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Effects of different polyvinylpyrrolidone concentrations on intracytoplasmic sperm injection

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Summary

To explore whether different polyvinylpyrrolidone (PVP) concentrations affect the results of intracytoplasmic sperm injection (ICSI), a prospective study was conducted for 194 couples undergoing 210 ICSI therapy cycles. These cycles were divided into three groups (10, 7 and 5% groups) using the corresponding concentration of PVP for sperm immobilization. The main outcome measures were analyzed. Results indicated that, with a decrease in PVP concentrations, all of the main outcome measures increased. In particular, the high-quality cleavage embryo rate in the 7% group was significantly lower than in the 5% group ($P < 0.01$), and the cleavage, high-quality cleavage embryo, and high-quality blastocyst rates in the 5% group were significantly higher than those in the 10% group (all $P < 0.001$). For high-/intermediate-quality semen, all of the main outcome measures were significantly increased with 5% PVP. For the poor-quality semen, only the high-quality cleavage embryo and high-quality blastocyst rates were significantly higher in the 5% group. Therefore, lowering PVP concentrations greatly promoted the development of embryos in ICSI cycles, with an optimal concentration of 5% for ICSI.

Introduction

Intracytoplasmic sperm injection (ICSI) is a crucial technology in assisted reproductive technology (ART), mainly for treatment of male factor infertility (Esteves *et al.*, 2017; Rosenwaks and Pereira, 2017). During ICSI, motile sperm with motility and normal morphology are selected, immobilized, and captured under an inverted microscope with a magnification of ×200. Sperm immobilization is considered to be the most critical procedure before sperm injection (Esteves and Varghese, 2012; Palermo *et al.*, 2014; Luna *et al.*, 2015). This is because after sperm are injected into an egg, this will promote decondensation of the sperm head and activation of oocytes (Gerris *et al.*, 1995; Velaers *et al.*, 2012; Vanden Meerschaut *et al.*, 2014; Yeste *et al.*, 2017). Ultimately, this affects the results of fertilization and embryonic development. Sperm immobilization involves two consecutive processes, including a reduction in speed of sperm movement and stopping a sperm by rubbing its tail with an ICSI injection needle (Hussain *et al.*, 2011). Polyvinylpyrrolidone (PVP) has been successfully used for ICSI to increase the viscosity of the operating solution and reduce the speed of sperm movement, therefore facilitating sperm immobilization (Kimura and Yanagimachi, 1995; Martin, 2000; Kato and Nagao, 2012). PVP is also applied to control the movement of sperm inside the injection needle and therefore it is also unavoidably injected into the oocyte (Hlinka *et al.*, 1998; Hernandez-Lopez *et al.*, 2005; Kato and Nagao, 2012). Although PVP has good biocompatibility and excellent water solubility (Hussain *et al.*, 2011), it delays the onset of calcium oscillations in oocytes and causes chromosomal abnormalities in embryos in ICSI cycles (Hlinka *et al.*, 1998; Kato and Nagao, 2012; Andersen *et al.*, 2008). Inhibition of fertilization and embryonic development by PVP is a concern for the academic community (Kato and Nagao, 2009). Therefore, we need to investigate methods to reduce or eliminate the adverse effects of PVP in human ART. In the present study, we analyzed 210 ICSI therapy cycles to assess fertilization, cleavage, high-quality cleavage embryo, and high-quality blastocyst rates. This study aimed to examine the overall effects of different PVP concentrations on ICSI results.

Research Article

Cite this article: Ding D *et al.* (2020) Effects of different polyvinylpyrrolidone concentrations on intracytoplasmic sperm injection. *Zygote*. **28**: 148–153. doi: 10.1017/S0967199419000820

Received: 22 September 2019
Revised: 7 November 2019
Accepted: 27 November 2019
First published online: 14 January 2020

Keywords:
Polyvinylpyrrolidone; concentration; intracytoplasmic sperm injection; semen; development

