Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2019, Vol. 47(2) 772–782

DOI: 10.1177/0300060518811228
journals.sagepub.com/home/imr
SAGE

Serum and follicular fluid levels of sirtuin 1, sirtuin 6, and resveratrol in women undergoing *in vitro* fertilization: an observational, clinical study

József Bódis[1], Endre Sulyok[2], Tamás Kőszegi[3], Krisztina Gödöny[1,4], Viktória Prémusz[1,2] and Ákos Várnagy[4]

Abstract

Objective: This observational, clinical study was designed to assess the role of sirtuin 1 (SIRT1), sirtuin 6 (SIRT6), and resveratrol in *in vitro* fertilization (IVF).

Methods: Paired serum and follicular fluid (FF) samples were obtained from 30 consecutive patients (age: 36.43 ± 4.17 years, body mass index: 22.90 ± 2.05 kg/m^2, duration of infertility: 5.10 ± 2.80 years) who received IVF treatment. SIRT1, SIRT6, and resveratrol levels were measured by enzyme-linked immunosorbent assay.

Results: Ovarian hyperstimulation resulted in significantly higher serum SIRT1 levels in pregnant women (8 patients) compared with non-pregnant women (22 patients). SIRT6 levels remained unchanged after ovarian hyperstimulation, but were significantly lower in pregnant women compared with non-pregnant women before and after hyperstimulation. Both SIRTs were detected in FF, but they appeared to be independent of their serum levels. After correction for confounders, FF SIRT6 levels were positively related to mature oocytes ($F = 6.609$), whereas serum SIRT1 and SIRT6 levels were related to clinical pregnancy ($F = 10.008$, $F = 5.268$, respectively).

Conclusions: Our study shows that SIRT1 and SIRT6, but not resveratrol, are involved in human reproduction and they may have a role in oocyte maturation and clinical pregnancy in IVF.

[1]MTA-PTE Human Reproduction Scientific Research Group, University of Pécs, Pécs, Hungary
[2]Doctoral School of Health Sciences, Faculty of Health Sciences, University of Pécs, Pécs, Hungary
[3]Department of Laboratory Medicine, Medical School, University of Pécs, Pécs, Hungary
[4]Department of Obstetrics and Gynaecology, Medical School, University of Pécs, Pécs, Hungary

Corresponding author:
Viktória Prémusz, Vörösmarty u. 4. H-7621, University of Pécs, Pécs, Hungary.
Email: premusz.viktoria@pte.hu

Keywords
In vitro fertilization, sirtuin, resveratrol, outcome measures, biomarker, ovarian hyperstimulation

Date received: 30 August 2018; accepted: 16 October 2018

Introduction

Sirtuins (SIRTs) are a highly conserved protein family of NAD-dependent histone deacetylases that confer protection against ageing and age-related disorders. In mammals, seven members of the SIRT family have been identified (SIRT1–SIRT7) with different subcellular localizations, tissue specificity, activity, and functions.[1–3] SIRT1, SIRT6, and SIRT7 are localized in the nucleus, SIRT2 is localized at the cytoplasm, and SIRT3, SIRT4, and SIRT5 are expressed in the mitochondria.[4,5] Generally, SIRTs regulate cellular metabolism, the redox state, stress signalling and the cell cycle, cell survival, and genome stability.[6–8]

A large body of evidence has shown that SIRTs are involved in regulating female reproductive function.[9] With advancing female age, there is a gradual decline in the size of the ovarian follicle pool, oocyte yield, and oocyte quality. This process is termed ovarian ageing. As a result, IVF outcomes deteriorate from 42.6% to 3.6% of live births when maternal age progresses from < 35 years to > 42 years.[10]

Studies that applied mouse models of ovarian ageing have clearly shown that SIRT1, SIRT3, and SIRT6 are closely related to ovarian reserve and can be regarded as markers of ovarian ageing.[11] Importantly, expression of SIRTs has been documented in mammalian ovaries, granulosa cells, oocytes, and embryos.[12–16] Furthermore, the SIRT1-deficient mouse has ovarian dysfunction and compromised oocyte developmental potential.[17] Furthermore, SIRT3 depletion resulted in downregulation of steroidogenic enzymes and caused decreased secretion of progesterone in human granulosa cells.[18]

