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# SEMEN QUALITY OF RAMS GROWN ON DRINKING SALINE WATER

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## SUMMARY

Twentry-four Barki male lambs (1.04 ± 0.1 months, age) were equally divided into two groups: the first represented the fresh water-treated (0.3 g/1 TDS) group (FW) and the second one used as saline-treated (13.1 g/1 TDS) group (SW). The experiment extended for about 22 months. During the last two months, animals trained for semen collection (using artificial vagina), then, semen quality of the first and second ejaculates, for nine rams from each group, was evaluated at 3-day intervals.

Long-term saline treated rams showed highly significant reduction ($P < 0.01$) in the sperm-cells concentration and the percentage of alive sperm-cells and advanced motility of spermatozoa. This was coincided with highly significant increase ($P < 0.01$) in the percentage of the sperm-cells primary and secondary abnormalities. Biochemical evaluation of semen reflected that SW-treated rams showed a highly significant increase ($P < 0.01$) in fructose content and a highly significant reduction ($P < 0.01$) in citric acid concentration and acetylcholinesterase enzyme activity. On the other hand, a highly significant negative correlation was found between fructose content and each of the sperm-cells concentration, percentage of the advances motility and percentage of the alive sperm-cells, however, acetylcholinesterase enzyme activity showed a highly significant positive correlation with each of the three physical parameters. The enzyme activity negatively correlated with the percentage of primary and secondary abnormalities of sperm-cells. The experiment showed a marked adverse effect on the semen quality of rams grown on drinking saline water.

## INTRODUCTION

It is well known that arid regions, such as north-western coast of Egypt, are characterized by a long dry season that may extend for few years. Under such drought conditions, heat stress and scarcity of fresh-water and food supplies is the main problem's influence the local animals. Wells that penetrate salt deposits may be the only available source of drinking water for sheep raised in such areas. It was found that the tolerable salt concentration by sheep ranges from 1.1 to 1.3 % of the chloride-type water (Pierce, 1966).

Few studies were concerned with the effect of drinking saline water on the reproductive performance of sheep. It was found that chloride (1.3 % TDS) or bicarbonate (0.5 % TDS) wate decreases the rate of reproduction in ewes an receiving chloride water under poor nutritiv conditions adversely affects the birth weight c lambs (pierce, 1968 a, b). However, Mittal an Ghosh (1983, 1984 a, b) observed no adver effect on the reproductive performance of ew offered underground water (0.20 - 0.35 % TD On the contrary, Assad and Bayoumi (199

found that drinking saline water (1.3 % TDS) promoted better reproductive performance in ewes, better lamb survival and did not affect lamb growth till weaning.

The present experiment was conducted to investigate the accumulative effect of saline drinking water (1.3 % TDS) on semen quality of the growing rams.

## MATERIAL AND METHODS

Twenty-four Barki male lambs, of 10.4 ± 0.1 month's age and 26.0 ± 0.8 kg body weight, were equally divided into two groups: the first used as the control group (FW) (fresh water-treated, 0.3 g/1 TDS) and the second one used as the saline-treated group (SW) (13.1 g/2 TDS). Drinking saline water prepared by daily dilution of the sea water (39.3 g/1 TDS). The experiment conducted at Maryout Station, 35 km. West of Alexandria, and extended from December 1992 to September 1994 (22 months).

During the last two months of the experiment, animals trained (using artificial vagina) for semen collection (1st & 2nd ejaculates) at 3-day intervals for about 5 periods of collection. Following the training period, semen quality of the first and second ejaculates, for nine animals from each group, evaluated at 3-day intervals for seven consecutive periods. Ejaculate volume (using graduated collection tubes) and pH (using Whatman indicator papers) were assessed rapidly for the fresh ejaculates. Ejaculate concentration was measured by diluting 100µl. Of raw semen in 7.9 ml of 2.9 % sodium citrate solution and counting sperms by using a haemocytometer. Percentage of the advanced motility was estimated to the nearest 10 % by diluting 10µ1, of raw semen in 2ml. of 5% egg-yolk citrate solution. One drop of the diluted semen spred on a warm slide and several microscopic fields (x 40) were examined. Percentage of dead and alive sperms (using eosin-analine stained smears) and primary and secondary sperm abnormalities (using Giemsa stained smears) (Saacke, 1970) were also determined for each ejaculat. Fresh samples of raw semen were also taken for the determination of fructose (Barakat and El-Sawaf, 1964), citric acid(Saffran and Denstedt, 1984) and acetylcholinesterase enzyme activity (Metcalf, 1951. Unfortunately, semen samples of most animals of both treated groups were finished which makes the determination of the concentration of some minerals and trace elements impossible.

