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META-ANALYSIS

Use of hyaluronic acid for sperm immobilisation and selection before intracytoplasmic sperm injection: A systematic review and meta-analysis

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Abstract

AIM: To appraise critically the published randomised controlled trials (RCTs) reporting on the effectiveness of using hyaluronic acid (HA) for sperm immobilisation and selection before intracytoplasmic sperm injection (ICSI).

METHODS: Two authors used the PICO Method in order to perform a comprehensive literature search of the standard medical databases in June 2015. Data from the included studies was extracted independently by two authors using a predefined pro-forma. Review Manager (RevMan) was used to calculate the combined outcomes where multiple studies contributed with their results. Risk ratio (RR) with a 95%CI using the Mantel-Haenszel method was calculated for binary data variables. Heterogeneity was measured using the χ^2 test and quantified using I^2. In case of substantial heterogeneity ($P < 0.10$ for χ^2 test or $I^2 > 50\%$) the combined outcome was calculated using the random effects model. The results from the meta-analysis were displayed as forest plots. The guideline of the Cochrane Collaboration was used to assess the risk of bias and it was illustrated as a risk of bias graph.

RESULTS: The systematic literature search identified 166 different studies related to sperm immobilisation and selection for ICSI. Eleven RCTs involving 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or polyvinylpyrrolidone (PVP)

were included in this systematic review and meta-analysis. There was low heterogeneity among the included trials (χ^2 = 16.86, df = 11, P = 0.11; I^2 = 35%). There was no statistical difference between HA and PVP groups in terms of fertilisation rate (RR = 1.01; 95%CI: 0.99-1.03; z = 0.75; P = 0.45), good embryos rate (RR = 1.01; 95%CI: 0.96-1.06; z = 0.30; P = 0.76), live birth rate (RR = 1.15; 95%CI: 0.86-1.54; z = 0.92; P = 0.36), clinical pregnancy rate (RR = 1.04; 95%CI: 0.92-1.17; z = 0.62; P = 0.53) and implantation rate (RR = 1.17; 95%CI: 0.94-1.46; z = 0.40; P = 0.16). The quality of most of the included studies was moderate to poor because of unclear randomisation technique, inadequate allocation concealment and blinding.

CONCLUSION: This systematic review and meta-analysis provides evidence of similar efficiency between using HA or PVP for sperm immobilisation and selection before ICSI.

Key words: Hyaluronic acid; Sperm; Intracytoplasmic sperm injection

Core tip: Hyaluronic acid (HA) has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP. We performed a systematic review and meta-analysis of eleven randomised controlled trials involving 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or PVP. There was no difference between HA and PVP groups in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates.

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INTRODUCTION

The success of intracytoplasmic sperm injection (ICSI), as a bypass of the natural selection processes taking place in the female reproductive tract, would be impossible without advances in the laboratory preparation and identification of sperm for use with ICSI. Several methods (ultramorphology, surface electric charge, apoptotic *vs* nonapoptotic, chromatin structure assay) have been recently proposed for optimising the sperm selection in order to reduce the risk of chromosomal anomalies associated with poor ICSI outcome[1,2].

Hyaluronic acid (HA) is found naturally in the women's reproductive tract and it forms a component of the cumulus-oocyte complex. It has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP[3,4].

Furthermore, it has been shown that sperm's capacity to bind HA is a biochemical marker of maturity and function, suggesting the selection of sperm by HA binding to be an alternative to microscopic assessment of motility and morphology[3].

Several descriptive reviews of the current advanced sperm selection methods support the use of HA for sperm immobilisation and selection for ICSI, but none of them report a quantitative measure of the effect it has on the ICSI outcome[5-8].

The objective of this study is to appraise critically the published randomised controlled trials (RCTs) reporting on the use of HA for sperm immobilisation and selection before ICSI.

MATERIALS AND METHODS

Literature search

We used the PICO Method[9] to formulate a specific and answerable clinical question following which we performed a comprehensive literature search based on a predefined protocol. The medical subject headings (MeSH) "sperm injections, intracytoplasmic", "semen", "hyaluronic acid", "infertility", "fertilization" and "live birth" were combined with free terms "hyaluronan", "sperm", "ICSI", "PICSI", "SpermCatch", "SpermSlow", "polyvinylpyrrolidone", "PVP", "embryo quality", "pregnancy", "implantation", "costs", "adverse events" in order to search Medline/PubMed/PMC, Cochrane Central Register of Controlled Trials (CENTRAL), EBSCOhost, ClinicalTrials.gov and Google Scholar from inception until June 2015. The "Related citations" function and hand search of references were used for all relevant studies in order to identify additional RCTs.

Study selection

We set our inclusion criteria as RCTs evaluating sperm immobilisation and selection using HA before ICSI with no filter for date, country or hospital of origin, publication language, sample size or blinding. For studies presented in more than one publication, we only included the most extensive and recent version in order to avoid overlapping data.

Endpoints

The primary endpoints of the present meta-analysis were defined as: fertilisation rate, embryo quality and live birth rate. Secondary endpoints were: clinical pregnancy and implantation rates, adverse events and costs.