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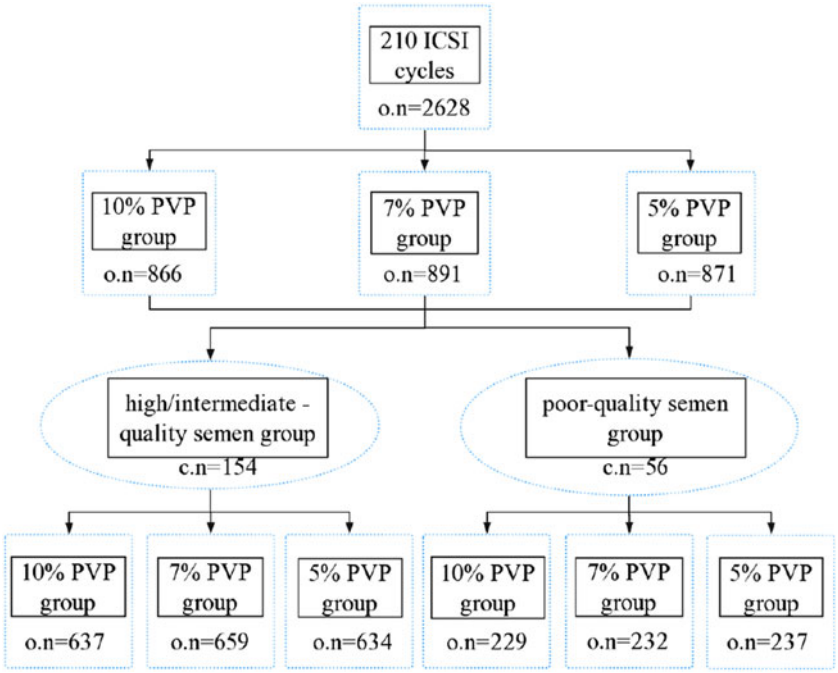

Figure 1. Experimental flow chart of the groups. c.n, cycle number; o.n, oocyte number.

Materials and methods

Study participants

In total, 194 couples who underwent 210 ICSI cycles from May 2018 to December 2018 in the Center for Reproductive Medicine of the First Affiliated Hospital of Anhui Medical University, China were included in this prospective study. All couples who were enrolled in this study met the following inclusion criteria: conventional *in vitro* fertilization (IVF) failure in a previous cycle; male infertility, but excluding percutaneous epididymal sperm aspiration, testicular sperm aspiration, or testicular sperm extraction microsurgery; and female infertility caused by fallopian tube factors, with exclusion of polycystic ovary, endometriosis, or uterine deformation to minimize the possible effect of oocyte quality on treatment outcomes. The age of the female patients ranged between 24 and 37 years. Clinical informed consent for ART was obtained from all patients prior to entering ICSI therapy cycles.

Grouping

In total, 194 couples experienced 210 ICSI therapy cycles. Prior to performing ICSI insemination, each patient was randomly assigned into one of three groups by lottery (10% group, 7% group and 5% group), in which the collected mature oocytes in each group underwent ICSI insemination with a corresponding concentration (10, 7 or 5%) of PVP solution for sperm immobilization (10% group: $n = 866$, 7% group: $n = 891$ and 5% group: $n = 871$). Subsequently, to assess the effects of different PVP concentrations on ICSI results for different quality semen, these 210 ICSI cycles were further divided into two subgroups (high-/intermediate-quality semen group: 154 cycles and poor-quality semen group: 56 cycles) based on each patient's semen quality parameters. Due to the very small number in the high-quality semen group (19 cycles), therefore the high-quality semen group and the intermediate-quality semen group were combined into one subgroup (the high-/intermediate-quality group). Numbers in each group are shown in Fig. 1.

Ovarian stimulation, oocyte retrieval, and preparation

All of the female patients received ovarian stimulation for superovulation using gonadotrophin-releasing hormone (Diphereline®; Ipsen Pharma Biotech, France) combined with recombinant human follicle-stimulating hormone (Gonal F®; Serono Barueri, SP, Brazil) in accordance with routine, established ovarian induction protocols used in our centre. The patients received administration of 10,000 IU human chorionic gonadotropin (Pregnyl®; AESCA Pharma, Austria) when the diameter of at least two to three follicles reached 18 mm or greater. At 36 h after human chorionic gonadotropin injection, transvaginal ultrasound-guided oocyte retrieval was conducted. Cumulus–oocyte complexes were isolated and cultured in IVF fertilization medium (Cook, Sydney, Australia) at 37°C in 6% CO_2 in air for 4–6 h. After ovum pick-up, the cumulus–oocyte complexes were completely removed from the cumulus and corona cells using 80 U/ml hyaluronidase solution (VitroLife, Gotebor, Sweden) and mechanical disruption with fine-bore glass pipettes. Each oocyte was identified for its maturity under an inverted microscope and only mature oocytes (metaphase II) were picked up for subsequent ICSI insemination.

