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Improvement of Semen Quality by Feed Supplement and Semen Cryopreservation in Swine

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Additional information is available at the end of the chapter

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1. Introduction

Artificial insemination in pig offers many advantages in swine production in terms of a better disease control through semen quality control, a diverse male genetic distribution and an easiness of management. It is accepted that in developing countries, AI helps to improve the genetic profile. A number of sows can be inseminated using the same ejaculate instead of only one from natural mating. The number of pig farms using AI has increased because of the technical improvement of semen extenders and equipments, and the technique can be performed on farm. In Thailand, AI in commercial pig farms is routinely used as a standard protocol in pig production. The results obtained by AI are quite similar or higher than that from natural. Because of the quality of insemination can be guaranteed by semen testing and evaluation before insemination. The improvement of semen quality can be acquired by feed supplement and semen freezing in boar can be used to genetic conservation. The feed supplement improving the semen quality have been imperatively used in the boars which have low libido and low semen quality, because these boars have been imported and are of superior genetic merit and so are perceived to have great value to their owners who, therefore, are very reluctant to cull them. Moreover, in tropical countries, cryopreservation of boar semen is nowadays performed in a limited scale and it has yet to be conducted in Thailand particularly for the commercial purpose. Concerning this point and obtained benefit in the future, the improvement of boar semen quality by feed supplement and boar semen cryopreservation are reviewed in this chapter.

2. Feed supplement to increase boar semen quality

The semen quality depends on individual, breed, season, confinement and boar health. It was found that the dietary supplements of antioxidants, vitamins and/or minerals can increase libido and semen characteristics in boars. Additions of antioxidants in seminal plasma or semen extender play an important role on boar semen storability. Semen with a normal motility contains higher polyunsaturated fatty acids (PUFAs) in cell membrane has that that having a low motility [1]. Short life span spermatozoa usually presented in low antioxidant condition resulting from the high lipid peroxidation of sperm plasma membrane. Spermatozoa in low antioxidants of seminal plasma also show a lower sperm motility, viability and normal morphology than spermatozoa in normal seminal plasma (Table 1) [1,2]. The feed supplements were expected to improve the semen quality by increasing the number of sperm per ejaculation, motility, viability and antioxidant in cell and seminal plasma. However, it depends on the initial performance of the boar influencing on successfully improving semen quality. Therefore, the key roles of feed supplement containing the rich of PUFAs, vitamins and minerals to improve the semen quality are increasing the antioxidant to reduce the plasma membrane damages from ROS and increase the amount of PUFAs in sperm plasma membrane that may increase the percentage of sperm motility and vitality.

3. Effect of Reactive Oxygen Species (ROS)

Boar sperm are highly sensitive to peroxidative damage due to the high content of unsaturated fatty acids in the phospholipids of the sperm plasma membrane [3,4] and the correlation of low antioxidant capacity of boar seminal plasma and lipid-peroxidation [5]. It has been reported in sperm freezing of human [6], bull [7] and mouse [8] that is associated with ROS level and oxidative stress. Moreover, the process of freezing and thawing bovine spermatozoa can generate the ROS [9], DNA damage [10], cytoskeleton alterations [11], inhibition of the sperm–oocyte fusion [12] and can affect the sperm axoneme that is influenced on the sperm motility [13].

The lipid-peroxidation of membrane phospholipid bound docosahexaenoic acid (DHA) has been presented as one of the major factors that limit the sperm motility in vitro. Semen samples show high sperm variability in lifespan and, consequently, in susceptibility toward lipid peroxidation. Therefore, it is postulated that there is also cell-to-cell variability in DHA content in human spermatozoa and that the content of the main substrate of lipid peroxidation (DHA) is critical and highly regulated during the sperm maturation process. Several studies have been performed to analyze the fatty acid content of germ cells and sperm at different stages of maturation, including in vivo studies in animal models, and in vitro approaches in human spermatozoa. One of the consequences of defective sperm maturation in the seminiferous epithelium is the retention of residual cytoplasm. This residual cytoplasm, which is attached to the midpiece and retronuclear area of the sperm head, has been shown to produce high levels of reactive oxygen species (ROS) [14-16]. In addition, the membranes

enclosing the residual cytoplasm are enriched in polyunsaturated fatty acids such as DHA [17,18]. The combination of high polyunsaturated fatty acid content and high ROS production in these immature sperm has been shown to lead to increased lipid peroxidation and subsequent loss of sperm function [14,15]. ROS-mediated damage to human spermatozoa was characterized in the early 1980s [19-24] and has been shown by many authors to be an important factor in the pathogenesis of male infertility [14,25-27].

