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Recent advances in preservation of boar semen in liquid state: An overview

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Abstract
Current century of artificial insemination (AI) of female pigs, knowledge about the preservation of boar semen is still very limited. In pig liquid semen preservation was mainly done at 15-20 ˚C for 5 days. The short-term and long-term extenders are used according to the time required for AI. In short-term extender both viability and fertility of semen was restored whether in long-term storage although the viability of semen was maintain but the fertility was extremely reduced with the time of preservation. The present study would try to give an overview about the preservation technique of boar semen in terms of extender, preservation time, preservation temperature, viability and fertility.

Keywords: Boar, semen preservation, method, extender, fertility

Introduction
Pigs are considered as scavenging animal and that the underprivileged are involved in pig production. Pig keeping is important in north-eastern states of India and particularly for the tribal communities [1]. Pig occupies a unique role among the meat-producing animals of the NE region. It is an animal of choice for meat especially to tribal population in northeast India as pig rearing is considered to be the most encouraging and appropriate livestock enterprise to narrow down the gap between the availability and requirement of animal meat in this part of the country and it also plays an important role in improving the socio-economic status of the weaker rural community and creates employment for the unemployed youth of the region [2]. According to the 19th livestock census, pig population in India stands at 10.29 million compared to world population of 977.02 million [3] which comprises 1.05% of world's pig population. Pig consisting of 2.01% of total livestock (512.05 million) in India and contribute only 0.95% of the global share in pig production [4].
The superior quality elite boar is exploited for artificial breeding of female pigs to maximum possible extent by artificially inseminating large number of female animals merely from a single ejaculate [5]. To achieve the said goal, extender of good quality is required for extending the boar semen which plays an crucial role in improving the post thaw semen quality in terms of spermatozoa viability, motility, plasma membrane and acrosomal integrity, mitochondrial membrane potential (MMP) etc. [5]
The temperature of semen storage is an important factor in retaining the fertilizing capacity of boar spermatozoa [6]. In modern piggery industry, the artificial insemination (AI) of sows is mostly based on liquid cooled semen [7, 8]. For preservation of liquid boar semen, the temperature 16-20 °C is still common practice as sperm cryopreservation remains suboptimal in this species [9]. To preserve spermatozoa for prolonged periods, their metabolic activity needs to be reduced by diluting it in an appropriate medium and lowering the temperature [8]. As boar spermatozoa very sensitive to cold shock, the boar sperm alters the viability (10). Storage time lower than 72 h, it is preferable to use a short-term extender and more than 4 days is recommended to use a long-term extender on a higher sperm concentration to compensate for reduced sperm viability due to ageing [11]. Therefore, an assessment of integrity of the sperm membrane of diluted semen is important for determining which extender to use and how long the semen can be stored [12].

Preserving of Boar Semen in Liquid State

Boar spermatozoa are particularly sensitive to cold shock, which induces changes in the plasma membranes of spermatozoa, resulting in a reduction in their biological properties [13]. The effect of cold shock on spermatozoa is dependent on the rate of their cooling, the time and temperature range of storage, as well as the type of applied extender (14). Extended boar semen is cooled to between 15° and 18°C and is maintained at this temperature for several days until use for AI [8, 15, 16]. Semen was preserved in 1.5 ml micro-centrifuge tubes at 15℃ in BOD incubator up to 72 h [17].

Extender Used for Preservation of Boar Semen in Liquid State

The fertilizing capacity of the stored boar semen depends mainly on fresh semen quality, spermatozoa dose used for insemination, and composition of extender [18]. The progressive motility is the most commonly used indicator of both viability and fertility of boar semen, though a high level of motility does not guarantee better reproduction results [7, 19]. Spermatozoa maintain their metabolism and motility, principally through glycolytic pathways [20]. Any extender must contain an energy source, which is often glucose, as other sources such as galactose, ribose and trehalose have been reported to give worse results [11]. Different combinations of sugars (glucose) and non-sugars energy substrates (citrate and lactate) during a 7-day period of storage at 17 ºC give suitable results [21]. A practical level and for current production purposes, diluents can be divided into two major groups: Diluents for short-term preservation (1-3 days) and diluents for long-term preservation (over 4 days). For example of short-term diluents are- Beltsville Liquid (BL-1), Beltsville Thawing Solution (BTS), Illinois Variable Temperature (IVT), Kiev, Vital etc. The long-term diluents are- Acromax, Androhep, Modena, MR-A, Mullberry III, Reading, X-cell, Zorlesco, ZORPVA etc. [8]. The first of the so-called long-term diluents was the Zorlesco medium (Gottardi*et al*., 1980).KOftOWO-3 extender was mostly used extender in Poland [22].

Many extenders are formulating to enhance preservation capabilities by providing compounds to protect the spermatozoa against cold shock [6]. Three different commercial extenders devised for short-term (BTS+) or long-term preservation (MR-A and X-Cell), were used to test the storage of semen from four mature, fertile boars at 17 ˚C for 96 hours [9]. The Safe cellplus extender is better than BTS to preserve boar semen in the liquid state at 16-18 °C in Large White Yorkshire boars [23].

Additives Used for Preservation of Boar Semen in Liquid State

Additives which impart anti-oxidant, sperm membrane stabilization properties and sperm motility enhancer are deliberately used with semen extender for better preserving quality. A number of additives have been studied over the past few years and their effect on improving the quality of boar semen. The additives like antioxidants, sugars, cholesterol and proteins, are effective for improving the fertilizing ability even in post thaw stage [5].

