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SEMEN CHARACTERISTICS AND PLASMA TESTOSTERONE OF NEW-ZEALAND MALE RABBITS AS AFFECTED BY ENVIRONMENTAL TEMPERATURES

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ABSTRACT

Sixteen adult New-Zealand White rabbit males at the age of 6 months and weight of 2.5 ± 0.14 kg were used to study semen characteristics and some biochemical and enzymatic properties of seminal plasma, and blood plasma testosterone during spring and summer seasons. The averages of maximum and minimum ambient temperature in both seasons were 27.1 °C and 18.9 °C in spring, versus 32.2 °C and 26.5 °C in summer, while the averages of relative humidity were 86.1 % in spring, and 89.5 % in summer. Results showed that sperm concentration, percentages of dead sperm, and sperm with intact acrosome were decreased ($P<0.01$) during summer, while percentages of live sperms and acrosome-reacted sperm were increased ($P<0.01$). Ejaculate volume and percentages of motile and abnormal spermatozoa were slightly affected by the season. Biochemical analysis of seminal plasma showed increase in total protein (TP), globulin ($P<0.01$) and albumin ($P<0.05$) during summer. Seminal plasma total lipids (TL) were decreased ($P<0.01$) while cholesterol was increased ($P<0.05$) during summer. Alkaline phosphatase (ALP) decreased ($P<0.01$) and lactate dehydrogenase (LDH) increased ($P<0.05$) during summer. Initial fructose and blood plasma testosterone were increased ($P<0.01$) during the summer season. Thus, changes in environmental conditions from spring to summer season were accompanied with marked alterations in some seminal plasma parameters and related reproductive functions of rabbits.

Key words: Semen, Testosterone, Rabbits, Environmental Temperatures

INTRODUCTION

In tropical and sub-tropical countries, climatic heat is the major factor restricting animal productivity. Growth, milk production and reproduction are impaired as a result of drastic changes in biological functions caused by heat stress (Marai et al., 1995). Rabbits are very sensitive to high environmental temperature, where the dense fur and lack of sweat glands make heat loss very difficult above the zone of thermal neutrality. Excess heat is dissipated mainly by expired air (evaporation of water), stretching out to increase body surface, and vasodilatation of ear veins (Harkness, 1988). Elevation of ambient temperature affects puberty deleteriously, leads to testicular degeneration and reduces percentages of normal and fertile spermatozoa in the ejaculate of males. The ability of male to mate and fertilize are also affected. Biological backgrounds of such phenomenon include disturbances in each of sexual activity, endocrine and testis functions; spermatogenesis and physical and chemical characteristics of the semen (Abdel-Samee et al., 1997).

Seminal plasma, an extracellular fluid that provides the medium and vehicle for spermatozoa, is a composite mixture of secretions that come from the male accessory reproductive organs. Determinations of biochemical constituents of seminal plasma are needed for semen evaluation, since physical characteristics of semen alone are not completely satisfactory for semen appraisal in the current practice (Mann and Lutwak- Mann, 1981). On the

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other hand, some investigators (Abdel-Samee and Marai, 1997) reported that lower fertility rate of rabbits during summer is mainly due to females rather than males. They suggested that fertility of bucks including sexual drive and semen quality were not greatly affected during hot seasons.

Thus, the present study was aimed at studying semen characteristics and the major biochemical constituents of seminal plasma and their relationship with some indicators of semen quality of the male New-Zealand rabbits during spring and summer seasons.

