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Research Article

Effect of isthmic and ampullary oviductal proteins on lipid peroxidation levels of buffalo spermatozoa

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ABSTRAT

A study was undertaken to find out the effect of oviductal proteins from different regions of oviduct and phases of estrous cycle on lipid peroxidation (LPO) of buffalo spermatozoa. Oviducts were collected from apparently healthy buffalo genital tracts (nonluteal and luteal phases of estrous cycle) and separated into isthmus and ampulla. Each segment of oviduct (nonluteal and luteal) was flushed with PBS (pH 7.4), centrifuged, filtered and frozen at -20°C. Proteins in pooled nonluteal isthmic and ampullary and luteal isthmic and ampullary fluids were precipitated overnight using ammonium sulphate, centrifuged and dialyzed (> 10kDa). Aliquots of 10 mg proteins were lyophilized in cryovials and stored at -20°C. Pooled good quality ejaculates (n=6) from two Murrah buffalo bulls were utilized for the study. Each pooled ejaculate was split into five parts, extended in Tris-Egg Yolk-Citrate extender and cryopreserved in 0.5 ml French straws at -196°C. Before freezing, the isthmic and ampullary (luteal and nonluteal) oviductal proteins were supplemented (1 mg per ml). Random semen straws from each group were evaluated for LPO levels in sperm and seminal plasma in equilibrated and frozen thawed semen. Results revealed that addition of nonluteal isthmic oviductal proteins reduced the LPO level in post thaw spermatozoa significantly ($p<0.05$) whereas, no significant differences in LPO levels among other proteins added and control groups was seen. Thus, it was inferred that oviductal proteins differentially affect LPO levels of sperm depending upon the region of oviduct and the phase of estrous cycle.

Key words: Buffalo, oviductal proteins, spermatozoa, lipid peroxidation

Buffalo semen being rich in polyunsaturated fatty acids is more susceptible to peroxidative damage than that of cattle (Sidhu and Guraya, 1985; Singh *et al.*, 1989). Lipid peroxidation results in membrane damage, respiration inhibition and intracellular enzymes leakage (White, 1993). Usually, melonaldehyde (MDA), an end product of lipid peroxidation is used to asses lipid peroxidation in spermatozoa. Addition of oviductal fluid/ protein to frozen-thawed semen prolonged the sperm motility, viability and *in vitro* fertilizing ability (Abe *et al*, 1995, Grippo *et al*, 1995, Boquest *et al*, 1999). Thus hypothesizing that oviduct secretes some factors that protect the spermatozoa from oxidative stress. In accordance, Lapointe *et al.* (1998) proved the existence of catalase activity in cow oviductal fluid, suggesting a mechanism by which the oviductal fluid reduces the sperm damage due to lipid peroxidation. Catalase levels of buffalo oviductal fluid at different phases of estrous cycle vary (Kumaresan *et al.*, 2003), so it may affect the LPO levels of spermatozoa during the course of cycle. The present study was thus planned to evaluate the effect of oviductal proteins obtained from different parts of oviduct during nonluteal and luteal phases of estrous cycle on lipid peroxidation levels.

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MATERIALS AND METHODS

Collection of slaughter house materials

Apparently healthy female buffalo genital tracts (with intact ovaries) were collected immediately after slaughter from the Corporation Slaughter House, Bareilly. The genitalia were brought to the laboratory on ice within 3-4 h of slaughter. Upon arrival, the ovarian morphology was examined and the estrous cycle phase

as either luteal or nonluteal was determined (Ireland *et al.*, 1980).

Collection of oviductal fluid

Oviducts were dissected free of the surrounding connective tissues and fimbria, thoroughly washed (PBS - pH 7.4) and flushed. Nonluteal and luteal oviducts were flushed separately. Ampullary - isthmic junction was removed to prevent cross contamination. Isthmus and ampulla were flushed with 2 to 4 ml of PBS using a 22-gauge needle. Pooled fluids from every part were centrifuged (3000 rpm for 20 min) and the cells/debris removed. Supernatent of different groups was filtered (Ministart, Sartorius, 0.2μ), labeled and frozen at –20°C till further use.

Extraction of oviductal proteins

Proteins in the concentrated oviductal fluid were separated by following the procedure given by Boquest *et al*, (1999). All the following procedures were performed at 4°C unless otherwise specified. The protein was precipitated using solid ammonium sulphate for overnight, pelleted by centrifugation (10000 rpm for 30 min.), resuspended in a two pellet volumes of PBS (pH 7.4) and dialyzed overnight against four litre of PBS in four changes. The retentates (>10 kDa) were filter sterilized (Sartorius - 0.2 μ) and protein content was estimated (Lowry *et al.* 1951). Aliquots of containing 10 mg of protein were dispensed in 1 ml cryovials and lyophilized to dryness and stored at –20°C.

Addition of oviductal proteins to semen

Six good quality pooled ejaculates from two Murrah buffalo bulls (six ejaculates each) were utilized for this study (Six replications). Each pooled ejaculate was divided into five parts and extended with Tris-Egg Yolk-Citrate extender (Davis *et al.*, 1963)) and frozen in 0.5 ml French straws (30 million sperms/straw) with an equilibration period of 4 h. The nonluteal isthmic & ampullary and luteal isthmic & ampullary oviductal proteins were added at the rate of 1 mg/ml of extended semen before freezing along with a control group (with no added protein). The concentration of oviductal proteins is selected based on our previous study (Kumaresan, 2002).

