# What research is needed to improve commercial pig reproduction

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Artificial insemination with fresh or stored semen is currently the only sperm technology used at a commercial scale in the pig industry. Attention should therefore be given for further improvement of the functionality and fertilizing ability of cryopreserved semen, as well as for sperm sorting for gender pre-selection. During the last two decades various proteins and polypeptides have been identified in boar seminal plasma, and the relevance of some of them to reproductive technologies has been discussed at this conference. The long- term goal should be to isolate/synthetize those seminal plasma proteins proven important for the spermatozoa, and use them as ingredients in media used for e.g. cryopreservation and sex sorting of semen. A close cooperation between biochemists, molecular biologists and animal scientists is necessary to reach this goal.

The genomic revolution has brought us transcriptional profiling, allowing for the identification of many genes involved e.g. in mammalian gametogenesis, fertilization, and preimplantation embryo development. We do expect further progress within this field of research during coming years. The new information has also capacity to revolutionize the genetic progress within animal breeding.

The time of insemination in relation to ovulation is of great importance for fertility. Promising results were presented when using single insemination with fresh semen at fixed-time ovulation. However, further research is needed to find out if the same model also works in females given deep insemination with lower sperm number, or inseminated with sexed semen.

Piglet mortality is multifaceted in nature. To be successful in improving piglet survival in commercial pig production, a balanced selection program should be coupled with environmental and nutritional interventions.

Welfare and ethical aspects of commercial production are of growing interest for the society and for consumer organisations and cannot be neglected. More attention should therefore be paid to introduction/ improvement of different loose-/group-housing systems for sows all over the world.

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## Introduction

Reproductive performance of the sow is a critical component of sustained production, and depends on several factors such as genetic background, environment, parity, season, and nutritional status. The relatively low heritability of reproductive performance traits such as ovulation rate, litter size, and prenatal survival and their expression only in females, limits improvement of these traits through traditional selective breeding programs. However, there is evidence of genetic variation in these traits between pig breeds, which can be exploited to improve reproductive performance as a whole. The genomic revolution has brought us transcriptional profiling, allowing for the identification of many genes involved e. g. in mammalian gametogenesis, fertilization, and preimplantation embryo development (Sutovsky 2009). Sequencing of the porcine genome has opened up new possibilities (Groenen *et al*. 2012) for commercial development.The major challenge facing reproductive biology is to apply meaningful approaches to analyze these gene products at the protein level. We know that the animals have the genetic flexibility to adapt to new environment. A theoretical framework to get around genotype-environment interactions is under development and will be discussed at this conference (Knol & Mathur 2013).

During the last two decades various proteins and polypeptides have been identified in boar seminal plasma (Strzezek *et al*. 2005; Mogielnicka-Brzozowska & Kordan 2011). The present knowledge of their relevance to reproductive technologies will be discussed at this conference (Flowers *et al*. 2013). Furthermore, a few seminal plasma proteins appear to have reasonable correlations with fertility in boars that normally produce spermatozoa with excellent motility and morphology. However, Flowers *et al*. conclude that a more holistic approach may be beneficial, than focusing on a single one/a few proteins in terms of predicting a boar´s reproductive potential.

The primary objective of this presentation is to present/discuss:

- The state of the art of some reproductive processes/phenomena, especially insemination/ fertilization, all of importance for commercial reproduction.
- Which reproductive technologies will be relevant in the near future or should be developed further.

## Can artificial insemination be further improved?

*Intracervical deposition of semen*

At mating as well as at conventional insemination, semen is deposited into the cervical canal of the sow. At the UTJ/isthmus a sperm reservoir is formed in order to escape reflux and phagocytosis in the uterine lumen and ensure that sufficient numbers of potentially fertile spermatozoa are present at the site of fertilization when oocytes are released.