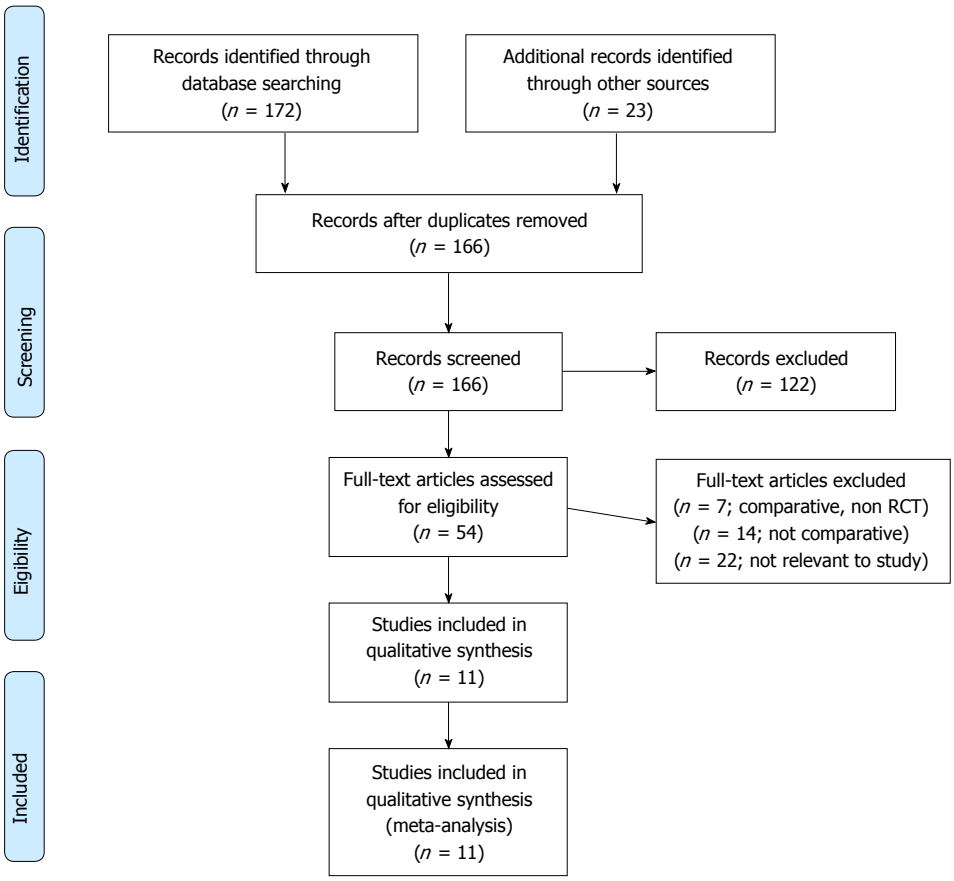


Figure 1 PRISMA flow chart showing trial selection methodology. RCT: Randomised controlled trial.

Data extraction

Two authors extracted the data following the literature search and study selection using predefined tables. Information related to first author, year of publication, country of origin, age of participants, inclusion criteria, the day of embryo transfer, number of embryos transferred, publication type, intervention protocols, number of participants, fertilisation rate, embryo quality, clinical pregnancy rate, implantation rate, live birth rate, adverse events, costs, randomisation technique, allocation concealment, blinding and data reporting was retrieved for each of the included studies. We contacted the study authors in order to obtain more data where it was required.

Statistical analysis

The software package RevMan 5.2.11[10], provided by the Cochrane Collaboration, was used for statistical analysis. We calculated the risk ratio (RR) with a 95%CI using the Mantel-Haenszel method[11] for binary data variables.

We measured the heterogeneity using the χ^2 test and quantified it[12] using I^2. In case of substantial heterogeneity ($P < 0.10$ for χ^2 test or $I^2 > 50\%$) we reported the combined outcome calculated using the random effects model[13]. Forest plots were used for the visual display of the results from the meta-analysis.

The guideline of the Cochrane Collaboration[14] was used to assess the risk of bias and it was illustrated as a risk of bias graph. GradePro (Version 3.2 for Windows) provided by the Cochrane Collaboration[15] was used to generate the summary of the evidence.

We performed subgroup analysis based on publication type (full text *vs* abstract) and type of reporting of the results (per women *vs* per cycle) for each of the variables with summated outcome.

RESULTS

The systemic literature search identified 166 different studies related to sperm immobilisation and selection for ICSI. The PRISMA flow chart to explain the RCTs selection is shown in Figure 1. The summary of the evidence is presented in Figure 2. Eleven RCTs[5,16-25] evaluating 13719 oocyte intracytoplasmatic injections with sperm immobilised and selected using HA or PVP were included in this systematic review and meta-analysis. There were 6926 injections in the HA group and 6793 injections in the PVP group. The characteristics of the included RCTs are shown in Table 1, and the procedure protocols used for the women in all of the RCTs are shown in Table 2. Variables used to achieve a combined outcome are shown in Table 3. One RCT[17] included four arms and we analysed the data as for two studies. Seven RCTs[5,16,18,20,22,23,25] were

