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Characterization of oocyte retrieval cycles with empty zona pellucida

Aki Oride | Haruhiko Kanasaki | Tomomi Hara | Hiroko Ohta | Satoru Kyo

Department of Obstetrics and Gynecology, Shimane University, Faculty of Medicine, Izumo City, Japan

Correspondence
Aki Oride, Shimane University Faculty of Medicine, Deptartment of Obstetrics and Gynecology, Izumo City, Japan.
Email: oride@med.shimane-u.ac.jp

Abstract

Purpose: To identify the factors that characterize cycles with empty zona pellucida (EZP).

Methods: Thirty-six oocyte retrieval cycles from which EZP were collected and another 36 cycles from which no EZP was collected were compared. The patients were divided into three groups: those with no EZP collected during any cycle, those with EZP collected during all cycles, and those experiencing cycles both with and without EZP.

Results: The mean number of oocytes collected per cycle was higher in the cycles with EZP than without EZP. The fertilization rate of the collected oocytes and the rate of good embryo formation were significantly lower in the cycles with EZP. No significant difference was observed between the three groups in terms of age, number of oocytes collected, or hormone levels before and after the oocyte retrieval. The fertilization and pregnancy rates were highest in the patients with no EZP being collected during any cycle, followed by those experiencing cycles both with and without EZP, and then by those with EZP collected during all cycles.

Conclusion: The observation of lower fertilization, poor embryo formation, and a low pregnancy rate in the patients with EZP suggests the poor quality of oocytes that were collected with EZP in the same cycle.

KEYWORDS

empty zona pellucida, fertilization, in vitro fertilization, infertility, oocyte retrieval

1 | INTRODUCTION

An empty zona pellucida (EZP) refers to an oocyte that contains only the zona pellucida (ZP). An EZP can be formed either during oocyte retrieval by manual aspiration, where high suction pressure could create a crack in the ZP, through which the cell exits to form an EZP, or as the result of shrinkage of the cell inside the ZP.[1,2] In order to identify the phase of the oocyte retrieval cycle in which an EZP oocyte is likely to occur during in vitro fertilization (IVF), the baseline values of gonadotropins and estradiol and the *P*-values per oocyte before and during oocyte retrieval were compared in relation to the development (or not) of an EZP during oocyte retrieval. Also examined were the differences in the rates of fertilization, good embryo formation, and pregnancy during oocyte retrieval cycles with and without an EZP.

2 | MATERIALS AND METHODS

2.1 | Patients

Of the 281 oocyte retrieval cycles that were performed at the authors' hospital between January, 2010 and December, 2012, 36 cycles from which an EZP were collected and 36 cycles from which

TABLE 1 Backgrounds and outcomes of the oocyte retrieval cycles containing empty zona pellucida (EZP)

Variable	EZP(−)	EZP(+)	*P*-value
No. of cycles	36	36	—
No. of patients	30	32	—
Mean age (y)	36.4 ± 4.4	35.8 ± 4.4	—
Mean no. of oocytes collected (excluding EZP)	6.6 ± 3.8	10.9 ± 7.0	<.01
LH (baseline: mIU/mL)	4.0 ± 3.0	3.7 ± 2.3	—
FSH (baseline: mIU/mL)	10.2 ± 6.1	8.8 ± 4.7	—
Estradiol (baseline: pg/mL)	29.1 ± 13.7	29.1 ± 12.3	—
Estradiol/total no. of oocytes collected (before hCG administration)	290.3 ± 166.4	318.1 ± 307.5	—
Estradiol/total no. of oocytes collected (on the day of oocyte retrieval)	196.0 ± 89.1	194.2 ± 124.5	—
Progesterone (before hCG administration: ng/mL)	1.2 ± 1.1	1.3 ± .9	—
Progesterone (on the day of oocyte retrieval: ng/mL)	8.6 ± 6.1	9.5 ± 8.7	—
Fetilization rate (%) (including ICSI and CON)	76.2 ± 20.4	65.0 ± 22.0	<.05
Good embryo formation rate (%) (G1 or G2)	68.3 ± 30.8	48.1 ± 37.3	<.01
Pregnancy rate (%)	23.3	21.9	—

CON, conventional insemination; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; LH, luteinizing hormone.

no EZP was collected (72 cycles in total) were compared. The 36 cycles without an EZP were randomly selected. The patients' age at oocyte retrieval was 31-43 years (mean: 36.4 ± 4.4 years) for the cycles without EZP and 24-43 years (mean: 35.8 ± 4.4 years) for those with an EZP. The patients who underwent multiple oocyte retrieval cycles and experienced cycles both with and without an EZP were counted in both cycles. In total, 55 patients were included in this study.