Stimulation by the boar during mating releases oxytocin in sows. The release of oxytocin can also be triggered by intense stimulation similar to that of the boar (Madsen *et al*. 2002; Madej *et al*. 2005). However, Gerritsen *et al*. (2005) showed that artificial boar stimulation does not mimic the real boar and it is unclear how boar stimuli should be imitated to elicit the optimal physiological response in the female pig (for review see Norrby 2010).

No changes of the plasma concentration of prostaglandin (measured as prostaglandin $F_2\alpha$-metabolite, PGFM) were seen at natural mating, while elevation always occurred in conventionally or intrauterine inseminated sows (Madsen *et al*. 2002; Madej *et al*. 2005; Wongtawan *et al*. 2006; Norrby *et al*. 2007). By incubating cells from the porcine cervix and uterus with seminal plasma prostaglandin synthesis was inhibited, providing a possible explanation for the differences in PGFM after natural mating (Madej *et al*. 2012).

*Distribution of semen within the genital tract*

Semen, deposited in the cervical canal or into the uterine body, is rapidly distributed along the uterine horns and the oviducts. However, it is since long time accepted that the volume and the number of spermatozoa inseminated influence sperm distribution/fertilization (Baker *et al*. 1968). Thus the percentage of fertilized oocytes was low (< 50%) when only $1x10^9$ spermatozoa independent of volume were inseminated and/or when the volume was as low as 20 mL independent of sperm numbers inseminated. The semen transport is effected by the contractile mechanism in the musculature of the reproductive tract. The myometrial contractions are of antiperistaltic (ad ovarian) as well as peristaltic (ad cervical) nature. Langendijk *et al*. (2002), measuring the uterine activity in sows using a non-surgical open-end catheter technique, found no effect on the uterine activity after infusion of 100 mL saline or seminal plasma during oestrus. Similar results were also earlier reported (Bower 1974; Viring & Einarsson 1980a).

Polge (1978) inseminating radio-opaque material in oestrous pigs could not trace it in the oviducts and therefore drew the conclusion that only spermatozoa but not seminal plasma enter the oviducts. For tracing low concentrations of seminal plasma constituents, radiolabelled compounds (molecular weight 335 – 69 000) suspended in seminal plasma were utilized to study the possible entrance of different sized molecules into the oviducts of oestrous pigs (Einarsson *et al*. 1980). All the compounds used were traced in all oviducts measured. After simultaneous insemination of two compounds there was a close agreement between their distribution in the genital tract. By using similar radiolabelled compounds as tracers, it was for the first time demonstrated that a transuterine transport takes place in female pigs after insemination (Viring *et al*. 1980a).

*Seminal plasma is usually removed/diluted for AI, doesn´t it have any effect on sperm distribution?*

In an experimental study performed under general anaesthesia, the uterine horns were ligated, preventing backflow as well as transuterine movement of semen. This ligation did not influence the uterine contraction pattern. After ligation concentrated fresh semen suspended in 10 mL seminal plasma or in 10 mL of a buffer were slowly deposited into the uterine horns anterior to the ligatures through a cannula. One hour after insemination the mean number of spermatozoa recovered from the oviducts was always higher when the adjacent horn was inseminated with spermatozoa suspended in seminal plasma (Viring & Einarsson 1980b). Therefore, it was supposed that the seminal plasma effect is mainly localized to the isthmus part of the oviduct, close to the UTJ. Recording the motility of the isthmus part of the oviduct by use of balloon-tipped catheters in it was also demonstrated a decreased spontaneous activity of boar seminal plasma after its deposition in the lumen (Viring & Einarsson 1980a). As the constricted lumen of the isthmus portion of the porcine oviduct during oestrus, together with the oedematous condition of the longitudinal folds, as ascribed a functional importance for sperm transport (Hunter 1975), it is assumed that the relaxative effect of seminal plasma facilitates sperm transport into the oviducts.

