Nidacon News

The news letter from your ART supplier • No 1 • 2017

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Vitrification in Japan

At the 7th Congress of the Asia Pacific Initiative on Reproduction (ASPIRE 2017), Kuala Lumpur, Malaysia, on 30 March – 2 April, Dr Tetsunori Mukaida presented his results on vitrification and what you can do to improve your results.

For the last 20 years, he has worked as clinical director of the Hiroshima HART Clinic. His main interest lies in vitrification of gametes and embryos: he is one of the pioneers in clinical application of vitrification including several reports of successful birth of day 2-3 embryos cryopreserved by vitrification with straw, and the blastocysts cryopreserved by ultra-rapid vitrification using cryoloop technique in 1998 and 2000.

> Artificial shrinkage is always performed.

Dr Mukaida has been using the products VitriBlast™ and ThermoBlast™ for several years and he played an important role in the development of the products themselves together with Dr. Thorir Hardarsson and the team at Fertilitetscentrum in Gothenburg.

In Japan a major part (77, 3%) of all ART babies today are born after vitrification.

The ART approach at Dr Mukaida's clinic is;

- Initial attempt – day 2-3 fresh transfer and culturing supernumerary embryos to blastocysts
- Second attempt –day 5-6 fresh transfer
- Third and after – vitrification of all blastocysts and warming cycles with controlled endometrial prep.

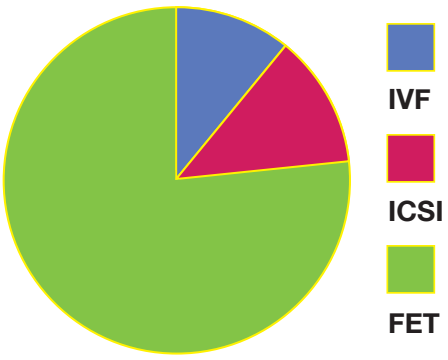
The secret to improvement of your vitrification program is through maximising the number of available blastocysts, improving the vitrification procedure and optimising the endometrial preparation.

He has introduced Piezo-ICSI in his lab, in which the piezo-electric effect (crystal deformation in response to an externally applied voltage) propels a microinjection needle tip forward in a precise and rapid movement, deformation of the oocyte during insertion of the needle is restrained by vibration of the piezo, and the oolemma is punctured readily and securely by the piezo pulse, at the site where the spermatozoon is injected (1). The technique has increased the fertilisation rate and reduced the degeneration rate. Fertilisation rate in the clinic today is about 82 % and number of degenerated oocytes is down to 2.3 %.

Time-lapse is used for all patients and pre-implantation genetic screening (PGS) is performed.

VitriBlast™ and ThermoBlast™ are used for vitrification/warming. The device used is a version of the cryo-loop (open-system).

2014 Total ART Babies in Japan



Artificial shrinkage is always performed. In 2003 the clinic added artificial shrinkage (AS), puncturing the blastocoele (2) with a laser or a micro-needle as a routine after observing that the efficiency of vitrification of human blastocysts was negatively correlated with the expansion of the blastocoele.

The study performed confirmed that AS of human expanded and hatching blastocysts before vitrification statistically improves the survival and pregnancy rate. It is important to make the hole big enough to help the whole blastocyst to escape during hatching.

Reference

1. Clinical efficiency of Piezo-ICSI using micropipettes with a wall thickness of 0.625 µm Hiraoka et al J Assist Reprod Genet 2015 32

2. Artificial shrinkage of blastocoeles using either a micro-needle or a laser pulse prior to the cooling steps of vitrification improves survival rate and pregnancy outcome of vitrified human oocytes. Mukaida T et al Human Reproduction Vol 21 2006

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What happens to sperm after ejaculation *in vitro*

These experiments are examples from the work I am doing for the PhD that I am currently working toward at the Karolinska Institute, in Stockholm.

Together with the andrologists at the Anova clinic, we are looking in detail at what happens in the semen sample after ejaculation and collection, and during preparation of the sperm for Assisted Reproductive Technologies.

When the ejaculate is presented to the lab it has already started on a journey of increasing osmolality. The different glandular secretions (prostate and seminal vesicle mostly) are mixed when the ejaculate is collected as opposed to during intercourse. The mixing of enzymes and large proteins from the glandular secretions start an enzymatic reaction that leads to an increase in the number of particles and also, therefore also osmolality. As can be seen in experiment 1, the increase in osmolality varies greatly between samples.

