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Biotechnological and clinical outcome of in vitro fertilization in non-obese patients with polycystic ovarian syndrome

Przemyslaw Ciepiela[1], Tomasz Baczkowski[1], Pawel Brelik[2], Anna Antonowicz[1], Krzysztof Safranow[3], Rafal Kurzawa[1]

[1]Department of Reproductive Medicine and Gynecology, Pomeranian Medical University, Szczecin-Police, Poland
[2]Ian Clwyd Hospital, Conwy And Denbighshire NHS Trust, Rhyl, Benbighshire, United Kingdom
[3]Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland

Abstract: Introduction: Polycystic ovarian syndrome (PCOS) is a hormonal and metabolic disorder which poses problems with controlled ovarian stimulation (COH). It has been also postulated that PCOS patients have oocytes and embryos with poorer quality which affects IVF results. Aim: To verify IVF outcome in non-obese patients with PCOS. Materials and methods: IVF results of 71 non-obese PCOS patients with 243 non-obese non-POCS patients, regardless of stimulation protocol, from years 2004-2006 were compared. Results: Biotechnological results of PCOS patients in opposition to non-PCOS patients were respectively as follows: higher average number (10.19 vs. 7.61; p=0.001) and percentage (82.34% vs. 76.25%; p=0.025) of retrieved mature M2 oocytes; similar (77.01% vs. 76.75%; p=0.835) fertilization rate with higher average number of embryos (7.633 vs. 5.650 p=0.003); higher average number (4.830 vs. 3.304; p=0.001) and percentage (65.66% vs. 60.57%; p=0.006) of embryos with optimal Z1 and Z2 pronuclei pattern according to Scott; higher average number of class A embryos (3.57 vs. 2.34; p=0.001). Similar number of embryos were transferred in both groups (2.408 vs. 2.485, p=0.552). Clinical results in PCOS and non-PCOS patients were as follows: similar stimulation duration (10.53 days vs. 10.31 days; p=0.639) with significant less gonadotropin total usage (1866.54 IU vs. 2276.18 IU; p=0.001). Also clinical pregnancy per transfer (57.75% vs. 41.98%; p=0.021) and delivery per transfer (45.07% vs. 32.51%; p=0.066) were more often in PCOS patients with comparable miscarriages (12,68% vs. 6,58%; p=0.131) and ectopic pregnancy (0.00% vs. 2.06%; p=0.591) rates, respectively. Conclusion: PCOS in non-obese patients is linked with good biotechnological and clinical IVF outcome.

Key words: Polycystic ovarian syndrome - IVF - ICSI - Oocyte quality - Embryo quality - IVF outcome

Introduction

Polycystic ovary syndrome (PCOS) is a endocrine disorder that affects 4% - 6% of females [1-3]. It is the most common hormone-metabolic disturbance among women of reproductive age and leading cause of anovulatory infertility. For PCOS patients that failed to ovulate with clomiphene citrate and following chronic low-dose gonadotropin protocols assisted reproductive techniques (ART) are proposed [4]. However, some authors indicated that obesity, independent of insulin resistance, may be associated with relative gonadotropin resistance [5]. Additionally, exaggerated response to gonadotropins associated with high risk of ovarian hyperstimulation syndrome (OHSS) potentially complicates controlled ovarian hyperstimulation (COH) in those patients. Excessive response to gonadotropins may often results in higher estradiol (E2) level on the day of hCG administration what can potentially affect oocyte maturity [6] and quality [7]. A negative correlation between BMI and number of collected oocytes in non-PCOS and PCOS patients was also reported [8,9]. Furthermore, abnormalities of folliculogenesis and granulose cell function have been observed in patients with PCOS [10].

The higher percentage of low-quality oocytes in PCOS may promote lower fertilization rates and embryos of poorer quality that have been reported in PCOS patients compared with control women under-

Correspondence: P. Ciepiela, Dept. of Reproductive Medicine and Gynecology, Siedlecka Str. 2, 72-010 Szczecin-Police, Poland; e-mail: ciepiela@sci.pam.szczecin.pl

Table 1. Clinical characteristics of study participants in both studied groups expressed as means ±SD; NS - not significant; a - Mann-Whitney test.

