EFFECT OF ZINC ON THE SEMEN CHARACTERISTICS AND HAEMATOLOGICAL PROFILE OF LARGE WHITE BOAR

M. A. OGUIKE; I. A. UKWENI and A. A. ANSA

College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia. Abia state, Nigeria.
E-mail: maoguike2000@yahoo.com Phone: +2348037426939

ABSTRACT

The study assessed the effect of zinc on the semen characteristics and some haematological profile of intensively managed large white boar. Eight 9-10 months old large white boars were randomized into two treatment groups of four animals per group. Semen characteristics were evaluated using semen volume, sperm motility, sperm concentration, sperm morphology and live/dead sperm cells. The result revealed significant influence ($P<0.05$) in sperm concentration ($45.06\pm1.79x10^7/mm^3$ and $32.04\pm2.57x10^7/mm^3$), percentage of bent tail (2.89±0.45% and 3.42±0.11%), percentage of tailless spermatozoa (2.61±0.29% and 4.00±0.12%) and percentage of cytoplasmic droplet (1.61±0.11% and 2.46±0.23%) for the zinc-fed and control group respectively. Other traits such as semen volume (93.17±5.06 ml and 99.42±5.70 ml), percentage progressive motility (78.56±3.29% and 78.21±1.00%), percentage of abnormal head (2.33±0.00% and 2.79±0.32%) and percentage of live spermatozoa (77.11±1.41% and 73.63±1.33%) were not affected significantly ($P>0.05$) on the zinc-fed and control group respectively. Haematological indices to determine the effect of zinc on the physiological status of the animals were also evaluated and the result showed significant effect ($P<0.05$) on Mean Corpuscular Haemoglobin (MCH) at 18.99±0.30 pg and 17.44±0.44 pg for zinc treated (T_2) and control group (T_1) respectively. Significant differences ($P<0.05$) were also detected on Packed Cell Volume (38.13±0.77% and 33.67±0.46% for T_2 and T_1 group respectively) and Haemoglobin (Hb) Concentration count (12.18±0.44 g/dl and 10.36±0.07 g/dl for T_2 and T_1 group respectively). Treatment effect on Red Blood Cell count ($6.40\pm0.17\times10^6/mm^3$ and $5.90\pm0.13\ x10^6/mm^3$), Mean Corpuscular Volume (59.48±0.30 fl and 57.15±0.86 fl), White Blood Cell count ($16.93\pm0.17\times10^3/mm^3$ and $15.89\pm0.22\times10^3/mm^3$), and Mean Corpuscular Haemoglobin Concentration (31.95±0.53% and 30.78±0.39%) for T_2 and T_1 group respectively, were not influenced significantly ($P>0.05$). The result of this study indicated that zinc oxide supplementation in the feed of breeding boar holds a promise of enhancing seminal traits towards a better semen output in the account of planning mating under artificial insemination programme.

Key words: Boar, haematology, Semen characteristics, zinc

INTRODUCTION

Dietary trace minerals are widely used as feed supplements to stimulate growth, improve feed efficiency, secure uniformity of performance and control infections (Ensminger and Olentine, 1980). Although half of the genetic pool of the drove resides in the boar (Alltech, 2002), there are several researches that have evaluated the effect of nutrition on boar semen quality. The use of micro minerals for the development and function of the cells of the testis is almost completely lacking. However, several micro minerals are needed for hormone production, whereas others play a role in the structure of the sperm and cells of the testis (Alltech, 2002). Kemp and Soede (2001) reported that determining the specific dietary needs of the boar may not be as critical as determining potential positive and negative effects of specific dietary factors on sperm production.

With increasing emphasis on fewer boars and the use of artificial insemination in many drove, the awareness of having structurally sound boars in good body condition that are producing semen of high quality is paramount to achieving high reproductive performance (Marin-Guzman *et al.*, 2000). The production of ejaculates of high quality subsequently allows a greater dilution of the semen to attain large number of

insemination dosages per animal (Alltech, 2002).

