

# Intrauterine instillation of diluted seminal plasma at oocyte pick-up does not increase the IVF pregnancy rate: a double-blind, placebo controlled, randomized study

M. von Wolff<sup>1,\*</sup>, S. Rösner<sup>2</sup>, A. Germeyer<sup>2</sup>, J. Jauckus<sup>2</sup>, G. Griesinger<sup>3</sup>, and T. Strowitzki<sup>2</sup>

<sup>1</sup>Department of Gynaecological Endocrinology, University Women's Hospital, and Reproductive Medicine, University of Berne, Effingerstrasse 102, Berne 3010, Switzerland <sup>2</sup>Department of Gynaecological Endocrinology and Reproductive Medicine, University Women's Hospital, University of Heidelberg, Vossstrasse 9, Heidelberg 69115, Germany <sup>3</sup>Department of Gynaecological Endocrinology and Reproductive Medicine, University Hospital of Schleswig-Holstein, Campus Luebeck, Ratzeburger Allee 160, Luebeck 23538, Germany

\*Correspondence address. Tel: +41-31-632-1303; Fax: +41-31-632-1305; E-mail: michael.vonwolff@insel.ch

Submitted on April 8, 2013; resubmitted on July 30, 2013; accepted on August 8, 2013

**STUDY QUESTION:** Does intrauterine application of diluted seminal plasma (SP) at the time of ovum pick-up improve the pregnancy rate by  $\geq 14\%$  in IVF treatment?

**SUMMARY ANSWER:** Intrauterine instillation of diluted SP at the time of ovum pick-up is unlikely to increase the pregnancy rate by  $\geq 14\%$  in IVF.

**WHAT IS KNOWN ALREADY:** SP modulates endometrial function, and sexual intercourse around the time of embryo transfer has been suggested to increase the likelihood of pregnancy. A previous randomized double-blind pilot study demonstrated a strong trend towards increased pregnancy rates following the intracervical application of undiluted SP. As this study was not conclusive and as the finding could have been confounded by sexual intercourse, the intrauterine application of diluted SP was investigated in the present trial.

**STUDY DESIGN, SIZE, DURATION:** A single-centre, prospective, double-blind, placebo-controlled, randomized, superiority trial on women undergoing IVF was conducted from April 2007 until February 2012 at the University Department of Gynaecological Endocrinology and Reproductive Medicine, Heidelberg, Germany.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** The study was powered to detect an 14% increase in the clinical pregnancy rate and two sequential tests were planned using the Pocock spending function. At the first interim analysis, 279 women had been randomly assigned to intrauterine diluted SP (20% SP in saline from the patients' partner) ( $n = 138$ ) or placebo ( $n = 141$ ) at the time of ovum pick-up.

**MAIN RESULTS AND THE ROLE OF CHANCE:** The clinical pregnancy rate per randomized patient was 37/138 (26.8%) in the SP group and 41/141 (29.1%) in the placebo group (difference:  $-2.3\%$ , 95% confidence interval of the difference:  $-12.7$  to  $+8.2\%$ ;  $P = 0.69$ ). The live birth rate per randomized patient was 28/138 (20.3%) in the SP group and 33/141 (23.4%) in the placebo group (difference:  $-3.1\%$ , 95% confidence interval of the difference:  $-12.7$  to  $+6.6\%$ ;  $P = 0.56$ ). It was decided to terminate the trial due to futility at the first interim analysis, at a conditional power of 62%.

**LIMITATIONS, REASONS FOR CAUTION:** The confidence interval of the difference remains wide, thus clinically relevant differences cannot reliably be excluded based on this single study.

**WIDER IMPLICATIONS OF THE FINDINGS:** The results of this study cast doubt on the validity of the concept that SP increases endometrial receptivity and thus implantation in humans.

