age were significantly different from those under the opportunity reference line (P < 0.05) (Table 4).

Subsequently, ROC curves of basic serum Sirt2 protein, AMH, AFC and age-predicted cumulative pregnancy outcomes were analyzed in three groups (Table 5). Unfortunately, there was no statistical significance in the three groups.

Discussion

Currently, most of the indicators predicting fertility outcomes are those predicting ovarian reserve and response, such as AMH, AFC and age. However, their value are not uncertain. Take OPOI patients for example, the decrease of the number of antral follicles is the main problem of OPOI patients, if OPOI patients could obtain eggs, and the egg quality is good enough to form high-quality embryos, the patient could get good clinical pregnancy rate of IVF cycle (13). In our study ,we found that though the AMH and AFC in OPOI group was less than the other groups ,the high quality embryo formation rate was higher than advanced age group and control group, live birth and continued pregnancy rate was also not lower than control group. Besides, patients in the advanced age group might be superior to the OPOI group in ovarian reserve, the BMI and spontaneous abortion rate were higher than OPOI group and control group. As the age increases, the body's metabolic function declines, and older women are more likely to become overweight and obese than younger women. But the biggest BMI in advanced age group is 27.5 kg/m2, mean is 23.7 ± 3.1 kg/m2 .Overweight and obesity, defined by World Health Organization (WHO) as a BMI of 25–30 kg/m2 and ≥ 30 kg/m2 (WHO, 2004).There is no obesity patient in our group. The sudy of Antonio MacKenna etc showed that BMI does not influence the outcome of ART performed in women of Latin America (14). Also some study (15) showed that among women undergoing first FET with high-quality embryo transfer, obesity was associated with worse IVF outcomes, but not overweight. In our study, BMI also did not show statistical differences between the pregnant and non-pregnant groups. And for advanced age patients, the main reason of lower fertility and higher abortion rate might not be low ovarian reserve, but the quality of eggs decreases, such as non-segregation of meiotic chromosomes of eggs, chromosome arrangement and the abnormal composition of spindle matrix appear (16). The cumulative pregnancy rate in control group was higher. This might be related to the higher ovarian reserve in the control group, which can obtain more eggs and transplantable embryos.

Sirt1 and Sirt2 proteins. are important members of the SIRTUIN protein family of deacetylases, and the role of energy receptors plays an irreplaceable role in various fields,such as cell biology, metabolism, fate and so on .Previous studies have shown that under CR (Calorie restriction) conditions, an increase in endogenous sirt1 reduces the expression of p53 (instead of foxo3a), reduces follicular apoptosis or atresia, and maintains ovarian reserve (17).Sirt2 is involved in the mitotic structure, from the central body of the pre-mitotic stage to the central body during the mid-term spindle and cell division, ensuring normal cell division (18) .The study in 2017 (11) further found that sirt2 participates in the regulation of normal oocyte meiosis devices by deacetylating BubR1 and mediates the effects of advanced age on mouse oocyte quality. To explore the relationship between sirt1 and sirt2 and fertility ,we tested serum and follicle fluid Sirt1 and Sirt2 protein in our study. Among the three groups, only the level of FFSirt2 in advanced age group was significantly lower than other groups. The results suggested that FFsirt2 may be associated with age and AFC, which was confirmed in the subsequent correlation analysis. And there were no difference between baseline serum sirt1/sirt2 and HCG Day serum sirt1/sirt2. This may be related to the highly conserved state of the gene, so it is relatively stable in serum and is not susceptible to ovulation induction drugs.

In the comparison between the cumulative pregnancy group and the cumulative non-pregnant group, the bSirt2 in the pregnancy group was significantly higher..This result is consistent with the ROC curve analysis of predicting the cumulative pregnancy value. The cut-off value was 29.95 IU/l. This may suggest that the bSirt2 may be superior to FFsirt2 in predicting overall reproductive outcomes. Pregnancy is related to ovarian reserve, egg quality, uterine environment, and general condition. Serum SIRT2 levels may be more responsive to a patient's whole body state than FFsirt2. The serum and follicular fluid SIRT1 did not show any difference between two groups. Because the sample size of this study is small, further research is needed to verify this.

