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Original Article

Effect of aflatoxin b1 dietary exposed male Wister rats; spermatogeneis, dynamics, kinetics and loss of rat epididymal sperm proteins

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ABSTRACT

Several studies have linked Infertility associated spermatotoxicty/spermato-mutagenesis of Adult male Wister dietary exposed to aflatoxin B, the dynamics may be as a result of chromosomal aberration or simply by its anti-spermatogenesis properties. To evaluate the protein composition of epididymal fluid and sperm extracts of the rats using a two-dimensional gel electrophoresis. Epididymal luminal fluid from the corpus and cauda regions of male animals rendered infertile by the anti spermal and mutagenic properties of aflatoxin B1 possessed a certain protein (contraception-associated protein 1, CAP1) which has a molecular mass of - 25 kDa and isoelectric point (pl) of 5.8; although it wasn't found in fluids, but was present in sperm, from fertile rats. Infrared matrix-assisted laser desorption/ionization mass spectrometry indicated that the molecular mass of CAP1 was 20 420 ± 120 daltons. Homology of the hormone from a known insect *(Acheta domesticus)* was carried out by analysing 17 amino acids which demonstrated 49 percentile homologous linkage Silver-recolored gels demonstrated the nearness of CAP1 in more prominent sums in cauda than in corpus liquid from the dietary uncovered creatures, while liquid from the rete testis needed CAP1. In vitro hatching periods of tissue from the caput, corpus, and cauda epididymidal areas with [35S] methionine gave no insight that CAP1 was a discharge result of the epididymal epithelium. The nonattendance of CAP1 from luminal liquid acquired from the sperm-drained corpus epididymidis of the dietary uncovered rats recommended this had a spermatozoa root. CAP1 was available in spermatozoa from the caput epididymidis however not from the rete testis in control creatures. less CAP1 was available in cleanser concentrates of cauda sperm from the dietary uncovered rats than in sperm from control creatures, proposing against spermatogenesis related dislodging of protein from sperm to liquid. The nearness of CAP1 in epididymal liquid, presumably beginning from spermatozoa, proposes that aflatoxin B1 initiate barrenness by a pre-previous protein in preparation.

Keyword: Aflatoxin, Speematotoxicity, Contraception associated proteins, Acheta domestica

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INTRODUCTION

Sperm development or treating limit are not natural for sperm themselves but rather they are gained amid their travel through the epididymis after the testis [1]. The epididymis is made out of a since quite a while ago convoluted tube, three primary areas are perceived for it, to be specific caput, corpus and cauda, and the epithelial cells of these particular epididymal locales make a one of a kind luminal liquid environment by emitting proteins and different liquid segments [2]. In this novel and persistently evolving milieu, spermatozoa bit by bit get their forward motility and limit with respect to preparation, and after that they are put away in a quiet state in the caudal epididymis. These liquids are especially appropriate to shield spermatozoa from various sorts of physiological and biochemical hostilities [3]. In spite of the fact that the part of the epididymis in sperm development has been entrenched four decades prior [1], just a couple of particular atom of epididymal source had been exhibited to be connected to this development procedure [4]. Notwithstanding the development in the epididymis, spermatozoa should in this manner experience various film changes before they communicate effectively with the oocyte, these procedures are by and large alluded to as capacitation and ordinarily occur inside the female conceptive tract [5,6]. Capacitated spermatozoa hence experience the acrosome response (AR) which brings about the discharge and actuation of acrosomal catalysts. The sperm cell is then ready to tie and infiltrate the zona pellucida and to wires with the oocyte plasma film [7–9]. Capacitation involves a progression of procedures, for example, adjustments in sperm surface protein dispersion, modifications in plasma layer qualities, changes in enzymatic exercises and tweak of intracellular constituents [10,11]. In spite of the way that the wonder of capacitation has been found over half century prior [5,12–14] and that much advance had been made in distinguishing sperm occasions required in capacitation, couple of particular atoms of epididymal birthplace have been recognized to be specifically required in this procedure in vivo. Already, we reported a newfound rodent epididymis caudal locale particular quality named CAP1 [15].

Aflatoxins are optional metabolites of two normal form species, Aspergillus flavus and Aspergillus parasiticus, known for the sullying of creature sustains and additionally staple sustenances, for example, groundnuts, corn, rice, flavors and seeds (1,2). This pollution is normal in creature encourage and additionally sustenance supplies in creating nations, where nourishment generation innovation is not grew enough to maintain a strategic distance from the conditions helpful for shape development (2). It is evaluated that around 4.5 billion individuals living in creating nations are routinely presented to this poison in uncontrolled fixations (2). In 2010, a study directed in Texas found that one-fourth of the members had exposures over the everyday restrain set by the FDA (11). This demonstrates even in more created ranges where aflatoxin cutoff points are set, huge introduction still happens. Aflatoxins have been observed to be a critical donor to numerous intense and ceaseless wellbeing issues, including liver malignancy, dietary impedance, and immunosuppression. A study in 2010 assessed that somewhere around 4.6% and 28.2% of liver growth cases worldwide can be credited to aflatoxin introduction (9). Far reaching assessments of the impacts of aflatoxins on other worldwide wellbeing issues have not been as altogether researched.

While the part of aflatoxins as a cancer-causing agent in liver growth has been all around concentrated, all the more as of late it has been researched as an immunosuppressant in clinical studies. In an investigation of Gambian kids, those whose blood tests yielded a positive result for aflatoxin introduction indicated diminished centralizations of safe proteins in salivation (4). Different studies have found that aflatoxins negatively affect cell-intervened resistance and on generation.

The current study reports that, aflatoxin B1 has a negating effect on CAP1 protein, the role of this protein in sperm capacitation has also been characterized. Furthermore strong evidence shows that CAP1 protein like the HongrES1 is a novel and critical molecule in regulation of sperm capacitation and male fertility.

