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REVIEW *Infertility*

Techniques used for IUI: is it time for a change?

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STUDY QUESTION: Are the guidelines for the technical aspects of IUI (WHO, 2010) still in accordance with the current literature?

SUMMARY ANSWER: In general, the laboratory guidelines of the World Health Organization (WHO) are a suitable protocol, although the evidence is not always conclusive and some changes are advisable.

WHAT IS KNOWN ALREADY: Lack of standardization of the technical procedures required for IUI might result in inter-laboratory variation in pregnancy rates. Most centers still use their own materials and methods even though some guidelines are available.

STUDY DESIGN, SIZE, DURATION: A structural review focusing on the association between pregnancy rates and the procedures of semen collection (e.g. ejaculatory abstinence, collection place), semen processing (e.g. preparation method, temperature during centrifugation/storage), insemination (e.g. timing of IUI, bed rest after IUI) and the equipment used.

PARTICIPANTS/MATERIALS, SETTING, METHODS: A literature search was performed in Medline and the Cochrane library. When no adequate studies of the impact of a parameter on pregnancy results were found, its association with sperm parameters was reviewed.

MAIN RESULTS AND THE ROLE OF CHANCE: For most variables, the literature review revealed a low level of evidence, a limited number of studies and/or an inadequate outcome measure. Moreover, the comparison of procedures (i.e. semen preparation technique, time interval between semen, collection, processing and IUI) revealed no consensus about their results. It was not possible to develop an evidence-based, optimal IUI treatment protocol.

LIMITATIONS, REASONS FOR CAUTION: The included studies exhibited a lack of standardization in inclusion criteria and methods used.

WIDER IMPLICATIONS OF THE FINDINGS: This review emphasizes the need for more knowledge about and standardization of assisted reproduction technologies. Our literature search indicates that some of the recommendations in the laboratory guidelines could be adapted to improve standardization, comfort, quality control and to cut costs.

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Introduction

At the moment, there is an ongoing discussion about the value of IUI. Recent Dutch studies showed a positive performance of the treatment especially in cases of mild andrological and unexplained infertility (Bensdorp *et al.*, 2015; Moolenaar *et al.*, 2015; Tjon-Kon-Fat *et al.*, 2015). On the other hand, the British guideline for infertility treatment strongly reduced the indications for IUI to sexual dysfunction, same sex relationship and special conditions (NICE, 2013). In this guideline, clinics are directed to apply in IVF or ICSI as a first line treatment in the majority of cases. However, the evidence cited to support this guideline was, not robust (Woodward *et al.*, 2016). Not only clinical, but also economical and financial evidence favors IUI over IVF in many cases (Bahadur *et al.*, 2016c). A lot of discussion is ongoing on this subject (Heneghan *et al.*, 2016; Spencer *et al.*, 2016; Bahadur *et al.*, 2017). Probably, for this reason, until now only a small proportion of clinics have made significant changes to their IUI practice (Kim *et al.*, 2015) and IUI is still performed on a large scale worldwide and it is remains worthwhile to try to improve the outcome.

The IUI procedure can roughly be separated in three steps: diagnosis and indication, cycle preparation and the technical stage. The third step, including the whole process between semen collection and insemination, is barely included in guidelines. Only the World Health Organization (WHO) laboratory manual (WHO, 2010) attempts to describe the process. This description is incomplete, because parts of the pre- and post-laboratory stages are missing.

This structural review focuses on the technical phase of IUI and we check whether the present guidelines are in concordance with available literature. As the WHO manual (WHO, 2010) is the only international guideline that describes a protocol for semen collection, analysis and preparation, we used this guideline as the main reference point for our study.

Methods

The available literature on the following procedures or variables of IUI was reviewed: ejaculatory abstinence (EA), semen collection place, time intervals (i.e. between semen collection and semen processing, between semen processing and insemination, and between semen collection and insemination), semen preparation methods, centrifugation medium, centrifugation and storage temperature, timing of IUI, use of different disposables (e.g. catheters) and duration of bed rest after IUI.

A computerized search was carried out in Medline and in the Cochrane library. Key words for the search were 'intrauterine insemination', 'IUI' or 'artificial insemination'. Specific key words used for the individual variables included: 'ejaculatory abstinence', 'ejaculatory frequency', 'time interval', 'collection to processing', 'collection to IUI', 'processing to IUI', 'semen purification', 'semen preparation', 'semen separation', 'density gradient', 'swim up', 'wash', 'buffer', 'zwitterion', 'bicarbonate', 'HEPES', 'MOPS', 'TEST', 'medium', 'temperature', 'centrifugation', 'incubation', 'storage', 'timing', 'insemination timing', 'disposable', 'devices', 'tube', 'glove', 'pipette', 'catheter', 'collection container', 'bed rest', 'supine positioning', 'immobilization' and 'mobilization'. The titles and abstracts were screened to exclude citations considered as irrelevant, thereafter full texts of potentially eligible studies were reviewed. Articles published before 1 November 2016 in peer reviewed journals in the English language were included. The references and related citations of these articles were used to identify extra potential articles of interest. Studies reporting the impact of used laboratory procedures on sperm parameters or IUI pregnancy rates (PRs) were included.

The recommendations on technical aspects of IUI stated in the WHO guideline (WHO, 2010) were used as reference and compared with the results of the literature search. The results were arranged in an evidence-level structure as described by NICE (2013). Finally, a summary is given of the recommendations, limitations of the available literature and knowledge gaps.

Ejaculatory abstinence

The WHO recommends an EA period of 2–7 days before semen collection (WHO, 2010), both for diagnostics and semen preparation. Although no explanatory literature is provided, studies on sperm parameters support this recommendation since an EA of 2–7 days resulted in a significantly higher semen volume (Carlsen *et al.*, 2004; De Jonge *et al.*, 2004; Marshburn *et al.*, 2014) and total motile sperm count (TMSC) (Jurema *et al.*, 2005; Levitas *et al.*, 2005; Marshburn *et al.*, 2010). On the contrary, a recent study reported a significant higher sperm motility for ejaculates of infertile men if they were produced within 40 min after an initial sample with <5 million motile spermatozoa (Bahadur *et al.*, 2016b). Moreover, an EA period of 0–2 days also resulted in higher percentages of morphologically normal spermatozoa (Levitas *et al.*, 2005, 2006; Bahadur *et al.*, 2016b).

The explanation of these observations can be found in the effect of reactive oxygen species (ROS). A certain level of ROS is required for the maturation of epididymal spermatozoa (Noblanc *et al.*, 2011). Excessive ROS, however, can induce oxidative damage which negatively affects the fertilization potential of spermatozoa (Marshburn *et al.*, 2014). The exposure time of spermatozoa to ROS is influenced in an EA time-dependent manner, thereby influencing the incidence of sperm DNA fragmentation, especially in infertile men (Sharma and Agarwal, 1996; Alkan *et al.*, 1997). As a consequence, a shorter period of EA will result in higher PRs both in natural and IUI cycles, especially in sub-fertile men (Gosalvez *et al.*, 2011; Sanchez-Martin *et al.*, 2013).

So far, the relationship between duration of EA and IUI PRs has been investigated in only two retrospective studies. These studies showed a negative impact of longer EA periods on PRs in cohorts of 372 (Marshburn *et al.*, 2010) and 417 couples (Jurema *et al.*, 2005). These studies reported highest PRs in the group with an EA up to 2 days and up to 3 days, respectively. In a retrospective pilot study, it was also found that in cases of oligozoospermia, the aggregation of consecutive ejaculates resulted in a higher PR (Bahadur *et al.*, 2016a). So, irrespective of a higher TMSC, the WHO recommendation of 2–7 days is debatable. A possible bias in the plea for a shorter EA is that these couples had intercourse shortly before insemination, thereby increasing the probability of a natural conception. More studies are needed to confirm these findings, both in normozoospermic and oligozoospermic men. For now, it can be advised to change the WHO recommendation into an EA period of maximum 3 days.