Sperm preparation

After 3–5 days of sexual abstinence for male patients, semen samples were collected through masturbation. After liquefaction at 37.0°C for 20–30 min, semen quality parameters, including sperm concentration, total motility, and percentage of normal morphology, were analyzed in strict accordance with

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Table 1. Comparison of patient characteristics

	10% PVP	7% PVP	5% PVP	*P*-value
Female age, years	29.18	29.50	28.76	ns
Male age, years	30.68	30.64	29.97	ns
Basic FSH level, mIU/ml	7.323	7.524	7.829	ns
Basic LH level, mIU/ml	5.109	7.494	6.574	ns
Basic E2 level, pmol/l	167.8	201.6	184.9	ns
Duration infertility, years	3.682	3.230	3.329	ns
Average no. oocyte retrieval	15.52	14.72	15.70	ns
BMI	21.98	22.18	22.14	ns

Data analyzed using Student's parametric *t*-test. Values are presented as means. ns, not significant.

World Health Organization standards (2010) (Ford, 2010). All of the semen samples were processed using density gradient centrifugation or swim-up technique depending on the concentration and activity of each sample.

PVP preparation

Prior to ICSI, a commercial 10% PVP solution (Sigma, St. Louis, MO, USA) was diluted to 7% or 5% with gamete medium (Cook). These three different concentrations of PVP solutions were used for subsequent ICSI operation.

ICSI and embryo culture

The ICSI procedure in this study was performed by a specific embryologist who had 10 years of experience in ICSI operation. The collected mature oocytes from each patient were picked up for subsequent ICSI insemination. Only the sperm with motility and normal morphology were selected for injection into the oocytes through an inverted microscope equipped with micromanipulators. After injection, the inseminated oocytes were placed into cleavage medium (Cook) at 37°C in 6% CO_2 for early embryo culture. In total, 16–18 h later, fertilization was assessed and the fertilized oocytes were picked up for a further 2 days of culture in the same medium. On day 3 post-ICSI, the embryos were moved into blastocyst medium (Cook) for an additional 2–3 days of blastocyst culture. During embryo culture, the number of high-quality cleavage embryos and high-quality blastocysts in each cycle was counted through observation under the invert microscope (Nikon, Tokyo, Japan).

Embryo grading

The quality of cleavage embryo was evaluated on the basis of the scoring criteria described by Tomas *et al.* (1998). On day 3 after ICSI, a cleavage embryo with 6–8 equal-sized blastomeres, as well as no fragments or <20% fragmentation, was designated as a high-quality cleavage embryo. The quality of blastocysts was scored in accordance with the Gardner blastocyst grading system (Gardner *et al.*, 2000). A blastocyst with >3BB grade on day 5 or >4BB on day 6 was considered as a high-quality blastocyst.

Semen classification

The semen specimens were classified as reported elsewhere (Damsgaard *et al.*, 2017; Wang *et al.*, 2019). In brief, if the total motility was <32%, and/or the concentration was <15 million/ml, and/or the normal morphology was <4%, these semen samples were categorized as the poor-quality semen; if the total motility >50%, the sperm concentration >40 million/ml and the morphology >9%, was classified as the high-quality semen. Other remaining semen samples were categorized as the intermediate-quality semen.

Statistical analysis

Statistical analysis was performed with SPSS for Window (version 21.0; SPSS Inc., Chicago, IL, USA). The differences in means between continuous variables (female age, male age, basic follicle-stimulating hormone (FSH) level, basic luteinizing hormone (LH) level, basic E2 level, duration infertility, average no. oocyte retrieval and body mass index (BMI)) were calculated using Student's parametric *t*-test. The categorical variables (rates of fertilization, cleavage, high-quality cleavage embryo, and high-quality blastocyst) in each group were analyzed using chi-squared test or Fisher's exact test. A *P*-value <0.05 was considered significant.

Results

The 194 couples underwent 210 ICSI therapy cycles, and these cycles consisted of three groups. The number of oocytes in these three groups (10% PVP, 7% PVP, and 5% PVP groups) was 866, 891 and 871, respectively. In addition, the majority of the basal parameters for all patients in each group are listed in Table 1. There was no significant difference in the baseline data among the three groups of patients.

The results of the study indicated that with a decrease in PVP concentrations, the fertilization, cleavage, high-quality cleavage embryo and high-quality blastocyst rates of ICSI cycles increased (Table 2). There were no significant differences in the fertilization rate, cleavage rate, and high-quality blastocyst rate between the 7% PVP and 5% PVP groups. However, the high-quality cleavage embryo rate was significantly lower in the 7% PVP group than in the 5% PVP group ($P < 0.01$). All of the above-mentioned parameters in either the 7% PVP or 5% PVP group were higher than those in the 10% PVP group (all $P < 0.05$; Table 1). In particular, the cleavage, high-quality cleavage embryo, and high-quality blastocyst rates in the 5% group were significantly higher than those in the 10% PVP group (all $P < 0.001$; Table 1).

