Relation between washed sperm cells and fluid from White Fulani Genitalia in some enzyme activities and lipid contents

V.A. Togun and G.N. Egbunike²

¹Department of Animal Production and Health, Lagoke Akintola University of Technology, Ogbomoso, Nigeria

²Animal Physiology Laboratory, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Abstract

Lipid contents and enzyme activities were studied in the fluid and washed sperm (WS) from the genitalia of 26 mature White Fulani (WF) bulls of mean live weight 213.96 ± 4.88 kg (range 140-260 kg) in a complete randomized design experiment. Tissue homogenization and biochemical assay method were employed after storing the tissue in deep freeze for 24 hours post slaughter. Total lipid contents did not differ significantly ($P > 0.05$) between WS and fluid of the testis, the caput epididymis and the cauda epididymis but was significantly ($P < 0.05$) higher in WS than the fluid in corpus epididymis and vas deferens. Phospholipid and phospholipid phosphorus contents of WS and fluid did not vary significantly ($P > 0.05$) between WS in the testis and epididymis. Alkaline phosphatase (ALP) activity was higher ($P < 0.05$) in the corpus and cauda epididymal fluid than in the WS. Glutamic Oxalate Transaminase (GOT) activity was significantly ($P < 0.05$) higher in the fluid than the WS in all segments but not in the testis. The generally higher values of lipids in the washed sperm over the fluid the need for lipids as protective cover and as substrates for sperm survival from its formation up to maturation. The generally higher enzyme activity in the fluid is an indicator of damage to sperm acrosome, with corresponding leakage of enzyme into the fluid consequent upon the various stress conditions encountered as sperm traverses the epididymis for the maturation process and finally sojourns in the epididymis.

Keywords: Enzyme activity, lipid content, washed sperm, fluid, bull genitalia.

Introduction

Efficient sperm storage is dependent on the maintenance as well as the survival of the sperm cells. The oxidation of fatty acid by ejaculated ram sperm during incubation in synthetic medium without added substrate has been reported (Hatree and Mann, 1961). Komarck *et al* (1965) reported bull sperm to contain 12% lipid with phospholipids constituting 73.3% of total lipid. The resistance of sperm to cooling was significantly correlated with lipid concentration (Antoyuk, *et al.*, 1982) which decreased with

age but was not affected by strain (Gilman *et al.*, 1977). Leakage of intracellular constituents from sperm have been reported to take place in the epididymis (Jones and Glover, 1975; Ozawa *et al.*, 1987). Increased lipid diffusion through the bull spermatozoa plasma membrane, as a result of progressive removal of seminal plasma by repeated centrifugation and re-suspension in media, has been reported by Wolfe *et al* (2001). According to Johnson *et al* (1969), the lipid content of plasma is lower than in sperm cells, while Douard *et al* (2000) implicated metabolic

process as a factor responsible for the difference between the lipid composition in seminal plasma and spermatozoa. Alkaline Phosphatase (ALP) activity in seminal plasma was higher than in the testis and epididymis (Antonov and Zakhariev, 1977) and also in the washed sperm cells (Stallcup, 1965; Roussel and Stallcup, 1965; Atonov and Zakhariev 1977). Pedroso *et al* (1977) reported significantly negative correlation of ALP activity with percent live sperm but positive correlation with sperm concentration. ALP concentration in genital organs of rats treated with antifertility substances, significantly increase above the control (Nidhi and Jacob 2001). According to Pursel *et al* (1971), Ueckert *et al* (1976) and Nauk *et al* (1991), cooling, freezing and cold shock (adverse conditions) caused loss Glutamic Oxalate Transaminase (GOT) and ALP activities from spermatozoa and concomitant increase of these activities in the plasma. The loss increase with storage time and was significantly negatively with sperm survival rate (Semkov *et al*, 1973), keeping quality (Dhami and Sahni, 1993), and sperm motility (Ueckert *et al*, 1976). According to Semkov *et al* (1973), GOT in seminal plasma was significantly higher than in spermatozoa.

There is paucity of information on the relationship between washed sperm cells and plasma from the various segments of the White Fulani (WF) bull genitalia in enzyme activities and lipid profile before the sperm becomes mature and capable of fertilizing an ovum after ejaculation. This study was therefore carried out to provide information on some lipid contents and enzyme activities of WS and the fluid from the different segments of the WF bull genitalia.

