EFFECT OF NITRIC OXIDE DONOR SODIUM NITROPRUSSIDE ON SPERM VOLUME OF DILUTED BULL SEMEN

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Keywords: nitric oxide, (SNP), osmolarity.

ABSTRACT

Study the effect of nitric oxide donor on sperms membrane integrity and volume and their relationship with viability and sperm motility. This study was done using two groups each one contained 10 samples first one exposed to eight different gradient of hypotonic solutions containing Sodium nitroprusside (SNP) and second 10 samples diluted with gradient hypotonic solutions without Sodium nitroprusside, Bull semen tris dilution treated with Sodium nitroprusside that protected the sperm from osmocellular changes stress. The results showed tolerance sperm to gradient hypotonic solution in sperm swelling and classic spermaticocrit marked significance by the relative volume shift volumetric data. In addition the SNP had sperm protection to osmolarity tested and give improvement viability and sperm motility. Hypotonic media tonicity that may be attributed to direct liberation of Nitric oxide that produced vital regulation of Na-K ATPase and Calcium channels of sperm membrane.

INTRODUCTION

When cells encounter hypo- or hyper-tonic solution, they tend to swell or shrink due to the influx or efflux of water during reestablishment of osmotic equilibrium. However, spermatozoa are able to maintain their volume after osmotic shock, thus avoiding the consequences of excessive volume changes (1). At expansion diluted semen, they transfer from the hypertonic epididymal environment to the isotonic conditions of seminal plasma (2), preservative dilution and the female genital tract fluids, at which time the spermatozoa experience a considerable osmotic gradient (3). Moreover, under the artificial conditions of semen cryopreservation, the cells are exposed to major osmotic challenges: during freezing, they become dehydrated and shrink due to local hypertonicity; during thawing, when rehydration takes place, they are submitted to hypotonic shock (4). Nitric oxide had provocation mechanism to sperm performance and has antioxidant effect membrane system osmotic demined. To be able to maintain cellular functionality in the face of such osmotic changes, through adjusted spermatozoa osmotic regulatory system of sperm had been found to exhibit volume regulatory abilities (5 and 6).
MATERIAL AND METHODS

Semen characteristics

Fresh semen was obtained from the artificial insemination center-Iraq, that assisted low grade fertility (~40% individual motility, ~57% Sperm viability and ~6.8 Sperm abnormality) which were assessed by (7).

Isotonic suspending medium

The medium considered to be isotonic to the spermatozoa was a (0-191M) NaCl tris diluents' solution. This consisted of Ringer solution, in which the 0-9 % (0-154 molality)NaCl had been replaced by 0-191 molality NaCl. The osmolality of this Ringer solution was became 0-353 (7). The tested media of semen solution was prepared as the following composition: (A) 100% NaCl-SNP Tris solution (nine parts of 0-191 M-NaCl Ringer + one part of 0.1 M-SNP Tris solution), (B) 80% and (C) 60% NaCl-SNP Tris solution being obtained by diluting (a) with distilled water; (D), (E), (F) and (G) 40%, 20%, 16.6% and 14.3% NaClTris solution, respectively which were had increase hypotonicity, determined from data given by Giese (8), While the control samples where same concentration of treated samples of NaClTris solutions without SNP.

Semen sampling protocol

Twenty Semen samples were collected from ten Holstein bulls (4-5 years old) imported from Australia divided in to two equal samples groups; control samples and Sodium nitroprossid (0. 35×10^5 %) treated samples, each one were separated in to tensub-tested trials with examined the seven samples replications according to NaCl and sodium nitroprusside concentrations. Bull semen sample quantities of stock suspension which, after extension with 1:10 of different hypotonic and isotonic solutions served as control, resulted in the required sperm concentration were calculated with the aid of a pre-test described earlier (7).

