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# Alleviation of Heat Stressed Egyptian Suffolk Rams by Treatment with Selenium, Melatonin or Prostaglandin $F_{2\alpha}$ During Hot Summer of Egypt

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**Abstract:** In the present work, the rams were divided into four groups of nearly equal average weights, during THE hot season of the year. The first group was kept without treatment as control. The second group was treated with selenium (0.1 mg / kg DM as sodium salenite) orally. The third group was injected with melatonin (25 $\mu$g / kg body weight, daily at sunrise; melatonin was dissolved in a minimum of absolute ethanol and diluted in 0.9 NaCL 1: 9) and the fourth was injected with Prostaglandin F2$\alpha$ (PGF2$\alpha$; 3 mg/head, one hour before collection of semen). The calculated temperature-humidity index (THI) value during the experimental period (25.6 with daylight length 14.05), indicated that the rams were exposed to very severe heat stress. The results showed that, in summer breeding season, rams injected with PGF2$\alpha$ and melatonin had significantly ($P<0.05$) lower rectal and skin temperature than in selenium and control groups, with the lowest values in PGF2$\alpha$ group.. The differences between selenium and control group were not significant in rectal temperature and between selenium and melatonin group in skin temperature. Scrotal-skin temperature was insignificantly affected by treatment. Rams injected with Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) surpassed ($P< 0.05$) the control group in sperm motility percentage and sperm-cell concentration ($x10^9$/ml), while the contrary occurred ($P< 0.05$) in reaction time, percentages of dead spermatozoa, sperm abnormalities and acrosomal damage. Rams treated with selenium had lower significantly ($P<0.05$) semen pH than in the other groups. Scrotal length was higher significantly ($P<0.05$) in rams treated with $PGF_{2\alpha}$ or selenium than in control or melatonin groups. The differences between $PGF_{2\alpha}$ and selenium and between melatonin and control groups, were not significant. Scrotal circumference and testes length were insignificantly affected by treatment. Blood components in rams, were insignificantly affected by selenium, melatonin or $PGF_{2\alpha}$ treatments.

**Key words:** Egyptian Suffolk rams, semen characteristics, scrotal measurements, blood components, selenium, melatonin, $PGF_{2\alpha}$, heat stress.

## Introduction

In Egypt, one of the early trials which was carried out to improve the Ossimi local sheep, was by grading up to 15 / 16 U.K Suffolk sheep, in Ministry of Agriculture. The crossbreeding programme was begun in the year 1957, then inter se mating was carried out. The obtained flock (Egyptian Suffolk) was of 70-90 % Suffolk blood (Aboul-Naga and Aboul-Ela, 1985).

The common breeding season in the country is practised during May-June, due to that lambing and growth of lambs occur during the mildest weather during the year (beginning of October), coincided with the availability of Egyptian clover, the main fodder crop in the country. However, the climate in such breeding season is hot with rapid and sudden fluctuations due to that the mentioned months are at the end of the spring season, which adversely affects the animals productive and reproductive abilities (Daader *et al.*, 1985, Kamal *et al.*, 1988, Habeeb *et al.*, 1992 and Marai *et al.*, 1996). Alleviation of heat stress may be achieved by ameliorating the environment, reducing the animal's heat production and/or helping the animals to dissipate the heat load. The latter includes, physical, physiological and nutritional techniques (Marai *et al.*, 1994 and Marai and Habeeb, 1997). In that respect, melatonin hormone and prostaglandins can be used as physiological techniques and selenium can be used as nutritional technique. This is attributed to that selinium acts as a component of the enzyme glutathione peroxidase activity, an enzyme that catalyzes the degradation of organic hydroperoxdase. Regarding the melatonin hormone, it can be used to increase fecundity. The prostaglandins have a role in increase of testicular contraction with consequent promotion in the release and progression of spermatozoa towards the epididymis (Hagrove and ELLis, 1976), in addition to that it

play important roles in control of blood pressure, lipolysis, gastric secretion, blood clotting and other general physiological processes including renal and respiratory functions (Hafez, 1987). Shawki *et al.* (1986) reported that rats subjected to 10 $\mu$g of $PGF_{2}\propto$ showed increase in sperm-cell concentration and motility and survival of spermatozoa conceded with activation of most of the somniferous tubules and decrease in the secretions of the seminal vesicles and prostate. The objectives of the present investigation were to study the effects of selenium dietary supplementation and injection of melatonin and $PGF_{2}\propto$ on reproduction of Egyptian Suffolk rams under stressful hot summer conditions of Egypt.

