Abstract FRM New York 2017

The REPROSTAR: A novel high security system for a safe and standardized density gradient separation of human semen

Study question
To evaluate a new gradient forming and harvesting tube allowing direct access to the pellet fraction whereby contamination of the pellet is eliminated.

Summary answer
The efficacy of the REPROSTAR was monitored by the use of human seminal plasma samples from postvasectomized patients and of human sperm samples both spiked with horse radish peroxidase. It could be shown that post contamination of the sperm pellet occurred significantly less as compared to conventional harvesting techniques. In addition, less wash steps were required to achieve the same cell purity as compared with the conventional gradient forming and harvesting technique.

What is known already?
Traditional density centrifugation is associated with the risk to post contaminate the separated pellet fraction due to the harvesting step. Access to the pellet can usually be enabled by aspiration or penetration of the entire gradient overlay leading to the fact, that several wash steps are necessary to observe the desired purity of the pellet. Tube systems which can avoid post contamination are commercially available, but not designed to guarantee a maximal risk reduction of pellet contamination since all steps (gradient forming, sperm overlay and pellet harvesting) have to be performed from the upper entrance of the tube.

Study design, size and duration
The REPROSTAR has been developed in order to allow direct access to the pellet by means of a leading channel conjoined with a side entrance of the tube. Thereby the contact with the upper layer of the gradient can by bypassed, whereby the screw cap of gradient tube remains closed during the harvesting step. Ten prototypes were used to evaluate both the efficacy of the harvesting- as well as the wash steps.

Participants/ materials, setting, method
Ten individual human semen samples derived from patients of the daily andrology routine were spiked with defined concentrations of horseradish peroxidase (HRP). After density gradient separation the pellet fractions were harvested using either the traditional aspiration of the gradient overlays (DG group) or using the REPROSTAR system (RS group). Data were monitored by determination of the O.D. values at 492 nm. To evaluate the efficacy of the washing steps thirteen individual sperm samples were spiked with HRP and analyzed.

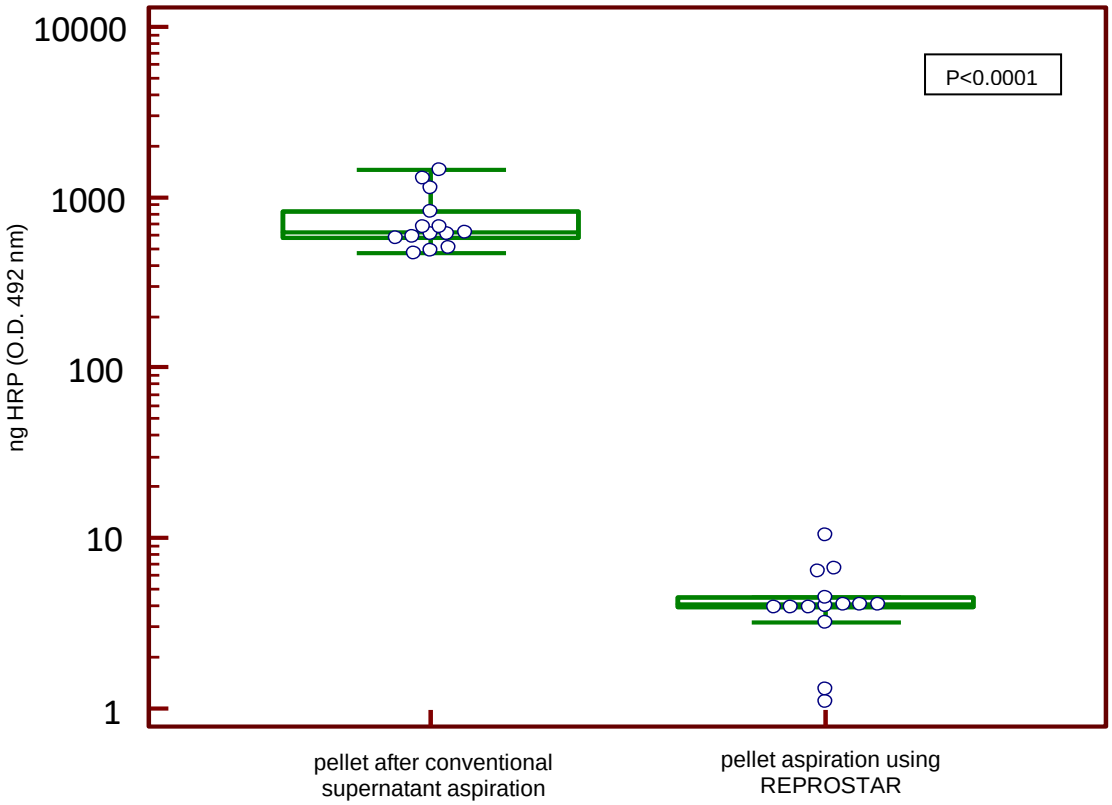
Main results and the role of chance
Data of mean values of the DG group where 1746 ng (range 544 - 2391 ng) and in the RS group 39 ng (range 10 - 39 ng). The mean value of the DG group was 106.2 times higher as compared to the RS group (4037.3 vs. 38.0; $p<0.0002$).
To illustrate the effect of washing steps spiked sperm samples were of both groups were compared and expressed as ratios pre-and post treatment. In the DG group three washing steps were necessary to achieve approx. the same cell purity as compared to the RS group without any washing steps.