Long ago, it was determined that a typical boar ejaculate contains many more sperm cells than are necessary to impregnate a single sow. Because natural mating systems dominated the industry, there was little incentive for investigating nutritional approaches for increasing the average number of fertile sperm cells produced in an ejaculate. It was common for swine producers to feed boars a gestating sow diet and assume that male reproductive efficiency would not be seriously impacted. Today, however, artificial insemination is the most common mating system in the swine industry and each additional quality dose of semen processed from an ejaculate has monetary value. Therefore, to examine the effects of trace mineral supplementation on reproduction in the boar should be a worthy research effort.

Zinc is considered essential for normal growth but few studies have been conducted to investigate the effect of zinc supplementation on the reproductive performance of boar. Reasons for this relative lack of attention probably include the fact that mature boars did and still do comprise a relatively small part of the entire swine population. Research conducted by Haln and Baker (1993), Carlson *et al.* (1999) and Hull *et al.* (2000) showed that feeding 3, 000 ppm zinc, added as zinc oxide, enhanced growth and health of nursery pigs. Carlson *et al.* (1998) reported that feeding 3, 000 ppm zinc as zinc oxide produced deeper crypts and greater total thickness in the duodenum. Katouli *et al* (1999) found that 2, 500 ppm zinc in diets of weaning pigs helped maintain the stability of intestinal microflora and diversity of the coliform for the first 2 weeks after weaning. Some researchers have speculated that pharmacological zinc levels may improve performance through a systemic effect via the blood rather than the enteric effects in the small intestine (Berger, 2003). However, recent data suggest that bioavailability of zinc is irrelevant to the performance response (Case and Carlson, 2002).

Objective of the study was to examine the effects of zinc on the semen parameters of the boar as well as on some of the haematological parameters of the boar.

MATERIALS AND METHODS

Management of experimental animals

The research work was carried out at the Piggery Unit of the Teaching and Research farm of Michael Okpara University of Agriculture, Umudike (MOUAU) in the South Eastern Nigeria. Umudike falls within latitude $05^{0}28^{1}$ North and Longitude $07^{0}35^{1}$ East and an altitude of 112 m above sea level in the tropical rainforest zone. It has an average rainfall of 2177 mm per annum, with relative humidity of about 72% and monthly temperature range of 17^{0}C to 36^{0}C (Meteorological station of NRCRI, Umudike, 2007).

Eight 9 - 10 months old large white boars having a weight range of 30- 40 kg obtained from swine herd in the College of Animal Science and Animal production, MOUAU Research farm were used for the study. They were randomized into two treatment groups of four animals per group. The animals were managed intensively in the piggery house that has dwarf concrete wall and sufficiently divided into pens of dimension 2.2 m x 4.5m per pen. The respective pens were provided with concrete wallow, feeder and drinker of dimension 127 cm x 60 cm x 23 cm, 100 cm x 30 cm x 12 cm, and 60 cm x 52 cm x 21 cm, respectively. The animals were tagged and housed singly in pens for ease of identification and management. Prior to the commencement of the experiment, the animals were dewormed with worm-care drench at the rate of 5 ml worm care/10 kg body weight.

Experimental diet

Two experimental diets were formulated (Tables 1).

One was used as control diet (T_1) and the other (T_2) was supplemented with zinc oxide at an inclusion level of 200 ppm. The zinc oxide was obtained from FINLAB Owerri in Imo State. Four boars were assigned to each of the treatment diets. The animals were fed the treatment diets twice daily at the rate of 2kg per boar per day over a period of 56 days before the start of data collection. Water was supplied *ad libitum* throughout the experimental period.

Table 1: Experimental diet fed to the boars (g/100 g Dm)

Feed Ingredient	T_1 (%)	T_2 (%)
Maize Offal	30.00	30.00
PKC	60.50	60.50
Soya Bean Meal	7.00	7.00
Oyster Shell	0.75	0.75
Bone Meal	1.25	1.25
Salt	0.25	0.25
Premix	0.25	0.25
Zinc Oxide (ppm)	-	200
Total	100	100

Calculated composition of the diet

Crude protein (%)	16.74
Digestible Energy (Kcal/kg)	2546.63
Methionine (mg)	0.28
Lysine (mg)	0.73
Calcium (mg)	0.83
Cysteine (mg)	0.33
Phosphorus (mg)	0.50

Semen collection and evaluation

The eight boars used in this experiment were placed on a semen collection schedule of two times per week such that semen was collected from each boar once daily between 0800 to 1000 hour (local time) on Mondays and Thursdays. This procedure started from the 57th day following the introduction of the test diets and was continued for three consecutive weeks. Semen collection from the boars was by the gloved hand technique. In this technique, the spiral end of the boar's penis was held with a gloved hand following stimulation by a teaser and the ejaculate was collected into a calibrated cylinder.

