Nidacon News

The news letter from your ART supplier • No 1 • 2018

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Basic semen sperm preparation workshop

Today most IVF labs understand that sperm is a vital part of the IVF process, the better you handle your preparations the better embryos you will have to transfer and freeze.

Previously, the role of spermatozoa in fertilization and embryo development was minimized to being a carrier that transports DNA to the oocyte. It is now proved that human spermatozoa play an extensive role that extends even beyond the early stages of fertilization to include abnormal embryogenesis leading to implantation failure (Barroso et al., 2009).

On the 4th of May we had a basic semen preparation workshop here at Nidacon and therefore in this issue of our newsletter, we are focusing on sperm preparation. A subject that we at Nidacon quite like and share with Dr David Mortimer, who is the author of the following two pages.

> It's a very informal day and a chance to discuss the small things.

We have these workshops twice a year; next one will be in October, also here in Göteborg, Sweden. With a small group of about 10-12 people we have hands-on training on sperm preparation using the density gradient, sperm freezing and vitality staining of sperm. Since it's a smaller group, we usually have quite a lot of discussions and sometimes I feel that we at Nidacon learn as much as we hope that the delegates do. It's a very informal day and a chance to discuss the small things in the lab that you don't find in the literature but is ever so important to help you improve the results in your lab.

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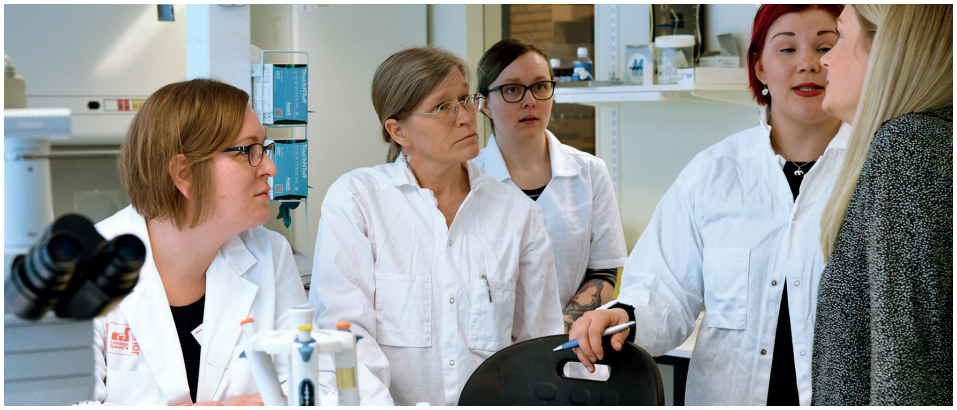
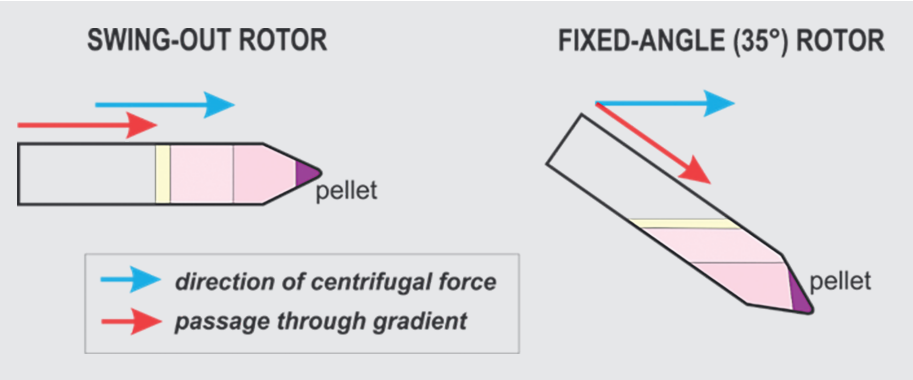


How does the centrifuge rotor affect the yield from a PureSperm® gradient?

The instructions for use of PureSperm® call for a bench top centrifuge with a swing-out rotor. I only have a fixed-rotor centrifuge – is there any difference in the outcome?

The centrifuge rotor can have a big influence on the outcome of your density gradient. The aim is to collect the sperm with the highest density (specific gravity), and they are the ones that make it all the way through the gradient, as their density is slightly higher than the density of 80% PureSperm®.

With a swing-out rotor, the tubes are vertical when they are placed in the centrifuge, but when the rotor starts to spin, the tubes swing out to be horizontal, so the sperm pass directly through the gradient layers, in the direction of the centrifugal force, and collect in a pellet at the bottom of the tube (as illustrated in the figure).



In contrast, the sperm that pass through the gradient in a fixed-rotor centrifuge are not in a tight pellet, rather the pellet is more of a smear across the side/bottom of the conical centrifuge tube. This is because the direction of movement through the gradient is not the same as the direction of the centrifugal force, so it is less efficient.