Lipid peroxidation assay

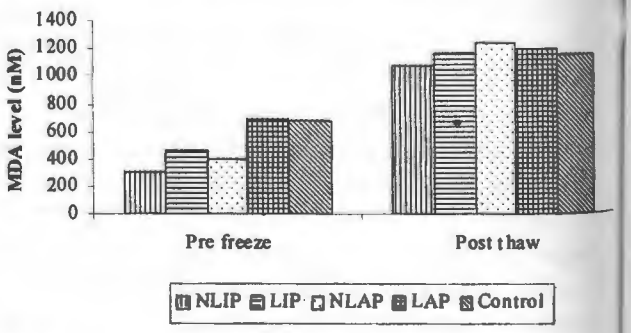
Lipid peroxidation level of sperm and seminal plasma was measured by determining the malonaldehyde (MDA) production, using thiobarbituric acid (TBA) as per the method of Buege and Aust (1978) as modified by Suleiman *et al.* (1996).

The data was analyzed as per the standard procedure given by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Significant beneficial effect of oviductal proteins was observed at pre-freeze evaluation itself. Isthmic and ampullary oviductal proteins obtained from nonluteal oviducts prevented lipid peroxidation effectively than the luteal oviductal proteins (figure 1). Among the proteins nonluteal isthmic proteins added group had significantly ($P<0.05$) lower MDA level (305.00±5.45) than all other groups, followed by nonluteal ampullary proteins (403.75±6.22) added group. There was no significant difference between the luteal ampullary proteins added group and control group.

The effect of stage of oestrous cycle on lipid peroxidation levels was not clear in post-thaw evaluation. Though the nonluteal isthmic proteins reduced lipid peroxidation levels significantly ($P<0.05$) than luteal isthmic proteins, the ampullary proteins did not. Similar



NLIP – Nonluteal isthmic oviductal proteins
LIP – Luteal isthmic oviductal proteins
NLAP – Nonluteal ampullary oviductal proteins
LAP – Luteal ampullary oviductal proteins

Fig 1: Effect of oviductal proteins on lipid peroxidation levels of spermatozoa (10^9) before and after freezing.

beneficial effects of oviductal proteins were also observed in terms of MDA level in seminal plasma in which, the differential effect of estrous cycle as well as region of oviduct from which the proteins were obtained was clearly visible.

Unsaturated fatty acids in the lipids of mammalian spermatoza are known to make them susceptible to the deleterious effects of lipid peroxidation (Mann and Lutwak-Mann, 1981, Halliwell and Gutteridge, 1984). Buffalo semen is rich in polyunsaturated fattyacids and hence more susceptible to oxidative damage (Sidhu and Guraya, 1985). Melonaldehyde, markers of lipid free radical peroxidation, were higher in spermatozoa in our study. The values are higher than what has already been reported in buffaloes (22.9±2.40 to 31.7±1.87 nM/10^9 sperm, Singh *et al.*, 1992, 180 nM/10^8 sperm, Sidhu and Guraya, 1985). However, these studies have been conducted with fresh semen. In our study we assessed lipid peroxidation levels in equilibrated and in frozen-thawed semen. Slaweta *et al.* (1988) reported that the lipid peroxidation levels in bull sperm was 2-3 times more in frozen-thawed spermatozoa than fresh semen. Similarly, Sidhu and Guraya (1985) observed higher MDA levels in cold shocked buffalo spermatozoa (180 nM/ 10^8 sperm) than in fresh spermatozoa (140 nM/10^8 sperm). Our results were also similar to earlier studies.

Results revealed that inclusion of oviductal proteins in extender before freezing have beneficial effects of spermatozoa in terms of reduced levels of lipid peroxidation. The MDA levels in all oviductal proteins included groups except the luteal ampullary group were significantly ($P<0.05$) lower than control group at pre-freeze evaluation itself. The secretion rate of motility maintaining factors has been reported to be higher in oestrogen dominated phase than the progesterone dominated phase of oestrous cycle (Lapointe *et al.*, 1995) and we had earlier reported that the catalase activity was higher in nonluteal stage of estrous cycle in buffalo oviductal fluid (Kumaresan, 2002). This might be a reason for low MDA levels in the nonluteal isthmic protein included group. Boquest *et al.* (1999) hypothesised that oviductal proteins may promote the viability of spermatozoa by protecting the sperm membranes from oxidative damage. Our findings support this hypothesis. It can be suggested that these beneficial effects of oviductal proteins on reduction of sperm lipid peroxidation levels may be due to the existence of catalase. This is further supported by the significant higher levels of catalase activity in nonluteal phase than the luteal phase in oviductal and uterine fluids (Kumaresan *et al*, 2003). The higher catalase activity in oviductal fluid may protect spermatozoa, which stays for longer period in oviduct by establishing reservoir until ovulation occurs. The catalase prevents the deliterious effects of H_2O_2 on spermatozoa. During extension of semen the existing little catalase is diluted several which is not sufficient to protect the spermatozoa from oxidative damage.

From this study, It may be inferred that oviductal proteins differentially affects LPO levels of sperm depending upon the region of oviduct and the stage of estrous cycle at which the proteins was obtained.

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