2.2 | Controlled ovarian stimulation, oocyte retrieval, and fertilization

Oocyte retrieval was performed during an ovarian stimulation cycle by one of the following methods: (1) the gonadotropin-releasing hormone (GnRH) antagonist method using clomiphene citrate (Clomid Tablets; Shionogi & Company, Ltd., Osaka, Japan) alone, human menopausal gonadotropin (hMG for intramuscular injection; Fuji Pharmaceutical Company, Ltd., Tokyo, Japan) alone, or a combination of both agents; or (2) the long protocol method of using buserelin acetate (Suprecur Nasal Spray; Mochida Pharmaceutical Company, Ltd., Tokyo, Japan) and hMG administered from 10 days and 3 days before the onset of menstruation, respectively. In the antagonist method, the administration of 0.25 mg Cetrotide (Shionogi & Company, Ltd., Osaka, Japan) was started when the dominant follicle reached 15 mm in diameter and was given daily until the day of human chorionic gonadotrophin (hCG) administration. When the follicle reached 18-20 mm in diameter, 10,000 IU hCG (Mochida Pharmaceutical Company, Ltd.) was injected i.m., followed by oocyte retrieval ~34 hours later. The ocytes were collected by manual aspiration with a syringe and an 18 gauge needle (HAKKO & Company, Ltd., Tikuma, Japan). The same research team members were involved in all the oocyte retrieval procedures. The follicular fluid was aspirated at an approximate rate of 25 mL/min. The oocytes were fertilized by conventional insemination or by intracytoplasmic sperm injection (ICSI) in modified or regular human tubal fluid medium (Irvine Scientific, Santa Ana, CA, USA).

2.3 | Plasma concentrations of estradiol and progesterone

The serum concentrations of estradiol, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured by chemiluminescent electro-immunoassay by using an IMMULYZE 1000 system (LSI Medience, Tokyo, Japan).[3]

2.4 | Other aspects

G1 and G2 oocytes were defined as good embryos, based on Veeck's classification.[4] Pregnancy was confirmed by visualizing a fetal sac on transvaginal ultrasonography.

2.5 | Statistical analysis

The data were transferred to Microsoft Excel 2016 for Mac or IBM SPSS Statistics for Windows (IBM Corporation, Armonk, NY, USA) for analysis. The results are presented as the mean ± standard deviation. The Student's *t*-test and the chi-square test were used for statistical analysis, with a significance level set at $P<.05$.

3 | RESULTS

3.1 | Patient background and outcome of the cycles with empty zona pellucida

Table1 shows the patients' background and outcome of the cycles with an EZP. The mean age of the patients with cycles without an EZP (n = 30) was 36.4 ± 4.4 years (range: 31-43 years) and that of the patients with cycles with an EZP (n = 32) was 35.8 ± 4.4 years (range: 24-43 years), with no significant difference between groups

(which included overlaps of the patients). The mean number of oocytes that was collected was significantly higher in the cycles with an EZP (10.9 ± 7.0) than in the cycles without an EZP (6.6 ± 3.8) (*P*<.01). No significant difference was found in the LH, FSH, or estradiol levels between cycles, with and without an EZP, during menstruation before ovarian stimulation. The estradiol and progesterone levels before hCG administration were divided by the total number of oocytes that had been collected to give the hormone level per oocyte. The mean estradiol level per oocyte was 290.3 ± 166.4 pg/mL for the cycles without an EZP and 318.1 ± 307.5 pg/mL for the cycles with an EZP, while the mean progesterone level before hCG administration was 1.2 ± 1.1 ng/mL without an EZP and 1.3 ± .9 ng/mL with an EZP. Thus, no significant difference was found in the estradiol or progesterone level between cycles with or without an EZP. The mean estradiol level per oocyte on the day of oocyte retrieval was 196.0 ± 89.1 pg/mL for the cycles without an EZP and 194.2 ± 124.5 pg/mL for the cycles with an EZP, while the mean progesterone level was 8.6 ± 6.1 ng/mL without an EZP and 9.5 ± 8.7 ng/mL with an EZP, again with no significant difference in either parameter between cycles.