MATERIALS/METHODS

Animals: 30 Male and 30 female rats (Wister (albino); males, 350-470 g Of reproductive age females, 250-350 g) were obtained. Animals were kept at normal room temprerature °C under a 12L:12D cycle and were provided with water and rat standard diet (Altromin GmbH, Lage,) ad libitum. The experimental studies were conducted in accordance with the ethics of the usage of laboratory animals.

Treatment of Male Rats: Aflatoxin B1 (Sigma,) was given by oral gavage at a dosage of (400 mg)/kg per day for a month in a volume of 0.14 ml vehicle (demineralized water containing 0.2% per 100g body mass.. Fertility of male rats after Aflatoxin B1 treatment was assessed by mating

with females as described in a previous study [2]. The fertility of the male rats used in

Collection of Fluid and Spermatozoa from the Epididymis and Rete Testis: At the end of drug administration, the animals were killed by CO 2 asphyxiation and the epididymides were removed. The cauda region and ductus deferens were cleaned of fat and blood vessels, and cauda epididymidal contents were collected by retrograde perfusion of PBS (pH 7.2; Gibco BRL, Berlin, Germany) through the vas deferens [4]. Spermatozoa were separated from epididymal fluid by centrifugation through 5 ml Ficoll (5% [w:v] in PBS; Sigma) for 5 min at 800 g. The sperm pellet was resuspended in a small volume of PBS and stored at -20 0C or used directly for sperm protein extraction. The diluted epididymal fluid above the Ficoll was stored at -20°C for analysis. Fluid from more proximal parts of the epididymis was collected by cannulation of parts of the corpus or caput epididymidal tubule with polyvinyl chloride tubing (0.5 mm i.d.; 0.8 mm o.d.; Dural Plastics, Dural, NSW, Australia) drawn out over a flame to a fine diameter, followed by perfusion with PBS and collection of the fluid at a more proximal site of the tubule. For collection of caput sperm, the caput epididymidis was decapsulated, cleaned of blood vessels, washed, and cut with fine scissors into small pieces to release the sperm in 1 ml PBS. The sperm suspension was transferred to an Eppendorf tube, and tissue debris was pelleted for 11 sec and then 14 sec at 500 x g. The sperm in the supernatant were washed through Ficoll (see above). Testicular fluid was obtained 18 h after ligation of the efferent ducts [7] by puncture of the rete testis with a drawnout, sharp glass capillary tube. Rete testis sperm were separated from the fluid by a Ficoll wash (see above).

Preparation of Sperm Extracts: Detergent-soluble proteins from spermatozoa were prepared by resuspension and incubation of the sperm pelletin PBS (pH 7.2) containing 5 mM Pefabloc (Boehringer, Mannheim, Germany) and 40 mM N-octyl-3-D-glucopyranoside (NOBG; Sigma) for 1 h at room temperature. The suspension was cleared of sperm fragments by centrifugation at 20 000 X g for 30 min (Ole-Dich centrifuge, Hvidore, Denmark), and the supernatant was dialyzed against 20 mM (NH4)2CO 3 (pH 8.2) for 24 h with at least three changes of dialysis buffer. The protein content of sperm extracts and epididymal fluid was measured by the method of Lane et al. [8].

Two-Dimensional SDS-PAGE: Two-dimensional (2D) SDS-PAGE analyses of luminal fluid and sperm extracts were performed using a modification of the method of O'Farrell [9]. Samples containing 75 RIg protein were denatured and reduced by addition of 20-30 Rl solubilization buffer (9.5 M urea, 2% [v:v] Nonidet P-40, 2% [v:v] ampholytes pH 3-10 [Pharmacia, Freiburg, Germany], 5% [w:v] -mercaptoethanol). They were subjected to isoelectric focusing (IEF) on 1.5-mm (i.d.) x 10-cm polyacrylamide gels containing 4% (v:v) ampholytes pH 5-7 and 1% (v:v) ampholytes pH 3-10. The anodic and cathodic buffers were, respectively, 25 mM H3PO4 and 50 mM NaOH. Samples were focused at 400 V for at least 14 h followed by 2 h at 800 V. The pH gradient was determined by using standards of known p (IEF-Mix 3.6-6.6; Sigma). After the first-dimension run, gels were extruded from the glass tube into 1 ml of sample buffer (0.125 M Tris-HCl pH 6.8, 4% [w:v] SDS, 20% [v:v] glycerol, 10% [w:v] -mercaptoethanol, 0.05% [w:v] bromophenol blue), equilibrated for 15 min, and then used for the second dimension or frozen at -700C until run. The second dimension was run on a 12.5% SDS-PAGE gel [10]. Molecular weight markers (LMW; Pharmacia) were also separated to allow molecular mass determination of the resolved proteins. Proteins were visualized by silver staining according to the procedure of Rabilloud et al. [11]. The relative quantitation of proteins was performed using a laser densitometer (resolution of beam 55 Rim) and appropriate software (Gelscan; Pharmacia), and was expressed in arbitrary units representing area under the curve (AUC) of the scan.

Preparative Electrophoresis: Cauda epididymidal fluid from dietary aflatoxin B1 treated animals was a source of an adequate amount of contraception nassociated protein 1 (CAP1) for amino acid sequence analysis and infrared matrix-assisted laser desorption/ionization mass spectrometry (IR-MALDI-MS; see below). Electrophoresed proteins with nonalkylated cysteines were electroblotted onto polyvinylidene fluoride (PVDF) membranes (Immobilon-P; Millipore, Bedford, MA) in a semi-dry blotting apparatus (Multiphor II; Pharmacia) in gel transfer buffer (48 mM Tris-HCl, pH 8.5; 39 mM glycine, 0.037% [w:v] SDS, 20% [v:v] MeOH). The PVDF membrane was stained with Coomassie blue (45% [v:v] MeOH, 10% [v:v] acetic acid, 0.1% [w:v] Coomassie blue R-250) for 5 min and washed for 15 min (20% [v:v] EtOH, 8% [v:v] acetic acid). The blots for MALDI analysis were incubated in a matrix instead of being stained.