This increase in osmolality is temperature dependant as can be seen in experiment 2.
As the sperm are exposed to selection media such as a density gradient or swim-up, they will encounter a drop in osmolality. The commercial media are often adjusted to an osmolality that is isotonic to body fluid (290mOsm/kg).

The response of the sperm to this hypo-osmotic change is to take up water which, in turn, causes the sperm to swell and the swelling causes the tails to coil. There are varying degrees of coiling and the categories we refer to can be seen in experiment 4. The hypo osmotic swelling test (HOS test) results in a complete coiling of the tail, the sperm looks more like a round cell. This is the results of exposure to very low osmolality.

The swelling of the tail directly affects the motility of the sperm, as can be seen in the results in experiment 3.

Experiment 1. Progressive increase in osmolality of semen after ejaculation

47 semen samples were incubated for 3hrs at 37°C during which the change in osmolality was measured by freeze-point depression.

Mean osmolality after 1h was 322 mOsm/kg (280-361). Meanwhile the mean change in osmolality after 3h was 57 mOsm/kg (13-105). The final mean osmolality was 379 mOsm/kg (292-454). As can be seen in the graph the change in osmolality varies considerably between samples but all increases.

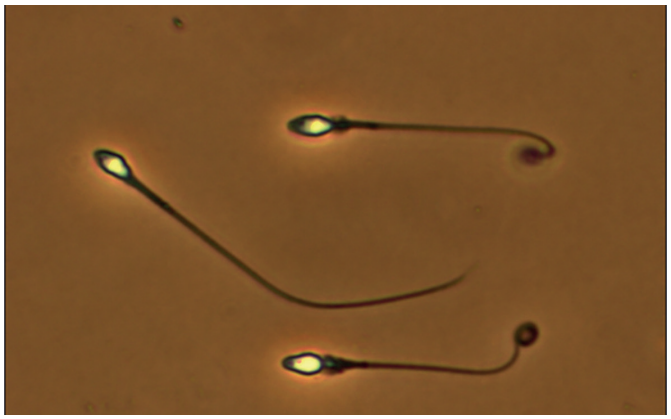


Photo by: Emma Holmes

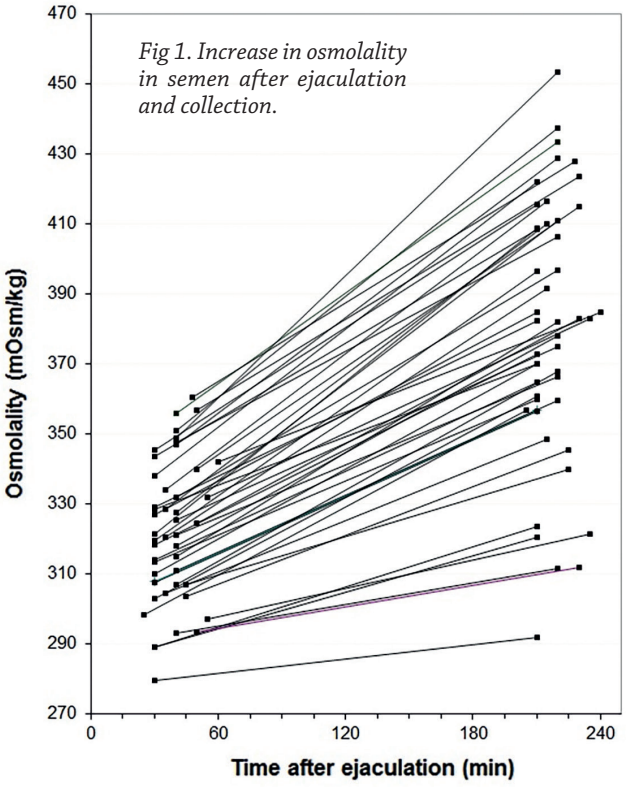


Fig 1. Increase in osmolality in semen after ejaculation and collection.

Experiment 2. The change in osmolality of semen in relation to storage temperature

We examined 6 pools of liquified semen which were then subdivided into 24 aliquots with 6 aliquots in each temperature group.