To a first approximation, the process of lipid peroxidation involves the initial abstraction of a hydrogen atom from the bis-allylic methylene groups of polyunsaturated fatty acids, mainly DHA, by molecular oxygen. This leads to molecular rearrangement to a conjugated diene and addition of oxygen, resulting in the production of lipid peroxide radical. This peroxyradical can now abstract a new hydrogen atom from an adjacent DHA molecule leading to a chain reaction that ultimately results in lipid fragmentation and the production of malonaldehyde and toxic shortchain alkanes (e.g., propane). These propagation reactions are mediated by oxygen radicals. DHA is the major polyunsaturated fatty acid in sperm from a number of mammalian species, including the human, accounting in this species for up to 30% of phospholipid-bound fatty acid and up to 73% of polyunsaturated fatty acids. At the same time, DHA is the main substrate of lipid peroxidation, accounting for 90% of the overall rate of lipid peroxidation in human spermatozoa [23,28].

Characteristics	Normal motility	Low motility
Sperm per ejaculate ($\times 10^9$)	88.6 \pm 41.7 ^a	76.9 \pm 36.2 ^a
Sperm motility, %	82.6 \pm 5.2 ^a	30.6 \pm 12.8 ^b
Sperm viability, %	86.7 \pm 5.8 ^a	31.5 \pm 14.9 ^b
Normal morphology, %	96.2 \pm 1.9 ^a	85.1 \pm 4.9 ^b
Normal plasma membrane, %	83.3 \pm 7.4 ^a	15.7 \pm 7.5 ^b
Total antioxidant status in seminal plasma (ng/ml)	1.54 \pm 0.35 ^a	0.80 \pm 0.56 ^b

Rows with different superscripts (a,b) differ $P \leq 0.05$ [1-2]

Table 1. Semen characteristics and antioxidant capacity in seminal plasma of boars having normal and low sperm motility (means \pm SD)

Lipid peroxidation has profound consequences in biological membranes. The generation of the polar lipid peroxides ultimately results in the disruption of the membrane hydrophobic packing, inactivation of glycolytic enzymes, damage of axonemal proteins (loss of motility), acrosomal membrane damage, and DNA alterations [29,30]. Oxidation of phospholipid-bound DHA has been shown to be the major factor that determines the motile lifespan of sperm in vitro [6,31,32]. Three basic factors determine the overall rate of lipid peroxidation of sperm in vitro: oxygen concentration and temperature in the medium (OXIDANT), the presence of antioxidant defenses (ANTIOXIDANT), and the content of membrane-bound DHA (SUBSTRATE). Thus, the higher the temperature and the concentration of oxygen in

solution, the higher the rate of lipid peroxidation as measured by malonaldehyde production [24]. In boar, total antioxidant in seminal plasma relates to percentage of normal sperm morphology and plasma membrane. The low storability semen has presented the high plasma membrane damage from ROS, which was resulted from low amount of antioxidant in seminal plasma [2]. Moreover, the semen which having poor normal sperm morphology has shown the low level of antioxidant in seminal plasma (Table 1) [1].

The balance between these key factors determines the overall rate of peroxidation in vitro. In this system, the substrate seems to play a key role. The main substrates for lipid peroxidation are polyunsaturated fatty acids, especially docosahexaenoic acid.

4. Effect of vitamins and minerals

The glutathione peroxidase is main intracellular antioxidant enzyme that catalyses to reduce the hydrogen peroxide and organic hydroperoxides to nontoxic metabolized compounds. The essential component of this enzyme is selenium. Vitamin E or alpha-tocopherol is the dominant antioxidant in cell plasma membranes. Many researches have shown a synergism of antioxidant activity between selenium in glutathione peroxidase and vitamin E. The effects of selenium supplementation on semen quality were more reported than the effects of vitamin E supplementation, and selenium supplementation improved in higher conception rates when gilts were serviced with extended semen from the boars [33]. However, feed additive on boar diet with high levels of vitamin C had no effects on semen quality or libido characteristics in healthy boars. U.S. Food and Drug Administration (FDA) regulations allow up to 136 g of selenium add on/pound of feed for pigs.

