FERTILIZING ABILITY OF BOAR SEMEN EXTENDED WITH GARLIC (*Allium sativum* L.) EXTRACTS

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ABSTRACT
Preservation of boar semen is associated with the production of reactive oxygen species which leads to a reduction of spermatozoa quality and decrease in fertilizing ability. The outcome of Artificial Insemination (AI) largely depends on the ability of the acrosome to penetrate the zona pellucida, for the deposition of DNA materials into the oocyte, for a successful fertilization and embryonic development. The objective of this study was to investigate the potential of Ethanolic Allium sativum Extracts (EASE) on acrosome integrity and pH of extended boar semen. Semen was collected using the gloved hand technique. Aliquot portions of semen were divided into sixty sample bottles comprising of five treatments with three replicates. T1 served as the control (Beltsville Thawing Solution (BTS) + semen) in ratio 1:3 semen-extender, while other treatments T2 to T5 contained semen-extender at the same ratio, but supplemented with EASE at varying concentrations of 50, 75, 100 and 125 µg/L respectively in a completely randomized design. Extended semen samples were stored at 17 °C . Data obtained were subjected to assess relationship between acrosome integrity and pH and one way ANOVA to evaluate extract effects.
Results suggest a strong and negative relationship between pH and acrosome integrity. Also, supplementation of BTS with EASE at 125 µg/L maintained the acrosome integrity of the spermatozoa compared to control.
Keywords: Acrosome Integrity, Boar semen, Ethanolic *Allium sativum* Extracts (EASE), semen quality, spermatozoa fertilizing potential.

INTRODUCTION
The outcome of Artificial Insemination (AI) largely depends on the semen quality and the insemination procedure. The fertilizing potential of a semen dose is inherently linked to the quality of the spermatozoa itself (Tsakmakidis *et al.*, 2010). But during preservation after collection, due to fluctuation and changes in temperature and other metabolic activities, the fertilizing potential, acrosome integrity inclusively of the spermatozoa is altered or impaired. The quality of boar semen stored in a liquid state is influenced by the type of extender and storage time (Johnson *et al.*, 2000). Currently, many different diluents are used for the storage of semen, which help maintain the viability of the spermatozoa for several days (Gadea, 2003). Research has shown that short and medium-term extenders (up to 5 days), as early as on the second day of storage, spermatozoa motility and the percentage of normal spermatozoa morphology decrease and DNA disintegration increases, which makes them unsuitable for insemination (Klimont *et al.*, 2015). It is well-known that for *in vitro* semen preservation, a good extender should also provide an environment that inhibits the formation of harmful reactive oxygen species (ROS) or lipid peroxide (Orok *et al*., 2010) and cold shock. Boar spermatozoa are very sensitive to cold-shocked temperature resulting in reduced survivability during preservation (Gączarzewicz *et al.*, 2015). The fertility enhancing capacity of plant extract has been reported in numerous studies. Plant derived antioxidants have been getting major focus due to their lower cytotoxicity and are considered to be better than synthetic antioxidants (Gupta and Sharma, 2006 and Sen *et al*., 2010).
Garlic (*Allium sativum*) has been shown to have varied biological properties, including anti-carcinogenic, anti-thrombotic, anti-microbial, anti-inflammatory, anti-atherosclerotic, anti-oxidative, and various other biological actions. Potential antioxidant properties of garlic are as a result of presence of phenolic and flavonoids fractions (Miller *et al*., 2000).
Hence, the study to investigate the effect of garlic (*Allium sativum*) on the acrosome integrity of extended boar semen.

MATERIALS AND METHODS

Experimental Location

Semen collection was carried out at the Piggery Unit of the Teaching and Research Farm, University of Ibadan (7º20'N, 3º50'E; 200 - 300 above sea level), while the analyses of semen were carried out at the Animal Physiology and Bioclimatology Laboratories of the Department of Animal Science of the same institution.

