ANTIOXIDATIVE EFFECT OF WATERMELON (*Citrullus lanatttus)* JUICE ON MORPHOLOGY OF EXTENDED BOAR SEMEN

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ABSTRACT
Boar semen preservation is associated with the production of reactive oxygen species which leads to reduction of sperm quality and decrease in fertilizing ability. The aim of this study was to replace conventional semen extender with watermelon juice (WMJ) and to determine the most effective dilution rate that will maintain the viability of the spermatozoa over a 48-hours extension period. Semen was collected from a boar at the piggery unit of the teaching and research farm University of Ibadan, Ibadan using the glove hand method. The experiment was divided into five treatments with Beltsville Thawing Solution being replaced by WMJ at the following rate; 0, 12.5, 25, 37.5 and 50% and designated as T1, T2, T3, T4 and T5 respectively. Diluted semen samples were stored at 17^0C and were evaluated at 0, 24 and 48 hours for morphology. The experimental design was a completely randomised Design; data obtained were analysed using descriptive statistics and ANOVA.
The results showed that 50 % of BTS can be replaced by watermelon juice. However the effect of watermelon juice was only ascertained not more than 48 hours of storage. This evaluation shows that extender developed with WMJ could be an extender of choice for a reliable short term preservation in swine artificial insemination programme.
Keywords: Boar, Watermelon Juice extension, Semen quality, Spermatozoa.

INTRODUCTION
Successful swine fertility programs can be achieved using extended cooled semen when compared to those using natural service. Although the former is a routine procedure, its use is limited because sperm cells remain viable for about 3 to 5 days (Gadea *et al.*, 2005). Many studies have been carried out on carotenoids, considered an important group of natural antioxidants (Bast *et al*., 1998, Møller *et al*., 2000, Schabath *et al*., 2004 and Rao and Rao, 2007). For over a decade, lycopene, a carotenoid present in tomatoes (*Lycopersicon esculentum*) and several ripe fruits and vegetables, such as watermelon, pink grapefruit and carrots (Nguyen and Schwartz, 1999, Perkins-Veazie *et al*., 2003 and Tadmor *et al*., 2005), has been considered in some studies related with human and animal health (Bhuvaneswari and Nagini, 2005, Rao *et al*., 2006, Avci and Durak, 2007 and Bhom, 2007) and reproductive physiology (Martino *et al*., 2006, Goyal *et al*., 2007, Mangiagalli *et al*., 2007, Turk *et al*., 2007 and Mendiola *et al*., 2010). In roosters, in particular, Mangiagalli *et al*. (2010) found positive effects of lycopene addition on fertility and qualitative characteristics of semen.
With AI expanding rapidly, the demands for semen increased. The simplest way to meet this demand was to "stretch" each ejaculate further by using fewer spermatozoa per insemination, provided that this could be accomplished without sacrificing fertility.
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). It is important to evaluate the effectiveness of natural antioxidants that are readily available, such as watermelon juice in reducing the oxidative stress in post ejaculated boar semen, to evaluate the effectiveness of antioxidant in watermelon juice on the boar semen quality and determine the proportions of *watermelon juice* that is suitable for preserving boar semen quality.

RESULTS

TABLE 1: Characteristics of semen collected from Boar.

Parameters	Value
Volume (ml)	50
Progressive Motility (%)	95
Mass activity	++++
Morphology (%)	95

Characteristics of semen collected

Table 1 shows the results from the initial evaluation of boar semen before extension was done. The result reveals that the sample was suitable for extension as the values fall within acceptable ranges for high quality semen.

Table 2: Effect of water melon juice fortification on extended boar semen quality at 0 hour (Mean ±SD)

Parameters (%)	T1	T2	T3	T4	T5
Morphology	94.33±0.57^{a}	90.00±0.00^{a}	85.00±5.00^{b}	91.67±5.77^{a}	86.67±2.88^{a}

abc = mean values in the same row with different superscripts are significantly different (P<0.05); SD = Standard deviation

Effect of water melon juice fortification of boar semen quality at 0 hours

Table 2 shows the mean of morphology and of extended boar semen supplemented with graded levels of watermelon juice refrigerated at 17 ^{0}c.

There was a significant reduction (P<0.05) in morphology in treatment 3 (25 % WMJ) which gave the least mean value.

