Artificial insemination in sows using Guelph and two local semen extenders

D.O. Umesiobi, M.U. Iloeje and N.A. Berepubo¹

Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria

¹*Department of Animal Science, Rivers State University of Science and Technology, Port Harcourt, Nigeria*

Abstract

Sixty two and half-year-old Large White sows and 12 boars of the same breed and age were used to study the comparative effects of conventional (Guelph) and local (coconut water and fresh raphia palm wine) extenders and storage (3 days) on the fertility of artificially inseminated sows. Semen was collected twice a week by gloved-hand method. Semen was either extended or unextended and evaluated on the day of collection (day 0) and during day 3 of storage for percentage sperm motility, acrosome morphology and fertility (Non-return rate, farrowing rate and litter size). Both Guelph and local extenders produced significant effects ($P < 0.01$) on boar semen characteristics and fertility. Fresh semen extended in Guelph extender had the highest percentage sperm motility (86.2%) whereas fresh semen extended with coconut water gave the highest normal apical ridge (NAR) (91%); farrowing rate (88.3%). Litter size (7.1 piglets/litter) and number of young born alive (5.8), with the highest number of non return to service sows. Guelph extended semen ranked second in influencing fertility in sows.

Keywords: Artificial insemination, semen extenders, sperm viability, sow fertility

Introduction

Since the development of appropriate buffer media from phosphates (Phillips, 1939), Sodium citrate (Salisbury and VanDemark, 1961), Carbon dioxide bicarbonate (VanDemark and Sharma, 1957) and trisbuffers (Foote, 1970) little development of other buffers and extenders for preservation of semen has been reported. Rather, attempts have been made to improve these basic buffers by the inclusion of various additive such as gelatin, peptone, glycerol, glycerine, glucose, honey, coconut milk, egg yolk and other materials.

Also, the discovery that semen could be successfully stored in extenders containing glycerol diverted researchers from studying the protective properties and effect of some undefined materials, including coconut water and raphia palm wine.

In Nigeria, for instance, the use of artificial insemination (AI) for genetic improvement of animals, especially pigs has suffered severe setbacks. This is partly due to the inability of unextended boar semen to store for more than 1 day without a significant reduction in fertility (Walters and Hooper, 1990) and the

incapacitating inflationary cost of conventional semen extenders which is beyond the affordability of Nigerian local farmers.

The purpose of this study was to ascertain the comparative effects of a conventional (guelph) and local extenders (coconut water and fresh raphia palm wine) on the fertility of boar semen based on progressive sperm motility, acrosome morphology, non-return rate, farrowing rate and litter size.

Materials and methods

The composition and pH of buffers and extenders is presented in Table 1. In this study, sixty two and half-year-old Large White sows were used for AI. The sows were part of the pigs used in routine. AI service in the Federal University of Technology Research Farm, Owerri, Nigeria.

Selection of Boars: Semen from 12 Large White boars was evaluated for storability by test-storing three different ejaculates from each boar. Acrosome morphology and percentage progressively motile sperm cells were the criteria used for evaluation. Semen from 10 boars was acceptable (minimum of 45% normal

apical ridge (NAR) and 35% sperm cell motility). Ten out of the 12 boars were finally selected for use. All boars were used routinely for AI research project and were assigned to a twice-a-week collection schedule.

Semen collection and evaluation. Semen was collected twice a week by the gloved-hand method into pre-sperm, sperm-rich and sperm poor fractions. The gel mass was separated immediately by filtration through double-layered cheese cloth. A part of the extended semen (at a ratio of 1:5 semen to diluents) sample was used for spot estimate of sperm motility on a warm stage at X400 base contrast microscope. Motility was evaluated microscopically in two ways:

- (1) as a percentage of progressively moving sperm and
- (2) by type of movement, graded on a scale of 0 to 9 (Boender, 1977). The remainder of the semen samples from each hour were diluted in individual insemination plastic bottles containing 100ml of extended semen (2×10^9 spermatozoa).

Table 1 *Composition of the various boar extenders for the experiment*

Ingredients	Unextended semen	Guelph extender	Coconut water extender	Raphia palm wine extender
Buffers				
Sodium hydrogen carbonate (g)	-	1.2	-	-
Distilled water, final vol. (ml)	100	-	100	100
Trisodium citrate (g)	-	3.7	-	-
Ethylene diamine tetracetic acid (g)	-	3.7	-	-
Glucose (g)	-	6.0	-	-
Extenders				
Buffer, % by vol.	10	10	10	10
Guelph, % by vol.	-	100	-	-
Coconut water, % by vol.	-	-	100	-
Raphia palm wine, % by vol.	-	-	-	100
Penicillin, units/ml	1000	1000	1000	1000
Streptomycin, mg/ml.	1000	1000	1000	1000
pHs of extenders	7.4	7.0	7.7	7.6

