Vitrification of blastocysts, experience from Hiroshima HART Clinic, Japan.

Dr Tetsunori Mukaida, head of the Hiroshima HART clinic in Japan, with main interest for vitrification of gametes and embryos: he is responsible for the two first reports in the world of successful birth from vitrified embryos.

Dr Mukaida presented results at the ESHRE Conference in London 2013 from the vitrification program at Hiroshima HART clinic (1). The title was; “the perinatal outcome of children born after vitrification of blastocyst (8440 cycles with 2757 babies in 14 years experience”.

These results were based on all patients who had vitrified blastocyst transfer cycles between 2000 and 2013. A total of 12941 blastocysts were vitrified, with a survival rate of 95.3%. The implantation rate was 38.6% and the pregnancy rate 47.3%.

Since 2010, the Hiroshima HART clinic has been using VitriBlast™ and ThermoBlast™ for all their blastocyst vitrification cycles. The results for the period 2010-2014 can be found in the table. The formulation for VitriBlast™ and ThermoBlast™ is actually based on Dr Mukaida’s own vitrification media. The method for performing vitrification recommended by Nidacon is also based on the vast experience of Dr Mukaida.

One example is the collapsing of the blastocoelic cavity using a laser pulse, results published in 2006 (2). With the help of a laser pulse, they increased the survival rate to 97.2% from 86%. In addition the pregnancy rate became significantly higher, 60.2% compared to 34.1%. The Hiroshima clinic has also published an article on using the cryoloop (3) which was first described by Lane et al in 1999 (4) showing a very high survival rate.

References
Semen decontamination for the elimination of semen pathogens

Earlier this year Jozef Fourie presented his thesis at the University of Pretoria, South Africa. He has been supervised by Prof. Carin Huyser with Prof. Naida Loskutoff as co-supervisor.

**Thesis summary**
The presence of pathogens in semen can compromise the outcome of assisted-reproduction treatment, together with the possibility of the female partner or offspring becoming infected. This is a cause of concern, especially in South Africa with a high prevalence of HIV-1. Most of these infected individuals are in their reproductive years with a desire to have their own, genetically related children.

However, during sperm preparation by standard discontinuous density gradient centrifugation, the supernatant is aspirated to allow access to the purified sperm pellet. Pathogens from the upper layers can adhere to the inside surface of the test tube and flow down to re-infect the purified sperm sample after the density gradient centrifugation.

Furthermore, seminal pathogens can bind specifically or non-specifically to spermatozoa, rendering semen decontamination procedures ineffective. Serine proteases, such as trypsin, have been demonstrated to effectively inactivate viruses and to break pathogen–sperm bonds. Serine proteases, such as trypsin, have been demonstrated to effectively inactivate viruses and to break pathogen–sperm bonds.

Evaluation of sperm parameters after semen processing indicated that trypsin and trypsin inhibitor did not have any impact on sperm mitochondrial membrane potential, vitality, motility and zona binding potential, or acrosine activity. Seminal bacteria were highly prevalent in patients wishing to participate in the Unit’s assisted reproductive program, with 49.5% of semen samples presenting with positive bacterial cultures.

Semen processing by means of discontinuous density gradient centrifugation with the tube insert, eliminated significantly more in vitro derived (spiked) bacteria and white blood cells from semen compared to processing without the insert. Furthermore, the semen decontamination procedure was effective in removing HIV-12 RNA from 100% of samples and proviral DNA from 98.1% of semen samples from HIV-1 positive patients.

The effectiveness of density gradient centrifugation for the elimination of seminal pathogens could be improved therefore by addition of trypsin to the upper density layer, without supplementing the bottom layer with trypsin inhibitor. Additionally, semen decontamination efficiency could also be improved by the prevention of re-contamination of processed sperm samples by the utilization of a tube insert during the density gradient centrifugation.
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North American Wood Bison project

In 2008, we started collaborating with a group of researchers from the University of Saskatchewan in Canada.

The aim was to develop a protocol for the removal of bacteria in the semen of wood bison. One such bacterium is brucella abortus, the cause of brucellosis. The materials supplied from Nidacon were BoviPure™ Pro and the ProInsert™.

The ProInsert™ was used in order to ensure that there was no recontamination of the semen during and after cleaning with a density gradient, in this case BoviPure™ Pro. Since the wood bison have been listed as a threatened species and several of the herds in Canada are infected, a National recovery plan has been implemented. An AI program that includes removal of bacteria from semen and production of healthy embryos holds great potential for both export and management of infected herds in Canada.

This program was even more important for Canada during a period when the transportation of live animals across the border to the United States was limited and essentially cut of their biggest export market.