Rath *et al*. (1989) inseminated gilts with either seminal plasma or buffer immediately prior to artificial insemination. At days 3-5 after insemination, the number of accessory spermatozoa in the zona pellucida was significantly ($P < 0.05$) higher in the gilts that were inseminated with seminal plasma. A component of seminal plasma that has been supposed to be responsible for this effect is oestrogen (for review see Soede 1993). However, the total quantity per ejaculate varies considerably depending on season and boar. When Weitze *et al*. (1989) inseminated gilts with frozen semen, seminal plasma application before insemination significantly increased the number of embryos with accessory spermatozoa, whereas oestrogen application resulted

in non-significant increase. Therefore it must be some other factor in seminal plasma affecting sperm transport.

*Seminal plasma influences the local production of prostaglandin E in isthmus*

Rodriguez-Martinez & Einarsson (1985) studied the oviductal motility in gilts under general anaesthesia using intraluminal pressure transducers. By injecting small doses of prostaglandin $E_2$ in an artery close to the oviduct, they obtained an inhibitory response on the motility of the isthmus during oestrus. Boar seminal plasma does not contain any measurable amounts of prostaglandins (M. Hamberg & S. Einarsson 1972, unpublished results). However, the oviductal fluid contains a low concentration of prostaglandin $E_2$ during oestrus (J.Å. Lindgren personal communication in Rodriguez-Martinez & Einarsson 1985). Recently Kaczmarek *et al.* (2010) published most interesting results showing that boar seminal plasma stimulated the local production of $PGE_2$ from porcine oviductal epithelial cells, but did not influence secretion of $PGF_2\alpha$. The same study demonstrated that $PGF_2\alpha$ synthase (PGFS) and prostaglandin 9-ketoreductase (CBR1), which converts $PGE_2$ to $PGF_2\alpha$, were down-regulated one day after infusion of seminal plasma into the uterine horns.

Noteworthy, it took 40 years from the first observation that seminal plasma affects the sperm transport into the oviducts until the mechanism was clarified. What should we learn from this? (1) That it is important to be familiar with earlier published observations and (2) a need of close cooperation between scientists doing clinical experiments and cell biologists, and (3) you should never give up.

*Fate of excess semen*

A significant volume or the naturally or artificially inseminated semen is lost within 30-60 minutes via vaginal reflux (e.g. Viring & Einarsson 1981; Steverink *et al.* 1998). Also a small trans-oviductal sperm passage occurs, especially during the preovulatory period (e.g. Viring 1980). The remaining part of the inseminated spermatozoa, not colonizing the uterotubal junction and the adjacent part of the isthmus (sperm reservoir, SR), is eliminated from the uterine lumen mainly by polymorphonuclear neutrophils (PMLNs) (e.g. Rodriguez-Martinez *et al.* 1990). The influx of PMNLs into uterus (e.g. Lovell & Getty 1968; Rodriguez-Martinez *et al.* 1990; Rozeboom *et al.* 2000; Matthijs *et al.* 2003) is accompanied by accumulation of macrophages, granulocytes and lymphocytes in the endometrial stroma (e.g. Kaeoket *et al.* 2003). The PMNLs and debris of spermatozoa are then eliminated from the uterine lumen by continuous vaginal discharge and epithelial phagocytosis (Rodriguez-Martinez *et al.* 1990), so that a new inseminate can enter a cleansed uterine lumen (Rodriguez-Martinez *et al.* 2008). In 2010 (Rodriguez-Martinez *et al.*) it was reported that the major seminal plasma glycoproteins (spermadhesins PSP-I/PSP-II heterodimer) are inducing the migration of PMLNs into the uterine cavity of the sow, initiating endometrial-released cascade of transient and long-lasting immunological events. Further studies are needed to elucidate the compounds in seminal plasma that interact with the sow´s reproductive tract.