## Materials and Methods

The present study was conducted in the Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The experimental work was carried out at El-Gemmaiza Experimental Station located in mid Nile Delta (30.5°N) and the lab work was conducted in the Department of Sheep and Goats Research, both belonging to Animal Production Research Institute, Ministry of Agriculture, Egypt.

A total number of 20 rams of Egyptian Suffolk sheep was used, during May – July months. The rams were of 1.5 - 2.5 years of age and 60 - 70 kg body weight. The animals were divided into four groups of nearly equal average weights. Eack group was of 5 rams. The first group was kept without treatment as control. The first group was kept without treatment as control. The second group was treated with selenium (0.1 mg / kg DM as sodium salenite) orally. The third group was injected with melatonin (25 $\mu$g / kg body weight, daily at sunrise; melatonin was dissolved in a minimum of absolute ethanol and diluted in 0.9 NaCL 1: 9) and the fourth was injected with Prostaglandin F2$\propto$ (PGF2$\propto$; 3 mg/head, one hour before collection of semen).

Rams were housed in soil floored, partially wood roofed in semi-open sheds during the experimental period. The partitions of the rams 3 x 8 metres. The surface area of each shed pen was surrounded by brick walls of 2 metres height.

Animals were offered their requirements according to NRC (1985). The quantity of selenium determined in the experimental diet was 0.09 ppm/kg DM which was nearly similar to the requirements for sheep according to N.R.C. (12985). The animals had access to water daily four times in summer at 9.00, 12.00, 15.00. Additional ration for rams were offered 15 days before and after beginning of the breeding seasons (total 35 days). The additional ration was composed of 1.0 kg concentrates, 0.25 kg barely and rice straw *ad lib.* Proximate analysis of the concentrates and roughages (Table 1) were carried out according to A.O.A.C. (1980).

### Measurements and procedures

**Semen traits:** Semen ejaculates were collected weekly by means of artificial vagina. The temperature of inner liner rubber sleeve of the artificial vagina was adjusted to be 41 - 43°C at the time of collection. At each collection time, sterile inner liner and graduated collecting tube, were used.

At collection of semen, libido (reaction time), pH, semen ejaculate volume, percentages of sperm motility, dead spermatozoa, sperm abnormalities and acrosomal damage, were measured.

Libido was assessed according to Chenoweth (1981). Hydrogen ion concentration was tested with pH paper. Semen ejaculate volume was measured directly in millilitres to the nearest 0.1 ml in a transparent graduated glass tube. Mass motility percentage was assessed in a drop of fresh semen examined under low power microscope, using a hot stage adjusted at 37°C. Mass motility was estimated as percentage score according to the procedure outlined by Melorse and Laing (1970). Live and dead sperm percentages were examined immediately after semen collection in a smear made from a drop of fresh ejaculated semen and stained by distilled water eosin (5 %) according to the method of Hancock (1951), then dried by warm air. For determination of percentage of abnormal spermatozoa, the slides of live sperm were used. Live and abnormal spermatozoa percentages were counted using hand counter. Acrosomal abnormalities were determined by using smears made from the row semen and stained by Gimsa stain according to Watson (1975). Two hundred spermatozoa were examined for each sample. Sperm-cell concentration ($x10^{9}$/ml) was determined by using Spectronic 20 machine of set wave length at 550 millimicrons. A spectronic 20 tube containing 7.9 ml of 2.9 % sodium citrate solution was inserted into machine and adjusted to read 100 % transmittance, then removed and 0.1 ml semen was added to the tube by using serological pipette. The tube (containing 7 - 9 ml of 2.9 % sodium citrate and 0.1 ml semen) was inserted into Spectrionc 20 machine and the percentage of transmittance after 10 seconds was recorded.

Table 1: Chemical analyses of the diets used in experimental trails.

| Items | DM (%) | CP (%) | CF (%) | EE (%) | NFE (%) | OM (%) | Ash (%) | Sel. (%) | Car.(%) |
|---|---|---|---|---|---|---|---|---|---|
| Concentrates | 90.46 | 18.50 | 13.46 | 4.85 | 53.69 | 90.50 | 9.50 | 0.095 | 1.46 |
| Barely | 90.00 | 7.78 | 10.00 | 2.23 | 76.56 | 96.57 | 5.34 | 0.097 | 2.00 |
| Rice straw | 92.30 | 3.47 | 35.10 | 1.41 | 39.65 | 39.65 | 20.37 | 0.084 | 2.00 |

Se. = Selenium and Car = Carotene.

