Journal of Animal and Veterinary Advances 8 (11): 2311-2317, 2009
ISSN: 1680-5593

Effects of Dietary Selenium and Vitamin E on Semen Quality and Sperm Morphology of Young Boars During Warm and Fresh Season

S. Echeverría-Alonzo, R. Santos-Ricalde, F. Centurión-Castro,
R. Ake-López, M. Alfaro-Gamboa and J. Rodríguez-Buenfil
Faculty of Veterinary Medicine and Animal Science, Universidad Autonoma de Yucatan,
Apdo. Postal 4-116, Itzimna, Merida, Yucatan, Mexico

Abstract: The objective of this study was to evaluate the effect of dietary Se or Vit. E on semen quality and sperm morphology during fresh and warm seasons in young boars. Four diets (0; 0.5 ppm of Se; 250 IU kg^{-1} diet of Vit. E; or 0.5 ppm of Se +250 IU kg^{-1} of Vit. E) were used. Significant effects of season and Vit. E or Se on semen quality and sperm morphology was found. Seminal volume was lower in fresh season ($p<0.05$). However, sperm concentration, Total Sperm number/Ejaculate (TSE) percentage of normal spermatozoa and motility were higher in fresh season ($p<0.05$). Spermatic motility, percentage of normal spermatozoa, head abnormalities and retention of cytoplasmic droplets were positively affected ($p<0.05$) by Se and Vit. E in both seasons. Combination of Se and Vit. E improved sperm concentration in fresh season ($p<0.05$), while Vit. E solely or combined with Se increased sperm concentration in warm season ($p<0.06$). Positive effect of Vit. E or combined with Se on TSE was observed in warm season ($p<0.05$). Reduction of tail abnormalities was observed only during the fresh season in boars fed fortified Se and Vit. E diets ($p<0.05$). These results suggested that semen quality and sperm morphology can be affected negatively during warm season and Vit. E and Se improve semen quality and strength fertility of boars during the warm season.

Key words: Season, selenium, vitamin E, semen quality, sperm morphology, boars

INTRODUCTION

Infertility in boars is a major concern for pig keepers in tropical countries, where there are hot temperatures during several months of the year. Heat stress during hot summer months can results in the inhibition of spermatogenesis or semen quality reduction (Cameron and Blackshaw, 1980; Larsson and Einarsson, 1984; Suriyasomboon *et al*., 2004; Kunavongkrit *et al*., 2005). The seasonal infertility may due to a combination of low motility, abnormal morphology and acrosome abnormality (Wetteman *et al*., 1976; Kunavongkrit *et al*., 2005; Suriyasomboon *et al*., 2005; Murase *et al*., 2007).

Several studies revealed that Selenium (Se) and Vitamin E (Vit. E) can affect positively boar semen quality. Selenium supplementation reduces seminiferous tubule degeneration, numbers of spermatozoa within the seminiferous tubules, low sperm motility and morphological anomalies in rats and boars (Wu *et al*., 1973; Behne *et al*., 1996; Marin-Guzman *et al*., 2000; Köhrle *et al*., 2005). The findings indicate that testicular morphology and functions are affected by Se deficiency and that the element is necessary for testosterone biosynthesis and the formation and normal development of spermatozoa (Behne *et al*., 1996; Köhrle *et al*., 2005). Have been reported loss of male fertility in Se deficiency results from the sequential development of sperm defects expressed during both spermiogenesis and maturation in the epididymis (Olson *et al*., 2004, 2005). According to Marin-Guzman *et al*. (1997, 2000), boars fed diets low in Se have structural abnormalities in the spermatozoal mitochondria and lower ATP concentrations.

The function of Vit. E is entirely associated with its antioxidant properties. Boar semen is extremely sensitive to peroxidative damage due to high content of unsaturated fatty acids in the phospholipids of the plasma membrane and the relatively low antioxidant capacity of seminal plasma (Peña *et al*., 2003; Jeong *et al*., 2009; Gliozzi *et al*., 2009). The structural damage to sperm takes place when lipid peroxidation occurred; this resulted in a decline in sperm motility (Jones and Mann, 1977; Gliozzy *et al*., 2009). Brzezinska-Slebodzinska *et al*. (1995) reported an increased concentration of spermatozoa in semen from boars supplemented with Vit. E as an effect associated to the antioxidant properties of this vitamin.

