

Excessive Hormones Concentration in Serum May Increase the Risk of Polyspermy for Single Oocyte

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Research

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Abstract

Background: Fertilization of an oocyte by multiple sperms is known as polyspermy. Here, the purpose of this work was to investigate the possible correlation between hormones concentration in serum and the fertilization outcome of single oocyte in IVF cycles.

Methods: In this retrospective analysis, the baseline characteristics and serum hormones levels of female patients retrieving single oocyte in IVF cycles were analyzed. Female age, duration of infertility, body mass index (BMI), basal follicle stimulating hormone (FSH), basal luteinizing hormone (LH), basal estradiol (E2), the level of FSH, LH, E2 and progesterone (P) on the day of Gonadotropin (Gn), the level of FSH, LH, E2 and P on the day of human chorionic gonadotropin (HCG) were measured.

Results: According to the number of pronucleus in single oocyte on Day1, patients were assigned to IVF-2PN group (two pronucleus) and IVF-3PN group (three or more pronucleus). There were extremely higher levels of basal E2, LH and E2 on the day of Gn in IVF-3PN group compared with IVF-2PN group ($P<0.01$). The significantly higher level of LH on the day of HCG was present in IVF-3PN compared to IVF-2PN group ($P<0.05$). Furthermore, the optimal cutoff value of basal E2 (70.5 IU/L), LH (6.315 IU/L) and E2 (128.5 IU/L) on the day of Gn and LH (3.28 IU/L) on the day of HCG were determined by ROC curve analysis. The results showed that the percentage of IVF-2PN significantly decreased and the percentage of IVF-3PN considerably increased when the basal E2 concentration was higher than the cutoff value ($P<0.05$), the same tendency were present in the analysis of LH concentration, E2 concentration on the day of Gn and LH concentration on the day of HCG ($P<0.01$).

Conclusions: Our data indicated for the first time that the excessive concentration of basal E2, excessive concentrations of LH and E2 on the day of Gn and excessive concentration of LH on the day of HCG may significantly increase the risk of polyspermy for single oocyte in IVF cycles.

Plain English Summary

Oocyte that fuses with more than a single sperm contain an excessive amount of genetic material contributed by the male and are said to be polyspermic, which is catastrophic to human embryonic development in vitro.

In this study, total of 433 patients from in vitro fertilization (IVF) cycles were analyzed. We have made comparisons on basic information and serum hormones levels between IVF-2PN group (two pronucleus) and IVF-3PN group (three or more pronucleus). We also determined the optimal cutoff value of basal E2, LH and E2 on the day of Gn and LH on the day of HCG by Receiver Operator Characteristic (ROC) curve analysis. The data indicated that the percentage of IVF-2PN significantly decreased and the percentage of IVF-3PN considerably increased when these hormones concentration were higher than the cutoff value.

In conclusion, for the patients retrieving single oocyte in IVF cycles, excessive hormones concentration may significantly increase the risk of polyspermy.

Background

Fertilization is an essential biological process in sexual reproduction and contains a series of molecular interactions between the sperm and oocyte [1, 2]. Mammalian fertilization has been studied as a sequence of steps: sperm bind and penetrate through the zona pellucida of the oocyte, adhere to the oocyte plasma membrane and finally fuse with the oocyte. Sperm-oocyte fusion is indispensable for completing mammalian fertilization. Among the various steps of fertilization, gamete membrane fusion must be an extremely robust and precise mechanism, as it is the climax of fertilization [3].

Fertilization of an oocyte by multiple sperms, a condition is known as polyspermy, is catastrophic to embryonic development in most sexually reproducing species [4]. The rate of polyspermy from in vitro fertilized human oocytes is around 10% and therefore remarkably high [5]. Two primary safeguards protect oocytes against polyspermy: the “oocyte membrane block” and the “zona reaction”. The former involves a depolarization of the oocyte membrane, polyspermy is prevented at the plasma membrane level, where fusion of the first sperm causes depolarization of the membrane and shedding of the oocyte’s sperm receptor Juno, thereby preventing fusion of further sperm [6, 7], transiently contributing to polyspermy block. The “oocyte membrane block” or so-called “fast block” to polyspermy occurs in seconds [8, 9]; The second proposed mechanism involves a Ca^{2+} oscillation event activated by attachment of the sperm to the oocyte membrane. The subsequent increase in intracellular Ca^{2+} concentration triggers the exocytosis of cortical granules from just below the oocyte membrane into the perivitelline space. Cortical granules contain enzymes such as hydrolase, proteinase, and peroxidase that modify and thereby harden the zona pellucida [10]. This “slow block” occurs within approximately 5 to 8 min of oocyte activation and is considered the main mechanism of polyspermy block in humans [11].

