

**LACTATE DEHYDROGENASE ACTIVITY AND CALCIUM
CONTENT IN WASHED SPERM AND TESTICULAR PLASMA
OF WHITE FULANI BULLS IN IBADAN**

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ABSTRACT

Lactate dehydrogenase (LDH) activity and calcium content in plasma and washed sperm (WS) from the testes of mature White Fulani (WF) bulls, divided into four body-weight groups and within the weight range of 140-260kg were studied in a complete randomize design. Animals were weighed at slaughter. Their testes were weighed immediately after slaughter. A part of each testis was stored in the freezer for 48hrs and later homogenized with a pair of scissors in physiological saline. Ultraviolet (UV) and colorimetric methods were employed to assay the homogenate and WS for LDH and calcium respectively. The overall mean LDH activity in the Testicular plasma and WS were 0.57 ± 0.13 and 0.38 ± 0.09 imoles/min in 500×10^6 sperm cells respectively. LDH activity in WS did not differ significantly from plasma of the testes. There were also no significant differences ($P > 0.05$) between the live weight groups. The mean calcium content in the plasma and WS were 0.81 ± 0.15 mg and 0.73 ± 0.11 mg respectively. Testicular WS did not differ significantly between different live-weight groups nor did the testicular plasma. Testicular plasma calcium was higher than in testicular washed sperm.

LDH activity in the plasma could be a good measure of the fertility status of stored sperm. Calcium status of the sperm environment can be determined from its measure in the plasma. Enhancing reproductive potential of bulls through dietary calcium supplementation requires caution to prevent partial intoxication.

Keywords: Lactate dehydrogenase, calcium, testis, plasma, washed sperm, White Fulani

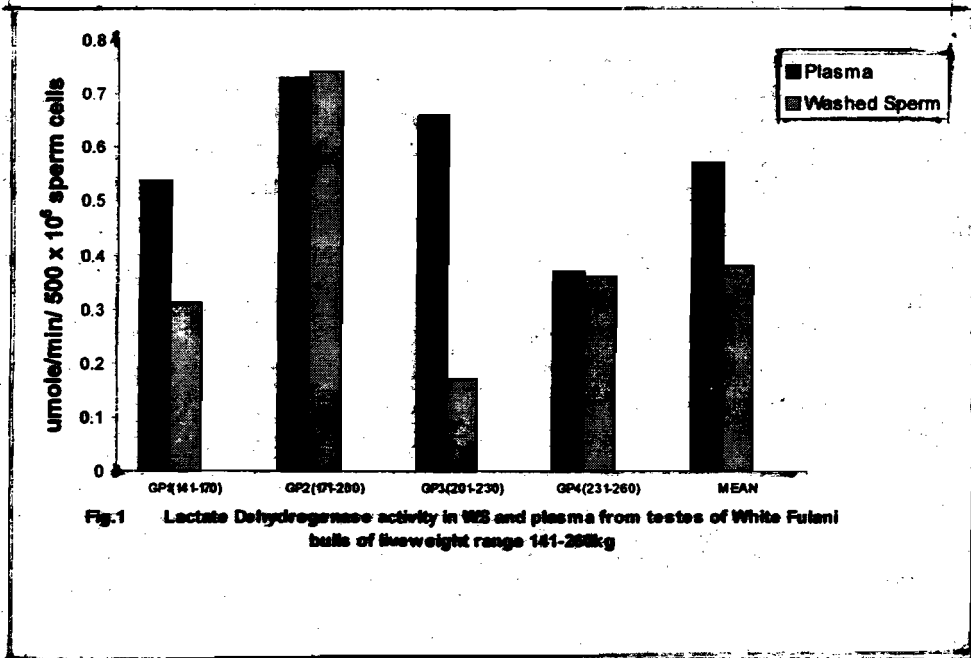
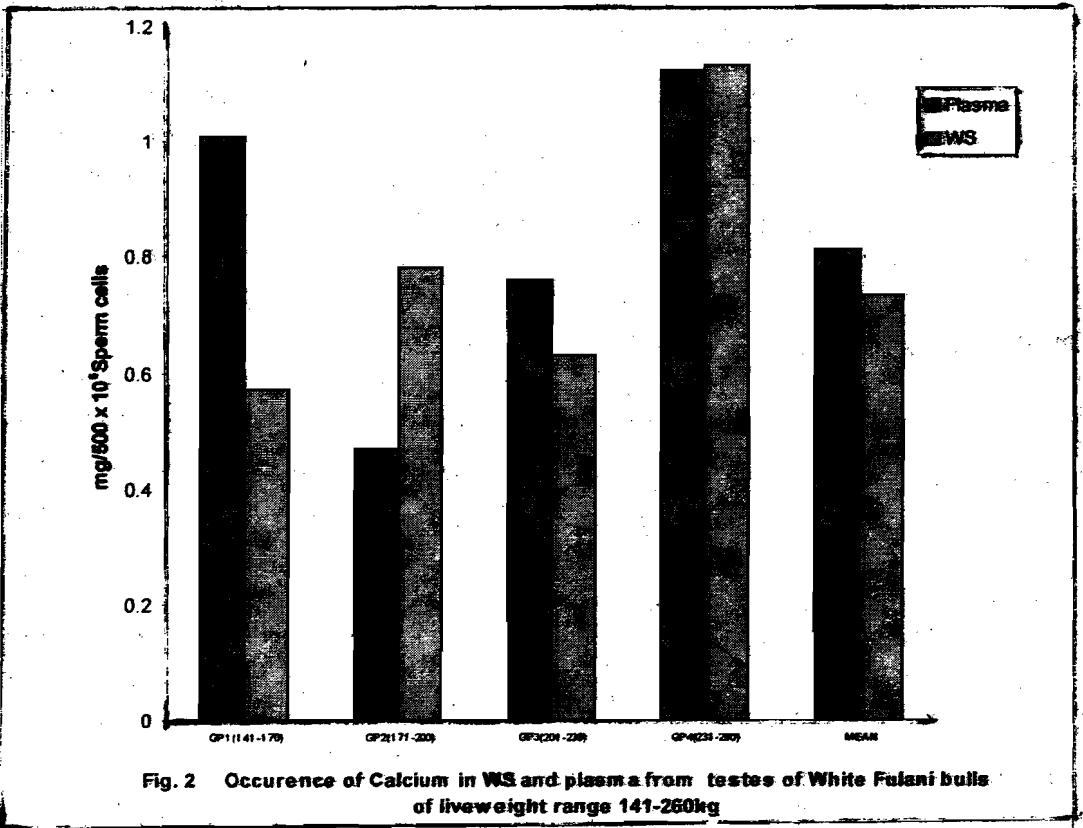