Measurement of Osmolality

A vapor pressure osmometer (model 5500; Wescor, Logan, UT-Canada) was calibrated with 100-, 300-, and 1000-mOsm standards and used to measure the osmolality of all solutions. Different concentrated experimental solutions were prepared, and the osmolality of each test solution was assayed in duplicate or triplicate (6 and 9).
Spermatocrit values and sperm functions test assessments

Semen suspension sample for determinations of spermatozoa in media of increasing hypo-tonicities were carried out at 2000 g for 15 minute at (23 to 24) C°(10). While in diluents expander of toxicity down to ~ 50 % of the isotonic one, the spermatozoa are capable to exhibit tail motion.

**Individual motility**

One drop of diluted semen in different hypotonic solution was transferred on wormed slide, the semen drops were covered by cover slip. The individual sperm motility was scored percent degree of motility under light microscope (10).

**Percentage of dead sperm**

The evaluation of live and dead sperms was identified by using one step eosin-nigrosin staining technique according to method of Petrunkina (2007)

**RESULTS**

Though these continuously decrease as the degree of swelling and of change of shape of the cells increase, the cells had first to be turned into immotile. The motility-activating substance used was Sodium nitroprusside (SNP).

**Spermatocrit on spermatozoa in media of increasing hypotonicity**

The quantities of sperm stock suspensions which were transferred to the different media (A to G) to give significant P<0.05 changes of spermatocrits were showed in table (1). There were gradual increases in the spermatocrits value under gradual decrease of diluent tonicity in control samples where the values of spermatocrits in treated samples displayed significant tolerance to decrease diluent tonicity until 40% of SNP-NaCltris diluent (sample D) as compared to control samples.

**Sperm motility and percentage of dead sperm:**

Controls samples displayed a significant (P<0.05) gradual decrease in sperm motility as corresponding decrement tonicity as compared with saline sample on the other hand the SNP treated sample showed significant (P≤0.05) maintenance of motility and activity in acceptable values at 20% (E) dilution and to the 14.3(G) as compared with isotonic of control samples (2). In contrast percentage of dead sperm (table 3) showed a significant (P<0.05) gradual increase in percentage of dead sperm as usual decrement tonicity as compared with saline sample whereas,
Table (1) the effect a single-step exposure to Sodium nitroprosid (SNP) on Spermatocrit (ml) in Bull semen samples in gradient of hypotonic dilution of NaCltris diluent.

<table>
<thead>
<tr>
<th>NaCltris hypotonic diluents</th>
<th>semen samples</th>
<th>Isotonic</th>
<th>A 100%</th>
<th>B 80%</th>
<th>C 60%</th>
<th>D 40%</th>
<th>E 20%</th>
<th>F 16.6</th>
<th>G 14.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.016</td>
<td>0.019</td>
<td>0.025</td>
<td>0.34</td>
<td>0.48</td>
<td>0.054</td>
<td>0.057</td>
<td>0.059</td>
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<tr>
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<tr>
<td></td>
<td></td>
<td>0.002 Aa</td>
<td>0.003 Ba</td>
<td>0.008 Ca</td>
<td>0.002 Da</td>
<td>0.007 Ea</td>
<td>0.003 Fa</td>
<td>0.004 FGa</td>
<td>0.005 Ga</td>
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<td></td>
<td></td>
<td>0.014</td>
<td>0.015</td>
<td>0.016</td>
<td>0.018</td>
<td>0.019</td>
<td>0.020</td>
<td>0.025</td>
<td>0.030</td>
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Values are expressed as Mean ± SE: n(10)
Small letters denote differences P<0.05 between samples
Capital letters denote differences P<0.05 between diluents

Table (2) the effect a single-step exposure to Sodium nitroprosid(SNP) on Individual motility (%) in Bull semen samples in gradient of hypotonic dilution of NaCltris diluent.