MATERIALS AND METHODS

This experiment was carried out at the Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University. Sixteen New-Zealand adult white rabbit males, 6 months old and 2.5 ± 0.14 kg of mean body weight were used to study the effect of spring and summer climates on semen characteristics, major biochemical constituents of seminal plasma and blood plasma testosterone. The averages of maximum and minimum ambient temperature in both seasons were 27.1 ºC and 18.9 ºC in spring (April-May), versus 32.2 ºC and 26.5 ºC in summer (July-August), while the averages of relative humidity were 86.1 % in spring and 89.5 % in summer. During each season 8 animals were maintained under these conditions for two months for each season. During the experimental period the rabbits were individually housed in universal galvanized wire batteries with feed and fresh tap water offered *ad libitum*. A commercial balanced pelleted ration for breeding rabbits containing 18% crude protein, 14% crude fiber, 2% fat and 2600 kcal DE/ kg feed was used.

Blood samples were collected once every two weeks from each animal through the ear vein in heparinized tubes, early in the morning before access to feed and water. Plasma was obtained by blood centrifugation at 3,000 rpm for 20 min and then stored at -20 ºC until used for analysis of testosterone concentration.

Semen collection was performed weekly using an artificial vagina at about 9 a.m. Volume of semen ejaculates was recorded and ejaculates were placed in water bath at 38 ºC. Determination of seminal initial fructose was carried out immediately after collection according to the method of Mann (1948). Percentages of spermatozoal progressive motility were subjectively estimated at 400x magnification using light microscope equipped with heating stage. Sperm concentration was measured by a hemocytometer slide. The percentages of dead, live and abnormal spermatozoa were assessed by the method reported by Blom (1983). Acrosome integrity was evaluated according to the method of Bryan and Akruk (1977).

Seminal plasma was separated from ejaculates by centrifugation at 5,000 rpm for 10 min. The recovered seminal plasma fraction was further centrifuged at 10,000 rpm for 15 min. at 4 ºC. Fractions of two-week seminal plasma were pooled and stored at -20 ºC until analysis. Total seminal plasma protein (TP) was measured by the Biuret method as described by Armstrong and Carr (1964) and total albumin concentration was determined by the method of Doumas et al., (1971). Total globulin concentration was calculated as the difference between seminal plasma total protein and seminal plasma albumin. Total lipids (TL) were determined as described by Frings et al., (1972) and total cholesterol concentration was measured according to Watson (1960). Transaminase activities (aspartate amino transaminase, AST, alanine amino transaminase, ALT) and alkaline phosphatase (ALP) were measured by the method of Reitman and Frankel (1957). All the previous biochemical parameters were determined using commercial kits obtained from Bio ADWIC, Egypt. Lactate dehydrogenase (LDH) was determined according to Stroev and Makarova (1989). Blood plasma testosterone was determined by Enzyme Linkage Immuno Sorbent Assay (ELISA) kits obtained from Bio-source Europe S.A. Belgium.

Statistical analysis of the obtained data was performed using the general linear model (GLM) produced by Statistical Analysis Systems Institute (SAS, 1989). Significant differences among means were evaluated using Duncan's Multiple Range Test of SAS (1989).

RESULTS AND DISCUSSION

Results presented in figure 1 indicated that there was insignificant decrease in ejaculate volume and sperm motility during exposure of male rabbits to summer conditions. However, sperms concentration decreased significantly ($P<0.01$) during summer compared to spring. The overall mean values for sperm concentration were $428.5x10^6$/ml during spring and $269.9x10^6$/ml during summer. Determination of the percentages of dead spermatozoa during spring and summer seasons indicated that dead spermatozoa were higher during spring, while live spermatozoa values showed opposite trend (Fig. 1). On the other hand the overall mean percentages of abnormal spermatozoa were not affected by the season. The same trend was reported by El-Azab (1980), who found that there is an increase in live spermatozoa in the summer season compared to winter. Also Tawfeek et al. (1994) showed no significant differences between seasons in percentages of live rabbit sperm.