Fig.1 Lactate Dehydrogenase activity in WS and plasma from testes of White Fulani bulls of live weight range 141-260kg

0.17 ± 0.01 imole/min in 500 x 10⁶ sperm cells for testicular plasma and WS respectively. The corresponding values in the other groups, without any significant but only marginal difference between plasma and WS in LDH activity were 0.54 ± 0.04 and 0.31 ± 0.05; 0.73 ± 0.19 and 0.74 ± 0.09; 0.37 ± 0.07 and 0.36 ± 0.06 imole/min in 500 x 10⁶ for plasma and WS of groups 1, 2 and 4 respectively. The LDH activity in the testicular washed sperm cells of group 2 bulls was significantly higher than in the washed sperm from the testes of all the other groups, while there was no significant difference between the LDH activity of the testicular plasma from the different live-weight groups.

The calcium contents of testicular plasma and washed sperm are shown in Figure 2. The overall mean calcium contents in

plasma and WS of mature WF bulls involved in the study were 0.81 ± 0.15 and 0.73 ± 0.11 mg/500x10⁶ sperm cell respectively. The calcium content of the testicular WS cells did not differ significantly between the different live weight groups nor did the contents in plasma as shown in figure 2. The calcium content of testicular plasma was generally higher than that of the WS cells in all the different live-weight groups as shown in figure 2, with the testicular plasma and WS values of 1.01 ± 0.35 and 0.57 ± 0.13; 0.47 ± 0.09 and 0.78 ± 0.20; 0.76 ± 0.15 and 0.63 ± 0.18 and 1.12 ± 0.22 and 1.13 ± 0.02 mg/500 x 10⁶ sperm cells for groups 1, 2, 3, and 4 respectively.