Corresponding Author: Ronald Santos, Faculty of Veterinary Medicine and Animal Science,
Universidad Autonoma de Yucatan, Apdo. Postal 4-116, Itzimna, Merida, Yucatan, Mexico

The aim of this study was to investigate the effect of dietary Se or Vit. E on semen quality and sperm morphology during two seasons in young boars reared under subtropical conditions in Yucatan, Mexico.

MATERIALS AND METHODS

The experiment was carried out in the facilities of the faculty of veterinary medicine and animal science of the University of Yucatan, Yucatan, Mexico.

Four experimental diets varying in dietary supplementation of Se (0 or 0.5 ppm) and Vit. E (0 or 250 IU kg^{-1} diet) were fed to 16 boars (PIC 337× Camborough 22) of 12 months old and 180 kg of BW.

Treatment diets were fed in meal form and formulated using sorghum and soybean meal as the major basal ingredients, with 16% CP and 3200 kcal of digestible energy/kg. Dietary Se was added as organic Se (Sel-plex®, Alltech, Nicholasville, KY) and Vit. E was dl α-tocopheryl acetate.

The experiment was carried out in two seasons. The fresh season was from January to April and warm season from May to July. The temperature was recorded every day using a max-min electronic thermometer (Oakton).

The boars were fed a restricted quantity (2.5 kg day^{-1}) of their treatments diets since 3 months before the experiment began.

Semen from the 16 treatment boars was collected once weekly. The ejaculates were collected using the gloved-hand method. A prewarmed (37°C) 1 L, insulated polyethylene bag in a thermos was used to collect the semen. The gel fraction was discarded and the subsequent volume of the ejaculate was calculated. Sperm motility was evaluated by placing a drop of semen on a prewarmed (37°C) microscope slide immediately after collection, using a light microscope. The percentage of motile spermatozoa was estimated by visual appraisal. Motility was identified as those sperm cells that demonstrated progressive motility.

To estimate the sperm concentration and total sperm number, semen was diluted 1:100 (vol vol^{-1}) ratio with a fixative solution containing 1% formaldehyde in a saline solution (0.85%). The diluted semen was placed on haemocytometer. The total number of sperm was determined by multiplying sperm concentration/mL with the volume of the strained ejaculate.

In addition, the percentage of normal sperm was calculated, 100 spermatozoa were counted and abnormalities of the head, tail and retention of cytoplasmic droplets of spermatozoa were studied using 1% formaldehyde saline preparation examined by oil-immersion phase-contrast microscopy and the percentage of each was calculated.

The data from January to April (fresh) and from May to July (warm) was used to evaluate the effect of season. The data was analyzed as a complete randomized experiment, comparing the fresh vs. warm season and using the statistical procedure for repeated measurements. The experimental data from each season was used to evaluate the effect of Se and vitamin E. The statistical analysis using the procedure for repeated measurements was performed as a complete randomized experiment in each season. The means were compared by lsmeans when necessary.

RESULTS AND DISCUSSION

Seasonal effect: The average temperature recorded during the fresh season was 25.3±1.60°C with a maximum of 31.8±2.60°C and a minimum of 18.8±0.70°C. During the warm season, the average temperature was 28.7±0.15°C with a maximum of 35.4±0.23°C and a minimum of 22.1±0.61°C.

There was a significant effect of the season on semen characteristics (Fig. 1). The seminal volume was 12.5% lower in fresh season than in warm season ($p<0.05$). The present results are not consistent with the results were the seminal volume was lower during the warm months (Suriyasomboon *et al*., 2004). Other experimental results have not found statistical effect of the season on semen volume (Wettemann *et al*., 1976; Cameron and Blackshaw, 1980; Okere *et al*., 2005; Murase *et al*., 2007). It is important to mention that the volume of whole ejaculate depend solely upon the volume of the sperm-poor fraction (Murase *et al*., 2007), so, even semen volume was higher in the boars during the warm season the sperm concentration and total sperm number per ejaculate was lower in this experiment. The results obtained in this experiment suggesting that sperm-poor fraction could be not affected by seasonality. To respect, Wettemann *et al*. (1976) did not find effect of elevated temperature on semen volume and gel weight, therefore, they conclude, that accessory gland function probably is not altered by the elevated temperature.