A variety of oocyte-related factors contribute to the pathogenesis of polyspermy, including the degree of oocyte maturation, changes in the molecular biology of the ZP, and alterations in the cortical reaction [6, 12–14]. It also has been shown that the phosphatidylinositol signaling pathway plays an important role in the cortical reaction, and alterations of this pathway might disrupt the membrane block leading to polyspermy [15]. Understanding the mechanisms that protect mammalian oocytes from polyspermy is not only of interest for fundamental research but also of direct medical relevance.

In human assisted reproductive technology, polyspermy is lethal to the embryo development in vitro, particularly to the patients who retrieved single oocyte which is so precious. Therefore, our study was the first time to focus on the possible correlation between hormones concentration in serum and the fertilization outcome of single oocyte in IVF cycles.

Methods

Study Population

In this retrospective study, total of 433 patients from conventional IVF between 2015–2019 were analyzed: The inclusion criteria: a) the female patients retrieved single oocyte during the operation; b) the fertilization outcome of single oocyte was 2PN or 3PN.

Controlled ovarian hyperstimulation protocol

In all of IVF cycles, the types of controlled ovarian hyperstimulation protocol were mini-stimulation cycles, natural/semi-natural ovulation cycles, superovulation cycles and antagonist cycles.

Criteria for grouping

In selected IVF cycles, according to the number of pronucleus in single oocyte on Day1, patients were assigned to two groups: a) IVF-2PN group: one female pronucleus and one male pronucleus were present in the oocyte; b) IVF-3PN group: three or more pronucleus were present in the oocyte.

Statistical analyses

Differences in age, duration of infertility, BMI, basal hormone levels and the levels of FSH, LH, E2 and P during medication were analyzed by *T-test* using a SPSS version 22 (IBM Corp., USA). The values were expressed as the mean \pm standard error. The values with $P < 0.05$ were considered statistically significant.

Results

Baseline characteristics of patients

Comparison of demographic information from the patients, such as age, duration of infertility, BMI and basal hormone levels between IVF-2PN group and IVF-3PN group were listed in Table 1. There was a significantly higher level of basal E2 in IVF-3PN group compared with IVF-2PN group ($P < 0.01$).

Information of patients during medication

In the period of controlled ovulation, the main information of female patients, such as total usage of Gn, the levels of FSH, LH, E2 and P in serum on the day of Gn; total usage of HCG, the levels of FSH, LH, E2 and P in serum on the day of HCG were comparative in the two groups (Table 2). There were considerably higher levels of LH and E2 in serum on the day of Gn in IVF-3PN group compared with IVF-2PN group ($P < 0.01$). In addition, a significantly higher level of LH in serum on the day of HCG was showed in IVF-3PN group compared to IVF-2PN group ($P < 0.05$).

The thresholds of several hormone concentrations in serum

The optimal cutoff value of basal E2 (70.5 IU/L), LH (6.315 IU/L) and E2 (128.5 IU/L) in serum on the day of Gn and LH (3.28 IU/L) in serum on the day of HCG were determined by ROC curve analysis. The percentage of IVF-2PN significantly decreased and the percentage of IVF-3PN considerably increased when the basal E2 concentration was higher than the cutoff value ($P < 0.05$) (Table 3), the same tendency

were present in the analysis of LH concentration–E2 concentration on the day of Gn (Table 4, Table 5) and LH concentration on the day of HCG ($P < 0.01$) (Table 6).

Discussion

Sexual reproduction culminates in fertilization and occurs when two haploid gametes recognize each other and fuse to form a single diploid zygote [16]. Eggs that fuse with more than a single sperm contain an excessive amount of genetic material contributed by the male and are said to be polyspermic, resulting in the formation of a nonviable embryo. Viable human embryo can only develop from an oocyte that is fertilized by a single sperm [17]. However, the incidence of polyspermy is 3–10% in IVF cycles [18, 19].

