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EFFECT OF BRADYKININ ON FREEZABILITY OF ABATTOIR DERIVED MURRAH BULL EPIDIDYMAL SPERMATOZOA

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ABASTRACT

The research was performed on thirty-six (36) pairs of buffalo bull tests obtained from an abattoir. This study aimed to assess the impact of bradykinin on seminal characteristics, including progressive motility, viability, abnormalities, and HOS reactivity, in post-dilution, pre-freeze, and post-thaw semen obtained from the epididymis. Seminal parameters were evaluated in relation to skim milk, tris, citrate, skim milk combined with bradykinin, tris combined with bradykinin, and citrate combined with bradykinin. Notable discrepancies were seen in all metrics, specifically post-thaw motility, viability, HOS reactivity, and sperm abnormalities. In conclusion, the inclusion of bradykinin in the dilutor markedly enhances post-thaw progressive motility, viability, and HOS responsiveness relative to the Control group. Bradykinin can be utilized to enhance the quality of frozen-thawed semen from Murrah bulls.

Keywords: *Bubalus bubalis*, buffaloes, additive, bradykinin, dilutors, epididymal spermatozoa, Murrah bull

INTRODUCTION

Buffalo exhibits a low fertility rate with frozen semen (Shukla and Misra, 2007) due to intrinsic issues in semen quality, including inadequate libido, seasonality, diminished epididymal sperm reserves, low sperm yield, poor freezability, and contamination by microbial agents and extraneous substances. The interest in utilizing epididymal sperm from both domestic and nondomestic species is growing due to the intrinsic value of individuals that perish prior to contributing to their genetic lineage, the need to mitigate the risk of elevated inbreeding coefficients in captive animal populations, and the imperative to preserve genetic diversity among endangered species within confined captive populations (Foote, 2000). Furthermore, Artificial Insemination (AI) might be employed in the preservation of uncommon breeds or endangered species (Morrell, 2011). To uphold the quality of epididymal sperm derived from slaughtered or post-mortem sources, preservation techniques must evolve through Artificial Reproductive Technologies (ART) to enhance the gene pool of both wild and

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captive animals, hence augmenting the utility of sperm (Yu and Leibo, 2002). Caudal epididymal spermatozoa exhibit motility and fertilization capability (Igboeli and Foote, 1968; Amann and Griel, 1974; Hammerstedt and Park, 1987) and can be efficiently utilized for the preservation of germplasm from critically damaged or deceased valuable animals (Dong *et al*., 2008). The presence of diluents in egg yolk, attributed to hormones (Hartmann *et al*., 1998), together with hygiene concerns, undermines the fertilizing capacity of spermatozoa (Muller-Schlosser *et al*., 2001). The kallikrein-kinin system is crucial for regulating sperm motility in humans (Mizutani and Schill, 1985; Sato and Schill, 1987) and bovines (Somlev, 1985). Bradykinin, a nonapeptide, is generated by the kininogenase kallikrein from its precursor kininogen and disrupts glucose and fructose transport through bradykinin receptors on the cell membrane (Schill *et al*., 1979; Schill *et al*., 1989). Miska and Schill (1994); Seims *et al.* (2003) determined that the action of bradykinin is not mediated by the particular B2 bradykinin receptor on the sperm plasma membrane. Contradictory reports exist (Somlev and Subdev, 1998) about the mechanism by which bradykinin stimulates the post-thaw motility of bovine spermatozoa. The potential of bradykinin to safeguard Murrah bull semen from cryo-damage has yet to be investigated. The literature on the application of bradykinin as a semen additive in Murrah bull semen is scarce. This study was done to compare the effects of bradykinin on the freezability of Murrah bull semen.