In Vitro Secretion of P5S]Methionine-Labeled Proteins from Epididymal Tissues: Protein synthesis was performed as described by Moore et al. [12]. Explants (around 1 mm3, -10 mg) were cut from the caput, corpus, and cauda epididymidis and incubated for 2 h at 35°C with 5% CO2 in 100 il

DMEM without methionine (Dulbecco's Modified Eagle's medium; ICN, Eschwege, Germany) pH 7.2 (buffered with 25 mM Hepes). The tissue was transferred to 100 gil fresh medium containing 50 ICi [3 5 S] methionine (Hartmann Analytics, Braunschweig, Germany) for 5 h at 35°C. Tissue was removed from the medium, washed, and centrifuged at 2000 x g for 5 min. The incubation media were subjected to 2D SDS-PAGE after free [3 5 S] methionine had been removed by a desalting step with Bio-spin 6 columns (Bio-Rad, Mtinchen, Germany). After the electrophoretic separation, the gels were impregnated with a solution of 2,5-diphenyloxazole (PPO, 22% [w:v]) in dimethylsulfoxide (DMSO) overnight after three 20-min incubations in fresh DMSO. Precipitation of PPO was achieved by shaking the gel for 1 h in water. Gels were vacuum dried and exposed to xray films (Sigma) for 2 wk.

Amino Acid Sequence Analysis: CAP1 separated by 2D gel electrophoresis from cauda epididymidal fluid from ornidazole-fed rats was transferred onto a PVDF membrane, stained with Coomassie blue (see above), and sent to TOPLAB, Gesellschaft fur angewandte Biotechnologie mbH (Miinchen, Germany) for sequencing. CAPi was enzymatically digested by incubation of the protein in the membrane with 1 g endoproteinase LysC (Boehringer) for 8 h at 37°C. The supernatant was separated by HPLC (Supersher 60 RP selectB; Merck, Darmstadt, Germany) using an acetonitrile gradient of 0-60% (v:v) generated by combining 0.1% (v:v) trifluoric acid (TFA) in acetonitrile and 0.1% (v:v) TFA at a flow rate of 300 jLl/ min (over 60 min). The sequence of a 17-amino acid fragment was determined with an automatic sequencer Portion 3600 (Beckman Instruments, High Wycombe, UK).

Sperm morphology and count

Sperm cell isolation: Sperm cells were collected from the vas deferens of the sacrificed rats, the following methods where used Sacrifice the Rat.

Locate the Vas deferens

Ligate both extremities of the Vas deferens to length of about 36mm minimum

Cut both extremities

Add 6µl of normal saline already adjusted to body temperature to a petri dish

Place the Ligated Vas deferens on the petri dish with normal saline

Tease the Vas deferens to allow the sperm cells diffuse

View under the microscope for Motility.

MOTILITY OF SPERMATOZOA

There is a good correlation between sperm motility soon after ejaculation and male fertility and there is evidence that motility is a very sensitive indicator of sperm function. The motility of the spermatozoa must always be evaluated with regard to three variables:

Progressive (or qualitative) motility. usually expressed in terms of a mean Progressive motility score, Progressive Motility (PM), (ii) Non Progressive Motility (NPM), (iii) Immotile (IM),Percentage of motile spermatozoa usually presented with 5% intervals since the precision is not higher; and

Spermatozoa can show good motility and viability in the seminal plasma 24 hours after ejaculation but in some semen samples the motility declines much faster

With a micro Pasteur pipette take a drop of sperm cell from the petri dish

Dispense the Sperm cell on a Grease free clean slide

Cover with a transparent cover slip

View under the microscope (X10 and X40) objective lens

Score the Motility in percentage according to their motile nature as progressively motile, Non progressively motile and Immotile

VITALITY TESTING

It is important to distinguish between live and dead spermatozoa when there is < 4-0% motile spermatozoa. This can be done with a supravital stain like eosin Y. Many laboratories are not equipped with a negative phase-contrast microscope and in this case the following technique can be used. One volume of semen (usually a drop) is milted with two volumes of an eosin Y solution (1% in distilled water). After 30 seconds three volumes of a 10% nigrosin solution (in distilled water) are added and the sample is mixed. A thin smear is then made immediately and air dried. Such a smear can be examined under normal oil immersion microscopy (100 x objective). Live spermatozoa are unstained (white) and the dead ones are red. [1, 3, 10] This usually done in circumstances where the percentage of immotile cells are higher to determine the live cells from the dead ones

MORPHOLOGY

To assess properly the sperm morphology one must employ a staining technique which visualizes not only the head (incl acrosome) but also the midpiece. protoplasmic droplet (if present) and tail. Acceptable staining techniques are for example the slight modification of the Papanicolaou. The Blom, and the Bryan-Leishman techniques. [10, 11] In the past, most studies have been restricted to the morphology of the sperm head. Furthermore. in some publications immature germ cells have

been included in defining the frequency distribution of the spermatozoa. This is unfortunate as illustrated by the following example. If a semen sample contains30% 'immature cells', *20"1.*'oval heads'i etc.. the observer has actually counted 70 sperm beads (= 70 spermatozoa) plus 30 'immature cells'. The true frequency of 'oval heads' per 100 *spermatozoa is* then 29% (20/70 = 0.29). It is therefore difficult to compare results from such studies with those in which 'immature cells' were not included in the frequency distribution of 'spermatozoa'.In the present review the term 'normal' morphology does not refer to anything other than 'a configuration which presently is defined as normal for head, midpiece, *and* tail'. To make comparisons between different laboratories possible, a common technique for assessment is necessary [11, 10] .Scoring can be done in percentage and all graded as Normal or Abnormal cells Normal cells has a Head, Axonem, mid piece and Tail, Abnormal Sperm cells are (Big headed, Headless, Tailess, Bulgy midpiece, Curved tail, Joined head)

