

Nidacon News

The news letter from your ART supplier • No 1 • 2014

Contents

Trichomonas vaginalis _____	1
Comparison of outcomes after slow freezing and vitrification using DMSO or PrOH of human blastocysts _____	1
Comparison of different buffers _____	2
Selection of spermatozoa _____	3
PS100-1000 ml _____	4
SpermCryo Protec™ _____	4
Upcoming events _____	4
Who to contact _____	4
Frequently asked questions _____	4

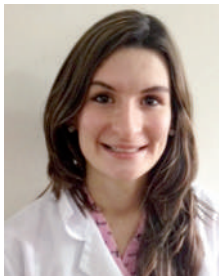
▶ Trichomona vaginalis a latent intruder in unexplained infertility?

SUMMARY

The incidence of T. vaginalis infection has been increasing around the world over the past decade. This has led to insight of the importance of this microorganism as a serious problem in reproductive health outcomes including pelvic inflammatory disease, pregnancy complications and an increased risk of HIV acquisition. For natural fertility, not only optimal semen sample that contains a normal and motile sperm cell number is needed but also to be free of any microorganism including T. vaginalis. Here, we present a case report of a male patient with unexplained



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ned infertility who underwent an IVF cycle. The ejaculated sample from this patient was collected for IVF purposes and immediately analyzed under microscopic examination. Contamination with T. vaginalis and several polymorphonuclear cells were shown in the semen sample. In addition, severe asthenozoospermia was observed which has a negative impact on achieving oocyte fertilization. A laboratory protocol using density gradients separation and a special device, the ProInsert™ from Nidacon, was performed as

a preparation method of the semen sample for capacitation and recovery of motile sperm cells needed for the IVF procedure. We found that the density gradients with the ProInsert™ effectively removed T. vaginalis microorganisms and polymorphonuclear cells, leaving only the motile sperm and giving rise to a successful in vitro oocyte fertilization and embryo development. Embryos were transferred but unfortunately, pregnancy was not achieved due to an embryo implantation failure that may be caused by endometrial T.vaginalis infection from asymptomatic female partner.

▶ Comparison of outcomes after vitrification using DMSO or PrOH.

More and more clinics are now discovering our products, VitriBlast™ and ThermoBlast™ for vitrification of blastocysts.

One of them is IVF clinic Falun, a private clinic in Sweden. They presented a study at the Nordic meeting earlier this year where they had compared slow freezing and vitrification using DMSO and vitrification using PrOH.

Background: The clinic has successfully used slow freezing for cryopreservation of blastocyst and before implementing a new met-

hod the methods were evaluated.

Study question: Does slow freezing of human blastocysts give comparable results to those of vitrification? Does vitrification with DMSO yield better results than with PrOH?

Main outcome measures: Cryosurvival, implantation and birth rates.



IVF clinic Falun.

with DMSO and vitrification with PrOH. Blastocysts were frozen or vitrified according to the clinic's protocol using a closed system. The frozen cycles were conducted with single embryo transfers.

Results: The results showed no significant difference between groups with regards to implantation and live birth rates. Blastocysts vitrified with DMSO showed significantly higher survival rates than blastocyst vitrified with PrOH.

Design and setting: Prospective randomized pilot study at a private IVF clinic.

Methods: 300 IVF cycles were randomized into three groups, slow freezing, vitrification

	Slow freezing	DMSO	PrOH
Number of cycles	100	100	100
Survival rate	93%	93%	87%
Positive hCG	41%	46%	40%
Born babies/Ongoing pregn.	28%	28%	26%

► Effect of various commercial buffers on sperm viability and capacitation.

Report from University of Padova, Italy.

Sperm preparation in washing buffers is a very important step in maintaining the correct physiological activities of cells. The procedure widely adopted to assess cell viability is normally restricted to evaluation of flagellum motility/hyperactivation, a parameter which is insufficient for sperm characterization.