MATERIALS AND METHODS

Thirty-six (36) pairs of buffalo bull testicles were procured from Allana Sons Ltd., Arora (AOV Ltd.), and Mass Food Agro Ltd., situated in Unnao, Uttar Pradesh, India. The testicles were transported to the laboratory in an airtight sterile cryobox at 4^{o}C and processed within 6 h of collection. The testis-epididymal complex was first isolated, and the cauda epididymis was subsequently washed with physiological saline at ambient temperature. Subsequently, the cauda was maintained in a vertical orientation, the inferior ligature was excised, and 0.2 ml of physiological saline was administered into the lumen, prompting the expulsion of sperm through the cauda.

Six combinations of diluents (3 ml each) *viz*. D1 (Skim milk), D2 (tris), D3 (citrate buffer), D4 (Skim milk+bradykinin 3 ng), D5 (tris+bradykinin 3 ng) and D6 (citrate buffer +bradykinin 3 ng) were prepared at 35^{o}C and each combination used separately to dilute the semen so that extended semen contain 40 million motile sperm cells per ml, afterwards, ten French mini straw (0.25 ml) were filled and maintained at sub-zero temperatures for 24 h and subsequently used for post thaw evaluation. The post-dilution (PD) and post thaw (PT) sperm motility, viability, sperm abnormality and HOS reactivity were assessed by counting 200 spermatozoa for each attribute.

Data was analyzed using two-way ANOVA to assess differences among the bulls and treatments when the F ratio is significant ($P<0.05$). Tukey's HSD test was employed to compare treatment means using GraphPad Instat Version 5 software.

RESULTS AND DISCUSSIONS

Least squares mean of motility (%), viability (%), abnormalities and HOS reactivity

(%) of epididymal semen of buffalo bull of different treatment groups at post diluted as well as post thawed semen samples are depicted in Table 1 and 2.

Sperm motility

The PD-motility (mean±SE) was 75.00+0.77, 80.00+0.58, 77.67+0.71, 75.83+0.87, 84.67+1.20 and 80.00+0.87% and differed significantly (P<0.05) among D1 to, D2, D3, D4, D5 and D6 groups respectively (Table 1). Similarly, Igboeli and Foote (1968) reported that tris serves as a superior diluent and preserves a higher percentage of motile caudal epididymal spermatozoa. It can be deduced that, under identical conditions, tris can maintain a greater quantity of progressive motile spermatozoa compared to egg yolk citrate or skim milk diluents. PD-motility exhibited a significant positive correlation with PD-viability (r=0.95, 0.61, 0.90, 0.89, 0.96, and 0.74) and PD-HOS reactivity (r=0.80, 0.70, 0.70, 0.94, 0.90, and 0.78), while demonstrating a significant negative correlation with PD-sperm abnormality percentage (r=-0.99, -0.76, -0.77, -0.78, -0.97, and -0.89) across D1, D2, D3, D4, D5, and D6 in the treatment groups, respectively. Similarly, Shukla and Misra (2007) observed comparable enhancement following the addition of bradykinin to the diluent.

The PT-motility (mean±SE) was 46.33±0.67, 53.00±0.86, 48.50±0.72, 51.50±0.43, 58.33±0.67, and 55.17±0.65%, exhibiting significant differences (P<0.05) among the D1, D2, D3, D4, D5, and D6 groups, respectively (Table 2). Lambrechts *et al*. (1999) documented a 50% reduction in post-thaw sperm viability following cryopreservation of epididymal semen from the African buffalo (Syncerus caffer). In contrast, certain investigations demonstrated favorable cryopreservation outcomes for epididymal spermatozoa in Sika deer (Cervus nippon) (Hishinuma *et al*., 2003), dogs (Ponglowhapan *et al*., 2006), cats (Hermansson and Axner, 2007), rams (Kaabi *et al*., 2003), and bucks (Blash *et al*., 2000). Moreover, the current findings align closely with the observations made by Njonge and Mutugi (2013). Cryopreservation induces cryo-injury and significantly impairs the viability and progressive motility of buffalo bull epididymal spermatozoa. The present study observed significantly elevated PT-motility (compared to control) at a concentration of 3 ng/ml bradykinin, potentially attributable to enhanced energy availability and utilization by sperm, particularly during the stress of cryopreservation or due to inefficient energy resource utilization following bradykinin degradation by Kininase II, which is present in seminal plasma (Somlev and Subev, 1998). Additionally, the role of Kallikrein, which facilitates the formation of bradykinin from its precursor Kininogen, is pertinent to spermatogenesis and the epididymal maturation of spermatozoa (Haidli and Schill, 1993). PT-motility exhibited a significant positive correlation with PT-viability (r=0.76, 0.81, 0.92, 0.62, 0.98, and 0.77) and PT-HOS reactive sperm (r=0.84, 0.82, 0.78, 0.61, 0.66, and 0.82). Conversely, it demonstrated a significant negative correlation with the percentage of PT-sperm abnormalities (r=-0.57, -0.97, -0.91, -0.42, -0.95, and -0.77) across treatment groups D1, D2, D3, D4, D5, and D6, respectively.