## Establishment of a sperm reservoir within the genital tract, a key phenomenon for fertility

A sperm reservoir is established in the deep furrows of the UTJ and the ad-uterine segment of the isthmus (Viring *et al.* 1980b; Hunter 1984), where the spermatozoa mostly showed a normal ultrastructure (Rodriguez-Martinez *et al.* 1990). Changes in the distribution, number and membrane integrity of spermatozoa in the UTJ-isthmic region were closely related to

ovulation, as monitored by transrectal ultrasound examination (Mburu *et al*. 1996). In another study Mburu *et al*. (1997) examined changes in morphology and in the precise localisation of boar spermatozoa in perfusion-fixed UTJ and isthmus around ovulation in sows, using scanning and transmission electron microscopy. Two sperm populations were evident, one with epithelial contact and one without such contact. Most of the sperm population with epithelial contact maintained intact plasma membrane during the pre-ovulatory period. Ovulation was accompanied by a relocation of spermatozoa from the mucosal crypts/interfolds towards the more central part of the mucosal surface. Inside the crypts (spaces between the terminal folds of the UTJ), the spermatozoa had straight or slightly curved tails, indicating that their level of activity was low. It seems probable that the epithelium of the UTJ produces an environment favourable for storage of spermatozoa without loss of their fertilizing capacity. The components of this environment are only in a limited degree identified and known and should therefore be dedicated for further research.

## Which spermatozoa colonize the sperm reservoir?

Saravia *et al*. (2008) suggested that the first 10 mL of the sperm-rich fraction ought to be considered as that primarily colonizing the sperm reservoir (SR) in the porcine oviducts. Rodriguez-Martinez *et al*. (2009) finally demonstrated in an elegant experimental study, inseminating spermatozoa in the same order as ejaculated in vivo, that the highest proportion of spermatozoa localized to the SR originated from the first sperm-rich fraction. These spermatozoa should also be considered as those mainly involved in fertilization (Rodriguez-Martinez *et al*. 2005). Moreover, several studies have suggested that boar spermatozoa from this fraction best survive manipulation, including cryopreservation (Pena *et al*. 2006; Morell *et al*. 2009).

From the SR restricted sperm numbers are gradually, but apparently continuously, released towards the presumed site of fertilization at the ampullar isthmic junction (AIJ). This occurs particularly when ovulations are approaching or have occurred (Rodriguez-Martinez *et al*. 2005).

Smith & Yanagimachi (1990), making observations through the oviductal wall in the hamster, noted that spermatozoa on the mucosal surface broke contact with the epithelium and swam for a short distance whereupon they reattached again. It should be interesting to know if the same thing happens in the pig. However, the method they used must be modified to be applicable on pig due to the difference in morphology of the oviduct wall.

## Intrauterine deposition of semen

Procedures for intra-uterine semen deposition have been presented during the last decade. The semen is placed either transcervically using a novel insemination device (Watson & Behan 2002) or deep into one uterine horn, using a long, flexible catheter (Martinez *et al*. 2001; Vazquez *et al*. 2005; Tummaruk *et al*. 2007). Deep uterine catheterization is performed after the insertion of a commercial AI spirette. The catheter is then inserted through the spirette, moved through the cervical canal, and propelled forward along the uterine body and uterine horn. Several trials have since then confirmed that it is possible to insert the flexible catheter into one uterine horn in more than 90% of the sows, taking approximately 3-4 minutes per insemination (for review see Vasquez *et al*. 2008). The semen dose is deposited deep into one uterine horn. However, spermatozoa are able to reach the contralateral oviduct. Vasquez *et al*. (2008) have made observations supporting two different pathways: transperitoneal and transuterine. However, the trans-peritoneal pathway is effective in only a very small percentage

of sows (< 5%), while the transuterine seems to be the predominant route (> 75 % of the sows) (Martinez *et al.* 2005).