Limitation, reasons for caution
The data represented by means of HRP can reflect the significance of the REPROSTAR system only in an indirect way. Clinical data observed from human sperm as well as CE-marking are in progress and will be available soon.

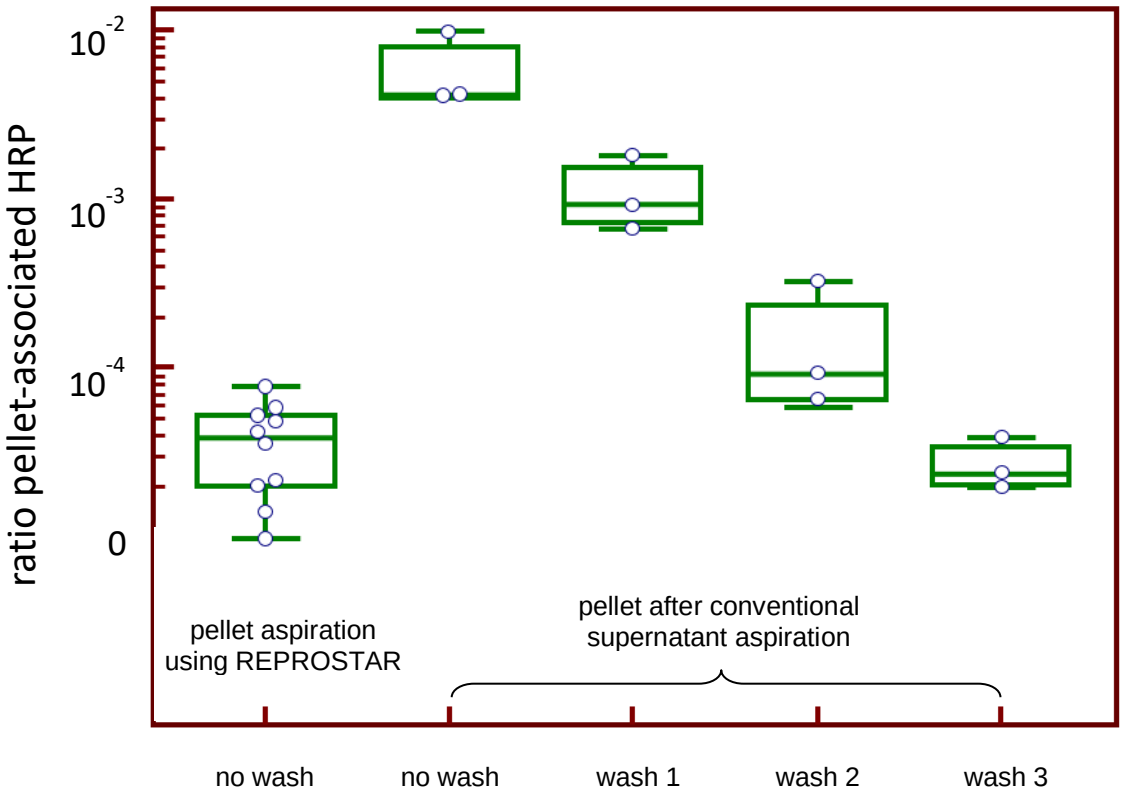
Wider implications of the findings
The REPRSTAR represents a novel high security system for a safe and efficient density gradient preparation on which direct access to the contamination-free pellet fraction is guaranteed. A reduction of wash steps leads not only to less cell damage but also to economies of wash buffer and time.