Materials and Methods:

Twenty-six mature White Fulani (WF) bulls of mean live-weight 213.96kg and of weight range

between 140-260kg, extensively managed by nomadic Fulani herdsmen without supplementary feeding programme, were involved in the completely randomized design study. Their testes and epididymides were removed immediately after slaughter and taken to the laboratory for processing and analysis. Each of the reproductive parts was stored in the freezer for 24hrs, and from these, weighed samples were later taken from the testes, the caput, corpus, and cauda epididymides as well as the vas deferens. These samples were each separately homogenized with a pair of scissors for 2 minutes in a Pyrex beaker containing a known volume of physiological saline solution at 200mg/ml. The resulting homogenate was filtered through double layer cheese cloth and from the filtered homogenate, 5mls was taken and centrifuged at 10°C and 400g for 10 minutes (Egbunike, 19980). The supernatant was removed as the fluid from that particular part of the reproductive organ, while the pellet was washed twice in 0.15m NaCl and centrifuged at 10°C and 400g for 10 minutes. The resultant sperm was designated washed sperm (WS) and re-suspended in 2ml physiological saline. Alkaline phosphatase (ALP) activity, total lipid, phospholipids phosphorous and phospholipids contents of the fluid and WS were determined by the colorimetric method of Boehringer (1979). Glutamic oxalate transaminase (GOT) activity was determined by the colorimetric method of sigma (1979).

The data were subjected to statistical analysis of variance using the SPSS computer package (Field 2000).

Results

The mean total lipid, phospholipids phosphorous and phospholipids contents of the fluid corresponding WS from the various segments of the WF bull genitalia are shown in

table 1. The mean ALP and GOT activities are shown in Table 2.

Total lipids, phospholipids phosphorous and phospholipids contents did not differ significantly ($P>0.05$) between WS cells and fluid of the testis, the caput epididymis and the vas deferens. Phospholipid phosphorous contents did not differ significantly between washed sperm cells and fluid of the corpus and cauda epididymides. Phospholipid content did not differ significantly ($P>0.05$) between WS cells and fluid of the corpus epididymis. Total lipids was significantly ($P<0.05$) higher in the sperm than fluid of corpus epididymis while both total lipid and phospholipids were significantly ($P<0.05$) higher in the WS of caudal epididymis than the fluid. The WS contents were however generally higher in both phospholipids phosphorous and phospholipids contents in all the genitalia segments studied.

ALP activity in fluid was significantly ($P<0.05$) higher than in WS of the testis and the corpus epididymis but not in the other parts of the genitalia studied. GOT activity was significantly ($P<0.05$) higher in the fluid of all the epididymal segments than the WS. Values in the testis were not significantly ($P>0.05$) different although the activity in the fluid was marginally higher.

Discussion

The significantly higher values recorded for total lipid in WS over the fluid in the corpus and cauda epididymis, as well as the non-significant difference between the values recorded in the testis and the caput epididymis agreed with the findings of Johnson *et al* (1969), that seminal plasma lipid was lower than in the sperm cell. This agreement is further strengthened by the generally lower values recorded for fluids in all other lipids studied in the different segments of the EF bull genitalia. However, the marginally

higher value recorded in the total lipid content of the testis fluid over the WS can be explained by the fact that the immature sperm cells from the testis were in the process of being fortified against acrosome leakage before leaving the testis. The testis environment contained a large quality of lipid to fortify the newly developing sperm cells against acrosome damage before traversing the epididymal segments (Guyton and Hall, 1996). The marginally higher value in total lipids of the fluid over the WS of the caput epididymis could be the result of the metabolic stress (Douard, *et al*, 2000), to which sperm cells exposed in the attempt to adjust to the caput epididymal environment. These processes would increase the stress on the sperm cells (Dhami, *et al*, 1995; Wolfe *et al.*, 2001; Dhanju *et al.*, 2002). The result was the leakage of intracellular substance (Ozawa *et al.*, 1987) from the sperm. With a concomitant increase in the plasma, hence the plasma value represented the result of additional contribution from sperm cells.

The significantly ($P<0.05$) higher values for WS in total lipids and phospholipids contents at the cauda epididymis would be due to the remodeling process, which the sperm plasma membrane has to undergo during the sperm sojourn and maturation in readiness for final capacitation protocol. Thus, less lipid is lost in the cauda epididymis.