Semen evaluation involved estimation of both microscopic and macroscopic indices. On-farm evaluation involved the determination of sperm motility aided by a field microscope immediately after collection, and semen viability and morphology using a drop of eosin nigrosin stain to a drop of semen. Semen volume was measured directly from the calibrated cylinder and recorded. Sperm concentration was however determined in the laboratory by a haemocytometer after dilution to 1:200 with buffer solution according to the methods described by Herbert *et al* (2005).

Blood collection and analysis

At the 7th day after commencement of the experiment and at the 56th day before the start of semen collection, blood samples were collected via the jugular vein into sample bottles containing Ethylenediaminetetra-acetic acid (EDTA) as anticoagulant at the rate of 3 mg/ml. Upon completion of the blood sample collection, the whole samples were transferred to the Animal Science Laboratory, MOUAU where they were analyzed for packed cell volume (PCV) using microhaematocrit method (Dacie and Lewis, 1991), Erythrocyte and leukocyte counts using improved Neubauer haemocytometer method described by Jain (1986), and haemoglobin (Hb) concentration using the colorimetric method. The erythrocytic indices Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were calculated from erythrocyte, haemoglobin and Packed Cell Volume as described by Jain (1986).

Data analysis

Means and standard error of means (SEM) were calculated from the data generated using T-test in accordance the methods of Steel and Torrie (1980)

RESULTS AND DISCUSSION

Semen evaluation

The result of semen characteristics of large white boars used in the study are shown in Table 3. The results revealed marked significant differences ($P < 0.05$) in semen concentration ($45.06\pm1.79 \times 10^7/mm^3$ and $32.042\pm2.57 \times 10^7/mm^3$), percentage of bent tail (2.89±0.15% and 3.42±0.11%), percentage of proximal cytoplasmic droplet (1.61±0.11% and 2.46±0.23%), and percentage of tail less spermatozoa (2.61±0.30% and 4.00±0.12%) between the zinc-treated (T_2) versus control group (T_1) respectively.

Table 3: Effect of zinc on semen characteristics of the experimental large white boars

Parameters	Zinc-treated Group (T_1)	Control Group (T_2)
Sperm Conc. ($x10^7/mm^3$)	$45.06^a\pm1.79$	$32.04^b\pm2.57$
Progressive motility (%)	78.56±3.29	78.21±1.00
Volume (ml)	93.17±5.06	99.42±5.70
Bent tail (%)	$2.89^b\pm0.45$	$3.42^a\pm0.11$
Abnormal Head (%)	2.33±0.00	2.79±0.32
Proximal Cytoplasmic Droplet (%)	$1.61^b\pm0.11$	$2.46^a\pm0.23$
Tailless (%)	$2.61^b\pm0.29$	$4.00^a\pm0.12$
% Live Spermatozoa (%)	77.11±1.41	73.63±1.33

(^{a,b}) Different superscripts indicate significant ($P < 0.05$) differences within row.

Other parameters which include semen volume, percentage of progressive motility, and percentage of live spermatozoa were not significantly different ($P > 0.05$).

Observations made on the mean sperm concentration in this study are consistent with the range reported by Rozeboom (2001). Also, the higher significant difference ($P < 0.05$) in sperm concentration observed in this study conforms with that of Liao *et al.*, (1985) who reported higher sperm concentration for boars fed diets supplemented with zinc. This suggests that the cellular components in the testis (Sertoli cells) responsible for nurturing the developing spermatids responded favourably to the dietary zinc. The implication of this result in sperm density governs the dilution of semen, which will influence the extent to which the semen can be diluted and therefore the number of insemination doses available.

Similarly, the percentage of progressive motile sperm in ejaculate is in agreement with the observations made for good quality semen by Dausend (1974), Rostel (1975), and Flowers (1998). Semen motility is an important index in reproductive assessment because it demonstrates the ability by which spermatozoa progressively propels to fertilize an ovum. However, there was no significant difference ($P > 0.05$) in progressive motile sperm between the zinc-treated and control groups.

