

Alleviation of Heat Stressed Egyptian Suffolk Rams by Treatment with Selenium, Melatonin or Prostaglandin F₂α During Hot Summer of Egypt

¹I.F.M. Marai, ¹A.A. El- Darawany, ²E.I. Abou-Fandoud and ²M.A.M. Abdel- Hafez

¹Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

²Department of Sheep and Goats, Institute of Animal Research,
Ministry of Agriculture, Dokki, Egypt

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 selenite) orally. The third group was injected with melatonin (25 µg / 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 F₂α (PGF₂α; 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 PGF₂α and melatonin had significantly (P<0.05) lower rectal and skin temperature than in selenium and control groups, with the lowest values in PGF₂α 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 F₂α (PGF₂α) surpassed (P<0.05) the control group in sperm motility percentage and sperm-cell concentration (x10⁹/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 PGF₂α or selenium than in control or melatonin groups. The differences between PGF₂α 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 PGF₂α treatments.

Key words: Egyptian Suffolk rams, semen characteristics, scrotal measurements, blood components, selenium, melatonin, PGF₂α, 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 selenium acts as a component of the enzyme glutathione peroxidase activity, an enzyme that catalyzes the degradation of organic hydroperoxidase. 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 μg of $\text{PGF}_2\alpha$ 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. The objectives of the present investigation were to study the effects of selenium dietary supplementation and injection of melatonin and $\text{PGF}_2\alpha$ 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. Each 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 selenite) orally. The third group was injected with melatonin (25 μg / 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 $\text{F}_2\alpha$ ($\text{PGF}_2\alpha$; 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 raw semen and stained by Gimsa stain according to Watson (1975). Two hundred spermatozoa were examined for each sample. Sperm-cell concentration ($\times 10^9/\text{ml}$) 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 Spectronic 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 (\pm S. E.) Of physiological parameters, libido, semen characteristics and scrotal measurements as affected by selenium, melatonin and prostaglandin PGF2 α during summer season, in Egyptian Suffolk rams

| Variables | Treatments | | | | Significance |
|---|-------------------|--------------------|--------------------|-------------------|--------------|
| | Control | Selenium | Melatonin | Prostaglandin | |
| Physiological parameters | | | | | |
| Rectal temperature (°C) | 39.95a \pm 0.11 | 39.56a \pm 0.11 | 39.14b \pm 0.11 | 38.14b \pm 0.11 | ** |
| Skin temperature (°C) | 38.46a \pm 0.07 | 38.17b \pm 0.07 | 38.08b \pm 0.07 | 37.71c \pm 0.07 | ** |
| Scrotal-skin temperature (°C) | 32.75 \pm 0.46 | 32.18 \pm 0.46 | 32.33 \pm 0.46 | 31.79 \pm 0.046 | NS |
| Libido and semen characteristics | | | | | |
| Reaction time (Libido, second) | 21.08a \pm 0.84 | 20.00ab \pm 0.84 | 18.04b \pm 0.84 | 15.04c \pm 0.84 | ** |
| Ph | 6.94b \pm 0.03 | 6.79b \pm 0.03 | 6.93a \pm 0.03 | 6.90a \pm 0.03 | ** |
| Ejaculate volume (ml) | 0.95 \pm 0.07 | 1.10 \pm 0.07 | 1.00 \pm 0.07 | 1.05 \pm 0.07 | NS |
| Sperm motility (%) | 67.29b \pm 1.88 | 70.63ab \pm 1.22 | 72.08ab \pm 1.88 | 75.83a \pm 1.88 | * |
| Dead spermatozoa (%) | 25.19a \pm 1.39 | 22.36ab \pm 1.39 | 21.21ab \pm 1.39 | 18.71b \pm 1.39 | ** |
| Sperm abnormality (%) | 19.54a \pm 1.15 | 18.08ab \pm 1.15 | 15.33ab \pm 1.15 | 14.38b \pm 1.15 | ** |
| Acrosomal damage (%) | 15.54a \pm 1.06 | 14.38ab \pm 1.06 | 11.33b \pm 1.06 | 10.54b \pm 1.06 | ** |
| Sperm cell concentration (x10 ⁹ /ml) | 0.68c \pm 0.09 | 1.12b \pm 0.09 | 1.12b \pm 0.09 | 1.58a \pm 0.09 | ** |
| Scrotal measurements | | | | | |
| Scrotal circumference (cm) | 23.33 \pm 1.05 | 22.17 \pm 1.05 | 24.96 \pm 1.05 | 25.02 \pm 1.05 | NS |
| Scrotal length (cm) | 19.19b \pm 0.44 | 21.40a \pm 0.44 | 19.78b \pm 0.44 | 21.50a \pm 0.44 | ** |
| Testes length (cm) | 11.75 \pm 0.40 | 11.63 \pm 0.40 | 11.63 \pm 0.40 | 11.71 \pm 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 (\pm S.E) of blood components as affected by selenium, melatonin and Prostaglandin F2- α treatments, during summer season, in Egyptian Suffolk rams.