The influence of high environmental temperature on semen ejaculate volume of males in farm animals is conflicting. The studies of Marai et al. (1991) and Lide et

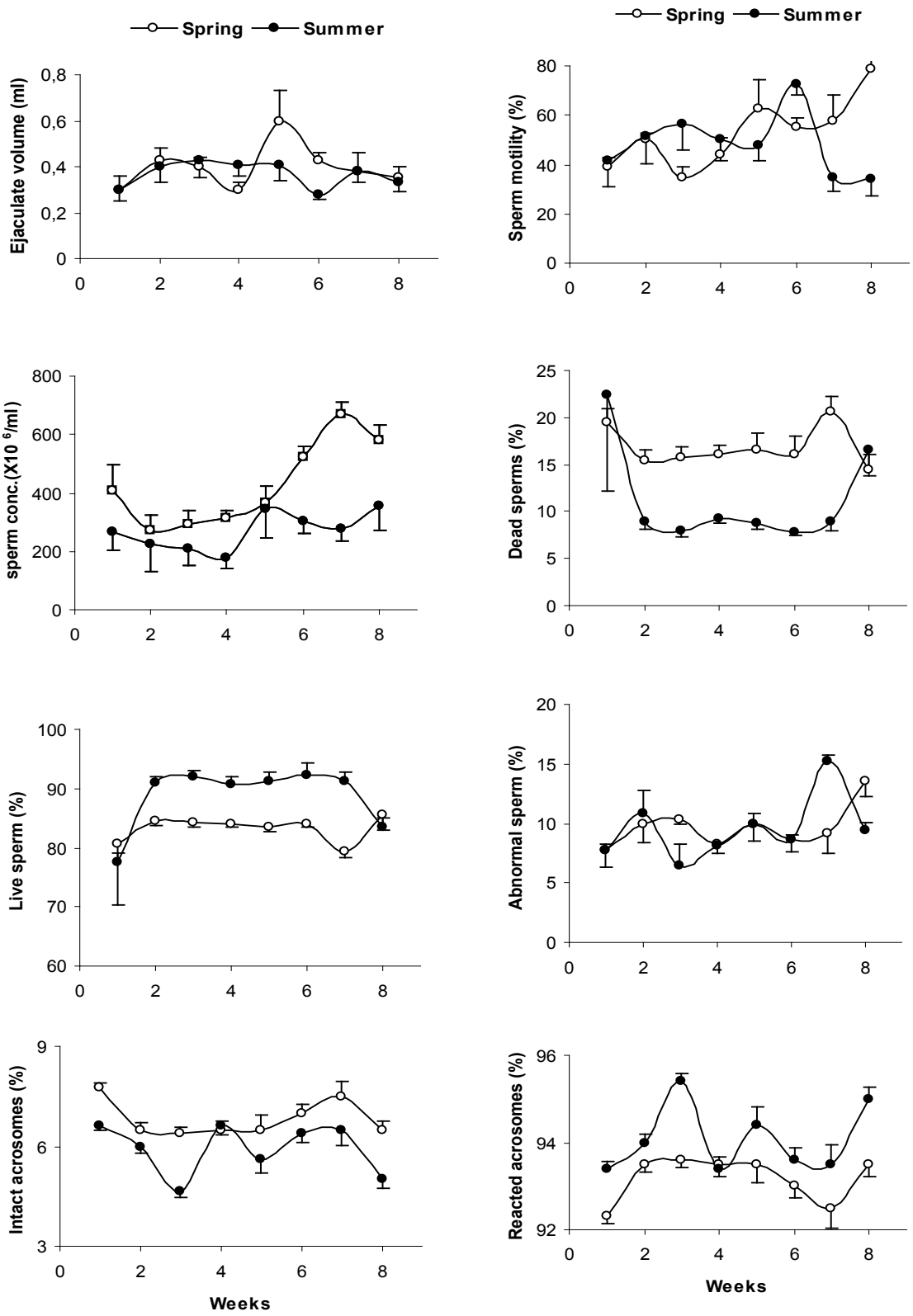

Fig. 1: Seasonal variations in ejaculate volume, sperm motility, concentration, and in the percentages of dead, live, abnormal sperm and sperm with intact or reacted acrosomes in New-Zealand male rabbits during spring (○) and summer (●) seasons

