African Journal of Biotechnology Vol. 8 (8), pp. 1984-1987, 20 April, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Motility and fertilizing capacity of boar semen stored in raffia palm (*Raffia hookeri*) sap extender at 15°C

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Accepted 24 February, 2009

Two separate experiments were conducted to test the ability of new semen extenders containing various levels of tropical raffia palm sap in sustaining the motility and fertilizing capacity of boar spermatozoa during storage at 15°C. Highly significant variations (P < 0.01) occurred in the ability of extenders to sustain progressive motility from days 1 to 4 of storage. One of the new extenders, raffia palm extender 2 (RPE–2) was superior to other trial extenders including the control (coconut milk extender, CME) in sustaining progressive motility of boar sperm from 24 - 72 h of storage; average motility scores at 24 and 72 h were 73.0 and 50.0% for RPE-2, 65.0 and 41.0% for CME (control), 55.0 and 36.0% for RPE-1 and 45.0 and 6.5% for RPE-0. Sperm tail vibratory movement was observed in sperm stored in RPE-1 and RPE-0 from day 2 of storage. Sperm in RPE-0 and RPE-1 lost motility completely at 96 h while motility of sperm in RPE-2 showed a sharp drop in motility values from 72 – 96 h relative to control. Average conception rates in 24 gilts inseminated per treatment with semen stored for 24, 48 and 72 h respectively were 83.3, 66.6 and 16.6% for RPE-2, 50, 16.6 and 0.0 percent for RPE-1, 16.6, 0.0 and 0.0% for RPE-0 and 75.0, 50.0 and 26.6% for CME (control). Average number of piglets born using semen stored for 24, 48 and 72 h respectively were 8.0, 8.0 and 6.5 for RPE-2 and 8.2, 8.2 and 6.0 for the control. RPE-2 supported better fetal survival rate than other trial extenders and control. Highly significant variations (P < 0.01) occurred among extenders in conception rate and number of piglets born. These results portray RPE-2 as a reliable short-term liquid semen storage medium for swine artificial insemination in the humid tropics.

Key words: Motility, fertilising capacity, boar semen, raffia palm, extender.

INTRODUCTION

The main objective of semen preservation is to extend the usefulness of genetically superior males for purposes of expanding the genetic base of breed, repopulating/ recreating breeds or lines and discovering new gene (Waberski et al., 1994; Purdy et al., 2008). This is usually optimized by maximizing the number of doses of semen obtainable from a given ejaculate, without reducing fertility and by extending the fertile life of the doses to facilitate their effective use in breeding (Foote, 1974; Saacke, 1983). The ideal semen preservation media would combine the important properties of nourishing the spermatozoa and protecting them from the stress of dilution and low temperature storage aimed at extending their viability and fertility (Johnson et al., 1988; Machaty et al., 1992; Weitze and Petzoldt, 1992). Thus normal liquid semen preservation medium should contain a buffering component (which may be inorganic or organic) to guard against pH fluctuations in stored semen, the basal nutritive component to supply metabolizable substrate, the antimicrobial agent and substances that would protect sperm from cold shock and effect of dilution (Norman, 1964; Igboeli, 1970; Pursel et al., 1973). Boar sperm unlike bull sperm rapidly loses fertilizing ability when stored for long periods in liquid form at temperatures between 15 and 20°C. As a result of this, studies are still on to identify the best of each component that would guarantee prolonged fertile life of stored boar semen and ensure widespread application of artificial

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insemination in swine production in different countries which is currently highly variable (Gadea, 2003). The present study was designed to test the ability of a new semen extender, containing tropical raffia palm sap as nutrient base, in maintaining the viability and fertilizing capacity of boar spermatozoa and to assess subsequent fetal survival rates when used after varying durations of storage.

MATERIALS AND METHODS

The raffia palm sap

The raffia palm sap is the fluid obtained by tapping the inflorescence or succulent part of stem of raffia palm (*Raffia hookeri*). The raffia palm is a type of palm found growing in swampy and semi-swampy areas of the equatorial rain forest or derived savanna. The primary purpose of tapping the sap of raffia palm is to obtain a whitish fluid which when left to stand for a few hours changes into a light alcoholic drink. Fresh palm sap is generally described as a 10 to 14% neutral sucrose solution (Okafor, 1978; Megwa 1984) containing various nutrients usually expected in typical plant sap. The biochemical constituents of fresh raffia palm sap are presented in Table 1.

Collection and processing of raffia palm sap and coconut milk

One litre of fresh raffia palm sap was collected from a local taper and immediately centrifuged at 1000 g for 5 min to sediment the yeast cells. The supernatant was transferred into a steel container, boiled for 10 min and filtered into sterile 500 ml flasks. After allowing to cool to room temperature, aliquots of 15, 10 and 5 ml of the processed sap were measured into separated sterile 100 ml volumetric flasks as basal component of the trial extenders. Other components of the trial extenders (Table 2) were respectively measured into the flasks dissolved and made up to 100 ml mark with distilled water.