Vitamin C or ascorbic acids are a dominant water-soluble antioxidant. Their action is scavenger to disable the function of any type ROS. Vitamin C is a powerful source of electron donor which reacts with hydroxyl radicals, peroxide and superoxide to form de-hydroxyl ascorbic acid. The level of ascorbic acid in seminal plasma is approximately 10-fold higher concentration comparing with blood plasma in human [30,34]. The level of ascorbic acid in seminal plasma has a positively correlation with the percentage of normal [35].

5. Effect of Polyunsaturated Fatty Acids (PUFAs)

Linoleic acid or omega-6 fatty acid is the only FA for which NRC has established requirements at least 0.1% of diet for sexually active boars. However, the effect of various fatty acids (FAs) top on diet, particularly the omega-3 fatty acids, on semen quality and libido characteristics in boars are more interesting. Nowadays, there are 3 types of omega-3 fatty acids that are linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA). The boar feed commonly consist of the large amounts of crops, with source of protein added in the form of soya-bean, fish powder, bone powder, etc. Thus, dietary fatty acids have a (n-6):(n-3) normal ratio of greater than 6:1 and do not contain long chain n-3 PUFAs. If 22:6(n-3) is essential for

optimal fertility in pig spermatozoa, as being in human sperm [28,36,37], then it is possible that supplement 22:6(n-3) PUFAs on boar diets to improve the spermatogenesis. This supplementation may increase from either a deficit of (n-3) fatty acids or an increasing synthesis of 22:6(n-3) from 18:3(n-3) to competition between (n-6) and (n-3) fatty acids [38]. The tuna oil supplementing on the boar diet can increase the percentages of sperm cells with progressive motility, the proportion of live sperm, normal acrosome head, and normal morphology [39]. It was found that boars fed with commercially available product containing DHA, vitamin E and selenium (PROSPERM®, Minitube America, Inc., Minneapolis, MN) for 16 weeks had a higher sperm concentration, number of sperm/ejaculate, and sperm motility comparing with control group [40]. In many experiments, 8-week period was used as the control period because spermatogenesis in boars requires 34–39 d and epididymal transport involves another 9–12 d [41]. It is not surprising that a 7–8 week period may be necessary after dietary supplementation [40,42].

6. Boar semen cryopreservation

The research on semen cryopreservation in boar is limited even though the procedures have been studied during the past 60 years [43–47]. The advantages for development of frozen semen include the preservation of the good genetic resource, the distribution of superior genetic boars, and the improvement of the transportation of sperm across countries [48]. However, the utilization of frozen-thawed (FT) semen prepared for artificial insemination (AI) at present is estimated to be less than 1% of all insemination worldwide. The most important reasons are the poor sperm quality after cryopreservation and a lower fertilizing capacity of FT semen, when used for conventional AI compared to fresh semen. Poor sperm quality frequently found in FT boar semen is partly due to a high sensitivity of the boar sperm to rapid cooling to a few degrees above 0°C, the so-called “cold shock”, which the sperm have to traverse during cryopreservation process. This is evidenced by the loss of viable sperm and by more capacitation-like changes in the viable sperm [49]. These changes result in a shorter survival time of the FT sperm in the female genital tract in comparison to its fresh and liquid-preserved counterparts [50,51].

7. Factors affecting the success of boar semen cryopreservation

Boar semen differs in several respects from the semen of other domestic animals. It is produced in large volume (200 to 250 ml) and is extremely sensitive to cold shock. The success of freezing boar semen depends on both internal and external factors. Internal factors include the inherent characteristics of sperm and the existing differences among boars and ejaculates, while external factors are composed of the composition of the extenders, freezing packages, and the method of freezing and thawing of the semen, for example [48].