Time intervals

The time intervals between semen collection to processing, processing to insemination and semen collection to insemination have impact on IUI PRs (Table I). However, the WHO provided only a recommendation for the time interval between collection and processing (WHO, 2010). They stated that semen sample collection for IUI should

Table I Summary of findings reported by literature when comparing the impacts of time intervals on the IUI pregnancy rate.

Time interval	Time intervals studied (min)	Study	Included couples (*n*)	Cause of infertility	Time interval with statistically significant highest pregnancy rates (min)
Semen production → processing	15–30, 31–60, >60	Yavas and Selub (2004)	62 (132 cycles)	Female, male, unexplained and combination	15–30*
	<30, 30–60, >60	Song *et al.* (2007)	335 (633 cycles)	Female cervical factor, male	No difference
Semen processing → IUI#	≤30, 31–60, >60	Yavas and Selub (2004)	62 (132 cycles)	Female, male, unexplained and combination	≤30 and 31–60*
	Not defined	Song *et al.* (2007)	335 (633 cycles)	Female cervical factor, male	No difference
	< 30, 30–59, 60–89, 90–119, 120–180	Kilicdag *et al.* (2005)	– (1125 cycles)	Mild male, unexplained	≥30
	<40, 40–80, >80	Fauque *et al.* (2014)	709 (862 cycles)	Female, male, unexplained	40–80
	<60, about 24 h	Jansen *et al.* (2016)	1135 (2154 cycles)	Female, male, unexplained	No difference
Semen production → IUI	≤90, 91–120, >120	Yavas and Selub (2004)	62 (132 cycles)	Female, male, unexplained and combination	≤90*
	Not defined	Song *et al.* (2007)	335 (633 cycles)	Female cervical factor, male	No difference

*Results reported in couples with human menopausal gonadotropin (hMG)-treated women, no differences in couples with clomiphene citrate (CC)-treated women, # samples were stored at body temperature.
– = not available in study.

preferably take place in a private room near the clinical laboratory, but when collection at home is preferred the semen should be delivered to the laboratory within 1 h after collection (while protected from extremes of temperature) (WHO, 2010).

When comparing PRs, a higher PR was reported when semen was collected in the clinic (Yavas and Selub, 2004). Another study found no difference in PRs between collection at home and in the clinic (Song *et al.*, 2007). Furthermore, semen collection in the clinic led to a time interval that was on average 26 min shorter than collection at home (Yavas and Selub, 2004). Nevertheless, no impact of time interval duration was found in a large study population (n = 633 cycles) (Song *et al.*, 2007). This was also shown in women treated with clomiphene citrate (n = 95 cycles) in another study (Yavas and Selub, 2004). On the contrary, a shorter time interval (i.e. 15–30 min) resulted in a higher PR in a small group of women treated with human menopausal hormone (hMG; n = 37 cycles) (Yavas and Selub, 2004). Lower PRs caused by longer time intervals, might be explained by decapacitating factors in the seminal plasma (Kanwar *et al.*, 1979; Rogers *et al.*, 1983; Mortimer *et al.*, 1998) or ROS-induced DNA damage (Marshburn *et al.*, 2014; Yavas and Selub, 2004).

Regarding storage time after processing (i.e. time interval between processing and insemination), a shorter time interval was related to a lower proportion of premature sperm chromatin decondensation (Hammadeh *et al.*, 2001a), to less sperm DNA fragmentation (Fauque *et al.*, 2014) and to a higher PR due to the storage time-dependent spontaneous acrosome reaction (Mansour *et al.*, 2008; Fauque *et al.*, 2014). In practice, however, no consensus was shown in reported ideal time intervals. PRs were comparable when IUI was performed within 30 min or after 31–60 min of storage, but decreased after >60 min, only in couples with hMG-treated women (Yavas and Selub, 2004). Others reported highest clinical PRs in the groups with a storage time of 40–80 min (Fauque *et al.*, 2014) and >30 min (Kilicdag *et al.*, 2005). With another approach, another study (Song *et al.*, 2007), found no differences in in storage time intervals between a group of pregnant and non pregnant couples. Moreover, a recent study reported no difference in ongoing PRs between immediate insemination and insemination one day after semen processing (Jansen *et al.*, 2016).

Two retrospective studies evaluated the impact of the total time interval between semen collection and insemination. In one study, higher PRs were found when insemination took place within 90 min after semen collection (i.e. compared to 91–120 min and >120 min) (Yavas and Selub, 2004), the other study found no differences (Song *et al.*, 2007).

In conclusion, literature on this subject is scarce and presented contradictory results. More information can be obtained in RCTs, but also in retrospective, well-designed multicenter studies, where standardized time intervals should be compared. For now, it is not possible to recommend one time interval over the other, nevertheless, the majority of the results propose to avoid long time intervals. Especially the time interval between semen collection and processing should not exceed 60 min, since no pregnancies were reported in this group (Yavas and Selub, 2004; Song *et al.*, 2007).

Semen preparation methods

After semen production and liquefaction, it is necessary to separate sperm from the seminal plasma, thereby preventing uterine cramps, extended ROS formation and inhibition of fertilization (Kanwar *et al.*, 1979; Agarwal *et al.*, 1992). Many separation techniques have been described. Compared to the initial semen sample, the use of all these techniques resulted in significantly better semen parameters

(Joshi et al., 1998; Chen and Bongso, 1999; Hammadeh et al., 2001b) and higher IUI PRs (Goldenberg et al., 1992).

According to the WHO, the choice of semen preparation technique should be based on the nature of the semen sample (WHO, 2010). It is recommended to use swim-up in cases of normozoospermia, while density gradients should be the method of choice in other cases.

Density gradient centrifugation started with the use of Percoll^{R}. In 1996, however, Percoll^{R} was withdrawn from the clinical market, since it was stated that the polyvinylpyrrolidone (PVP)-coated silica in Percoll^{R} contained endotoxins (Svalander et al., 1995). Since then, several endotoxin-free products with silane-coated silica particles were introduced. In first instance, research concentrated on comparing these new products to Percoll^{R} and conflicting results were found with respect to sperm motility and recovery rate (Centola et al., 1998; Claassens et al., 1998; Chen and Bongso, 1999; Sharma et al., 1999; McCann and Chantler, 2000; Ren et al., 2004; Tsai et al., 2004). Despite these disagreements, silane-coated products are now widely used (Henkel and Schill, 2003).

There is consensus that the swim-up technique resulted in lower recovery rates compared to density gradient centrifugation, making it suitable only in cases of normozoospermia (Van Voorhis et al., 2001; Henkel and Schill, 2003). As swim-up selects spermatozoa based on their motility, one would expect that it would result in a high fraction of motile spermatozoa. Some studies, however, reported a comparable or even lower motility if swim-up was compared to gradients (Le Lannou and Blanchard, 1988; Almagor et al., 1993; Dodson et al., 1998). The same is true for the percentage morphologically normal spermatozoa (Le Lannou and Blanchard, 1988; Chan et al., 1991; Chen et al., 1995; Erel et al., 2000).

