View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Media Peternakan

Media Peternakan, August 2013, pp. 101-105
ISSN 0126-0472 EISSN 2087-4634
Accredited by DGHE No: 66b/DIKTI/Kep/2011

Online version:
http://medpet.journal.ipb.ac.id/
DOI: 10.5398/medpet.2013.36.2.101

The Success Rate of Artificial Insemination Using Post-Thawed Spotted Buffaloes Epididymal Sperm

Y. Yulnawati[a,b,*,#], H. Maheshwari[c,#], M. Rizal[d], & A. Boediono[c,#]
[a]Graduate School, Bogor Agricultural University
[b]Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI)
Jln. Raya Bogor km. 46, Cibinong, 16911, West Java, Indonesia
[c]Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine,
Bogor Agricultural University
[#]Jln. Agatis, Kampus IPB Darmaga, Bogor 16680, Indonesia
[d]Department of Animal Science, Faculty of Agriculture, Lambung Mangkurat University
Jln. Jenderal Ahmad Yani km 36 Banjarbaru, South Kalimantan 70714, Indonesia
(Received 14-06-2013; Reviewed 01-07-2013; Accepted 15-07-2013)

ABSTRACT

Spotted buffalo, an exotic species that exists in Tana Toraja, South Sulawesi, Indonesia, is getting extinct due to high number of slaughtered during a funeral ceremony, called Rambu Solo', as well as special treatments that do not allow the male spotted buffaloes perform natural mating activity. According to that, the research was trying to start conservation program by collected the cauda epididymal sperm soon after slaughtered. Two egg yolk-based extenders with different buffers, tris hydroxyl amino methane (TEY20) and trisodium citrate dehydrate (CEY20), were used as comparison to evaluate the post-thawed epididymal sperm quality and fertilizing capacity in artificial insemination program. The results showed that the post-thawed progressive motility of epididymal sperm was 40% and 39.17%, while viability was 65.99% and 63.26% and membrane integrity was 65.43% and 63.03% in TEY20 and CEY20 extenders, respectively. The success rate of pregnancy was 46.67% using post-thawed epididymal sperm in TEY20 and 40% using the one in CEY20 extenders. In conclusion, tris-based and citrate-based extenders have similar ability to maintain the epididymal sperm quality and its fertilizing capacity.

Key words: artificial insemination, epididymal sperm, spotted buffalo

ABSTRAK

Kerbau belang merupakan jenis kerbau unik yang ditemukan di Tana Toraja, Sulawesi Selatan. Populasi ternak ini menurun secara drastis karena tingginya laju pemotongan pada upacara adat Rambu Solo', serta keyakinan dalam adat yang tidak memperbolehkan kerbau belang jantan melakukan aktivitas reproduksi. Berdasarkan hal tersebut, studi ini dilakukan sebagai langkah awal proses konservasi dengan menggunakan cauda epididimis sebagai sumber sperma potensial. Studi ini menggunakan dua jenis bufer dalam bahan pengencer berbasis kuning telur, yaitu tris hidroxil amino metana (TEY20) dan trisodium sitrat dehidrat (CEY20), untuk menguji kualitas dan daya fertilitas sperma pasca-*thawing* dalam proses inseminasi buatan. Hasil yang diperoleh menunjukkan bahwa persentase motilitas progresif adalah sebesar 40% dan 39.17%, dengan persentase hidup mati sebesar 65.99% dan 63.26%, serta persentase membran plasma utuh sebesar 65.43% dan 63.03% dalam bahan pengencer TEY20 dan CEY20 secara berturut-turut. Dapat disimpulkan bahwa bahan pengencer berbasis tris dan sitrat memiliki kemampuan yang sama dalam mempertahankan kualitas dan daya fertilitas sperma epididimis kerbau belang pasca-*thawing*.