The mean fertilization rate, including both the conventional insemination and ICSI, was significantly lower in the cycles with an EZP (65.0 ± 22.0%) than in the cycles without an EZP (76.2 ± 20.4%) (*P*<.05). The mean rate of good embryo formation was significantly lower in the cycles with an EZP (48.1 ± 37.3%) than in the cycles without an EZP (68.3 ± 30.8%) (*P*<.01). The mean pregnancy rate per patient was 23.3% for the cycles without an EZP and 21.9% for the cycles with an EZP.

3.2 | Comparison of the patient background and outcome of cycles with empty zona pellucida according to the ovarian stimulation method

Table2shows patients' background and outcome of cycles with an EZP according to the ovarian stimulation method that was used. When the cycles with an EZP were analyzed for various parameters in relation to the ovarian stimulation methods (namely, the long protocol method [14 cycles, 14 patients] and the GnRH antagonist method [22 cycles, 20 patients]), the mean age of the patients was significantly higher for those who had been treated with the GnRH antagonist method (37.2 ± 4.5 years) than for those who had been treated with the long protocol method (33.6 ± 3.4 years) (*P*<.01). The mean number of oocytes that was collected, excluding EZP oocytes, was 12.6 ± 7.9 with the long protocol method and 9.8 ± 6.3 with the GnRH antagonist method, with no significant difference between methods.

The baseline LH, FSH, and estradiol levels, respectively, before ovarian stimulation were 3.7 ± 2.4 mIU/mL, 7.0 ± 3.3 mIU/mL, and 28.9 ± 13.3 pg/mL in the patients who had been treated with the long protocol method and 3.8 ± 2.4 mIU/mL, 10.1 ± 5.3 mIU/mL,

TABLE 2 Comparison of the backgrounds and outcomes of cycles with empty zona pellucida (EZP) by the ovarian stimulation method

Variable	Long protocol	GnRH antagonist	*P*-value
No. of cycles	14	22	—
No. of patients	14	20	—
Mean age (y)	33.6 ± 3.4	37.2 ± 4.5	<.01
Mean no. of oocytes collected (excluding EZP)	12.6 ± 7.9	9.8 ± 6.3	—
LH (baseline: mIU/mL)	3.7 ± 2.3	3.8 ± 2.4	—
FSH (baseline: mIU/mL)	7.0 ± 3.3	10.1 ± 5.3	<.05
Estradiol (baseline: pg/mL)	28.9 ± 13.3	29.3 ± 12.0	—
Estradiol/total no. of oocytes collected (before hCG administration)	462.0 ± 441.6	226.5 ± 119.3	<.05
Estradiol/total no. of oocytes collected (on the day of oocyte retrieval)	241.6 ± 158.1	164.1 ± 88.9	—
Progesterone (before hCG administration: ng/mL)	1.0 ± .8	1.4 ± 1.2	—
Progesterone (on the day of oocyte retrieval: ng/mL)	12.0 ± 11.1	7.8 ± 6.5	—
Fetilization rate (%) (including ICSI and CON)	73.2 ± 16.2	59.8 ± 23.9	<.05
Good embryo formation rate (%) (G1 or G2)	46.0 ± 39.3	49.5 ± 37.0	—
Pregnancy rate (%)	28.6	15.0	—

CON, conventional insemination; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; LH, luteinizing hormone.

and 29.3 ± 12.0 pg/mL in those who had been treated with the GnRH antagonist method; the FSH level was significantly higher in those who had been treated with the GnRH antagonist method ($P<.05$).

The mean estradiol level per oocyte before hCG administration was significantly higher with the long protocol method (462.0 ± 441.6 pg/mL) than with the antagonist method (226.5 ± 119.2 pg/mL) ($P<.05$). The respective progesterone levels were 1.0 ± .8 ng/mL and 1.4 ± 1.2 ng/mL, with no significant difference between ovarian stimulation methods. The mean estradiol level per oocyte on the day of oocyte retrieval was 241.6 ± 158.1 pg/mL and 164.1 ± 88.9 pg/mL in those who had been treated with the long protocol and antagonist methods, respectively, and the respective progesterone levels were 12.0 ± 11.1 ng/mL and 7.8 ± 6.5 ng/mL, with no significant difference in either parameter between the stimulation methods.