**STUDY FUNDING/COMPETING INTEREST(S):** Funding was provided by the department's own research facilities.

**TRIAL REGISTRATION NUMBER:** DRKS00004615.

**Key words:** endometrium / seminal plasma / *in vitro* fertilization / implantation

## Introduction

Besides the quality of the embryo, endometrial receptivity plays an important role in the establishment of pregnancy. Endometrial functional disorders are likely of little relevance in a physiological context, e.g. spontaneous conception in a natural cycle. However, under non-physiological conditions such as high-dose gonadotrophin stimulation and absence of sexual intercourse, as it is typically the case in IVF treatment, endometrial dysfunction is likely to play a role in the non-achievement of pregnancy despite the transfer of vital embryos. Horcajades *et al.* (2005) were able to detect a dysregulation of endometrial gene expression under high-dose gonadotrophin stimulation. Furthermore, Gutsche *et al.* (2003) and von Wolff *et al.* (2007) demonstrated that seminal plasma (SP) has a direct effect on endometrial cytokine expression.

In order to improve endometrial receptivity under IVF treatment, numerous treatments have been proposed, such as the administration of acetylsalicylic acid and/or heparin, as well as active and passive immunotherapy. The efficacy of these treatments, however, has never been unequivocally proved.

Increasing knowledge regarding the high immunomodulatory potential of SP on endometrial function has motivated various study groups to use SP as a physiological endometrial stimulant. Tremellen *et al.* (2000) showed that the proportion of transferred embryos that were viable at 6–8 weeks was significantly higher in women exposed to semen through intercourse compared with those who abstained. Von Wolff *et al.* (2009) carried out a prospective randomized double-blind study, where SP was applied intracervically at the time of follicle puncture. This pilot study showed that there was a higher clinical pregnancy rate in the patients treated with SP compared with those who received isotonic sodium chloride as placebo, although the difference was not statistically significant. The pregnancy rate per embryo transfer was ~12% higher in the SP group than in the placebo group and the pregnancy rate per randomized patient was ~8% higher than in the placebo group. Another recently published paper supported the result of this study. Chicea *et al.* (2013) performed an almost identical study but without the use of sodium chloride as placebo. They found a non-significantly higher pregnancy rate in 346 treated patients in the SP group than in the group without SP (55.5 versus 44.0%,  $P = 0.09$ ) and a significant higher implantation rate (34.7 versus 27.5%,  $P = 0.026$ ).

Despite inconclusive results, the study from von Wolff *et al.* gave a first estimate of potential treatment effect sizes on which subsequent study designs can be based. Accordingly, the present study was designed as a superiority study, employing a similar methodological rigour, e.g. a prospective, randomized, placebo-controlled, double-blind, single-centre study design. In contrast to the pilot study (von Wolff *et al.*, 2009), SP was instilled into the lower part of the uterine cavity at the time of follicle aspiration. It was hypothesized that intrauterine instillation would potentially increase the effect when compared with intra-cervical instillation. Furthermore, the risk of protocol violation by patients ignoring the advice not to engage in intercourse required a protocol, which was less dependent on such effects, especially as absence from intercourse can hardly be proved (Gallo *et al.*, 2006).

As the intrauterine instillation of undiluted SP can cause uterine contractions, SP was used in a diluted form in the present trial. The *in vitro* studies mentioned above have not investigated whether the endometrial effects can also be achieved by using diluted SP.

## Materials and Methods

This is a single-centre, two-armed, randomized, placebo-controlled, double-blind clinical study investigating superiority of SP administration at the time of oocyte pick-up in a German university affiliated IVF programme.

### Subjects

All couples undergoing IVF or ICSI treatment at the Department of Gynaecology and Reproductive Medicine, Heidelberg, between April 2007 and February 2012 were considered for enrolment in the trial. Of these, 279 women (25.2–43.5 years of age) were eligible for enrolment and consented to participate in the study. Participation was limited to one treatment cycle per couple. The exclusion criteria included the presence of hepatitis B, C or human immunodeficiency virus, leukocytospermia or other signs of infection and men with <300  $\mu$ l of SP. The study was approved by the local ethical committee and each patient's approval was given by written consent.

### Clinical IVF protocol

Either a long agonist ( $n = 267$ ), short agonist ( $n = 2$ ) or antagonist ( $n = 10$ ) protocol was applied for ovarian stimulation.