In 2011, we started collaborating with a research group, this time in the United States, at Colorado State University. They were also having problems with bacteria shed in semen, such as brucella abortus, from bison this time in Yellowstone National Park. These wild bison pose a threat to livestock on and near the border of Yellowstone National Park.

The aim of their project was to stop the spread of brucellosis by introducing an AI program similar to the one in Canada, while at the same time, protect the threatened Wood Bison. BoviPure™ Pro and the ProInsert™ proved again to be the perfect complement to such an AI program.

The results from both projects have been very good so far and we are very pleased every time new results come in!

Workshop on oocyte vitrification with SafeSpeed™ closed system.

Overview:
SafeSpeed™, a novel, ultra-rapid and safe vitrification system for cryopreservation of human gametes and embryos; not just another vitrification device.

It is the closed vitrification system with the best heat transfer rates, allowing it to achieve the results of the best open systems, with survival rates superior to 90% for any operator.

SafeSpeed™ has been developed by Safe Preservation, a spin-off originated at Seville Engineering School, Spain, with the objective to apply the principles of physics and cryobiology to gamete and embryo cryopreservation: a 10 year project that is now brought to the market by Nidacon.

Workshop Objectives:
Main Objective: The main goal of this workshop is to provide the theoretical and practical competencies to perform successfully the vitrification of human gametes and embryos with SafeSpeed™ technique. Once the training is complete, the embryologist will be able to implement the technique in their own clinical practice with the best results from the first day.

Specific Goals:
- Acquire the theoretical knowledge that allows developing the practical abilities necessary for the optimal vitrification of gametes and embryos with the SafeSpeed™ system.
- Identify and familiarize with the main variables which are most determining for the survival of oocytes and embryos, to have them under control, establishing a robust vitrification protocol to achieve reproducible results.
- Identify the critical points in the process of vitrification and warming, and develop the abilities for their resolution.
- Proficiency in the process of sample loading in the SafeSpeed™ device and sample recovery.

Contact Nidacon for the next available workshop
contact@nidacon.com
New employee

My name is Manisha Olausson and I started at Nidacon International in August 2014 as RA manager.

In September 2012 I began a 2-year Higher Vocational Education course called "Quality and Process developer – Life Science", which I finished in June this year. Previously I have worked as a Math and Science teacher for 16 years in Swedish secondary and upper secondary schools.

My intention and aim is to work with high quality standards, in the same spirit as everyone at Nidacon always has done. I will ensure that we continue to make safe and effective products by following and keeping up-to-date with valid directives and requirements for all different countries.

My journey through the regulatory field has just begun and I am eager to find out what is awaiting around the corner. I am also looking forward to getting to know the people and companies linked to Nidacon.

Translation of website

For the benefit of our customers, we have incorporated a new button to view our content in your own language into our website.

You will find the button in the lower right corner ("Translate"). Click the button and you will find the language you want to use.

We know that these translations are not the best since this system comes directly from google translator. However, I think it is a help since it gives a general idea about the content.

I hope this will help, and that you will benefit from it.

Who to contact

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Frequently asked questions

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

Why is NidOil™ packed in amber bottles?
There have been several reports of other commercially available paraffin oils becoming embryo–toxic after exposure to light on the laboratory bench. Therefore, NidOil™ is packed in amber bottles as a precaution against light-induced changes to the product.

Should I equilibrate NidOil™ in the CO₂ incubator before use?
Yes, NidOil™ should be equilibrated in the same way as the culture medium before use to avoid differences in temperature and gaseous content between the components of the culture system.

Do I need to wash Nidoil™ before use?
You don’t need to wash Nidoil™ before use. Studies performed at Nidacon have shown equal or better results for embryo culture compared to washed oils.

How is Nidoil™ tested before a batch is released?
Nidoil™ is tested quite rigorously, we do density controls, sterility, endotoxin, mouse embryo assay, human sperm survival assay and we also measure the peroxide level. All these tests are done on both the final product and on the raw material.

Upcoming events

ASRM Annual meeting, Oct 18th-22nd, 2014, Honolulu, Hawaii.
ANDRO Sexta Edición, Dici 9-14, 2014 Riviera Maya – Mexico.
The Nordic IVF Laboratory Society (NILS), Jan 9-10 2015, Copenhagen, Denmark.

Workshop

We would like to invite you to the following lectures sponsored by Nidacon and MedTech during ASRM - Hawaii 2014

Monday October 20th at 7:00pm to 8:45pm
Ala Moana Hotel - Honolulu

1. What will be the future of the IVF lab?
Dr. Elkin Lucena
Cecofís, Colombia.

Kathleen Miller
IVF, Florida.

3. Clinical outcome of Vitrified Blastocyst Transfer Program (more than 8000 cycles with 14 years of experience).
Dr. Tetsunori Mukaida
Hiroshima HART Clinic, Japan.

For more information, please contact Nidacon.