8. The semen donors

Variation between individuals in the extent to which their sperm are damaged by freeze-thawing has been reported in many species including pig [52-55]. For instance, some study assigned individual boars into good, average and poor freezability groups on the basis of their post-thaw sperm viability using a system of multivariate pattern analysis, and suggested that cryosurvival of the sperm was not necessarily related to the observed quality of the semen sample. In addition to inter-animal variation, intra-animal variation such as difference between ejaculate fractions has also been described as a source of difference in boar sperm freezability [56,57]. Some researcher found that sperm present in the first 10 ml of the sperm-rich fraction (portion I) better sustain cooling and freeze-thawing compared to those present in the rest of the ejaculate (portion II) [56]. These differences were manifested by motility patterns, the maintenance of membrane integrity and capacitation-like changes of sperm after thawing. However, variation between ejaculate fractions is dependent of individual boars, with some boars differing in the ability of the two ejaculate portions to sustain cryopreservation, while in other boars such differences were not detected [57]. The mechanisms underlying differences in cryosensitivity between different individuals and different ejaculate portions have yet to be elucidated, but there is some evidence for physiological differences between sperm from individual boars. Harrison and co-workers demonstrated that the stimulatory effects of bicarbonate on the process of capacitation differ among individual boars [58]. Also, the existence of differences in seminal plasma composition and sperm morphology has been hypothesized as a possible explanation for the distinct ability of different boars and different ejaculate portions to sustain cryopreservation [59,60]. In general, boar sperm heads present in portion I were significantly shorter and wider than those present in portion II, detected by using computer-assisted sperm head morphometry analysis (ASMA) [57]. It has been hypothesized that such differences could be genetic in origin. Thurston and co-workers using Amplified fragment length polymorphism (AFLP) technology to analyze genome of 22 Yorkshire (Y) boars indicated that 16 candidate genetic markers linked to genes controlling sperm freezability and these genomes varied among individual boars. Consequently, they may be useful for the prediction of both post-thaw semen quality and fertility of individual boars [55].

9. The composition of freezing extenders

A number of substances have been added to boar semen during cryopreservation in order to improve FT sperm quality. It has been investigated that egg yolk added to boar semen could protect sperm acrosomes during cold shock and hence reduce cryodamage of FT boar sperm [61]. Protection has been claimed to be due to both phospholipids and the low density lipoprotein fraction in egg yolk [62,63]. The mechanism of action is unclear but could be mediated by either a less intense cellular dehydration or by stabilization of the sperm plasma membrane [51].

Cryoprotective agents (CPAs) have been divided into those that penetrate the cell and those which remain extracellular. Glycerol considered as penetrating agents and other non-penetrating agents such as various sugars have been evaluated for cryoprotective effect in boar sperm [64,65]. Glycerol in low concentrations (3 to 4%) has been utilized in various techniques of sperm cryopreservation [47,66]. At these concentrations, glycerol gives maximum post-thaw viability and also in vitro fertilizing capacity of sperm [43]. Both post-thaw motility and acrosome integrity of boar sperm would be decreased when glycerol concentration reached 5%. Glycerol and other penetrating agents could improve FT sperm survival by penetrating sperm and reduce the shrinkage of the cells developed during cooling [8]. They could also lower the freezing point of extra-cellular fluid via action of non-penetrating CPAs [67]. Therefore, the damage of sperm from the formation of intracellular ice occurred during freezing is reduced.

The success of the boar sperm cryopreservation was dramatically increased when the detergent Sodium Dodecyl Sulphate (SDS; later known as Equex STM paste) was included in the cryopreservation protocol [68,69]. The addition of SDS to semen extenders decreases freeze-thaw damage to sperm in several species, including boar [70-72]. Pursel and co-workers stated that the use of 0.5% Orvus Es Paste, a commercial preparation of SDS, in the BF5 extender significantly enhanced the preservation of fertilizing capacity concomitant with an increase in post-thaw percentages of normal acrosome morphology and motility of boar sperm [69]. The beneficial effect of SDS on the sperm membrane is not fully understood, but it has been suggested that its protective effect is mediated through a change in the extending medium, by solubilization of the protective lipids in the egg yolk contained in the extenders. This effect enhanced the cold shock resistance of sperm [73,74]