For the long agonist protocol, pituitary desensitization was performed by intranasal application of the GnRH agonist 0.4 mg nafarelin acetate (Synarela<sup>®</sup>, Merck Serono, Darmstadt, Germany), starting in the luteal phase of the previous cycle. After down-regulation of the pituitary gland, patients received in addition either 150–300 IU of recombinant FSH (Gonal F<sup>®</sup>, Merck Serono) or highly purified HMG (Menogon HP<sup>®</sup>, Ferring, Hamburg, Germany) per day. Dosages were adjusted according to estradiol concentrations and vaginal ultrasound scan.

For the short agonist protocol, nasal application of 0.4 mg nafarelin acetate was started on Day 1 or 2 of the menstrual cycle and was continued until ovulation induction. FSH or HMG stimulation was started on Day 3 of the cycle using the dosages as described above.

For the antagonist protocol, FSH or HMG was initiated between Days 3 and 5 of the menstrual cycle using the dosages as described above. GnRH antagonists (Orgalutran<sup>®</sup>, MSD, Haar, Germany or Cetrotide<sup>®</sup>, Merck Serono) were started between Days 6 and 7 of the menstrual cycle and continued until ovulation induction.

Once an adequate ovarian response had been confirmed, FSH and HMG were discontinued and 10 000 IU of urinary hCG (Predalon<sup>®</sup>, MSD, Haar, Germany) or 250  $\mu$ g of recombinant hCG (Ovitrelle<sup>®</sup>, Merck Serono) was administered to induce ovulation. Transvaginal oocyte retrieval was scheduled 34–36 h after hCG administration and was performed under general anaesthesia. Fertilization was achieved by standard IVF or ICSI. One to three oocytes at the pronuclear stage were selected and cultured for another 1–2 days, when they were transferred back into the uterus. Selection of embryos for transfer was not performed due to the restrictions of the German embryo protection law. Intravaginal progesterone (600 mg/day) was used as luteal support.

### Randomization

Physicians reported a scheduled oocyte pick-up with the information relevant for randomization to the IVF laboratory once the stimulation was started. The technician (J.J.) in the laboratory randomized the patient 1 day before the oocyte aspiration was scheduled. The technician took SP or the placebo to the operating theatre on the day of aspiration. The blinded sample was handed over to the physician to inject the sample into the uterine cavity under general anaesthesia.

For randomization, two randomization lists (Stratums I and II) with block randomization for every 20 patients were prepared before the onset of the

study. Patients were consecutively added to the list on the day that follicle aspiration was scheduled. Accordingly patients were allocated to one of the treatment arms.

Stratums I and II were introduced as the pregnancy rate of IVF/ICSI treatments is highly dependent on the age of the patient and on the number of previous treatment cycles. Stratum I (expected low pregnancy rate) was defined as:  $\geq 36$  or  $< 36$  years of age and transfer of  $\geq 6$  embryos in  $\geq 3$  embryo transfers without a pregnancy in previously performed IVF/ICSI cycles. All other patients were allocated to Stratum II (expected a high pregnancy rate).

## Preparation and application of SP

Semen samples were obtained  $\geq 1/2$  weeks before follicle aspiration by masturbation from the patient's partner and were collected in sterile flasks. SP was extracted by centrifugation of ejaculates at 600 g for 15 min. A second round of centrifugation was performed at 10 000 g for 15 min to extract all spermatozoa. SP was analysed several times to exclude remaining contamination with spermatozoa. None of the samples contained any spermatozoa after centrifugation. Then 400  $\mu$ l of supernatant were diluted with 1600  $\mu$ l of sterile sodium chloride and mixed. From this solution, 1500  $\mu$ l was stored in 2 ml syringes at  $-20^{\circ}\text{C}$  (verum). For the placebo, 1500  $\mu$ l of sterile sodium chloride was also stored in 2 ml syringes at  $-20^{\circ}\text{C}$ . Blinding of the samples was achieved by using a placebo (sodium chloride), which could not be differentiated from SP due to the same optical appearance. SP and placebo samples were stored in separate freezers in exactly the same syringes with the names of both partners attached for later allocation.