al. (1992) on rabbits showed that semen-ejaculate volume decreased with the elevation of temperature. However, in the present study semen-ejaculate volume did not vary significantly between spring and summer seasons, which is in agreement with the results of Tawfeek et al. (1994), who reported no effect of heat elevation on the semen-ejaculate volume in rabbits. The above conflictions could be attributed to the type and duration of heat exposure, intensity of environmental heat, breed and age of the experimental animals (Abdel-samee et al., 1997). The same confliction was observed concerning the effect of season of the year on sperm motility. Some studies showed that the initial motility of rabbit spermatozoa decreased in hot climatic conditions (Tawfeek et al., 1994). Other studies indicated that motility of spermatozoa either increased or did not show any change due to elevation of temperature (Mittal, 1982), and this later observation was in agreement with the present findings. On the other hand, the present results indicated that sperm concentration was markedly decreased during summer conditions, and coincide with other studies showing detrimental effects on the concentration of spermatozoa in rabbits exposed to high ambient temperature (Marai et al., 1991, Lide et al., 1992 and El-Maghawry and Soliman (2002). A decrease in sperm concentration may be due to degeneration of germinal epithelium and partial atrophy in the seminiferous tubules (El-Sherry et al., 1980).

The percentages of sperm with intact acrosome were decreased ($P<0.01$) and that of the acrosome-reacted sperm were increased ($P<0.01$) during the summer season (Fig. 1). The critical temperature that inhibits spermatogenesis in Hereford bulls was estimated to be 29.4 ºC under conditions of continuous exposure (Rhynes and Ewing, 1973). Viability and true acrosome reaction of bovine spermatozoa are impaired at 40 ºC (Lenz et al., 1983). Seasonal changes in temperature are associated with changes in percentages of abnormal spermatozoa. Heat causes detrimental effects even on indigenous stock in hot climatic regions, including abnormalities in head, mid-piece, tail or proximal cytoplasmic droplets (Akpokodje et al., 1985). However, some investigators found no significant effect of season of the year on buffalo bulls (Gili et al., 1974), and this observation agrees with the present results.

Changes in concentration of seminal plasma TP, albumin and globulin during spring and summer seasons are shown in (Fig. 2). There were significant effects of season on TP, globulin ($P<0.01$) and albumin ($P<0.05$). The overall mean values for TP, albumin and globulin concentrations showed lower values during spring than during summer (1.38, 0.77 and 0.61gm/dl vs. 2.52, 1.00 and 1.52 gm/dl, respectively) (Fig. 2). Seminal plasma proteins are mainly composed of albumin and globulin, in addition to small quantities of non-protein nitrogen amino acids and peptides (Kulkarni et al., 1996). These compounds make up the amphoteric property of seminal plasma proteins and, thus, low protein content in seminal plasma reduces its buffering capacity and in turn semen quality (Dhami et al., 1994). Many studies showed that low content of seminal plasma proteins is associated with poor semen quality (Verma et al., 1985, Dhami and Kodagali, 1989). Strezek et al. (1985) found that during the period of reproductive activity globulin was the major seminal plasma protein, while during the period of reproductive quiescence the level of globulin gradually decreased in red deer males. Therefore, the rise in seminal plasma proteins during summer, which was reported in the present study, suggests that the semen quality was slightly affected in summer.

Total lipids were significantly ($P<0.01$) affected by season, where higher values were recorded in spring (2.36 gm/dl) compared to that in summer (1.48 gm/dl) months (Fig. 2). Kelso et al. (1997) reported that the reduction in sperm concentration and motility were associated with a decrease in seminal plasma content of lipids. On the other hand, seminal plasma cholesterol was higher ($P<0.05$) during summer (114.2 mg/dl) than during spring (91.6 mg/dl). Thyroid hormones were reported to stimulate cholesterol synthesis as well as the hepatic mechanisms that remove cholesterol from circulation. Cholesterol is the precursor in the biosynthesis of sex hormones. The decline in plasma cholesterol level during the spring season may be due to the rate of the later process exceeds that of the former, the plasma cholesterol level drops before the metabolic rate rises. In addition, it was reported that male sex hormones (androgens) increase blood cholesterol (Guyton, 1981).