10. Freezing packages

Boar sperm have been frozen in many forms of packages. Pellet, a form of freezing bull semen on dry ice, was adapted to freeze boar semen and first reported as in [47]. Boar sperm have also been frozen in 5-ml maxi-, 0.5-ml medium- and 0.25-ml mini-straws, as well as different types of 5-ml flat plastic bags [67,75]. All package forms have their own advantages and drawbacks. The 5-ml maxi-straw contains one insemination dose but has a relatively small surface-to-volume ratio, which constrains optimal freezing and thawing throughout the sample. The plastic bags allow even more homogeneous freezing and thawing and also contain a whole insemination dose, but they are not suited for storage in standard liquid nitrogen containers, and therefore are not in commercial use [71]. Pellets and the small straws (0.25- and 0.5-ml straws) have a cryobiologically suitable shape with a large surface-to-volume ratio; thus theoretically, FT sperm in pellets and small straws are less damaged than those in maxi-straws [76,77]. However, with pellets, there are difficulty in the identification of the doses and a risk of cross-contamination during storage, and the thawing procedure is rather complicated as well [71]. Also, the small packages could contain relatively few sperm such as 250 to 500 $\times 10^6$ sperm per straw, which are not enough for a single dose of conventional AI in pigs. Eriksson and Rodriguez- Martinez developed a new flat plastic container

(the FlatPack®) for freezing boar semen. This package could contain a complete insemination dose, allows a quick and uniform freezing and thawing due to its large surface-to-volume ratio, and fits into any conventional liquid nitrogen container. Nonetheless, insemination with large numbers of sperm, such as 5 to 6×10^9 sperm per dose, reduces the number of AI doses per ejaculate. Achieving successful AI with fewer sperm is more important if using boars of superior genetic merit [71].

Fertility after transcervical deep AI of FT boar semen.

11. Conventional AI in pigs

Three techniques of AI can be performed by conventional, intrauterine and deep intrauterine. The conventional AI is common in fresh semen practice, while intrauterine with a reduced concentration of semen is increasing with a satisfying result. The deep intrauterine insemination is used for special kind of semen such as frozen semen or sexed semen with a reduced concentration and semen can be deposited near the junction of uterine-oviductal junction.

Conventional AI in domestic pigs is practiced with doses of approximately 3×10^9 sperm extended to a volume of 80 to 100 ml. Semen doses are stored at temperatures ranging from 16 to 20°C, usually for up to 3 days in simple extenders, but longer when using other extenders [78,79]. The semen is deposited into the posterior region of the cervix by using a disposable, often an intra-cervical, catheter whose tip stimulates the corkscrew shape of the boar penis and engages with the posterior folds of the cervix as it occurs during natural mating. In general, the AI process starts 12 h after detection of standing estrus and it is repeated every 12 to 18 h until standing estrus is no longer shown. When proper detection of estrus is performed, the farrowing rate (FR) and litter size (LS) are comparable with those achieved by natural mating, reaching over 90% of FR and mean LS of 14 piglets [80].

12. Use of FT semen in porcine AI

Contrary to what occurs in cattle, where FT semen is routinely used for AI [81], cryopreserved boar semen is used in less than 1% of the AIs performed around the world. The reasons behind this restricted use of FT boar semen are the low survivability of sperm after the freeze-thawing process and the shorter lifespan of the surviving sperm. These result in lower FR and small LS compared with AI using semen preserved in liquid form [48]. Furthermore, owing to the restricted lifespan of the FT boar sperm, excessive sperm numbers are used often 5 to 6×10^9 sperm per dose. Moreover, at least two AIs are usually performed per estrus in order to reach acceptable fertility rates in the field [82]. Altogether, few doses can be obtained from a single ejaculate and too many sperm are used to ensure fertilization. A decrease in the number of sperm per dose is therefore required to improve the use of ejaculates, so that the production will be cheaper and the use of genetically superior sires more effective.

13. Transcervical deep AI

Although few sperm are required for fertilization within the oviduct, this reduced number is the product of a sequential and very effective reduction along the process of sperm transport in the female reproductive tract (i.e., 25 to 40% of inseminated sperm are lost with the backflow and 50% of the rest of the sperm are ingested by leukocytes in the uterus; Matthijs et al., 2003). The problem to be overcome during AI is to get an adequate number of sperm to the uterotubal junction (UTJ) that could ensure the establishment of the functional sperm reservoir with enough viable, potentially-fertile sperm to ensure maximal fertilization. One strategy proposed to accomplish this is to decrease the number of sperm per AI-dose, by depositing the semen directly in the uterus, and get sufficient sperm into the UTJ. Such deep AI with reduced sperm numbers is a relatively new reproductive practice that has attracted the attention of the swine industry. Such a method could also be advantageous for the spreading of AI with FT semen.