On the day of oocyte collection, blinded SP or placebo samples were taken out of the freezer by the technician around 30–60 min before the aspiration of the follicles and were transferred to the operating theatre. Follicles were aspirated and bleeding was stopped. An insemination catheter (Laboratoire C.C.D., Paris, France) was introduced just above the end of the cervix and 1500  $\mu$ l SP or placebo was injected.

## Outcome measures, statistics and sample size

The study was based on the hypothesis of superiority of SP administration in terms of clinical pregnancy likelihood. Based on an increase in pregnancy rates found in the pilot study (von Wolff *et al.*, 2009), it was calculated that group sample sizes of 269 in the SP group and 269 in the placebo group could achieve 90% power to detect a difference in clinical pregnancy rate of 14%. This difference of 14% was considered as clinically meaningful by the authors. The clinical pregnancy rate in the SP group was assumed to be 24% under the null hypothesis and 38% under the alternative hypothesis. The clinical pregnancy rate in the placebo group was expected to be 24%. The test statistic is set at an alpha level of 0.05 using a two-sided Z-test with continuity correction. These results assume that two sequential tests are made using the Pocock spending function to determine symmetrical test boundaries (Software program used for this calculation: PASS 2005, Kaysville, Utah, USA). Thus, an interim analysis was planned when 50% of the total sample had been randomized. If that interim analysis revealed a positive effect of SP administration at an alpha of 0.031, the study had to be prematurely stopped at a power of 60% and superiority had been proved. If this was not the case, the study had to be continued.

The primary outcome measure is the clinical pregnancy rate (fetal heart beat) per randomized patients 4–5 weeks after follicle aspiration. The SP and placebo groups are described in terms of age, etiology of infertility, stimulation protocol, proportion of patients undergoing IVF or ICSI, average number of transferred embryos and average number of blastomeres per transferred embryo. All data are presented descriptively as means ( $\pm$  standard deviation or ranges), or proportions with 95% confidence intervals, as appropriate. Fisher's exact test was used to compare dichotomous

characteristics of the SP and placebo groups, as well as pregnancy rates in both groups. The significance level alpha was set to 5%.

## Results

A pre-planned interim analysis was performed at 50% recruitment, e.g. when 279 patients had been enrolled and randomized. The clinical pregnancy rate per randomized patient was 37/138 (26.8%) in the SP group and 41/141 (29.1%) in the placebo group (difference:  $-2.3$ , 95% confidence interval of the difference:  $-12.7$  to  $+8.2\%$ ;  $P = 0.69$ ). The live birth rate per randomized patient was 28/138 (20.3%) in the SP group and 33/141 (23.4%) in the placebo group (difference:  $-3.1$ , 95% confidence interval of the difference:  $-12.7$  to  $+6.6\%$ ;  $P = 0.56$ ).

Contrary to the hypothesis and the pilot study, no trend toward a positive treatment effect was seen with SP administration. The z-score value of  $-0.2858$  was exceeding the lower boundary of  $-2.1570$  as determined by the Pocock spending function for the interim analysis. At a conditional power of 62%, it thus was decided to terminate the trial due to its futility. If the comparator pregnancy rate had remained stable at 29% till the end of the trial (e.g. when the pre-planned sample size had been reached), a pregnancy rate of 59.7% would have been necessary to reach statistical significance in the remaining 130 patients to be allocated to the intervention.

Table I describes the demographics and outcomes of the two groups. Characteristics of the 279 trial participants were similar and not statistically different in the SP and placebo groups. There were 143 patients allocated to Stratum I and 136 patients allocated to Stratum II. In 239 patients, embryos were transferred. Of those, 122 patients received SP and 117 placebo. Even though diluted SP was injected into the uterine cavity, neither painful cramps nor infections were reported at oocyte pick-up or 2 weeks later (Fig. 1).