Authors: Roth, F.; Hollenstein, S.; Häberlin, F.; Kuwayama, M.

REPROSTAR reduces pellet contamination



REPROSTAR avoids excessive wash steps



fiore LAB
FACHLABOR FÜR REPRODUKTIONSMEDIZIN
LABORATORY SERVICES FOR ASSISTED CONCEPTION

REPROLIFE

The REPROSTAR: A novel high security device for a safe and standardized density gradient separation

F. Roth[1], M. Suerdieck[1], F. Haeberlin[1], M. Kuwayama[2]
[1]fiore: Institute of Reproductive Medicine, St. Gallen, Switzerland
[2]REPROLIFE Research Centre, Development, Tokyo, Japan

Introduction

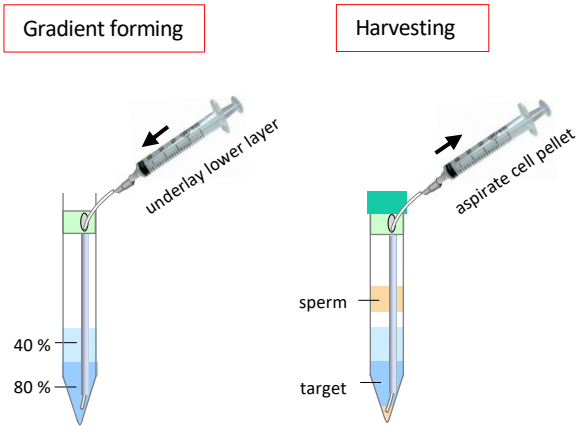
Density gradient separation of human sperm is the method of choice in most of the ART Laboratories worldwide. Standardized gradient forming is difficult to achieve since the overlay can easily be mixed up with the lower layer of the gradient. Furthermore the target fraction, enriched with high quality sperm, is located on the bottom of the tube. Both penetration and aspiration of load and gradient will lead to contamination of the target fraction. Multiple washing steps after harvesting are time and cost intense and lead to cell damage.

Double tubes which allow direct access to the cell pellet fraction are available, but the risk of contamination still exists since sperm loading and harvesting compartment are localized close to each other.

The presented "secure sperm tube for assisted reproduction" (STAR) involves both the possibility of standardized gradient forming as well as a high security harvesting step of the pellet fraction by means of a leading tube joined to a side entrance.

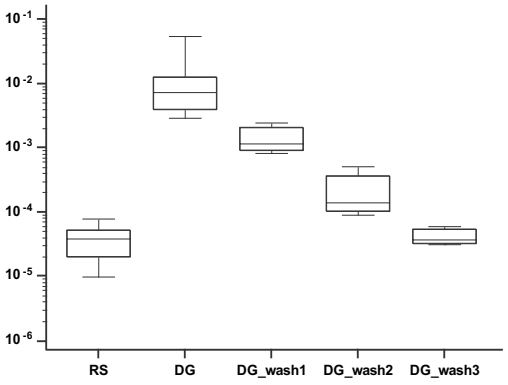
Materials and Methods

Ten individual sperm samples from the daily Andrology routine were spiked with defined amounts of horse radish peroxidase (HRP), split into two groups and quantified before and after density gradient centrifugation. O.D. values at 492 nm were monitored after classical harvesting by aspiration of the whole supernatant (DG) followed by three wash steps (DG_wash 1-3) and compared with data generated using the REPROSTAR due to standardized gradient forming and bypassing the sperm overlay (RS).



