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Training course on cryoprotectant-free vitrification of human spermatozoa in Cologne, Germany



Beginning from October 2015

Medical Faculty, Maternal Hospital of Cologne University,
Cologne, Germany

<http://frauenklinik.uk-koeln.de>

Dear Colleagues,

We would like to call your attention to the following One-on-One Training Course (Hands-on workshop) on “Aseptic cryoprotectant-free vitrification of human spermatozoa: four modifications of two different technologies for ICSI (small volume) and for intrauterine insemination (large volume).” This is a training program featuring an exclusive one-on-one individual training to facilitate learning. This approach is anticipated to provide value for cost for participants as this is more effective than group training. We extend a cordial invitation to you to this event and would be pleased to welcome you face-to-face in Cologne.

Course Design:

This workshop is intended for postgraduate students and specialists in the field of mammalian cells cryopreservation in reproductive medicine and veterinary medicine.

At present for business to produce something for clinical use it is much more difficult than some time ago and this process needs the time. We announce our workshop only today because only to the present the plastic elements of the kits will be for sure manufactured by firms and presented on this workshop.

Our course will contain short basic lectures and film regarding the technologies. The central part of our workshop is hands-on manipulations.

Each element of the technology is a result of our own research that we have been already published [1-17].

The comparative cryopreservations of spermatozoa by slow freezing with permeable cryoprotectants vs. our technology will be offered to your attention. For this comparison it will be possible to use here, during our workshop, your home technology of sperm freezing. If you prefer to use your home freezing solutions, you can bring it (as well as instruments, plastic, containers etc.) to our workshop.

A few concise paragraphs on the technology and the workshop:

1. Technology of cryoprotectant-free vitrification of human, canine, cat (<http://blog.cincinnati.org/2015/05/14/glass-glass-baby-birth-of-healthy-kittens-following-sperm-vitrification-for-artificial-insemination/>) and fish spermatozoa is now reality [1-23].
2. Technology we have developed is original. Spermatozoa, vitrified by "capillary" and "one large straw" methodologies, immediately after thawing are ready for further use without any additional treatment (centrifugation for removal of cryoprotectants, separation in the gradient and others). Technology allows obtaining the following: we vitrify 0.3 ml or 0.01 ml suspension of spermatozoa, thaw this 0.3 ml or 0.01 ml and the same 0.3 ml or 0.01 ml use for ICSI, IVF or insemination. Spermatozoa after the warming are free from sperm plasma.
3. Technology of cryoprotectant-free vitrification for intrauterine insemination (large volume) is presenting in two forms: “straw-in-straw” [10] and “one large straw” [7]. Technology of cryoprotectant-free vitrification for ICSI and IVF in small volumes is presenting in two forms: “capillary technology” [12] as well as “open pulled [OPS] straw technology” [24].
4. Vitrification kit includes only one solution without permeable cryoprotectants.
5. We recommend our technology not only for its simplicity, but above all, because our technology better preserves spermatozoa (especially for ejaculates of bed quality, like asthenozoospermia and cryptozoospermia) compared to traditional “slow” freezing with permeable cryoprotectants.

Date: Beginning from October 2015 (any date at the participant convenience).

Duration: 2 days.

Number of participants: One, maximum two per session.

Location: Cologne University Maternal Hospital, Research Laboratory for Reproductive Medicine, Kerpener Str. 34, 50931 Cologne, Germany.

Language: English as well as German, Persian, or Spanish upon individual request.

Course directors:

Prof. Dr. med. Peter Mallmann, Director of University Maternal Hospital.

Dr.(SU) Evgenia Isachenko, Biological Director of IVF-Laboratory (http://frauenklinik.uk-koeln.de/reproduktionsmedizin-kryokonservierung?set_language=de).

Assoc. Prof. Dr. med. Gohar Rahimi, Medical Director of IVF-Laboratory.

Prof. Dr. med. Raul Sanchez, Director of Biomedical Center, La Frontera University, Temuco, Chile (during October 2015 only).

Dr.(SU) Vladimir Isachenko, Leader of Research Group for Reproductive Medicine (http://frauenklinik.uk-koeln.de/klinik_fuer_frauenheilkunde/forschung/research-group-for-reproductive-medicine?set_language=en).

PROGRAM

First day

8:00-8:45 Lecture: Cryobiology as field of knowledge and application in reproductive medicine and oncology.

V. Isachenko.

9:00-9:45 Lecture: Cryoprotectant-free vitrification of human spermatozoa: theory and practical application.

E. Isachenko

10:00-10:45 Film with comments: Cryoprotectant-free vitrification of mammalian spermatozoa: two aseptic technologies for ICSI and IVF in micro volumes and for intrauterine insemination.