Results showed that samples stored at 37°C increased more than samples stored at room temperature (20 to 22°C) as well as samples stored in refrigerator (4 to 8°C) and samples stored in the freezer (-18 to -20°C). This means that keeping the semen samples in a lower temperature will lower the osmotic difference between sample and preparation media.

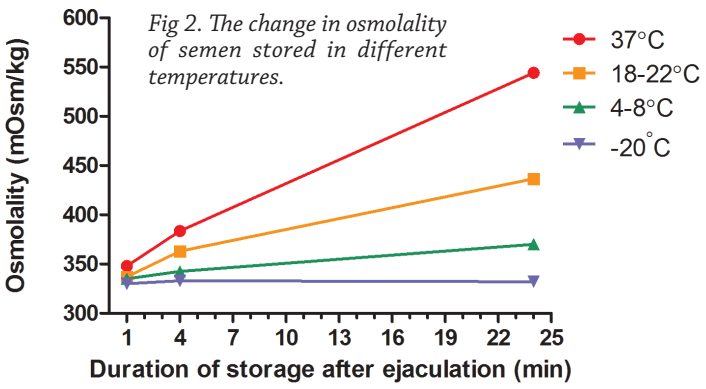


Fig 2. The change in osmolality of semen stored in different temperatures.

Experiment 3. Changes in osmolality affect sperm volume and motility

10 semen samples were washed according to a standard density gradient protocol; the media used had an osmolality of 400mOsm/kg. The sperm pellets were then mixed in washing medium with one of two different osmolalities. Final osmolalities were either 400 mOsm/kg (control no difference in osmolality for the sperm) or 290 mOsm/kg (treatment group, a change in osmolality from 400mOsm/kg to 290mOsm/kg). The motility was analysed at time 0, 15, 30, 45 and 60 min

Results: The VCL (Curvilinear Velocity), VAP (Average Path Velocity) and VSL (Straight Line Velocity) analysed on a Computer Assisted Sperm Analyser from Hamilton Thorne Bioscience can be seen in the graph below. The control group, were the osmolality stayed the same throughout the experiment, had a higher VCL than the treatment group. The VAP and VSL were also higher in the control group but not as high as the VCL. The motility is affected by a change in osmolality and the VCL seems to be more affected than VAP and VSL.