In practice, the clinical outcome of IUI is of more importance than the value of semen parameters. In 2007, a systematic Cochrane review (Boomsma et al., 2007) included six RCTs in their meta-analysis, comparing the effectiveness of density gradient techniques versus swim-up techniques and versus wash-only. They concluded that there is no evidence to choose for one technique over the other. The included studies, however, were characterized by low numbers of patients, diversity in the cause of infertility and diversity in the techniques that were compared. Only one study (Carrell et al., 1998) included a larger study population ($n = 363$). Still, this study is of limited value, since five different techniques were studied in a population with all causes of infertility. Since Boomsma's review, only one suitable RCT has been performed. A significantly higher PR (both per cycle and per couple) was found using density gradient centrifugation (SpermGrad^{R}) compared to the swim-up technique, in couples with unexplained infertility (Karamahmutoglu et al., 2014). An overview of all studies is given in Table II (Zavos and Centola, 1992; Carrell et al., 1998; Dodson et al., 1998; Ragni et al., 1998; Xu et al., 2000; Tsai et al., 2004; Grigoriou et al., 2005; Posada et al., 2005; Soliman and Goyal, 2005; Karamahmutoglu et al., 2014). Additional studies with standardized patient inclusion criteria and study designs are necessary to confirm the results obtained from these studies.

pH buffer of washing and storage medium

To maintain an optimal pH level, the WHO recommends to select a buffer medium based on the used incubator: a zwitterion-buffered medium (e.g. HEPES, TEST, MOPS) if the incubator contains atmospheric air and a bicarbonate-based medium if the incubator contains an atmosphere of 5% CO_2 (and if gas exchange is allowed) (WHO, 2010). Meanwhile, most commercially available sperm wash media contain zwitterions for pH buffering, although a certain level of bicarbonate is present as key capacitating agent for spermatozoa (Gadella and van Gestel, 2004). Although these media are effective, there are concerns that zwitterion buffers may interfere with some important processes in different cell types and, consequently, have negative effects on gametes and embryos (Swain, 2010).

As far as we know, only one RCT (Byrd et al., 1991) compared the PRs of sperm prepared with bicarbonate buffer and with HEPES buffer, in IUI with cryopreserved donor sperm ($n = 324$ cycles). This study reported significantly higher PRs when sperm was prepared using HEPES buffer. It has to be stated, however, that the effect might not be attributed to HEPES alone as two different culture media were used (HTF and HAM's F10). More RCTs are necessary on this subject, with stratification for normozoospermic and oligozoospermic men, and with temperature as important factor as the pH of buffers is temperature-dependent.

Temperature during centrifugation

It was suggested that the impact of the centrifugation temperature on sperm capacitation might mimic the impact of the storage temperature (Marin-Briggiler et al., 2002), as reported later in this review. In a group of 50 normozoospermic men, however, no significant difference was found in the level of DNA damage between samples centrifuged at controlled (testis or body temperature: 35 or 37°C, respectively) and non-controlled temperature (room temperature: ~25°C) (Repalle et al., 2013). In another small group of normozoospermic men ($n = 10$), the percentage of motile sperm cells was higher after centrifugation at 34°C compared to centrifugation at room temperature (Franken et al., 2011). The samples centrifuged at 34°C were reported with a higher sperm yield, but only when they were also stored at this temperature before semen processing. Both studies provided no explanations for the temperature-dependent influences of semen centrifugation.

Only one RCT ($n = 671$) evaluated the impact of centrifugation temperature on IUI PRs. Included were couples with unexplained infertility and no differences were found in sperm parameters and IUI PRs between controlled and non-controlled centrifugation temperature (Selvaraj et al., 2013). Based on this RCT and since non-controlled centrifugation is commonly used for reasons of ease, we conclude that further evaluation is not needed at this moment.

Temperature during storage

Usually, the storage of semen samples after preparation takes place at body temperature. Long-term storage (≥24 h) of spermatozoa at body or testis temperature, however, resulted in reduced motility and sperm quality (Calamera et al., 2001; Thijssen et al., 2014). In general, reduced motility is observed both at room and body temperature in a time-dependent manner, but to a greater extent and more rapid at 37°C (Matsuura et al., 2010). Moreover, long-term storage at 37°C resulted in an increased incidence of large vacuoles in sperm nuclei (Peer et al., 2007). The positive impact of lower storage temperatures is explained by the switch of spermatozoa to a resting state, where better energy

Table II Main results of the randomized controlled trials comparing the IUI pregnancy rates between semen preparation techniques.

Study	Included couples (*n*)	Cause of infertility	Mean post-wash TMSC (million)	Compared preparation techniques (PR per cycle)	Main results according to pregnancy rates
Grigoriou *et al.* (2005)*	52	Unexplained	20	-Sperm wash with PAF in medium (23%)	Sperm treated with PAF significant higher clinical pregnancy rate than direct swim-up technique
			20	-Swim-up (8%)	
Posada *et al.* (2005)*	82 (121 cycles)	Not available	10.9	-Density gradient centrifugation (8%)	Significant increased clinical pregnancy rates in swim-up technique compared with density gradient centrifugation
			16.2	-Swim-up (26%)	
Soliman and Goyal (2005)*	63	Not available	Not available	-Density gradient centrifugation (11%)	No superior technique
				- Wash-only (14%)	
Xu *et al.* (2000)*	60	Male factor	41.4	-Wang tube sperm separation (45%)	Wang tube sperm separation methods significantly higher pregnancy rate than other two methods in oligoasthenoteratozoospermic men
			24.3	-Swim-up (15%)	
			32.3	-Percoll^{R} density gradient centrifugation (20%)	
Carrell *et al.* (1998)*	363 (898 cycles)	Female, male, unexplained	≥20	-Sperm washing (7%)	Swim-up and Percoll^{R} density gradient higher chance (not statistically significant) of pregnancy than other techniques. Swim-down significantly lower pregnancy rate than swim-up and Percoll^{R} technique
			≥20	-Swim-up (13%)	
			≥20	-Swim-down (6%)	
			≥20	-Percoll^{R} density gradient centrifugation (13%)	
			≥20	-Refrigeration/heparin incubation (8%)	
Dodson *et al.* (1998)*	80 (153 cycles)	Female, male, unexplained	29	-Double centrifugation (15%)	No superior technique
			6	-Multiple-tube swim-up (14%)	
			27	-Percoll^{R} density gradient centrifugation (20%)	
Karamahmutoglu *et al.* (2014)	223 (338 cycles)	Unexplained, mild male	Not available	-SpermGrad^{R} density gradient centrifugation (17%)	Higher pregnancy rates in density gradient centrifugation compared to swim-up in couples with unexplained infertility
				-Swim-up (7%)	
Tsai *et al.* (2004)	121	Female ovulation dysfunction	Not available	-PureSperm^{R} density gradient centrifugation (13%)	No significant differences in pregnancy rate
				-Percoll^{R} density gradient centrifugation (14%)	
Ragni *et al.* (1998)	121 (194 cycles)	Male, unexplained	8.5	-Swim-up (14%)	Test yolk buffer significantly increased pregnancy rate compared with standard swim-up
			7.6	-Swim-up with test yolk buffer (26%)	
Zavos and Centola (1992)	148 (307 cycles)	Female, unexplained	29.4	-Double wash (10%)	SpermPrep^{R} filtration significantly higher clinical pregnancy rate per cycle than sperm wash
			26.7	-SpermPrep^{R} filtration (21%)	

*Randomized controlled trials (RCTs) reported by Boomsma *et al.* (2007) comparing the effectiveness of gradient technique versus swim-up technique versus wash technique.
TMSC = total motile sperm count, PR = pregnancy rate, PAF = platelet-activating factor.

preservation might result in longer survival (Thijssen *et al.*, 2014). This hypothesis is supported by the reported influence of storage temperature on some cellular mechanisms involved in sperm capacitation: a temporary blockage of capacitation-related events was present during storage at 20°C, but not at 37°C (Marin-Briggiler *et al.*, 2002).

Clinical studies about the impact of storage temperature on PRs are missing. Furthermore, the above studies included small groups of men (*n* = 12–41) and did not specify the impact separately for fertile and infertile men. Further research is needed to evaluate the impact of storage temperature on IUI PRs. As literature is scarce, we can only recommend to avoid long-term storage at body temperature.