RESULTS

CAUDAL AND RETE SPERMATOZOA

Two-dimensional separation of proteins in luminal fluid from the corpus and cauda epididymidis revealed many changes in the protein composition of the fluids due to dietary Aflatoxin B1. Our studies concentrated on a prominent protein with a molecular mass of -25 kDa and a pI of 5.8 (named contraception-associated protein 1, **CAP1)** that was present in the corpus and cauda epididymidal fluid of Aflatoxin B1-treated rats but was not detected in fluid from the control animals (plate. 1 and 2). A similar molecular mass for CAP1, protein 2, and protein 3 (proteins 2 and 3 from the nomenclature for cauda epididymidal fluid proteins separated by 2D gel electrophoresis [13]) was found in 2D gels: 23.4 ± 0.43 kDa (mean ± SEM). The pIs of protein 2 (5.7 ± 0.05; mean ± SEM, n = 7) and protein 3 (6.1 ± 0.06) tended towards a basic than the PI of CAP1 which I (5.8±0.05) Measurements of the absorbance of these three silver stained protein spots by a gel scanner revealed a correlation between their presence in the fluid and the treatment received by the rat (Fig. 3). The absorbance of protein 3 in cauda epididymidal fluid from control fertile rats was about 60% greater, and statistically significantly higher ($p < 0.01$, n = 7), than that of protein 2 (ratio protein 3:protein 2 of fertile rats 1.66 0.27 vs. infertile rats 0.46 ± 0.13; mean + SEM; n = 15). In Aflatoxin B1 animals, protein 3 decreased concomitantly with the appearance of CAP to only half the level of protein 2 (plate 1, 2). As observed CAP1 was absent from rete testis fluid (RTF) from control and Aflatoxin B1 fed animals (Fig. 4) and from caput fluid from control rats (not shown). Proteins 3 and 2 were detectable in RTF which appeared as a faint spot.

SPERM CELL MORPHLOGY AND COUNT

The results revealed the deleterious effect of Aflatoxin B1 on sperm cell quality and spermatogenesis on the male rats, there was an observable significance with the total sperm cell count with the control subject having an increased sperm cell count of 281.1±10.1_x10^6cells/mm^3 as compared with those dietary exposed to Aflatoxin B1 with a total SEM of 125.3± x10^6cells/mm^3 with a significance of (0.00) P=(<0.05), there was a decreased progressive motility from the control animal compared to the dietary fed animals (81.3±1.9, 57.3±3.1). Apart from spermatogenesis, dietary fed rats with Aflatoxin B1 had abnormal spermatozoa, ranging from headless, tailless and abnormal axonem formation, this proves that Aflatoxin B1 has a toxic effect on sperm cell formation not only affect CAP1 associated with contraception, for the control subject normal morphology were (86.1±1.3, 14.0±1.3 as to the study subject 55.3±2.6/44.6±2.6) P=(<0.05) which is shown in tables 1 and 2, and plate 4,5 and 6).

DISCUSSION

Two-dimensional separations of epididymal proteins in the luminal fluid of the corpus and cauda epididymidis from ornidazole-treated rats revealed differences in fluid from control, fertile males. One major protein in particular was reproducibly present (in this paper termed contraception associated protein 1 or CAP1) that was not found in any of the fluids of control rats so far reported in the literature. The protein had a molecular mass of 23-25 kDa according to its electrophoretic mobility and a pI of 5.8 by IEE Proteins 2 and 3 (nomenclature in [13]) migrated the same distance as CAP1 in every run in the second dimension but had slightly more basic pIs (5.7 and 6.1, respectively). The gel-electrophoretic pattern of these three proteins is characteristic of isoforms of the same protein, which are often separated in 2D gels as a series of protein spots at the same molecular weight with different pI. Densitometric evaluation of these proteins showed a treatment-related change in their staining on the 2D gels. Whereas control epididymal fluid did not contain CAP1, it contained threefold more protein 3 than that of the treated animals. The amount of protein 3 was higher than that of protein 2 in the control

Since proteins that possess sugar residues or fatty acids cannot be saturated with SDS, the determination of their molecular masses with

marker proteins is inaccurate [15, 16]. A prominent example is a sequenced and biochemically well-characterized sperm surface glycoprotein, SMemG [17]. Its apparent molecular mass has varied from about 37 kDa [18] to 32 kDa [19], 26 kDa [20], and 16 kDa [17] because of discrepancies due to the sugar residues of this protein [21]. To circumvent these obstacles, the molecular mass of CAPI was determined by IR-MALDI mass spectrometry, a technique described by Bahr et al. [22] and Hillenkamp et al. [23]. The MALDI analysis was carried out directly after blotting of CAP1 onto a PVDF membrane and incubation in the infrared matrix succinic acid [24, 25]. Measurements with this method generated a molecular mass of 20 420 120 daltons. This is 4000-5000 daltons lower than the molecular mass estimated from the electrophoretic runs in the second dimension and is probably indicative of glycosylation. It should also be noted that the mass determined by IR-MALDI-MS could be too high, since cysteine residue were not alkylated and Eckerskorn et al. [25] have recently demonstrated the importance of quantitative alkylation prior to gel electrophoresis for correct mass determination. Enzymatic digestion of CAP1 and partial amino acid sequence analysis of a derived 17-amino acid sequence gave no hints for a relationship with other already fully characterized proteins from the epididymis or other tissues of the rat. Comparisons between a 17-amino acid sequence from CAP1 with sequenced proteins in the EMBL library revealed a 49% homology to a diuretic hormone from the house cricket *(Acheta domesticus).* Interestingly, Osterhoff et al. [26] reported cloning of a human epididymis-specific mRNA (HE6) coding for a seven-transmembrane receptor that showed closest similarity to the diuretic hormone receptor from *Acheta domesticus.* The absence of CAPI from the luminal fluid of control rats, a protein with the same gel-electrophoretic coordinates was found on epididymal sperm in Aflatoxin B1 treated and control animals. The similarity of these proteins from individual 2D gels of the two sources was difficult to ascertain using only pI and electrophoretic mobility values; and in the absence of sequence data or specific antibodies, identity cannot be proved. A similarity of size and charge was demonstrated by joint electrophoretic separation of proteins from epididymal fluid and sperm extracts from control and vehicle-treated fertile rats. The protein from control sperm appeared at the exact location anticipated for CAPI found in fluid from treated males, and when co-electrophoresed, the protein from both sources presented only one protein spot.