DISCUSSION

The generally non-significant ($P > 0.05$) difference between the different live-weight groups in both LDH activity and calcium contents of both plasma and WS from the testis indicated some stability in the physiology of the testes of these animals. Such stability would be consistent with the assumption that this body-weight range coincided with the mature weight of WF bulls in Ibadan. This result is in agreement with Bostedt *et al* (1976) that age has a significant effect on enzyme activity. The extensive system of management, to which these bulls were exposed, without feed supplementation could also have largely downplayed some of the individual variations, which could have affected the

final physiological responses of the testes as it was observed in this study.

The higher values recorded for LDH activity in the testicular plasma over the WS could be an indication of loss of LDH from the immature testis sperm into the surrounding medium as a result of damage to the acrosome. This is due to the adverse conditions to which the sperm cells were exposed during the storage period, when the testes were frozen before homogenization. This is in agreement with Upreti *et al* (1996), and Patel *et al* (2000). In adjusting to the new environment,

sperm cells would increase metabolic activities. Dave (1975) and Dube et al (1982) had reported that LDH activity was associated with sperm metabolism.

The leakage from sperm would account for the increase in plasma value (Pursel et al., 1971), with a corresponding reduction in the fertility due to the adverse effect on the quality of

spermatozoa (Stamatiadis et al., 1984).

Dhami and Kodagali (1990) had reported that motility and fertility of post-thawed sperm were negatively correlated with LDH activity of seminal plasma, which was positively correlated with Glutamic Oxalate Transaminase (GOT) and Alkaline Phosphatase (ALP) activities. Thus the leakage of LDH from sperm into plasma, observed in this study indicated a loss in the quality of sperm. LDH could thus be used as an enzyme, whose level in the plasma could reflect the quality of spermatozoa in stored semen. This is in line with the observation of Pursel et al (1971) that the loss of LDH in sperm resulted in a concomitant increase in the seminal plasma, having been established that LDH activity is related to metabolic process in spermatozoa (Dave, 1975; Dube et al., 1982; Patel et al., 2000). Such information would therefore be relevant in the development of semen processing protocols and semen diluents as earlier suggested by Upreti et al (1996).

The higher calcium content of testicular plasma over the washed sperm cell is a

Pointer to the need for calcium by developing sperm cells in the testis. Higher calcium content than normal in seminal plasma has been implicated with sperm abnormality (Bostedt et al., 1976), poor sperm motility (Leonhard-Marek, 2000) and testicular degeneration (Pedroso et al., 1978). The results of this study therefore, implied that the testis environment on its own carries some level of calcium, which has been implicated with the enhancement of spermatogenesis and fertility. This is of importance to farms, where the breeding efficiency is paramount in their management procedures. In view of the emphasis on breeding efficiency, diets given to breeding males are often supplemented in order to improve the semen quality of their males.

Sometimes, some of the extensively managed bulls find their way into breeding

herds of private farmers, who might be tempted to utilize dietary supplementation with calcium as a mineral, for the enhancement of their spermatogenic potential and fertility. Under this circumstance, such farmers should be cautious in order to avoid the partial toxicity of calcium (Leonhard-Marek 2000) due to the implications itemized, above.

CONCLUSION

It could be concluded from this study that when spermatozoa are exposed to adverse conditions, the leakage of LDH from sperm acrosome occurs. This leakage often accounts for the increased LDH activity in the surrounding plasma

Medium. The testis environment Should be determined before the contains a level of calcium, which is utilization of dietary calcium as a means required for spermatogenesis. Such level of enhancing fertility in extensively managed bulls.