## Intraoviductal deposition of semen

The first successful pregnancy with frozen-thawed boar semen was reported by Polge *et al.* in 1970. They used a surgical procedure (laparotomy) placing the spermatozoa locally in the oviducts. When they inseminated the frozen-thawed spermatozoa via cervix no pregnancy was obtained. Using intra-oviductal deposition they demonstrated that boar spermatozoa survived the freezing-thawing procedure. A few years ago surgical procedure (laparoscopy) was successfuly used for intra oviductal insemination of cryopreserved and sex-sorted spermatozoa (Garcia *et al.* 2007; Vazquez *et al.* 2008). Surgical techniques can of course not be utilized in commercial pig production, but can be valuable for scientists to take the first step in proving fertilizing capacity of manipulated semen.

## Cryopreservation of boar semen is still not optimal for commercial use

Frozen-thawed boar spermatozoa have a shorter life span than freshly maintained spermatozoa (Einarsson & Viring 1973; Pursel *et al.* 1978). Differences in fertilizing capacity of frozen-thawed-spermatozoa between boars has been known for long time (Larsson & Einarsson 1976; Polge 1978). However, when gilts were inseminated with frozen-thawed spermatozoa a few hours before ovulation, the fertilizing capacity of the spermatozoa was equal for good as well as for bad freezers (Larsson 1976). Thus, the maintenance of fertilizing capacity of the spermatozoa from the "bad freezers" was shorter than from the "good freezers". Therefore, to obtain maximum fertilization with frozen-thawed boar semen, sows should be inseminated close to the time of ovulation. Achievement of acceptable fertility was reported to be obtained when frozen-thawed spermatozoa were deposited 4-6 hours before ovulation (Waberski *et al.* 1994). The short lifespan of boar spermatozoa following cooling and freezing-thawing processes is one of the major drawbacks to a successful application of cryopreserved boar spermatozoa in commercial AI programmes. Efforts have over the years been dedicated to reducing the negative effects of the freezing procedures on boar spermatozoa by introducing improvements in the sperm cryopreservation protocols (Eriksson *et al.* 2001; Carvajal *et al.* 2004; Saravia *et al.* 2005, 2009; Rath *et al.* 2009). Despite the improvements post-thaw viability, lifespan and fertility of cryopreserved spermatozoa are still reduced as a consequence of injuries arising during the cryopreservation/thawing procedures (Parrilla *et al.* 2009).

There is also a variability in the seminal plasma composition among boars as well as between ejaculates or fractions of the same ejaculate (Rodriguez-Martinez *et al.* 2005, 2008; Saravia *et al.* 2010). Moreover addition of seminal plasma from boars with good freezability to freezing extenders improved sperm cryosurvival (Hernandez *et al.* 2007). However, the specific components of the seminal plasma improving survival at freezing must be further identified and maybe then in the future be added to extenders for cryoconservation of boar spermatozoa. This field needs closer cooperation between chemists, molecular biologists and animal scientists.

Artificial insemination with fresh or stored semen is currently the only sperm technology used at a commercial scale in the pig industry. Cryopreservation of spermatozoa as well as sperm sorting for gender pre-selection are still associated with too low reproductive performance. Due to the great variability in sperm freezability among boars and the large number of spermatozoa required per AI dose, frozen boar semen is today only used at international exchange of genetic

lines without transporting livestock, the long-term conservation of superior genetic individuals in resource banks, or the testing for presence of pathogens before use (Rodriguez-Martinez *et al*. 2009). Special attention should therefore be given during the coming years for further improvement of the functionality and fertilizing ability of the treated semen.

## Can ovulation time be predicted under commercial conditions for suitable insemination time?

Knowledge of oestrous symptoms and time of ovulation is important in order to achieve a high fertility at artificial insemination. Ultrasonographic examination of the porcine ovaries, using the transcutaneous method, was originally described by Weitze *et al*. (1989). However, the transrectal ultrasonography, introduced in sows by Soede *et al*. (1992), is the most appropriate non-surgical method of studying ovarian activity and ovulation time. By this technology was demonstrated that the duration from onset of oestrus to ovulation increased when the duration of oestrus increased (Soede *et al*. 1994; Mburu *et al*. 1995). The interval from onset of oestrus to ovulation varied between 35-42 hours (Mburu *et al*. 1995). However, to predict the exact time of ovulation the oestrus observation must be very careful and the ultrasonography performed every 4 hours when ovulation time approaches. This is of course not for practical use in commercial pig production, but it is an excellent tool at animal experimentation.