Seminal plasma initial fructose was significantly ($P<0.01$) higher during summer (245.9 mg/dl) than during spring (137.0 mg/dl) (Fig. 3). Mann and Lutwak-Mann (1981) reported a close relationship between fructose and citric acid concentrations in the semen and the androgenic activity of the male. In the present study fructose concentration showed lower levels during spring months, when seminal plasma testosterone were low (Fig. 3). Similar trend of blood testosterone profile was reported by Ayoub et al. (2000). Therefore, level of fructose in seminal plasma reflects testosterone activity and quality of semen. The present results are in agreement with those of Amir and Volcani (1965), and of Taha et al. (2000) in sheep, where minimum fructose concentrations in seminal plasma were found during spring months and the highest values were noted during August till November.

Measurements of some enzymatic activity in seminal plasma during spring and summer indicated that transaminases were slightly affected by season (Fig. 3). Meanwhile, alkaline phosphatase (ALP) was significantly ($P<0.01$) affected by season (Fig. 3) showing elevated overall mean values (3,984 IU/L) in spring compared

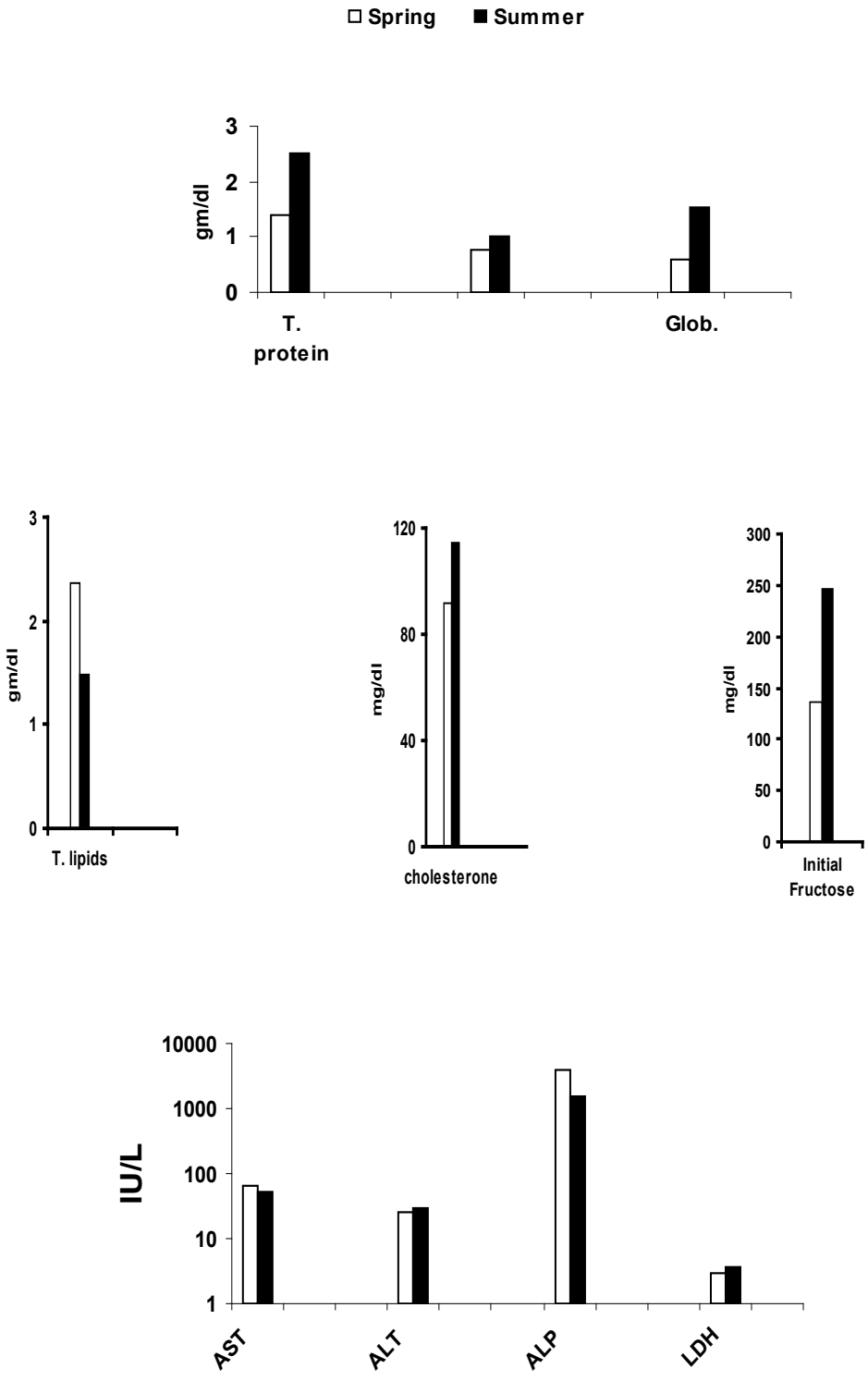

Fig. 2: Seasonal variations in T. protein, albumin, globulin, T. lipids, cholesterol, initial fructose and AST, ALT, ALP, and LDH in seminal plasma of New - Zealand rabbits during spring (□) and summer (■) seasons

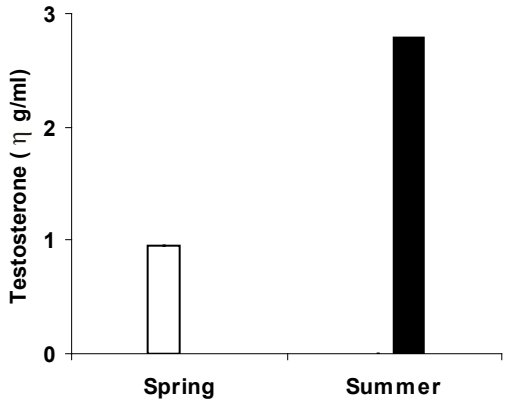

Fig. 3: Changes in testosterone concentrations in New-Zealand male rabbits during spring (□) and summer (■) seasons