Sperm concentration and Total Sperm number/ Ejaculate (TSE) were higher in fresh season in comparison to warm season ($p<0.05$). These finding corresponds to previous results by Suriyasomboon *et al*. (2004) and Trudeau and Sanford (1986). In contrast some researcher were unable to demonstrate a significant seasonal effect on sperm concentration in their studies (Cameron and Blackshaw, 1980; Cameron, 1985). This discrepancy might be due to the differences in the ambient temperatures where the boars were reared or time kept in heat stress.

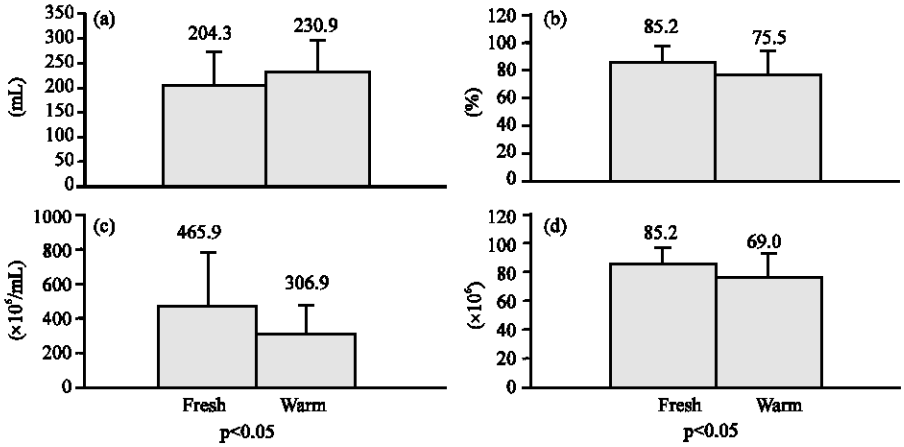

Fig. 1: Effect of season on semen characteristics of young boars; a) volume, b) motility, c) concentration and d) total sperm number/ejaculate

As has been mentioned before, even volume of ejaculate was higher in warm period TSE was lower than in fresh season. These results are in agreement with other studies, where total sperm number per ejaculate in boars reared under tropical conditions was seasonality affected (Suriyasomboon *et al*., 2005; Trudeau and Sandord, 1986).

Suriyasomboon *et al*. (2004) observed a reduction of total sperm production in boars kept with either conventional open air system or evaporative cooling system during the warm months of Thailand. In contrast, previous studies have reported that the total number of spermatozoa per ejaculate did not change during the year (Murase *et al*., 2007) or did not affected by exposure boars to temperatures of 35°C (Larsson and Einarsson, 1984). Probably, elevated temperature may not be the only factor that affect total sperm number per ejaculate, also, humidity have a great effect (Suriyasomboon *et al*., 2004).

Clear seasonal effect, with a significant reduction of 10% during warm season, was evident for the percentage of motile spermatozoa ($p<0.05$), which corresponded to a previous report (Murase *et al*., 2007). In dissimilarity, no significant seasonal effect has been observed in the percentage of spermatozoa motility from landrace boars, although, the motility type changed seasonally (Trudeau and Sanford, 1986). Thus, sperm motility in boars can be negative affected seasonality.

The morphological alter found in the spermatic cells in the 2 seasons are shown in Fig. 2. There was a reduction of normal spermatozoa in the warm season ($p<0.05$). Among the abnormalities observed, retention of cytoplasmic droplets was significantly higher in warm season ($p<0.05$). The percentage of abnormal heads and tails of spermatozoa were not statistical different between seasons ($p>0.05$). Previous study of large white boars demonstrates that the percentage of spermatozoa with abnormalities increases during summer (Murase *et al*., 2007). Similarly, Suriyasomboon *et al*. (2005) observed a temperature significant effect on the percentage of morphological normal spermatozoa. Other than, no significant effect of season was observed in the percentage of spermatozoa with normal heads, normal tail or cytoplasmic droplets in Chinese breeds and Duroc boars (Borg *et al*., 1993), while, the maximum percentage of abnormal spermatozoa was observed in late winter (March) in landrace boars (Trudeau and Sanford, 1986). However, the increased in spermatozoa abnormalities at higher ambient temperatures is in accordance with the results of several previous experimental studies by Wettemann *et al*. (1976), Cameron and Blackshaw (1980), Larsson and Einarsson (1984) and Suriyasomboon *et al*. (2005). Thus, there is a general tendency for periodic increased of spermatozoa abnormalities during warm season.