<table>
<thead>
<tr>
<th>NaCltris hypotonic diluents</th>
<th>semen samples</th>
<th>Isotonic</th>
<th>A 100%</th>
<th>B 80%</th>
<th>C 60%</th>
<th>D 40%</th>
<th>E 20%</th>
<th>F 16.6</th>
<th>G 14.3%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>47.37</td>
<td>44.72</td>
<td>36.12</td>
<td>30.34</td>
<td>23.95</td>
<td>20.53</td>
<td>12.81</td>
<td>8.40</td>
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<tr>
<td>SNP</td>
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<td>±</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.42 Aa</td>
<td>1.53 Ba</td>
<td>1.17Ca</td>
<td>1.16 Da</td>
<td>0.78 Ea</td>
<td>0.95 Fa</td>
<td>0.31 Ga</td>
<td>1.29 Ha</td>
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<tr>
<td></td>
<td></td>
<td>80.72</td>
<td>79.38</td>
<td>74.10</td>
<td>70.05</td>
<td>66.27</td>
<td>63.85</td>
<td>59.00</td>
<td>44.50</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SE: n (10)
Small letters denote differences P<0.05 between samples
Capital letters denote differences P<0.05 between diluents
Table (3) the effect a single-step exposure to Sodium nitroprosid(SNP) on Viability (dead sperms) (%) in Bull semen samples in gradient of hypotonic dilution of NaCltris diluent.

<table>
<thead>
<tr>
<th>NaCltris hypotonic diluents</th>
<th>Semen samples</th>
<th>Isotonic</th>
<th>A 100%</th>
<th>B 80%</th>
<th>C 60%</th>
<th>D 40%</th>
<th>E 20%</th>
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<tr>
<td>Values are expressed as Mean ± SE: n(10)</td>
<td></td>
<td>1.53Aa</td>
<td>0.78Aa</td>
<td>1.14 Ba</td>
<td>0.75Ca</td>
<td>0.92 Da</td>
<td>1.36 Da</td>
<td>1.40Ea</td>
<td>1.46Fa</td>
</tr>
<tr>
<td>SNP</td>
<td></td>
<td>15.00</td>
<td>14.48</td>
<td>15.28</td>
<td>16.00</td>
<td>17.50</td>
<td>19.17</td>
<td>20.51</td>
<td>21.65</td>
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<td></td>
<td>±</td>
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<tr>
<td></td>
<td>0.52ABb</td>
<td>0.81Aa</td>
<td>1.32 C ABb</td>
<td>0.88ABCb</td>
<td>0.63CDb</td>
<td>0.72DEb</td>
<td>0.38Eb</td>
<td>1.88Eb</td>
<td></td>
</tr>
<tr>
<td>Small letters denote differences P&lt;0.05 between samples</td>
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<td>Capital litters denote differences P&lt;0.05 between diluents</td>
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</table>
the SNP treated samples showed significant (P<0.05) conservation of dead sperm percentage in lower rate at all osmotic gradient dilution as compared with isotonic of control samples.

**DISCUSSION**

This finding of our results is consistent with earlier studies demonstrating that even motility of sperm from nitric oxide donor treated samples of bull were reduced compromised in tonicity changes tolerance of ability in vitro \(12\), beginning of shape and swelling \(4\), and regulation of sperm volume \(9\), during a hypotonic stress, cell typically respond by swelling, a response that can result in lysis if a maximum volume is exceeded control samples. Critical tonicities for the normospermic sodium nitroprussid treated samples were lower than that for the control samples reported for bull spermatozoa, \(13\). However, the values for critical tonicity of spermatozoa from the treated samples bull's semen far lowest the values for control samples in spermatocrits, Surprisingly, there was a double-fold magnitude decrease between the treated samples and control bull sperm, and duplicated and triplicated increase of both SNP treated samples and control respectively as compared with isotonic sample. Which reinforced our previous assertions that so and function are markedly powerful integrity of sperm membrane due to Sodium nitroprussid as NO donor \(13\).