Table 3: Effect of water melon juice fortification on extended boar semen quality at 24 hours (Mean ± SD)

Parameter	T1	T2	T3	T4	T5
Morphology	87.66±2.51^{a}	87.66±0.57^{ab}	85.30±0.57^{ab}	85.30±0.00^{ab}	81.66±0.88^{b}

abc = mean values in the same row with different superscripts are significantly different (P<0.05); SD = Standard deviation.

Semen quality of extended (17 ^{0}c) boar semen fortified with water melon juice, (24 hours)

Table 3 shows the mean values of morphology of extended boar semen supplemented with graded level of watermelon juice refrigerated at 17^{0}C. There was a significant difference (P<0.05) in mean value at 24 hours. Significant reduction (P<0.05) in morphology was observed with the increase in concentration of watermelon juice extract.

Table 4: Effect of water melon juice fortification on extended boar semen quality at 48 hour (Mean ± SD)

Parameters	T1	T2	T3	T4	T5
Morphology	83.66±1.15^{a}	82.00±0.00^{ab}	80.00±0.57^{b}	80.33±0.57^{b}	79.33±1.15^{b}

abc = mean values in the same row with different superscripts are significantly different (P<0.05); SD = Standard deviation

Effect of water melon juice fortification on extended boar semen quality at 48 hours
Table 4 shows the mean values of morphology of extended boar semen supplemented with graded level of watermelon juice refrigerated at 17^0C. Significant differences in mean values was observed. It was significantly lower ($P<0.05$) in T5 (50 % WMJ).

DISCUSSION

Morphology

For sperm morphology, it was observed that significant amount of spermatozoa were normal according to Shipley *et al.* (1999) that the percentage spermatozoa should be at least 70 %. Also, Cerolini *et al*. (2000) reported that inclusion of antioxidant into storage diluents prevented deterioration of boar spermatozoa quality and provided protection to the cells up to 5 days of storage through its prevention of oxidative reduction in the levels of major polyunsaturated fatty acid.
The highest mean value recorded for spermatozoa morphology in treatment 2 can be accrued to inclusion of antioxidant as a supplement in the extender. This is in agreement with the report of Cerolini *et al*. (2000) that the inclusion of antioxidant into storage diluents prevented deterioration of boar semen quality and provided protection to cells up to 5 days of storage through its prevention of oxidative reduction in levels of major polyunsaturated fatty acid.

CONCLUSION

This study was carried out to investigate the effect of watermelon juice fortification of extended boar semen. Semen was collected from a mature landrace boar and extended with BTS (Beltsville thawing solution). The control treatment contained only BTS with a semen- extender ratio of 1 to 4, while the other treatments contain BTS with different concentrations of watermelon juice. The semen was stored using liquid storage (17^0C). After collection and assessment for odour, colour, volume, morphology and satisfactory results were obtained, the semen was added to the extender and placed in a regulated refrigerator. Semen evaluation was carried out at every 24 hours for three days. Semen characteristics evaluated include morphology. Mean value for morphology was reduced but falls within the normal range that can support fertilisation. The results obtained in the study suggest that 50 % of BTS can be replaced by watermelon juice; however the effect of WMJ anti-oxidative properties is inconclusive up to 48 hours of storage. Watermelon juice extract has proven to have potentials to supply exogenous antioxidant thus inhibiting oxidative stress condition in boar semen. Even at a high level of inclusion in extended semen, it was able to give a percentage motility that was capable of fertilising the oocyte. From this study, extended semen (17^0C) at 12.5% inclusion level of watermelon juice extract gave the best percentage in morphology throughout the experimental period because all mean values are within acceptable range of normal values indicative of good semen quality.

REFERENCES

Avci A., Durak I. 2007. Tomato and cancer. In: Durak I. (Eds.). Nutraceuticals and natural products in cardiovascular diseases, cancer and hemorrhoids. 41-46.

Bast, A. Haenan, G. R, Van Den Berg, R, and Van Den Berg H. 1998. Antioxidant effect of carotenoids. *International journal for vitamin nutrition research, 68: 3999-403.*

Bohm ,F., Tinkler, J.H., and Truscott, T.G, 2007. Carotenoids protect against cell membrane damage by nitrogen dioxide radical. *Natural Medicine.* 1: 98-99.

Bhuvaneswari V., Nagini S. 2005. Lycopene: a review of its potential as an anticancer agent. *Current Medical Chemistry. Anti-Cancer Agents*, 5: 627-635.