In recent years, important studies have shown that resveratrol (3, 4, 5–trihydroxy-trans-stilbene) protects against age-associated infertility[19] and improves oocyte maturation and subsequent development in various mammals.[19–23] This polyphenolic compound has anti-ageing, anti-oxidant, anti-inflammatory, and chemopreventive properties. The beneficial effects of resveratrol on female reproduction are thought to be achieved mainly by inducing SIRT1 expression and by promoting telomerase activity with subsequent attenuation of ageing-associated telomere shortening.[20–22,24]

The present study aimed to investigate involvement of nuclear SIRT1, SIRT6, and resveratrol in the process of IVF in women receiving this treatment. We examined the response pattern of these biologically active compounds with ovarian hyperstimulation and assessed the relationship between serum and follicular fluid (FF) levels of these compounds. Furthermore, we attempted to establish the effects of SIRT1, SIRT6, and resveratrol individually or in combination on reproductive performance as estimated by the number of mature oocytes and embryos, chemical and clinical pregnancy, and live-born infants.

Methods

Patients

We carried out an observational, clinical study between 1 September 2016 and 1 October 2016 in the Assisted Reproduction Unit, Department of

Obstetrics and Gynaecology, University of Pécs, Hungary. During this time, we initiated 30 randomly chosen IVF cycles and performed transvaginal ultrasound-guided aspiration of FF.

The patients were consecutively selected. No patients suffered from any metabolic or vascular diseases, such as obesity, diabetes mellitus, metabolic syndrome, fatty liver diseases, or atherosclerosis, during the study period. The patients had the following main infertility diagnoses: male factors (13, 43.33%), damaged or blocked fallopian tubes (5, 16.67%), severe endometriosis (5, 16.67%), other female factors (6, 20.00%), and unexplained infertility (1, 3.33%).

We performed superovulation treatment after completing some basic examinations. These examinations included a cervical smear, serum hormone measurements (follicle-stimulating hormone [FSH], luteinizing hormone [LH], prolactin, oestradiol, progesterone, testosterone, thyroid-stimulating hormone) on the 3rd and 21st days of the unstimulated cycles, human immune deficiency virus and hepatitis-B surface antigen screening, hysteroscopy or hysterosalpingo-contrast sonography, and an andrological examination. Two independent gynaecologists approved participation of the patients in the IVF procedure.

Controlled ovarian hyperstimulation

The gonadotropin-releasing hormone agonist triptorelin (Gonapeptyl; Ferring, Kiel, Germany) was used in a long or short protocol. Stimulation was performed with individual dosages of recombinant FSH (Gonal-F; Serono, Aubonne, Switzerland), which varied from 150 to 250 IU per day depending on follicular maturation. The starting dose was adapted according to the body mass index (BMI) and age. For patients with a previously known low response, recombinant FSH was increased to a maximum dose of 300 IU daily. Follicular maturation was determined by ultrasound examination from the 6th day of the cycle every other day. We changed the amount of administered gonadotropins individually according to the size of the follicles. Final oocyte maturation was induced by injection of 250 μg of human chorionic gonadotropin (hCG) (Ovitrelle; Serono) when at least two follicles exceeded 17 mm in diameter. Aspiration of FF was performed 36 hours later by ultrasonography-guided transvaginal puncture under routine intravenous sedation. FF aspirates were only obtained from mature follicles.

Collection of blood serum and FF

Blood samples were obtained from the patients before the stimulated cycle on the 21st day of their menstrual cycle and in the morning of follicular puncture before sedation. Oocyte and FF collection was performed using the Sonoace 6000C two-dimensional real-time ultrasound scanner equipped with a 4- to 8-MHz endovaginal transducer in G-MOPS^{TM} medium (Vitrolife, Göteborg, Sweden). The fluid was centrifuged for 10 minutes at $252 \times g$ and the supernatants were frozen and stored at $-80°C$ for future analysis.

Fertilization procedures

The fertilization process was carried out with intracytoplasmic sperm injection (ICSI) depending on the andrological status (sperm count <15 M/mL), maternal age (>35 years), and the number of previous IVF cycles the patients had undergone previously (>2).

The oocytes that were selected for ICSI and denuded with hyaluronidase were checked for maturity. Only metaphase II oocytes that showed the presence of the first polar body were selected for fertilization. ICSI was performed 3 to 6 hours later in G-MOPS^{TM} medium (Vitrolife). The

other oocytes were fertilized with the conventional IVF method in a bicarbonate-buffered medium (G-IVF^{TM}, Vitrolife). Fertilization was checked in G-Iv5^{TM} medium (Vitrolife) 24 hours later.