There are basically two non-surgical procedures for depositing sperm into the pig uterus. These include semen deposition either in the uterine body [49,75,83] or into the uterine horn [84,85].

Intra-uterine insemination (IUI) (Figure 1a)

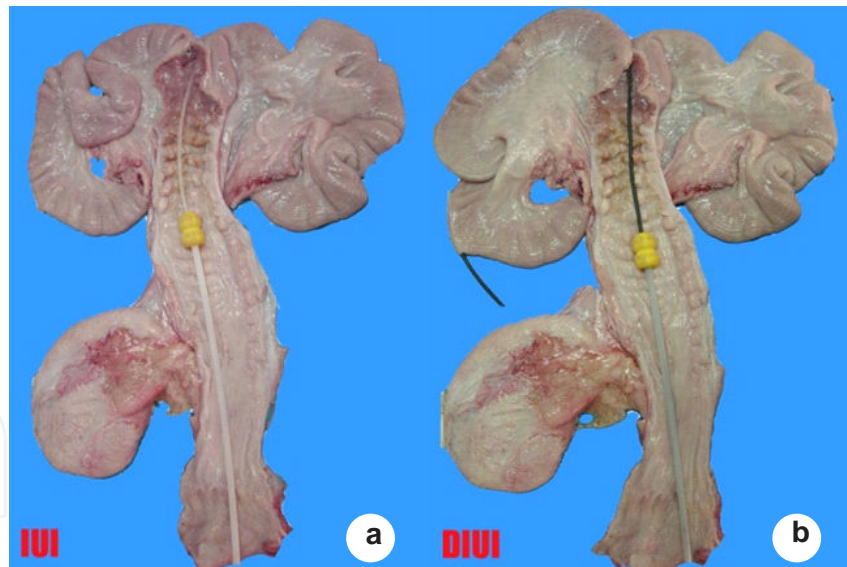


Figure 1. Sperm can be deposited in different procedures: (a) intra-uterine insemination (IUI) and (b) deep intra-uterine insemination (DIUI)

A non-traumatic transcervical catheter that allows an easy penetration of the cervix and deposition of semen in the uterine body of the sow has been designed. Briefly, a conventional catheter (outer catheter) is placed toward and locked into the cervix. An inner tube (around 4 mm outer diameter) is passed through the outer catheter, along the cervical lumen, to reach the uterine body or the posterior part of one of the uterine horns (about 200 mm be-

yond the tip of the outer catheter). The IUI catheter can be used with minimal training and it does not seriously delay the process of insemination, although it can only be safely used in sows [83]. Under commercial conditions, use of the IUI catheter with extended fresh semen can reduce sperm numbers to 1×10^9 sperm per insemination dose and results in a comparable effect on both FR and LS (89% FR and 12 LS) compared with 91% FR and 12.5 LS after conventional AI with 3×10^9 sperm. However, in the field trials carried out by references [86,87], FR were similar between IUI with 1×10^9 sperm and conventional AI with 3×10^9 sperm, but IUI sows had significantly less piglets born per litter (1.5 to 2 smaller LS). The reasons for the loss in LS have not been clarified. Rozeboom and co-workers suggested that several factors such as aged sperm, improper semen handling or insemination-ovulation interval can cause decreases in reproductive performances when low numbers of sperm are used, and in order to obtain consistently high fertility results, a slightly higher number of sperm should be considered.

14. Deep intra-uterine insemination (DIUI) (Figure 1b)

Non-surgical DIUI has been performed in non-sedated pigs using a flexible fiber optic endoscope (1.35 m length, 3.3 mm outer diameter) inserted via the vagina and cervix to reach the upper segment of one uterine horn [84]. The procedure required 3 to 5 min in 90% of the females. After this DIUI, only 1% of the sows showed signs of uterine infection. However, the endoscope is a highly expensive instrument and unpractical for routine use. A flexible catheter was therefore developed on the basis of the propulsion force and flexibility of the fibro-endoscope [85]. The method allows deposition of low sperm doses of either fresh or FT sperm. Moreover, the technology can be successfully used to produce piglets with sex-sorted sperm [88], or for embryo transfer [89].