Subsequently, to assess the effects of different PVP concentrations on ICSI results in patients with different semen quality, we further divided the 210 cycles of 194 male patients into the high/intermediate-quality and poor-quality semen groups on the basis of each patient's semen parameters. ICSI results in the high-/intermediate-quality semen group are shown in Fig. 2. The fertilization and cleavage rates in the 7% PVP group were equivalent to those in the 5% PVP group, with no significant difference between the groups. However, the fertilization and cleavage rates in these two groups were significantly higher than those in the 10% PVP group (all $P < 0.05$, respectively). The high-quality cleavage embryo and high-quality blastocyst rates at low PVP concentrations were higher than those at high PVP concentrations as follows. The high-quality cleavage embryo rate in the 5% PVP group was significantly higher than that in the 7% PVP and 10% PVP groups ($P < 0.001$, $P < 0.0001$, respectively). The high-quality blastocyst rate in the 5% group was significantly higher than that in the 10% PVP group ($P < 0.001$).

https://doi.org/10.1017/S0967199419000820 Published online by Cambridge University Press

Table 2. Effects of different polyvinylpyrrolidone concentrations on the results of ICSI

	10% PVP	7% PVP	5% PVP
Number of mature oocytes	866	891	871
Fertilization rate	77.94% (675/866)	82.94% (739/891)[a]	82.89% (722/871)[c]
Cleavage rate	92.00% (621/675)	95.67% (707/739)[a]	96.26% (695/722)[d]
High-quality cleavage embryo rate	69.40% (431/621)	75.53% (534/707)[b]	81.73% (568/695)[e,f]
High-quality blastocyst rate	42.67% (265/621)	49.93% (353/707)[a]	54.96% (382/695)[e]

Data analyzed using chi-squared test or Fisher's exact test.
Different symbols within columns and different letters within columns and within rows indicate significant differences. [a]$P < 0.01$, compared with the 10% PVP group; [b,c]$P < 0.05$, compared with the 10% PVP group; [d]$P < 0.001$, compared with the 10% PVP group; [e]$P < 0.0001$, compared with the 10% PVP group; [f]$P < 0.01$, compared with the 7% PVP group.