Recent studies emphasized the importance of the evaluation of novel parameters, such as biochemical analysis of the endogenous content of reactive oxygen species (ROS) and the level of Tyr-phosphorylation of the cells, as mostly efficacy features depicting the correct status of sperm. (Donà et al., 2011). In fact, only when the above mechanisms are correctly satisfied, sperm may be considered capacitated and can undergo acrosome reaction. Capacitation is a set of alterations leading to the acrosome reaction (AR), an exocytotic process mediated by hydrolytic enzymes (e.g acrosin) that are released to allow sperm to fertilize the oocyte.

What is to be underlined is that one of the most delicate step in the ART procedure is represented by the correct sperm preparation, including initial washing and final incubation leading to the precious achievement of the capacitated status.

A recent study (Andrisani et al, 2014.) compared the effect of different commercial buffers according to their capacity to induce ROS production, the Tyr-P of the head and consequently, AR, paying parti-

cular attention to the cell survival at the end of 2h incubation. Interestingly, PSW-Nidacon was by far the best medium for sperm preparation: in fact, cells reaching the AR (67.2±7.9%) was almost three-five fold compared with results obtained with other commercial buffers. PSW-Nidacon also preserved cells from apoptosis (only 3.5±1.4% of total cells were not viable) compared with the great number observed with the other ones (Fig. 1).

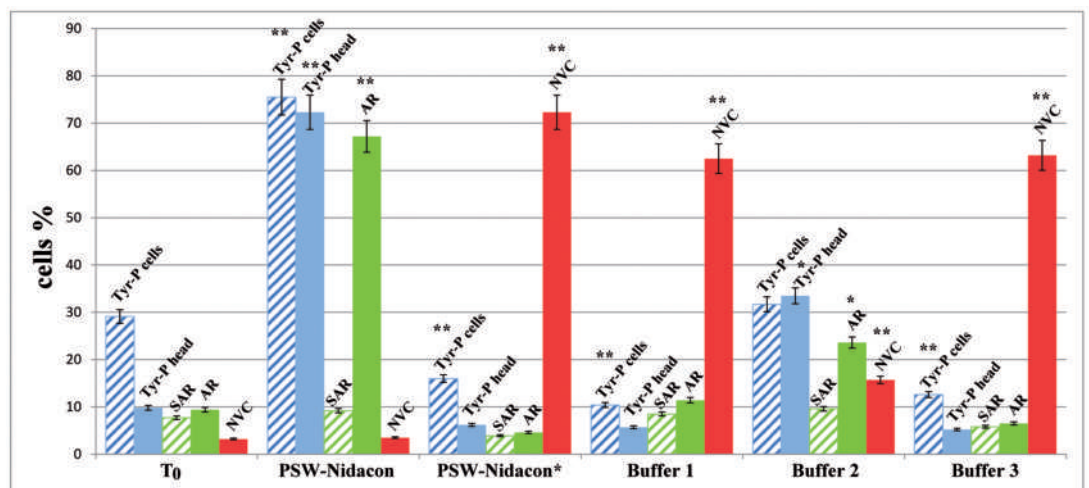
The study also addressed the well-known problem on how buffers must be stored, since human serum albumin, glucose, lactate etc. promote bacterial/fungal contamination, thus resulting in increased buffer ROS production with a consequent fast sperm denaturation and inability to reach the capacitated state.

Capacitation implies marked reorganization of membrane architecture, due to the activity of extracellular proteins which

have the task of extracting cholesterol and reorganizing membrane in specialized microdomains, also called rafts, capable of remodelling and reorganizing themselves to undergo acrosome reaction.

Once capacitation has occurred, these rafts migrate from the flagellum, where they are found extensively, to the periacrosomal region where they presumably allow interaction with the oocyte. The buffers used in the ART for sperm preparation must induce optimal conditions to achieve AR, an, once more, PSW clearly showed the best membrane reorganization to achieve the potential AR. PSW-Nidacon is an optimal sperm medium to prepare sperm to undergo the potential successive AR. And to prevent time-dependent denaturation.

In order to avoid any complications, it is recommendable to use fresh buffer avoiding prolonged storage.