The absence of CAP1 from luminal fluid of ornidazole-treated efferent duct-ligated rats suggested that CAPI present in epididymal fluid of ornidazole-fed rats originates from spermatozoa. Proteins 8 and 9 were also absent from the electrophoretic profile of efferent duct-ligated aflatoxin B1 dietary fed rats, indicating their origin from spermatozoa. The absence of CAP1 from testicular sperm, however, suggests either that it is of proximal epididymal/ efferent duct origin or that it may be derived by modification of preexisting testicular sperm proteins of differing molecular weight and charge. CAP1 was detected in a higher amount on control cauda sperm than on sperm from treated rats in which the protein is present in luminal fluid. This suggests a loss of CAP1 from spermatozoa and transfer to the fluid in the lumen of the treated rats.

CONCLUSION

Oral dietary exposed rats to aflatoxin B1 leads to a specific release of a protein (here contraception associated protein 1, CAP 1) from spermatozoa into the fluid of the corpus and cauda epididymidis. Comparison of the protein profiles from fluid and sperm suggests an association between the presence of CAPI and the capacity of sperm to fertilize an egg. Whether the decreasing amount of this protein on sperm is responsible for reduced fertility, and what the role of this protein in fertilization remains to be investigated.

CONFLICT OF INTEREST

There was no conflict of interest during this study.

ACKNOWLEDGEMENT

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Table 1. SEM mean Motility and Sperm cell count distribution across the study and control subject

	CONTROL	*TEST*	*t*	*sig*
PM %	81.3±1.9	57.3±3.1	5.150	0.00
NPM %	7.3±1.5	14.6±1.6	3.214	0.06
IM %	11.3±0.9	28.0±2.7	-5.493	0.00
TSC x10^{6}cells/mm^3	281.1±10.1	125.3±10.5	0.856	0.00

Key: PM=progressive motility, NPM=non progressive motility, IM=immotile, TSC=total sperm cell count, P=<0.05

Table 2. SEM mean morphological analysisdistribution across the study subject and control

	Control	*Test*	*t*	*sig*
Normal %	86.1±1.3	55.3±2.6	10.64	0.00
Abnormal %	14.0±1.3	44.6±2.6	-10.64	0.00

(A)

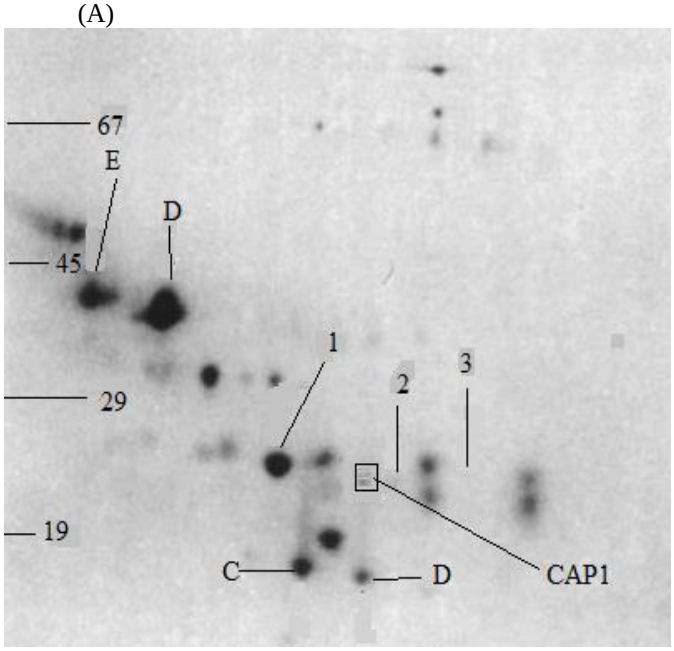

Plate (1) 2D SDS PAGE protein separation from the corpus epididymis fluid, Ofthe rats dietary fed on Aflatoxin B1, from the proteins shown, 1, with 2 slightly shown,There is the CAP1 protein which is in a box approximately 25kda from the rats under dietary exposure to Aflatoxin B1

(B)

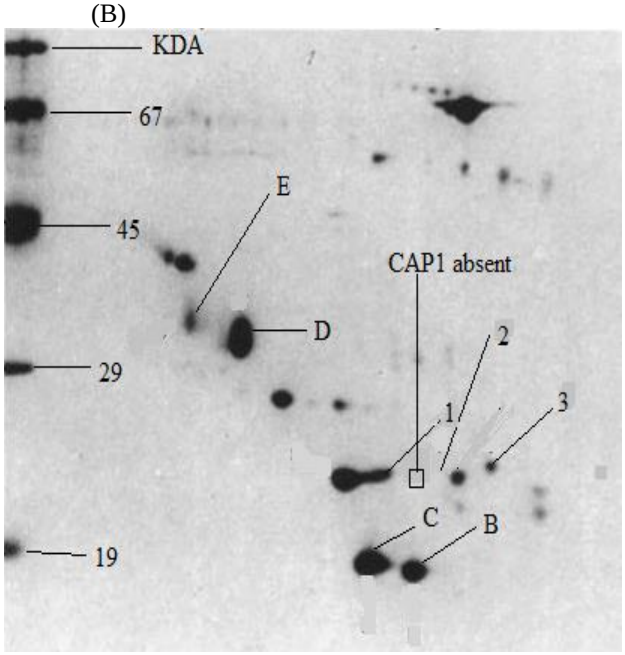

Plate 2. 2D SDS PAGE protein separation from the corpus epididymis of the control subject, there was no presence of the CAP1 proteinsbut other proteins where expressed, protein 3, E, D C and B with 1 also expressed.