In the correlation analysis, we found the correlation between FFsirt2 and age and AFC, and also found that the serum level of Sirt1(HCG day) was negatively correlated with blastocyst formation but was positively correlated with the number of abortions. The serum levels of Sirt2(HCG Day)was positively correlated with AFC, the number of eggs, the number of matured eggs, the number of fertilization, the number of blastocyst formation and the number of frozen embryos but negatively correlated with age and basal estradiol levels($P < 0.05$). Besides bSirt2 was positively correlated with the cumulative number of pregnancies. SIRT1 promotes cell proliferation and also inhibits cell proliferation too (19). Xiong et al (20) showed that down-regulation of SIRT1 expression inhibited apoptosis of mouse granulosa cells. This may also partially explain the results of HCG day serum Sirt1 in our study. Sirt2 not only participates in oocyte meiosis, but also participates in a variety of inflammatory and oxidative pathways.Sirt2 interacts with NF-κB in oxidative stress (21), regulates intracellular ROS levels which is also closely related to the quality of eggs and embryos (22).Those may be the reasons why serum and follicular fluid Sirt2 have much more positive results in our study. But much more similar researches are still needed.

In the ROC curve analysis of predicting the ovarian reserve value, all the levels of Sirt1, Sirt2 did not show significant predictive value like AFC, which was inconsistent with the results of mice model. This may be due to the lower sensitivity of Sirt1 and Sirt2 in serum and follicular fluid. However, due to the lack of relevant research, it is currently unclear.

The main limitations of the study is that the sample size is not large enough, we only use one method of ELISA to detect the serum and follicular fluid concentration of Sirt1 and Sirt2. Since there was no such related research before, longitudinal studies with larger sample size, and more accurate detection methods were needed to clarify the correlation between serum and follicular fluids Sirt1 and Sirt2 and ovarian reserve, egg quality, embryo quality, and reproductive outcome in the future.

Conclusions

In this study, the correlation between serum and follicular fluid Sirt1 and Sirt2 and the assisted reproductive outcomes of patients of different ages and ovarian reserve was studied for the first time. The results suggested that FFsirt2 and HCG day serum Sirt2 may be a candidate biomarker to evaluate the ovarian reserve and bSirt2 may be the predictive parameter of the pregnancy outcome of ART. .Since there is no such related research at present, further

studies are needed to clarify the correlation between serum and follicular fluids Sirt1 and Sirt2 and ovarian reserve, egg quality, embryo quality, and reproductive outcome. We still need to expand the sample size, and use a variety of kits for testing, evaluation, and comparison, so that the clinical and scientific value of Sirt1 and Sirt2 are further discovered and confirmed.

Abbreviations

Follicular fluid Sirt1 (FFSirt1)

Follicular fluid Sirt2 (FFSirt2)

occult form of premature ovarian insufficiency (OPOI)

oocyte pick up (OPU)

diminished ovarian reserve (DOR)

antral follicle count (AFC)

anti-Mullerian hormone (AMH)

assisted reproductive technology (ART)

diminished ovarian reserve (DOR)

in vitro fertilization (IVF)

intracytoplasmic sperm injection (ICSI)

Polycystic Ovary Syndrome(PCOS)

enzyme-linked immunosorbent assay (ELISA)

body mass index (BMI)

World Health Organization (WHO)

Calorie restriction (CR)

Declarations

Ethics approval and consent to participate

This study was approved by Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (Reference Number:2017-734) and written consent was obtained from all the patients.

Consent for publication

All presentations of reports in the present study have consent for publication.

Availability of data and materials

All data and materials is available at the consent of corresponding author.

Competing interests

The Authors declare that there is no conflict of interest.

Funding

This work was supported by Medical and Health Science Project of Zhejiang Province, People's Republic of China (No.2017192063). This work was supported by Natural Science Foundation project of zhejiang province, China (No.LQ15H030001).