Antioxidants are the agents, which break the oxidative chain reaction-eliminating, taking up, or reducing the formation of ROS (24) and thereby reduce the oxidative stress (25, 26). Based on their chemical property, antioxidants may also be categorized as enzymatic (e.g. glutathione reductase or, GSH, SOD, and catalase), and non-enzymatic (e.g. vitamins C, E, and B, carotenoids, carnitine, cysteines, pentoxifylline, metals, taurine, hypotaurine, and albumin) [24]. BSA was included in the extenders as they maintain sperm motility, membrane integrity and reproductive performances [27].

Funahashia and Sano [28] reported that addition of glutathione and cysteine can improve the viability and functional status of boar spermatozoa during liquid preservation and boar spermatozoa penetrated *in vitro* even after preservation in the presence of cysteine at 10 ˚C for 29 days.

Polyunsaturated fatty acids and antioxidants supplementation of boars had a beneficial effect on the biological characteristics of the spermatozoa, which could be useful for semen preservation at different temperatures (29). Heparin induces *in vitro* capacitation changes in the boar spermatozoa as evident by highest hyper activation of spermatozoa at 4 hours of incubation in crossbred Hampshire boar as reported by Haque *et al.* [30] and Talukdar *et al*. [31]. Addition of lipoprotein fractions in semen extender enhanced cold shock resistance of boar spermatozoa, particularly during storage at 5 °C [7].

Temperature and Storage of Preserved Liquid Boar Semen

Buhr *et al*. [32] stored boar semen at 25 ˚C for 160 minutes. Strzezek *et al*. [29] had preserved the Hampshire boar semen diluted with Kortowo-3 extender at 5 ˚C and 16˚C for 5-6 days. Althouse *et al.* [6] preserved the boar semen after diluting with Androhep extender at 5 different storage temperatures of 8, 10, 12, 14 and 17 ˚C for 48 hours. Perez *et al.* [33] incubated boar semen in a water bath at 37 ˚C for 2 hours. Kumaresan *et al*. (34) have used BTS for preservation of Hampshire boar semen at 18˚C up to 96 hours. Kanshi *et al*. [35, 36] have used Lactose egg yolk (LEY) dilutor for preservation of boar spermatozo at 15˚C in a BOD incubator upto 96 hours. Khan and Kumar [37] have used BTS solution for boar semen preservation at 18 ˚C up to 120 hours in 24 mature fertile boars of four different genetic groups (HS, CB-50%, CB-75% and CB-87.5%). The BTS, Kiev and Modena extender was used by Gobindasamy *et al*. (38) for preservation of Hampshire boar semen at 17˚C up to 5 days. Patra *et al*. (39) preserved the Large White Yorkshire, Tamworth and T & D (cross of Tamworth and Desi) semen up to 72 hours by using BOD incubator. By using Modena extender, Chutia *et al*. [17] have used GEPS extender for preservation of boar semen at 15 ˚C for 72 hours. Reddy *et al*. [40] have used Farmer friendly Boar Semen Extender (FBSE) stored at refrigerator temperature (5-8 ˚C) and Beltsville Thawing Solution (BTS) stored at BOD incubator (15-18 ˚C) for 24 hours of Large White Yorkshire boar semen. Karunakaran *et al*. [23] preserved the semen of Large White Yorkshire boar by using BTS and SAFE cell plus extender at 16-18˚C over a period of 7 days. Haque *et al*. [41] preserved the Hampshire boar semen at 15 ˚C up to 120 hours.

Fertility of Preserved Liquid Boar Semen

We should consider that the relationship between semen quality (which the diluent preserves) and resultant fertility of semen [11]. While comparing between the diluents used for short-term and long-term, there is no significant differences in fertility or litter size in the first 3-4 days of storage [42]. The Kiev extender provides better fertility than BL-1 after 1 day (74.5% vs 64.7%) or 3 days (65.9 vs. 60.5%) of storage [43]. The fertilization rate with Androhep and control was 82% and 81% respectively [16]. Frangez *et al*. [44] reported that there is

no significant difference in fertility rate between BTS and Merck extender. The diluent MR-A was able to maintain the same fertility rate and number of live births using semen stored for up to 5 days as reported by Martinez *et al.* [45]. Subsequently, similar fertility rate was found when compared the diluents BTS, MR-A and Modena [18]. No difference was recorded in terms of fertility when compared the diluent Androhep, BW25, or Kiev stored for 3 or 5 days [46]. The diluents MR-A, BTS, Modena and Androhep in comparison were found that there are no significant differences in fertility neither among the diluents nor according to the time of preservation (1-2 days vs. 3-4 days) as reported by Laforest and Allard [47].

Conclusion

After almost a century of artificial insemination in swine, knowledge about the preservation of boar semen is still very limited. The demands for future studies should be take place with the following directions. The design of new diluents has been based on an empirical model, and the new models are needed to be evaluated and optimize with its components. There is a possibility of preservation of boar semen at temperatures below than 15℃ would help to reduce the metabolic activity and protect against the detrimental effects of microbial contamination. Deep intrauterine insemination technique would change the scenario of preservation such that very less spermatozoa per dose and reduced insemination volume could require new preservation conditions and better diluent need to be assessed. The choice of diluent should depend on its proposed use. If the time of collection and insemination is less than 3 days then short-term diluent preferred or if more than 4days then long-term diluent is mostly the choice. But the precaution will be taking care that any loss of sperm viability, fertility and litter size should not affect much every possible effort should be made to ensure the best semen quality. So the choice of dilution should be always aimed to optimize the subsequent effects in a particular condition of each farm.

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