(C)

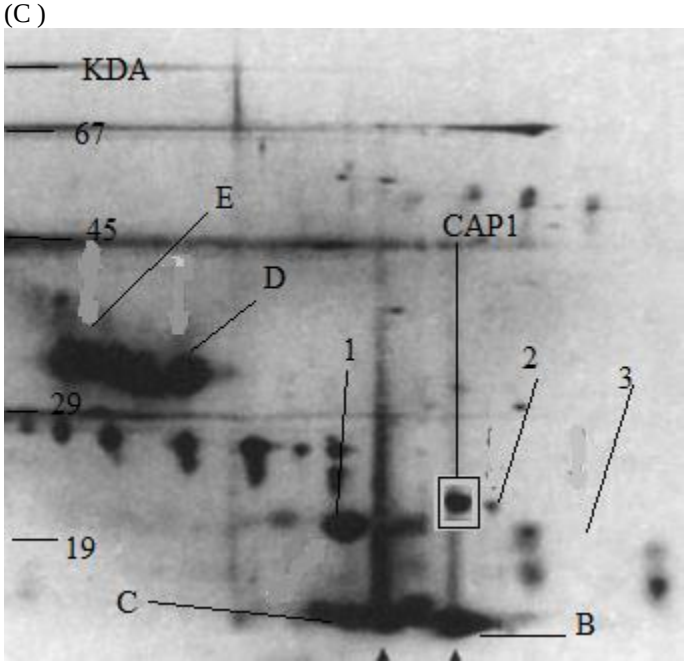

Plate 3. 2D SDS PAGE separation of proteins from the luminal fluid of the caudal epididymis from the rats dietary fed with aflatoxin B1 400mg/kg for 31 days, there is the presence of protein 2 and in the box we have the CAP1 proteins present

(D)

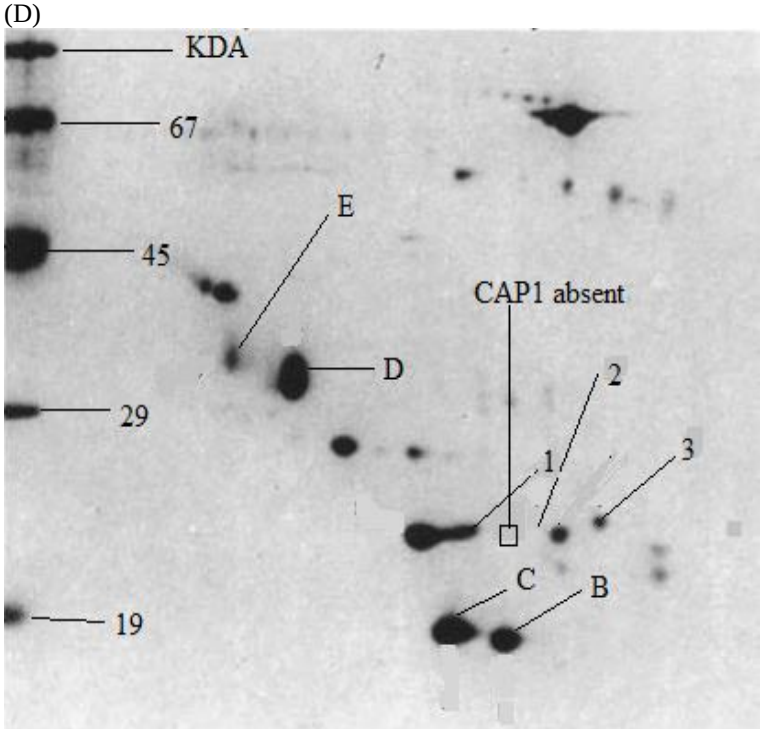

Plate 4. 2D SDS PAGE separation of proteins from the luminal fluid of the caudal epididymis from the control subject, there is absence of the CAP1 protein and also the presence of 3 protein and absence of Protein 1

(E)

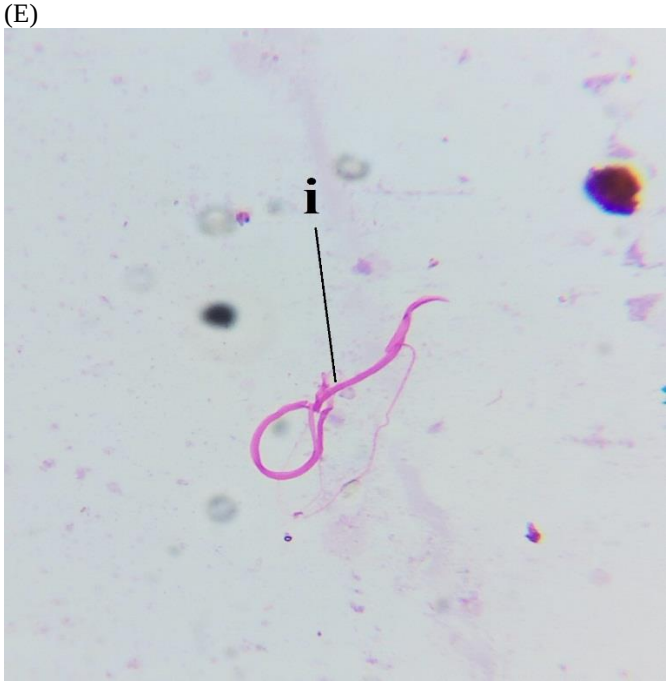

Plate 5. (i) abnormal spermatozoa, tailless sperm cells from the dietary fed rats With aflatoxin B1