Embryo transfer was carried out 3 to 5 days after follicular puncture. G-21Mv5 medium (Vitrolife) was used from day 3 to the blastocyst stage. Only the best quality embryos were transferred (according to the consensus embryo scoring system of the European Society of Human Reproduction and Embryology). Upon the patient's request, one, two, or three embryos were transferred. The remaining embryos were frozen in compliance with Hungarian law. A total of 300 mg of progesterone was prescribed to support the luteal phase (Utrogestan; Lab. Besins International S. A., Paris, France). Evaluation of treatment was performed by a transvaginal ultrasound examination to detect a gestational sac 21 days after embryo transfer.

Laboratory measurements

Laboratory measurements were performed by using commercially available enzyme-linked immunosorbent assay (ELISA) kits. SIRT1, SIRT6, and resveratrol kits were produced by Cloud-Clone Corporation (Houston, TX, USA) The intra- and inter-assay coefficients of variation were $<10\%$ and $<12\%$, respectively, for each kit. The detection limits for SIRT1, SIRT6, and resveratrol levels were 0.27, 0.115, and 246.9 ng/mL, respectively.

Ethics approval and consent to participate

The study was reviewed and approved by the Human Reproduction Committee of the Hungarian Medical Research Council (5273-2/2012/EHR). Informed consent was signed by the patients who participated in the study. The investigation conformed to the principles outlined in the Declaration of Helsinki.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). Normality of data distribution was tested by the Kolmogorov–Smirnov test. Depending on distribution of data, the Student's paired or independent t-test was used to compare continuous variables. The association between two continuous variables was tested by using Spearman's or Pearson's correlation coefficients. Analysis of covariance was used to examine the effect of SIRT1, SIRT6, and resveratrol levels on outcome measures in patients with IVF. The Bonferroni test was used for multiple comparisons. Data are expressed as mean $\pm$ standard deviation. A value of $p < 0.05$ was considered statistically significant.

Results

The clinical parameters of the patients are shown in Table 1. A total of 30 patients were included in the study. In response to ovarian hyperstimulation, serum SIRT1 levels tended to increase ($p = 0.378$) and SIRT6 levels remained unchanged, but resveratrol levels were significantly increased ($p = 0.009$) compared with those before ovarian hyperstimulation. FF levels of SIRT1 and resveratrol were markedly lower than those in serum (both $p < 0.001$; Table 2), while FF SIRT6 levels were similar to serum levels.

Patients who underwent successful IVF treatment and progressed to clinical pregnancy and delivery at term (8 patients, pregnant group) were compared with those who failed to become pregnant (22 patients, non-pregnant group) (Table 2). A similar response pattern was observed after

Table 1. Clinical characteristics of the patients.

Characteristics	n = 30
Age, years	36.43 ± 4.17
Nulligravid, n (%)	24 (80.0)
Nulliparous, n (%)	22 (73.3)
Duration of infertility, years	5.1 ± 2.8
Body mass index, kg/m^2	22.9± 2.05
Cause of infertility, n (%)	
Poor semen quality	13 (43.33)
Tubal	5 (16.67)
Endometriosis	5 (16.67)
Other female causes	6 (20.00)
Unexplained	1 (3.33)
No. of stimulation procedures initiated previously	
Cycle 0	13
Cycle 1	9
Cycle 2	4
Cycle 3	4
Serum oestradiol, pmol/L	1645.3 ± 2046.3
Progesterone, nmol/L	24.5 ± 16.3
Total dose of gonadotropin, IU	1505.3 ± 605.1
Duration of stimulation, days	11.8 ± 2.9
No. of mature oocytes (metaphasis II)	5.03 ± 4.78
No. of Grade 1 embryos	2.93 ± 2.23
No. of transferred embryos (fresh only)	1.69 ± 0.47
hCG on day 12, IU	248.4 ± 485.3
No. of chemical pregnancies, n (%)	8 (26.6)
No. of clinical pregnancies, n (%)	8 (26.6)

Values are n (%) or mean ± standard deviation. hCG = human chorionic gonadotropin.

Table 2. Serum and follicular fluid levels of sirtuin 1, sirtuin 6, and resveratrol during IVF.