	Non-PCOS	PCOS	P value
No. of patients	243	71	
Age (years)	32.44 ± 4.17	30.77 ± 3.63	0.003[a]
Male cause of infertility			
Normal semen parameters [%]	20.99	21.13	NS
Coexisting male factor (according to WHO) [%]	79.01	78.87	NS

going ART [7]. However, others noted comparable general implantation and pregnancy rates in PCOS patients when compared to controls [11].

The aim of this study was to verify oocyte and embryo quality as well as clinical outcome in PCOS non-obese patients undergoing IVF. To avoid influence of obesity on general in vitro fertilization (IVF) outcome we decided to exclude obese patients in our study.

Materials and methods

Patients. A total of 314 infertile women, between 23 and 44 years of age, who underwent intracytoplasmic sperm injection (ICSI) treatment in the Department of Reproductive Medicine and Gynecology, Pomeranian University of Medicine in Szczecin in years 2004-2006 were retrospectively analyzed. General inclusion criteria for all study participants included: body mass index (BMI) <28 kg/m^2. Additionally, for PCOS patients: meeting of 2003 Rotterdam PCOS criteria. General exclusion criteria for all study participants included: ≥2 miscarriages, ≥3 unsuccessful IVF cycles, anatomical abnormalities of the uterus on laparoscopy or hysteroscopy. Group I consisted of 243 non-PCOS patients with mean age of 32.44 ± 4.17 (SD) years. Group II included 71 PCOS patients with a mean age of 30.77 ± 3.63 (SD) years. The clinical characteristics of the patients in both examined groups are shown in Table 1.

Protocol for controlled ovarian hyperstimulation. To synchronize menstrual cycle before starting IVF patients were receiving oral monophasic contraceptives pills (Cilest; Janssen-Cilag, Belgium) for a month. In both protocols, only two clinicians and two embryologists were involved in the study. GnRH antagonist and agonists protocol were used in both studied groups. During ovarian hyperstimulation human menopausal gonadotropin hMG (Menopur, Ferring Pharmaceuticals, Holland) or recombinant human FSH (Gonal F; Serono Pharma, Switzerland) were used. PCOS patients were stimulated only with recombinant human FSH (recFSH, Gonal F; Serono Pharma, Switzerland) as it was also the case in GnRH antagonist protokols.

GnRH antagonist protocol. From the 2nd day of the cycle women were given regular daily recFSH subcutaneous injections (usually between 18.00 and 20.00 hours). With a starting dose of a 150IU per day which was adjusted individually depending on patients response measured by transvaginal ultrasonography and the levels of E2. Ultrasound and E2 levels monitoring started from the 7th day of the cycle (6th day of COH) after 5 doses of recFSH and was continued every second day and the day of hCG administration. A GnRH antagonist - cetrorelix (Cetrotide; Merck Serono, Germany) was administered subcutaneously between 8.00 and 12.00 hours when at least two ovarian follicles reached 14mm in diameter. The protocol consisted of daily Cetrotide 0,25 mg subcutaneous injections, average 4, until the criteria for recombinant human chorionic gonadotropin (hCG) administration were met. For final oocyte maturation, when the dominant follicle reached ≥18mm with the following two ≥16mm and E2 levels between 1000-4000 pg/mL, an intramuscular (Pregnyl; Organon, Holland) injection of 10.000 IU hCG or subcutaneous (Ovitrelle; Merck Serono, France) injection of 250 μg hCG was given.

GnRH agonist protocol. During oral contraception (OC) on days 16-18 of the preceding cycle, after transvaginal ultrasonographic screening of ovaries, an intramuscular injection of GnRH agonist triptorelin (Diphereline SR 3.75; Boufor Ibsen Pharma, France) was given. After 14 days, if the level of LH was <2 mIU/mL and E2 <50 pg/mL the administration of gonadotropins commenced. Women were given regular daily recFSH or hMG subcutaneous injections (usually between 18.00 and 20.00 hours). Also in this protocol starting dose was 150 IU per day, which was adjusted individually, depending on patients response. Similarly, ultrasound and E2 level monitoring started from the 7th day of the cycle (6th day of COH) after 5 doses of gonadotropins and was continued every second day and the day of hCG administration. The GnRH antagonist protocol final oocyte maturation was induced by an intramuscular (Pregnyl) injection of 10.000 IU hCG or subcutaneous (Ovitrelle) injection of 250 μg hCG when the dominant follicle reached ≥18 mm with the following two ≥16 mm and E2 level was between 1000-4000 pg/mL.