At the former conference held in Canada in 2009, excellent reviews were presented on "nutritional and lactational effects on follicular development in the pig" (Quesnel 2009),"intra-follicular regulatory mechanisms in the porcine ovary" (Hunter & Paradis 2009), "ovarian responses to lactation management strategies" (Soede *et al*. 2009) as well as on "studies on fixed-time ovulation induction in the pig" (Brüssow *et al*. 2009). It was summarized that our knowledge is still far too incomplete for predicting time of ovulation during postweaning oestrus under commercial conditions. Even at fixed-time ovulation using hormones it was recommended to inseminate twice during oestrus with fresh semen (Brüssow *et al*. 2009). The time of insemination in relation to ovulation is of great importance for the fertility results. This is especially the case when using cryopreserved spermatozoa and/or when inseminating with a low number of fresh spermatozoa. At the present conference Driancourt (2013) presented interesting results on fixed time artificial insemination in gilts and sows involving ovulation induction with busereline at the end of altrenogest treatment. However, Driancourt concluded that "further research is needed to check whether buserelin + a single fixed time insemination will perform well in females given deep inseminations with lower sperm number, or inseminated with sexed semen".

## Litter size and piglet mortality must be further considered

For commercial pig production number of weaned piglets per sow and year is important from an economical point of view. One way to reach this goal has been to create breeding programs for large litter sizes. Such programs have been successful especially in some European countries (Christiansen 2010; Pedersen *et al*. 2010). However, a large litter size is often combined with high piglet mortality. The higher mortality seems mainly to be due to a large rate of stillborn piglets or piglets that die within the first hours after birth. The biology of factors that are crucial for piglet survival shows that large litter sizes result in more piglets being born small, weak or underdeveloped. These piglets have an increased risk of dying during or after birth. Mortality after birth is caused by crushing, starvation, cold and disease. The high proportion of dead piglets

is both an ethical and a welfare problem. The large litter size has also necessitated the use of nursing sows due to lack of teats to all piglets by their biological mother. Possible strategies for reduction of piglet mortality need further consideration. Inclusion of piglet mortality in the breeding index is one opportunity that has been practised for instance in Denmark during the last years (Pedersen *et al*. 2010) . There is also a need for improved management to reduce the mortality such as increasing birth surveillance and birth assistance of the sow, improvement of microclimate at the birth site, improvement of sow health (e.g. udder health) (reviewed by Oliviero at the present conference). Quesnel et al. (2013) underlined at the present conference that neonatal survival largely depends on adequate colostrum intake by the newborn piglets. A review presented at this conference by Baxter and Edwards (2013) concluded that piglet mortality is multifaceted in nature. The best chance of success in improving piglet survival is "coupling environmental and nutritional interventions with a balanced selection program".

The pre-weaning mortality occurring within two days of birth happens most often to the small and weak piglets. If these piglets should survive, their growth rates are anyhow impaired (Tilley *et al*. 2007). According to Vallet *et al*. (2009) the weight of the piglet is correlated with the weight and function of the foetal placenta. However, Baxter *et al*. (2008) found that stillborn piglets were disproportionately long and thin, with lower ponderal index (PI) and body mass index (BMI) compared with surviving littermate. They found that PI and BMI were more predictive than birth weight alone of whether or not a piglet would be stillborn. Rootwelt *et al*. (2012) associated the placental area with piglet vitality, thus emphasizing the influence of placental quality on postnatal viability. Therefore, it seems important to further increase our knowledge on how the foetal placenta develops in terms of functionality and size.