	Sirtuin 1 (ng/mL)			Sirtuin 6 (ng/mL)			Resveratrol (ng/mL)		
	1	2	3	1	2	3	1	2	3
All patients (n = 30)									
Mean	4.66	5.65	**1.02**°°	0.27	0.28	0.33	2036.2	**2380.8**°	**1080.1**°°
SD	3.80	3.20	**0.57**	0.19	0.18	0.17	543.1	**941.2**	**398.9**
Non-pregnant group (n = 22)									
Mean	4.07	4.72	**0.96**°°	0.31	0.31	0.29	2066.5	2422.0	**1184.9**°
SD	2.71	2.69	**0.44**	0.19	0.19	0.17	491.8	889.1	**354.8**
Pregnant group (n = 8)									
Mean	6.04	**7.93***	**1.31**°°	**0.14***	**0.19***	0.45	1956.7	2272.4	**791.7***°
SD	5.64	**3.36**	**1.07**	**0.07**	**0.09**	0.06	691.9	1125.3	**389.9**

Numbers 1, 2, and 3 represent serum samples before (1) and after ovarian hyperstimulation (2), and follicular fluid samples (3).

*$p < 0.05$, compared with the non-pregnant group; °significant intra-group difference between values before and after ovarian hyperstimulation: °$p < 0.05$, °°$p < 0.01$.

IVF = *in vitro* fertilization, SD = standard deviation.

hyperstimulation in serum and FF levels of SIRT1 and resveratrol, but no difference was detected in SIRT6 levels in either group. Pregnant patients had significantly higher post-hyperstimulation SIRT1 levels compared with those in non-pregnant patients ($p = 0.039$). In pregnant patients, pre-hyperstimulation serum and FF SIRT1 levels appeared to be higher than those in non-pregnant patients, but this did not reach significance. In contrast to SIRT1, serum SIRT6 levels were significantly lower in pregnant women (before and after hyperstimulation, $p = 0.002$ and $p = 0.049$, respectively) compared with non-pregnant women. FF resveratrol levels were also significantly lower in pregnant women compared with non-pregnant women ($p = 0.014$), but serum resveratrol levels were similar in the two groups.

To examine the possible contribution of circulating SIRTs and resveratrol to their respective FF levels, we examined the relationship between the corresponding parameters that were measured simultaneously in serum and FF. A significant positive correlation was found between post-hyperstimulation serum and FF resveratrol levels ($r = 0.555$, $p = 0.002$). However, we failed to demonstrate such an association for SIRT1 and for SIRT6 levels. This finding suggested that local ovarian production of these SIRTs predominates over their serum-derived fraction.

Maternal age negatively affected serum SIRT1 and resveratrol levels (SIRT1 after hyperstimulation: $r = -0.427$, $p = 0.029$; resveratrol before hyperstimulation: $r = 0.400$, $p = 0.032$, Table 3). A further negative association was found between FSH dosage and serum SIRT1 levels ($r = -0.379$ and -0.488 before and after hyperstimulation, both $p < 0.05$). However, serum SIRT1 levels were

Table 3. Correlations of clinical and laboratory parameters with serum and follicular fluid levels of sirtuins and resveratrol during IVF ($n = 30$).

	Sirtuin 1 (ng/mL)			Sirtuin 6 (ng/mL)			Resveratrol (ng/mL)		
	1	2	3	1	2	3	1	2	3
Age (years)									
r	−0.195	**−0.427***	0.107	−0.147	−0.194	0.019	**−0.400***	−0.123	0.142
p	0.310	**0.029**	0.610	0.448	0.313	0.925	**0.032**	0.526	0.453
BMI (kg/m^2)									
r	**−0.479***	0.240	0.170	−0.085	−0.163	0.161	−0.074	0.043	0.061
p	**0.013**	0.258	0.428	0.679	0.427	0.451	0.704	0.829	0.752
No. of cycles									
r	−0.007	0.038	**0.472***	−0.296	−0.179	0.003	−0.241	0.086	0.024
p	0.972	0.853	**0.017**	0.118	0.354	0.989	0.209	0.659	0.898
E2 (pmol/L)									
r	**0.497****	0.022	−0.090	−0.081	−0.046	0.055	0.333	0.300	0.124
p	**0.007**	0.916	0.668	0.683	0.818	0.786	0.078	0.121	0.521
FSH dosage (U/L)									
r	**−0.379***	**−0.488***	−0.120	−0.020	−0.192	0.238	−0.171	−0.026	−0.327
p	**0.046**	**0.013**	0.569	0.920	0.327	0.233	0.376	0.894	0.084

IVF = *in vitro* fertilization, BMI = body mass index, E2 = oestradiol, FSH = follicle-stimulating hormone.
Numbers 1, 2, and 3 represent serum samples before (1) and after ovarian hyperstimulation (2), and follicular fluid samples (3).
r = correlation coefficient; *$p < 0.05$, **$p < 0.01$.

positively related to oestradiol before hyperstimulation ($r = 0.497$, $p = 0.007$).