Management of the Boar and Semen collection

Semen was collected from a mature boar with a proven semen quality and fertility for this study. The boar was kept in an intensively managed, clean and well ventilated pen. Feed and clean water were supplied *ad libitum.* Semen was collected into a boar semen collection cup; lined with a disposable plastic bag and covered with a disposable milk filter. The gel-fraction was separated from the sperm-rich fraction by the milk filter covering the semen collection cup. The gloved-hand technique/method was used to collect semen from the boar. Before the ejaculate collection, the boar was thoroughly washed to eliminate urine and other materials that could lead to semen contamination during collection. As the boar initiates extension of the penis on a standing heat sow, the end of the penis, which is cork screw shaped was held strongly by gloved hand. Collection procedure started with strong force to the coiled part of the penis by hand. Collection continued until the boar dismounted.

Preparation of Extracts from Garlic *(Allium sativum L.)*Bulb

Fresh garlic bulbs were purchased from a location, about 2 km from the study site. The fresh garlic bulbs were peeled and 150g was weighed out and fully chopped to increase its surface area, then oven dried at 45 ºC, for seven days and later ground into a powdery form using a ceramic mortar. Ethanol (absolute) was used for the extraction using a Sohxlet extractor. Rotary evaporator was further used to obtain a concentrated garlic extracts. Afterwards, the garlic extracts was dried up in an incubator at 45 ºC for 24 hours. The garlic extracts was recovered and reconstituted using dimethyl sulfoxide (DMSO) and stored (4 ºC) for subsequent use.

Semen Processing

Semen samples collected were immediately evaluated for mass activity, progressive motility, liveability, percentage normal spermatozoa, pH and the temperature was checked 15 minutes after collection. The volume of collected ejaculate was estimated by weighing on a top loader balance. Semen aliquots were diluted in each of experimental extenders (treatments). Diluted semen was preserved in a thermo regulated fridge at 17 ºC and evaluated for acrosome integrity.

Acrosome integrity assessment:

Ejaculate smears of all samples were prepared for confirmation of acrosome integrity condition utilizing eosin-nigrosin stain. All the slides were assessed under oil immersion at X1000 magnification using a bright field microscope. A total of one hundred spermatozoa per slide were assessed, and normal acrosomes (spermatozoa without apical shifts) were expressed as percentages.

Experimental Treatment and Design

Aliquot portions of diluted semen were allotted to five treatments with three replicates per treatment in a completely randomized design (CRD) and spermatozoa of acrosome integrity was evaluated .

Treatment description:

Treatment 1: Semen+ Beltsville Thawing Solution (BTS®)
Treatment 2: Semen+ BTS + 50 µg/L Ethanolic *Allium sativum* Extract (EASE)
Treatment 3: Semen+ BTS + 75 µg/L EASE
Treatment 4: Semen+ BTS + 100 µg/L EASE
Treatment 5: Semen+ BTS + 125 µg/L EASE