An  $\sim 8\%$  lower pregnancy rate in Stratum I (pregnancies: 34/143 = 23.8%), including patients  $\geq 36$  years or those with previous implantation failure, than in Stratum II (pregnancies: 44/136 = 32.4%), confirmed the adequacy of stratification. Adequacy of randomization was confirmed by the similar characteristics of trial participants in the two trial arms (Table I).

## Discussion

The study revealed that intrauterine application of SP at the time of follicle aspiration in 279 IVF treatment cycles did not increase the pregnancy rate compared with placebo. It is important to emphasize that at the outset of this study some over-enthusiasm about a supposed effect of SP on implantation likelihood led to the decision to design a superiority trial, which could be prematurely terminated in favour of the alternative hypothesis using a common alpha spending function approach to group sequential testing. It is also important to emphasize that no distinct lower boundary of the alpha spending function to determine futility was pre-specified. With the results at the first interim analysis in hand, it was then decided, on both clinical and statistical reasoning, to terminate the study early. There are, however, important drawbacks to this decision: first, there is still a chance, albeit very small, that SP instillation would be superior to placebo had the trial been continued. Secondly, the true effect of SP instillation into the uterine cavity could well be negative and with the limited sample size such a phenomenon cannot be ruled out with confidence.

**Table 1** Characteristics of trial participants and outcome of treatment assignment

	SP (n = 138)	Placebo (n = 141)	Difference (95% CI)	P
Mean age (years)	34.6 (25.2–43.5)	34.9 (25.2–41.3)		
Etiology of infertility (n/total)				
Male factor	49/138	50/141		
Tubal factor	19/138	15/141		
Idiopathic	5/138	10/141		
Others	8/138	7/141		
Unknown	57/138	59/141		
Long agonist protocol	134/138	133/141		
Short agonist protocol	0	2/141		
Antagonist protocol	4/138	6/141		
Patients with previous implantation failure <sup>a</sup> (n/total)	70/138 (Stratum I)	72/141 (Stratum I)		
IVF (n/total)	56/138	65/141		
ICSI (n/total)	82/138	76/141		
Median no. of transferred embryos	1.83 (1–3)	1.86 (1–3)		
No. of blastomeres/transferred embryo	3.8	3.8		
Clinical pregnancy rate: Stratum I (n/total) <sup>b</sup>	15/70 (21.4%)	19/73 (26.0%)	–4.6% (–18.3 to 9.4)	0.56
Clinical pregnancy rate: Stratum II (n/total) <sup>b</sup>	22/68 (32.4%)	22/68 (32.4%)	0% (–15.4 to 15.4)	1.00
Total clinical pregnancies (n/total)	37/138 (26.8%)	41/141 (29.1%)	–2.3% (–12.7 to 8.2)	0.69
Abortions (n/total)	8/138 (5.8%)	6/141 (4.3%)	1.5% (–4.9 to 7.2)	0.59
Tubal pregnancies (n/total)	1/138 (0.7%)	1/141 (0.7%)	0% (–3.2 to 3.3)	1.00
Deliveries (n/total)	28/138 (20.3%)	33/141 (23.4%) <sup>c</sup>	–3.1 (–12.7 to 6.6) <sup>c</sup>	0.56

<sup>a</sup>Previous implantation failure: transfer of  $\geq 6$  embryos in  $\geq 3$  embryo transfers without a pregnancy.

<sup>b</sup>Stratum I: expected low pregnancy rate:  $\geq 36$  or  $< 36$  years of age and transfer of  $\geq 6$  embryos in  $\geq 3$  embryo transfers without a pregnancy in previously performed IVF/ICSI-cycles. Stratum II: expected high pregnancy rate: all other patients.

<sup>c</sup>Information about whether live birth occurred is missing for one patient with a clinical pregnancy in the placebo group. This pregnancy was not counted as a live birth. If this pregnancy resulted in live birth, the placebo group live birth rate is 34/141 (24.1%) and the difference between treatments is –3.8% (95% CI: –13.5 to 6.0;  $P = 0.47$ ).