The effect of SIRT1, SIRT6, and resveratrol, as well as some selected clinical and laboratory parameters, on outcome measures in our patients with IVF were also investigated. The number of oocytes, mature oocytes, and embryos, as well as serum hCG levels on day 12 and clinical pregnancy, were used as indices of outcome (Table 4). Univariate linear regression showed that none of these outcome measures, except for hCG levels on day 12 ($r = 0.436$, $p = 0.042$), were affected by SIRTs or by resveratrol. Serum oestradiol positively affected the number of oocytes ($r = 0.604$, $p = 0.001$), mature oocytes ($r = 0.538$, $p = 0.003$), and embryos ($r = 0.435$, $p = 0.018$). Maternal age had a negative effect on all of these parameters (number of oocytes: $r = -0.375$, $p = 0.054$; mature oocytes: $r = -0.444$, $p = 0.020$; and embryos: $r = -0.457$, $p = 0.016$). Analysis of covariance was applied to examine the effect of different markers on clinical pregnancy using maternal age as a covariate. We found that serum SIRT1 levels ($F = 10.008$, $p = 0.005$) and serum SIRT6 levels ($F = 5.268$, $p = 0.031$) had a significant effect on the occurrence of clinical pregnancy. Moreover, FF SIRT6 levels were significantly higher in patients with a mature oocyte number of ≥ 10 than in those of ≤ 9 (0.515 ± 0.193 ng/mL versus 0.288 ± 0.176 ng/mL; $F = 6.609$, $p = 0.016$).

Discussion

The present study showed that in women who underwent IVF, ovarian hyperstimulation resulted in an elevation in serum SIRT1 levels in patients who progressed to clinical pregnancy. In contrast, SIRT6 levels remained unchanged after ovarian hyperstimulation and they were markedly reduced in pregnant women compared with non-pregnant women. Both SIRTs were detected in FF, but they appeared to be independent of their serum levels, which suggested that FF SIRT1 and SIRT6 levels were mostly derived from local ovarian production. With measurement of resveratrol in serum and FF samples, we could not provide evidence for resveratrol stimulation of SIRT1 and SIRT6. Univariate linear regression showed that the outcome measures of IVF were independent of SIRTs. However, when corrections were made for confounders, serum SIRT1 and SIRT6 levels were positively related to clinical pregnancy and FF SIRT6 levels were related to the number of mature oocytes.

With regard to function in female reproduction, nuclear SIRT1 and SIRT6 and mitochondrial SIRT3 are the most extensively studied SIRTs. SIRT1 protects cells against oxidative stress, regulates glucose/lipid metabolism, promotes DNA stability, and extends the lifespan.[25,26] SIRT1 regulates the metabolic response to caloric restriction, which is the main factor in slowing down ageing and in reducing age-related disorders.[27] SIRT1 may mediate diverse cellular responses because its substrates can be histone-modifying enzymes, transcription factors, regulators of cell cycle progression/survival under stress, cell signalling components, DNA repair modulators, and regulators of metabolism.[28] SIRT1 has also been identified as a positive regulator of telomere length because it attenuates telomere shortening associated with ageing.[29] Importantly, post-translational modification of SIRT1 by different kinases may exert either stimulatory or inhibitory effects on its activity.[30]

Involvement of SIRT1 in female reproductive function has also been established. In addition to various mammalian species[11–15] SIRT1 mRNA abundance has been detected in human granulosa cells, and its regulatory role in progesterone secretion, aromatase expression, and

Table 4. Effect of sirtuins and resveratrol, as well as some selected clinical parameters, on outcome measures in patients with IVF (n = 30).