Outcome	Illustrative comparative risks[1] (95%CI)		Relative effect (95%CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk Polyvinylpyrrolidone (PVP)	Corresponding risk Hyaluronic acid (HA)				
Fertilisation rate RR Follow-up: mean 8 wk	Study population 740 per 1000 Moderate 745 per 1000	747 per 1000 (733 to 762) 752 per 1000 (738 to 767)	RR 1.01 (0.99 to 1.03)	13719 (12 studies)	⊕⊕⊕⊖ moderate[1]	
Good embryos rate RR Follow-up: mean 8 wk	Study population 466 per 1000 Moderate 367 per 1000	471 per 1000 (447 to 494) 371 per 1000 (352 to 389)	RR 1.01 (0.96 to 1.06)	5834 (8 studies)	⊕⊕⊕⊖ moderate[1]	
Clinical pregnancy rate RR Follow-up: mean 8 wk	Study population 417 per 1000 Moderate 400 per 1000	433 per 1000 (383 to 487) 416 per 1000 (368 to 468)	RR 1.04 (0.92 to 1.17)	1367 (9 studies)	⊕⊕⊕⊖ moderate[1]	
Implantation rate RR Follow-up: mean 8 wk	Study population 150 per 1000 Moderate 164 per 1000	175 per 1000 (141 to 219) 192 per 1000 (154 to 239)	RR 1.17 (0.94 to 1.46)	1637 (4 studies)	⊕⊕⊕⊖ moderate[1]	
Liver birth rate RR Follow-up: mean 37 wk	Study population 253 per 1000 Moderate 263 per 1000	291 per 1000 (218 to 390) 302 per 1000 (226 to 405)	RR 1.15 (0.86 to 1.54)	473 (3 studies)	⊕⊕⊕⊖ moderate[1]	

[1]Selection, performance and detection bias due to inadequate concealment technique, blinding of personnel and outcome assessment. RR: Risk ratio.

Figure 2 Summary and strength of the evidence from trials analysed on GradePro®.

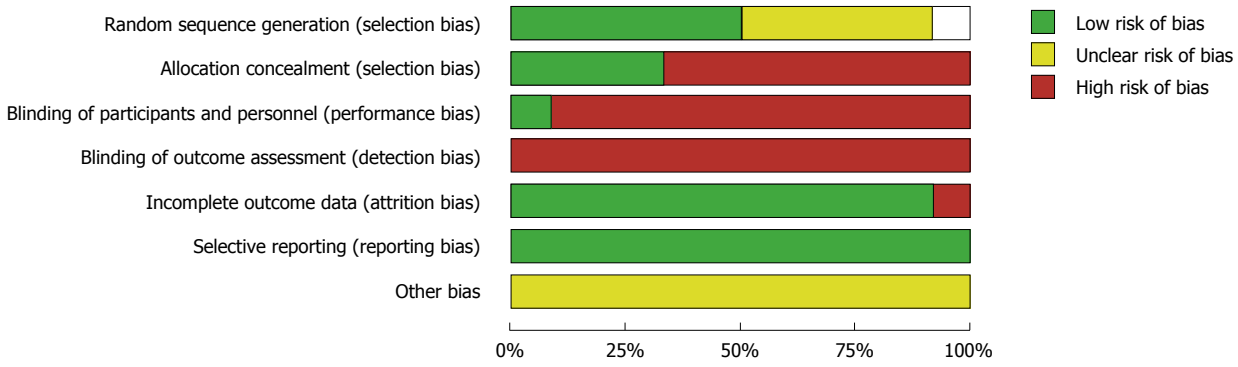


Figure 3 Risk of bias generated using cochrane risk of bias assessment tool.

published as full articles and four RCTs were published as abstracts[17,19,21,24]. Two RCTs[5,22] reported the results per ICSI cycle not per woman randomised. There was complete agreement between authors in terms of included studies and extracted data.

Methodological quality of included studies

Based upon the guidelines suggested by the Cochrane Collaboration, the quality of most of the included studies was moderate to poor because of unclear randomisation technique, inadequate allocation concealment and blinding (Figure 3).

The combined outcome of all of the variables is given below.

Fertilisation rate per oocyte injected

All the included RCTs reported on this outcome with low heterogeneity [χ^2 = 16.86, df = 11 (P = 0.11); I^2 = 35%] among them. The fertilisation rate was similar (RR = 1.01; 95%CI: 0.99-1.03; z = 0.75; P = 0.45; Figure 4A) in the HA group compared to the PVP group. The result did not change when we excluded the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.47) or when we used the random effects

Table 1 Characteristics of included trials

Trial	Country	Mean age	Inclusion criteria	Day of embryo transfer	Number of embryos transferred	Publication type
Balaban *et al*[16]						
HA	Sweden	NA	Male factor	Day 2-3	3.1	Full text
PVP					3.1	
Barak *et al*[5]						
HA	Israel	31.0	Male factor	Day 2-3	3.8	Full text
PVP		30.9			3.8	
Castillo-Baso *et al*[17]						
HA	Mexico	35.1	NA	Day 2-3 or day 5-6	NA	Abstract
PVP		35.3				
Choe *et al*[18]						
HA	Korea	35.4	Previous low fertilization rate Multiple IVF failures	Day 3 or day 5	NA	Full text
PVP						
Gandhi *et al*[19]						
HA	Spain	NA	NA	NA	NA	Abstract
PVP						
Majumdar *et al*[20]						
HA	India	31.7	Unexplained infertility, normal semen analysis	Day 2-3	2.49	Full text
PVP		31.5			2.39	
Moon *et al*[21]						
HA	Korea	NA	NA	Day 3 or day 5	NA	Abstract
PVP						
Parmegiani *et al*[22]						
HA	Italy	37.5	Sperm number > 10^6 and sperm motility > 5%	Day 2-3	2.25	Full text
PVP		37.1			2.15	
Van Den Bergh *et al*[23]						
HA	Switzerland	NA	Women younger than 38 yr with at least four metaphase Ⅱ oocytes	Day 2-3	NA	Full text
PVP						
Worrilow *et al*[24]						
HA	United States	NA	NA	NA	NA	Abstract
PVP						
Worrilow *et al*[25]						
HA	United States	33.3	Women younger than 40 years with at least four metaphase Ⅱ oocytes; Men with sperm count > 10000/mL	Day 2-3 or day 5-6	NA	Full text
PVP		33.7				

HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone; NA: Not available.

model for calculation (P = 0.83).

Good embryos rate per oocyte injected

Eight RCTs reported on this outcome with low heterogeneity [χ^2 = 12.08, df = 7 (P = 0.10); I^2 = 42%] among them. There were 2714 good quality embryos obtained from 5835 oocytes injected. No statistically significant difference was found between the HA and PVP groups (RR = 1.01; 95%CI: 0.96-1.06; z = 0.30; P = 0.76; Figure 4B). Similar results were obtained when we excluded the RCT[22] reporting the outcomes per ICSI cycle (P = 0.56) or when we used the random effects model for calculation (P = 0.59).

Live birth rate per cycle

Three RCTs reported on this outcome with no heterogeneity [χ^2 = 0.67, df = 2, (P = 0.73); I^2 = 0%] among them. The live birth rate was similar in the HA group compared to the PVP group (RR = 1.15; 95%CI: 0.86-1.54; z = 0.92; P = 0.36; Figure 4C). Same results were obtained after excluding the RCT[22] reporting the outcomes per ICSI cycle (P = 0.68) or when we calculated using the random effects model (P = 0.41).

Clinical pregnancy rate per transfer

Nine RCTs reported on this outcome with no heterogeneity [χ^2 = 5.50, df = 8, (P = 0.70); I^2 = 0%] among them. The clinical pregnancy rate was similar between HA group and PVP group (RR = 1.04; 95%CI: 0.92-1.17; z = 0.62; P = 0.53; Figure 5A). No difference was found by excluding the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.98) or by calculating the combined outcome using the random effects model (P = 0.68).

Implantation rate per embryo transferred

Four RCTs reported on this outcome with no heterogeneity [χ^2 = 1.12, df = 3, (P = 0.77); I^2 = 0%] among them. Similar implantation rates were obtained in the HA and PVP groups (RR = 1.17; 95%CI: 0.94-1.46; z = 0.40; P = 0.16; Figure 5B). Excluding the two RCTs[5,22] reporting the outcomes per ICSI cycle (P = 0.67) or using the random effects model for calculation (P = 0.17) did not change the result.