Oocytes retrieval. Oocytes were retrieved with transvaginal ultrasound guided aspiration needle (OPS Single lumen: without tap; Laboratoire C.C.D, France) 36 hours after hCG administration to a collection tubes containing HEPES-buffered medium (FertiCult Flushing medium, FertiPro, Belgium), washed, and then cultured in ISM1 medium (Medicult, Denmark). Before ICSI, a 10% hyaluronidase solution (Hyaluronidase solution in Flushing Medium, FertiPro, Belgium) dose was used to remove cumulus cells. After that, oocytes were washed in flushing medium (FertiCult Flushing medium, FertiPro, Belgium) until the ICSI procedure.

Embryological assessment. Retrieved oocytes were classified in metaphase II (M2) as mature and in metaphase I (M1) or germinal vesicle stage (GV) as immature. Fertilizations were checked during routine non-invasive examination 16-18 hours after ICSI [12,13]. Embryo grading was assessed on the pronuclear stage according to the Scott criteria and on the day of the Embryo transfer (ET) according to the internal laboratory cumulative embryo score standards [14,15]. We also distinguished A class embryos on the third day of the culture defined as an embryo with ≥8 symmetrical, non-fragmented blastomers. Decision on number of transferred embryos was taken according to 2006 ASRM embryo-transfer guidelines [16]. Embryos were transfer to uterine cavity was in catheter (Frydman Soft 4.5 with guide, Laboratoire C.C.D., France) 72 hours after ICSI.

Luteal support. Luteal phase support included simultaneous oral 30 mg/day of dydrogestreon (Duphaston; Solvay Pharma, Belgium) and intravaginal 150 mg/day of progesterone (Luteina; Adamed, Poland).

Pregnancy was confirmed by pregnancy test 14 days after ET and by vaginal ultrasound scan at 5 weeks gestation. Subsequently after 11th week of gestation. Biochemical gestation was not taken into consideration at any stage of the trial.

Outcome measures.

Biotechnological endpoints:

- number and percentage of retrieved matured oocytes (M2), defined as proportion of metaphase II (M2) to total number of retrieved oocytes;
- fertilization rate defined as proportion of two pronuclei oocytes to number of injected oocytes;
- analysis of quality of all zygotes on 1st day of the culture and of transferred embryos on 3rd day of culture

Clinical endpoints:

- number of days of gonadotropin treatment;
- gonadotropin use;
- clinical pregnancy per attempt, defined as an ongoing pregnancy at 12 weeks of gestation;
- delivery per attempt, defined as proportion of deliveries to number of patients in group
- multiple pregnancy per attempt,
- miscarriages per attempt.

Statistics. Chi-square test or Fisher's exact test for 2×2 tables were used to analyze statistical significance for qualitative variables. Non-normally distributed variables were analyzed by non-parametric Mann-Whitney test. P value <0,05 was considered as significant. All calculations were performed using Statistica for Windows 7.1 (StatSoft Inc., Tulsa;USA).

Ethical issues. All women signed informed consent before study participation according to local ethical committee at Pomeranian University of Medicine.

Results

Three hundred fourteen enrolled in the study patients underwent embryo transfer (ET). The comparison of biotechnological outcome measures in non-PCOS and PCOS patients are shown in Table 2.

Total number of retrieved oocytes (p=0.002), number (p=0.003) and percentage (p=0.034) of immature GV oocytes retrieved as well as number (p=0.014) and percentage (p=0.060) of immature M1 oocytes retrieved as well as number (p=0.001) and percentage (p=0.02) of mature oocyte (M2) and number of fertilized oocytes (p=0.001) were significantly lower in non-PCOS patients comparing with PCOS patients. However, fertilization rate was similar in both studied groups (p=0.835).