Effect of selenium and vitamin E: The results of dietary Se and Vit. E on seminal characteristics are presented in Fig. 3. The seminal volume was not affected by Se or Vit. E dietary supplementation in both season ($p>0.05$). However, there was a tendency to improve seminal volume when Se and Vit. E were simultaneously in warm season. The results obtained are in agreement with Segerson *et al*. (1981) and Marin-Guzman *et al*. (1997), who did not find any effect of Se and Vit. E on seminal volume of young boars. However, increases in seminal volume of rabbits fed with Vit. E fortified diets have been reported by Yousef *et al*. (2003).

Spermatic motility was positively affected ($p<0.05$) by Se and Vit. E in both seasons, being more evident this effect in warm season (Fig. 3). The motility responses of sperm observed in this experiment are consistent with the report by Marin-Guzman *et al*. (1997), who evaluate similar treatments in young boars. They found that boars fed either the nonfortifed Se or vitamin E diets had sperm with

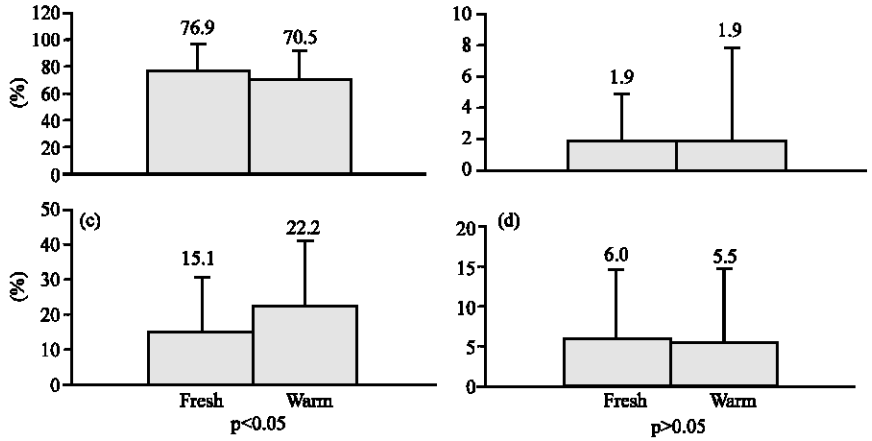

Fig. 2: Effect of season on spermatozoa morphology in young boars; a) normal spermatozoa, b) abnormal heads, c) cytoplasmic droplets and d) abnormal tails