Table 2 Treatment protocol adopted in included trials

Ref.	HA group	PVP group
Balaban *et al*[16]	Oocytes were injected with sperm exposed to, SpermCatch (NidaCon International, Gothenburg, Sweden), a viscous liquid containing hyaluronate and human serum albumin	Oocytes were injected with sperm exposed to the PVP-containing product
Barak *et al*[5]	Sperm suspension which contained motile spermatozoa was introduced into the center of viscous suspension of hyaluronic acid. Motility of the sperm cells was slowed down. After breaking the sperm tail with the injection pipette, the spermatozoa were injected into the oocytes	MII oocytes were injected with sperm cells which were immobilized and aspirated for injection in 10% PVP solution
Castillo-Baso *et al*[17]	Sperm selected by PICSI (Mid Atlantic Diag. Inc.)	Sperm selected by conventional ICSI
Gandhi *et al*[19]	2 μL droplet with suspension of spermatozoa was placed to a 5 μL droplet of HA-containing medium (SpermSlow; MediCult, Jyllinge, Denmark) and incubated for 15 min at 37 ℃ under oil. Spermatozoa bound to HA in the junction of the two droplets were identified and carefully detached by injecting pipette (ICSI Micropipette; TPC, Thebarton, Adelaide, South Australia) and subsequently injected into a MII oocyte	Before injection, 3 μL of sperm suspension was transferred to 7 μL of 7% polyvinylpyrrolidone (PVP; SAGE) solution to remove debris and get better control. Spermatozoa with best morphology were selected for injection into a MII oocytes using inverted microscope equipped with micromanipulators
Gandhi *et al*[19]	Donated oocytes to avoid female infertility as a bias factor, randomly carried out with SpermSlow for sperm selection	Donated oocytes to avoid female infertility as a bias factor randomly carried out with PVP for sperm selection
Majumdar *et al*[20]	Sterile PICSI dishes (Origio MidAtlantic Devices, United States) with three hyaluronan microdots attached to the interior bottom, were used. 10 μL droplets of culture medium (GMOPS, Vitrolife) were placed over the hyaluronan microdots and an elongated 10 μL drop of PVP was made below the drops, before covering the dish with oil. 1-2 μL of sperm suspension was then added to the hyaluronan microdot containing droplets. After 5 min of incubation at 37 ℃, HA bound sperm with normal morphology were removed with an injecting micropipette (TPC, Australia) to the adjacent PVP droplet, immobilized and subsequently injected	An elongated 10 μL poly vinyl pyrolidone drop (PVP, Medicult, Denmark) under oil, was used to select spermatozoa with normal morphology for subsequent injection
Moon *et al*[21]	ICSI were performed with husband spermatozoa which immobilized in 2.5 mg/mL hyaluronic acid	ICSI were performed with husband spermatozoa which immobilized in 5% PVP
Parmegiani *et al*[22]	Spermatozoa were selected for their ability to bind to HA: A 2-mL droplet with suspension of spermatozoa was connected with a pipette tip to a 5-mL droplet of HA-containing medium (SpermSlow; MediCult) and allowed to incubate for 15 min at 37 ℃ under oil (Liquid Paraffin; MediCult). Spermatozoa bound to HA in the junction zone of the two droplets were selected and easily detached by injecting pipette (ICSI Micropipette; Humagen Fertility Diagnostics) and subsequently injected into oocytes	Conventional PVP-ICSI procedure
Worrilow *et al*[24]	At the time of injection, drops were prepared in the lid of a Falcon Petri dish (353004; Becton Dickinson, Franklin Lakes, United States). In the middle of the dish a 10 μL drop of Spermslow (Medicult) and a 10 μL Flushing Medium drop (Medicult) were connected by a 3-4 mm junction bridge of medium and consecutively encircled by five 10 μL drops of Flushing medium. This setup was covered with liquid paraffin (Medicult). A 2 μL volume of prepared semen was added to the medium part of the Spermslow/Flushing medium central mixture. The spermatozoa were allowed to migrate towards the junction for a period of 15-20 min at 37 ℃. Spermatozoa were carefully selected near the junction between the sperm droplet and the SpermSlow droplet	Non-bound, forward-moving spermatozoa were taken from the SpermSlow droplet
Worrilow *et al*[24]	PICSI embryos created using Hyaluron Bond-sperm	Standard sperm selection criteria
Worrilow *et al*[25]	The final sperm suspension of HYAL patients was placed upon microdots of hyaluronan in the PICSI Sperm Selection Device (Biocoat, Inc., Horsham, PA) and overlaid with oil. Following a 5-10 min incubation period, HB sperm were selected following the manufacturer's instructions	The final sperm suspension of patients in the control group was placed into standard ICSI dishes for selection

PVP: Polyvinylpyrrolidone; ICSI: Intracytoplasmic sperm injection; HA: Hyaluronic acid.

Adverse events and costs

None of the studies reported on these outcomes.

DISCUSSION

Main findings

This systematic review and meta-analysis based on eleven moderate to low quality RCTs provides evidence of similar efficiency between using HA or PVP for sperm immobilisation and selection before ICSI in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates. None of the studies reported on costs hence we could not perform a cost-effectiveness analysis.

Strengths and limitations

By performing a comprehensive literature search of the standard medical databases and grey literature

Table 3 Variables used for systematic review and meta-analysis *n* (%)

Trial	Transfers (*n*)	Fertilisation rate	Good embryos	Clinical pregnancy	Implantation rate	Live birth
Balaban *et al*[16]	Women					
HA	48	360 (72.14)	226 (50.33)	20 (41.66)	27 (18.12)	19 (39.58)
PVP	44	337 (75.05)	211 (46.99)	19 (43.18)	27 (19.14)	18 (40.90)
Barak *et al*[5]	Cycles					
HA	58	525 (72.61)	NA	29 (50.00)	41 (18.55)	NA
PVP	65	484 (74.57)		25 (38.46)	35 (14.00)	
Castillo-Baso *et al*[17]	Women					
HA	30	143 (49.14)	87 (29.89)	16 (53.33)	-34	NA
PVP	30	134 (50.95)	84 (31.93)	12 (40.00)	-24	
Castillo-Baso *et al*[17]	Women					
HA	30	140 (56.91)	105 (42.68)	14 (46.66)	-25	NA
PVP	30	163 (61.97)	99 (37.64)	13 (43.33)	-22	
Choe *et al*[18]						
HA	18 women	81 (75.70)	13 (12.14)	NA	NA	NA
PVP		93 (83.03)	15 (13.39)			
Gandhi *et al*[19]	Women					
HA	77	909 (82.33)	675 (61.14)	41 (53.24)	NA	NA
PVP	77	923 (82.48)	698 (62.37)	47 (61.03)		
Majumdar *et al*[20]	Women					
HA	71	353 (64.65)	154 (43.62)	25 (35.21)	39 (22.03)	22 (30.98)
PVP	80	371 (65.66)	170 (45.82)	28 (35.00)	36 (18.84)	21 (26.25)
Moon *et al*[21]						
HA	1 woman	18 (81.81)	11 (50.99)	NA	NA	NA
PVP		22 (78.57)	10 (35.71)			
Parmegiani *et al*[22]	Cycles					
HA	125	304 (91.56)	101 (30.42)	31 (24.80)	35 (12.41)	29 (23.20)
PVP	105	236 (85.81)	55 (20.00)	22 (20.95)	23 (10.17)	19 (18.09)
Van Den Bergh *et al*[23]						
HA	44 women	154 (75.49)	NA	NA	NA	NA
PVP		142 (69.95)				
Worrilow *et al*[24]	Women					
HA	7	77 (61.11)	NA	4 (57.14)	NA	NA
PVP	8	98 (66.66)		2 (25.00)		
Worrilow *et al*[24]	Women					
HA	237	2105 (77.21)	NA	112 (47.25)	NA	NA
PVP	245	2024 (74.41)		117 (47.75)		
Total						
HA		5169 (74.63)	1372 (46.44)	292 (42.75)	142 (17.12)	70 (28.68)
PVP		5027 (74.00)	1342 (46.59)	285 (41.66)	121 (14.97)	58 (25.32)

HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone; NA: Not available.

with no filters for date, country or hospital of origin, publication language, sample size or blinding we were able to identify eleven RCTs including conference abstracts in order to calculate the combined outcomes. Where the reported data was insufficient we contacted the study authors to gain extended reports.

The heterogeneity was low among the included RCTs and lead to consistent results by using both fixed effect and random effects models for calculations.

Our study is limited by the moderate to poor methodological quality of the included studies because of unclear randomisation technique, inadequate allocation concealment and blinding.

Some might argue in relation to the inclusion of the two RCTs reporting the results per ICSI cycle and not per women randomised, but we performed calculations excluding them for each of the primary and secondary outcomes, without identifying any significant difference.

Comparison with other studies and further research

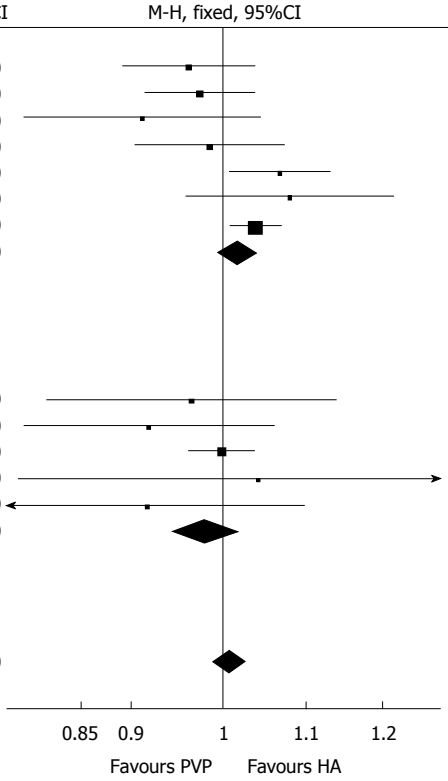
To our knowledge this is the first meta-analysis comparing HA with PVP for sperm immobilisation and selection before ICSI. By observing the forest plot for each end point one can easily notice the similarities between the RCTs as most of them cross the vertical line meaning there is no statistical difference between the groups.

Future trials should be conducted according to the CONSORT guidelines. Due to the nature of the intervention, it would be difficult to achieve blinding of the embryologist performing the sperm selection, but the risk of bias could be reduced by blinding the outcome assessors and the personnel performing the embryo transfers.

It is important to report on the possible adverse events related to less physiological immobilisation and selection of the sperm and to set live birth rate as a primary outcome for the comparisons.