Although we noticed significantly lower number of zygotes with optimal pronuclear morphology classified as Z1 (p=0.016) and Z2 (p=0.001) as well as Z1 and Z2 together (p=0.001) in non-PCOS patients, the percentage of mentioned pronuclear patterns Z1 (p=0.459), Z2 (p=0.137), both Z1 and Z2 (p=0.237) was similar in both groups. Nevertheless, zygotes with optimal pronuclear morphology dominated in a cohort of fertilized oocytes in both studied groups. There were no significant differences in number (p=0.09) and percentage (p=0.660) of zygotes with suboptimal pronuclear morphology described as Z3 and number (p=0.441) and percentage (p=0.228) of zygotes with non-optimal described as Z4.

Embryos qualified to the ET originated primarily from Z1 or Z2 zygotes. However, significantly lower number (p=0.024) and percentage (p=0.006) of embryos which had a history of optimal pronuclear Z1+Z2 pattern and considerably higher number (p=0.014) and percentage (p=0.012) of embryos which had suboptimal Z3 pattern were transferred in non-PCOS women. We observed significantly lower number of high quality class A embryos in a cohort of all embryos of non-PCOS patients (p=0.001). Similar number of embryos were transferred in both groups (p=0.552) with comparable mean number of blastomers per transfer (p=0.176). Needless to say, the biotechnological data did not differ in relation to stimulation protocol within the studied groups of patients (data not included).

The results originating from clinical data are shown in Table 3. The number of stimulation days did not differ (p=0.639) but total gonadotropin dose was significantly higher (p=0.001) in a non-PCOS group. Significantly higher (p=0.001) serum E2 level on the day of hCG was observed in PCOS group.

Pregnancy rate per attempt was significantly lower (p=0.021) but the delivery rate per attempt did not differ statistically (p=0.066) in non-PCOS vs. PCOS group. There were no statistical differences in multiple pregnancy (p=0.179), miscarriage (p=0.131) and ectopic pregnancy (p=0.591) rates.

Discussion

There are many studies assessing IVF outcome among PCOS patients. Generally, where IVF or ICSI have been performed for PCOS-associated infertility, the outcome is similar to other forms of infertility [17]. When comparing PCOS patients to controls, more follicles are produced and more oocytes are collected with a lower dose of total gonadotropins, general implantation and pregnancy rates are noted to be comparable [8,18]. Nevertheless, studies with comprehensive assessment of oocytes and embryos, especially in PCOS women, are lacking.

Available trials concentrates mainly on oocyte quality and clinical outcome such as fertilization, implantation and pregnancy rate. Numerous authors reported poorer quality of collected oocytes with lower fertilization capacity in PCOS cycles [6,19,20]. Ludwig *et al.* [18] observed among PCOS women who underwent ICSI similar rate of metaphase II oocytes,

Table 2. Comparison of biotechnological outcome measures in non-PCOS and PCOS patients. Values are expressed as means ±SD; NS - not significant; Z1+Z2 - optimal pronuclei pattern. a - Mann-Whitney test.