Kata kunci: inseminasi buatan, sperma epididimis, kerbau belang

*Corresponding author:
E-mail: yulnawati@yahoo.com

INTRODUCTION

Spotted buffalo (*Bubalus bubalis carabanensis*) is a sub-breed of Asiatic swamp type, which is belong to water buffalo classification. Male spotted buffaloes have strong bound with the culture and tradition in Tana Toraja, where this animal exists. Male spotted buffaloes have been used as prestigious symbol in social status that showed in funeral ceremony, called *Rambu Solo'*. In this tradition, hundred of animals, mainly male spotted buffaloes and including cattle, pig, horse, deer, chicken, *etc*., are slaughtered as an expression of sorrow and condolence. It is believed that the more male spotted buffaloes slaughtered the faster ride to reach paradise. Because of this myth and cultural value, male spotted buffaloes get special treatments while they are alive, any mating activities are not allowed. Consequently, the population is significantly decreased and the cost of a fully-grown male spotted buffaloes is 10 times higher than the normal ones, neither solid nor white bulls.

An overcoming problem solution is absolutely needed to protect the existence of male spotted buffaloes in the world. One possible technique that can be applied to conserve male spotted buffalo is artificial insemination (AI) using sperm from cauda epididymal tissues that collected from slaughtered male spotted buffalo during the funeral ceremony. It has been known in other species that cauda epididymis sperm has similar capability to fertilize the eggs and have resulted normal births (Lone *et al*., 2012). This technique has been considered for utilization of gamete from valuable animals or animals in risk of extinction (Kaabi *et al*., 2003). It is confirmed in our previous study that fresh male spotted buffalo's epididymal sperm has equal quality with the one from ejaculation (Yulnawati *et al*., 2010). That study was using commercial extender with some modification. Chemically defined is cheap, easy to prepare and fresh extender. It is more applicable to be used for spotted buffalo semen cryopreservation, because there are some limitations of the commercial extenders, i.e. expensive price with short period of using and limitation access for the product, since the location of male spotted buffaloes are far from the lab. In this study, our objective was to evaluate the post-thawed epididymal sperm quality in chemically defined extenders and its correlation to success rate of pregnancy and parturition rate in AI program.

MATERIALS AND METHODS

Cryopreservation of Epididymal Sperm

Six pairs of cauda epididymis tissues from six to ten years old slaughtered male spotted buffaloes were collected during the funeral ceremonies in North Toraja, South Sulawesi. Scrota-coated of testes-epididymus tissues were transported in NaCl 0.9%, room temperature for 1-2 h. The cauda epididymal sperm was collected using both slicing and flushing methods in two different extenders, tris hydroxyl aminomethane-20% egg yolk (TEY20) or trisodium citrate dehydrate-20% egg yolk (CEY20). The composition of TEY20 and CEY20 extenders were referred to Singh *et al*. (2007) and Lone *et al*. (2011), respectively (Table 1). Fresh and post-thawed sperm quality evaluation, sperm dilution, equilibration, cryopreservation and thawing procedure were referred to Yulnawati *et al*. (2010).

Artificial Insemination

Thirty female spotted buffaloes were injected using double dosage of prostaglandin ($PGF_{2\alpha}$) with 11 d of interval to synchronize the estrous periods. The sperm used in this experiment was collected from the same bull to avoid bias of AI results because of male individual factor. Estrous detection was performed 72-84 h after second $PGF_{2\alpha}$ injection. Female spotted buffaloes were inseminated twice within 8-12 h intervals in between using single straw per insemination (concentration 60.10^6 sperm/straws). The pregnancy rate was confirmed by rectal palpation at day 90-120 after insemination. Success rate of AI was proved by number of offspring that delivered in AI program.

Data Analysis

All individuals were considered as the replications in these experiments. Results were expressed as the means ± standard error mean (SEM) and analyzed using t-test within 5% ($P<0.05$) level of confidence to determine statistical differences.

RESULTS AND DISCUSSION

Fresh sperm that obtained from cauda epididymal tissues of male spotted buffaloes has good quality according to the requirements for artificial insemination and also for the cryopreservation process (Indonesian National Standard, SNI 01-4869.2-1998). The excess using from cauda epididymis is high concentration of sperm that could be collected since it is the depository before ejaculation. As it has shown in our results, the obtain concentration of male spotted buffaloes epididymal sperm was high with good quality in motility and other parameters (Table 2).

Table 1. Extenders composition

Chemicals	TEY20	CEYF20
Tris hydroxyl amino methane (mol/L)	0.025	-
Citric acid (mol/L)	0.009	-
Trisodium citrate dehydrate (mol/L)	-	0.008
Fructose (mol/L)	0.005	0.006
Glycerol (% v/v)	6	6
Egg yolk (% v/v)	20	20
Penicillin (IU)	500	500
Streptomycin (μg/mL)	500	500
H_2O (mL)	100	100

Note: TEY20: tris hydroxyl amino methane-20% egg yolk; CEY20: trisodium citratedehydrate-20% egg yolk. All chemicals are from Merck, Germany.