			Oocyte number	Mature oocyte number	Embryo number	hCG levels (IU) on day 12
Hormonal parameters						
Sirtuin 1 (ng/mL)	1	r	0.309	0.221	0.218	0.228
		p	0.125	0.279	0.285	0.283
	2	r	0.130	0.125	0.045	0.376
		p	0.545	0.560	0.833	0.093
	3	r	−0.029	−0.101	−0.079	0.136
		p	0.892	0.638	0.713	0.557
Sirtuin 6 (ng/mL)	1	r	0.064	0.087	0.184	−0.275
		p	0.757	0.673	0.369	0.204
	2	r	−0.234	−0.097	−0.090	0.154
		p	0.250	0.639	0.663	0.484
	3	r	−0.214	−0.042	−0.172	**0.436***
		p	0.315	0.844	0.421	**0.042**
Resveratrol (ng/mL)	1	r	0.246	0.182	0.130	−0.137
		p	0.199	0.346	0.502	0.504
	2	r	0.113	0.107	0.050	−0.209
		p	0.568	0.587	0.802	0.316
	3	r	−0.025	−0.070	−0.110	−0.385
		p	0.897	0.718	0.571	0.052
Clinical parameters						
Age (years)		r	−0.375	**−0.444***	**−0.457***	−0.274
		p	0.054	**0.020**	**0.016**	0.195
BMI (kg/m^2)		r	0.018	0.076	0.157	−0.199
		p	0.927	0.696	0.417	0.331
No of cycles		r	0.006	-0.051	−0.061	−0.256
		p	0.976	0.794	0.751	0.206
E2 (pmol/L)		r	**0.604****	**0.538****	**0.435***	0.008
		p	**0.001**	**0.003**	**0.018**	0.968
FSH dosage		r	−0.158	−0.128	−0.076	0.148
		p	0.412	0.509	0.695	0.472
hCG levels (IU) on day 12		r	−0.123	0.011	−0.081	
		p	0.550	0.956	0.695	

IVF = *in vitro* fertilization, BMI = body mass index, E2 =oestradiol, FSH = follicle-stimulating hormone, hCG = human chorionic gonadotropin.
Numbers 1, 2, and 3 represent serum samples before (1) and after ovarian hyperstimulation (2), and follicular fluid samples (3).
r = correlation coefficient, *$p < 0.05$; **$p < 0.01$.

oestrogen synthesis has been demonstrated.[28,31] Most recently, Zhao et al.[32] provided evidence that all seven members of the SIRT family were present in human germinal vesicles and in mature oocytes *in vivo* or *in vitro*. However, the relative abundance of SIRTs in *in vivo* mature oocytes exceeded that of mature oocytes *in vitro*. Our study also provided evidence for local ovarian production of SIRT1 and it showed an association with clinical pregnancy.

Importantly, an association has been reported between telomere length in the cumulus–oocyte complex and oocyte/embryo quality in women undergoing IVF.[33] There is also convincing evidence that SIRT1 and SA1/SA2 cohesion proteins modulate telomere homeostasis in cumulus cells.[34] SIRT1 expression correlates with cohesion proteins, telomere length, response patterns to ovarian stimulation (oocyte number >6 versus <4), maternal age (>38 versus <34 years), and BMI (>25 versus <25 kg/m^2).[34]

SIRT6 has multiple enzymatic activities and regulates DNS repair and telomere maintenance when challenged by oxidative stress. Furthermore, SIRT6 functions as a nutrient sensor and controls glucose and lipid metabolism.[35] In mouse oocytes, SIRT6 depletion resulted in inadequate histone deacetylation, chromosome missegregation, and aneuploidy with subsequent defects in embryonic development.[36,37] In humans, SIRT6 was also found to be expressed in germinal vesicles, and SIRT6 was upregulated during maturation in *in vivo* and *in vitro* mature oocytes.[32]

The present study showed SIRT6 levels in the maternal circulation and FF, which suggested a role in oocyte maturation and in early embryonic development. Unexpectedly, serum levels of SIRT6 were markedly reduced in women who progressed to clinical pregnancy compared with those of non-pregnant women. However, FF SIRT6 levels were positively related to the mature oocyte number and serum SIRT6 levels had a positive effect on successful pregnancy when adjustments were made for relevant clinical and biochemical confounders.

On the basis of compelling evidence on the fertility-sparing effects of resveratrol in mammalian females, we anticipated a positive association of serum and/or FF resveratrol levels with SIRTs and IVF outcome measures.[38] The protective effects of resveratrol are due to induction of progesterone secretion and to its anti-oxidant properties that are closely related to an increase in SIRT1 expression.[19,20] Our failure to reveal such an association might be explained by the relatively low concentration of resveratrol present to exert its dose-dependent stimulation of expression/action of SIRT1.