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Stored semen evaluation

An aliquot (50ml) of semen from each boar was removed for 3 days storage. The remainder (150ml) of the ejaculate was diluted with local extenders (2×10^9 spermatozoa - 100ml/inseminate) and used for routine AI. The 50ml semen taken for storage from each boar was maintained at refrigeration temperature (5°C) for 3 days. After 3 days of storage, each semen sample was removed, warmed (20°C and evaluated for sperm motility. Minimum requirements for keeping the stored semen were 35% progressively motile sperm, otherwise all the sorted semen from that ejaculate was discarded. Sperm motility was estimated microscopically on a warm stage at 400x. Sperm motility was graded in increment of 1 on a scale of 0 to 10 (convertible to 0 to 100%) and the type of movement on a scale of 0 to 9 as described by Boender (1977). Acrosome morphology was determined as described by Foote (1970). Acrosome were rated by morphological damage and placed into the following categories: normal apical ridge (NAR), damaged apical ridge (DAR), missing apical ridge (MAR) and loose acrosomal cap (LAC) as described by Pursel *et al.* (1972). If after 3 days, any stored sample from each boar did not have at least 45% NAR and 35% motile sperm, all stored semen from that boar was discarded.

Artificial insemination

Sows to be inseminated were assigned at random across 6 treatments to the various boars with 2 replicates for each treatment.

Since the acceptance of the boar was the best indicator of oestrus in sows (Glossop, 1991), and oestrus expectancy list, based on previous oestrus of 21 days was used in detecting sows in oestrus. Sows in oestrus were detected by either exerting pressure on the sacrolumber region or by sitting in a riding position on the sow's hindquarters. Sows were inseminated 12 to 24 hours after onset of the first oestrous after

weaning. A long catheter (Polyanstey and Podbereznyi, 1990) was used for all the inseminations. A 100-ml of diluted semen was warmed to about 20°C and then injected slowly as far as possible into the cervix, with a pad of cotton inserted into the vagina so as to prevent the semen from flowing back. Inseminations started on April 15, 1996 and ended on May 20, 1999).

Statistical evaluation

Data were analysed using a 3 x 2 factorial experiment in a completely randomized design and fertility estimates were tested by Chi-square analysis (Snedecor and Cochran, 1980). Other variables were analysed by the Analysis of Variance procedure of the Statistical Analysis System (Snedecor and Cochran, 1980).

Results and discussion

The effects of Guelph and local extenders on quality of fresh or stored boar semen and fertility of sows are summarized in Tables 2 and 3 respectively. Percentage of motile spermatozoa, type of sperm movement graded on a scale of 0.9 and acrosome morphology (Table 2) slightly differed between the various fresh extended and stored unextended semen ($P < 0.05$).

After the 3 day storage at 5°C, values for motile sperm and type of sperm movement were significantly ($P < 0.01$) higher for semen extended in Guelph extender than for those extended in coconut water and raphia palm wine. The presence of some important buffers such as glucose and Trisodium citrate in Guelph extender may be responsible for the maintenance of motility in Guelph extended semen. Acrosome morphology differed significantly, with coconut water extended semen exhibiting the highest NAR (58%). Unextended semen sample produced the least NAR (46%). Organic acids, reducing sugars and vitamins in coconut water (Opeke, 1992)

Table 2 *Effects of extenders on stored boar semen quality*

Item	FRESH SEMEN				STORED SEMEN (3 DAYS)			
	Unextended semen	Extended semen	Guelph	Coconut water	Raphia palm wine	Unextended semen	Extended semen	Semen
<i>Sperm motility</i>								
Motility, %	60.8 ^a	86.2 ^b	80.5 ^b	78 ^b	45.5 ^{ab}	64.5 ^d	63.7 ^e	60.3 ^f
Type of Movement, 0 to 9	5 ^a	8 ^b	7.3 ^b	7 ^b	3.5 ^{ac}	8 ^{dc}	7.5 ^{dc}	7.2 ^c
<i>Acrosome morphology</i>								
NAR, %	80 ^a	90 ^b	91 ^b	89 ^b	46 ^{ac}	55 ^e	48 ^e	50 ^{af}
DAR, %	10 ^a	5 ^b	4 ^b	8 ^c	20 ^d	14 ^c	13 ^b	16 ^{af}
MAR, %	1 ^a	2 ^b	1 ^a	1 ^a	17 ^b	14 ^c	14 ^c	15 ^c
LAC, %	9 ^a	2 ^b	4 ^b	2 ^b	16 ^c	14 ^c	14 ^c	19 ^d