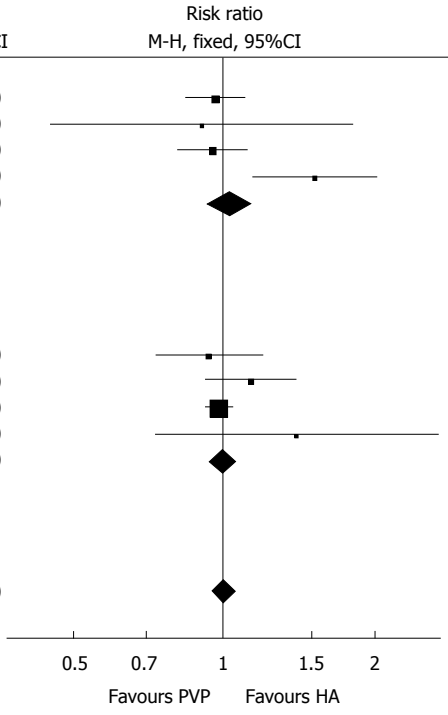
A

Study or subgroup	HA Events	HA Total	PVP Events	PVP Total	Weight	Risk ratio M-H, fixed, 95%CI
1.1.1 Full texts						
Balaban 2003	360	499	337	449	7.0%	0.96 (0.89, 1.04)
Barak 2001	525	732	484	649	10.1%	0.97 (0.91, 1.04)
Choe 2012	81	107	93	112	1.8%	0.91 (0.80, 1.04)
Majumdar 2013	353	546	371	565	7.2%	0.98 (0.90, 1.07)
Parmegiani 2010	304	332	236	275	5.1%	1.07 (1.01, 1.13)
Van Den Bergh 2009	154	204	142	203	2.8%	1.08 (0.96, 1.22)
Worrilow 2013	2105	2726	2024	2720	39.9%	1.04 (1.01, 1.07)
Subtotal (95%CI)		5137		4973	73.9%	1.02 (0.99, 1.04)
Total events	3882		3687			
Heterogeneity: Chi^2 = 12.23, df = 6 (P = 0.06); I^2 = 51%						
Test for overall effect: z = 1.48 (P = 0.14)						
1.1.2 Abstracts						
Castillo-Baso 2011 a	143	291	134	263	2.8%	0.96 (0.82, 1.14)
Castillo-Baso 2011 b	140	246	163	263	3.1%	0.92 (0.79, 1.06)
Gallego 2013	909	1104	923	1119	18.1%	1.00 (0.96, 1.04)
Moon 2000	18	22	22	28	0.4%	1.04 (0.79, 1.37)
Worrilow 2006	77	126	98	147	1.8%	0.92 (0.77, 1.10)
Subtotal (95%CI)		1789		1820	26.1%	0.98 (0.94, 1.02)
Total events	1287		1340			
Heterogeneity: Chi^2 = 2.40, df = 4 (P = 0.66); I^2 = 0%						
Test for overall effect: z = 1.03 (P = 0.30)						
Total (95%CI)		6926		6793	100.0%	1.01 (0.99, 1.03)
Total events	5169		5027			
Heterogeneity: Chi^2 = 16.86, df = 11 (P = 0.11); I^2 = 35%						
Test for overall effect: z = 0.75 (P = 0.45)						
Test for subgroup differences: Chi^2 = 2.67, df = 1 (P = 0.10); I^2 = 62.6%						



B

Study or subgroup	HA Events	HA Total	PVP Events	PVP Total	Weight	Risk ratio M-H, fixed, 95%CI
1.2.1 Full texts						
Balaban 2003	226	499	211	449	16.5%	0.96 (0.84, 1.11)
Choe 2012	13	107	15	112	1.1%	0.91 (0.45, 1.82)
Majumdar 2013	154	353	170	371	12.3%	0.95 (0.81, 1.12)
Parmegiani 2010	101	332	55	275	4.5%	1.52 (1.14, 2.03)
Subtotal (95%CI)		1291		1207	34.3%	1.03 (0.93, 1.14)
Total events	494		451			
Heterogeneity: Chi^2 = 9.02, df = 3 (P = 0.03); I^2 = 67%						
Test for overall effect: z = 0.59 (P = 0.55)						
1.2.2 Abstracts						
Castillo-Baso 2011 a	87	291	84	263	6.5%	0.94 (0.73, 1.20)
Castillo-Baso 2011 b	105	246	99	263	7.1%	1.13 (0.92, 1.40)
Gallego 2013	675	1104	698	1119	51.4%	0.98 (0.92, 1.05)
Moon 2000	11	22	10	28	0.7%	1.40 (0.73, 2.68)
Subtotal (95%CI)		1663		1673	65.7%	1.00 (0.94, 1.06)
Total events	878		891			
Heterogeneity: Chi^2 = 2.96, df = 3 (P = 0.40); I^2 = 0%						
Test for overall effect: z = 0.11 (P = 0.91)						
Total (95%CI)		2954		2880	100.0%	1.01 (0.96, 1.06)
Total events	1372		1342			
Heterogeneity: Chi^2 = 12.08, df = 7 (P = 0.10); I^2 = 42%						
Test for overall effect: z = 0.30 (P = 0.76)						
Test for subgroup differences: Chi^2 = 0.31, df = 1 (P = 0.57); I^2 = 0%						



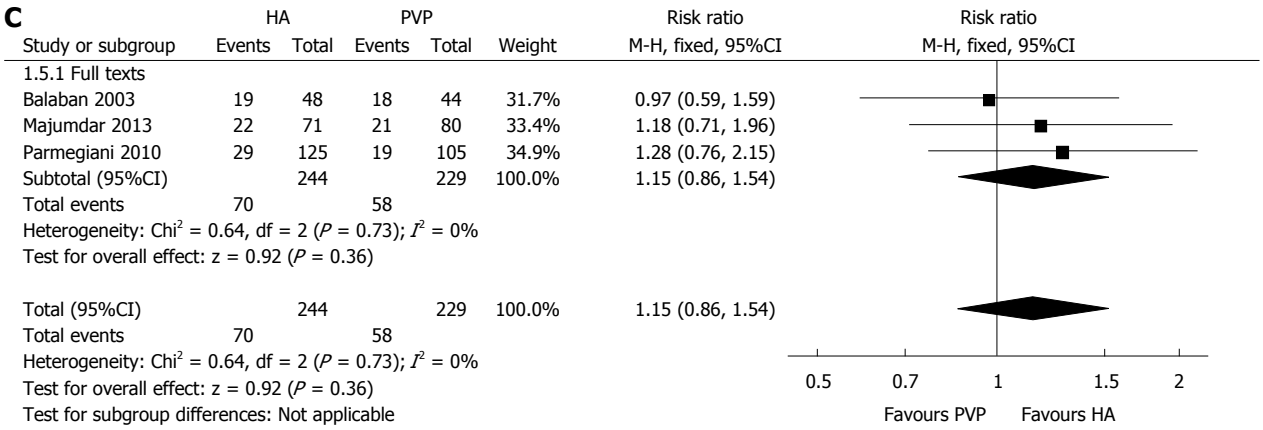


Figure 4 Primary endpoints. A: The forest plot showing fertilisation rate per oocyte injected. Risk ratio is shown with 95%CI; B: The forest plot showing good embryos rate per oocyte injected, risk ratio is shown with 95%CI; C: The forest plot showing live birth rate per cycle. Risk ratio is shown with 95%CI. HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone.