## Housing affects reproduction

The management procedures in modern pig production include a number of events, which might act as stressors on the animals. Due to welfare consideration, systems with loose-housed sows instead of system with crates/stalls for the sows have become common, at least for non-lactating sows. In the EU it is from 2013 not allowed to keep non-lactating sows in crates/stalls from 4 weeks of pregnancy until one week before expected farrowing. This new directive has several advantages: i.e. the animals can perform their natural behaviour. However, there are also factors in these systems which might affect reproduction (for reviews see Einarsson *et al*. 2008a; Kemp & Soede 2012). In systems with group-housed sows, the number of sows are in most cases higher than in groups formed in the wild. The female pig form family units of one or several sows and their offsprings (Graves 1984), and the individuals in the family unit avoid contact with other unfamiliar sows (Meynhardt 1990). Therefore, in wild pigs (Sus scrofa) confrontation between unfamiliar female pigs happens very rarely. In commercial group housing systems, however, mixing of unfamiliar sows is difficult to avoid. A drawback with the group-housing system is also the difficulty to avoid regrouping. In most lactation units the sows are housed individually, and grouping of unfamiliar sows usually takes place at least once after weaning. Elevated stress levels in a newly formed small group of sows may persist for approximately two days until a ranking order is established among the animals (Tsuma *et al*. 1996), but might continue for additional days in large groups of sows.

Several experimental studies have been performed to find out if reproduction is impaired due to grouping/regrouping (for review see Einarsson *et al*. 2008b). Important factors which already have been identified are time of grouping, group size, age/size of the sows grouped together, bedding, feeding systems. The effect of housing on pregnancy rate 28 days post-service, early disruption of pregnancy and behavior, was presented by Munsterhjelm *et al*. in 2008. Half of

the dry sows were kept in stalls, and half were group-housed on deep litter bedding. Behavioural indicators proposed a lower welfare in stalled animals compared with group-housed ones. However, reproduction, in terms of weaning-to oestrus interval, rebreeding rate and irregular rebreeding (% of rebreedings), was negatively affected in the group-housed sows compared to the individual housed sows. This shows that it is not easy to predict reproductive consequences of different housing conditions. Therefore, more attention in terms of experimental animal studies as well as of field studies should be paid to different loose-/group-housing systems for sows all over the world. Welfare and ethical aspects are of growing interest for the society and for consumer organisations and cannot be neglected in the future.

## Some concluding remarks together with future views

- During the last two decades various proteins and polypeptides have been identified in boar seminal plasma, and the relevance of some of them to reproductive technologies has been discussed at this conference. A more holistic approach should be beneficial and be a priority in the future.
- The long- term goal should be to isolate/synthetize seminal plasma proteins proved to be of importance for the spermatozoa to survive cryopreservation with preserved fertilizing capacity. The long-term goal should then be to synthezise these products and use them as ingredients in media used for e.g. cryopreservation and sex sorting of semen.
- Other fields of reproductive technologies which need further development for being available for commercial pig reproduction are IVF and non-surgical embryo transfer.
- The genomic revolution has brought us transcriptional profiling, allowing for the identification of many genes involved e.g. in mammalian gametogenesis, fertilization, and preimplantation embryo development. We do expect further progress within this field of research during coming years. The new information has also capacity to revolutionize the genetic progress within animal breeding.
- The time of insemination in relation to ovulation is of great importance for the fertility results. Promising results were presented when using single insemination with fresh semen at fixed-time ovulation (using hormones). However, further research is needed to find out if the same model also works in females given deep insemination with lower sperm number, or inseminated with sexed semen.
- An excellent review presented at this conference concluded that piglet mortality is multifaceted in nature. The best chance of success in improving piglet survival is coupling environmental and nutritional interventions with a balanced selection program. This program must be considered in commercial pig production in the near future.
- More attention in terms of experimental animal studies as well as of field studies should be paid to different loose-/group-housing systems for sows all over the world. Welfare and ethical aspects are of growing interest for the society and for consumer organisations and cannot be neglected in the future.

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