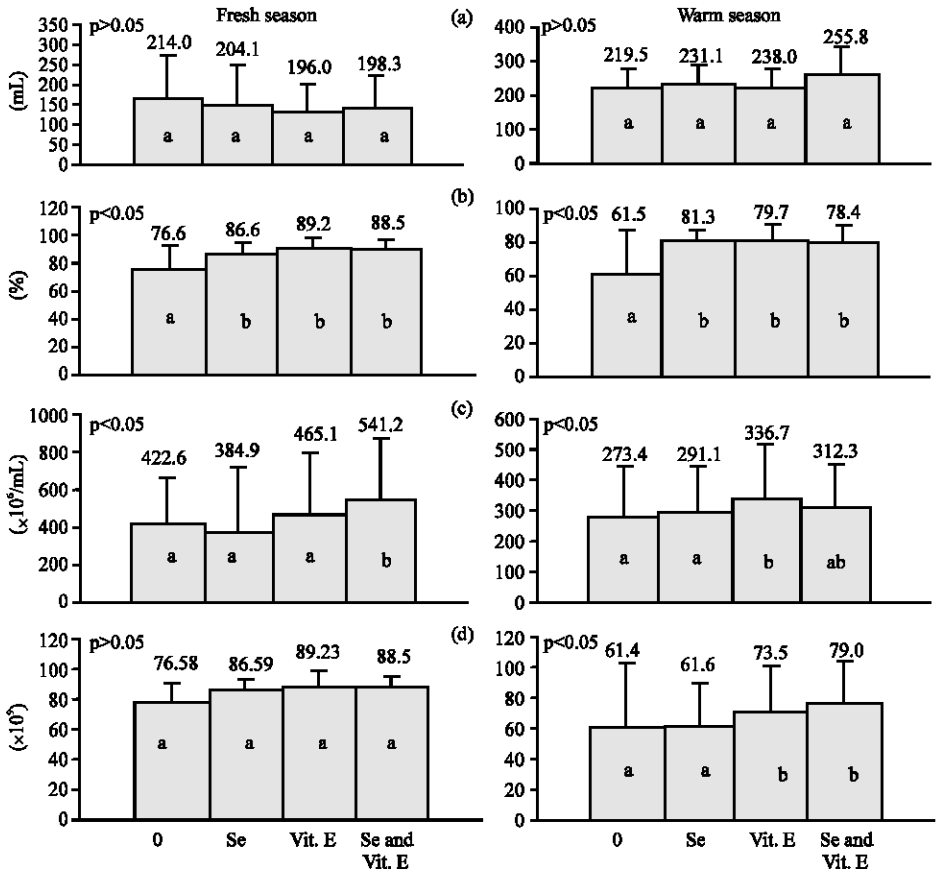

Fig. 3: Seminal characteristics of young boars fed fortified selenium and vitamin E diets, during fresh and warm season; a) volume, b) motility, c) concentration and d) total sperm number/ejaculate

lower motilities. This finding was more remarkable in warm season in this experiment. These observations are in accordance with our results, where a reduction of abnormal spermatozoa was observed in boars fed fortified Se or Vit. E in both seasons ($p<0.05$). In agreement with previous reports, fortified Vit. E or Se diets reduced spermatozoa abnormalities and consequently increases spermatic motility (Wu *et al*., 1973; Behne *et al*., 1996;

Marin-Guzman *et al*., 1997; Olson *et al*., 2005). There is evidence that Vit. E may directly protect the sperm from morphological damage by binding endoperoxides, which may therefore, affect the percentage of normal and motile sperm cells (Marin-Guzman *et al*., 1997). Likewise, Se reduces incidence of spermatozoa abnormalities, thus, increase sperm motility (Wu *et al*., 1973; Marin-Guzman *et al*., 1997; Olson *et al*., 2005). Therefore, the results obtained in this experiment evidence the protective effect of Se and Vit. E on spermatozoa integrity and motility, being more evident this effect in warm season.

Combination of Se and Vit. E improved sperm concentration in fresh season ($p<0.05$), while, Vit. E solely or combined with Se increased sperm concentration in warm season ($p<0.06$) (Fig. 3). Also, significant effect of Vit. E or combined with Se on TSE was observed in warm season ($p<0.05$), but, not in fresh season ($p>0.05$) (Fig. 3). The positively affected of Vit. E solely or with Se on seminal concentration and total sperm number/ejaculate observed mainly in warm season in this experiment denote that Vit. E and Se played an important role in spermatogenesis (Marin-Guzman *et al*., 1997, 2000). These findings are in conformity with the report of Brzezinska-Slebodzinska *et al*. (1995), who found that vitamin E supplementation in boars significantly increased the number of spermatozoa per 1 cm^3 of ejaculate. Similarly, increases in sperm concentration and total sperm output were found in rabbits (Yousef *et al*., 2003). According to Cooper *et al*. (1987) the effect of vitamin E occurs directly or indirectly on the regulation of intratesticular factors, which regulate specific steps of germ cell development. The improvement of total sperm number/ejaculate mainly in warm season could be related to the discussed effect of Vit. E on germ cell proliferation and an effect possibly linked to the antioxidant properties of this vitamin (Marin-Guzman *et al*., 1997; Audet *et al*., 2004). Similarly, the importance of Se for testicular germ cell development has been reported (Behne *et al*., 1996; Köhrle *et al*., 2005; Olson, 2005). Selenium inadequate diets reduce spermatogenesis, decrease diameter of