Timing of insemination

The timing of insemination comprises two variables: the detection/induction of ovulation and the time interval from this point to insemination. The WHO guideline provides no recommendations for one timing method over the other. According to the NICE, however, the use

of basal body temperature charts does not reliably predict ovulation (NICE, 2013). In 2014, a review (Cantineau et al., 2014) included 18 RCTs about the effectiveness of different timing methods in natural and stimulated IUI cycles. When comparing hCG administration and LH surge as timing method for IUI, no differences in PRs were found, albeit the quality of evidence was low or very low. Additionally, double inseminations (e.g. at 24 and 48 h after ovulation induction) and the use of different types and dosages of hCG and GnRH-a resulted in no differences in IUI PRs (Polyzos et al., 2010; Cantineau et al., 2014).

Next to ovulation timing method, the timing of insemination can be discussed. The NICE guideline stated that insemination should be performed around ovulation (NICE, 2013). In literature, the comparison of different time intervals between ovulation induction and insemination showed no statistically significant differences in PRs (Huang et al., 2000; Claman et al., 2004; Wang et al., 2006; AboulGheit, 2010; Rahman et al., 2011; Aydin et al., 2013; Weiss et al., 2015). The majority of these studies compared time intervals between 24 and 48 h after ovulation induction. From a biological view, however, the insemination of sperm before ovulation might be favorable, i.e. at the time of ovulation induction (Pacey et al., 1995; Suarez and Pacey, 2006). After intercourse, spermatozoa attach to the isthmus epithelium, where this binding keeps them viable and prevents capacitation (Suarez and Pacey, 2006). Moreover, this interaction results in de novo protein synthesis (Holt and Fazeli, 2010). Once ovulation occurs, a cascade of signals results in a hyperactivated sperm movement towards the oocyte (Aitken and Nixon, 2013). This ovulation-related timing mechanism is important, since an early start of capacitation resulted in apoptosis of the spermatozoa (Aitken, 2011), while a late start of capacitation resulted in spermatozoa that were not equipped to recognize oocytes (Aitken and Nixon, 2013). Although the majority of these processes was found in animal studies with healthy subjects, it would be worthwhile to set up clinical studies to tests this theory in humans. Only one study compared PRs between injection simultaneously with administration and 34–36 h after hCG administration, but found no statistically significant differences (Aydin et al., 2013).

Other treatment related factors might affect the correct moment of insemination. For example, embryos might be affected by premature luteinization, due to an early rise in progesterone at the end of the follicular phase in controlled ovarian hyperstimulated IUI cycles. This early rise of progesterone was observed in 22% of the cycles and led to reduced PRs from 23 to 8% (Requena et al., 2015). Also, human papillomavirus positivity was found to have a negative impact on IUI PRs (Depuydt et al., 2016).

Additional (multicenter) RCTs are recommended on all of these aspects.

IUI devices

The most important devices of influence on IUI results are laboratory and clinical disposables and media, like semen containers, wash media and catheters. Two possible impacts of these products can be distinguished: function and toxicity. With respect to function, the type of catheter and ultrasound guidance can be of influence. A soft tip catheter was found to cause less trauma to the endometrium compared to a hard tip catheter (Lavie et al., 1997), but was not superior in PRs in a Cochrane review (van der Poel et al., 2010). Ultrasound guidance during insemination makes it possible to visualize the movement of the catheter inside the endometrial cavity and could so avoid endometrial trauma and uterine contractions (Oztekin et al., 2013). This ultrasound guidance did not result in higher PRs in comparative studies (Ramon et al., 2009; Oruc et al., 2014; Polat et al., 2015), it will only result in more complexity and higher costs.

For both laboratory and clinical equipment, cytotoxicity is a problem. Nijs and colleagues (Nijs et al., 2009) state that toxicity can be caused by the composition of materials, the production process, the handling and packaging or the sterilization and transport processes. Using a human sperm survival assay (HSSA), these authors demonstrated that one type of sterile Pasteur pipette was related to a delayed manifestation of toxicity and that the inside lid of one type of sperm container caused an immediate negative impact on sperm motility. Others reported toxicity of certain ART products by use of a mouse embryo assay (MEA). The set up and validation of both assays is however poorly described, both biologically (Ainsworth et al., 2017) and statistically (Punt-van der Zalm et al., 2009). Also, pre-release clinical safety and effectivity tests of devices is missing in many cases and European legislation is unclear on this point (Wetzels et al., 2010). We conclude that additional well-described tests are needed before introduction of IUI and ART devices on the market.

Bed rest after IUI

The WHO guideline provides no recommendations for bed rest after IUI (WHO, 2010). The rationale for a positive impact of a short period of supine positioning after insemination is that the spermatozoa may reach the fallopian tube within only 10 min (Settlage et al., 1973). Immediate mobilization might counteract this movement due to gravity (Ledger, 2009).

Few RCTs evaluated the impact of 10–15 min of supine positioning on IUI PRs compared to direct mobilization. In two of these RCTs, with inclusion of 391 and 95 couples, supine positioning led to higher PRs (Saleh et al., 2000; Custers et al., 2009). In disagreement with these findings, a recent RCT found no significant positive effect of bed rest after IUI. This study was performed in 479 couples with idiopathic or mild male subfertility (van Rijswijk et al., 2016). Possible explanations for these differences might be found in the indications for IUI and the number of treatments. For this moment, it is not possible to advise one policy over the other.

Levels of evidence

Table III gives a summary of the impact of different laboratory procedures on IUI success. Also, the corresponding levels of evidence (LOE) according to the NICE guideline (NICE, 2013) are shown. These LOEs are however more or less misleading, especially in the assignment of level 1a and 1b. In these cases, the included RCTs are most of the times characterized by the absence of standardized methods or small sample sizes, resulting in contradictive results. In general, it is remarkable that the procedures for IUI are characterized by a low level of evidence or insufficient literature even though IUI has been performed for decades. Even well-designed retrospective studies are missing, while these could be performed relatively simply and would lead to valuable information for efficiently setting up the more complex RCT's.

Table III Main conclusions of the included literature about the impact of different laboratory procedures on IUI pregnancy rates and summary of the recommendations and suggested next steps in research. Presented are the levels of evidence according to the NICE guideline (NICE, 2013) and the number of studies.

Variable	Level of evidence	Number of studies	Main conclusions in literature; reported procedure with highest PRs	Recommendations based on literature and WHO guideline	Next steps in research
Ejaculatory abstinence	3	2	EA up to 2 // 3 days	EA ≤ 3 days	Evaluation in RCTs, with stratification for oligo- and normozoospermic men
Collection place (clinic versus at home)	3#	2	Collection in the clinic // no difference	Either in the clinic or at home	Evaluation in RCTs, with stratification for oligo- and normozoospermic men
Time intervals	3#	4	Avoid short and long TIs // no impact	Sample delivered within 1 h after collection, avoid long TIs between semen collection-insemination and semen processing-insemination	In first instance in multi-center retrospective studies, separately for oligo- and normozoospermic men
Semen preparation technique	1a#	6 †@	No superior method	Method selection should be based on semen sample	Identification of methodologies with best IUI results in retrospective studies (e.g. number of layers, volume of medium)
Buffer of wash medium	1b	1	HEPES buffer better than bicarbonate buffer	Selection of the medium buffer should be based on used incubator	Additional evaluation in RCTs, with stratification for oligo-and normozoospermic men
Centrifugation temperature	1b	1	No difference between body // testis and room temperature	Non-controlled centrifugation temperature, for reasons of ease	None
Storage temperature	2*	4	Storage at room temperature better than body temperature*	Avoid body temperature, especially during long-term storage	Evaluation of impact on PRs in RCTs, with stratification for oligo- and normozoospermic men
Method of timing IUI	1a	18 †	No superior method	No recommendable method	Evaluation in RCTs with standardized methods
Time between ovulation and insemination	1b	7	No superior time interval	Insemination 24–48 h after ovulation induction	Evaluation in RCTs with standardized methods, including insemination <24 h after ovulation induction. With stratification for oligo-and normozoospermic men
IUI devices	–	–	Some devices were reported as cytotoxic	Avoid the use of IUI devices that cause reprotoxicity	Development of well-described tests to identify safe and effective devices
Bed rest after IUI	1b	3	Bed rest of 10 // 15 min // no difference between bed rest and immediate mobilization	Either bed rest of 10–15 min or direct mobilization	Additional evaluation in RCTs, with stratification for oligo-and normozoospermic men

*Based on the impact on sperm parameters instead of pregnancy outcomes.
†Number of studies included in systematic review; # studies show contradictive results; @ number of studies in the meta analysis; // results of different studies.
EA = ejaculatory abstinence, TI = time interval, PR = pregnancy rate, RCT = randomized controlled trial.