For the control extender, 200 ml of coconut milk was collected from mature nuts, heated for 10 min at 92 - 95°C filtered into sterile 100 ml flasks and used in preparing the coconut milk extender (CME) according to procedures outlined by Norman (1964). All extenders were adjusted to pH 7.4 with 10% sodium hydroxide.

Experiment I: Semen storage

Semen was obtained from 4 normal and sexually mature Large White x Landrace (F_1) boars using the dummy and artificial vagina at weekly interval. Ejaculates were visually separated into sperm-rich and sperm-poor fractions but only the sperm-rich fractions used in storage experiments were assessed for volume, progressive motility and sperm concentration.

Portions of sperm-rich fraction were diluted to a high concentration of $10x10^6$ based on number of motile sperm in the fraction to facilitate easy microscopic examination. Four milliliter portions of diluted semen were stored in 5 ml glass tubes with plastic corks at 15°C for 4 days. Motility scores were used to evaluate sperm viability and scores taken immediately after dilution was regarded as scores for day 0, while experimental motility scores were recorded from days 1 to 4. Three storage trials were conducted for each extender.

Table 1. The biochemical composition of fresh raffia palm sap.

Constituent	Composition*
Total sugar (%)	10.5 (9 – 13)
Total protein (%)	0.38 (0.10 – 0.50)
Glucose (%)	1.31 (0.95 – 2.40)
Fructose (%)	1.19 (1.0 – 2.5)
Acetic acid (%)	0.24 (0.20 – 0.35)
Vitamin C (mg/100 ml)	10.60 (8.7 – 12.5)
Vitamin B_{12} (mg/100 ml)	160.0 (150 – 175)
pH	7.0 (6.5 – 7.2)

*Values in parenthesis are ranges. Source: Onyemaechi (1991).

Experiment II: Fertility trials with stored semen

In the fertility experiments, semen stored for periods described in experiment I were used. Inseminations were carried out with semen stored in various diluents for 24, 48 and 72 h. For each storage period, 24 gilts with consistent cycling characteristics weighing between 75 and 90 kg were used. They were sorted into 4 groups of 6 gilts each to correspond with the three trial extenders and control. The estrus of the animals were synchronized by progesterone administration which was regulated in such a way that estrus of the four groups of gilts corresponded with the appropriate storage age of semen to be used. Semen used for insemination were stored in aliquots of 50 ml in corked narrow plastic vials of equivalent capacity. The sperm concentration adopted for the inseminations was $3.5x10^9$. Inseminations were carried out with a 50 ml syringe fitted with disposable Swedish swine catheta. All inseminations took place on the second day of heat. Animals that conceived were allowed to farrow based on which fetal survival rates were assessed.

All data collected were subjected to standard analysis of variance as described by Steel and Torrie (1980) using a statisgraphic computer package. Means separation was carried out by Duncan's New Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

In experiment I (Table 3) highly significant ($P < 0.01$) variations occurred in sperm motility of the new extenders and CME (control). RPE-2 containing 5 ml raffia palm sap had the highest motility values all through the storage period compared to other trial extenders and control. The differences in motility scores of RPE-2 and other new extenders (RPE-0 and RPE-1) were highly significant ($P < 0.01$) at all storage periods. The motility scores for RPE-2 and CME from 24 – 72 h of storage were higher than values obtained for various types of Tris-buffered extenders (Igboeli, 1970) for corresponding storage durations.

Boar sperm stored in RPE-0 and RPE-1 lost motility completely after 72 h while sperm stored in RPE-2 exhibited abrupt drop in motility between 72 and 96 h with average value that was statistically lower than that of control (CME). The complete loss of motility by RPE-0

Table 2. Composition of the raffia palm sap (RPE) and control (CME) extenders.

Ingredient	Amount contained in 100 ml of dilute			
	RPE-0	RPE-1	RPE-2	CME (control)
Raffia palm-sap (ml)	15.0	10.0	5.0	-
Coconut milk (ml)	-	-	-	15.0
Sodium citrate (g)	2.2	2.2	2.2	2.2
Sulphalinamide (mg)	300.0	300.0	300.0	300.0
Sodium penicillin (mg)	60.0	60.0	60.0	60.0
Dihydrostrep, sulphate (mg)	135.0	135.0	135.0	135.0
Catalase (units)	1000	1000	1000	1200
Myocostatin (Units)	1500	1500	1500	1000
Egg yolk (ml)	5.0	5.0	5.0	5.0

Table 3. Progressive motility (%) of boar semen stored in raffia palm – sap (RPE) extenders at 15°C.