(Andrisani et al., 2014) Sperm, incubated for 180 min in capacitating conditions in different buffers, were analysed for Tyr-P pattern, AR and viability (NVC) by immunofluorescence cytochemistry as described in Methods. Number of cells expressed as % of the total amount of cells showing Tyr-P in any part of the cell-body, or in the head, were detected and reported as Tyr-P cells and Tyr-P head, respectively. Percentage of cells undergoing acrosome reaction or not viable cells (AR and NVC, respectively) were also reported. Spontaneous AR percentages were also evaluated in each sample in the absence of A23187 and values reported (SAR). ** $p < 0.0001$ and * $p < 0.02$, comparing each sample against T0 (ANOVA followed by Dunnett's post hoc test). Values are expressed as the mean \pm SD.

References Andrisani A, Donà G, Ambrosini G, Bonanni G, Bragadin M, Cosmi E, Clari G, Armanini D, Bordin L. Effect of various commercial buffers on sperm viability and capacitation. *Syst Biol Reprod Med.* 2014 Mar 27. [Epub ahead of print] Donà G, Fiore C, Andrisani A, Ambrosini G, Brunati A, Ragazzi E, Armanini D, Bordin L, Clari G. Evaluation of correct endogenous reactive oxygen species content for human sperm capacitation and involvement of the NADPH oxidase system. *Hum Reprod.* 2011;26(12):3264-73. Donà G, Fiore C, Tibaldi E, Frezzato F, Andrisani A, Ambrosini G, Fiorentini D, Armanini D, Bordin L, Clari G. Endogenous reactive oxygen species content and modulation of tyrosine phosphorylation during sperm capacitation. *Int J Androl.* 2011;34(5):411-9.

➤ Selection of spermatozoa for ART involves an often ignored and uncontrolled hypoosmotic shock that affects basic sperm volume control and sperm motility.

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Introduction

Spermatozoa used for assisted reproduction are challenged by an increased osmolality in the seminal fluid. The increase is due to enzymatic degradation that start when the whole semen sample is collected in a container and mixed and not during normal intercourse. The increments in osmolality vary

The objective of this investigation was to study how and why these iatrogenic challenges affect sperm motility.

greatly from one man to another. At 3 hours after ejaculation the osmolality was 384mOsm (284-544; N=35) (Holmes at al unpublished). The spermatozoa are then challenged again by an abrupt hypotonic shock when transferred into isotonic sperm selection media.

Although sperm selection medium is standardized with its specific composition. The response to the media becomes a non-standardized unique event for every semen sample if changes in osmolality are not taken into account. The objective of this investigation was to study how and why these iatrogenic challenges affect sperm motility.

Objective

The objective of the study was to mimic what happens to spermatozoa during preparation and handling in the IVF (In Vitro Fertilisation) lab and investigate how basic function of the sperm tail, motility will be affected.

Material and Methods

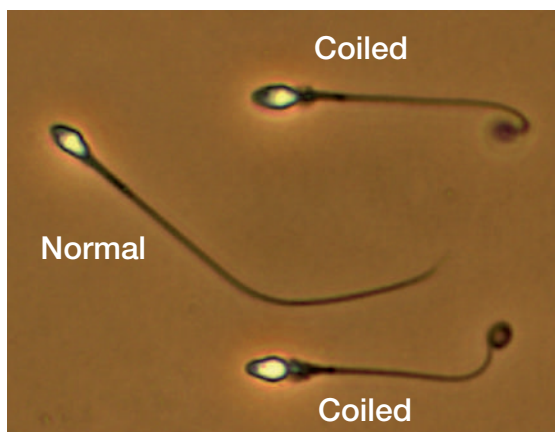
The media used for washing and extending the semen were prepared ahead of time. They are well tested commercial media, PureSperm®40, PureSperm®80 and PureSperm®Wash from Nidacon AB. The varying osmolality's were achieved by adjusting the glucose, NaCl and KCl levels in the media to get the desired osmolalities 290-450mOsm.

Samples were collected by masturbation prior to coming to the laboratory. The samples were kept at room temperature (20-22°C) until liquefaction had taken place.

The samples were then split into different aliquots for the different osmolality treatments. Each aliquot were then cleaned by density gradient centrifugation, washed in washing medium and then resuspended. The osmolality was kept the same throughout the cleaning process.