Sperm viability

The PD-viability (mean±S.E) was 79.00±0.82, 85.17±1.05, 83.50±0.62, 81.50±0.62, 89.67±0.84, and 87.17±1.05, exhibiting significant differences (P<0.01) among D1, D2, D3, D4, D5, and D6 treatments, respectively (Table 1). Njonge and Mutugi (2013) similarly noted a greater live count in tris diluent compared to milk and citrate

buffer. The PD-viability (mean±S.E.) exhibited a significant positive correlation (P<0.01) with the percentage of PD-HOS reactive spermatozoa (r=0.75, 0.95, 0.81, 0.78, 0.89, and 0.98), while demonstrating a significant negative correlation (P<0.01) with PD-sperm abnormality (r=-0.94, -0.84, -0.92, -0.74, -0.97, and -0.92) across treatment groups D1, D2, D3, D4, D5, and D6, respectively. A notable (P<0.01) positive connection with PD-motility (r=0.90, 0.74, 0.89, 0.88, 0.86, and 0.95) and PD-HOS reactivity (r=0.98, 0.96, 0.93, 0.91, 0.84, and 0.90) was identified in D1, D2, D3, D4, D5, and D6 across the treatment groups, respectively. The current findings align with those of Dong *et al.* (2008), who documented a comparable decline in sperm viability during the pre-freeze stage.

The PT-viability (mean±SE) was 60.33±0.61, 66.50±1.20, 61.83±1.14, 61.83±0.95, 72.00±1.13, and 69.50±1.09 percent, exhibiting significant variation (P<0.05) among the D1, D2, D3, D4, D5, and D6 diluters, respectively (Table 2). Likewise, Shukla and Misra (2007) noted an elevated live count (81.60±1.80) percent in the bradykinin-treated groups. This may be attributable to the protective function of bradykinin for epididymal spermatozoa. Higher cryopreservation damage is observed in buffalo semen compared to cow semen when contrasted with ejaculated semen. Tiplady *et al.* (2002) observed that cryopreservation of cooled equine epididymal spermatozoa resulted in a reduction in motility, although viability remained unchanged from the pre-freeze to post-thaw stage. The PT viability exhibited a significant positive correlation with PT motility (r=0.76, 0.81, 0.92, 0.62, 0.98, and 0.77) and PT-HOS reactivity (r=0.84, 0.90, 0.95, 0.85, 0.76, and 0.91), while demonstrating a significant negative correlation with PT sperm abnormality (r=-0.74, -0.89, -0.91, -0.83, -0.85, and -0.92) across D1, D2, D3, D4, D5, and D6, respectively. The current data indicate that the reduction in PT-motility across all treatments during freezing and thawing may be attributed to elevated solute concentrations and the generation of intracellular and extracellular ice crystals during cryopreservation (Mazur, 1984).