Discussion

The general conclusion of this review is that evidence is poor on most technical aspects of the IUI procedure. Different studies show contradictive results, mainly due to a low degree of standardization, low statistical power and inaccuracy in handling confounding factors. Nevertheless, some advise can be given to change the current guidelines.

We state that an EA period of up to 3 days is preferable to the 2–7 days described in the WHO manual. Furthermore, we advised avoiding long time intervals between semen production and processing. It is easier to perform centrifugation and storage at room temperature and this yields good results. Finally, zwitterion-buffered media might be preferred over bicarbonate-buffered media and IUI devices should be validated using HSSA.

Although only a part of these recommendations are really evidence-based, we think that they could be introduced for reasons of standardization, comfort (ease), quality control and costs. This does not mean that further research on these items is expendable. As literature is scarce, every new study can influence the recommendations. This was also the case for two items in this review: the time between sperm preparation and insemination and bed rest after insemination. In these cases, a recent retrospective study and RCT, respectively, reported different study results than the former literature, which resulted in a last-minute change of the recommendations.

Although RCTs can be preferred, multicenter retrospective studies could be informative as well in some cases, because these studies can give us the possibility to include the many variables that are present in the IUI process over the different clinics. In these studies, it is important that the participating clinics share the same definitions on their data, e.g. for cycle number, pregnancy outcome and underlying diagnosis. Furthermore, a good registration of all technical variables is important. Next to clinical studies, for some variables, it could also be useful to perform biological experiments as alternative, like zona binding assays or measurement of DNA damage after different time intervals of incubation of prepared semen.

Based on the results of this review and in agreement with other IUI-related reviews (Keel *et al.*, 2002; Boomsma *et al.*, 2007), we emphasize the importance of standardization in IUI (study) protocols and guidelines. Here we meet another problem, since the readiness of clinics to follow existing guidelines is low (Riddell *et al.*, 2005; Haagen *et al.*, 2010), even when two different implementation strategies were used (Mourad *et al.*, 2011). An overview of guideline adherence of the laboratory stage of IUI is missing. We assume that this will be low also, because (older) studies ascribe limited willingness to follow guideline recommendations for processes related to IUI, like semen analysis (Helmerhorst *et al.*, 1995; Ombelet *et al.*, 1997; Souter *et al.*, 1997; Keel *et al.*, 2002; Riddell *et al.*, 2005) and to the vagueness and incompleteness of the recommendations, since supporting evidence is missing (Helmerhorst *et al.*, 1995; Penn *et al.*, 2011). We agree with earlier statements (Haagen *et al.*, 2013) that efforts should be made to improve guideline development and implementation by means of clinical results and economic consequences of IUI care.

This review indicates that further research on many IUI-related factors is necessary. We suggest to start with evaluating the current adherence to laboratory guidelines, e.g. by sending a questionnaire to fertility laboratories. This is also relevant with respect to semen analysis, as highlighted before (Bahadur *et al.*, 2016c); only a fraction of the laboratories is ISO 15189 accredited to the WHO standards for semen analysis (WHO, 2010). This may lead to wrong classification of semen samples and therefore disproportionate use of IVF and ICSI treatments.

Next, further research to update the current recommendations should include RCTs focusing on the impact on IUI success of wash medium buffers, storage temperature, timing of insemination (<24 h after ovulation induction) and bed rest. A first multicenter RCT could focus on two aspects of the sperm preparation method: gradient centrifugation compared to swim-up and bicarbonate compared to HEPES buffer. This study can be performed with enough power within a limited period of time. Whether aspects like EA and collection place should be studied in RCTs is point of discussion. In these cases, multicenter retrospective studies including patient and treatment characteristics (e.g. female age, cycle number, ovarian stimulation protocol) can be helpful instead.

With the results of these studies, guidelines can be updated and implementation strategies (e.g. educational materials or standardized training visits) can be drawn up. Subsequently, the effectiveness of the implementation strategy can be evaluated, both in pregnancy results and in costs.

Authors' roles

L.L., S.K., C.B. and A.W. searched the databases and wrote the first and final draft of this review. W.N. and D.B. contributed to the review by helping with the interpretation of the data, revising the article critically and writing the final draft.

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Conflict of interest

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References

AboulGheit S. Pregnancy rates following three different timings of intrauterine insemination for women with unexplained infertility: a randomised controlled trial. *Middle East Fertil Soc J* 2010;**15**:265–268.

Agarwal A, Manglona A, Loughlin KR. Improvement in semen quality and sperm fertilizing ability after filtration through the L4 membrane—comparison of results with swim up technique. *J Urol* 1992;**147**:1539–1541.

Ainsworth AJ, Fredrickson JR, Morbeck DE. Improved detection of mineral oil toxicity using an extended mouse embryo assay. *J Assist Reprod Genet* 2017;**34**:391–397.

Aitken RJ. The capacitation-apoptosis highway: oxysterols and mammalian sperm function. *Biol Reprod* 2011;**85**:9–12.

Aitken RJ, Nixon B. Sperm capacitation: a distant landscape glimpsed but unexplored. *Mol Hum Reprod* 2013;**19**:785–793.

Alkan I, Simsek F, Haklar G, Kervancioglu E, Ozveri H, Yalcin S, Akdas A. Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: relationship to seminal plasma antioxidants. *J Urol* 1997;**157**:140–143.

Almagor M, Benbenishti D, Rosenak D, Mogle Y, Yaffe H. Simultaneous swim-up/swim-down of sperm in assisted reproduction procedures. *J Assist Reprod Genet* 1993;**10**:261–265.

Aydin Y, Hassa H, Oge T, Tokgoz VY. A randomized study of simultaneous hCG administration with intrauterine insemination in stimulated cycles. *Eur J Obstet Gynecol Reprod Biol* 2013;**170**:444–448.

Bahadur G, Almossawi O, Ilahibuccus A, Al-Habib A, Okolo S. Factors leading to pregnancies in stimulated intrauterine insemination cycles and the use of consecutive ejaculations within a small clinic environment. *J Obstet Gynaecol India* 2016a;**66**:513–520.

Bahadur G, Almossawi O, Zeirideen Zaid R, Ilahibuccus A, Al-Habib A, Muneer A, Okolo S. Semen characteristics in consecutive ejaculates with short abstinence in subfertile males. *Reprod Biomed Online* 2016b;**32**:323–328.

Bahadur G, Homburg R, Muneer A, Racich P, Alangaden T, Al-Habib A, Okolo S. First line fertility treatment strategies regarding IUI and IVF require clinical evidence. *Hum Reprod* 2016c;**31**:1141–1146.

Bahadur G, Woodward B, Homburg R, Al-Habib A, Muneer A. Pitfalls of NICE recommendations on fertility treatment. *Br Med J* 2017;**356**:j751.

Bensdorp AJ, Tjon-Kon-Fat RI, Bossuyt PM, Koks CA, Oosterhuis GJ, Hoek A, Hompes PG, Broekmans FJ, Verhoeve HR, de Bruin JP *et al.* Prevention of multiple pregnancies in couples with unexplained or mild male subfertility: randomised controlled trial of in vitro fertilisation with

single embryo transfer or in vitro fertilisation in modified natural cycle compared with intrauterine insemination with controlled ovarian hyperstimulation. *Br Med J* 2015;**350**:g7771.