| Variables | Treatments | | | | Significance |
|------------------------------|--------------------|---------------------|---------------------|---------------------|--------------|
| | Control | Selenium | Melatonin | Prostaglandin | |
| Total protein (g/dl) | 6.25 \pm 0.84 | 7.59 \pm 0.84 | 6.92 \pm 0.84 | 6.83 \pm 0.84 | NS |
| Albumin (g/dl) | 3.15 \pm 0.28 | 3.97 \pm 0.28 | 3.97 \pm 0.28 | 3.97 \pm 0.28 | NS |
| Globulin (g/dl) | 3.10 \pm 0.39 | 3.62 \pm 0.39 | 2.95 \pm 0.39 | 2.79 \pm 0.39 | NS |
| Creatinine (mg/L) | 27.70 \pm 1.45 | 17.95 \pm 1.45 | 21.12 \pm 1.45 | 21.35 \pm 1.45 | NS |
| Glucose (mg/dL) | 7.12 \pm 2.56 | 10.06 \pm 2.56 | 9.11 \pm 2.56 | 5.57 \pm 2.56 | NS |
| Alkaline phosphatase (u/L) | 691.36 \pm 0.714 | 451.89 \pm 107.14 | 724.10 \pm 107.14 | 757.24 \pm 107.14 | NS |
| Lactate dehydrogenase (u/L) | 318.74 \pm 92.62 | 398.02 \pm 92.61 | 301.13 \pm 92.61 | 475.45 \pm 92.61 | NS |
| GOT (u/L) | 75.33 \pm 5.51 | 90.33 \pm 5.51 | 86.67 \pm 5.51 | 92.0 \pm 5.51 | NS |
| OPT (u/L) | 18.67 \pm 4.95 | 27.00 \pm 4.95 | 18.33 \pm 4.95 | 33.0 \pm 4.95 | NS |
| Inorganic phosphorus (ng/dl) | 3.59 \pm 0.93 | 3.69 \pm 0.92 | 5.85 \pm 0.92 | 4.49 \pm 0.921 | NS |
| Zinc (ug/dl) | 214.84 \pm 42.76 | 376.58 \pm 42.76 | 358.99 \pm 42.76 | 216.40 \pm 42.76 | NS |
| Testosterone (ng/ml) | 3.07 \pm 2.23 | 3.47 \pm 2.23 | 4.21 \pm 2.23 | 6.18 \pm 2.23 | NS |
| Estradiol (pg/ml) | 12.12 \pm 5.61 | 13.30 \pm 5.61 | 16.59 \pm 5.61 | 20.44 \pm 5.61 | NS |
| T3 (ng/dl) | 27.93 \pm 7.45 | 50.12 \pm 7.45 | 42.87 \pm 7.45 | 41.50 \pm 7.45 | NS |
| Costisol (ug/100ml) | 4.23 \pm 1.62 | 4.70 \pm 1.62 | 4.70 \pm 1.62 | 3.99 \pm 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. Creatinine 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 I¹²⁵ Radio-immunoassay (RIA) kits purchased from Diagnostic Products Corporation, Los Angeles, 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 (I¹²⁵-Estradiol). The kit was purchased from DPC, Los Angeles, California, USA. Triiodothyronine (T₃) hormone in plasma was measured with the coat-A-count T₃ Radio-immunoassay by kits purchased from Diagnostic Products Corporation, Los Angeles, 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 spectrophotometry 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): $THI = db^{\circ}C - \{(0.31 - 0.31 RH) (db^{\circ}C - 14.4)\}$, 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: $y_{ij} = \mu + T_i + e_{ij}$, where: μ = 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 $\text{PGF}_{2\alpha}$ and melatonin groups than in selenium and control groups, with the lowest values were recorded in $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ may be due to the beneficial role of either $\text{PGF}_{2\alpha}$ or melatonin, in the physiological functions of the animals. Hafez (1987) reported that $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ 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 μg of $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ and selenium than in the control group. The differences between $\text{PGF}_{2\alpha}$ 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 $\text{PGF}_{2\alpha}$ 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, creatinine, glucose, alkaline phosphatase, lactate dehydrogenase, SGOT, SGPT, inorganic phosphorus, zinc, testosterone, estradiol, T_3 and cortisol were insignificantly affected by selenium, melatonin or $\text{PGF}_{2\alpha}$ 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.