The potential effect of ejaculate or SP on endometrial function has been the subject of numerous investigations for several years, mainly in animal models. However, when interpreting these studies, it should be remembered that in many species such as mouse, horse and pig, the ejaculate reaches the uterine cavity physiologically, whereas probably only very small amounts ascend through the cervical canal in the human system.

In the animal model it has been shown that SP or sperm in the cervix and endometrium induce the synthesis of proinflammatory cytokines (Robertson, 2007). The cytokines and the paternal antigens such as sperm and seminal leukocytes recruit and activate inflammatory cells in the endometrium, including macrophages, dendritic cells and granulocytes. Furthermore, paternal antigens have been suggested to trigger a beneficial tolerant immune response towards paternal antigens that then favours the successful implantation of the resultant embryo sharing those same paternal antigens (Robertson et al., 2013). Even though SP has significant molecular and cellular effects on the endometrium in animals, SP does not appear to be essential for the establishment of a pregnancy in these animals. Tests have shown that intrauterine insemination, even after extraction of SP, leads to pregnancy in horses and pigs (Katila, 2012).

In humans, investigations have been largely limited to *in vitro* experiments. Gutsche et al. demonstrated that SP stimulates the expression

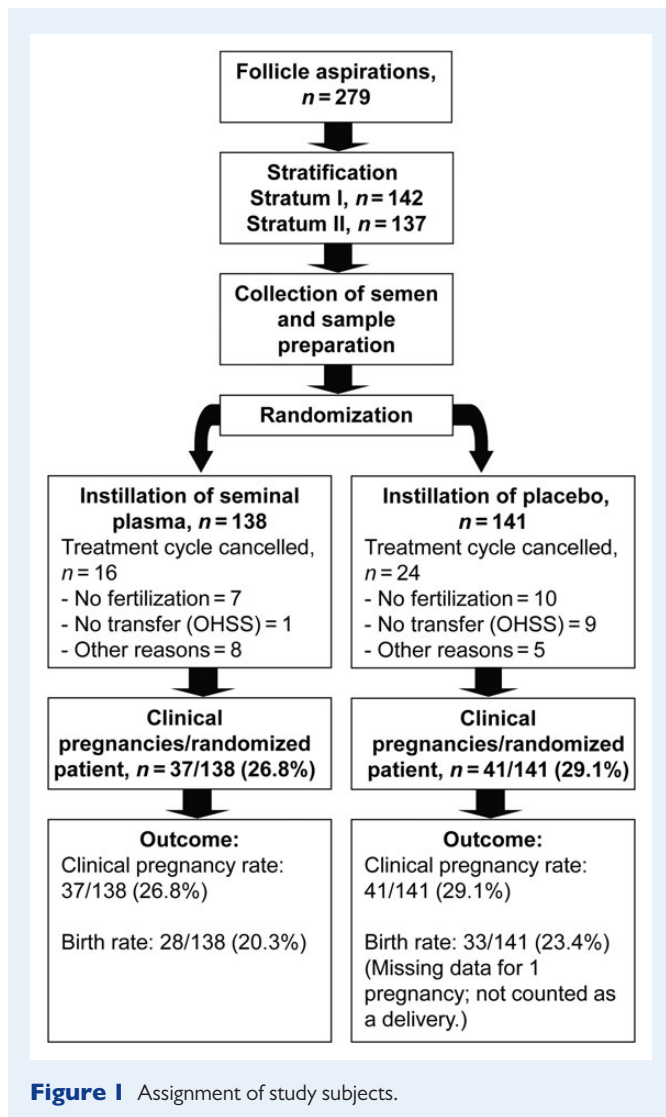
of leukaemia inhibitory factor (LIF) and interleukin 6 (IL-6) *in vitro* (Gutsche et al., 2003). Furthermore, a recent study demonstrated that SP increases the decidualization of stromal cells (Doyle et al., 2012). The concept of this study implies that active SP factors reach the endometrial stromal cells through the luminal epithelium. Based on investigations by Kucharski et al. (2008), who detected a transfer of cytokines administered into the uterine cavity into the bloodstream of pigs, such a mechanism is conceivable. The above study also implies that accelerated decidualization leads to an improvement in the endometrial implantation efficiency. However, recent studies have shown, by using antagonist protocols, that an early rise in progesterone and the probable resulting decidualization leads to a reduced pregnancy rate (Ochsenkühn et al., 2012).