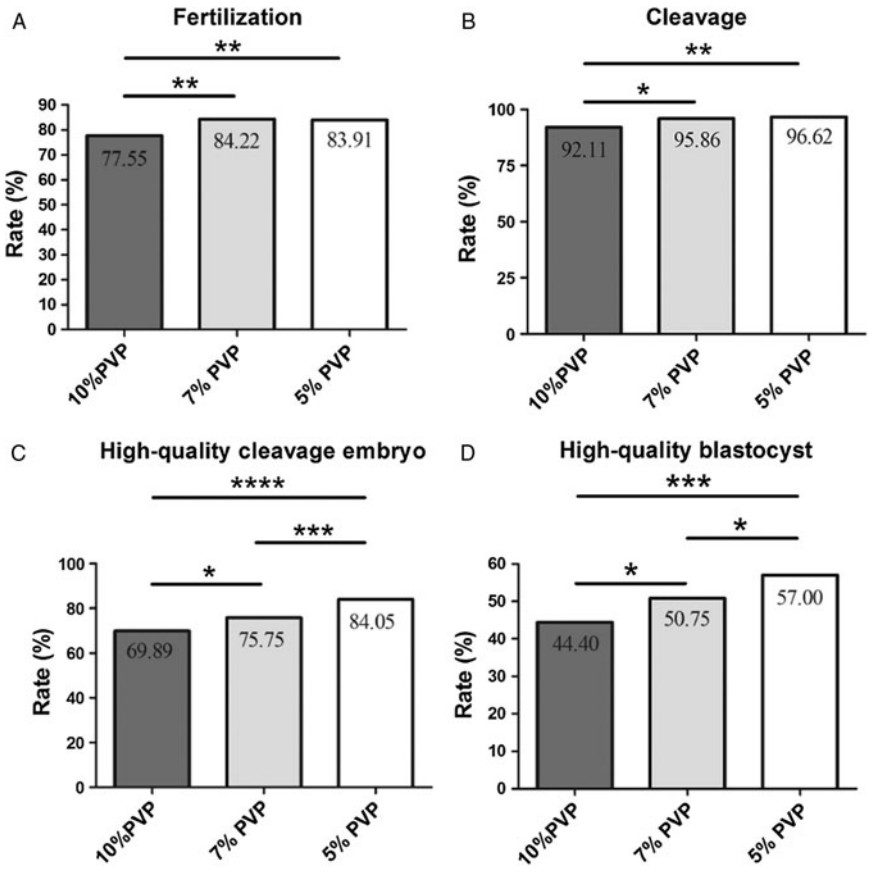

Figure 2. For sperm in the patients with high/intermediate-quality semen, comparisons among three groups of the rates of fertilization (*A*), cleavage (*B*), high-quality cleavage embryo (*C*) and high-quality blastocyst (D). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

The ICSI results in the poor-quality semen group are shown in Fig. 3. Only the high-quality cleavage embryo rate and high-quality blastocyst rate in the 5% PVP group were significantly higher than those in the 10% PVP group (both $P < 0.05$). However there were no significant differences in the other parameters among the groups.

Discussion

ICSI is an important ART and an invasive method of artificial insemination. ICSI is mainly used for therapy of male factor infertility or IVF fertilization disorder in which sperm are directly injected into the egg to complete the process of insemination (Schwarzer *et al.*, 2003; Yoeli *et al.*, 2008; Ward and Yanagimachi, 2018). However, this invasive insemination method inevitably causes mechanical or chemical damage to oocytes. Therefore, the safety of ICSI technology must be considered (Simopoulou *et al.*, 2016; Rubino *et al.*, 2016). Mechanical damage is mainly caused by injection of the ICSI needle into the cytoplasm, while chemical damage is caused by the unavoidable residue of PVP in the cytoplasm. In conventional ICSI procedures, 10% PVP is widely used as an essential reagent for reducing the speed of sperm movement. The operator can efficiently use the ICSI needle to wipe the sperm tail to achieve sperm immobilization. Furthermore, the operator can smoothly control the movement of the sperm in the injection needle during ICSI injection. However, even for the most skilled operator, ensuring that PVP is not left in the cytoplasm is difficult.

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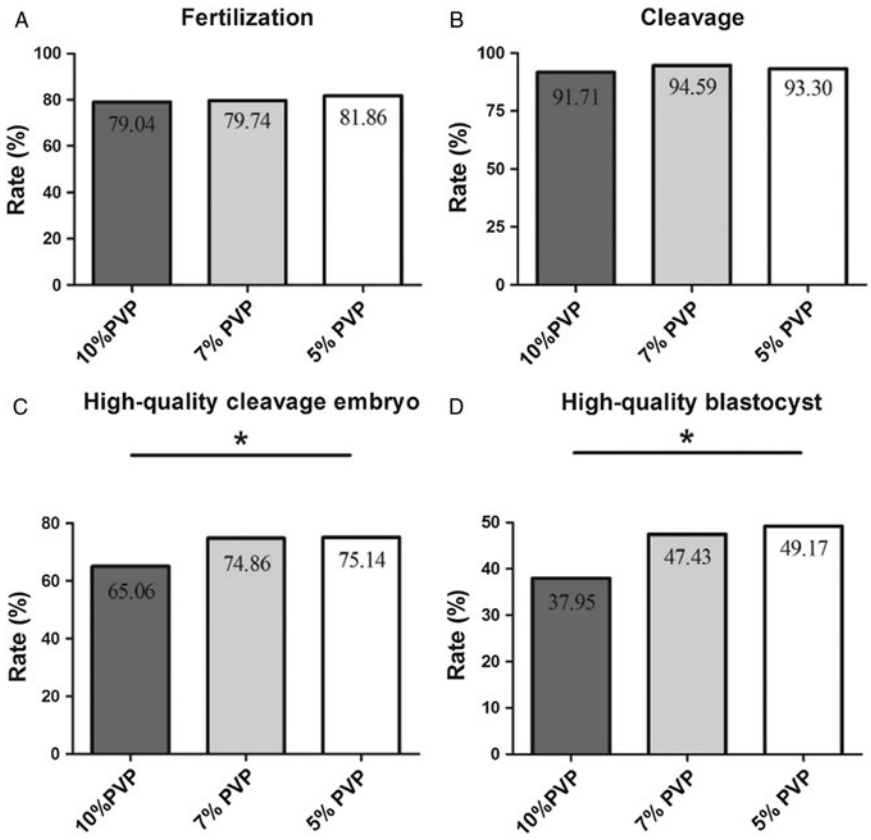