The phospholipase C (PLC)-induced cascade of events was one of the earliest known signaling pathways initiated by fertilization. Fast polyspermy block after fertilization in *X. laevis* eggs is mediated by activation of PLC, which increases inositol 1,4,5-trisphosphate (IP3) and evokes Ca^{2+} release from the endoplasmic reticulum (ER). The calcium rise is caused by the opening of IP3-gated calcium channels in the ER [20]. This ER-derived Ca^{2+} then activates a Cl^- channel to induce the fast polyspermy block [21]. So, a major question remains to be elucidated. How does the sperm-egg interaction activate PLC to increase IP3 and release of Ca^{2+} from the ER in the oocyte? Our results indicated that the significant differences in the level of LH and E2 on the day of Gn, the level of LH on the day of HCG between IVF-2PN group and IVF-3PN group may be relative to the fertilization outcome of single oocyte.

During the development of primary follicles, gap junctions construct channels for material and information exchange between granulosa cells and oocytes. Some hormones, ions and signal molecules (such as cAMP, Ca^{2+} , IP3, etc.) can communicate between cells and cause important biological effects. For example, the signal molecules produced by oocytes can be transported to granulosa cells to maintain the continuous proliferation and development of granulosa cells and prevent the premature differentiation of granulosa cells. In the secondary follicular stage, the follicular membrane began to differentiate into two layers: the inner and outer membranes. At this time, LH receptor has been expressed on endometrial cells. LH can act on endometrial cells and promote the synthesis and secretion of androgen. Androgen diffuses into granulosa cells, and then they are transformed into E2 by the aromatase in granulosa cells. There are receptors for E2 in the cytoplasm and nucleus of granulosa cells. The synthesized E2 has a positive feedback effect on granulosa cells and can stimulate the proliferation of granulosa cells. Combined with the above professional knowledge and our previous research results, we put forward such a scientific hypothesis: the more LH content, the more androgens synthesized by the endometrial cells, and the more E2 synthesized by the granulosa cells, then E2 promotes the proliferation of granulosa cells. At this time, in order to maintain the continuous proliferation and development of granulosa cells, the signal molecule IP3 produced by oocytes may be transported to granulosa cells through gap junctions, so the number of IP3 binding to endoplasmic reticulum ligands is reduced, which

leads to the insufficient release of Ca^{2+} in endoplasmic reticulum, the blocking mechanism of polyspermy can not be started, and which eventually results in the occurrence of polyspermy (Fig. 1).

The limitation of this paper is to analyze the differences of hormone levels in a retrospective way. In the next prospective cohort study, we will detect the hormones concentration secreted by granulosa cells from the cell level, and discover the gene variation in the peripheral blood from the genetic point of view.

Conclusion

The study was the first time to focus on possible correlation between hormones concentration in serum and the fertilization outcome of single oocyte in IVF cycles. Our data indicated that excessive concentrations of LH (≥ 6.315 IU/L) and E2 (≥ 128.5 IU/L) on the day of Gn and excessive concentration of LH (≥ 3.28 IU/L) on the day of HCG may increase the risk of polyspermy for single oocyte. Our findings also provide a strong evidence and a clear clinical guidance for clinicians: when the patient retrieving single oocyte suffered a 3PN fertilization fate in IVF, ICSI is recommended for the next cycle to avoid polyspermy if the relevant hormone concentrations were above the threshold.

Abbreviations

IVF: in vitro fertilization

ICSI: intracytoplasmic sperm injection

E2: Estradiol

LH: Luteinizing Hormone

FSH: Follicle Stimulating Hormone

P: Progesterone

Gn: gonadotropin

HCG: human chorionic gonadotropin

ROC: Receiver Operator Characteristic

BMI: body mass index

ZP: zona pellucida

PLC: phospholipase C

IP3: inositol 1,4,5-trisphosphate

ER: endoplasmic reticulum

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Medical Ethics Committee of Sun Yat-Sen Memorial Hospital affiliated with SunYat-sen University. Participants gave written informed consent.

Consent for publication

The article was approved for publication by Institutional Medical Ethics Committee of Sun Yat-Sen Memorial Hospital.