Authors' contributions

YAO L and LIN W collected and detected the samples, JIANG N and LI C analyzed and interpreted the data, CAO H helped the statistic analysis, LI H helped the detection of samples, QIAN J supervised the project.

Acknowledgements

Not applicable.

References

1.
Levi AJ, Raynault MF, Bergh PA, Drews MR, Miller BT, Scott RJ. Reproductive outcome in patients with diminished ovarian reserve. FERTIL STERIL. 2001;76:666–9.
2.
Thum MY, Abdalla HI, Taylor D. Relationship between women's age and basal follicle-stimulating hormone levels with aneuploidy risk in in vitro fertilization treatment. FERTIL STERIL. 2008;90:315–21.
3.
Baker VL. Primary ovarian insufficiency in the adolescent. Curr Opin Obstet Gynecol. 2013;25:375–81.
4.
Izhar R, Husain S, Tahir S, Husain S. Occult Form of Premature Ovarian Insufficiency in Women with Infertility and Oligomenorrhea as Assessed by Poor Ovarian Response Criteria. J Reprod Infertil. 2017;18:361–7.
5.
Yao L, Zhang W, Li H, Lin W. The role of serum AMH and FF AMH in predicting pregnancy outcome in the fresh cycle of IVF/ICSI: a meta-analysis. INT J CLIN EXP MED. 2015;8:1755–67.
6.
Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun. 2000;273:793–8.
7.
Grabowska W, Sikora E, Bielak-Zmijewska A. Sirtuins, a promising target in slowing down the ageing process. BIOGERONTOLOGY. 2017;18:447–76.
8.
Zhang J, Fang L, Lu Z, et al. Are sirtuins markers of ovarian aging? GENE. 2016;575:680–6.
9.
North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. MOL CELL. 2003;11:437–44.
10.
Zhang L, Hou X, Ma R, Moley K, Schedl T, Wang Q. Sirt2 functions in spindle organization and chromosome alignment in mouse oocyte meiosis. FASEB J. 2014;28:1435–45.
11.
Qiu D, Hou X, Han L, Li X, Ge J, Wang Q. Sirt2-BubR1 acetylation pathway mediates the effects of advanced maternal age on oocyte quality. AGING CELL 17, 2018.
12.
Kumar R, Chaterjee P, Sharma PK,, et al. Sirtuin1: a promising serum protein marker for early detection of Alzheimer's disease. PLOS ONE. 2013;8:e61560.
13.
Thum MY, Abdalla HI, Taylor D. Relationship between women's age and basal follicle-stimulating hormone levels with aneuploidy risk in in vitro fertilization treatment. FERTIL STERIL. 2008;90:315–21.
14.
MacKenna A, Schwarze JE, Crosby JA, Zegers-Hochschild F. Outcome of assisted reproductive technology in overweight and obese women. JBRA Assist Reprod. 2017;21:79–83.
15.
Zhang J, Liu H, Mao X, et al. Effect of body mass index on pregnancy outcomes in a freeze-all policy: an analysis of 22,043 first autologous frozen-thawed embryo transfer cycles in China. BMC MED. 2019;17:114.
16.
Qiao J, Wang ZB, Feng HL,, et al. The root of reduced fertility in aged women and possible therapentic options: current status and future perspects. MOL ASPECTS MED. 2014;38:54–85.
17.