The mean fertilization rate was significantly higher with the long protocol method (73.2 ± 16.2%) than with the antagonist method (59.8 ± 23.9%) ($P<.05$). The rate of good embryo formation was 46.0 ± 39.0% with the long protocol method and 49.5 ± 37.0% with the antagonist method, with no significant difference between stimulation methods. The pregnancy rate was higher with the long protocol method (28.6%) than with the antagonist method.

3.3 | Comparison between patients with and without empty zona pellucida oocytes

Table3 shows a comparison between the patients with and without EZP oocytes. No EZP was collected during any oocyte retrieval cycle from 23 patients, while an EZP was collected during all cycles from 25 patients, and seven patients experienced cycles both with and without an EZP. The respective mean age for these groups was 36.9 ± 4.0 years, 36.2 ± 4.1 years, and 34.6 ± 5.4 years, with no significant difference found between them. The mean number of normal oocytes that were collected was highest in those with an EZP that had been collected during all cycles ($P<.01$). The estradiol level per collected oocyte, measured before and after hCG administration, showed no significant difference between the three groups. A similar result was obtained for the progesterone level both before and after hCG administration.

The fertilization rate for the collected oocytes was significantly lower in those with an EZP that had been collected during all cycles: 75.7 ± 19.8% in those with no EZP collected during any cycle, 63.0 ± 20.8% in those with an EZP collected during all cycles, and 74.2 ± 24.6% in those experiencing both cycles. The rate of good embryo formation was significantly higher in those with no EZP being collected during any cycle: 71.6 ± 28.3%, 53.1 ± 37.8%, and

TABLE 3 Comparison of patients with and without empty zona pellucida (EZP) oocytes

Variable	EZP(−)	EZP(+) in all cycles	EZP(−) and (+)	*P*-value
No. of cycles	29	28	15	—
No. of patients	23	25	7	—
Mean age (y)	36.9 ± 4.0	36.2 ± 4.1	34.6 ± 5.4	—
Mean no. of oocytes collected (excluding EZP)	6.5 ± 3.9	11.9 ± 7.5	7.3 ± 3.1	<.01
LH (baseline: mIU/mL)	3.9 ± 3.0	3.8 ± 2.5	4.0 ± 2.3	—
FSH (baseline: mIU/mL)	9.9 ± 6.7	8.5 ± 5.2	10.3 ± 3.1	—
Estradiol (baseline: pg/mL)	29.6 ± 14.7	27.9 ± 12.4	30.4 ± 11.1	—
Estradiol/total no. of oocytes collected (before hCG administration)	311.6 ± 171.5	295.9 ± 238.6	305.3 ± 371.0	—
Estradiol/total no. of oocytes collected (on the day of oocyte retrieval)	211.3 ± 89.6	193.8 ± 118.4	166.3 ± 118.7	—
Progesterone (before hCG administration: ng/mL)	1.4 ± 1.2	1.3 ± .8	1.1 ± 1.1	—
Progesterone (on the day of oocyte retrieval: ng/mL)	9.0 ± 6.2	9.0 ± 7.2	9.2 ± 10.3	—
Fetilization rate (%) (including ICSI and CON)	75.7 ± 19.8	63.0 ± 20.8	74.2 ± 24.6	<.05
Good embryo formation rate (%) (G1 or G2)	71.6 ± 28.3	53.1 ± 37.8	53.5 ± 54.9	<.05
Pregnancy rate (%)	30.4	20.0	28.6	—

CON, conventional insemination; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; LH, luteinizing hormone.

53.5 ± 54.9%, respectively ($P<.05$). The pregnancy rate per patient was also significantly higher in those with no EZP being collected during any cycle: 30.4%, 20.0%, and 28.6%, respectively.