The marginally value of ALP activity in fluid over WS, in most of the segments, is in agreement with the report in exotic cattle (Stallcup 1965; Antonov and Zakhariae, 1977). It is indicative of loss of ALP through the sperm acrosome into the fluid brought about by metabolic activity. The significantly higher ($P<0.05$) values in the fluid GOT over the WS in all segments of the epididymis is in support of the report of Pursel *et al* (1971). It explained the fact the sperm metabolism increase as sperm continue its

Table 1
Relation Between Washed Sperm and Fluid From Different Parts of Bull Genitalia in Lipid Contents and Enzyme Activities (Mean $\pm$ Sem)

GENITALIA SEGMENT	TOTAL LIPIDS (MG)		PHOSPHOLIPIDS PHOSPHOROUS (MG)		PHOSPHOLIPIDS (MG)	
	Fluid (adjusted to 500 $\times 10^6$ sperm cells)	Washed Sperm (500 $\times 10^6$ sperm cells)	Fluid (adjusted to 500 $\times 10^6$ sperm cells)	Washed Sperm (500 $\times 10^6$ sperm cells)	Fluid (adjusted to 500 $\times 10^6$ sperm cells)	Washed Sperm (500 $\times 10^6$ sperm cells)
Testis	40.73 $\pm$ 8.53	38.49 $\pm$ 4.99	10.34 $\pm$ 0.84	10.83 $\pm$ 8.531.05	253.36 $\pm$ 21.01	269.31 $\pm$ 26.06
Epididymis:						
Caput	19.54 $\pm$ 8.532.05	17.02 $\pm$ 2.72	21.65 $\pm$ 0.84	24.44 $\pm$ 2.96	541.14 $\pm$ 21.07	610.85 $\pm$ 74.04
Corpus	36.17 $\pm$ 6.32b	85.74 $\pm$ 11.37a	34.61 $\pm$ 2.94	35.05 $\pm$ 3.29	864.62 $\pm$ 73.62	873.88 $\pm$ 82.44
Cauda	17.63 $\pm$ 4.09b	33.98 $\pm$ 4.94a	7.14 $\pm$ 2.93	7.30 $\pm$ 5.31	73.30 $\pm$ 66.37b	188.72 $\pm$ 13.95a
Vas Deferens	84.15 $\pm$ 17.59	52.08 $\pm$ 11.49	46.55 $\pm$ 7.39	51.26 $\pm$ 4.68	1162.71 $\pm$ 185.15	1281.49 $\pm$ 116.96

^{ab}Means on the same row of each lipid type with different superscript differ significantly.

Table 2:
Enzyme activities in fluid and Washed Sperm Cells from the Different segments of White Fulani bull Genitalia (Mean $\pm$ Sem)

GENITALIA SEGMENT	Alkaline Phosphatase (ALP)		Glutamic Oxaloacetate transaminase (GOT)		Washed Sperm
	Fluid	Washed Sperm	Fluid	Washed Sperm	
Testis	40.73 $\pm$ 8.53	38.49 $\pm$ 4.99	10.34 $\pm$ 0.84	10.83 $\pm$ 8.531.05	253.36 $\pm$ 21.01
Epididymis:					
Caput	19.54 $\pm$ 8.532.05	17.02 $\pm$ 2.72	21.65 $\pm$ 0.84	24.44 $\pm$ 2.96	541.14 $\pm$ 21.07
Corpus	36.17 $\pm$ 6.32b	85.74 $\pm$ 11.37a	34.61 $\pm$ 2.94	35.05 $\pm$ 3.29	864.62 $\pm$ 73.62
Cauda	17.63 $\pm$ 4.09b	33.98 $\pm$ 4.94a	7.14 $\pm$ 2.93	7.30 $\pm$ 5.31	73.30 $\pm$ 66.37b
Vas Deferens	84.15 $\pm$ 17.59	52.08 $\pm$ 11.49	46.55 $\pm$ 7.39	51.26 $\pm$ 4.68	1162.71 $\pm$ 185.15

^{ab}Means on the same row of each lipid type with different superscript differ significantly.

maturation process, which becomes very noticeable as the sperm approaches and sojourns in the cauda epididymis. Maturation process increases metabolism, which could result in damage to acrosome and leakage of GOT from the sperm into the surrounding fluid. The GOT of the fluid is a result of contribution by epididymal fluids (Eugenia and Schindler, 1965; Stallcup, *et al.*, 1968) as well as leakage from the sperm, during the maturation processes.

Conclusion

The results of this study indicate that WS from the various parts of the genitalia contained higher quantities of the various lipids than the surrounding milieu. This confirms the need for lipids to serve as substrates as well as a protective cover for sperm cells during the maturation process. The generally higher enzyme activities in the fluid is an indication of the leakage of these enzymes as a result of sperm acrosome damage consequent upon the acclimatization and maturation process.