(F)

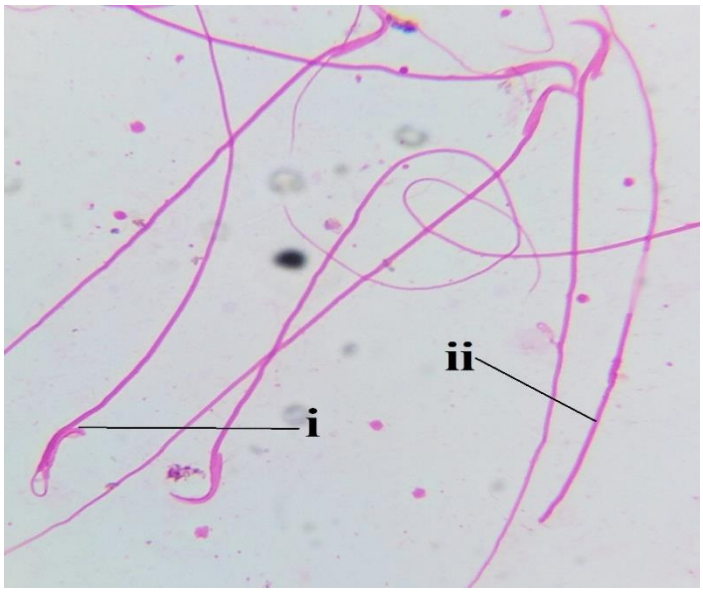

Plate 6. (i) and (ii) abnormal spermatozoa, headless sperm cells from the dietary fed rats With aflatoxin B1

(G)

Plate 7. Normal morphology of a rat spermatozoa with (i) Head, axonem, and tail from the control group (H)

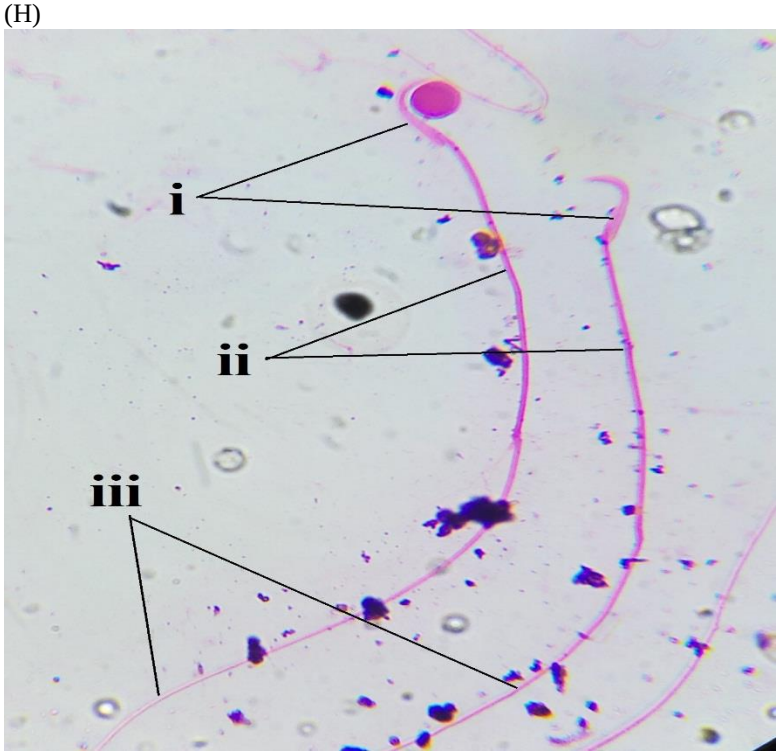

Plate 8. Normal spermatozoa, normal count and morphology from the control group. (i) Head, (ii) body (iii) Tail