Sperm abnormality

The PD-sperm abnormalities (mean±S.E.) were 42.33±0.95, 38.00±0.68, 40.50±1.03, 41.50±0.76, 36.17±0.48, and 37.67±0.61%, exhibiting significant differences (P<0.01) among the D1, D2, D3, D4, D5, and D6 Treatment groups, respectively (Table 1). Our results aligned well with the observations of Ball and Peters (2004). Additionally, a sperm anomaly rate of 3 to 26% was documented (EL-Menoufy, 1974), occurring less frequently during the summer (Oloufa *et al.*, 1959). A significant (P<0.01) negative correlation between sperm abnormalities and PD-motility (r=-0.99, -0.76, -0.77, -0.78, -0.97, and -0.89), PD-HOS reactivity (r=-0.81, -0.75, -0.94, -0.83, -0.78, and -0.90), and viability (r=-0.94, -0.84, -0.92, -0.74, -0.97, and -0.92) was observed in Treatment groups D1, D2, D3, D4, D5, and D6. These findings corroborate the observations made by Srivastava and Kumar (2014).

The PT-sperm abnormality (mean±S.E.) was 33.33±0.49, 26.83±0.60, 29.33±0.61, 27.67±0.92, 24.00±0.58, and 26.33±0.95 percent, exhibiting significant differences (P<0.05) across Treatment groups D1, D2, D3, D4, D5, and D6, respectively (Table 2). The reduced PT-sperm abnormalities in tris diluents compared to milk and citrate buffer indicates that tris is the superior diluent for the cryopreservation of buffalo epididymal spermatozoa. The PT thaw sperm abnormalities was reduced in the bradykinin-treated group compared to the untreated dilutors,

potentially due to bradykinin's protective effect during spermatozoa cryopreservation (Shukla and Misra, 2007). PT-sperm abnormality exhibited a significant negative correlation (P<0.01) with PT-motility (r=-0.57, -0.97, -0.91, 0.42, -0.95, and -0.77), PT-viability (r=-0.95, -0.81, -0.94, -0.74, -0.97, and -0.99), and PT-HOS reactivity (r=-0.74, -0.89, -0.91, -0.83, -0.85, and -0.92) across Treatment groups D1, D2, D3, D4, D5, and D6, respectively.

Hos reactive sperm percent

The PD-HOS reactivity was measured at 29.17±0.70, 38.83±0.91, 35.00±0.93, 34.17±0.65, 46.50±1.03, and 41.33±0.80%, exhibiting significant differences (P<0.01) among the D1, D2, D3, D4, D5, and D6 groups, respectively (Table 1). In contrast, Selvaraju *et al.* (2008) documented elevated values in pure semen; additionally, diminished values were noted by Srivastava and Kumar (2014). The response of spermatozoa to the HOS solution is contingent upon the animal species, solution osmolality, and duration of incubation (Correa *et al*., 1997b; Neild *et al*., 1999; Amorim *et al*., 2009). Moreover, not all spermatozoa with an intact plasma membrane respond to moderate osmotic pressure; rather, the swelling response predominantly occurs within the first minute of incubation in a hypotonic solution (Correa *et al*., 1997a). The integrity of the cytoplasmic membrane is essential not only for metabolic activities that facilitate sperm motility but also for critical functions in fertilization, including capacitation, the acrosomal response, and sperm adhesion to the ovum's surface (Jeyendram *et al*., 1994). An intact cytoplasmic membrane ensures regular metabolic functions, facilitating the appropriate transfer of substrates and electrolytes across the membrane (Rizal *et al*., 2003). The intact cytoplasmic membrane exhibited a positive association with PD-motility, PD-viability, and PD-membrane integrity of the acrosome head in spermatozoa. A significant positive correlation (P<0.01) between PD-HOS reactive sperm and PD-motility percentage was noted (r=0.80, 0.70, 0.70, 0.94, 0.90, and 0.78) as well as with PD-viability (r=0.75, 0.95, 0.81, 0.78, 0.89, and 0.98). Conversely, a significant negative correlation was found with PD-sperm abnormality (r=-0.81, -0.75, -0.94, -0.83, -0.78, and -0.90) across D1, D2, D3, D4, D5, and D6 treatments, respectively. Similarly, same associations were discovered by Srivastava (2011) in the ejaculated semen of Murrah bulls.