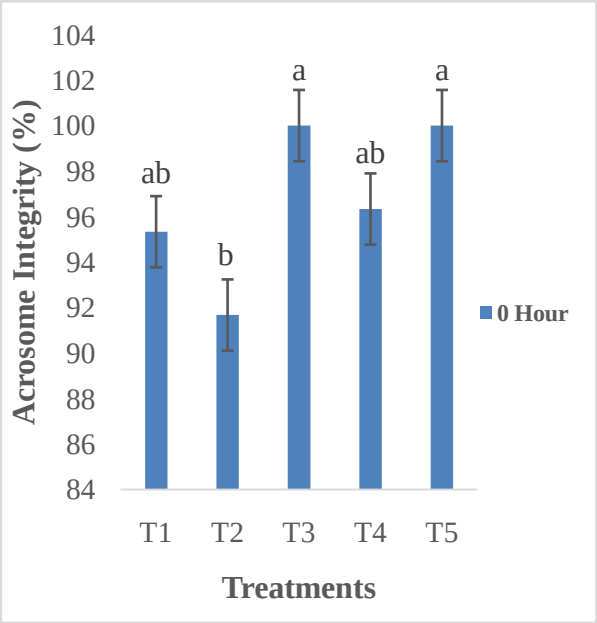
Statistical Analyses

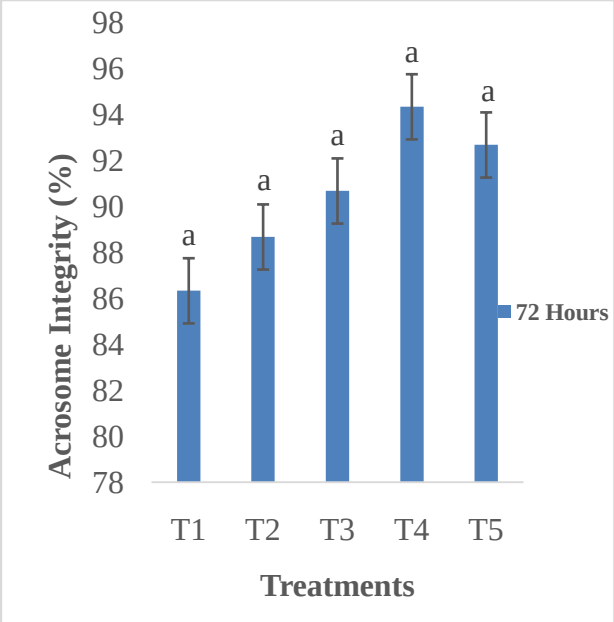
Data obtained were analyzed using one-way analysis of variance (ANOVA) procedure of SAS (2010) and means were compared using Duncan's multiple range test of the same software.

RESULTS

Table 1: Correlations between pH and Acrosome integrity

Parameter	Acrosome integrity
pH	-0.585





T1: Control; T2: 50µg EASE; T3: 75µg EASE; T4: 100µg EASE; T5: 125µg EASE; EASE: Ethanolic *Allium sativum* Extract

a,b: bars with different superscripts are significantly different (P<0.05)

Figure 1&2: Effect of EASE on acrosome integrity of extended boar semen at 0 and 72 hours respectively

DISCUSSION

Effect of EASE on Acrosome Integrity of Extended Boar Semen

Acrosome integrity of spermatozoa in extended boar semen, supplemented with EASE at 17 °C is presented in Figures 1 and 2. Significant difference (P<0.05) occurred at 0 hour. Acrosome integrity is an important parameter useful in predicting the fertilizing capacity of

spermatozoa *in vitro*. From this study, it was observed that acrosome integrity was intact across the treatments from 0 to 72 hours of observation, except for a little fluctuation in T4 at 48 hours observation. This is an indication that EASE has the capacity to maintain the integrity of the acrosome. However, acrosome integrity decline with storage time at 17 °C. This finding supports Gadea *et al.* (2005) report that reduced gluthathione could reduce acrosome integrity, improving the function and fertilizing capacity of frozen boar spermatozoa. This aligns with Orok *et al*. (2010) storage of semen for longer period causes ultra-structural, biochemical and function damage to the spermatozoa, resulting in a decline in motility, viability, fertility and impaired acrosome integrity and transport. Also, reduction in acrosome integrity will result in induced acrosome reaction of greater portion of spermatozoa before reaching the site of fertilization. This scenario is suggested in the negative relationship in pH and acrosome integrity. Reduced pH indicates more metabolic activity in the system and possibility of acrosome reaction before spermatozoa arrival at fertilization site. This finding indicates that EASE inclusion at 125µg competes favourably in preservation of the acrosome integrity better than a conventional extender (T1).

CONCLUSION

From this study, it was observed that BTS supplemented with 125 µg/L EASE, has the potential of maintaining the acrosome integrity of extended boar semen for 72 hours of preservation.

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