When sperm are exposed to hypertonicity, the first response is to shrink from intracellular water loss, with extent of shrinkage depending on intracellular free water volume \(3\). The sperm apparently have an innate resistance to hypotonic stress \(2\) and \(6\) in limited and critical value. In contrast; likewise, little or no membrane damage is detectable, when bull sperm are exposed to osmolalities up to 800 mOsm, with higher osmolalities causing extensive membrane damage \(6\). This contrasted with our present observations in bulls, where sperm maintained high proportions of intact membranes even in solutions down tonicities. \(14\) Reported that the plasma membrane of the human spermatozoon undergoes extensive reorganization during hypotonic swelling that places excessive demands on the cellular cytoskeleton due to donation of nitric oxide by SNP, with most of the related Na-K ATPase that control integrity of cell membrane and sperm osmolarity \(15\). First, when cells are exposed to hypotonic solutions, it may cause a net leak/influx of non-permeating solutes into the cells. This alteration is presumed to arise from the solute's ability to change the conformation and stability of the hydrophilic portions of the plasma membrane \(8\). Subsequently, when cells are established to lowering tonicity solutions, the difference in the osmolality within the cell and the external
environment trigger the cells to swell beyond their normal isotonic volume and lyse or rupture of bilayer phospholipids of sperm membrane which coincided with results in table (3) that maintain the cell integrity by NPS treated samples reduce lysis and sperm death direct acting on Ca$^{2+}$ ion pivotal activity in through Nitric oxide control fast channel of Calcium (16) Our findings further confirm this negative relationship between sperm motility and hypotonic changes in control samples whereas reduce these negatively effect on motility by accelerating dynine head hydrolysis of ATP and influx of Ca$^{2+}$ ion due to Nitric oxide liberated from nitric oxide which were reduced that act to osmotic stress. To fully understand the side of usefulness in the cryobiological properties of cells, it is critical to: 1-SNP as nitric oxide donor judge the effect of permeating cryoprotectants on sperm motility and membrane integrity; and 2-govern the plasma membrane penetrability to water (osmotic controlling ions conductivity) during cryoprotectant, as well as the respective maintain their fertility inseminated vagina (3). Knowledge of these biophysical properties of adding SNP would allow calculating the number of steps, as well as the volumes of the diluent to be added, to minimize the effects of osmotic stress on cells to be cryopreserved. Studies are in progress examining additional characteristics of spermatozoa activating by nitric oxide donor in sperm membranes, effect of permeating cryoprotectants on sperm motility and membrane integrity, and permeability characteristics of water and cryoprotectants (6). This is the next logical phase in developing a reliably effective sperm cryopreservation method for bull semen.

Taher Wabeh Awais Al-Terikik (SNP) في حجم الثقبة لمني الثيران المخفف

كماران رسول عبد الشافي
كلية الطب البيطري، جامعة بغداد، العراق

الخلاصة

دراسة تأثير الواهب لوكسيد النيتروجين في صلامة وديمومة غشاء النطفه وحجمها وعلاقاتها بلجوية وحركة النطف. أجريت التجربة للمقارنة بين عشرون نموذج من السائل المنوي مقسم إلى مجموعتين متساويتين عشرة نماذج تم تخفيضها بمخفف الترس والمضادات اليه تثامن تراكيز مختلفة الأوزوموزكية الناقصة التوتر معامل بالصوديوم نايتروبروسيد وعشرة نماذج في المجموعة الثانية من السائل المنوي المخفف بمخفف الترس والغير مضادات اليه الصوديوم نايتروبروسيد من التراكيز الناقصة التوتر، مخفف الترس معامل بالصوديوم نايتروبروسيد في المني أظهر حماية عالية للنطف من التغييرات الأوزوموزكية. وأظهرت النتائج أن درجة حماية عالية للسائل المنوي من المخفف بنتانق تركز المحل الناقصة التوتر على التوالي في اختيار حجم النطف المضغوطه والتورم التي تنتصب بأهمية البيانات لحجم التحول النسبي الحجمي. بالإضافة أن اضافة الصوديوم
نايتروسبايدانثور دورا وقائيا لاختبار الأوزموزية وأبيات فلئية على الاختلاف بصلاحيه النطفه وقرنيا على الحركة في المحلول ناقص التوتر. وقد يعزى ذلك للتحكم المباشر لأوكسيد النتريرك مؤديا إلى التنظيم الحيوي لخيميرة الصوديوم-بوتاسيوم (Na-K ATPase) وقوائت الال kalciوم في غشاء خليه النطفه.

REFERENCE