REFERENCE

1. Abdin M, Ahmad MM, Javed S (2010) Advances in molecular detection of Aspergillus: an update. Arch Microbiol 192: 409-425.
2. Adekunle AA, Elegbe RA (1974) Lysosomal activity in aflatoxin Bl - treated avian embryo. Lancet. 2: 991.
3. AgroBioTek International (2009) Las Micotoxinas en la Agroindustria – Aflatoxinas. AgroBioTekDominicana.
4. AkÅŸit, Caglayan S, Yaprak I, Kansoy S (1997) Aflatoxin: is it a neglected threat for formula-fed infants? Acta Paediatr Jpn 39: 34-36.
5. Alavanja MC, Rush GA, Stewart P, Blair A (1987) Proportionate mortality study of workers in the grain industry. J Natl Cancer Inst 78: 247-252.
6. Alimentação Animal (Revista de la Associação Portuguesa dos Industriais deAlimentos Compostospara Animais(IACA)) 49: 32-44.
7. Arafa AS, Bloomer RJ, Wilson HR, Simpson CF, Harms RH (1981) Susceptibility of various poultry species to dietary aflatoxin. Br Poult Sci 22: 431-436.
8. Arangurén EM, Argüelles MJ (2009) Detección de aflatoxina M1 en quesos frescos comercializa dose nelmunicipiode Yopal,Casnare,mediantelatécnicade Elisa. Thesis, PontificiaUniversidad Javeriana,Bogotá, Colombia: 25.
9. Bababunmi EA, Bassir O (1972) Effects of aflatoxin B 1 on the swelling and adenosine triphosphatase activities of mitochondria isolated from different tissues of the rat. FEBS Lett 26: 102-104.
10. Bahr U, Karas M, Hillenkamp E (1994) Analysis of biopolymers by matrix assisted laser desorption/ionisation (MALDI) mass spectroscopy. Fresenius' J Anal Chem; 348:783-791.
11. Barbieri G, Bergamini C, Ori E, PescaP (1994) AflatoxinM1inparmesancheese: HPLC determination. Journal of Food Science 59: 1313–1331.
12. Becroft DM (1966) Syndrome of encephalopathy and fatty degeneration of viscera in New Zealand children. Br Med J 2: 135-140.
13. Bennett JW, Kale S, Yu J (2007) Aflatoxins: Background, Toxicology, and Molecular Biology. In: Simjee S. Infectious Disease: Foodborne Diseases. HumanaPress Inc, TotowaNJ: 355-373.
14. Bilgrami KS, Sinha KK, Premlata S (1981) Inhibition of aflatoxin produced by ferulic acid on some cereals and oil seeds. Current. Sc. 50: 997 – 998
15. Brooks DE, Higgins SJ (1980). Characterization and androgen-dependence of proteins associated with luminal fluid and spermatozoa in the epididymis. J Reprod Fertil; 59:363-375.
16. Brooks DE (1983). Effect of androgens on the protein synthesis and secretion in various regions of the rat epididymis, as analyzed by two-dimensional gel electrophoresis. Mol Cell Endocrinol; 29:255-270.
17. Castegnaro M, McGregor D (1998) Carcinogenic risk assessment of my cotoxins. Revue de Médecine Vétérinaire 149: 671-678.
18. Dunn MJ. Gel electrophoresis: proteins. In: Graham JM, Billington D (1993), Introduction to Biotechniques. Oxford: BIOS Scientific Publishers;: 57-58.
19. Eccleston ED, White TW, Howard JB, Hamilton DW. Characterization of a cell surface glycoprotein associated with maturation of rat spermatozoa. Mol Reprod Dev 1994; 37:110-119.
20. Eckerskom C, Strupat K, Schleuder D, Hochstrasser D, Sanchez JC, Lottspeich F, Hillenkamp E (1997) Analysis of proteins by Direct-Scanning Infrared/MALDI Mass Spectrometry after 2D-PAGE separation and electroblotting. Anal Chem; 69:2888-2892.
21. Hamilton DW, Wenstrom JC, Baker JB (1986). Membrane glycoproteins from spermatozoa: partial characterization of an integral *Mr* = -24,000 molecule from rat spermatozoa that is glycosylated during epididymal maturation. Biol Reprod ; 34:925-936.
22. Hillenkamp E Karas M, Beavis RC, Chait BT (1991) MALDI-MS of biopolymers. Anal Chem; 63:1193A-1201A.
23. Kirchhoff C(1996). CD52 is the "major maturation-associated" sperm membrane antigen. Mol Hum Reprod; 2:9-17.
24. Klinefelter GR, Laskey JW, Kelce WR, Ferrell J, Roberts NL, Suarez JD, Slott V. Chloro-ethyl-methane-sulfonate-induced effects on the epi- 1264 Chemical Carcinogenesis. ACS monograph 182) Washington D.C. : 945 -1136.
25. McClain RM, Downing JC (1988). Reproduction studies in rats treated with ornidazole. Toxicol Appl Pharmacol; 92:480-487.
26. Moore A, Hall L, Hamilton DW (1990). An 18 kDa androgen-regulated protein that modifies galactosyltransferase activity is synthesized by the rat caput epididymis, but has no structural similarity to rat milk alphalactalbumin. Biol Reprod ; 43:497-506.

27. Moore A, White TW, Ensrud KM, Hamilton DW (1989) . The major maturation glycoprotein found on rat cauda epididymidal sperm surface is linked to the membrane via phosphatidylinositol. Biochem Biophys Res Commun 160:460-468.
28. Oberlander G, Yeung CH, Cooper TG (1994). Induction of reversible infertility in male rats by oral ornidazole and its effects on sperm motility and epididymal secretions. J Reprod Fertil; 100:551-559.
29. Oberlander G, Yeung CH, Cooper TG (1996). Influence of oral administration of ornidazole on capacitation and the activity of some glycolytic enzymes of rat spermatozoa. J Reprod Fertil; 106:231-239.
30. Olson GE, Danzo BJ (1981). Characterization of the surface glycoproteins of rat spermatozoa. Biol Reprod; 24:431-443.
31. Olson GE, Hinton BT (1985) Regional difference in luminal fluid polypeptides of the rat testis and epididymis revealed by two-dimensional gel electrophoresis. J Androl; 6:20-34.
32. Osterhoff C, Ivell R, Kirchhoff C (1997). Cloning of a human epididymis specific mRNA, HE6, encoding a novel member of the seven transmembrane- domain receptor family. DNA Cell Biol; 16:379-389.
33. Patterson DS (1973) Metabolism as a factor in determining the toxic action of the aflatoxins in different animal species. Food Cosmet Toxicol 11: 287-294.
34. Rabilloud T, Carpentier G, Tarroux P (1985). Improvement and simplification of low-background silver staining proteins by using sodium dithionite. Electrophoresis; 9:288-291.
35. Strupat K, Karas M, Hillenkamp F, Eckerskorn C, Lottspeich E (1994) Matrix- assisted laser desorption ionisation mass spectroscopy of proteins electroblotted after polyacrylamide gel electrophoresis. Anal Chem; 66:464-470.
36. Yeung CH, Oberlinder G, Cooper TG (1995). Effects of the male antifertility agent ornidazole on sperm function in vitro and in the female genital tract. J Reprod Fertil; 103:257-264.
37. Zeheb R, Orr GA (1984). Characterization of a maturation-associated glycoprotein on the plasma membrane of rat caudal epididymal sperm. J Biol Chem; 259:839-848.