The PT-HOS reactivity (mean±S.E.) was 22.83±0.79, 32.50±1.09, 28.00±0.89, 29.33±0.76, 40.83±1.01, and 36.50±1.03%, exhibiting significant differences (P<0.01) among D1, D2, D3, D4, D5, and D6 treatments, respectively (Table 2). Likewise, Shukla and Misra (2007) reported increased PT HOS responsiveness in the bradykinin treatment group; nevertheless, the efficacy of bradykinin in safeguarding epididymal spermatozoa from cryodamage remains to be investigated. The PT-HOS reactive spermatozoa exhibited a significant positive correlation (P<0.01) with PT-motility (r=0.84, 0.83, 0.78, 0.61, 0.66, and 0.82) and PT-viability (r=0.84, 0.90, 0.95, 0.85, 0.76, and 0.91), while demonstrating a significant negative correlation (P<0.01) with PT-abnormality (r=-0.74, -0.89, -0.91, -0.83, -0.85, and -0.92) across the D1, D2, D3, D4, D5, and D6 treatment groups, respectively.

CONCLUSION

In conclusion, tris buffer was comparatively better as compared to skim milk and citrate

Table 1. Post diluted motility (%), viability (%), sperm abnormality (%) and HOS reactive sperm (Mean±S.E.) in epididymal semen of Murrah bull.

Group	PD-motility (%)	PD-viability (%)	PD-sperm abnormality (%)	PD-HOS reactive sperm (%)
D1	75.00±0.77^{a}	79.00±0.82^{a}	42.33±0.95^{a}	29.17±0.70^{a}
D2	80.00±0.58^{b}	85.17±1.05^{b}	38.00±0.68^{b}	38.83±0.91^{b}
D3	77.67±0.71^{c}	83.50±0.62^{bc}	40.50±1.03^{a}	35.00±0.93^{c}
D4	75.83±0.87^{a}	81.50±0.62^{c}	41.50±0.76^{a}	34.17±0.65^{c}
D5	84.67±1.20^{d}	89.67±0.84^{d}	36.17±0.48^{c}	46.50±1.03^{d}
D6	80.83±0.87^{b}	87.17±1.05^{e}	37.67±0.61^{b}	41.33±0.80^{e}

Mean with different superscript (a-f) differed significantly (P<0.05) within a column.

Table 2. Post thaw motility (%), viability (%), sperm abnormality (%) and HOS reactive sperm (Mean±S.E.) in epididymal semen of Murrah bull.

Group	PT-motility (%)	PT-viability (%)	PT-sperm abnormality (%)	PT-HOS reactive sperm (%)
D1	46.33±0.67^{a}	60.33±0.61^{a}	33.33±0.49^{a}	22.83±0.79^{a}
D2	53.00±0.86^{b}	66.50±1.20^{b}	26.83±0.60^{b}	32.50±1.09^{b}
D3	48.50±0.72^{c}	61.83±1.14^{c}	29.33±0.61^{c}	28.09±0.89^{c}
D4	51.50±0.43^{b}	61.83±0.95^{c}	27.67±0.92^{b}	29.33±0.76^{c}
D5	58.33±0.67^{d}	72.00±1.13^{d}	24.00±0.58^{d}	40.83±1.01^{d}
D6	55.17±0.65^{e}	69.50±1.09^{e}	26.33±0.95^{b}	36.50±1.03^{e}

Mean with different superscript (a-f) differed significantly (P<0.05) within a column.

buffer, to cope up cryo-damage during freezing and thawing. The significant improvement was observed with bradykinin fortified skim milk, tris and citrate buffer than those of unfortified contemporaries. Furthermore, most promising results were observed with bradykinin fortified tris dilutor as compared to other dilutors under study. Thus, Bradykinin as a semen additive in tris buffer can be supplemented in diluter before freezing of murrah bull semen.

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