Figure 3. For sperm in the patients with poor-quality semen, comparisons among three groups of the rates of fertilization (*A*), cleavage (*B*), high-quality cleavage embryo (*C*) and high-quality blastocyst (*D*). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Studies have shown that PVP residues in the cytoplasm inhibit the decondensation of the sperm head and activation of oocytes, and ultimately affect the outcomes of fertilization and embryonic development (Kato and Nagao, 2009). This is also an important reason why the ICSI fertilization rate cannot reach 100%. Therefore, avoiding the use of PVP during ICSI operation appears to be a reasonable choice. However, in the conventional ICSI operation, if PVP is abandoned, the operator has difficulty in quickly and accurately identifying and capturing morphological normal sperm, as well as complete subsequent ICSI insemination. Therefore, lowering PVP concentrations is an effective method for alleviating the harmful effects of PVP on oocyte development.

In the present study, we used three concentrations (10, 7, and 5%) of PVP to immobilize sperm for subsequent ICSI. Differences in the rates of fertilization, cleavage, high-quality cleavage embryo, and high-quality blastocyst after ICSI were examined. We found that, with a decrease in PVP concentrations, the rates of fertilization, cleavage, high-quality cleavage embryos, and high-quality blastocyst of the ICSI cycle were increased. No significant differences were found in the fertilization, cleavage, and high-quality blastocyst rates between the 7% PVP and 5% PVP groups. However, the high-quality cleavage embryo rate was significantly lower in the 7% PVP group than in the 5% PVP group. All of the above-mentioned parameters in either the 7% PVP or 5% PVP group were higher than those in the 10% PVP group. In particular, the cleavage, high-quality cleavage embryo, and high-quality blastocyst rates in the 5% PVP group were much higher than those in the 10% PVP group. These results suggest that lowering PVP concentrations can greatly promote development of embryos in ICSI cycles. The reason for our findings may be because the detrimental effects of PVP are alleviated as the PVP concentration decreases.

However, if PVP concentrations are further reduced, immobilization and capturing operations for sperm become difficult, which greatly extends the operation time of ICSI. Previous studies have shown that the longer the oocytes exist *in vitro*, the greater the damage to their development potential, leading to formation of few high-quality cleavage embryos (Li *et al.*, 2018). Our data showed that 5% was the optimal PVP concentration for ICSI. A concentration of 5% PVP greatly improved the development potential of embryos in our study, but it did not extend the *in vitro* operation time for eggs during ICSI performance.

Subsequently, we further explored the effects of different PVP concentrations on ICSI results for different semen quality. In the high-/intermediate-quality semen group, we found that 5% PVP significantly increased the rates of fertilization, cleavage, high-quality cleavage embryo, and high-quality blastocyst. However, in the poor-quality semen group, only the high-quality cleavage embryo and high-quality blastocyst rates were higher in the 5% PVP group than in the 10% PVP group. These results indicated that 5% PVP promotes development of oocytes, regardless of the semen quality, and is the optimal concentration for ICSI.

In conclusion, our study indicated that lowering PVP concentrations greatly promoted development of embryos in ICSI cycles, and that 5% is the optimal concentration for ICSI. Our study provides a theoretical basis for optimizing the ICSI technology system. Large-scale trials are still required in the future to confirm

https://doi.org/10.1017/S0967199419000820 Published online by Cambridge University Press

the effect of different PVP concentrations on the rates of clinical pregnancy and live birth through ICSI therapy.

Acknowledgements. We thank all the staff of the IVF centre at the First Affiliated Hospital of Anhui Medical University for their support for this study. We thank Ellen Knapp from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Author contributions. Designed the study: ZGZ. Acquisition of data and performed clinical assessments: DD, XYL, BLC, WWZ, DMJ, YH, HJZ and RFX. Analyzed the data: DD, QSW and ZGZ. Wrote the manuscript: DD, QSW and ZGZ. Supervised the study: ZGZ, ZLW, ZP and YXC. All authors read and approved the final manuscript.

Financial support. This study was supported by special funds for development of Science and technology of Anhui province (YDZX20183400004194), Research Fund Project granted from Anhui Research Institute of Translational Medicine (2017zhyx30), and 2018 Anhui Key Research and Development Project (1804a07020128).

Conflicts of interests. The authors declare that they have no competing interests.

Ethical standards. This study was approved by the institutional ethics review board of the First Affiliated Hospital of Anhui Medical University.

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