References

- Andtonov, S. and Z. Zakhariev (1977) Alkaline phosphatase activity in semen, testis and accessory sexual glands of bulls. *Veterinarnomeditsinski Nauki* 14:3, 61-67.
- Antonov, V.S., Bezlyudnikov, L.G., Il'nskaya, T.P. and L.V. Zhukovskaya (1982) Improving methods of freezing boar semen. *Sbornik Trudov, Belorusskii Nauchnoissledovatel'skii Institut Zhivotnovodstva*. 22:36-38.
- Boehringer Mannheim gmbH Diagnostica (1979)
- Dhami, A.J., Sahni, K.L. and G. Mohan, (1995) Effect of various extenders and Additives on deep freezing, enzyme and fertility of bovine semen under tropical climate. *Indian Journal of Animal Sciences*. 65(1) 20-27.
- Dhanju, C.K.m, Cheema, RS. and S.P. Kaur, (2002) Effect of freezing on biochemical constituents of sperm membrane extracts and seminal plasma of buffalo bulls. *Annals of Biology*. 18:1 71-76
- Douard, V., Hermier, D and E. Blesbois, (2000) Changes in turkey semen lipids. During in-vitro storage. *Biology of Reproduction*. 63(5): 1450-1456.
- Egbunike, G.N. (1980) Changes in Acetylcholinesterase Activity of Mammalian Spermatozoa during Maturation. *International Journal of Andrology* 3:459-468.
- Field, A. (2000) Discovering Statistics using SPSS for Windows. Standard Version. SAGE Publ. London p243-322.
- Gilman, D., Vasin, U.T. and V. P. Kolesen, (1977) Indices of lipid metabolism and their relation to economically valuable characteristic of pigs. *Vesstsi Akademii Navuk BSSR., Ser'ia Kagaspadarchykh, Navuk* 3:106-110.
- Guyton, A.C and J.E. Hall, (1996) textbook of Medical Physiology (9th Edition W.B. Saunders Company. Philadelphia, pp.

-
- Hatree, E.F. and T. Mann, (1961).** Phospholipids in ram semen. *Biochem. J.* 80:464-476
- Johnson, L.A., Gerritis, R.J. and E.P. Young, (1969)** Quantitative analysis of Porcine pholipids as affected by frequency of ejaculation. *J. Reprod. fertil.* 19:95-102.
- Jones, R. and T.D. Glover, (1975)** Interrelationships between spermatozoa. the epididymis and epididymal plasma. The biology of male gamete. Academic Press Inc. (London) Limited, London. U.K. pp 367-384.
- Komaraek, R.J., PiCkett, B.W., Gibson, E.W. and R.G. Jensen, (1964).** Lipid Composition.
- Nauk, V.A., Boronchuk, G. V. Granach, V. G and I.V. Balan, (1991)** maker Enzyme activity on the sperm plasma membrane of farm livestock and Fowl after freezing. *Izvestiya Akademiyi Nauk SSR Moldova. Biologicheskije i Khimicheskije Nauk* 1:45-48.
- Nidhi, S. and D. Jacob, (2001)** Inhibition of fertility and functional alteration in the Genital organs of male Swiss albino mouse after administration of Calotropis procera flower extract. *Pharmaceutical Biology* 39(6):403-407.
- Ozawa, Y., Ashizawa, K., and K. Okauchi, (1987)** Changes in elemental Concentrations around/on the rat sperm plasma membrane during Maturation in the male genital tract. *Gamete Research* 18 4:311-318.
- Pedroso, R., Kredl, F., Montes, Mrva, V. and A. Cuesta, (1977)** Biochemistry of seminal plasma and sperm count in bulls with reproductive disorders Memorial Association Latinoamericana de production Animal 13:174-175.
- Pursel, V.G., Johnson, L.A and R.G. Gerrits, (1971)** Distribution of GOT and LDH activities in boar semen after cold shock and freezing. *Cryobiology* 7:2-3), 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 (Astr.).
- Semkov, M., Zahriev, Z. and I. Ivanov (1973)** Studies on the transaminase Activity of bulls semen. L 1. Glutamic - Oxalate - transaminase activity in Certain qualitative values of the semen. *Comptes Rendus de L'Academie Agricole Georgi Dimitrov* 6(4): 387-388.
- Sigma Technical Bulletin No. 505 (1979).** Sigma Chemical Company. USA
- Stallcup, O.T. (1965)** Acid andd Alkaline phosphatase activity in bovine semen as Related to fertility. *J. Dairy Sci.* 48: 752-754.
- Ueckert, H.m, Mudra, K., Schmidt, D. and C. Kunze, (1976)** Freezing of bar Semen. 4. Estimation of viability in vitro storage. *Archiv fur Tierzucht* 19(3): 141-148.

Wolfe, C. A., James, P. S., Gunning, A. P., Shab, Ladha, Christova, Y and R. Jones (2001). Lipid dynamics in the plasma membrane of ram and bull spermatozoa after washing and exposure to

macromolecules Bovine Serum Albumin (BSA) and Polyvinylpyrrolidone (PVP). *Molecular Reproduction and Development* 59:3, 306-313.

(Received 6th Oct. 2004; Accepted 9th Jan., 2007).