The mean volume of semen recorded (Table 3) was lower than the findings of Rozeboom (2001) and Larsson (1998) who reported an ejaculate volume of 100 - 500 ml for normal value of an ejaculate. However, this was higher than the value of 50 ml reported by Flowers (1998). The semen volume recorded in this study showed no significant differences ($P > 0.05$) between the various experimental groups.

The percentage of abnormal spermatozoa (9.44% and 12.67% for T_2 and T_1, respectively) obtained in this study conforms with earlier reports (Holst, 1949; Singleton and Shelby, 1972; Dausend, 1974; and Flower,

1998) for normal ejaculate. The major morphological differences revealed in this study were the percentages of bent tail, tailless spermatozoa, and proximal cytoplasmic droplets.

These morphological characteristics which were significantly affected ($P < 0.05$) contrasted with the observations of Liao *et al.*, (1985) since these authors reported no effect of zinc treatment on the percentage spermatozoa with regards to morphological abnormalities. The significant decrease ($P < 0.05$) in spermatozoa abnormalities in the zinc fed group in this study suggests that zinc caused a slow release of immature spermatozoa and enhanced maturation of the sperm cells. However, Herbert and Acha (1995) attributed the causes of spermatozoa abnormalities to deformities or accidents and radiations. These workers further elucidated that abnormalities may also occur as artifact caused by the staining procedure.

The leydig cells of the testis are primarily responsible for testosterone production which maintains libido. In zinc deficiency, it has been shown that leydig cells are abnormal with a concurrent loss of epithelial tissue in the seminiferous tubules in the testis (Hesketh, 1982). This ascertains the assertion that zinc enhances libido in animals. Contrastingly, in the present study, semen collection was relatively difficult within the zinc treated group. Longer reaction time was observed, which may be indication of reduced libido.

Although the mean percentage of live spermatozoa in the zinc treated group (77.11±1.41%) and control group (73.63±1.34%) were not significantly influenced ($P > 0.05$), the percentage of live spermatozoa in the zinc fed group was numerically higher.

Haematology

The results of the effect of zinc on the haematology of large white boar are shown in Table 4.

Table 4: Effect of zinc on haematology of the experimental large white boar

Parameters	Zinc-treated Group	Control Group
Hb (g/dl)	12.18^{a}±0.44	10.36^{b}±0.07
RBC ($X10^{6}/mm^{3}$)	6.40±0.17	5.90±0.13
PCV (%)	38.13^{a}±0.77	33.67^{b}±0.46
MCV (fl)	59.48±0.30	57.15±0.86
MCH (pg)	18.99^{a}±0.30	17.44^{b}±0.44
MCHC (%)	31.95±0.53	30.78±0.39
WBC ($X10^{3}/mm^{3}$)	16.93±0.17	15.89±0.22

(^{a,b}) Different superscripts indicate significant ($P<0.05$) differences within row.

Miller *et al.* (1961) reported a mean value of 12.1±0.2g/dl Hb which compares favourably with 12.18±0.44g/dl obtained in this study for the zinc treated group but slightly lower than 13.0±1.19g/dl reported by Glawischnig *et al.* (1977). The mean Hb value obtained for the control group was lower and did not agree with the works of Miller *et al.* (1961) and Glawischnig *et al* (1977). Nevertheless, the result of Haemoglobin concentration revealed a significant difference ($P<0.05$)

Haemoglobin is a carrier of oxygen in the blood and this is released into the tissues from the capillaries. The higher Hb in the zinc-fed group indicates sufficient supply of oxygen to the body tissues of the zinc-fed group resulting in increased metabolism which must have enhanced spermatogenesis and the overall physiological processes in the boar.

RBC counts obtained in this study for both the zinc treated and control group did not differ. This agrees with the reports of Wachtel (1963). Although the results were not significantly affected ($P > 0.05$), the slight increase in the RBC value of the zinc-treated group tended to further support the assertion of an increased metabolism in their body tissues resulting from increased haemoglobin raising oxygen availability. Therefore,

increased RBC in the blood translates into increased Hb and a consequent increase in tissue oxidation.