References

- Abou-Fandoud, M. E.I., Abdel-Momin and A. M. A. Abdine, 1996. Effect of oxytocin, prostaglandin $F_{2\alpha}$ and adrenaline on semen output of $\frac{1}{2}$ Finn x $\frac{1}{2}$ Rahmani sheep. *J. Agric. Sci., Mansoura University, Egypt*, 21: 3499 – 3509.
- Aboul-Naga, A.M. and M. B. Aboul-Ela, 1985. The performance of Egyptian breeds of sheep, European breeds and their crosses. 2. European breeds and their crosses. *Proceedings of 36th Annual Meeting of the European Association for Animal Production, Kallithea, Greece*. pp: 1-20.
- A.O.A.C., 1980. Association of Official Agriculture Chemists. *Official Methods of Analysis*. Washington, D.C.
- BMDP, 1984. *Biomedical Programme for Statistical Analysis*. Mc-Grow-Hill Book Company, New York.

Marai *et al.*: Alleviation of Heat Stressed Egyptian Suffolk Rams by Treatment with Selenium

- Chenoweth, P.G., 1981. Libido and mating behaviour in bulls, boars and rams. A review. *Theriogenology*, 16: 155-177.
- Daader, A.H., F. El-Keraby, I. F. M. Marai and S. A. H. El-Jibouri, 1985. Ram semen characteristics as affected by some climate elements in sub-tropical conditions. *Egyptian Journal of Animal Production*, 25: 105-116.
- Indian J. Animal Sci., 69: 560 - 563.
- El-Darawany, A.A., 1999. Improving semen quality of heat stressed rams in Egypt. *Indian J. Animal Sci.*, 69: 1020 - 1023.
- Fatoh, E.M., 1981. Effect of prostaglandins ($F_2\alpha$ and E1) on some aspects of spermatozoal activity of Balady buck semen. Ph.D. Thesis, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.
- Fisk, G.H. and Y. Subbarow, 1925. The colourimetric determination of phosphorous. *J. Biological Chemistry*, 66: 376- 382.
- Habeeb, A.A., I. F. M. Marai and T. H. Kamal, 1992. Heat stress. In *Farm Animals and the Environment*, edited by C. Phillips and D. Piggins C.A.B. International. pp: 27- 47.
- Hafez, E.S.E., 1987. Reproduction in Farm Animals. 5th Edition, LEA & Febiger, Philadelphia.
- Hagrove, J.A. and L. C. Ellis, 1976. Autonomic nerves versus prostaglandins in the control of rat and rabbit testicular capsular contraction *in vitro* and *in vivo*. *Biotechnological Reproduction*, 14: 651 - 558.
- Hancock, J.I., 1951. A staining technique for the study of temperature shock in semen. *Nature*, London, 197: 323-343.
- Henricks, D.M., J. W. Copper, J. C. Spitzer and L. W. Grimes, 1984. Sex differences in plasma cortisol and growth in the bovine. *J. Animal Sci.*, 59: 376 - 381.
- Henry, R.J., D. C. Cannon and J. W. Winklemn, 1974. *Clinical Chemistry, Principles and Techniques*. 2nd Edition, Harper and Row, New York. pp: 884 - 889.
- Huggett, A.S.G. and D. A. C. Nixon, 1957. Enzymic determination of blood glucose. *Biochemical J.*, 66: 12 - 24.