Results

Fig. 1 REPROSTAR reduces pellet contamination and washing steps



Significantly lower HPR contaminants could be monitored in the RS group as compared to the DG group ($P<0.001$). Three washing steps were necessary to achieve approx. the same cell purity in the DG group as compared to the RS group without washings.

Discussion

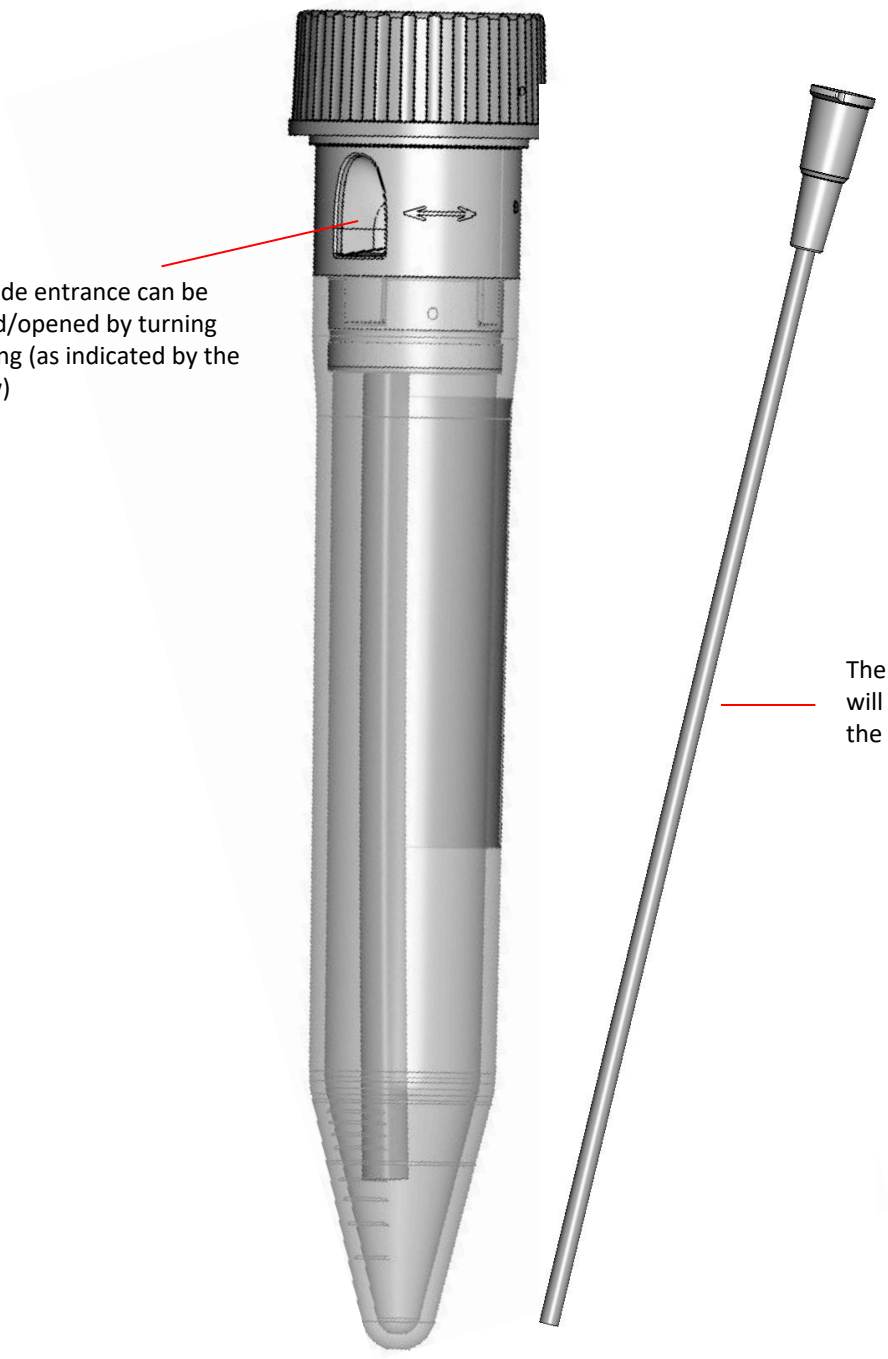
The REPROSTAR represents a novel possibility for high security density gradient separation. Exclusion of contamination during the harvesting step is given by a lateral entrance of the tube, whereby the main cap of the tube remains closed.