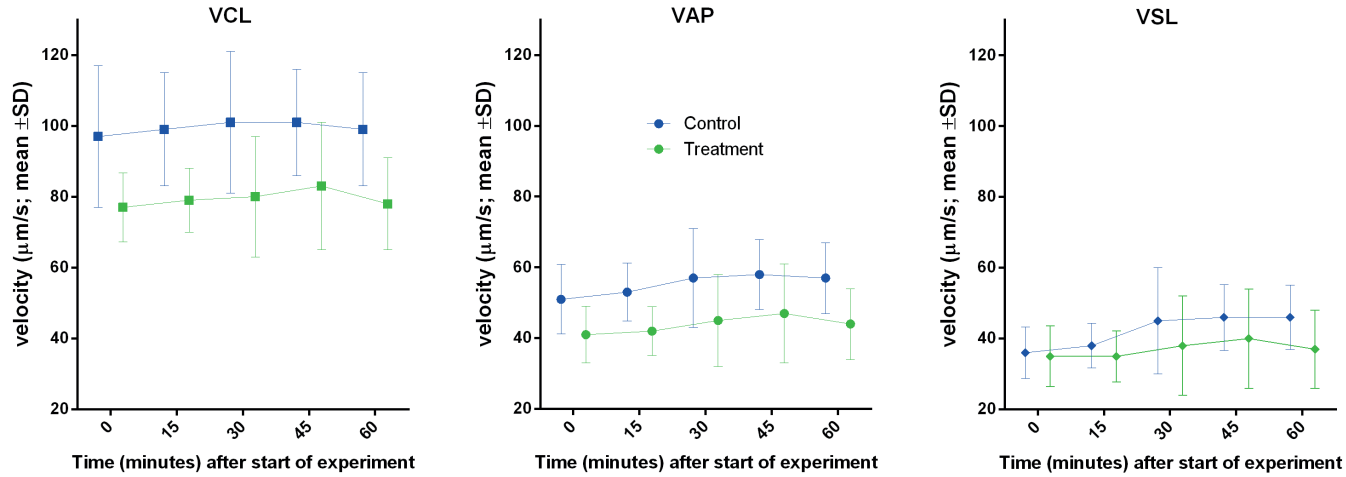


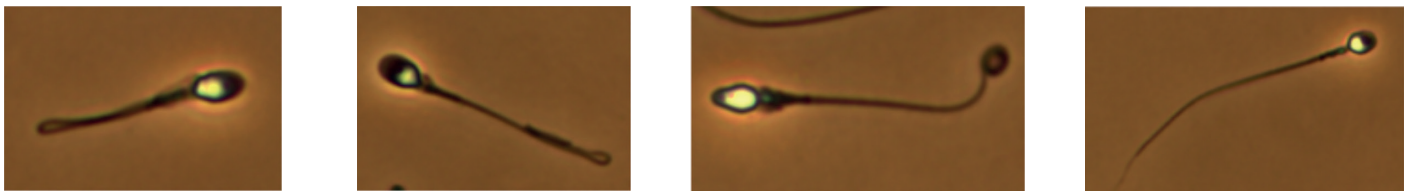
Fig 3. VCL, VAP and VSL for sperm exposed to different osmolality.

Experiment 4. Sperm Tail coiling

9 semen samples were prepared using density gradients with 4 different osmolalities. The gradients had osmolalities of 290 mOsm/kg, 310 mOsm/kg, 330 mOsm/kg, and 350 mOsm/kg. The samples were washed with washing medium with the same osmolality as the gradient.

The selected sperm suspension was divided in two and mixed with washing medium to obtain a final osmolality of 290 mOsm/kg or the same as it was being kept at (control). Samples were then fixed and analyzed under phase contrast microscopy and, thereafter, judged based on the 4 criteria of tail coiling seen here.

Photo by: Emma Holmes



1. ≥50% of tail folded
2. <50% of tail folded
3. Tip of tail coiled
4. Normal tail

The results were as follows:

- 330 mOsm to 290 mOsm; 18% coiled tails (9-43) P<0,01
- 350 mOsm to 290 mOsm; 57% coiled tails (21- 86), P<0,001
- 400 mOsm to 290 mOsm; 89% coiled tails (75-95) P<0,0001

These are just examples of some of the experiments we have done. Furthermore, we have observed evidence that the yield after a density gradient preparation is negatively affected by the change in osmolality that the sperm endures.

We have also seen evidence that diluting the sample with a hSA free buffer (such as PureSperm Buffer) will prevent much of the increase in osmolality that occurs.

In conclusion, there are many small adjustments in sperm handling protocols that can be used to minimize inflicting any negative impact on the sperm. We are dedicated to develop a protocol that is optimized first and foremost for the sperm but also for the one handling the samples.

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Calculate the correct RPM for your centrifuge

It is important to ensure that you have the correct revolutions per minute (RPM) for your centrifuge.

In order to make this as easy as possible we have now an easy formula to use on our website which will do the calculation for you.

Radius of rotor = rotational radius, the distance (mm) from the centre of the rotor to the bottom of a centrifuge tube in the bucket when raised to horizontal position.

Revolutions Per Minute (rpm)
Relative Centrifugal Force (RCF) or G-force
Radius of rotor (mm)
rpm 1000

Relative Centrifugal Force (RCF) or G-force
Revolutions per minute of rotor (rpm)
Radius of rotor (mm)
RCF or G-force 112

Upcoming events

eshre European Society of **Human Reproduction and Embryology**

- The 33rd Annual Meeting of ESHRE will be held in Geneva, Switzerland, 2 – 5 July 2017

Nordic Fertility Society

- Nordic Fertility Society – Nyborg, Denmark: 2 – 5 August 2017

asrm American Society for Reproductive Medicine | Impacting Reproductive Care Worldwide

- ASRM – 73nd ASRM Scientific Congress & Expo, San Antonio, Texas, USA October 28 – November 1, 2017

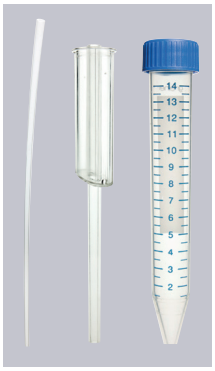
Frequently asked questions

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 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.



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