# EFFECT OF NITRIC OXIDE DONOR SODIUM NITROPRUSSIDEON SPERM VOLUME OF DILUTED BULL SEMEN

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## 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 usingtwo groupseach 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 nitroprussidein that protected the sperm fromosmocellular changes stress. The results showed tolerance sperm to gradient hypotonic solution in sperm swelling and classic spermatocrit 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 orefflux of water during reestablishment of osmotic equilibrium. However, spermatozoa are able to maintain their volume after osmotic shock, thus avoiding theconsequences 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 inseminationcenter-Iraq, that assisted low grade fertility (~40% individual motility, ~57% SpermViability 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) NaCltrisdiluents'solution. This consisted of Ringer solution, in which the 0-9 % (0-154 molality)NaCl had been replaced by 0-191 molalityNaCl. 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 (4-5years old) imported from Australia divided in to twoequal samples groups; control samples and Sodium nitroprossid(0. $35\times10^{5}$ %) treated samples, each one were separated in to tensub-testedtrialswith examined the seven samples replications according to NaCland responsible media used concentrations.Bull semen samplequantities 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 werecarried out at 2000 g for 15 minute at (23 to 24) C°(10).While in diluents expander of tonicity downto ~ 50 % of the isotonic one, the spermatozoa are capable to exhibittail motion.

**Individual motility**

Onedrop 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 spermatocritswere 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:**

Controlsamplesdisplayed a significant ($P<0.05$) gradual decrease in sperm motility as corresponding decrement tonicity as compared with saline sampleon the other hand the SNP treated sample showed significant ($P\leq0.05$) 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) showeda 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.

| | NaCltris hypotonic diluents | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Semen samples** | **Isotonic** | **A 100%** | **B 80%** | **C 60%** | **D 40%** | **E 20%** | **F 16.6** | **G 14.3%** |
| **Control** | 0.016 ± 0.002 Aa | 0.019 ± 0.003 Ba | 0.025 ± 0.008 Ca | 0.34 ± 0.002 Da | 0.48 ± 0.007 Ea | 0.054 ± 0.003 Fa | 0.057 ± 0.004 FGa | 0.059 ± 0.005 Ga |
| **SNP** | 0.014 ± 0.004 Ab | 0.015 ± 0.005 ABb | 0.016 ± 0.002 Bb | 0.018 ± 0.006 Cb | 0.019 ± 0.006 CDb | 0.020 ± 0.003 Db | 0.025 ± 0.009 Eb | 00.030 ± 0.007 Eb |

**Values are expressed as Mean ± SE: n(10)**
**Small letters denote differences P<0.05 between samples**
**Capital litters 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.

| | NaCltris hypotonic diluents | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Semen samples** | **Isotonic** | **A 100%** | **B 80%** | **C 60%** | **D 40%** | **E 20%** | **F 16.6** | **G 14.3%** |
| **Control** | 47.37 ± 0.42Aa | 44.72 ± 1.53 Ba | 36.12 ± 1.17Ca | 30.34 ± 1.16 Da | 23.95 ± 0.78Ea | 20.53 ± 0.95Fa | 12.81 ± 0.31Ga | 8.40 ± 1.29 Ha |
| **SNP** | 80.72 ± 1.67Ab | 79.38 ± 2.26 Bb | 74.10 ± 1.20Cb | 70.05 ± 3.68Cb | 66.27 ± 0.99Db | 63.85 ± 1.35Eb | 59.00 ± 1.14Fb | 44.50 ± 3.33 Gb |

**Values are expressed as Mean ± SE: n (10)**
**Small letters denote differences P<0.05 between samples**
**Capital litters denote differences P<0.05 betweendiluents**

**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.

| | NaCltris hypotonic diluents | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Semen samples** | **Isotonic** | **A 100%** | **B 80%** | **C 60%** | **D 40%** | **E 20%** | **F 16.6** | **G 14.3%** |
| **Control** | 14.95 ± 1.53Aa | 14.51 ± 0.78Aa | 18.90 ± 1.14 Ba | 21.92 ± 0.75Ca | 26.57 ± 0.92 Da | 30.58 ± 1.36 Da | 36.66 ± 1.40Ea | 40.10 ± 1.46Fa |
| **SNP** | 15.00 ± 0.52ABb | 14.48 ± 0.81Aa | 15.28 ± 1.32 C ABb | 16.00 ± 0.88ABCb | 17.50 ± 0.63CDb | 19.17 ± 0.72DEb | 20.51 ± 0.38Eb | 21.65 ± 1.88Eb |

**Values are expressed as Mean ± SE: n(10)**
**Small letters denote differences P<0.05 between samples**
**Capital litters denote differences P<0.05 between diluents**

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 withearlier studies demonstrating that even motility of sperm from nitric oxide donor treated samplesof bullwerereducedcompromised 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, cellstypically respond by swelling, a response thatcan result in lysis if a maximum volume isexceeded control samples. Critical tonicities for the normospermicin sodium nitroprussid treated sampleswerelower than that for the control samples reported for bull spermatozoa, (Table 1). However, the values for critical tonicity of spermatozoa from the treated samplesbull's semen far lowest the values for control samples in spermatocrits, Surprisingly, there was a double-fold magnitude decrease between the treated samplesand controlbull 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 nitroprossid as NO donor (13).