PCV is a measure of the proportion of blood volume that is occupied by RBC. A PCV value of 38.13±0.77% obtained in the zinc fed group is in consonance with the mean value of 36.8±0.50% reported by Miller *et al.* (1961) and a range of 32.0 - 50.0% observed by Banerjee (2005) but lower than the mean value of 43.1±3.42% observed by Wegner *et al.* (1975). On the other hand, the mean PCV value of 33.67±0.45% in the control group only falls within the range of normal values reported by Banerjee (2005) but lower than 36.8±0.50% and 43.1±3.42% independently observed by Miller *et al.* (1961) and Wegner *et al.* (1975) respectively.

PCV is used as an index of physiological status of the body and the significant difference ($P<0.05$) in the PCV index suggests an enhanced physiological balance (Esonu *et al.* 2001) in the zinc-fed group relative to the control group having a lower value.

RBC indices (MCHC, MCV and MCH) are helpful in the initial classification of anaemia. The mean values of these parameters obtained in this study were slightly lower than the values of MCHC (32.3±0.28 to 34.2±0.30%), MCV (58.2±0. 60 to 62.0±1. 90 fl), and MCH (19.2±0. 18 to 20.0±0.70 pg) obtained in literature (Miller *et al.*, 1961: McTaggart and Rowntree, 1969; and Leman *et al.* 1981). Although MCH (18.99±0.30 pg and 17.44±0.44 pg for T_2 and T_1 group respectively) indicated a significant difference ($P<0.05$), the results compare with the range reported by Banerjee (2005). This shows that the animals (both zinc treated and control groups) were not anaemic, having adequate volume of red corpuscles in the blood and therefore receiving sufficient oxygen in their tissue as needed to carry out normal physiological functions in the body.

The result of the haematological study further demonstrated no significant differences ($P>0.05$) in the WBC index. Although WBC count in the zinc fed group were slightly numerically higher than the control group ($16.93\pm0.17 \times 10^3/mm^3$ and $15.89\pm0.22 \times 10^3/mm^3$ for T_2 and T_1 respectively), the result agrees with the range of normal values observed by McTaggart and Rowntree (1969); Leman (1981) and Banerjee (2005) for pigs. On the other hand, Gudat and Shnell (1970) and Glawischnig *et al.*, (1977) observed lower WBC count of $14.260\pm1.09 \times 10^3 mm^3$ for boars.

CONCLUSION

The results obtained in this study on semen characteristics of large white boars showed that seminal traits and haematological parameters which determine the physiological status of an animal were enhanced in the zinc-fed group. The significant difference in sperm concentration should be taken into account in planning mating and artificial insemination (AI) programmes in swine. It is therefore recommended that zinc should be supplemented in the diet of boar for better semen output. Further research will however be necessary to determine the optimum dose of zinc at graded levels in boar's diets and its effects on other species of domestic animals.

REFERENCES

Alltech (2002). Mineral metabolism and boar. Animal Science Department. The Ohio State University, Columbus, OH, USA.

Banerjee, G. C. (2005). *A textbook of Animal Husbandry*. 8th ed. Oxford and IBH Publishing Co. PVT. Ltd. New Delhi.

Berger, L. L. (2003). Nutritional and pharmacological role of zinc: PhD. University of Illinois. *Www.saltinstitute.org/47m.html.*

Carlson, M. S; Hoover, S. L; Hill, G. M; Link, J. E. and Turk, J. R. (1998). Effect of pharmacological zinc on intestinal metallothionein concentration and morphology of the nursery pig. J. Anim. Sci. 76: (suppl.1): 57.

Case, C. L. and Carlson, M. S. (2002). Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. J. Anim. Sci. 80: 1917.

Dausend, C. H. P. (1974). Occurrence and importance in fertility diagnosis of morphologically abnormal sperm

in the boar with special reference to protoplasmic droplets. Diss, Coll. of Veterinary Medicine, Hannover, Germany.

Ensminger, M. E. and Olentine, C. G. (1980). *Feed and Nutrition complete.* The Ensminger Publishing Company. Clovis, California.