The results of the experiment were statistically analysed by using the Statgraphics Statistical Graphics system, version 5.0, copyright 1985-1991. Values expressed as percentage were subjected to arc-sine transformation before analysis.

## RESULTS AND DISCUSSION

The experiment reflected that long term drinking saline water (13.1 g/1 TDS) led to a significant increase ($P < 0.05$) in pH value of ram semen (7.16 vs/ 7.22 for FW and SW-treated groups, respectively), a highly significant reduction ($p < 0.01$) in sperm cells concentration and percentage of alive sperm-cells (29.4 % and 37.2 % from FW- treated group values, respectively) (Table 1). Graves (1978) reported that good-quality semen is usually on the acid side of neutrality than semen with lower sperm-cells concentration, and semen containing many dead spermatozoa may evolve ammonia, which will increase the pH value. In the present investigation, the significant increase in pH value in SW-treated group may be due to the increased percentage of dead cells.

Saline treatment had no significant effect on the ejaculate volume, however, successive ejaculation

**Table ( 1 ) – Analysis of variance and Duncan's multiple range test results for the semen physical parameters (Mean ± SED) of control and saline-treated rams.**

| Sources of variation | d.f. | Mean Square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | pH | Volume (ml) | Concentration ($x10^9$/ml) | % Alive | % Motility | % Primary Abnormalities | % Secondary Abnormalities |
| Treatment | 1 | 0.262 * | 0.0002 | 88.36 ** | 14900.2 ** | 26235.4 ** | 7402.1 ** | 13152.8 ** |
| Ejaculate | 1 | 0.073 | 1.43 ** | 11.11 ** | 157.5 ** | 1014.8 ** | 35.4 | 473.5 ** |
| Interaction | 1 | 0.067 | 0.01 | 4.09 * | 23.4 | 567.1 ** | 98.8 * | 42.5 |
| Error | 248 | 0.041 | 0.12 | 0.69 | 10.4 | 58.7 | 16.9 | 15.1 |
| | | Least Significant Difference | | | | | | |
| Treatment | FW | 7.16 ± 0.02 a | 0.89 ± 0.03 a | 4.02 ± 0.07 a | 69.95 ± 0.47 a | 77.5 ± 1.05a | 15.60 ± 0.56 a | 13.03± 0.53 a |
| | SW | 7.22 ± 0.02 b | 0.89 ± 0.03 a | 2.84 ±0.07 b | 43.93 ± 0.47 b | 45.2 ± 1.05 b | 31.39 ± 0.56 b | 33.89 ± 0.53 b |
| Ejaculate | 1st | 7.21 ± 0.02 a | 0.97 ± 0.03 a | 3.64 ± 0.07 a | 55.68 ± 0.47 a | 64.3 ± 1.05 a | 23.04 ± 0.56 a | 21.41 ± 0.53 a |
| | 2nd | 7.17 ± 0.02 a | 0.82 ± 0.03 b | 3.22 ± 0.07 b | 58.21 ± 0.47 b | 58.3 ± 1.05 b | 23.94 ± 0.56 a | 25.51 ± 0.53 b |

N.B.: Means in the same column in each block and followed by the same letter are not significantly different from each other. SED = Standered error of difference of means; d.f. = Degree of freedom; FW = Fresh-water treated group; SW = Saline-water treated group; * = $p< 0.05$; ** = $p< 0.01$.

**Table (2) – Analysis of variance and Duncan's multiple range test results for the semen biochemical parameters (Mean ± SED) of control and saline-treated rams.**

| Sources Of Variation | d.f. | Mean Square | | |
|---|---|---|---|---|
| | | Fructose (mg/100ml) | Citric Acid (mg/100ml) | ACHE (µmole/ml) |
| Treatment | 1 | 4892829.3 ** | 84202.1 ** | 579.4 ** |
| Ejaculate | 1 | 7238.6 | 378.4 | 42.5 ** |
| Interaction | 1 | 1411.5 | 6530.1 | 31.9 * |
| Error | 248 | 30048.4 | 3198.7 | 5.8 |
| | | Least Significant Difference | | |
| Treatment | FW | 687.1 ± 15.4 a | 194.8 ± 5.04 a | 13.21 ± 5.04 a |
| | SW | 965.8 ± 15.4 b | 158.2 ± 5.04 b | 10.18 ± 5.04 b |
| Ejaculate | 1st | 821.1 ± 15.4 a | 177.7 ± 5.04 a | 12.10 ± 5.04 a |
| | 2nd | 831.8 ± 15.4 a | 175.3 ± 5.04 a | 11.28 ± 5.04 b |

N.B.: Means in the same column in each block and followed by the same letter are not statistically different from each other. SED = Standered error of difference of means; d.f. = Degree of freedom; FW = Fresh-water treated group; SW = Saline-water treated group; ACHE = Acetylcholinesterase; * = $p< 0.05$; ** = $p< 0.01$.