- Jaff, B.M. and N. R. Behrman, 1974. Method of Hormone Radioimmunoassay. Academic Press. Washington DC.
- Kamal, T.H., A. A. M. Habeeb, A. Abdel-Samee and I. F. M. Marai, 1988. Milk production of heat stressed Friesian cows and its improvement in the sub-tropics. Proceedings of International Symposium on Constraints and Possibilities of Ruminant Production in the Dry Sub-tropics, Cairo, Egypt
- Marai, I.F.M., M. S. Ayyat, H. A. Gabr and U. M. Abdel-Monem, 1996. Effects of heat stress and its amelioration on reproduction performance of New Zealand White adult female and male rabbits, under Egyptian Conditions. Proceedings of 6th World Rabbit, Congress. Toulouse, France, 2: 197- 207.
- Marai, I.F.M., L. B. Bahgat, T. H. Shalaby and M. A. Abdel-Hafez, 2000. Fattening performance, some behavioural traits and physiological reactions of male lambs fed concentrates mixture alone with or without natural clay, under hot summer of Egypt. *Annals of Arid Zone*, 39: 449 - 460.
- Marai, I.F.M., K. A. El-Masry and A. S. Nasr, 1994. Heat stress and its amelioration with nutritional, buffering, hormonal and physical techniques for New Zealand White rabbits maintained under hot summer conditions of Egypt. *Options Mediterranee*, 8(Supplement): 475 - 487.
- Marai, I.F.M. and A. A. M. Habeeb, 1997. Management practices to ameliorate heat stress. *Options Mediterranee*, Seriee A., 17: 127 - 134.
- Melorse, D.R. and J. A. Laing, 1970. The Characteristics of Normal Semen, edited by J.A. Laing. Baillier Tindall and Cassel. London.
- N.R.C., 1985. Nutrient Requirements of Sheep. 6th Edition National Academy Press, 2120 Constitution, NW, Washington DC 20418.
- Owen, J.A., B. Iggo and F. J. Scandrett, 1954. The determination of creatinine in plasma and serum and urine, a critical examination. *Biochemical J.*, 58: 426 - 437.
- Ratliff, C.R. and F. F. Hall, 1973. Laboratory Manual of Clinical Biochemistry. Scott Office Temple, T.X. (Kit for Alkaline Phosphatase, Bio Analysis).
- SAS, 1995. Statistical Analysis System. Users Guide: Statistics. Version. 5th Edition. SAS Institute Inc. Cary, NC.
- Shawki, M.M., M. A. I. El-Sayed, M. A. Omar and A. G. Hegazi, 1986. Effect of Prostaglandin $F_2\alpha$ on semen cell characteristics in rats. *Egyptian J. Veterinary Science*, 23: 205 - 218.
- Steel, G.D. and H. Torrie, 1960. Principles and Procedures of Statistics. Mc-Graw-Hill Book, Company Inc. New York.
- Watson, P.P., 1975. Use of a Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Veterinary Res.*, 97: 12-15.
- Whiteside, P.G., 1979. An Introduction to Atomic Absorption Spectrophotometry 1st Edition Pye Unicam Ltd, Cambridge, U.K.
- Wooton, I.D.P., 1982. Microanalysis in Medical Biochemistry. 6th Edition. Churchill LTD. London.