Table 2: Least square means (±S. E.) Of physiological parameters, libido, semen characteristics and scrotal measurements as affected by selenium, melatonin and prostaglandin PGF2 ∝ during summer season, in Egyptian Suffolk rams

| Variables | Treatments | | | | Significance |
|---|---|---|---|---|---|
| | Control | Selenium | Melatonin | Prostaglandin | |
| Physiological parameters | | | | | |
| Rectal temperature (°C) | 39.95a±0.11 | 39.56a±0.11 | 39.14b±0.11 | 38.14b±0.11 | ** |
| Skin temperature (°C) | 38.46a±0.07 | 38.17b±0.07 | 38.08b±0.07 | 37.71c±0.07 | ** |
| Scrotal-skin temperature (°C) | 32.75±0.46 | 32.18±0.46 | 32.33±0.46 | 31.79±0.046 | NS |
| Libido and semen characteristics | | | | | |
| Reaction time (Libido, second) | 21.08a±0.84 | 20.00ab±0.84 | 18.04b±0.84 | 15.04c±0.84 | ** |
| Ph | 6.94b±0.03 | 6.79b±0.03 | 6.93a±0.03 | 6.90a±0.03 | ** |
| Ejaculate volume (ml) | 0.95±0.07 | 1.10±0.07 | 1.00±0.07 | 1.05±0.07 | NS |
| Sperm motility (%) | 67.29b±1.88 | 70.63ab±1.22 | 72.08ab±1.88 | 75.83a±1.88 | * |
| Dead spermatozoa (%) | 25.19a±1.39 | 22.36ab±1.39 | 21.21ab±1.39 | 18.71b±1.39 | ** |
| Sperm abnormality (%) | 19.54a±1.15 | 18.08ab±1.15 | 15.33ab±1.15 | 14.38b±1.15 | ** |
| Acrosomal damage (%) | 15.54a±1.06 | 14.38ab±1.06 | 11.33b±1.06 | 10.54b±1.06 | ** |
| Sperm cell concentration ($x10^9$/ml) | 0.68c±0.09 | 1.12b±0.09 | 1.12b±0.09 | 1.58a±0.09 | ** |
| Scrotal measurements | | | | | |
| Scrotal circumference (cm) | 23.33±1.05 | 22.17±1.05 | 24.96±1.05 | 25.02±1.05 | NS |
| Scrotal length (cm) | 19.19b±0.44 | 21.40a±0.44 | 19.78b±0.44 | 21.50a±0.44 | ** |
| Testes length (cm) | 11.75±0.40 | 11.63±0.40 | 11.63±0.40 | 11.71±0.40 | NS |

Means bearing different letter within the same raw, differ significantly (P<0.05)
** P<0.01, *P<0.05 and NS = Not significant. Sign. = Significance.

Table 3: Least square means (±S.E) of blood components as affected by selenium, melatonin and Protaglandin F2∝ treatments, during summer season, in Egyptain Suffolk rams.

| Variables | Treatments | | | | Significance |
|---|---|---|---|---|---|
| | Control | Selenium | Melatonin | Prostaglandin | |
| Total protein (g/dl) | 6.25±0.84 | 7.59±0.84 | 6.92±0.84 | 6.83±0.84 | NS |
| Albumin (g/dl) | 3.15±0.28 | 3.97±0.28 | 3.97±0.28 | 3.97±0.28 | NS |
| Globulin (g/dl) | 3.10±0.39 | 3.62±0.39 | 2.95±0.39 | 2.79±0.39 | NS |
| Createnine (mg/L) | 27.70±1.45 | 17.95±1.45 | 21.12±1.45 | 21.35±1.45 | NS |
| Glucose (mg/dL) | 7.12±2.56 | 10.06±2.56 | 9.11±2.56 | 5.57±2.56 | NS |
| Alkaline phosphatase (u/L) | 691.36±0.714 | 451.89±107.14 | 724.10±107.14 | 757.24±107.14 | NS |
| Lactate dehydrogenase (u/L) | 318.74±92.62 | 398.02±92.61 | 301.13±92.61 | 475.45±92.61 | NS |
| GOT (u/L) | 75.33±5.51 | 90.33±5.51 | 86.67±5.51 | 92.0±5.51 | NS |
| OPT (u/L) | 18.67±4.95 | 27.00±4.95 | 18.33±4.95 | 33.0±4.95 | NS |
| Inorganic phosphorus (ng/dl) | 3.59±0.93 | 3.69±0.92 | 5.85±0.92 | 4.49±0.921 | NS |
| Zinc (ug/dl) | 214.84±42.76 | 376.58±42.76 | 358.99±42.76 | 216.40±42.76 | NS |
| Testosterone (ng/ml) | 3.07±2.23 | 3.47±2.23 | 4.21±2.23 | 6.18±2.23 | NS |
| Estradiol (pg/ml) | 12.12±5.61 | 13.30±5.61 | 16.59±5.61 | 20.44±5.61 | NS |
| T3 (ng/dl) | 27.93±7.45 | 50.12±7.45 | 42.87±7.45 | 41.50±7.45 | NS |
| Costisol (ug/100ml) | 4.23±1.62 | 4.70±1.62 | 4.70±1.62 | 3.99±1.62 | NS |

All differences were not significant, NS = Not significant and Sign. = Significance.