Boomsma CM, Heineman MJ, Cohlen BJ, Farquhar C. Semen preparation techniques for intrauterine insemination.*Cochrane Database Syst Rev* 2007;(4):CD004507.

Byrd W, Ackerman GE, Bradshaw KD, Maddox MA, Svendsen BA, Carr BR. Comparison of bicarbonate and HEPES-buffered media on pregnancy rates after intrauterine insemination with cryopreserved donor sperm. *Fertil Steril* 1991;**56**:540–546.

Calamera JC, Fernandez PJ, Buffone MG, Acosta AA, Doncel GF. Effects of long-term in vitro incubation of human spermatozoa: functional parameters and catalase effect. *Andrologia* 2001;**33**:79–86.

Cantineau AE, Janssen MJ, Cohlen BJ, Allersma T. Synchronised approach for intrauterine insemination in subfertile couples. *Cochrane Database Syst Rev* 2014;**12**:CD006942.

Carlsen E, Petersen JH, Andersson AM, Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. *Fertil Steril* 2004;**82**:358–366.

Carrell DT, Kuneck PH, Peterson CM, Hatasaka HH, Jones KP, Campbell BF. A randomized, prospective analysis of five sperm preparation techniques before intrauterine insemination of husband sperm. *Fertil Steril* 1998;**69**:122–126.

Centola GM, Herko R, Andolina E, Weisensel S. Comparison of sperm separation methods: effect on recovery, motility, motion parameters, and hyperactivation. *Fertil Steril* 1998;**70**:1173–1175.

Chan SY, Chan YM, Tucker MJ. Comparison of characteristics of human spermatozoa selected by the multiple-tube swim-up and simple discontinuous Percoll gradient centrifugation. *Andrologia* 1991;**23**:213–218.

Chen MJ, Bongso A. Comparative evaluation of two density gradient preparations for sperm separation for medically assisted conception. *Hum Reprod* 1999;**14**:759–764.

Chen SU, Ho HN, Chen HF, Chao KH, Lin HR, Huang SC, Lee TY, Yang YS. Comparison between a 2-layer discontinuous percoll gradient and swim-up for sperm preparation on normal and abnormal semen samples. *J Assist Reprod Genet* 1995;**12**:698–703.

Claassens OE, Menkveld R, Harrison KL. Evaluation of three substitutes for Percoll in sperm isolation by density gradient centrifugation. *Hum Reprod* 1998;**13**:3139–3143.

Claman P, Wilkie V, Collins D. Timing intrauterine insemination either 33 or 39 hours after administration of human chorionic gonadotropin yields the same pregnancy rates as after superovulation therapy. *Fertil Steril* 2004;**82**:13–16.

Custers IM, Flierman PA, Maas P, Cox T, Van Dessel TJ, Gerards MH, Mochtar MH, Janssen CA, van der Veen F, Mol BW. Immobilisation versus immediate mobilisation after intrauterine insemination: randomised controlled trial. *Br Med J* 2009;**339**:b4080.

De Jonge C, LaFromboise M, Bosmans E, Ombelet W, Cox A, Nijs M. Influence of the abstinence period on human sperm quality. *Fertil Steril* 2004;**82**:57–65.

Depuydt CE, Verstraete L, Berth M, Beert J, Bogers JP, Salembier G, Vereecken AJ, Bosmans E. Human papillomavirus positivity in women undergoing intrauterine insemination has a negative effect on pregnancy rates. *Gynecol Obstet Invest* 2016;**81**:41–46.

Dodson WC, Moessner J, Miller J, Legro RS, Gnatuk CL. A randomized comparison of the methods of sperm preparation for intrauterine insemination. *Fertil Steril* 1998;**70**:574–575.

Erel CT, Senturk LM, Irez T, Ercan L, Elter K, Colgar U, Ertungealp E. Sperm-preparation techniques for men with normal and abnormal semen analysis. A comparison. *J Reprod Med* 2000;**45**:917–922.

Fauque P, Lehert P, Lamotte M, Bettahar-Lebugle K, Bailly A, Diligent C, Cledat M, Pierrot P, Guenedal ML, Sagot P. Clinical success of intrauterine insemination cycles is affected by the sperm preparation time. *Fertil Steril* 2014;**101**:1618–U1465.

Franken DR, van Wyk R, Stoumann C, Avari K. Temperature controlled centrifugation improves sperm retrieval. *Andrologia* 2011;**43**:217–221.

Gadella BM, van Gestel RA. Bicarbonate and its role in mammalian sperm function. *Anim Reprod Sci* 2004;**82–3**:307–319.

Goldenberg M, Rabinovici J, Bider D, Lunenfeld B, Blankstein J, Weissenberg R. Intra-uterine insemination with prepared sperm vs. unprepared first split ejaculates. A randomized study. *Andrologia* 1992;**24**:135–140.

Gosalvez J, Gonzalez-Martinez M, Lopez-Fernandez C, Fernandez JL, Sanchez-Martin P. Shorter abstinence decreases sperm deoxyribonucleic acid fragmentation in ejaculate. *Fertil Steril* 2011;**96**:1083–1086.

Grigoriou O, Makrakis E, Konidaris S, Hassiakos D, Papadias K, Baka S, Creatsas G. Effect of sperm treatment with exogenous platelet-activating factor on the outcome of intrauterine insemination. *Fertil Steril* 2005;**83**:618–621.

Haagen EC, Nelen WL, Grol RP, Braat DD, Hermens RP, Kremer JA. Variation in guideline adherence in intrauterine insemination care. *Reprod Biomed Online* 2010;**20**:533–542.

Haagen EC, Nelen WLDM, Adang EM, Grol RPTM, Hermens RPMG, Kremer JAM. Guideline adherence is worth the effort: a cost-effectiveness analysis in intrauterine insemination care. *Hum Reprod* 2013;**28**:357–366.

Hammadeh ME, Strehler E, Zeginiadou T, Rosenbaum P, Schmidt W. Chromatin decondensation of human sperm in vitro and its relation to fertilization rate after ICSI. *Arch Androl* 2001a;**47**:83–87.

Hammadeh ME, Zavos PM, Rosenbaum P, Schmidt W. Comparison between the quality and function of sperm after semen processing with two different methods. *Asian J Androl* 2001b;**3**:125–130.

Helmerhorst FM, Oei SG, Bloemenkamp KW, Keirse MJ. Consistency and variation in fertility investigations in Europe. *Hum Reprod* 1995;**10**:2027–2030.

Heneghan C, Spencer EA, Bobrovitz N, Collins DR, Nunan D, Pluddemann A, Gbinigie OA, Onakpoya I, O'Sullivan J, Rollinson A *et al*. Lack of evidence for interventions offered in UK fertility centres. *Br Med J* 2016;**355**:i6295.

Henkel RR, Schill WB. Sperm preparation for ART. *Reprod Biol Endocrinol* 2003;**1**:108.

Holt WV, Fazeli A. The oviduct as a complex mediator of mammalian sperm function and selection. *Mol Reprod Dev* 2010;**77**:934–943.

Huang FJ, Chang SY, Lu YJ, Kung FT, Tsai MY, Wu JF. Two different timings of intrauterine insemination for non-male infertility. *J Assist Reprod Genet* 2000;**17**:213–217.

Jansen CHJR, Dijksman LM, Elisen MGLM, Leenstra CW, Kaaijk EM, van Stralen K,Verhoeve HR. Intra uterine insemination pregnancy outcome is not affected by a longer time interval between semen processing and insemination. *ESHRE Annual Meeting*, 2016, Helsinki, Finland.