			Non-PCOS	PCOS	p value
Total no. of retrieved oocytes			9.38 ± 4.11	13.22 ± 4.92	0.002[a]
No. of immature GV oocytes retrieved			1.12 ± 1.44	2.12 ± 2.43	0.003[a]
Percentage of immature GV oocyte (%)			11.30 ± 14.01	15.99 ± 16.76	0.034[a]
No. of immature M1 oocytes retrieved			0.65 ± 1.19	0.91 ± 1.15	0.014[a]
Percentage of immature M1 oocyte (%)			6.35 ± 11.16	7.74 ± 10.15	NS
No. of mature M2 oocytes retrieved			7.61 ± 4.05	10.19 ± 4.86	0.001[a]
Percentage of mature oocyte M2 (%)			82.34 ± 17.80	76.25 ± 20.60	0.02[a]
No. of fertilized oocytes			5.65 ± 3.32	7.63 ± 3.82	0.001[a]
Fertilization rate (%)			76.75 ± 22.04	77.01 ± 20.09	NS
Pronuclear morphology according to Scott (16-18h after ICSI)					
Pronuclear morphology of all embryos		Z1	1.00 ± 1.1	1.54 ± 1.55	0.016[a]
		Z1%	19.24 ± 21.58	19.71 ± 18.46	NS
		Z2	2.29 ± 1.84	3.28 ± 1.87	0.001[a]
		Z2%	41.33 ± 26.47	45.94 ± 21.37	NS
		Z1+Z2	3.30 ± 2.22	4.83 ± 2.68	0.001[a]
		Z1+Z2%	60.57 ± 27.53	65.66 ± 22.05	NS
		Z3	1.9 ± 1.85	2.39 ± 2.12	NS
		Z3%	32.28 ± 26.01	29.31 ± 20.05	NS
		Z4	0.43 ± 0.78	0.40 ± 0.91	NS
		Z4%	7.13 ± 12.36	5.01 ± 10.73	NS
Pronuclear morphology of transferred on day 3 embryos		Z1	0.66 ± 0.73	0.76 ± 0.81	NS
		Z1%	27.12 ± 30.86	31.92 ± 35.15	NS
		Z2	1.04 ± 0.83	1.19 ± 0.82	NS
		Z2%	43.41 ± 34.39	51.40 ± 35.60	NS
		Z1+Z2	1.69 ± 0.88	1.95 ± 0.64	0.024[a]
		Z1+Z2%	70.54 ± 33.81	83.33 ± 24.23	0.006[a]
		Z3	0.68 ± 0.83	0.40 ± 0.62	0.014[a]
		Z3%	26.44 ± 31.63	15.25 ± 22.84	0.012[a]
		Z4	0.09 ± 0.30	0.04 ± 0.20	NS
		Z4%	3.01 ± 10.19	1.40 ± 6.75	NS
Characteristics of embryos (72h after ICSI)					
Class A embryos among all embryos			1.18 ± 1.32	1.98 ± 1.81	0.001[a]
No. of transferred embryos			2.48 ± 0.71	2.40 ± 0.54	NS
Mean no. of blastomers per transfer			16.50 ± 4.51	17.25 ± 3.93	NS
Mean no. of blastomers in transferred embryo			6.78 ± 1.37	7.29 ± 1.26	0.022[a]
Quality of transferred embryos	Symmetry	A	1.84 ± 0.80	1.98 ± 0.70	NS
		B	0.61 ± 0.88	0.42 ± 0.73	NS
		C	0.02 ± 0.18	0	NS
	Fragmentation	None	1.71 ± 0.90	1.73 ± 0.79	NS
		< 20%	0.73 ± 0.94	0.64 ± 0.92	NS
		20% - 50%;	0.4 ± 0.21	0.028 ± 0.16	NS
		> 50%	0	0	-

Table 3. Comparison of clinical outcome measures in non-PCOS and PCOS patients. Where appropriate values are means (±SD); NS - not significant; E2 - estradiol; a - Mann-Whitney test; b - Fisher exact test.

	Non-PCOS	PCOS	P value
No. of gonadotropin stimulation days	10.31 ± 2.11	10.53 ± 3.07	NS
Total gonadotropin dose (IU)	2276.18 ± 883.02	1866.54 ± 756.45	0.001[a]
Serum E_2 level on the day of hCG (pg/mL)	1404.63 ± 693.29	2419.40 ± 1286.20	0.001[a]
Pregnancy per attempt (%)	102/243 (42%)	41/71 (57.75%)	0.021[b]
Delivery per attempt (%)	79/243 (32.5%)	32/71 (45%)	NS
Multiple pregnancy per attempt (%)	21/243 (8.64%)	10/71 (14%)	NS
Miscarriages per attempt (%)	18/243 (7.4%)	9/71 (12.7%)	NS
Ectopic pregnancy per attempt (%)	5/243 (2.1%)	0	NS

fertilization and embryo development rates in comparison to controls. Heijnen *et al.* [21], in a meta-analysis of nine studies which compared conventional IVF outcomes in PCOS patients with matched controls, reported significantly more oocytes per oocyte retrieval obtained in PCOS group comparing with controls but higher fertilization rate in the control group which resulted in an equal number of fertilized oocytes in both studied groups. In the current study PCOS patients comparing to non-PCOS had more retrieved oocytes. Although we observed higher number of immature (GV and M1) oocytes in PCOS patients, we also noticed higher number of mature (M2) oocytes, what with comparable fertilization rate in both studied groups resulted in higher mean number of fertilized oocytes comparing with non-PCOS patients. We also observed that despite significantly lower FSH consumption, serum E2 level on the day of hCG administration was higher in PCOS patients. But that did not affect M2 oocyte quality, in terms of fertilization and embryo development in PCOS patients. Similarly, in Esinler *et al.* [22] study where PCOS patients had significantly higher E2 level on hCG day and higher number of metaphase II oocytes comparing with non-PCOS patients.