Table 2. Quality of fresh spotted buffalo epididymal spermatozoa*

Parameters	Values
Concentration (x10^6 cells/mL)	3578.33±740.33
Progressive motility (%)	74.17± 1.86
Viability (%)	85.02± 2.35
Abnormality (%)	7.10± 1.16
Membrane integrity (%)	86.22± 1.94

Note: *Presented at CRU Lunch Seminar, Uppsala, May 3th, 2012 (Yusnizar, 2012).

It is well known that the important the physiological characteristic differences of epididymal versus ejaculated spermatozoa is in the membranes composition that affect the survival rate of sperm cell after cooling and freezing (Martinez-Pastor *et al*., 2006). Reports in some species indicated that epididymal spermatozoa are less tolerant to cryopreservation process than ejaculate spermatozoa (Martins *et al*., 2009). Furthermore, epididymal spermatozoa has not been exposed to the complex secretions of the accessory sex glands that also important for during chilling and freezing process (Yu & Leibo, 2002).

There was no significant different (P>0.05) of sperm quality in all parameters, *i.e*. progressive motility, viability and membrane integrity during cryopreservation process (Table 3), as well as the success rate of pregnancy in TEY20 versus CEY20 groups (Figure 1). Both tris- and citrate-based extender seems to have equal maintenance ability as well as fertilizing capacity that was described on the pregnancy and delivery rate following artificial insemination program.

The decreasing temperature that happened extremely during cryopreservation process induces the formation of intracellular ice crystals and the osmotic and chilling injury of sperm cells (Isachenko, 2003). Ice crystal formation caused sperm cells injured and being damage to the cytoplasmic fracture; negative effects on the cytoskeleton and genome related structures; negative effect on sperm tail and its motile ability as well as reducing the fertility due to compromising the integrity of plasma membrane (Harshan *et al*., 2005; Andrabi *et al*., 2008). Cold shock influences the ratio of cholesterol/phospholipids, the content of lipids in the bilayer membrane, degree of hydrocarbon chain saturation and ratio of protein/phospholipid on the plasma membrane (Medeiros *et al*., 2002), reduces membrane permeability to water and solutes and injures acrosomal membranes (Purdy 2006), as well as mitochondrial sheath and axoneme (Salamon & Maxwell, 2000). A good extender is a combination of compositions that maintains the pH balance to protect membrane intactness from ice crystal damage and suitable for cell nutrition needs during cryopreservation process and storage. Membrane integrity is important for motility and viability of cells and also as indicator of fertilizing ability (Mehmood *et al*., 2009).

Tris-egg yolk extender has been widely recommended for routine use to cryopreserve male buffalo semen. The progressive motility of post-thawed ejaculated sperm of Nili-Ravi buffalo in tris-based extender was 48.8% (Waheed *et al*., 2012; Akhter *et al*., 2011a), while in post-thawed epididymal sperm of African buffalo (*Synceraus caffer*) were 56.4% in Triladyl and 44.1% in AndroMed (Herold *et al*., 2004). It is known that tris- and milk-based extender could maintain sperm quality better that citrate-based extender during cryopreservation (reviewed by Andrabi 2009; Sansone *et al*., 2000). Nevertheless, we still believe that the citrate-based extender capacity could be improved by some modification of its chemicals composition. The advantages of citrate-based extender were found sufficiently clear for motility assessment compare to commercially milk-based extender (reviewed by Sansone *et al*., 2000).

Several reports of previous studies showed that sugar addition into extender was beneficial as extracellular cryoprotectant, as well as energy source for the sperm itself. As a non-permeable cryoprotectant, sugar

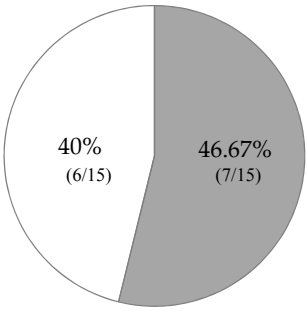

Figure 1. The success rate of pregnancy from artificial insemination program using spotted buffalo frozen-thawed epididynal sperm; TEY20 (■): tris hydroxyl amino methane-20% egg yolk; CEY20 (□): trisodium citratedehydrate-20% egg yolk.