Using fresh semen, FR and LS were not statistically different between DIUI with 150×10^6 sperm per dose and conventional AI with 3×10^9 sperm, ranging from 83 to 87% FR and 9.2 to 10.4 LS [88]. Nonetheless, LS was always lowest in the DIUI sows. Similarly, although no differences in FR were found (83% and 90% for DIUI and conventional AI, respectively), DIUI sows had less LS (10.5 and 12.9, respectively). The low LS achieved in the DIUI sows inseminated with 150×10^6 sperm probably resulted from the high incidence of unilateral or incomplete bilateral fertilization, and could be overcome by increasing the number of inseminated sperm to 600×10^6 sperm per dose [90]. On the other hand, when a single DIUI with 150×10^6 sperm was performed in hormonally induced ovulating sows, both FR and LS of DIUI sows (83% and 9.7) were not different from those of conventional AI sows (83% and 10) [85]. When FT semen (1×10^9 sperm per dose) was used for DIUI, promising results were obtained. With hormonally induced ovulation and a single DIUI, the FR was 77.5% and LS was 9.3, while with spontaneous ovulation and two DIUIs, the FR was 70% and LS was 9.3. The lower fertility obtained in the latter group resulted from the suboptimal insemination-ovulation period [91]. Bolarin and staff working with spontaneously ovulating sows ($n=407$) obtained FR of over 80% and about 10 piglets born per litter when two DIUIs, at 6 h interval, with only 1×10^9 FT sperm per dose were conducted at the peri-ovulatory period [92]. It has

been suggested that DIUI should be carried out ≤ 8 h before spontaneous ovulation when FT sperm are used [93].

15. Boar semen cryopreservation, experiences in Thailand

In tropical countries including Thailand, cryopreservation of boar semen is nowadays performed in a very limited scale and it has yet to be conducted for the commercial purpose. Our studies undertaken between 2004 and 2009 therefore aimed to develop boar semen cryopreservation in Thailand. Effects of straw volume, Equex STM paste added to a freezing extender and of the individual differences on boar sperm quality after cryopreservation were investigated. In addition, *in vivo* fertility results such as fertilization rate, FR and LS of FT boar semen after DIUI and IUI in multiparous sows were evaluated.

Using a lactose-egg yolk extender with 9% glycerol as a freezing extender of boar semen, it was demonstrated that after thawing the motility, viability and NAR of sperm evaluated with conventional methods were improved when 1.5% Equex STM paste was added into the freezing media [94]. This finding confirms beneficial effects of the detergent on preventing/diminishing cell damage during the freeze-thawing process [68,95]. Equex STM paste improves post-thaw survival of sperm by acting as a surfactant to stabilize cell membranes, particularly acrosomal membranes, and to protect sperm against the toxic effects of glycerol during cryopreservation [73]. However, since the positive effects of this substance are only observed in the presence of egg yolk in the semen extender, it is suggested that Equex STM paste exerts its beneficial action through the alteration of low-density lipoproteins in egg yolk rather than directly affects sperm membranes [69].

In theory, post-thaw sperm loaded in 0.5-ml straws which have smaller surface-to-volume ratio should not have a better quality than those in 0.25-ml straws. Nevertheless, based on the results of 12 ejaculates from 4 boars evaluated in our study [94], the viability and normal morphology of FT sperm packaged in 0.5-ml straws were superior to those in 0.25-ml straws despite being frozen and thawed with their own optimal protocols. The reason behind this is unknown, but it is interesting that similar results have also been observed in dog semen [96]. Therefore, in order to find the reason and draw conclusions with boar sperm, more investigations in this aspect might have to be performed.

With regard to effect of individual variations on the FT sperm quality, 45 ejaculates of 15 boars from three breeds (Landrace (L), Y and Duroc (D); 5 boars each) were studied [97]. It was found that the breed of boar and the individual boars within the same breed significantly influenced most of the FT sperm parameters evaluated. For instance, the post-thaw sperm viability in D and L boars was significantly higher than Y boars. The motility and the normal morphology of FT sperm were lowest in Y boars. L boars seemed to have the most variations in many of the FT sperm parameters. The difference in sperm quality among individual boars that was found in our study was in agreement with previous findings [52,98], suggesting that such individual variation may be correlated with difference in physiological characteristic of the sperm plasma membrane among boars. Additionally, the genomic dif-

ferences between individual boars may be responsible for freezability and post-thaw quality of their sperm [55].