Joshi NJ, Raj JP, Sundaram GS. Evaluation of quality of spermatozoa prepared by SpermPrep method as compared to those prepared by MiniPercoll. *Andrologia* 1998;**30**:79–83.

Jurema MW, Vieira AD, Bankowski B, Petrella C, Zhao Y, Wallach E, Zacur H. Effect of ejaculatory abstinence period on the pregnancy rate after intrauterine insemination. *Fertil Steril* 2005;**84**:678–681.

Kanwar KC, Yanagimachi R, Lopata A. Effects of human seminal plasma on fertilizing capacity of human spermatozoa. *Fertil Steril* 1979;**31**:321–327.

Karamahmutoglu H, Erdem A, Erdem M, Mutlu MF, Bozkurt N, Oktem M, Ercan DD, Gumuslu S. The gradient technique improves success rates in intrauterine insemination cycles of unexplained subfertile couples when compared to swim up technique; a prospective randomized study. *J Assist Reprod Genet* 2014;**31**:1139–1145.

Keel BA, Stembridge TW, Pineda G, Serafy NT. Lack of standardization in performance of the semen analysis among laboratories in the United States. *Fertil Steril* 2002;**78**:603–608.

Kilicdag EB, Bagis T, Haydardedeoglu B, Tanm E, Aslan E,SE. The prognostic factors that could be effect pregnancy rates in intra uterine insemination (IUI) cycles. *J Turk Soc Obstet Gynecol* 2005;**2**:223–228.

Kim D, Child T, Farquhar C. Intrauterine insemination: a UK survey on the adherence to NICE clinical guidelines by fertility clinics. *BMJ Open* 2015; **5**:e007588.

Lavie O, Margalioth EJ, GevaEldar T, BenChetrit A. Ultrasonographic endometrial changes after intrauterine insemination: a comparison of two catheters. *Fertil Steril* 1997;**68**:731–734.

Le Lannou D, Blanchard Y. Nuclear maturity and morphology of human spermatozoa selected by Percoll density gradient centrifugation or swim-up procedure. *J Reprod Fertil* 1988;**84**:551–556.

Ledger WL. Supine positioning after intrauterine insemination. *Br Med J* 2009;**339**:b4274.

Levitas E, Lunenfeld E, Weiss N, Friger M, Har-Vardi I, Koifman A, Potashnik G. Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples. *Fertil Steril* 2005;**83**: 1680–1686.

Levitas E, Lunenfeld E, Weisz N, Friger M, Har-Vardi I, Potashnik G. Relationship between sexual abstinence duration and the acrosome index in teratozoospermic semen: analysis of 1800 semen samples. *Andrologia* 2006;**38**:110–112.

Mansour RT, Serour MG, Abbas AM, Kamal A, Tawab NA, Aboulghar MA, Serour GI. The impact of spermatozoa preincubation time and spontaneous acrosome reaction in intracytoplasmic sperm injection: a controlled randomized study. *Fertil Steril* 2008;**90**:584–591.

Marin-Briggiler CI, Tezon JG, Miranda PV, Vazquez-Levin MH. Effect of incubating human sperm at room temperature on capacitation-related events. *Fertil Steril* 2002;**77**:252–259.

Marshburn PB, Alanis M, Matthews ML, Usadi R, Papadakis MH, Kullstam S, Hurst BS. A short period of ejaculatory abstinence before intrauterine insemination is associated with higher pregnancy rates. *Fertil Steril* 2010; **93**:286–288.

Marshburn PB, Giddings A, Causby S, Matthews ML, Usadi RS, Steuerwald N, Hurst BS. Influence of ejaculatory abstinence on seminal total antioxidant capacity and sperm membrane lipid peroxidation. *Fertil Steril* 2014;**102**: 705–710.

Matsuura R, Takeuchi T, Yoshida A. Preparation and incubation conditions affect the DNA integrity of ejaculated human spermatozoa. *Asian J Androl* 2010;**12**:753–759.

McCann CT, Chantler E. Properties of sperm separated using Percoll and IxaPrep density gradients. A comparison made using CASA, longevity, morphology and the acrosome reaction. *Int J Androl* 2000;**23**:205–209.

Moolenaar LM, Cissen M, de Bruin JP, Hompes PG, Repping S, van der Veen F, Mol BW. Cost-effectiveness of assisted conception for male subfertility. *Reprod Biomed Online* 2015;**30**:659–666.

Mortimer ST, Swan MA, Mortimer D. Effect of seminal plasma on capacitation and hyperactivation in human spermatozoa. *Hum Reprod* 1998;**13**: 2139–2146.

Mourad SM, Hermens RPMG, Liefers J, Akkermans RP, Zielhuis GA, Adang E, Grol RPTM, Nelen WLDM, Kremer JAM. A multi-faceted strategy to improve the use of national fertility guidelines; a cluster-randomized controlled trial. *Hum Reprod* 2011;**26**:817–826.

NICE. National Institute for Health and Clinical Excellence. *Fertility: Assessment and Treatment for People with Fertility Problems*, 2013, London.

Nijs M, Franssen K, Cox A, Wissmann D, Ruis H, Ombelet W. Reprotoxicity of intrauterine insemination and in vitro fertilization-embryo transfer disposables and products: a 4-year survey. *Fertil Steril* 2009;**92**:527–535.

Noblanc A, Kocer A, Chabory E, Vernet P, Saez F, Cadet R, Conrad M, Drevet JR. Glutathione peroxidases at work on epididymal spermatozoa: an example of the dual effect of reactive oxygen species on mammalian male fertilizing ability. *J Androl* 2011;**32**:641–650.

Ombelet W, Pollet H, Bosmans E, Vereecken A. Results of a questionnaire on sperm morphology assessment. *Hum Reprod* 1997;**12**:1015–1020.

Oruc AS, Yilmaz N, Gorkem U, Inal HA, Seckin B, Gulerman C. Influence of ultrasound-guided artificial insemination on pregnancy rates: a randomized study. *Arch Gynecol Obstet* 2014;**289**:207–212.

Oztekin D, Ozcinar E, Kose C, Gulhan I, Ozeren M, Tinar S. The use of ultrasound during intrauterine insemination in unexplained infertility may improve pregnancy outcomes. *Med Princ Pract* 2013;**22**:291–294.

Pacey AA, Hill CJ, Scudamore IW, Warren MA, Barratt CLR, Cooke ID. The interaction in-vitro of human spermatozoa with epithelial-cells from the human uterine (fallopian) tube. *Hum Reprod* 1995;**10**:360–366.

Peer S, Eltes F, Berkovitz A, Yehuda R, Itsykson P, Bartoov B. Is fine morphology of the human sperm nuclei affected by in vitro incubation at 37°C? *Fertil Steril* 2007;**88**:1589–1594.

Penn HA, Windsperger A, Smith Z, Parekattil SJ, Kuang WW, Kolettis PN, Nangia AK. National semen analysis reference range reporting: adherence to the 1999 World Health Organization guidelines 10 years later. *Fertil Steril* 2011;**95**:2320–2323.

Polat I, Ekiz A, Yildirim G, Sahin O, Ulker V, Alkis I, Tekirdag AI. Ultrasound-guided intrauterine insemination versus blind intrauterine insemination: a randomized controlled trial. *Clin Exp Obstet Gynecol* 2015;**42**:657–662.

Polyzos NP, Tzioras S, Mauri D, Tatsioni A. Double versus single intrauterine insemination for unexplained infertility: a meta-analysis of randomized trials. *Fertil Steril* 2010;**94**:1261–1266.

Posada MN, Azuero AM, Arango AM, Raigosa GC, Cano JF, Perez AL. Sperm washing with swim up versus gradients in intrauterine insemination (IUI): results of a prospective randomized study comparing pregnancy rates and costs. *Fertil Steril* 2005;**84**:S361–S361.