Availability of data and material

Data will not be shared, because the study contains human data, it is private.

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Authors' contributions

Haijing Zhao conceived of the manuscript and wrote the first draft, **Qi Qiu** and **Ping Yuan** performed the statistical analyses, **Nengyong Ouyang** and **Yuqin Zhu** collected and proofread data, **Wenjun Wang** participated in its design and helped to draft the manuscript. All authors reviewed the final manuscript. All authors read and approved the final manuscript.

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Not applicable.

Competing Interest

The authors declare no conflict of interest.

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Tables

Table 1

Baseline characteristics of patients from IVF-2PN group and IVF-3PN group.

	IVF-2PN group	IVF-3PN group	P
Female			
Mean age (y)	38.72 ± 0.28	39.06 ± 0.41	0.546
Duration of infertility	5.08 ± 0.24	5.06 ± 0.37	0.964
The total cycles	3.17 ± 0.15	3.80 ± 0.31	0.054
Infertility type	primary/secondary	primary/secondary	—
	95/236	33/74	
BMI (Kg/m ²)	22.42 ± 0.17	22.23 ± 0.36	0.608
Basal FSH (IU/L)	12.81 ± 0.49	12.30 ± 0.68	0.596
Basal LH (IU/L)	5.26 ± 0.26	6.05 ± 0.58	0.159
Basal E2 (IU/L)	69.29 ± 3.73	102.47 ± 14.46	0.002
Basal PRL (IU/L)	14.24 ± 1.11	17.06 ± 3.30	0.299
Male			
Mean age (y)	40.28 ± 0.34	40.91 ± 0.56	0.354
BMI (Kg/m ²)	24.52 ± 0.21	24.01 ± 0.29	0.196
Acrosomal enzyme	65.40 ± 2.27	74.12 ± 3.99	0.060
P < 0.05: significant difference			

Table 2

Information of patients from IVF-2PN group and IVF-3PN group during medication.

	IVF-2PN group	IVF-3PN group	P
Gn			
Total usage	1311.56 ± 63.03	1060.98 ± 82.52	0.038
Total day	7.08 ± 0.21	6.25 ± 0.39	0.058
Start usage	163.78 ± 3.70	161.33 ± 5.55	0.735
FSH (IU/L)	11.96 ± 0.38	12.34 ± 0.66	0.628
LH (IU/L)	5.64 ± 0.24	7.15 ± 0.55	0.004
E2 (IU/L)	123.16 ± 7.35	170.65 ± 19.58	0.006
P (IU/L)	1.12 ± 0.13	1.04 ± 0.17	0.738
HCG			
Total usage	9048.19 ± 123.27	9163.55 ± 217.87	0.644
FSH (IU/L)	25.95 ± 4.85	20.45 ± 1.17	0.521
LH (IU/L)	7.46 ± 0.35	9.22 ± 0.66	0.016
E2 (IU/L)	565.10 ± 22.16	508.48 ± 28.71	0.181
P (IU/L)	1.09 ± 0.13	1.32 ± 0.37	0.463
P < 0.05: significant difference			

Table 3

Comparison of the percentage of IVF-2PN and IVF-3PN between Low concentration group and High concentration group according to basal E2 level.

	Basal E2 (IU/L)		P
	Low concentration	High concentration	
Fertilization outcome	(< 70.5 IU/L)	(≥ 70.5 IU/L)	
IVF-2PN number (%)	232 (78.4)	94 (68.6)	0.028
IVF-3PN number (%)	64 (21.6)	43 (31.4)	0.028
P < 0.05: significant difference			

Table 4

Comparison of the percentage of IVF-2PN and IVF-3PN between Low concentration group and High concentration group according to LH level on the day of Gn.

LH (IU/L) (Gn)			
	Low concentration	High concentration	P
Fertilization outcome	(< 6.315 IU/L)	(\geq 6.315 IU/L)	
IVF-2PN number (%)	230 (79.9)	96 (66.2)	0.002
IVF-3PN number (%)	58 (20.1)	49 (33.8)	0.002
P < 0.05: significant difference			

Table 5

Comparison of the percentage of IVF-2PN and IVF-3PN between Low concentration group and High concentration group according to E2 level on the day of Gn.