Table 3. Epididymal sperm quality on post dilution and post equilibrationstage

Parameters	Post-dilution		Post-equilibration		Post-thawing	
	TEY20	CEY20	TEY20	CEY20	TEY20	CEY20
% PM	72.50±2.50	73.33±2.36	60.00±4.08	60.83±3.44	40.00±2.89	39.17±5.34
% Live	81.58±1.12	82.19±1.22	74.41±3.43	74.34±5.05	65.99±3.37	63.26±3.95
% MI	81.54±1.44	82.09±1.17	75.77±2.42	75.30±4.58	65.43±4.16	63.03±3.61

Note: TEY20: tris hydroxyl amino methane-20% egg yolk; CEY20: trisodium citratedehydrate-20% egg yolk; PM: progressive motility; Live: viability; MI: membrane integrity.

induces the increasing of osmotic pressure that could let the cell dehydration and reduce the incidence of intracellular ice crystal formation (reviewed by Andrabi 2009). Based on this information, fructose was added into citrate-based, as well as tris-based extender and it is shown in this study that both extenders could maintain post-thawed epididymal sperm quality and produce equal success rate of pregnancy (Table 3 and Figure 1). Parallel to our result, egg yolk-citrate-fructose extender also has similar potential in maintaining the viability and acrosomal integrity of ovine epididymal sperm during preservation at 4 ºC for 72 h (Lone *et al.,* 2012).

Compared to post-thawed ejaculates sperm quality of Nili-Ravi buffaloes (Akhter *et al*., 2010; 2011a), the quality and fertility of post-thawed epididymal sperm of spotted buffalo was not so much different. The delivery rate was 46.67% in TEY20 versus 40% in CEY20 in this study. The conception rate in river buffalo using post-thawed ejaculate sperm in different extenders was reported in widely range, i.e 44% in tris-egg yolk and 47% in Bioexcell (soya milk-based) extenders (Akhter *et al*., 2010), 41.5% in egg yolk-based and 56% in soya bean-based extenders (Akhter *et al*., 2011a), 41.5% in egg yolk-based and 53.5% in LDLs-based extenders (Akhter *et al*., 2011b).

The success rate of pregnancy in mares that were inseminated either hysteroscopically or conventionally using frozen-thawed epididymal sperm was 18% versus 8% (Morris *et al*., 2002). Pregnancy rate in mares could only be increased by deposited the epididymal sperm surgically in the region of utero-tubal junction in order to achieve equal success rate of pregnancy using ejaculate sperm (Morris *et al*., 2000). Eventhough, recent report showed a similar pregnancy rate using epididymal sperm (55.8%) versus ejaculate sperm (53.8%) in sheep (Álvarez *et al*., 2012).

Some problems in buffalo AI program are from female and male sides. Silent heat phenomenon that is common in female is caused by low hormonal level. This condition was generated the difficulties in estrous detection, un-appropriate time for insemination, and the failure of fertilization. Small uterine body size prevent the sperm to be introduced into one uterine horn could also be a reason for low conception rates to AI (Sansone *et al*., 2000). Due to that we understand that high quality of sperm is absolutely needed to increase the success rate of AI. Although, we optimist that frozen-thawed epididymal sperm is suitable to be used in spotted buffalo AI program.

CONCLUSION

Both tris-based and citrate-based extenders have equal ability in maintaining the post-thawed spotted buffalo epididymal sperm quality and fertilizing capacity in artificial insemination program. It is suggested that these two chemically defined extenders could be used to increase the efficiency and reduce the cost in sperm processing and cryopreservation in spotted buffalo conservation program.

ACKNOWLEDGEMENT

This study was funded by Indonesian Ministry of Education and Culture, Directorate General of Higher Education for Foreign Research Networking Project No. 447/SP2H/PP/DP2M/VI/2010. The authors wish to acknowledge the head and staff of Animal Division of North Toraja Government for supporting this study and collecting samples. Finally, we wish to thank all of the farmers and owners that have provided the buffalo samples for this study. Without their help, this study wouldn't be possible.