As it is harder to retrieve sperm from a more diffuse pellet than a tight pellet, centrifugation using a swing-out rotor is more likely to give a better yield of motile sperm.

Why should you use PureSperm®Buffer to dilute PS100® and also to wash the post-gradient pellet?

Protection against metal ion contaminants

Because some slight metal contamination is inherent in the manufacturing process of the colloidal silica used in PureSperm, the PureSperm family of products were all designed to include EDTA to chelate these ions. This protection will be reduced if culture media not containing EDTA are used to dilute PS100, or even lost if other media are also used to wash the pellets. Also, the risk of damage will be magnified if a medium containing iron were to be used.

Protection against osmotic shock

Low osmolarity is another mechanism that can promote the generation of ROS by sperm during washing, and some osmotic shock is inherent in almost all sperm washing protocols since the seminal plasma at half an hour after ejaculation is around 340 mOsm, and ART culture media are mostly between 285 and 295 mOsm.

Interestingly, the use of a hypertonic density gradient has been reported to produce better sperm preparations [1]. In those experiments a 6-step discontinuous gradient was used, ranging from 335 mOsm in the uppermost layer to 394 mOsm in the bottom layer.

Except for the PureSperm family of products, all commercial density gradient media are formulated to be in the same range as ART media. When used as a coherent system, PureSperm will create an intermediate osmolarity of 310 mOsm, reducing the osmotic shock the sperm will experience.

Conclusion

Always use the PureSperm family of products as they are intended. This will protect the sperm from possible transition metal-induced ROS damage, as well as limit any harmful effects caused by osmotic shock.

References
[1] Velez de la Calle JF. (1991) Human spermatozoa selection in improved discontinuous Percoll gradients. Fertil Steril 56:737-742.

What's the effect of changing the layer volumes on a density gradient's performance?

Most protocols for human sperm density gradient centrifugation recommend 1.5–2.0 ml upper and lower layers, and three common questions we hear are:

- Can't I use less volume to save money?
- Could I add proportionately more semen to the gradient?
- The sample has very few sperm – if I use the usual large volume layers won't they get lost?

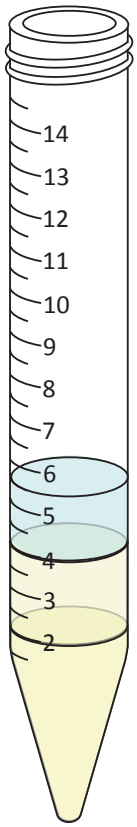
Answering such questions requires an understanding of how cells behave on a density gradient.

When centrifugal force is applied cells move down through the uppermost specimen layer (sperm suspended in seminal plasma) and then down the density gradient until they reach a point where the gradient matches their density ("density" being expressed in terms of mass per unit volume, e.g. in g/ml), this is called their isopycnic point. The greater the difference between a cell's density and the surrounding gradient material the faster the cell will move downwards. Consequently, in the upper layers cells will move towards the next interface: first the semen: upper layer interface, then the interface between the upper and lower layers. Cells that are not dense enough to pass through an interface into the more dense next layer will accumulate above the interface, forming a "raft". The more cells that accumulate there, the more dense will be the raft – eventually blocking the interface and preventing other cells from passing through into the next layer down.

With smaller volume layers the distance between the two interfaces will be shorter, and so cells will reach the lower interface between the two gradient layers faster, building up the raft faster and risking blocking the gradient. As a result, the risk is that the number of sperm that can be recovered from the pellet (the "yield" of the washing procedure) will be reduced. So while reduced volume layers might reduce costs by, say, 33% (going from 1.5 ml to 1.0 ml), the yield of the washing procedure will be prejudiced for some patients, especially those with more poor quality sperm, or dirtier semen samples (i.e. those with more debris and other cells).

> This would, of course, be true regardless of the gradient layer volumes or the size of the tube.

Similarly, if more semen is loaded onto a gradient then there will be more cells passing down the gradient, effectively risking overloading it by causing the raft(s) to build up faster. This is the basis for recommending that the volume of semen put onto a gradient should not exceed that of the upper layer (and might be reduced in the case of dirty specimens).



As for "losing" sperm on a gradient, this is not that much of a risk as all mature human sperm will pass through both layers of the gradient because the lower layer still has a density below that of a mature human spermatozoon (1.1 g/ml versus >1.12 g/ml); so the mature sperm reach the bottom of the tube before they reach their isopycnic point – and form the pellet. Therefore, whatever mature sperm there are in the sample will end up in the pellet – although it might be rather small and require the harvesting of an essentially impossible to see by eye "virtual" pellet. This would, of course, be true regardless of the gradient layer volumes or the size of the tube.