Some authors focused on abnormalities of folliculogenesis and granulose cell function in patients with PCOS [10]. In one study, anomalous expression of important oocyte-derived factor - growth differentiation factor 9 (GDF-9), which plays significant role in early follicular development and appear to have a part in controlling cumulus expansion in the preovulatory follicle, was found in oocytes from PCOS patients [23]. Also abnormal endocrine in vivo milieu, exposure to increased levels of LH and insulin has been postulated to contribute abnormal granulose cell function [10]. Laven *et al.* [24] suggests that normal inhibin B level produced by pre-antral and small antral follicles founded in PCOS patients is probably responsible for not increased number of healthy non-atretic follicles. Sengoku *et al.* [25] compared the chromosomal normality of unfertilized oocytes from PCOS patients vs. patients with tubal infertility and observed reduced fertilization rate but there was no differences in oocyte aneuploidy rates between two groups. Authors come to a conclusion that the reduced fertilization rate is not caused by chromosomal aberrations or immaturity of oocytes collected from PCOS patients [25]. On the other hand, Wood *et al.* reported recently molecular abnormalities in PCOS oocytes which could account for reduced fecundity [26].

Non-invasive methods of embryo evaluation has been widely employ to help assess embryos without damage [15]. We found the zygote scoring by Scott [14] to be simple and useful as a predictive factor for further development of the embryos and for pregnancy [27]. In current study zygotes with optimal (Z1+Z2) pronuclear morphology dominated in comparison to sub and non-optimal configuration of pronuclei in both studied groups. Nevertheless, patients with PCOS had statistically more zygotes with optimal pronuclear pattern than non-PCOS patients. In consequence, statistically more embryos with most favorable pronuclear morphology were available to transfer on 3rd day of the culture in PCOS group. We identify significantly more class A embryos among all embryos in PCOS group. However, regardless mean number of blastomers in transferred embryo, symmetry and fragmentation of transferred embryos was similar. Good quality of embryos in PCOS patients could be related to our strict inclusion criteria. The risk of metabolical disturbances and its negative influence of the oocyte and further and the embryo quality in this group is relatively lower in compare to obese PCOS patient [8,9].

Heijnen *et al.* [21] reported significantly longer duration of stimulation and no significant difference in the amount of gonadotropins used in PCOS group compared with controls. Conversely, in our study the number of stimulation days did not differ between both studied groups but total gonadotropin dose was significantly higher in a non-PCOS group comparing to PCOS group. This could be associated with homogeny

of PCOS group in our study comparing with Heijnen *et al.* analysis which included all PCOS patients regardless of BMI. Maheshwari *et al.* [28] as well as Fedorcsak *et al*. [9] reported that obese patient need more gonadotropins during COH comparing to non-obese.

In current study, similarly to Esinler *et al.* [22], pregnancy rate per attempt was significantly higher in PCOS group than in non-PCOS group and delivery rate per attempt was higher in PCOS group, but probably because of little group did not differ statistically. This results may indicate that excluding obesity improved oocyte and embryo quality and in consequence overall outcome. Also analogously to Esinler *et al.* [22] we did not observe statistical differences comparing non-PCOS patients and PCOS patients in multiple pregnancy rate. Heijnen *et al.* [21] did not observed significant difference for pregnancy attempt, delivery per attempt and the number of miscarriage between PCOS and non-PCOS patients. Miscarriages rate among PCOS patients in our study were also similar in both studied groups. Also Esinler *et al.* [22] reported comparable miscarriage rate in PCOS and non-PCOS group.

We noted that patients with PCOS may have more favorable ICSI outcome compared to non-PCOS patients. Our study shows, that the higher number of available embryos increases the chances of more good quality embryos development and allows to transfer top-quality embryos in the PCOS group which may result in significantly higher pregnancy rates and higher delivery rates in the PCOS group comparing to the non-PCOS patients. Concluding, the presence of PCOS in non-obese patient may be a favorable prognostic factor before considering IVF.

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