Storage time (h)	Progressive motility %			
	RPE-0	RPE-1	RPE-2	CME (control)
0	74.0	73.0	75.0	75.0
24	45.0^{c}	55.0^{b}	73.0^{a}	65.0^{b}
48	27.0^{c}	49.0^{b}	63.0^{a}	59.0^{b}
72	6.5^{c}	36.0^{b}	50.0^{a}	41.0^{b}
96	0.0^{c}	0.0^{c}	10.0^{a}	5.0^{b}

$^{a, b, c}$ Means with different superscripts in a row are different ($P<0.01$).

and RPE-1 as well as the abrupt drop in motility of sperm in RPE-2 are rather not worthy and point to the relative loss of stability of these extenders in terms of buffering capacity, osmolarity regulation and probably nutrient content. In the case of RPE-0 and RPE-1, it is felt that the higher levels of raffia palm sap included may have dis-stabilized the regulatory mechanisms of the extenders at that stage of storage (72 h). Harrison et al. (1978) and Gadea (2003) had earlier suggested that alterations in the concentrations of compounds or ions (e.g. plasma proteins and K^{+}) in diluted semen may result to adverse effects on the viability of sperm therein. On the other hand, the observed sharp drop in motility of sperm stored in RPE-2 may not have been as a result of failure of the regulatory systems of the extenders but could be due to abrupt depletion of the substrate that provides energy for motility. This appears evident since 10% of the sperm stored were motile at 96 h which does not imply a faulty regulatory system. It is therefore likely that the substrate provided by 5% of raffia palm sap in RPE-2 was not sufficient to carry the high concentration of sperm stored through till end of 96 h. Further work is therefore needed to improve these aspects.

The results on conception rates (Table 4) indicate highly significant ($P < 0.01$) variations in the ability of raffia palm sap extenders and CME (control) to sustain fertility of boar sperm at various storage periods. RPE-0 and RPE-1 sustained the fertilizing capacity of boar sperm for 24 and 48 h, respectively, while RPE-2 sperm retained fertilizing capacity for 72 h. The conception rates of 83.3 and 66.6% recorded for RPE-2 in 24 and 48 h storage periods were respectively higher than 79.4% reported for BTS-diluted boar sperm (Martin et al., 1984) and 64.6% recorded by Hofmo (1991) for BTS-stored semen of similar ages. However, at 72 h storage period, our average value for conception rate (16.6%) was far lower than 50% reported in preliminary studies on boar semen storage (Dzuik and Henshaw, 1958) as well as 82.2 and 82.6% reported more recently for Kiev and MR-A extenders (Ratto and Jokinen, 1990). It is likely that what caused the drop in motility of sperm stored in this extender, as indicated earlier, may have affected their fertilizing capacity even though the motility value (50%) was appreciably high at that stage of storage.

The average number of piglets born alive (Table 5) by gilts inseminated with RPE-0 and RPE-1 stored semen aged 24 h were significantly ($P < 0.01$) lower than average values recorded for RPE-2 and CME semen of similar age. Conversely, the number of piglets born alive by gilts inseminated with RPE-2 semen aged 24, 48 and 72 h were statistically similar with values recorded for the control.

Table 4. Conception rate (%) of gilts inseminated with semen stored in raffia palm sap extender (RPE) from 24 – 72 h.

Age of semen (h)	No gilts	Conception rate (%)			
		RPE-0	RPE1	RPE-2	CME(control)
24	24	16.6[c]	50.0[b]	83.3[a]	75.3[a]
48	24	0.0[c]	16.6[c]	66.6[a]	50.0[b]
72	24	0.0[c]	0.0[c]	16.6[b]	26.6[c]

[a, b, c] Means with different superscript in a row are different (P < 0.01).

Table 5. Average number of piglets born by gilts inseminated with semen stored in raffia palm sap extender from 24 – 72 h.

Age of semen (h)	No gilts	Number of piglets born			
		RPE-0	RPE-1	RPE-2	CME (control)
24	24	5.0[b]	7.0[b]	8.0[a]	8.2[a]
48	24	0.0[c]	5.0[b]	8.0[a]	8.2[a]
72	24	0.0[c]	0.0[c]	6.5[a]	6.0[a]

[a, b, c] Means with different superscripts in a row are different (P<0.01).

Based on these results, it can therefore be said that RPE-2 was better than other raffia palm sap extenders in sustaining higher fetal survival rates at various durations of storage. Although the numbers of piglets born alive in the present study were lower than average values reported by Kiev and MR-A extenders in previous studies (Ratto and Jokinen, 1990), the present results appear satisfactory for pigs reared in the humid tropics where exogenous and endogenous heat load are known to exert negative influence on feed utilization, reproductive efficiency and growth performance of pigs (Steinbach, 1985). In conclusion therefore, the observed satisfactory ability of RPE-2 to sustain boar sperm viability and fertility for 72 h, indicate its reliability as a short-term liquid semen diluent for swine artificial insemination in the humid tropics.

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