Esonu, B. O; Emenalom, O. O; Udedibie, A. B. I.; Herbert, U; Ekpor, C. F; Okoli, E. C. and Iheukwumere, F. C. (2001). Performance and blood chemistry of weaner pigs fed raw mucuna bean (*Velvet bean*) meal. Trop. Anim. Prod. Invest. 4:48-54.

Flowers, W. L. (1998). Management of reproduction. In: Progress in pig science, eds. Wiseman, J., Varley, M. and J. Chadwick. 18: 383-405.

Glawischnig, E., Schlerka, G., Schuller, W. and Baumgarther, W. (1977). *Aebeitswerte in der laboratoriumsdiagnostik beim schwein, wein tieraerztl monatsschr* 64: 341-346. In: Diseases of swine. Ed. Leman, A. D; Glock, R. D; Mengeling, W. L; Penry, R. H. C; Scholl, E. and Straw, B. (1981). The Iowa State University Press, Ames, Iowa, USA.

Gudat, E. and Schnell, U. (1970). *Zur Beeinflussung einiger Blutwerte von Besamungserbern durch den Deckakt.* Fort-pfi Haust 6: 36-40. In: Diseases of swine. Ed. Leman, A. D; Glock, R. D; Mengeling, W. L; Penry, R. H. C; Scholl, E. and Straw, B. (1981). The Iowa State University Press, Ames, Iowa, USA.

Hahn, J. D. and Baker, D. H. (1993). Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. J. Anim. Sci. 71: 3020.

Herbert, U. and Acha, C. O. (1995). Preliminary observations on the seminal characteristics of rabbits raised in two locations in south eastern Nigeria. Nigerian Journal of Animal Production, 22(2): 108-112.

Herbert, U.; Ozoje, M.O. and Adejumo, D.O. (2005). Effect of Leucaena and Gliciridia Leaf Meals on the Seminal Characteristics, Testes Weights and Seminiferous Tubule Diameters of Rabbits. Anim. Res.54:173-178.

Hesketh, J. E. (1982). Effects of dietary zinc deficiency on Leydig cell ultrastructure in the boar. J. Comp. Path. 92: 239-247.

Hill, G. M., Cronwell, G. L., Crenshaw, T. D., Dove, C. R., Ewan, R. C., Nabe, D. A., Lewis, A. J., Libal, G. W., Mahan, D. C., Shurson, G. C., Southern, L. L and Veum, T. L. (2000). Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). J. Anim. Sci. 78: 1010.

Holst, S. J. (1949). Sterility in boars. Nord Vet. Med. 50: 87-120.

Katouli, M; Meliin, L; Jensen-waern, M; Walgren, P; and Mollby, R. (1999). The effect of zinc oxide supplementation on the stability of the intestinal flora with special reference to composition of coliforms in weaned pigs. J. Appl. Microbiol. 87: 564.

Kemp, B. and Soede, N. M. (2001). Feeding of developing and adult boars. Swine Nutrition. 34: 771-782.

Larsson, K. (1998). Current therapy in theriogenology. In: Animal Science Facts. Pub. No. AN500-8125. North Carolina State university, College of Agricultural and Life Science.

Leman, A. D; Glock, R. D; Mengeling, W. L; Penry, R. H. C; Scholl, E. and Straw, B. (1981). *Diseases of swine.* The Iowa State University Press, Ames, Iowa, USA.

Liao, C. W., S. C. Chyr, and T. F. Shen (1985). The effect of dietary zinc content on reproductive performance of the boars. In: Proc. Of the third EAAP Animal Science Congress, Seoul, Korea Republic, 2: 613-615.

Marin Guzman, J. Mahan, D. C; and pate, J. L. (2000). Effect of dietary selenium and vitamin E on spermatogenic development in boars. J. Anim. Sci. 78: 1537.

McTaggart, H. S. and Rowntree, P. G. M. (1969). The haematology of "minimal disease" Bacon pigs: A comparison with genetically related conventionally reared pigs. Br. Vet. J. 125: 240-247.

Meterological station of NRCRI, Umudike (2007). Agroclimatology Report.

Miller, E. R; Ullrey, D. E; Ackermann, I; Schmidt, D. A; Luecke, R. W. and Hoefer, J. A. (1961). Swine haematology from birth to maturity. II. Erythrocyte population, size and haemoglobin concentration. J. Anim. Sci. 20: 890-897.