Figures differently superscripted within a row are significantly different ($P < 0.050$)

Table 3 *Effect of extenders on the fertility of sows artificially inseminated with fresh or stored boar semen*

Item	FRESH SEMEN				STORED SEMEN (3 DAYS)			
	Unextended fresh semen	Extended fresh semen	Guelph extender	Coconut water extender	Raphia palm wine extender	Unextended stored semen	Extended stored semen	Guelph extender
Nonreturn rate, %	56.0 ^a	32.4 ^b	20.50 ^c	40.7 ^d	85.0 ^e	68.0 ^f	29.25 ^{cg}	81.0 ^{ah}
Farrowing rate, %	49.20 ^a	70.0 ^b	88.30 ^c	60.37 ^d	16.75 ^e	50.0 ^{af}	87.50 ^{cg}	28.25 ^h
Litter size, No./litter	6.5 ^a	5.0 ^b	7.1 ^c	4.6 ^{bd}	1.25 ^e	3.50 ^f	5.25 ^{ag}	2.0 ^{eh}
Total piglets	3.5 ^a	4.0 ^b	5.8 ^c	2.6 ^d	1.0 ^e	3.50 ^{af}	5.50 ^g	1.50 ^{eh}
Live piglets	3.0 ^a	4.0 ^b	1.3 ^c	2.0 ^c	0.25 ^e	0 ^f	0.74 ^g	0.50 ^h
Dead piglets								

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have been implicated to be responsible for the protection of spermatozoa against cold shock. This is accomplished by minimizing the loss of lipids from the sperm during storage. (Foote, 1970; Evans and Ko, 1990).

The fertility estimates of sows artificially inseminated with fresh or stored boar semen is presented in Table 3. Both Guelph and the two local extenders significantly ($P < 0.05$) affected the nonreturn rate (NRR) while the fresh extended semen producing a lower NRR. This result is in agreement with earlier reports by Umesubi (2000) who observed that decreases in nonreturn rate of sows inseminated with fresh extended semen were not only due to the protective effects of the chemical components of the extenders, but also as a result of the physical characteristics of the fluid.

Coconut water extended semen gave the lowest NRR and the highest proportion of pigs farrowing in both the fresh and stored semen samples. It is therefore, probable that early embryonic mortality which was reported to occur due to such factors as time of insemination, chromosomal/genetic imbalance, uterine environment, and nutritional deficiencies (Reuse, 1990, Flowers and Alhusen, 1992) were prevented through the beneficial effects of essential nutrients in coconut water, especially the reducing sugars and vitamins (Glossop, 1991), hence the survival of spermatozoa *in vitro*.

Farrowing rate was significantly ($P < 0.05$) affected by extenders used. However, the farrowing rates of sows inseminated with stored extended semen were well above the minimum requirement as outlined by Reuse (1990). Sows inseminated with coconut water extended semen had the highest farrowing rate from both fresh and stored semen. The ability of the sperm cells to survive both during storage and during sperm capacitating in the sow's reproductive tract are good indications that the

chemical constituents (organic acids, reducing sugars and vitamins) of coconut water enhance fertility.

Sows inseminated with coconut water extended semen (both fresh and stored) farrowed the highest number of piglets and the greatest proportion born alive ($P < 0.05$). Since boar semen extended in coconut water capable of producing the highest farrowing rate in the artificially inseminated sows, it is reasonable to suggest that the largest litter size farrowed by the sows is a good indication of the functional integrity of the chemical nutrients (organic acids, reducing sugars and vitamins) contained in coconut water. However, embryonic mortality has been reported as one of the major factors that influence litter size. According to Summermatter and Schawaldner (1990), approximately 30% of the ova are not presented by live embryos on the 25th day old gestation and an additional 15% decrease in litter size occurs when based on the number of living young farrowed. Other factors known to affect litter size are age, parity, and inherent reproductive ability (Lai *et al.*, 1993).

Conclusion

The results show that fresh or stored extended boar semen is suitable for use in AI services. This conclusion rests on the observation that fresh or stored extended semen produced a higher farrowing rate and a larger average litter size (about eight piglets) than those of fresh or stored unextended control semen ($P < 0.01$). Overall motility and sperm acrosome morphology results produced by fresh or stored extended semen were well above the minimum requirements 45% NAR and 35% sperm cell motility.

The presence of some vital buffers such as glucose and trisodium citrate in Guelph extender has been implicated to be responsible for the highest sperm motility noticed in Guelph extended semen, whereas the highest

NAR, farrowing rate and number of young born alive obtained in sows artificially inseminated with coconut water were suggested to be as a result of the cushioning effects of organic acids; reducing sugars and vitamins found in coconut water.

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