REFERENCES

Akhter, S., M. S. Ansari, B. A. Rakha, S. M. H. Andrabi, N. Ullah, & M. Khalid. 2011a. Soya-lecithin in extender improves the freezability and fertility of buffalo (Bubalus bubalis) Bull Spermatozoa. Reprod. Dom. Anim. 47: 815-819. http://dx.doi.org/10.1111/j.1439-0531.2011.01973.x

Akhter, S., M. S. Ansari, B. A. Rakha, S. M. H. Andrabi, M. Khalid, & N. Ullah. 2011b. Effect of low-density lipoproteins in extender on freezability and fertility of buffalo (Bubalus bubalis) bull semen. Theriogenology 76: 759–764. http://dx.doi.org/10.1016/j.theriogenology.2011.04.009

Akhter, S., M. S. Ansari, B. A. Rakha, S. M. H. Andrabi, S. Iqbal, & N. Ullah. 2010. Cryopreservation of buffalo (Bubalus bubalis) semen in Bioxcell® extender. Theriogenology 74: 951-955. http://dx.doi.org/10.1016/j.theriogenology.2010.04.024

Álvarez, M., J. Tamayo-Canula, C. Martínez-Rodríguez, E. López-Urue-a, S. Gomes-Alvesa, L. Anela, L. Martínez-Pastor, & P. de Paz. 2012. Specificity of the extender used for freezing ram sperm depends of the spermatozoa source (ejaculate, electroejaculate or epididymis). Anim. Reprod. Sci.132: 145-154. http://dx.doi.org/10.1016/j.anireprosci.2012.05.006

Andrabi, S. M. H. 2009. Factors affecting the quality of cryopreserved buffalo (Bubalus bubalis) bull spermatozoa. Reprod. Dom. Anim. 44: 552–569. http://dx.doi.org/10.1111/j.1439-0531.2008.01240.x

Andrabi, S. M. H., M. S. Ansari, N. Ullah, M. Anwar, A. Mehmood, & S. Akhter. 2008. Duck egg yolk in extender improves the freezability of buffalo bull spermatozoa. Anim. Reprod. Sci. 104: 427–433. http://dx.doi.org/10.1016/j.anireprosci.2007.07.003

Harshan, H. M., L. P. Singh, A. Arangasamy, M. R. Ansari, & S. Kumar. 2006. Effect of buffalo seminal plasma heparin binding protein (HBP) on freezability and in vitro fertility of buffalo cauda spermatozoa. Anim. Reprod. Sci. 93: 124–133. http://dx.doi.org/10.1016/j.anireprosci.2005.07.010

Herold, F. C., J. E. Aurich, & D. Gerber. 2004. Epididymal sperm from the African buffalo (Synceruscaffer) can be frozen successfully with AndroMed® and with Trilady™but the addition of bovine seminal plasma is detrimental. Theriogenology 61: 715–724. http://dx.doi.org/10.1016/S0093-691X(03)00256-5

Isachenko, E., V. Isachenko, I. I. Katkov, S. Dessole, & F. Nawroth. 2003. Vitrification of human spermatozoa in the absence of cryoprotectants: from practical difficulties to present success. Reprod. Biomed. online 6: 191-200. http://dx.doi.org/10.1016/S1472-6483(10)61710-5

Kaabi, M., P. Paz, M. Alvarez, E. Anel, J. C. Boixo, H. Rouissi, P. Herraez, & L. Anel. 2003. Effect of epididymis handling conditions on the quality of ram spermatozoa recovered post-mortem. Theriogenology 60: 1249–1259. http://dx.doi.org/10.1016/S0093-691X(03)00139-0

Lone, F. A., R. Islam, M. Z. Khan, & K. A. Sofi. 2012. Effect of different egg yolk-based extenders on the quality of ovine cauda epididymal spermatozoa during storage at 4ºC. Reprod. Dom. Anim. 47: 257-262. http://dx.doi.org/10.1111/j.1439-0531.2011.01847.x

Lone, F. A., R. Islam, M. Z. Khan, & K. A. Sofi. 2011. Effect of transportation temperature on the quality of cauda epididymal spermatozoa of ram. Anim. Reprod. Sci. 123: 54–59. http://dx.doi.org/10.1016/j.anireprosci.2010.10.012