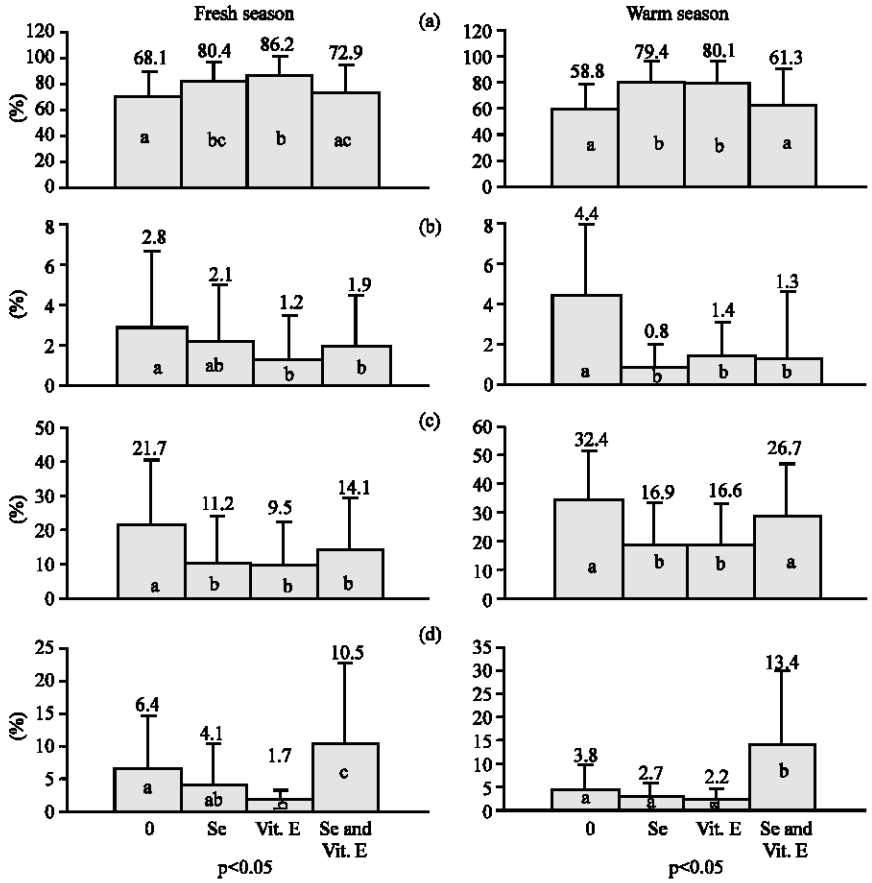

Fig. 4: Spermatozoa abnormalities observed in young boars fed fortified selenium and vitamin E diets during fresh and warm season; a) normal spermatozoa, b) abnormal heads, c) cytoplasmic droplets and d) abnormal tails

seminiferous tubules, reduce Sertoli cell development and cause testicular atrophy (Behne *et al*., 1996; Marin-Guzman *et al*., 2000; Köhrle *et al*., 2005). Probably, the antioxidant effect of Vit. E and the effect of Se on spermatogenesis seem important for boars, but could be more relevant when they are under heat stress. These results suggested that Vit. E and Se might reduce the seasonal effect on boar fertility.

The effect of dietary Se and vitamin E on spermatozoa characteristics are reported in Fig. 4. There was a significant positive effect ($p<0.05$) of Se and Vit. E on percentage of normal spermatozoa and occurrence of head abnormalities in both seasons. In both seasons, there was a significant reduction of cytoplasmic droplets in spermatozoa of boars fed fortified Se or Vit. E diets ($p<0.05$). However, Se and Vit. E together did not reduces cytoplasmic droplets in warm season ($p>0.05$). Similarly, Se and Vit. E reduces occurrence of tail abnormalities during the fresh season ($p<0.05$), while, during the warm season did not occurred ($p>0.05$). The positive effect of Se and Vit. E on incidence of abnormal spermatozoa is in concordance with previous studies by Wu *et al*. (1973) Behne *et al*. (1996), Köhrle *et al*. (2005) and Olson (2005). The most important effect of Se or Vit. E on reduction of abnormal heads and cytoplasmic droplets observed in this study are in discrepancy with other results where spermatozoa of Se deficiency rats, exhibit principally a specific set of flagellar structural defects (Wu *et al*., 1973; Olson *et al*., 2005). Similarly, boars fed low-Se diets produce a higher percentage of sperm with disrupting tail morphology (Marin-Guzman *et al*., 1997). However, in mice the most frequent abnormality was in the sperm head (Watanabe and Endo, 1991). Nevertheless, the information obtained in this experiment and in others supported the assumption that fortified diets with Se and Vit. E reduce abnormalities in spermatozoa. This effect could be more relevant in warm weather under tropical conditions.