V. Isachenko

11:00-12:45 and 13:40-16:00 Hands-on:

1. Preparation of vitrification medium.
2. Dilution of spermatozoa by vitrification medium.

3. Vitrification of spermatozoa for intrauterine insemination (with up to 500 μ l per straw ("one large straw" modification):

3.1. Aspiration of spermatozoa into plastic straw, hermetic straw sealing and plunging into liquid nitrogen.

3.2. Warming of spermatozoa in water-bath and expelling from straw for evaluation of quality and insemination.

4. Vitrification of spermatozoa for intrauterine insemination (with up to 100 μ l per straw ("straw in straw" modification):

4.1. Aspiration of spermatozoa in inner open straw, hermetic straw sealing and plunging into liquid nitrogen.

4.2. Warming of spermatozoa in tube, centrifugation of spermatozoa and evaluation of quality.

R. Sanchez, E. Isachenko, G. Rahimi, V. Isachenko.

Second day

8:00-8:45 Lecture: Integration of spermatozoa in oocytes: assisted technology for artificial activation and correction of position of the pronuclei.

P. Mallmann.

9:00-12:50 and 13:40-16:00 Hands-on:

1. Vitrification of 10 μ l spermatozoa for ICSI and IVF in micro volumes ("capillary technology"):

1.1. Aspiration of spermatozoa into capillary, relocation of capillary into isolating straw, sealing of isolating straw and plunging of capillary into liquid nitrogen (cooling).

1.2. Thawing of spermatozoa by plunging of capillary into warming medium.

2. Vitrification of 10 μ l spermatozoa for ICSI and IVF in micro volumes (open pulled straw [OPS] technology):

2.1. Aspiration of spermatozoa into OPS, relocation of OPS into isolating straw, sealing of isolating straw and plunging of capillary into liquid nitrogen (cooling).

2.2. Thawing of spermatozoa by plunging of OPS into warming medium.

3. Quality control of warmed spermatozoa with principle "it is better to do and see it once than read about it ten times":

3.1. Cryoprotectant-free vitrification of spermatozoa vs. traditional slow freezing with glycerol: comparison of effectiveness. For this type of comparative cryopreservations spermatozoa will be distributed onto two parts, for cryoprotectant-free vitrification and for conventional freezing with permeable cryoprotectants.

V. Isachenko, E. Isachenko, G. Rahimi, R. Sanchez.

16:00-17:00 Extensive discussions and repeated hands-on manipulations.

17:00-17:15 Obtaining of certificates and departure.

P. Mallmann, E. Isachenko, V. Isachenko, G. Rahimi, R. Sanchez.

Place:

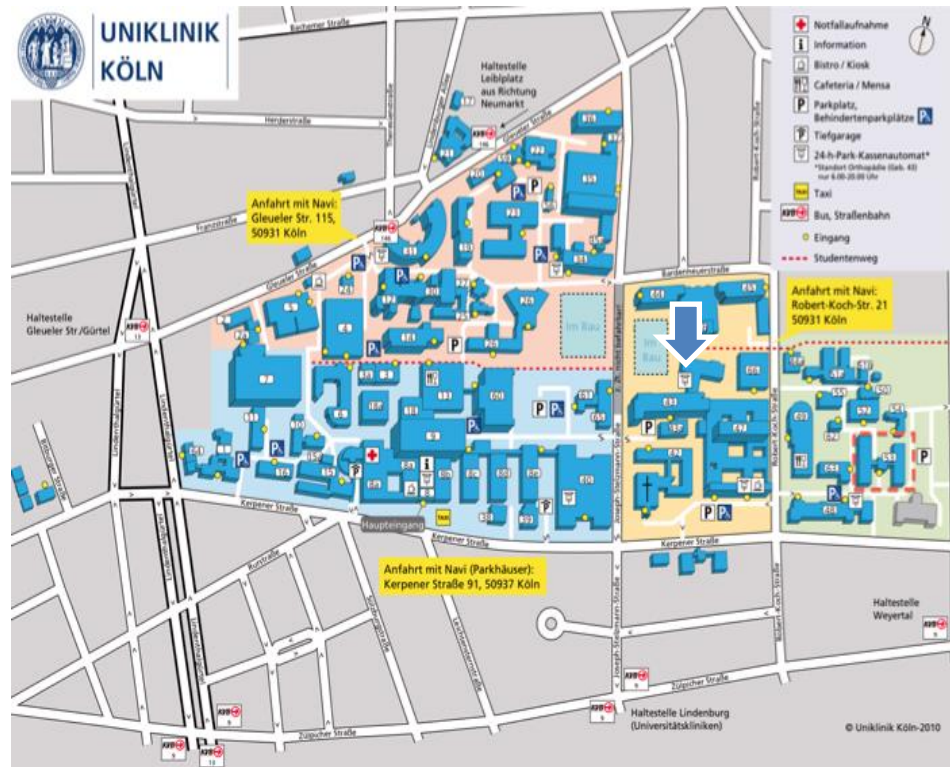
Cologne is famous tourist place (www.koeln-tourismus.de). Your staying in Cologne allows easily getting to Holland, Belgium, Luxemburg and France. For example, the tour to Amsterdam with 5-6 h staying in this city with transport Cologne-Amsterdam-Cologne occupies a little bit more than one light-day and costs to 25 EUR, or the 4 d tour to Paris (3 days in this city) including transport und hotel costs from 90 to 180 EUR.