Because studies on animal models and *in vitro* studies can only be applied to the clinical situation to a limited extent, only clinical studies can ultimately clarify whether SP has a relevant effect on pregnancy rates.

On the basis of this, the present study was carried out in succession of a study published in 2009 (von Wolff et al., 2009). The trend of the previous study which showed a positive effect of SP could not be confirmed in this study.

There are several possible reasons for the diverging results of these studies.





Firstly, the treatment concepts are different. In the study published in 2009, pure SP was instilled into the cervix, which is very close to the physiological situation during sexual intercourse. In our new study in which SP was injected into the uterine cavity, a treatment concept was chosen which is largely independent, but which represents a non-physiological form of treatment. Indeed, SP has never been proved to ascend in substantial volumes through the cervix into the uterine cavity. In humans, endometrial inflammatory responses induced by SP have only been shown *in vitro* (Gutsche *et al.*, 2003), whereas cervical inflammatory responses following exposure to ejaculate have been demonstrated *in vivo* (Sharkey *et al.*, 2001a). In addition, *in vitro* experiments have revealed that this effect is at least in part due to SP and not just due to semen (Sharkey *et al.*, 2012b). However, even though the intracervical and intrauterine treatment approach seems to be very different, in practice, they are possibly not. During the cervical instillation of SP, as performed in the first study, a substantial amount of SP probably also enters the uterine cavity. It can therefore be speculated that in both studies the effect of undiluted intracervical SP and diluted intrauterine SP would exert the same effect on the uterine endometrium. As the effect on the cervix and the vagina was however much more pronounced

in the first study and in the study by Chicea *et al.* (2013), this suggests, that in humans SP does act but more via the cervix and vagina than via the uterine cavity.

Furthermore, it could be speculated that the passage of the transfer catheter through the cervix already would have a positive effect, through which the positive effect of the SP was overshadowed. Meta-analyses have shown that a hysteroscopy, even without proof of any pathophysiological results, significantly increases the pregnancy rate of a subsequent IVF treatment (El-Toukhy *et al.*, 2008). In addition, several studies have demonstrated an increased pregnancy rate after endometrial injury before IVF treatment (Nastri *et al.*, 2012). This effect was not seen after endometrial injury at the time of follicle puncture. However, as the introduction of a transfer catheter possibly also causes some cervical trauma, it might be speculated that this would have a positive effect in our new study, although in reality such an effect was not observed.

Finally, there is the possibility that intracavitary treatment with SP does not have any clinically relevant immunomodulatory effects on the endometrium at all or that the dilution of SP reduced the effect to such an extent that it was not pronounced enough to have a substantial effect on endometrial function.

Indeed, the present study suggests that SP instillation into the uterine cavity is unlikely to increase the chance of clinical pregnancy, at least not by a rate  $\geq 14\%$  as initially hypothesized.

## Authors' roles

M.V. was responsible for the study conception and design, acquisition, analysis and interpretation of data and drafting the article. S.R., A.G. and J.J. were responsible for acquisition and analysis of data and revising the article. G.G. was responsible for analysis and interpretation of data and revising the article. T.S. was responsible for the study conception and revising the article. All authors gave final approval of the manuscript.

## Funding

Funding was exclusively limited to the department's own financial research facilities.

## Conflict of interest

There is no conflict of interest of any of the authors.