CONCLUSION

Semen quality and spermatozoa morphology from young boars can be affected negatively during the warm season. The main effect is on sperm motility, sperm concentration and TSE. The results obtained suggest that the effect of Vit. E and Se on semen quality and spermatozoa integrity strength the boar's fertility, being, more relevant when they are reared in warm weathers.

REFERENCES

Audet, I., J.P. Laforest, G.P. Martineau and J.J. Matte, 2004. Effect of vitamin supplements on some aspects of performance, vitamin status and semen quality in boars. J. Anim. Sci., 82: 626-633. PMID: 14974564.

Behne, D., H. Weiler and A. Kyriakopoulos, 1996. Effects of selenium deficiency on testicular morphology and function in rats. J. Rep. Fert., 106: 291-297. DOI: 10.1530/jrf.0.1060291. PMID: 8699413.

Borg, K.E., D.D. Lunstra and R.K. Christenson, 1993. Semen characteristics, testicular size and reproductive hormone concentrations in mature duroc, meishan, fengjing and minzhu boars. Biol. Reprod., 49: 515-521. PMID: 8399844.

Brzezinska-Slebodzinska, E., A. B. Slebodzinski, B. Pietras and G. Wieczorek, 1995. Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. Biol. Trace. Elem. Res., 47: 69-74. PMID: 7779577.

Cameron, R.D., 1985. Factors influencing semen characteristics in boars. Aust. Vet. J., 62: 293-297. PMID: 4074214.

Cameron, R.D. and A.W. Blackshaw, 1980. The effect of elevated ambient temperature on spermatogenesis in the boar. J. Reprod. Fert., 59: 173-179. PMID: 7401033.

Cooper, D.R., O.R. Kling and M.P. Carpenter, 1987. Effect of vitamin E deficiency on serum concentrations of follicle-stimulating hormone and testosterone during testicular maturation and degeneration. Endocrinology, 120: 83-90. PMID: 3096703.

Gliozzi, T.M., L. Zaniboni, A. Maldjian, F. Luzi, L. Maertens and S. Cerolini, 2009. Quality and lipid composition of spermatozoa in rabbits fed DHA and vitamin E rich diets. Theriogenology, 71: 910-919. PMID: 19121864. DOI: 10.1016/j.theriogenology.2008.10.022.

Jeong, Y.J., M.K. Kim, H.J. Song, E.J. Kang, S.A. Ock, B.M. Kumar, S. Balasubramanian and G.J. Rho, 2009. Effect of α-tocopherol supplementation during boar semen cryopreservation on sperm characteristics and expression of apoptosis related genes. Cryobiology, 58: 181-189. DOI: 10.1016/j.cryobiol.2008.12.004. PMID: 19141297.

Jones, R. and T. Mann, 1977. Toxicity of exogenous fatty acid peroxides towards spermatozoa. J. Reprod. Fertil., 50: 255-260. PMID: 881663.

Köhrle, J., F. Jakob, B. Contempré and J. E. Dumont, 2005. Selenium, the Thyroid and the Endocrine System. Endocr. Rev., 26: 944-984. DOI: 10.1210/er.2001-0034. PMID: 16174820.

Kunavongkrit, A, A. Suriyasomboon, N. Lundeheim, T.W. Heard and S. Einarsson, 2005. Management and sperm production of boars under differing environmental conditions. Theriogenology, 63: 657-667. DOI: 10.1016/j.theriogenology.2004.09.039. PMID: 15626423.