Information and registration:

Dr. V. Isachenko, vladimir.isachenko@googlemail.com
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Course fee: 800 EUR for single participant, 1200 EUR for two participants.

Plan of the clinic:



Publications mentioned in this announce:

1. F. Nawroth et al. Vitrification of human spermatozoa without cryoprotectants. *CryoLetters*, 23: 93-102 (2002)
2. E. Isachenko et al. Vitrification of mammalian spermatozoa in the absence of cryoprotectants: from practical difficulties to present success. *Reprod. Biomed. Online*, 6:191-200 (2003)
3. V. Isachenko et al. Cryoprotectant-free cryopreservation of human spermatozoa by vitrification and freezing in vapor: effect on motility, DNA integrity, and fertilization ability. *Biol. Reprod.*, 71:1167-1173 (2004)
4. E. Isachenko et al. DNA integrity and motility of human spermatozoa after standard slow freezing versus cryoprotectant-free vitrification. *Hum. Reprod.*, 19:932-939 (2004)
5. V. Isachenko et al. Clean technology for cryoprotectant-free vitrification of human spermatozoa. *Reprod. Biomed. Online*, 10:350-354 (2005)
6. E. Isachenko et al. Acrosomal status and mitochondrial activity of human spermatozoa vitrified with sucrose. *Reproduction*, 136:167-73 (2008)
7. V. Isachenko et al. Cryoprotectant-free vitrification of human spermatozoa in large (up to 0.5 mL) volume: a novel technology. *Clin. Lab.*, 57:643-650 (2011)
8. V. Isachenko et al. Human spermatozoa vitrified in the absence of permeable cryoprotectants: birth of two healthy babies. *Reprod Fertil Dev.*, 24:323-326 (2011)
9. O. Merino et al. Fish (*Oncorhynchus mykiss*) spermatozoa cryoprotectant-free vitrification: stability of mitochondrion as criterion of effectiveness. *Anim. Reprod. Sci.*, 124:125-131 (2011)
10. R. Sanchez et al. Live birth after intrauterine insemination with spermatozoa from an oligo-astheno-zoospermic patient vitrified without permeable cryoprotectants. *J Androl.*, 33:559-562 (2011)
11. R. Sánchez et al. Canine sperm vitrification with sucrose: effect on sperm function. *Andrologia*, 43:233-241 (2011)
12. V. Isachenko et al. Vitrification of human ICSI/IVF spermatozoa without cryoprotectants: new capillary technology. *J. Androl.*, 33:462-468 (2012)
13. O. Merino et al. Cryoprotectant-free vitrification of fish (*Oncorhynchus mykiss*) spermatozoa: first report. *Andrologia*, 44 Suppl. 1:390-395 (2012)
14. R. Sánchez et al. Vitrified sperm banks: the new aseptic technique for human spermatozoa allows cryopreservation at -86 °C. *Andrologia*, 44:433-435 (2012)
15. R. Sánchez et al. Vitrificación de espermatozoides: una alternativa a la inyección intracitoplasmática de espermatozoides en paciente con oligoastenozoospermia severa. *Rev Int Andrologia*. 11: 36-39 (2013).
16. E. Figueroa et al. Effect of seminal plasma on Atlantic salmon (*Salmo salar*) sperm vitrification. *Theriogenology*, 83:238-245 (2015)
17. O. Merino et al. Protective effect of butylated hydroxytoluene on sperm function in human spermatozoa cryopreserved by vitrification technique. *Andrologia*, 47:186-193 (2015)
18. Y. Chen et al. Small-volume vitrification for human spermatozoa in the absence of cryoprotectants by using Cryotop. *Andrologia*, doi: 10.1111/and.12320 (2014)
19. M. A. Khalili et al. Vitrification of neat semen alters sperm parameters and DNA integrity. *Urol. J.*, 11:1465-1470 (2014)
20. A. Agha-Rahimi et al. Vitrification is not superior to rapid freezing of normozoospermic spermatozoa: effects on sperm parameters, DNA fragmentation and hyaluronan binding. *Reprod. Biomed. Online*, 28:352-358 (2014)
21. M. A. Mansilla et al. High temperature is essential for preserved human sperm function during the devitrification process. *Andrologia*, doi: 10.1111/and.12406 (2015)
22. V. Kuznyetsov et al. Vitrification of a small number of spermatozoa in normozoospermic and severely oligozoospermic samples. *Syst Biol Reprod Med.*, 61:13-17 (2015)
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