Just prior to analysis the aliquots were extended with medium of differing osmolality's (50-450mOsm) in order to achieve a final osmolality of 112, 150, 290, 450mOsm.

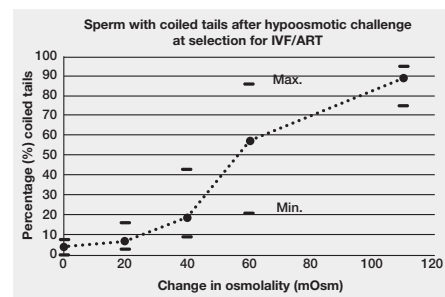
Motility was assessed with a Hamilton Thorn IVOS system and values for curvilinear velocities (VCL) and percentage of progressively motile spermatozoa are given. Observations were made every 5min for 60min.



Coiled tails after treatment with low osmolality
Photo by: Emma Holmes

Percent tail coiling was assessed by image analysis (Picsara Image system) of living sperm suspensions in which osmolality was reduced with 0, 20, 40, 60 and 110 mOsm to a final osmolality of 290 mOsm.

Results



Hyperosmotic change from 300 to 450 mOsm had no effect on motility. Hypoosmotic change from 300 to 150 mOsm decreased sperm velocity from 100 to 45µm/s and at 112 mOsm no motile sperm were found.

Hypoosmotic change from 400 mOsm to physiological 290 mOsm decreased sperm velocity permanently from 97 to 77µm/s (p<0.01) and decreased the percentage progressive spermatozoa from 80% to 68% (p<0.001). 89% of the sperm had coiled tails.

Conclusion

Selection of ejaculated sperm in the IVF lab often involves an hitherto ignored hypoosmotic shock. The severity varies between men and permanently affects the velocity and proportion of motile sperm. This may affect the outcome of the IVF treatment. However, the problem may be bypassed if sperm are selected at ejaculation as in natural selection.

Currently looking further into the effects of osmolality changes on spermatozoa. By using SCSA and flowcytometry we can study damages on the DNA.

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› PS100-1000 ml › 4 x PS100-250 ml



Nidacon has decided to terminate the production of PS100-1000 ml due to lower customer demand for PureSperm®100 in 1-liter bottles. During a transition period (June to Dec, 2014) we will offer our customers a package of four 250 ml bottles of PS100 to the same price as the 1000ml bottle.

For ordering, the item number for this kit will be the same, PS100-1000.

› SpermCryoProtec™ in a new bottle

We shall change the packaging of SpermCryoProtec™. Instead of the glass bottle with a stopper we shall have a plastic bottle with a screw cork. This will be easier for the customer to use, cheaper to transport and still give the same good result.

The new bottle will contain 25 ml instead of 20 ml as we have today. However, the price will remain the same.

With the change of bottle there will also be a small name change. We are removing the "II" after SpermCryoProtec™, back to only using SpermCryoProtec™.

The new bottles will be available later in May.

› Upcoming events



SSRM (Swedish Society for Reproductive Medicine)
May 16-17th, 2014. Umeå, Sweden



ESHRE, Annual meeting,
June 29th – July 2nd 2014. Munich, Germany



ASRM Annual meeting, October 18th - 22nd,
2014, Honolulu, Hawaii.

› Who to contact



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› Frequently asked questions

Some recent questions and answers that might be useful to you.

I have a very viscous sample; will it go through the ProInsert™?

It will go through but you can improve the outcome by treating your viscous sample with PureSperm® Buffer before use. Dilute your sample with PureSperm® Buffer 1 + 3 (1ml PSB + 3 ml sample) incubate in 37°C for 15-30 minutes, mix and it's ready for the density gradient preparation. You can find more information on our website.

For how long can I use Nidacon products after opening?

All Nidacon products except MorfoStain™ and SpermVitalStain™ should be stored in the fridge after opening. The same shelf- life applies even after opening if handled in an aseptic way. MorfoStain™ and SpermVitalStain™ should be stored in room temperature after opening.

Do I need to add egg-yolk to Sperm CryoProtec™ before use?

No you don't. SpermCryoProtec™ is a ready to use freezing medium which doesn't require any additives. Just follow the instructions and your sperm will be safely frozen and thawed.



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