Cerolini, S, Maldjian, A, Surai, P and Noble, R. 2000. Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. *Animal Reproduction Science*, 58: 99-111.

Gadea J, García-Vazquez F, Matás C, Gardón JC, Cánovas S, 2005 Cooling and freezing of boar spermatozoa: supplementation of the freezing media with reduced glutathione preserves sperm function. *Journal of Andrology* 26: 396-404.

Gagnon C, Iwasaki A, De Lamirande E, Kovalski N,1991 Reactive oxygen species and human spermatozoa. *New YorkAcademy of Science*.; 637: 436-444.

Gil , J, Rodriguez-Irazoqui, M, Lundeheim,N, Soderquist, L, and Rodriguez- Martinez, H,2003. Fertility of ram semen frozen in bioexcell and used for cervical artificial insemination. *Theriogenology.*59:1157-1170.

Goyal A., Chopra M., Lwaleed B.A., Birch B., Cooper A.J, 2007. The effects of dietary lycopene supplementation on human seminal plasma. BJU International., 99: 1456-1460.

Mangiagalli M.G., Martino P.A., Smajlovic T., Guidobono Cavalchini L., Marelli S.P. 2010. Effect of lycopene on semen quality, fertility and native immunity of broiler breeder. *British Poultry Science.*, 51: 152-157.

Martino P.A., Mangiagalli M.G., Marelli S.P., Smajlovic T., Guidobono Cavalchini L. 2006. Effect of lycopene on semen bacteriological status and native immunity in roosters. *World Poultry Science Journal*., XII European Poultry Conference, Verona, Italy. 62 *Supplement*.: 415.

Mendiola J., Torres-Cantero A.M., Vioque J., Moreno-Grau J.M., Ten J., Roca M., Moreno-Grau S., Bernabeu R. 2010. A low intake of antioxidant nutrients is associated with poor semen quality in patients attending fertility clinics. *Fertility Sterility*, 93: 1128-1133.

Møller, A.P., Biard C., Blount J.D., Houston D.C., Ninni P., Saino N., Surai P.F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poultry Biology Rev*., 11: 137-159.

Nguyen M.L., Schwartz S.J. 1999. Lycopene: chemical and biological properties. *Food Technology*, 53: 38-45

Perkins-Veazie P., Collins J. K., Edwards A., Wiley E., Clevidence B. 2003. Watermelon: rich in the antioxidant lycopene. *Acta Horticulture*., 628: 663-668.

Purdy , P.H.R.,Knox, W., Singleton, K, Spencer,S.F, Spiller, T, Stewart, I, Tharp, N, Welsh, C.S, and Blackburn, H.D,2008. Preservatives on cryopreservation , quality control and artificial insemination of frozen-thawed boar sperm in a natural repository. *Symposium proceedings, Mid west Boar and Manager Conference,* August 7-8, St Louis, MO, USA.

Rao A.V., Rao L.G. 2007. Carotenoids and human health. *Pharmacology Research*., 55: 207-216.

Rao A.V., Ray M.R., Rao L.G. 2006. Lycopene. *Advanced Food Research*., 51: 99-164.

Rigau, T, Piedrafita, J, Reverter, J, Canal, M, Rodriguez-Gil, J.E,1996. The rate of l-lactate production, A feasible parameter for the fresh diluted boar semen quality analysis. *Animal Reproductive Science* 43:161-172.

Schabath M.B., Grossman H.B., Delclos G.L., Hernandez L.M., Sue Day R., Davis B.R., Lerner S.P.,Spitz M.R.,Wu X. 2004. Dietary carotenoids and genetic instability modify bladder cancer risk. *Journal of Nutrition*, 134: 3362-3369.

Shipley C.F., Clifford F., Hurtgen J.P, 1999. Breeding soundness examination of the Boar, *Swine Health Production*, 3: 117-120

Tadmor Y., King S., Levi A., Davis A., Meir A., Wasserman B., Hirschberg J., Lewinsohn E. 2005. Comparative fruit colouration in watermelon and tomato. *Food Research International.*, 38:837-841

Turk G., Atessahin A., Sonmez M., Yuce A., Ceribasi, A.O. 2007. Lycopene protects against cyclosporin A-induced testicular toxicity in rats. *Theriogenology*, 67: 778-785.

Waberski, D, Weitze, K.F, Lietmann, C, 1994. The initial fertilizing capacity of long term stored liquid boar semen following pre and post ovulatory insemination. *Theriogenology 41, 1367-1377.*