Xiang Y, Xu J, Li L, et al. Calorie restriction increases primordial follicle reserve in mature female chemotherapy-treated rats. GENE. 2012;493:77–82.
18.
North BJ, Verdin E. Interphase nucleo-cytoplasmic shuttling and localization of SIRT2 during mitosis. PLOS ONE. 2007;2:e784.
19.
Morita Y, Wada-Hiraike O, Yano T, et al. Resveratrol promotes expression of SIRT1 and StAR in rat ovarian granulosa cells: an implicative role of SIRT1 in the ovary. Reprod Biol Endocrinol. 2012;10:14.
20.
Xiong F, Hu L, Zhang Y, Xiao X, Xiao J. miR-22 inhibits mouse ovarian granulosa cell apoptosis by targeting SIRT1. BIOL OPEN. 2016;5:367–71.
21.
Pais TF, Szego EM, Marques O, et al. The NAD-dependent deacetylase sirtuin 2 is a suppressor of microglial activation and brain inflammation. EMBO J. 2013;32:2603–16.
22.
Keefe DL, Marquard K, Liu L. The telomere theory of reproductive senescence in women. Curr Opin Obstet Gynecol. 2006;18:280–5.

Figures

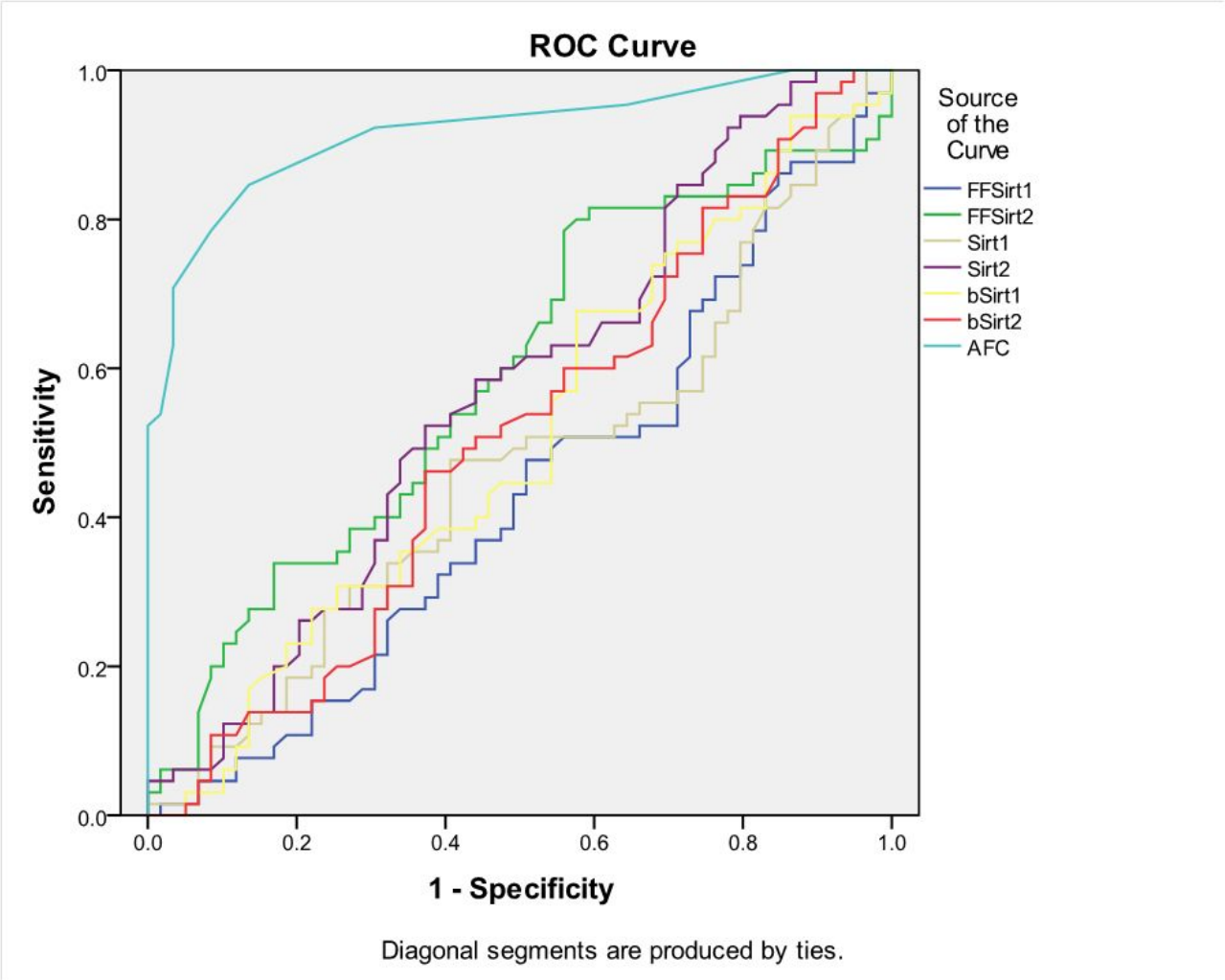

Figure 1

ROC curve in predicting ovarian reserve. ROC curve in predicting ovarian reserve. The diagnostic criteria of DOR were AMH less than 1.1ng/ml. All the biomarkers except AFC have no value in predicting DOR.

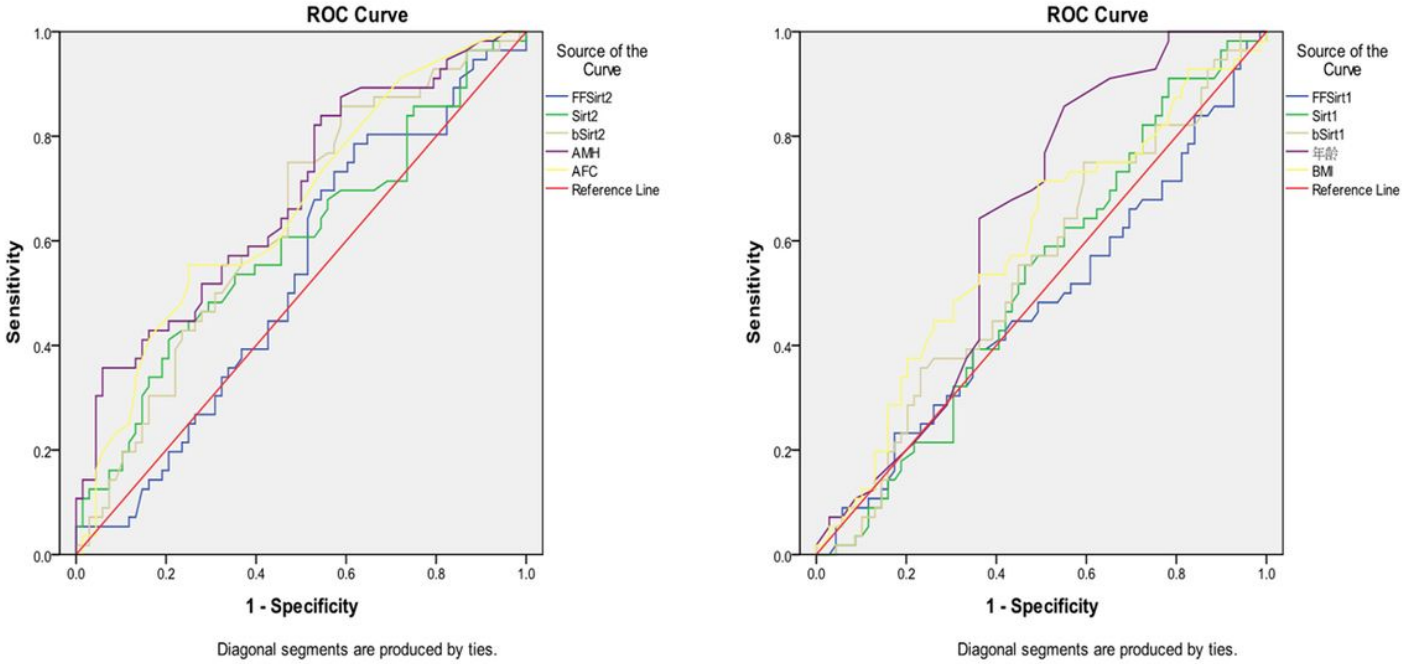

Figure 2

ROC curve in predicting pregnancy. AUC values of bsirt2, AFC, AMH and age were significantly different from those under the opportunity reference line.