**Physiological measurements:** Physiological measurements in rams (rectal, skin and scrotal skin temperatures and scrotal measurements (scrotal circumference, abdominal scrotal tip distance and testes length were estimated at 12.00 h in the same day of semen collection once every week, during the experimental period. Rectal temperature was measured to the

nearest 0.1°C by inserting electronic telethermometer 13 - 166 - 11 Probe (designed for measuring rectal temperature) to the depth of 5 - 6 cm into the rectum. The skin and scrotal-skin temperatures were measured using 15 - 176 -324 Probe which was of 30 mm long with a plate of 10 mm in diameter to be put between skin folds. Scrotal measurements such as testes length, scrotum circumference and scrotal length (distance between abdominal margin and scrotal tip), were measured with a flexible metal tape.

**Blood components:** Blood samples (5 ml) were collected from the jugular vein at 12.00 h from 3 animals chosen randomly from each group of rams into 10cc evacuated glass tubes, one of which contained heparin as anticoagulant. The other samples were put in refrigerator for half an hour without heparin and were allowed to clot. These samples were centrifuged at 3000 rounds/minute for 15 minutes. Plasma and serum were collected and stored at -10°C until analyzed (within one week).

Total protein and albumin in plasma were estimated using Biuret reaction and bromocresol green reagent kits, respectively, manufactured by Pasteur Lab, Egypt. Globulin values were calculated by subtraction of albumin values from their corresponding total protein values. Createnine in plasma was estimated in a protein-free supernatant of plasma, following the procedure of Owen *et al.* (1954). Glucose in plasma was measured by using the glucose oxidase method of Huggett and Nixon (1957).

Total testosterone concentration in blood plasma was estimated by the method of Jaff and Behrman (1974) using coat-A-count $I^{125}$ Radio-immunoassay (RIA) kits purchased from Diagnostic Products Corporation, Los Angelos, California, 90045, USA. Estradiol hormone in blood plasma was estimated by RIA technique using estradiol antibody coated tubes kit. The tracer was labelled with iodine 125 ($I^{125}$-Estradiol). The kit was purchased from DPC, Los Angelos, California, USA. Triiodothyronine ($T_3$) hormone in plasma was measured with the coat-A- count $T_3$ Radio-immunoassay by kits purchased from Diagnostic Products Corporation, Los Angelos, CA 90045, USA. Cortisol hormone in plasma was assayed using RIA technique by cortisol antibody coated tubes kit according to Henricks *et al.* (1984). Glutamic oxaloacetic transaminase (GOT) and glutamic pyrovic transaminase (GPT) in blood serum were determined using the method described by Henry *et al.* (1974), by kits of King Diagnostics, Indiana Polis, Indiana, USA. Alkaline phosphatase (ALP) in serum was determined according to Ratliff and Hall (1973) by using kits of Bio-Analytics. Lactate dehydrogenase (LDH) in serum was detected kinetically as explained in Stanbio kit according to Wooton (1982).

Inorganic phosphorus in serum was measured by direct method with ammonium molybdate reagent kits manufactured by Quimica Clinica Aplicada S.A. Amposta, Spain, according to Fisk and Subbarow (1925). Zinc in serum was estimated by using the atomic absorption spectrophotmetry according to Whiteside (1979).

**Temperature-humidity index:** Ambient air temperature, floor temperature and relative humidity (RH %) were recorded at the times of carrying out the physiological and scrotal measurements and semen collection. Ambient air and floor temperatures were recorded using Mercury thermometer to the nearest 0.1°C. Maximum and minimum temperatures were recorded using thermometer. Relative humidity was recorded using hair-hygrometer to the nearest 1 %. The averages of ambient temperatures and relative humidity values estimated in the present study were 28.2 and 61.4% during the period of the studysummer season, respectively.