Density gradient forming using the lateral entrance is facilitated allowing a clear separation of the gradient layers.

The data observed by means of HRP quantification indirectly reflect the high application potential of the REPROSTAR. A clinical trial is intended.

A reduction of wash steps not only safes costs and time, but especially leads to less cell damage which enables a more powerful fertility treatment for men.

The REPROSTAR



The side entrance can be closed/opened by turning the ring (as indicated by the arrow)

The harvesting canulla will be delivered within the tube

Annual 2018 Conference Of The Foundation For Reproductive Medicine
TRANSLATIONAL REPRODUCTIVE BIOLOGY AND CLINICAL REPRODUCTIVE ENDOCRINOLOGY
"Paradigms you will not hear about elsewhere!" and "Think differently!"
NOVEMBER 15-18, 2018 • NEW YORK, USA

SEMEN PROCESSING –SWIM-UP VS SWIM-DOWN TECHNIQUE: RE-EVALUATION OF THE BENEFITS

Author Block: F. Roth[1] B. Wusk[1] R. Redolfi[1] F. Haeberlin[2]; [1]fiore lab, St. Gallen, Switzerland, [2]Institute of Reproductive Medicine, St. Gallen, Switzerland

Abstract:

OBJECTIVE: The ideal sperm separation technique should (i) be quick, easy and cost-effective, (ii) isolate as much motile spermatozoa as possible, (iii) not cause sperm damage or non-physiological alterations of the separated sperm cells, (iv) eliminate dead spermatozoa and other cells (eg. Leukocytes, bacteria), (v) eliminate toxic or bioactive substances (e.g. decapacitation factors, reactive oxygen species), and (vi) allow processing of larger volumes of ejaculates.
Since none of the methods available meets all these requirements, a variety of sperm separation techniques is mandatory in clinical practice to obtain an optimal yield of functionally competent spermatozoa for insemination purposes (Henkel, Schill 2003).
Here, we re-evaluate the simultaneous swim-up/ swim-down sperm processing technique.

DESIGN: We developed in-house a tube prototype, called Reprostar (secure sperm tube for assisted reproduction), for gentle semen processing with focus on the swim-down method. The smart design of an additional, lateral entrance of the tube enables direct access to the motile spermatozoa fraction at the bottom of the tube.

MATERIALS AND METHODS: This prospective, mono-centric, non-randomized study assessed seminal parameters before and after semen processing. Random, surplus semen aliquots derived from routine andrology analysis patients, (n=33, mean age: 36.2±4.7) were prepared with a simultaneous swim-up/ swim-down sperm processing technique followed by one washing/ centrifugation step. Swim-down was achieved by overlaying the sperm fraction on 2ml of a 40% gradient solution (PureSperm, Nidacon). The tube was incubated 1hr at 34.5°C. Results of both procedures were compared before and after processing.