Conclusions

Although our study provides some circumstantial evidence for involvement of SIRTs in control of folliculogenesis, oocyte maturation, and developmental potential of early embryos, definitive conclusions cannot be drawn. We measured immunoreactive SIRT1 and SIRT6 protein levels, which are not reliable measures of their function. SIRT proteins may dissociate from their mRNA expression because they may undergo post-translational modifications. Epigenetic damage by phosphorylation or by small interfering RNA may alter conformation of SIRT protein, binding to their substrates, catalytic activity, and immunoreaction.[30,36] Because these modifications cannot be detected by ELISA, further studies need to be conducted to determine the molecular mechanism of SIRT function in patients receiving IVF.

Acknowledgements

We thank the women who underwent IVF treatment at the Assisted Reproduction Unit, Department of Obstetrics and Gynaecology, University of Pécs, for participating in our study.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This work was supported by EFOP-3.6.3-VEKOP-16-2017-00009 (“Development of

scientific workshops of medical, health sciences and pharmaceutical educations") and GINOP-2.3.2-15-2016-00021 ("The use of chip-technology in increasing the effectiveness of human in vitro fertilization"). The funding sources did not have any role in the study design, in collection, analysis and interpretation of data, or in writing and submitting this manuscript.

ORCID iD

Viktória Prémusz http://orcid.org/0000-0002-4059-104X

References

1. Imai S, Armstrong CM, Kaeberlein M, et al. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 2000; 403: 795–800.
2. Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. *Biochem Biophys Res Commun* 2000; 273: 793–798.
3. North BJ and Verdin E. Sirtuins: sir2-related NAD-dependent protein deacetylases. *Genome Biol* 2004; 5: 224.
4. Imai S and Guarente L. 10 years of NAD-dependent SIR2 family deacetylases–implications for metabolic diseases. *Trends Pharmacol Sci* 2010; 31: 212–220.
5. Finkel T, Deng CX and Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. *Nature* 2009; 460: 587–591.
6. Satoh A, Stein L and Imai S. The role of mammalian sirtuins in the regulation of metabolism, aging, and longevity. *Handb Exp Pharmacol* 2011; 206: 125–162.
7. Park S, Mori R and Shimokawa I. Do sirtuins promote mammalian longevity?: a critical review on its relevance to the longevity effect induced by calorie restriction. *Mol Cells* 2013; 35: 474–480.
8. Giblin W, Skinner ME and Lombard DB. Sirtuins: guardians of mammalian healthspan. *Trends Genet* 2014; 30: 271–286.
9. Tatone C, Di Emidio G, Vitti M, et al. Sirtuin functions in female fertility: possible role in oxidative stress and aging. *Oxid Med Cell Longev* 2015; 2015: 659687.
10. Gleicher N, Kushnir VA, Albertini DF, et al. Improvements in IVF in women of advanced age. *J Endocrinol* 2016; 230: F1–F6.
11. Zhang XM, Li L, Xu JJ, et al. Rapamycin preserves the follicle pool reserve and prolongs the ovarian lifespan of female rats via modulating mTOR activation and sirtuin expression. *Gene* 2013; 523: 82–87.
12. Kawamura Y, Uchijima Y, Horike N, et al. Sirt3 protects in vitro-fertilized mouse preimplantation embryos against oxidative stress-induced p53-mediated developmental arrest. *J Clin Invest* 2010; 120: 2817–2828.
13. Manosalva I and González A. Aging changes the chromatin configuration and histone methylation of mouse oocytes at germinal vesicle stage. *Theriogenology* 2010; 74: 1539–1547.
14. Luo LL, Chen XC, Fu YC, et al. The effects of caloric restriction and a high-fat diet on ovarian lifespan and the expression of SIRT1 and SIRT6 proteins in rats. *Aging Clin Exp Res* 2012; 24: 125–133.
15. Wang N, Luo LL, Xu JJ, et al. Obesity accelerates ovarian follicle development and follicle loss in rats. *Metabolism* 2014; 63: 94–103.
16. Zhang J, Fang L, Lu Z, et al. Are sirtuins markers of ovarian aging? *Gene* 2016; 575: 680–686.
17. Bordone L, Cohen D, Robinson A, et al. SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell* 2007; 6: 759–767.
18. Fu H, Wada-Hiraike O, Hirano M, et al. SIRT3 positively regulates the expression of folliculogenesis- and luteinization-related genes and progesterone secretion by manipulating oxidative stress in human luteinized granulosa cells. *Endocrinology* 2014; 155: 3079–3087.
19. Liu M, Yin Y, Ye X, et al. Resveratrol protects against age-associated infertility in mice. *Hum Reprod* 2013; 28: 707–717.
20. Wang F, Tian X, Zhang L, et al. Beneficial effect of resveratrol on bovine oocyte maturation and subsequent embryonic development after in vitro fertilization. *Fertil Steril* 2014; 101: 577–586.
21. Sugiyama M, Kawahara-Miki R, Kawana H, et al. Resveratrol-induced mitochondrial