to that in summer (1,543 IU/L). LDH was significantly ($P<0.05$) affected by season and showed a higher overall mean values in summer months (3.75 IU/L) than in spring (2.92 IU/L) (Fig. 3). Pursel et al. (1968) reported that one of the consequences of acrosomal damage is the leakage of enzymes from the sperm. The leakage of ALP and LDH revealed a positive correlation between enzyme release and sperm cell integrity and acrosomal damage (Chauban et al., 1993).

The hormonal profiles of blood plasma testosterone during spring and summer seasons are presented in figure (3). There was a significant ($P<0.01$) effect of season on blood plasma testosterone, where the overall mean value was higher during summer (2.79 ηg/ml) than during spring (0.96 ηg/ml). This trend coincided with the increase in both ambient temperature and photoperiod in the summer season (Taha et al., 2000). It was reported that thyroid hormones play a key role in the expression of seasonal reproductive cycle in ewes, because they increase responsiveness to estradiol negative feedback that cause termination of the breeding season (Webster et al., 1991).

The infertility of rabbits during summer might be due to the changes in biological functions caused by thermal stress which negatively affects reproductive performance of rabbits, especially on females. Physiological backgrounds of such phenomenon include drawbacks in thermal balance, endocrine status, conception, uterine function, uterine blood flow, early embryonic development, fetal growth and milk production (Abdel- Sammee and Marai, 1997).

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