RESULTS: As compared to the native semen (65±7.3 Mio/ml), both techniques swim-up and swim-down revealed a clearly lower sperm concentration (14±2.6 Mio/ml vs 23±3.4 Mio/ml). The proportion of sperm with fast forward progression (WHO A) was significantly increased in the swim-up (68±1.3) and the swim-down (65±1.1) procedure vs the native semen (43±2.5).
In addition, the overall yield of the swim-down was about 1.5x higher as compared to the yield after swim-up (52.7% vs 34.2%).

	native semen	swim up	swim down
conc (Mio/ml)	65±7.3	14±2.6	23±3.4
mot (WHO A)	43±2.5	68±1.3	65±1.1
conc recovery (%)	100	21.3	34.6
yield WHO A (%)	100	34.2	52.7

Table 1: mean values±SEM

CONCLUSIONS: Spermatozoa will be selected by their ability to swim out of seminal plasma into culture medium and/or 40% gradient solution. To date, the direct swim-up method is the preferred and most gentle technique for separating motile spermatozoa (Mortimer 1994), accepting the

drawback of a low yield .
Using the Reprostar tube we show that both techniques reveal a high proportion of motile sperm. The additional benefit of the higher yield using the swim-down procedure could be useful for ART treatments, where an increased number of spermatozoa is required (eg. IVF or IUI).
Keywords: semen processing, swim-up, swim-down, Reprostar

abstract accepted 17.08.2018

SEMEN PROCESSING - IMPROVING HANDLING, SAFETY AND EFFICACY IN ROUTINE ART LABORATORIES

Authors: F. Roth,[1] B. Wusk,[1] R. Redolfi,[1] M. Kuwayama,[2] F. Haeberlin[3]; [1]fiore, St. Gallen, Switzerland, [2]ReproSupport Medical Research Centre, Tokyo, Japan, [3]Institute of Reproductive Medicine, St. Gallen, Switzerland

Abstract:
OBJECTIVE: Semen samples should be handled with special care as a biohazard by laboratory personnel (WHO, 2010). Statistics have indicated the positive identification of microbes in approximately 50% of all semen samples obtained for ART procedures, with gram-negative species present in only a fraction of samples (Fourie et al., 2015).
Today, laboratory's staff safety but economic considerations as well are key factors in routine ART laboratories.
DESIGN: We developed a new device for semen processing to improve handling, safety and efficacy. Reprostar (secure sperm tube for assisted reproduction), a local prototype, is a novel tube for high security **density gradient separation**. Amongst fewer centrifugation steps, safe harvesting (lateral entrance of the tube) of the semen pellet is of most importance, especially for contaminated semen samples.
MATERIALS AND METHODS: Both techniques - standard vs Reprostar density gradient separation - have been processed in 30 semen samples. Benefits have been evaluated and compared according to: (1) simplicity, (2) gradient forming, (3) sperm pellet harvesting, (4) overall consumables and (5) sample processing time.
RESULTS: Reprostar density gradient separation combines several improvements compared to the current standard procedure as recommended by WHO: (1) fewer handling steps, eg. washes, centrifugations, tube changes, (2) more precise and sharp separation between gradient layers, (3) a reduced risk of cross- and post-contamination while sperm pellet harvesting due to the lateral entrance, (4) less buffer solution, wash tubes and pipetting steps and (5) an overall time saving of about 50%.
CONCLUSIONS: Biosafety training and utilization of practical procedures such as sperm decontamination are fundamental tools in any laboratory's risk-reduction to prevent, reduce or eliminate infectious or sperm-harming elements.
By implemetation of the Reprostar density gradient separation, we profit from a safe handling of sperm processing, while saving time and consumables.

Financial Support & References:
Financial Support: none
References: WHO, Examination and processing of human semen, 5th edition, 2010
FourieJM. et al. Reprod Biomed Online. 2015 Mar;30(3):296-302

Category (Complete): Procedures and Techniques-Laboratory: ART
Topic (Complete): Sperm Preparation