synthesis and autophagy in oocytes derived from early antral follicles of aged cows. *J Reprod Dev* 2015; 61: 251–259.
22. Itami N, Shirasuna K, Kuwayama T, et al. Resveratrol improves the quality of pig oocytes derived from early antral follicles through sirtuin 1 activation. *Theriogenology* 2015; 83: 1360–1367.
23. Mukherjee A, Malik H, Saha AP, et al. Resveratrol treatment during goat oocytes maturation enhances developmental competence of parthenogenetic and hand-made cloned blastocysts by modulating intracellular glutathione level and embryonic gene expression. *J Assist Reprod Genet* 2014; 31: 229–239.
24. Li Y, Wang J, Zhang Z, et al. Resveratrol compares with melatonin in improving in vitro porcine oocyte maturation under heat stress. *J Anim Sci Biotechnol* 2016; 7: 33.
25. Guarente L. Franklin H. Epstein lecture: sirtuins, aging, and medicine. *N Engl J Med* 2011; 364: 2235–2244.
26. Longo VD and Kennedy BK. Sirtuins in aging and age-related disease. *Cell* 2006; 126: 257–268.
27. Masoro EJ. Caloric restriction and aging: an update. *Exp Gerontol* 2000; 35: 299–305.
28. Gonfloni S, Iannizzotto V, Maiani E, et al. P53 and Sirt1: routes of metabolism and genome stability. *Biochem Pharmacol* 2014; 92: 149–156.
29. Palacios JA, Herranz D, De Bonis ML, et al. SIRT1 contributes to telomere maintenance and augments global homologous recombination. *J Cell Biol* 2010; 191: 1299–1313.
30. Flick F and Lüscher B. Regulation of sirtuin function by posttranslational modifications. *Front Pharmacol* 2012; 3: 29.
31. Zhang J, Liu J, Zhu K, et al. Effects of BMAL1-SIRT1-positive cycle on estrogen synthesis in human ovarian granulosa cells: an implicative role of BMAL1 in PCOS. *Endocrine* 2016; 53: 574–584.
32. Zhao HC, Ding T, Ren Y, et al. Role of Sirt3 in mitochondrial biogenesis and developmental competence of human in vitro matured oocytes. *Hum Reprod* 2016; 31: 607–622.
33. Cheng EH, Chen SU, Lee TH, et al. Evaluation of telomere length in cumulus cells as a potential biomarker of oocyte and embryo quality. *Hum Reprod* 2013; 28: 929–936.
34. Valerio D, Luddi A, De Leo V, et al. SA1/SA2 cohesion proteins and SIRT1-NAD+ deacetylase modulate telomere homeostasis in cumulus cells and are eligible biomarkers of ovarian aging. *Hum Reprod* 2018; 33: 887–894.
35. Kugel S and Mostoslavsky R. Chromatin and beyond: the multitasking roles for SIRT6. *Trends Biochem Sci* 2014; 39: 72–81.
36. Akiyama T, Nagata M and Aoki F. Inadequate histone deacetylation during oocyte meiosis causes aneuploidy and embryo death in mice. *Proc Natl Acad Sci USA* 2006; 103: 7339–7344.
37. Han L, Ge J, Zhang L, et al. Sirt6 depletion causes spindle defects and chromosome misalignment during meiosis of mouse oocyte. *Sci Rep* 2015; 5: 15366.
38. Takeo S, Kawahara-Miki R, Goto H, et al. Age-associated changes in gene expression and developmental competence of bovine oocytes, and a possible countermeasure against age-associated events. *Mol Reprod Dev* 2013; 80: 508–521.