Temperature-humidity index (THI) was estimated according to the following equation (Marai *et al.*, 2000): THI = db°C - {(0.31 - 0.31 RH) (db°C - 14.4)}, where db°C = dry bulb temperature in Celsius and RH = RH % / 100. Then the obtained values of THI were classified as follows: <22.2 = absence of heat stress, 22.2 - < 23.3 = moderate heat stress, 23.3 - <25.6 = severe heat stress and 25.6 and more = very severe heat stress. The calculated THI was 25.6 during the period of the study.

**Statistical analysis**

**The statistical analysis for rams data was carried out by using the following model:** $y_{ij} = u + T_i + e_{ij}$, where: $\mu$ = overall mean, $T_i$ = fixed effect of $i^{th}$ treatment (i = 1, 2, 3 and 4) and $e_{ij}$ = residual. The statistical analysis was computed using analysis of variance procedure. Significant differences between means were separated by Duncan's Multiple Range test procedure described in SAS (1995). Multivariate analysis including correlation coefficients between different traits was performed according to Steel and Torrie (1960), using computer program BMDP (1984).

## Results and Discussion

**Temperature-humidity index:** The calculated temperature-humidity index (THI) value was 25.6 (with daylight length 14.05) during summer, indicating exposure of the animals to very severe heat stress.

**Physiological parameters:** Table 2 shows that rectal and skin temperatures were lower significantly ($P<0.05$) in rams injected with $PGF_{2\alpha}$ and melatonin groups than in selenium and control groups, with the lowest values were recorded in PGF2$\alpha$ group. The differences were not significant between selenium and control group in rectal temperature and between selenium and melatonin group in skin temperature. Scrotal-skin temperature was insignificantly affected by treatment. Improvement of rectal and skin temperatures in rams treated with melatonin during summer season was in agreement with the results obtained by El-Darawany (1999). Improvement of rectal and skin temperatures in heat stressed rams that were injected with $PGF_{2\alpha}$ may be due to the beneficial role of either $PGF_{2\alpha}$ or melatonin, in the physiological functions of the animals. Hafez (1987) reported that $PGF_{2\alpha}$ is involved in control of blood pressure, lipolysis, gastric secretion and other general physical processes including renal and respiratory function.

**Libido and semen characteristics:** Rams injected with $PGF_{2\alpha}$ surpassed ($P<0.05$) the control group (without treatment) in sperm motility percentage and sperm-cell concentration, while the contrary occurred ($P<0.05$) in reaction time, percentages of dead spermatozoa, sperm abnormality and acrosomal damage. Rams treated with selenium were lower significantly ($P<0.05$) in semen pH than in the other groups. Such results were similar to those obtained by Fatoh (1981) and Abou-Fandoud *et al.* (1996). Improvement in libido (lowest reaction time) and semen traits in rams injected with $PGF_{2\alpha}$ during summer season may be due to the increase of testicular contraction with consequent promotion in the release and progression of spermatozoa towards the epididymis (Hagrove and ELLis, 1976), in addition to that it play important roles in control of blood pressure, lipolysis, gastric secretion, blood clotting and other general physiological processes including renal and respiratory functions (Hafez, 1987). Shawki *et al.* (1986) reported that rats subjected to 10 $\mu$g of $PGF_{2\alpha}$ showed increase in sperm-cell concentration and motility and survival of spermatozoa conceded with activation of most of the somniferous tubules and decrease in the secretions of the seminal vesicles and prostate.

**Scrotal measurements:** Table 2 illustrates that scrotal length was higher significantly ($P<0.05$) in rams treated with $PGF_{2\alpha}$ and selenium than in the control group. The differences between $PGF_{2\alpha}$ and selenium and between melatonin and control groups were not significant. Scrotal circumference and testes length were insignificantly affected by treatment. The increase in scrotal length during summer (hot condition) in rams treated with $PGF_{2\alpha}$ and selenium may be due to the improvement in tunica dartos function in rams.

**Blood components:** Data in Table 3 shows that plasma total protein, albumin, globulin, createnine, glucose, alkaline phosphatase , lactate dehydrogenase, SGOT, SGPT, inorganic, phosphorus, zinc, testosterone, estradiol, $T_3$ and cortisol were insignificantly affected by selenium, melatonin or $PGF_{2\alpha}$ treatments, during summer season.

## Conclusions

Injection with Prostaglandin $F_{2\alpha}$ during the hot summer of Egypt, seemed to alleviate the heat-stressed Egyptian Suffolk rams exposed to very severe heat stress, since such injection resulted in depression of rectal and skin temperatures and realized favourable changes in sperm motility, sperm cell concentration, reaction time, percentages of dead spermatozoa, sperm abnormalities and acrosomal damage.

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