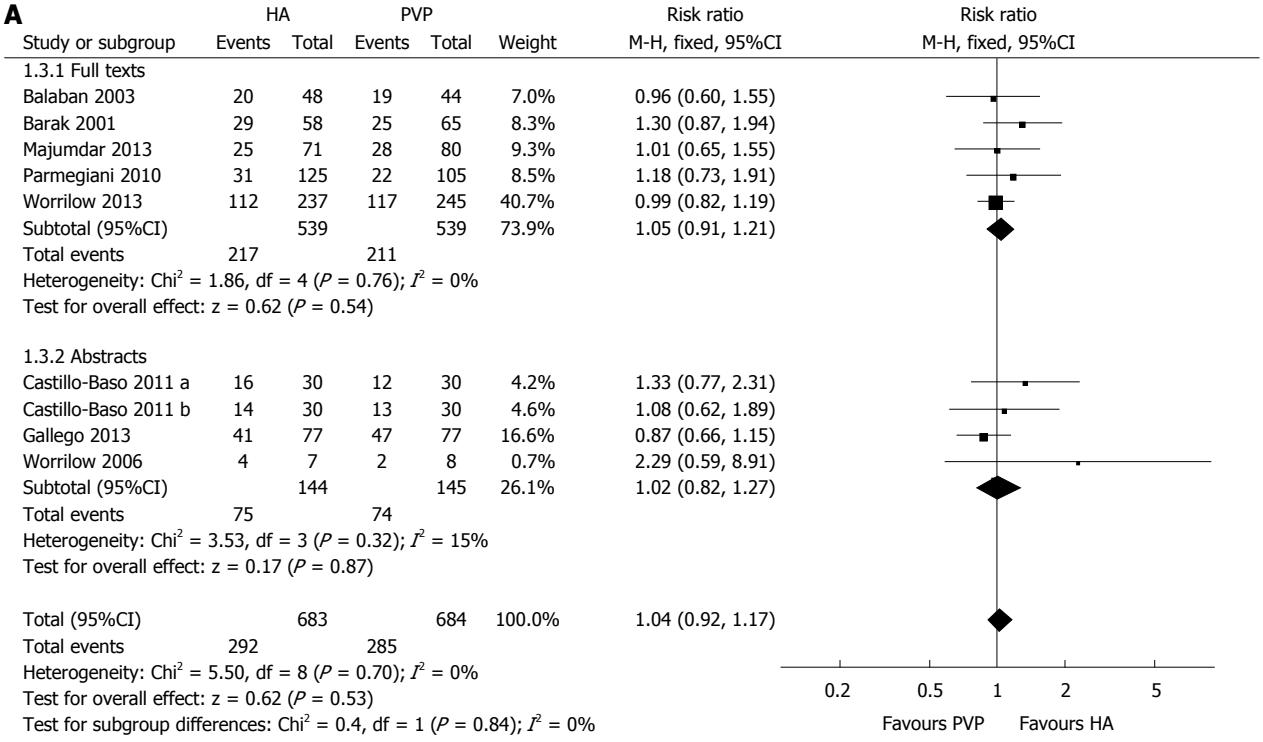


Figure 5 Secondary endpoints. A: The forest plot showing clinical pregnancy rate per transfer. Risk ratio is shown with 95%CI; B: The forest plot showing implantation rate per embryo transferred. Risk ratio is shown with 95%CI. HA: Hyaluronic acid; PVP: Polyvinylpyrrolidone.

Sub-group analysis considering the quality of the sperm, previous failed ICSI cycles, infertility cause, number and quality of transferred embryos should be considered in order to eliminate confounding factors.

Until then, this review may provide reassurance of non inferiority of one method over another for embryologists and laboratory staff involved in the acquisition of laboratory materials.

COMMENTS

Background

The majority of patients undergoing intracytoplasmic sperm injection (ICSI) treatment will reach the stage of embryo transfer due to important improvements of ovarian stimulation protocols and laboratory technology, but only a small proportion of transferred embryos implant leading to an overall success rate of 10%-40%.

Research frontiers

Several methods (ultramorphology, surface electric charge, apoptotic *vs* nonapoptotic, chromatin structure assay) have been recently proposed for optimising the sperm selection in order to reduce the risk of chromosomal anomalies associated with poor ICSI outcome.

Innovations and breakthroughs

Hyaluronic acid has been proposed as a physiological alternative to polyvinylpyrrolidone (PVP) for use as a selection medium to reduce sperm motility as a solution for the reported toxicity and unknown long term effects of PVP. Several studies investigated this method, but this is the first meta-analysis to assess the effect of using hyaluronic acid compared to PVP.

Applications

The results of this meta-analysis combining the outcomes from 11 randomised controlled trials concluded that there is no difference between hyaluronic acid and PVP for sperm immobilisation and selection before ICSI in terms of fertilisation, embryo quality, clinical pregnancy, implantation and live birth rates.

Terminology

Sperm immobilisation and selection is an important step in the ICSI process and refers to the use of a medium in the laboratory for reducing the speed of sperm in order to allow its manipulation in the ICSI process.

Peer-review

The peer-reviewers appreciated the completeness of this meta-analysis. The manuscript was assessed as being well prepared, interesting, clear and well defined.

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