Larsson, K. and S. Einarsson, 1984. Seminal changes in boars after heat stress. Acta Vet. Scand., 25: 57-66. PMID: 6464925.

Marin-Guzman, J., D. C. Mahan, Y. K. Chung, J. L. Pate and W. F. Pope, 1997. Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality and subsequent fertilization rates in mature gilts. J. Anim. Sci., 75: 2994-3003. PMID: 9374315.

Marin-Guzman, J., D.C. Mahan and J.L. Pate, 2000. Effect of dietary selenium and vitamin E on spermatogenic development in boars. J. Anim. Sci., 78: 1537-1543. PMID: 10875636.

Murase, T., N. Imaeda, H. Yamada and K. Miyazawa, 2007. Seasonal changes in seminal characteristics, composition of seminal plasma and frequency of acrosome reaction induced by calcium and calcium ionophore A23187 in large white boars. J. Reprod. Dev., 53: 853-865. DOI: 10.1262/jrd.19026. PMID: 17519520.

Okere, C., A. Joseph and M. Ezekwe, 2005. Seasonal and genotype variations in libido, semen production and quality in artificial insemination boars. J. Anim. Vet. Adv., 4: 885-888. http://medwelljournals.com/fulltext/java/2005/885-888.pdf.

Olson, E.G., V.P. Winfrey, K.E. Hill and R.F. Raymond, 2004. Sequential development of flagellar defects in spermatids and epididymal spermatozoa of selenium-deficient rats. Reproduction, 127: 335-342. DOI: 10.1530/rep.1.00103. PMID: 15016953.

Olson, G.E., V.P. Winfrey, S.K. NagDas, K.E. Hill and R.F. Burk, 2005. Selenoprotein P is Required for Mouse Sperm Development. Biol. Rep., 73: 201-211. DOI:10.1095/biolreprod.105.040360.PMID:15744015.

Peña, F.J., A. Johannisson, M. Wallgren, H. Rodriguez-Martinez, 2003. Antioxidant supplementation *in vitro* improves boar sperm motility and mitochondrial membrane potential after cryopreservation of different fractions of the ejaculate. Anim. Reprod. Sci., 78: 85-98. DOI:10.1016/S0378-4320(03)00049-6. PMID: 12753785.

Segerson, E.C., W.R. Getz and B.H. Johnson, 1981. Selenium and reproductive function in boars fed a low selenium diet. J. Anim. Sci., 53: 1360-1367. PMID: 7319974.

Suriyasomboon, A., N. Lundeheim, A. Kunavongkrit and S. Einarsson, 2005. Effect of temperature and humidity on sperm morphology in Duroc boars under different housing systems in Thailand. J. Vet. Med. Sci., 67: 777-785. DOI: 10.1292/jvms.67.777. PMID: 16141664.

Suriyasomboon, A., N. Lundeheim, A. Kunavongkrit and S. Einarsson, 2004. Effect of temperature and humidity on sperm production in Duroc boars under different housing systems in Thailand. Livest. Prod. Sci., 89: 19-31. DOI: 10.1016/j.livprodsci.2003.12.008.

Trudeau, V. and L.M. Sanford, 1986. Effect of season and social environment on testis size and semen quality of the adult Landrace boar. J. Anim. Sci., 63: 1211-1219. PMID: 3771402.

Watanabe, T. and A. Endo, 1991. Effects of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice. Mutat. Res., 262: 93-99. PMID: 2000100.

Wettemann, R.P., M.E. Wells, I.T. Omtvedt, C.E. Pope and E.J. Turman, 1976. Influence of elevated ambient temperature on reproductive performance of boars. J. Anim. Sci., 42: 664-669. PMID: 1262278.

Wu, S.H., J.E. Oldfield, P.D. Whanger and P.H. Weswig, 1973. Effect of selenium, vitamin E and antioxidants on testicular function in rats. Biol. Rep., 8: 625-629. PMID: 4736545.

Yousef, M.I., G.A. Abdallah and K.I. Kamel, 2003. Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim. Reprod. Sci., 76: 99-111. DOI: 10.1016/S0378-4320(02)00226-9. PMID: 12559724.