Cervical AI with FT semen usually results in suboptimal fertility; thereby, deep AI using IUI and DIUI procedures was developed. We evaluated fertility (fertilization rate, FR and LS) of FT boar semen after IUI, with 2×10^9 total sperm per dose, and DIUI, with 1×10^9 per dose, in spontaneously ovulating weaned sows. The results revealed that at approximately 2 days following inseminations either with IUI or DIUI, embryo(s) could be recovered from both sides of the oviducts. This observation, the first report in FT semen [99], was consistent with previous studies where the extended fresh semen was used [85,100,101]. It was demonstrated that both transuterine and transperitoneal migrations were involved in transport of sperm inseminated using DIUI to reach the other side of the oviduct [85]. Nonetheless, comparing between techniques, fertilization rate in the IUI group was significantly higher than the DIUI group. The reason for this finding might not associate with the insemination techniques, but rather it was a result of insemination time relative to the moment of ovulation which was not appropriate in the DIUI group (≥ 8 h before ovulation).

After AI using the same procedures (IUI and DIUI) and same numbers of FT sperm (1 to 2×10^9 per dose), acceptable fertility (67% FR and 7.7 to 10.5 LS) were obtained in both groups ($P > 0.05$); however, TB in the DIUI group was about 3 piglets fewer than the IUI group. This was probably the consequence of inadequate numbers of functional sperm used for DIUI (400×10^6 motile sperm) which led to the unilateral and/or incomplete bilateral fertilization and resulted in the low LS [102] (Table 2)

	Insemination procedure	
	IUI	DIUI
No. of sows	9	9
Parity number (mean \pm SD)	5.0 \pm 1.9	4.8 \pm 1.9
Weaning to estrus interval (days) (mean \pm SD)	4.9 \pm 0.9	5.1 \pm 1.5
Sows inseminated within 6 h before/after ovulation (%)	8/9 (89)	9/9 (100)
Non-return rate at 24 days (%)	8/9 (89)	6/9 (67)
Sows return-to-estrus after 24 days (%)	2/8 (25)	0 (0)
Farrowing rate (%)	6/9 (67)	6/9 (67)
Number of total piglets born per litter (mean \pm SD)	10.5 \pm 2.9	7.7 \pm 3.0
Number of piglets born alive per litter (mean \pm SD)	9.5 \pm 3.0	7.5 \pm 3.0

Table 2. Non-return rate, farrowing rate, number of total piglets born per litter and number of piglets born alive per litter after intra-uterine insemination (IUI) and deep intra-uterine insemination (DIUI) with frozen-thawed boar semen [102]

According to the results of our studies, it could be indicated that timing of insemination in relation to ovulation and sperm numbers per insemination dose are important factors for successful insemination regardless of insemination procedures and types of semen used. The time of insemination factor becomes more essential when using FT semen because the life span of FT sperm in the female reproductive tract is relatively short compared with the fresh cells, i.e. 4 to 8 h vs about 24 h after insemination, respectively [103,104]. It has been demonstrated that the number of sperm per insemination dose is related to both the number of functional sperm colonized in the oviductal sperm reservoir and fertilization rate [49,101]. Insufficient sperm numbers in the DIUI group might account for the lower fertilization rate [99] and thus smaller LS [102].

16. Conclusion

The feed supplement containing the rich of PUFAs, vitamins and minerals can improve the sperm motility, vitality and number of sperm per ejaculation in boar. The success of feed supplement depends on the initial performance of the boar. They may not improve the semen quality if the boars are the good performance of semen producers. Moreover, taking all of our researches, we can conclude that the production of cryopreserved boar semen and AI with FT boar semen could be successfully performed in Thailand and its application in commercial farm is undergoing. An IUI procedure was considered to be suitable for FT boar semen to produce acceptable fertility rates. This is very useful for the conservation and/or production of animal with high genetic merits.

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