REFERENCES

- Blass, G. de., M. Michaut, C. L. Trvino, C. N. Tonus, R. Yunes, A. Darszon and L. S. Mayorga, (2002). The intraacrosomal calcium pool plays a direct role in acrosomal exocytosis. *Journal of Biological Chemistry*. 277 (51): 49326-49331
- Boehringer Mannheim GmbH Diagnostica (1979)
- Bostedt, H., R. Gastauer and R. Hahn, (1976) Enzyme activity and electrolyte concentration in blood and semen plasma from bulls. *Zuchthygiene* 11:2, 83
- Breitbark, H. (2002) Intracellular calcium regulation in sperm capacitation and acrosomal reactions. *Molecular and Cellular Endocrinology* 187: 139-144
- Dave, B. (1975) Bovine follicular fluid and its effect on sperm metabolism. *Dissertation Abstracts International*, B. 36:1-3
- Dhami, A.J. and S.B. Kodagali, (1990) Freezability, enzyme leakage and fertility of buffalo spermatozoa in relation to the quality of semen ejaculated and Extenders. *Theriogenology* 34 (5) : 853- 863
- Dube, G.D., P.K. Pareek, P.K. Dwaraknath and K.K. Vyas, (1982) Lactic dehydrogenase in relation to semen quality. *Indian Journal of Dairy Science* 35(1): 80-82
- Egbunike, G.N. (1980) Changes in Acetylcholinesterase activity of mammalian spermatozoa during maturation. *International Journal of Andrology* 3: 459-468
- Esper. C. R., S. H. Gabaldi, A. Wolf and J. A. Oliveira, (2002). Invitro fertility of golden hamster spermatozoa capacitated with calcium ion channel blockers. *Revista Brasileira de Reproducao Animal*. 26 (3): 204-206
- Field (2000) *Discovering Statistics using SPSS for Windows, Standard Version*. SAGE Publ. London pp. 243-322

- Ho, H. C., K. A. Granish and S. S. Suarez, (2002) Hyperactivated motility of bull sperm is triggered at the axoneme by Ca^{2+} and not cAMP. *Developmental Biology* 205 (1): 208-217
- Jana, K., P. K. Samanta and D. Ghosh, (2002). Dose dependent response to an intratesticular injection of calcium chloride for induction of chemo sterilization in adult albino rats. *Veterinary Research Communications* 26 (8): 651-673.
- Kovacs, E. (1972) The effect of Calcium and Phosphorus levels in the diet on spermatogenesis in the gander. *Baromfiipa* 19 (1): 1-5
- Kovacevic, K., S. Veselinovic, D. Kosarcic, S. Vaseleinovic, M. Jovicin, and I. Sibalic, (1990). Improving the fertility of bulls with supplementary copper. *Veterinarski Glasnik* 44: 12, 1057-1059.
- Leonhard-Marek, S. (2000) Why do trace elements have an influence on fertility? *Tierarztliche Praxis. Usgabe G, GrossTiere Nutztiere* 28(2): 60-65
- Machal, L.; G. Chladek and E. Strakova, (2002) Copper, phosphorous and calcium in bovine blood and seminal plasma in relation to semen quality. *Journal of Animal and Feed Sciences*. 11(3): 425-435
- Patel, K.V., A.J. Dhami, M.K. Chauhan, A.S. Dave, V.P. Vadodaria and Y.G. Dugwekar, (2000) Freezability, enzyme leakage and fertility of bovine spermatozoa. *Indian Journal of Dairy Science*. 53(4): 284-290
- Pedroso, R., F. Kredl, I. Montes, V. Mrva and A. Guesta, (1978) Biochemistry and seminal plasma of sperm count in bulls with reproductive disorders. *Memoria Asociacion Latinoamericana de Produccion Animal* 13:174-175
- Pursel, V.G., L.A. Johnson and R.G. Gerrits, (1971) Distribution of GOT and LDH activities in boar semen after cold shock and Freezing. *Cryobiology* 7: 141-144
- Roussel, J.D. and O.T. Stallcup, (1965) Influence of age on transaminase and phosphatase in male Holstein blood serum. *J. Dairy Sci.* 48: 841 (abstr.)
- Stamatiadis, K., A. Karayannidis and P. Tsakalif, (1984) The Relationship between LDH activity in seminal plasma and Viability of ram spermatozoa. *The male in farm animal reproduction*. Martinus Nijhoff, Boston USA pp. 257-263
- Upreti, G.C., S.R. Payne, D.M. Duganzich, J.E. Oliver and J. F. Smith, (1996) Enzyme leakage during cryopreservation of ram spermatozoa. *Animal Reproduction Science* 41(1): 27-36

Wong Wai Yee., G. Flik, P.M.W. Groenen, D.W. Swinkels, C.M.G. Thomas, J.H.J. Copius-Peereboom, H.M.W.M. Merkus and R.P.M. Steegers-Theunissen, (2001) The impact of calcium magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. *Reproductive Toxicology* 15 (2): 131-136