Punt-van der Zalm JP, Hendriks JC, Westphal JR, Kremer JA, Teerenstra S, Wetzels AM. Toxicity testing of human assisted reproduction devices using the mouse embryo assay. *Reprod Biomed Online* 2009;**18**:529–535.

Ragni G, De Lauretis L, D'Ambrogio G, Pellegrini S, Maggioni P, Vegetti W, Perotti L, Dalla Serra A, Parazzini F, Crosignani PG. Semen preparation by standard swim-up versus swim-up with test yolk buffer incubation in intrauterine insemination: a randomized study. *Hum Reprod* 1998;**13**: 1859–1863.

Rahman SM, Karmakar D, Malhotra N, Kumar S. Timing of intrauterine insemination: an attempt to unravel the enigma. *Arch Gynecol Obstet* 2011;**284**:1023–1027.

Ramon O, Matorras R, Corcostegui B, Meabe A, Burgos J, Exposito A, Crisol L. Ultrasound-guided artificial insemination: a randomized controlled trial. *Hum Reprod* 2009;**24**:1080–1084.

Ren SS, Sun GH, Ku CH, Chen DC, Wu GJ. Comparison of four methods for sperm preparation for IUI. *Arch Androl* 2004;**50**:139–143.

Repalle D, Chittawar PB, Bhandari S, Joshi G, Paranjape M, Joshi C. Does centrifugation and semen processing with swim up at 37°C yield sperm with better DNA integrity compared to centrifugation and processing at room temperature? *J Hum Reprod Sci* 2013;**6**:23–26.

Requena A, Cruz M, Pacheco A, Garcia-Velasco JA. Ongoing pregnancy rates in intrauterine insemination are affected by late follicular-phase progesterone levels. *Fertil Steril* 2015;**104**:879–883.

Riddell D, Pacey A, Whittington K. Lack of compliance by UK andrology laboratories with World Health Organization recommendations for sperm morphology assessment. *Hum Reprod* 2005;**20**:3441–3445.

Rogers BJ, Perreault SD, Bentwood BJ, McCarville C, Hale RW, Soderdahl DW. Variability in the human-hamster in vitro assay for fertility evaluation. *Fertil Steril* 1983;**39**:204–211.

Saleh A, Tan SL, Biljan MM, Tulandi T. A randomized study of the effect of 10 minutes of bed rest after intrauterine insemination. *Fertil Steril* 2000; **74**:509–511.

Sanchez-Martin P, Sanchez-Martin F, Gonzalez-Martinez M, Gosalvez J. Increased pregnancy after reduced male abstinence. *Syst Biol Reprod Med* 2013;**59**:256–260.

Selvaraj P, Selvaraj K, Kalaichelvi S, Mahalakshmi R. Semen preparation techniques in intrauterine insemination: a comparison of non-temperature and temperature controlled centrifugation in cases of unexplained infertility. *J Hum Reprod Sci* 2013;**6**:241–244.

Settlage DS, Motoshima M, Tredway DR. Sperm transport from the external cervical os to the fallopian tubes in women: a time and quantitation study. *Fertil Steril* 1973;**24**:655–661.

Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. *Urology* 1996;**48**:835–850.

Sharma RK, Seifarth K, Garlak D, Agarwal A. Comparison of three sperm preparation media. *Int J Fertil Womens Med* 1999;**44**:163–167.

Soliman S, Goyal A. RCT comparing two different sperm preparations for intrauterine insemination. *Fertil Steril* 2005;**84**:S156–S156.

Song GJ, Herko R, Lewis V. Location of semen collection and time interval from collection to use for intrauterine insemination. *Fertil Steril* 2007;**88**:1689–1691.

Souter VL, Irvine DS, Templeton AA. Laboratory techniques for semen analysis: a Scottish survey. *Health Bull (Edinb)* 1997;**55**:140–149.

Spencer EA, Mahtani KR, Goldacre B, Heneghan C. Claims for fertility interventions: a systematic assessment of statements on UK fertility centre websites. *BMJ Open* 2016;**6**:e013940.

Suarez SS, Pacey AA. Sperm transport in the female reproductive tract. *Hum Reprod Update* 2006;**12**:23–37.

Svalander PC, Lundin K, Holmes PV. Endotoxin levels in Percoll® density-gradient media used to prepare sperm for human IVF treatment. *Hum Reprod* 1995;**10**:130.

Swain JE. Optimizing the culture environment in the IVF laboratory: impact of pH and buffer capacity on gamete and embryo quality. *Reprod Biomed Online* 2010;**21**:6–16.

Thijssen A, Klerkx E, Huyser C, Bosmans E, Campo R, Ombelet W. Influence of temperature and sperm preparation on the quality of spermatozoa. *Reprod Biomed Online* 2014;**28**:436–442.

Tjon-Kon-Fat RI, Bensdorp AJ, Bossuyt PMM, Koks C, Oosterhuis GJE, Hoek A, Hompes P, Broekmans FJ, Verhoeve HR, de Bruin JP *et al*. Is IVF-served two different ways-more cost-effective than IUI with controlled ovarian hyperstimulation? *Hum Reprod* 2015;**30**:2331–2339.

Tsai YC, Lin MY, Chen SH, Kang CY, Tsai YT, Lin LY, Chuan LT, Huang KF. Comparing the clinical outcomes of intrauterine insemination by two different density gradient preparation methods. *J Chin Med Assoc* 2004; **67**:168–171.

van der Poel N, Farquhar C, Abou-Setta AM, Benschop L, Heineman MJ. Soft versus firm catheters for intrauterine insemination. *Cochrane Database Syst Rev* 2010;(11):CD006225.

van Rijswijk J, Caanen M, Ammi Y, Mijatovic V, Vergouw C, Lambalk C, Schats R. Should patients be immobilised after intrauterine insemination? A randomised controlled comparison between 15 minutes of immobilisation and direct mobilisation. *ESHRE Annual Meeting*, 2016, Helsinki, Finland.

Van Voorhis BJ, Barnett M, Sparks AE, Syrop CH, Rosenthal G, Dawson J. Effect of the total motile sperm count on the efficacy and cost-effectiveness of intrauterine insemination and in vitro fertilization. *Fertil Steril* 2001;**75**:661–668.

Wang YC, Chang YC, Chen IC, Cnien HH, Wu GJ. Comparison of timing of IUI in ovarian stimulated cycles. *Arch Androl* 2006;**52**: 371–374.

Weiss A, Beck-Fruchter R, Lavee M, Geslevich Y, Golan J, Ermoshkin A, Shalev E. A randomized trial comparing time intervals from HCG trigger to intrauterine insemination for cycles utilizing GnRH antagonists. *Syst Biol Reprod Med* 2015;**61**:44–49.

Wetzels AM, Kastrop PM, Group NT-W. Dutch technical specification (NTA 8070) on devices for assisted reproductive technologies. *Reprod Biomed Online* 2010;**21**:252–258.

WHO. World Health Organization. In: *Laboratory Manual for the Examination and Processing of Human Semen*, 5th edn. Geneva: World Health Organization, 2010.

Woodward B, Tomlinson M, Kirkman-Brown J. Replacing IUI with IVF for initial treatment of unexplained infertility: why this NICE recommendation is cause for concern. *Hum Fertil (Camb)* 2016;**19**:80–84.

Xu L, Lu RK, Chen L, Zheng YL. Comparative study on efficacy of three sperm-separation techniques. *Asian J Androl* 2000;**2**:131–134.

Yavas Y, Selub MR. Intrauterine insemination (IUI) pregnancy outcome is enhanced by shorter intervals from semen collection to sperm wash, from sperm wash to IUI time, and from semen collection to IUI time. *Fertil Steril* 2004;**82**:1638–1647.

Zavos PM, Centola GM. Methods of semen preparation for intrauterine insemination and subsequent pregnancy rates. *Tohoku J Exp Med* 1992; **168**:583–590.