When sperm are exposed to hypertonicity, the first response is to shrink from intracellularwater loss, with extent of shrinkage depending on intracellular free water volume (3). The sperm apparently have an innate resistance tohypertonic stress (2 and 6) in limited and critical value. In contrast;likewise,little or no membrane damage is detectable, when bull sperm are exposed toosmolalities up to 800 mOsm, with higher osmolatiescausing extensive membrane damage(6). This contrasted with our present observationsin bulls, where sperm maintained highproportions of intact membranes even in solutionsdown tonicities.(14) Reported that the plasma membraneof the human spermatozoon undergoesextensive reorganization during hypotonicswelling that places excessive demands on thecellular cytoskeleton due to donation of nitric oxide by SNP, with most of the relatedNa-K ATPase that control integrity of cell membrane and sperm osmolarity (15). First, when cellsare exposed to hypotonic solutions, it maycause a net leak/influx of non-permeating solutesinto the cells. This alteration is presumed toarise from the solute's ability to change theconformation and stability of the hydrophilicportions of the plasma membrane (8). Subsequently,when cells are established to lowering tonicity solutions,the difference in the osmolality withinthe cell and the external

environment triggersthe cells to swell beyond their normal isotonicvolume 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 spem death direct acting on $Ca^{+2}$Ion pivotal activity in through Nitric oxide control fast channel of Calcium (16) Our findings furtherconfirm this negative relationshipbetween sperm motility andhypotonic changes in control samples whereas reduce these negatively effecton 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 propertiesof cells, it is critical to: 1-SNP as nitric oxide donor judge theeffect of permeating cryoprotectants on sperm motility andmembrane integrity; and 2-govern theplasma membrane penetrability to water (osmotic controlling ionsconductivity) during cryoprotectant, aswell as the respective maintain their fertility inseminated vagina (3). Knowledge of thesebiophysical properties of adding SNP would allow calculatingthe number of steps, as well as the volumes ofthe diluent to be added, to minimize the effectsof osmotic stress on cells to be cryopreserved.Studies are inprogress examining additional characteristics ofspermatozoa activating by nitric oxide donor in sperm membranes, effect ofpermeating cryoprotcctants on sperm motilityand membrane integrity, and permeability characteristicsof water and cryoprotcctants (6). This isthe next logical phase in developing a reliablyeffective sperm cryopreservation methodfor bull semen.

**تاثير واهب اوكسيد النتريك صوديوم نايتروبروسايد (SNP) في حجم النطفه لمني الثيران المخفف**

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**الخلاصة**

دراسة تأثير الواهب لاوكسيد النيتريك في سلامة وديمومة غشاء النطف ه وحجمها وعلاقتها بالحيوية وحركة النطف. أجريت التجربه للمقارنه بين عشرون نموذج من السائل المنوي مقسمه إلى مجموعتين متساويتين عشرة نماذج تم تخفيفها بمخفف الترس والمضاف اليه ثمان تراكيز مختلفة الاوزموزيه الناقصة التوتر معامله بالصوديوم نايتروبروسيدو ,وعشرة نماذج في المجموعة الثانيه من السائل المنوي المخففه بمخفف الترس والغير مضاف اليه الصوديوم نايتروبروسيد من التراكيزالناقصة التوتر، مخفف الترس المعامل بالصوديوم نايتروبروسايد في المني اظهر حماية عالية للنطفه من التغييرات الاوزموزيق. وأظهرت النتائج أن درجة حماية عالية للسائل المنوي من المخفف بتناقص تركيز المحلول الناقص التوتر على التوالي في اختبار حجم النطف المضغوطه والتورم التي تتسم بأهمية البيانات لحجم التحول النسبي الحجمي. بالإضافة أن اضافة الصوديوم

نايتروبروسايداظهر دورا وقائيا لاختبار الأوزمزيه وابدىقابلية على الا حتفاظ بصلاحية النطفهوقدرتها على الحركة في المحلول ناقص التوتر. و قد يعزى ذلك للتحرر المباشر لاوكسيد النتريك مؤديا الى التنظيم الحيوي لخميرة الصوديوم-بوتاسيوم الادينوسين ثلاثي الفوسفاتيز (Na-K ATPase) وقنوات الكالسيوم في غشاء خلية النطفة.

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