**Table (3) – Correlation coefficients between biochemical parameters and some semen characteristics.**

| | Fructose (mg/100ml) | Citric Acid (mg/100ml) | Acetylcholinesterase (µmole/ml) |
|---|---|---|---|
| Concentration ($x10^9$ /ml). | - 0.504 ** | 0.076 | 0.829 ** |
| % Motility. | - 0.481 ** | 0.237 ** | 0.556 ** |
| % Alive. | - 0.541 ** | 0.319 ** | 0.429 ** |
| % Primary Abnormalities. | 0.484 ** | - 0.249 ** | - 0.542 ** |
| % Secondary Abnormalities. | 0.564 ** | - 0.246 ** | - 0.532 ** |

Degrees of freedom= 250.; ** = $p< 0.01$.

led to a highly significant reduction ($P < 0.01$) in the ejaculate volume (Table 1).

Semen of SW-treated group showed a highly significant reduction ($P < 0.01$) in advanced motility of sperms (41.7% from FW-treated group value). This result was coincided with a highly significant increase ($P < 0.01$) in the percentage of the primary and secondary abnormalities (101.2 % and 160.1 % from FW-treated group values, respectively) (Table 1). The reduced sperm-cells concentration and percentage of alive sprm-cells, in addition to the increased percentage of sperm-cells abnormalities, may reflect an adverse effect of saline on the spermatogenic process. The long term saline treatment of rams may cause a stress on the pituitary gland and led to an alteration in the gonadotropins releasing cells activity which controls the androgenic secretion by testes. It has been reported that hypophysectomy results in cessation of spermatogenesis which can be restorated by treatment with both FSH and LH or with FSH and testosterone (Garner and Hafez, 1993).

The experiment reflected also that long term drinking saline water significantly increased ($P < 0.01$) the concentration of fructose (40.6 % from FW-treated group value) (Table 2). It is well known that fructose is the principal reducing sugar of the ram semen (Mann, 1946) and the formation of fructose in the male accessory organs is a hormone-dependent process (Mann, 1964). In addition, Hiroe et al. (1963) stated that the increased fructose content in ram semen is considered to be due to increased androgen secretion by the testes. In the present investigation, the increased fructose content in SW-treated ram semen may be due to the marked reduction in the sperm cells concentration and percentage of the alive (active) cells. Moss, et al. (1979) stated that the rate at which fructose is metabolized can give an indication of the activity of spermatozoa. It can be noticed from table (3) that fructose concentratiion showed a highly significant negative correlation with each of the sperm-cells concentration, pecentage of alive sperm-cells and percentage of advanced motility.

Saline-treated rams showed a highly significant reduction ($P < 0.01$) in seminal citric acid content (about 19% from FW-treated group value) (Table 2). It was found that most of the citric acid, the major organic compound in semen, is derived from the vesicular gland (Hess et al., 1960 and Shah et al., 1968). On the other hand, Lindner and Mann (1960) found that vesicular gland content of citric acid was significantly correlated ($r = 0.89$, $P < 0.01$) with the level of testicular testosterone. In the present study, the marked reduction in seminal citric acid content may give an indication of an alteration in the androgenic secretion activity of the testes due to the long term saline treatment stress on the pituitary gland.

Saline-treated rams showed a highly significant reduction ($P < 0.01$) in the acetylcholinestrase enzyme activity (about 23% from FW-treated group value) (Table 2). Nelson (1972) stated that acetylcholinesterase is thought to be responsible for the propulsion of the spermatozoa by controlling the intracellular level of acetylcholine, the estter of great significance in the generation and conductance of bioelectric currents along fibers. In addition, the enzyme activity probably plays a significant part in coordinating the motility function of spermatozoa (Briscoe, 1955). In the present investigation, the enzyme activity showed a highly significant positive correlation with the percentage of advanced motility (Table 3). On the other hand,the highly positive correlation between the enzyme activity and each of the sperm-cells concentration and percentage of the alive sperm-cells, in addition to its highly significant negative correlation with the percentage of each of the primary and secondary abnormalities, may offer an evidence for its intracellular localization. Similar results were also

found in bulls by Abdou et al. (1977).

In conclusion, long term saline treated rams exhibited a marked reduction in their semen quality as judged by the significant reduciton in the sperm-cells concentratiбn, percentage of alive sperm-cells and percentage of advanced motility of spermatozoa. Primary and secondary abnormalities of spermatozoa were significantly increased. The results may be indicative of some alteration in the spermatogenic precess following the long term saline treatment. Further studies are needed to clarify the exact direct effect of salinity on the male gonadal functions.

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