4 | DISCUSSION

Empty zona pellucida formation is considered to occur through two mechanisms: (i) the prolapse of the cell through a crack that is created in the ZP during oocyte retrieval; or (ii) denuding or shrinkage of the cell in the ZP.[1,2] Empty zona pellucida are often found during oocyte candling during IVF or the denuding of a cumulus oocyte complex. Although EZP formation is a well-known phenomenon, only a few studies have described the mechanism underlying it. It was thought that the ratio of empty ZP (36/281 cycles = 12.8%) was relatively higher than in the usual setting. The oocyte retrieval was performed by manual aspiration with a syringe and this method was a cause of the high rate of EZP.[2] As an aspiration pump was not available and sometimes the flushing of the follicles is performed after the initial aspiration, aspiration usually was performed manually. When aspirating by syringe, the authors are usually careful not to make the aspiration pressure become too strong. For this reason, the aspiration speed was set at ~25 mL/min.

The oocyte retrieval cycles without an EZP were associated with significantly higher rates of fertilization and good embryo formation and a higher rate of pregnancy than the oocyte retrieval cycles with an EZP. These results suggest a poorer quality of oocytes being collected if they have an EZP during the same cycle. However, no significant difference was observed between groups with and without an EZP in terms of age or ovarian residual of an EZP, based on the results of the ovarian function tests alone. Moreover, the observation of no significant difference in estradiol levels per oocyte before and after hCG administration or in the estradiol hormone-producing capacity per follicle or oocyte, with or without an EZP, also suggest the maintenance of the steroid-producing capacity in an environment where EZP oocytes can be formed.

In total, the number of oocytes that was collected during the cycles with an EZP was significantly higher than that for the cycles without an EZP. This is probably because patients with polycystic ovarian syndrome (PCOS) were included in the cycles with an EZP. Although no patient with PCOS was included in the cycles without an EZP, three of the 36 cycles with an EZP occurred in patients with PCOS, suggesting that an EZP is more likely to occur in patients with PCOS.

Then, it was attempted to determine the effective ovarian stimulation method for cycles (patients) with the potential for EZP formation (Table2). No significant difference was observed in the estradiol or progesterone level per oocyte that was collected by either the long protocol or antagonist method, suggesting an equivalent hormone-producing capacity following both stimulation methods. Both the fertilization and pregnancy rates were higher in the patients who were treated with the long protocol method. However, this does not mean that the long protocol method is more effective in EZP-forming patients; rather, it suggests that the long protocol method is more frequently chosen for younger patients, therefore resulting in higher fertilization and pregnancy rates.

When the patients were divided into those with an EZP that had been collected during all oocyte retrieval cycles, those with no EZP that had been collected during any cycle, and those experiencing cycles both with and without an EZP and their background factors were compared, no significant difference was observed in the baseline hormone levels or estradiol and progesterone levels per collected oocyte, while the mean number of oocytes that had been collected was significantly higher in those with an EZP that had been collected during all cycles. These findings suggest that it is possible to predict the occurrence of EZP oocytes in patients with a higher oocyte yield, such as those with PCOS, with a certain degree of accuracy. The fertilization rate was significantly lower in the patients with an EZP that had been collected during all cycles, while the rates of good embryo formation and pregnancy were higher, although not significantly, in those with no EZP being collected during any cycle, suggesting the potential for the presence or absence of EZP oocytes as a predictor of a good outcome. This is further supported by the fact that the rates of fertilization, good embryo formation, and pregnancy for the seven patients who experienced cycles both with and without an EZP were intermediate between the rates of the patients without an EZP and the rates for the patients with an EZP.

The presence of EZP oocytes appears to indicate a reduced overall capacity for obtaining good oocytes. Thus, oocytes that are collected during EZP-producing cycles are likely to be of originally poor quality, to have a ruptured ZP, and to form an EZP.

DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. *Human and Animal Rights*: All the procedures were followed in accordance with the ethical standards of the institutional ethical committee and with the Helsinki Declaration of 1964 and its later amendments. The institutional review board of Shimane University, Izumo City, Japan, approved this study to collect data from the medical records of the patients who underwent IVF treatment. Written informed consent was obtained from each patient. This article does not contain any study with animal participants that was performed by any of the authors.

ORCID

Aki Oride http://orcid.org/0000-0001-6609-2487
Haruhiko Kanasaki http://orcid.org/0000-0001-7011-8109

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