Martins, C. F., K. Driessen, P. Melo Costa, J. O. Carvalho-Neto, R. V. de Sousa, R. Rumpf, & M. N. Dode. 2009. Recovery, cryopreservation and fertilization potential of bovine spermatozoa obtained from epididymides stored at 5ºC by different periods of time. Anim. Reprod. Sci.116: 50–57. http://dx.doi.org/10.1016/j.anireprosci.2008.12.018

Martínez-Pastor, F., F. Martínez, V. García-Macías, M. C. Esteso, E. Anel, M. R. Fernández-Santos, A. J. Soler, P. de Paz, J. Garde, & L. Anel. 2006. A pilot study on post-thawing quality of Iberian red deer spermatozoa (epididymal and electroejaculated) depending on glycerol concentration and extender osmolality. Theriogenology 66: 1165–1172. http://dx.doi.org/10.1016/j.theriogenology.2006.03.027

Medeiros, C. M., F. Forell, A. T. Oliveira, & J. L. Rodrigues. 2002. Current status of sperm cryopreservation: why isn't it better? Theriogenology 57: 327-344. http://dx.doi.org/10.1016/S0093-691X(01)00674-4

Mehmood, A., M. Anwar, & S. M. Saqlan-Naqvi. 2009. Motility, acrosome integrity, membraneintegrity and oocyte cleavage rate of sperm separated by swim-up or Percoll gradient method from frozen–thawed buffalo semen. Anim. Reprod. Sci. 111: 141–148. http://dx.doi.org/10.1016/j.anireprosci.2008.02.011

Morris, L., C. Tiplady& W. R. Allen. 2002. The *in vivo* fertility of cauda epididymal spermatozoa in the horse. Theriogenology 58: 643-646. http://dx.doi.org/10.1016/S0093-691X(02)00742-2

Morris, L. H. A., C. Tiplady, S. Wilshe& W. R. Allen. 2000. Hysteroscopic utero-tubal insemination of mares with low numbers of frozen-thawed ejaculated and epididymal spermatozoa. Proceedings of Havemeyer Foundation Workshop: the European Equine Gamete Group. Saari, Finland. p 4-5.

Purdy, P. H. 2006. A review on goat sperm cryopreservation. Small Rum. Res. 6: 215–225. http://dx.doi.org/10.1016/j.smallrumres.2005.02.015

Salamon, S. & W. M. Maxwell. 2000. Storage of ram semen. Anim. Reprod. Sci.62:77–111. http://dx.doi.org/10.1016/S0378-4320(00)00155-X

Sansone, G., M. J. F. Nastri, & A. Fabbrocini. 2000. Storage of buffalo (Bubalus bubalis) semen. Anim. Reprod. Sci. 62: 55–76. http://dx.doi.org/10.1016/S0378-4320(00)00154-8

Singh, L. P., H. M. Harshan, & M. R. Ansari. 2007. Effect of egg yolk and seminal plasma heparin binding protein interaction on the freezability of buffalo cauda epididymal spermatozoa. Anim. Reprod. Sci. 99: 395–400. http://dx.doi.org/10.1016/j.anireprosci.2006.08.018

Waheed, S., N. Ahmad, N. Rahman, H.J. Rahman, M. Younis, & S. Iqbal. 2012. Evaluation of duck egg yolk for the cryopreservation of Nili-Ravi buffalo bull semen. Anim. Reprod. Sci. 131: 95-99. http://dx.doi.org/10.1016/j.anireprosci.2012.02.011

Yu, I. & S. P. Leibo. 2002. Recovery of motile, membrane-intact spermatozoa form canine epididymides stored for 8 days at 4 ºC. Theriogenology 57: 1179–1190. http://dx.doi.org/10.1016/S0093-691X(01)00711-7

Yulnawati, M. Gunawan, H. Maheshwari, M. Rizal, Herdis, &, A. Boediono. 2010. Quality of epididymal and ejaculated sperms of spotted buffalo in dextrose supplemented extender. Hayati J. Biosciences 17: 27-30. http://dx.doi.org/10.4308/hjb.17.1.27

Yusnizar, Y. 2012. Assisted reproductive technology in spotted buffalo conservation program. CRU Lunch Seminar, Uppsala, May 3th, 2012. [Abstract].