E2 (IU/L) (Gn)			
	Low concentration	High concentration	P
Fertilization outcome	(< 128.5 IU/L)	(\geq 128.5 IU/L)	
IVF-2PN number (%)	225 (79.5)	101 (67.3)	0.005
IVF-3PN number (%)	58 (20.5)	49 (32.7)	0.005
P < 0.05: significant difference			

Table 6

Comparison of the percentage of IVF-2PN and IVF-3PN between Low concentration group and High concentration group according to LH level on the day of HCG.

LH (IU/L) (HCG)			
	Low concentration	High concentration	P
Fertilization outcome	(< 3.28 IU/L)	(\geq 3.28 IU/L)	
IVF-2PN number (%)	98 (86.7)	228 (71.3)	0.001
IVF-3PN number (%)	15 (13.3)	92 (28.7)	0.001
P < 0.05: significant difference			

Figures

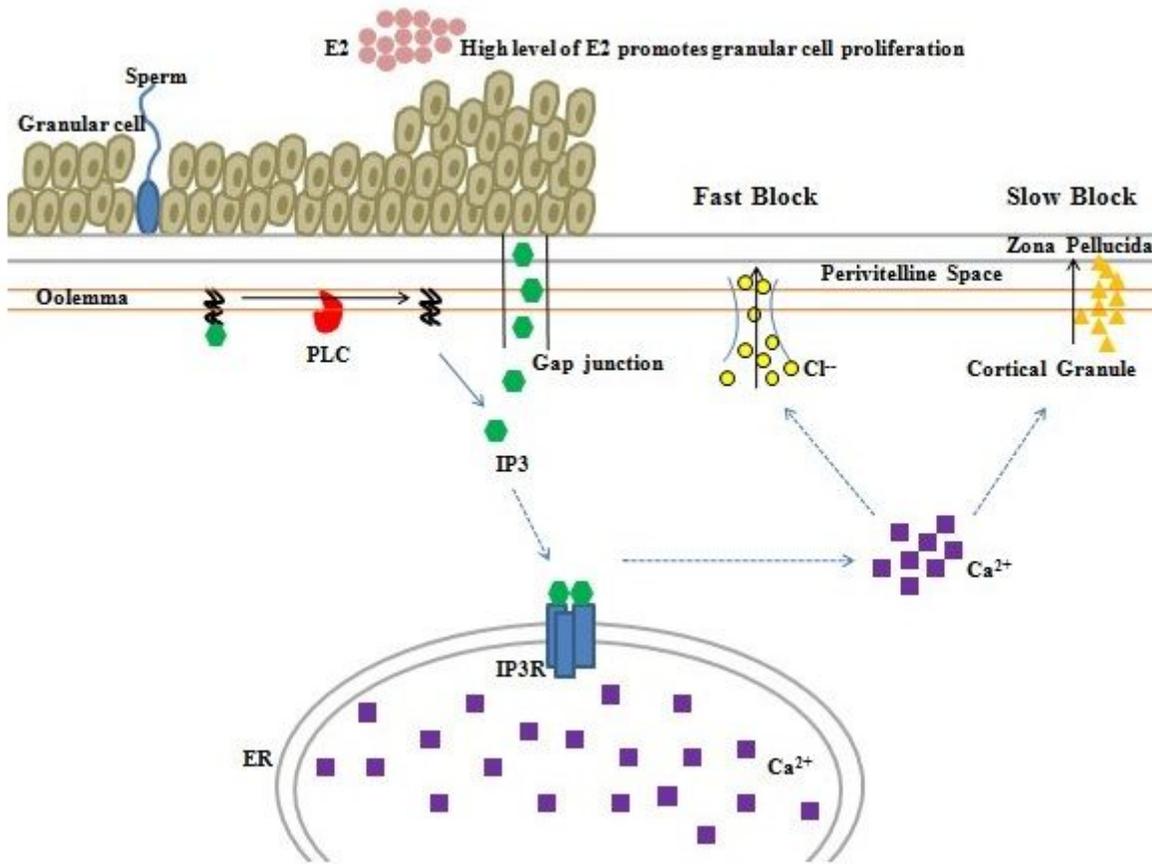


Figure 1

Excessive concentration of E2 negatively regulates the phosphatidylinositol signaling pathway and affects the blocking mechanism of polyspermy.