## References

- Chicea R, Ispasoiu F, Focsa M. Seminal plasma insemination during ovum-pickup—a method to increase pregnancy rate in IVF/ICSI procedure. A pilot randomized trial. *J Assist Reprod Genet* 2013;**30**:569–574.
- Doyle U, Sampson N, Zenzmaier C, Schwärzler P, Berger P. Seminal plasma enhances and accelerates progesterone-induced decidualisation of human endometrial stromal cells. *Reprod Fertil Dev* 2012;**24**:517–522.
- El-Toukhy T, Sunkara SK, Coomarasamy A, Grace J, Khalaf Y. Outpatient hysteroscopy and subsequent IVF cycle outcome: a systematic review and meta-analysis. *Reprod Biomed Online* 2008;**16**:712–719.
- Gallo MF, Behets FM, Steiner MJ, Hobbs MM, Hoke TH, Van Damme K, Ralimamonjy L, Raharimalala L, Cohen MS. Prostate-specific antigen to ascertain reliability of self-reported coital exposure to semen. *Sex Transm Dis* 2006;**33**:476–479.

- Gutsche S, von Wolff M, Strowitzki T, Thaler CJ. Seminal plasma induces mRNA expression of IL-1 $\beta$ , IL-6 and LIF in endometrial epithelial cells *in vitro*. *Mol Hum Reprod* 2003;**9**:785–791.
- Horcajades JA, Riesewijk A, Polman J, Van Os R, Pellicer A, Mosselmann S, Simón C. Effect of controlled ovarian hyperstimulation in IVF on endometrial gene expression profiles. *Mol Hum Reprod* 2005;**11**:195–205.
- Katila T. Post-mating inflammatory responses of the uterus. *Reprod Domest Anim* 2012;**47** (Suppl. 5):31–41.
- Kucharski J, Stefańczyk-Krzyszowska S, Jana B. Absorption of proinflammatory cytokines from inflamed porcine uterus into the uterine venous blood—preliminary data. *Pol J Vet Sci* 2008;**11**:9–16.
- Nastri CO, Gibreel A, Raine-Fenning N, Maheshwari A, Ferriani RA, Bhattacharya S, Martins WP. Endometrial injury in women undergoing assisted reproductive techniques. *Cochrane Database Syst Rev* 2012; **11**:CD009517.
- Ochsenkühn R, Arzberger A, von Schönfeldt V, Gallwas J, Rogenhofer N, Crispin A, Thaler CJ, Noss U. Subtle progesterone rise on the day of human chorionic gonadotropin administration is associated with lower live birth rates in women undergoing assisted reproductive technology: a retrospective study with 2,555 fresh embryo transfers. *Fertil Steril* 2012; **98**:347–354.
- Robertson SA. Seminal fluid signaling in the female reproductive tract: lessons from rodents and pigs. *J Anim Sci* 2007;**85** (Suppl. 13):E36–E44.
- Robertson SA, Prins JR, Sharkey DJ, Moldenhauer LM. Seminal fluid and the generation of regulatory T cells for embryo implantation. *Am J Reprod Immunol* 2013;**69**:315–330.
- Sharkey DJ, Tremellen KP, Jasper MJ, Gemzell-Danielsson K, Robertson SA. Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus. *J Immunol* 2012a; **188**:2445–2454.
- Sharkey DJ, Macpherson AM, Tremellen KP, Mottershead DG, Gilchrist RB, Robertson SA. TGF- $\beta$  mediates proinflammatory seminal fluid signaling in human cervical epithelial cells. *J Immunol* 2012b; **189**:1024–1035.
- Tremellen KP, Valbuena D, Landeras J, Ballesteros A, Martinez J, Mendoza S, Norman RJ, Robertson SA, Simon C. The effect of intercourse on pregnancy rates during assisted human reproduction. *Hum Reprod* 2000; **15**:2653–2658.
- von Wolff M, Nowak O, Pinheiro RM, Strowitzki T. Seminal plasma—immunomodulatory potential in men with normal and abnormal sperm count. *Eur J Obstet Gynecol Reprod Biol* 2007; **134**:73–78.
- von Wolff M, Rösner S, Thöne C, Pinheiro RM, Jauckus J, Bruckner T, Biolchi V, Alia A, Strowitzki T. Intravaginal and intracervical application of seminal plasma in *in vitro* fertilization or intracytoplasmic sperm injection treatment cycles—a double-blind, placebo-controlled, randomized pilot study. *Fertil Steril* 2009;**91**:167–172.