A final word of caution for labs with a formal quality management programme in place. If the sperm preparation protocol allows operators to select between 2 of more variations in the method then the lab's quality framework would expect regular audits of the choices made to ensure proper application of the protocol. Standardizing the method to a single, optimized protocol (i.e. 2 × 2.0 ml layers, or 2 × 1.5 ml layers) eliminates the need for this extra administrative work.

Our author – the sperm expert
Dr David Mortimer

is President and co-owner of Oozoa Biomedical and has many years of experience from the ART world.

Dr Mortimer is well-known in the field of reproductive biology, having authored or co-authored over 120 scientific publications, including the book *Practical Laboratory Andrology* (Oxford University Press, 1994) and Quality and Risk Management in the IVF Laboratory (Cambridge University Press, 2005)

He has been the Scientific Director of Sydney IVF, a position he held until he moved to Canada and formed Oozoa Biomedical Inc. in 2000.

During the last 20 years he has undertaken a wide range of consultancy and advisory work in various areas of reproductive biology and medicine in human, domesticated and laboratory species covering products, equipment and services from both the diagnostic and therapeutic perspectives.

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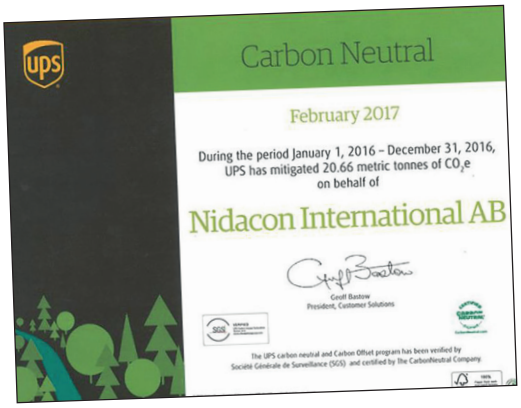
Every small step matters

In ecology, sustainability is the capacity to endure; it is how biological systems remain diverse and productive indefinitely.

In more general terms, sustainability is the endurance of systems and processes. The principle for sustainability is sustainable development, which includes the four interconnected domains: ecology, economics, politics and culture.



Nidacon believes in the importance of our legacy and works actively to make a positive imprint and to take responsibility for our impact on our world. A more conscious use of resources is essential in order to maintain healthy ecosystems and environments. Having a well-functioning system for waste recycling was therefore a natural step for us at Nidacon.

We investigated our express deliveries and came to the conclusion that we should compensate for carbon emissions. Therefore we now pay an extra fee for every package that is shipped from us. UPS has a program called "UPS Carbon Neutral" which we have joined and, for all other deliveries, we compensate by planting trees through WWF.

Nidacon and GDPR

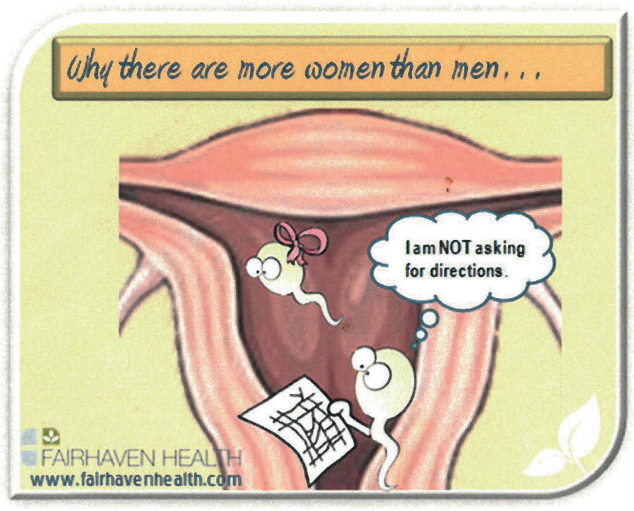
The General Data Protection Regulation (GDPR) is a regulation in EU law on data protection and privacy for all individuals within the European Union.

It also addresses the export of personal data outside the EU. The GDPR aims primarily to give control to citizens and residents over their personal data and to simplify the regulatory environment for international business by unifying the regulation within the EU.

It becomes enforceable on 25 May 2018 and of course we at Nidacon are working to have all data and registers up to date to comply with the new regulation.

Conferences, congresses & workshops

- ALPHA 12 Biennial Conference, May 17-20, 2018. Reykjavik, Iceland.
- ESHRE The 34th Annual Meeting, July 1-4, 2018 Barcelona, Spain.
- NILS Nordic IVF Laboratory Society 18th annual meeting, Sep 7-9, 2018 Oslo, Norway.
- Basic semen workshop, Nidacon, October 2018, Göteborg, Sweden.



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