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Superovulation and Factors Affecting Ovarian Functions in Barki Ewes

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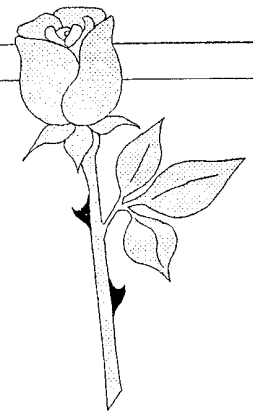
To My Husband

To my Son Mohamed

To my Father

and

To my Mother



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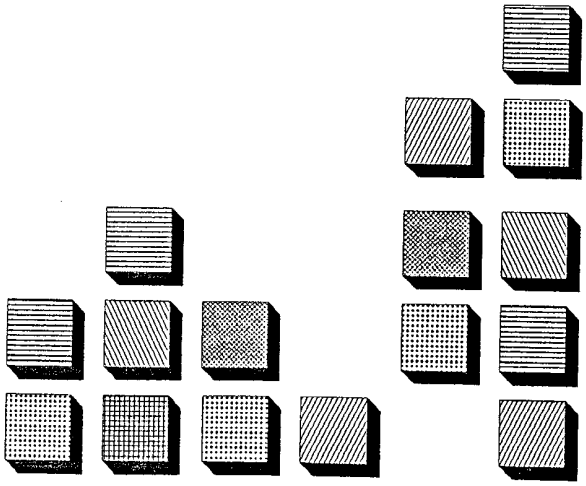


General Introduction

The endogenous breeds of sheep raised in Egypt include Barki, Rahmany and Osimi. Each of these breeds thrive favourably in environmental conditions differing from each other. Barki sheep, in particular are well adapted to newly reclaimed areas of our north western coast. Sheep have the ability to convert and diversify different types of forage into valuable products for mankind, such as mutton, milk and wool. It was estimated that, 1.498 million heads of sheep in Egypt are capable to produce 8000 tons of milk, 227 tons of wool and 21,000 tons of mutton (statistical Report, 1981).

Barki sheep belong to the northern desert. It is a fat tail, carpet wool breed with average body weight of 40 kg. Its milk contains high total solids and solid non fat (16.81% and 10.98% respectively) and the fat content averages 8.84% (Fahmy, Sirry and Safwat, 1969). Barki ewes have almost the same possibility to be bred allover the year (Badawy, El-Bashary and

Mohsen, 1973). Moreover, Barki ewes have 87.30% fertility, 89.38% prolificacy and the gestation period averages 152.66 days under the desert conditions (Omar and Shalash, 1980). Furthermore, it was established that Barki sheep have some sort of resistance against Brucella infection, as it eliminates the causative agent during a period of 5 months after infection (Ismail, 1971). Also, the mortality rate is lower than the other local breeds (Omar and Shalash, 1980).

Barki ewes are mainly raised for mutton, therefore, it is exported to nearly Arab countries especially to Saudi Arabia at Islamic festivals and pilgrimage (The Hajj).

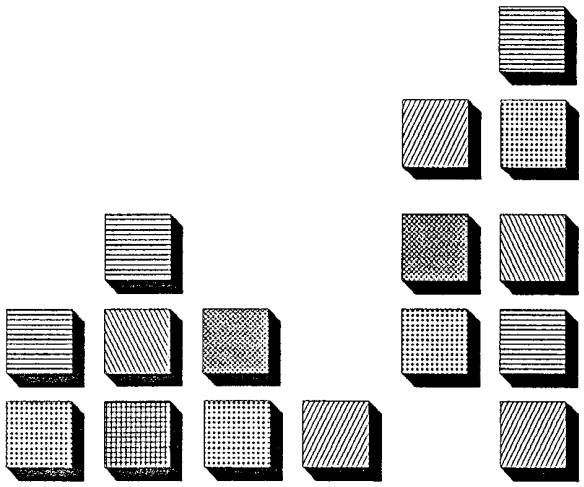
The main purposes of this study are to obtain information on:

1- Oestrous cycle of Barki ewes throughout the year, hormonal profile (oestradiol-17B and progesterone) and Blood chemistry (minerals and proteins).

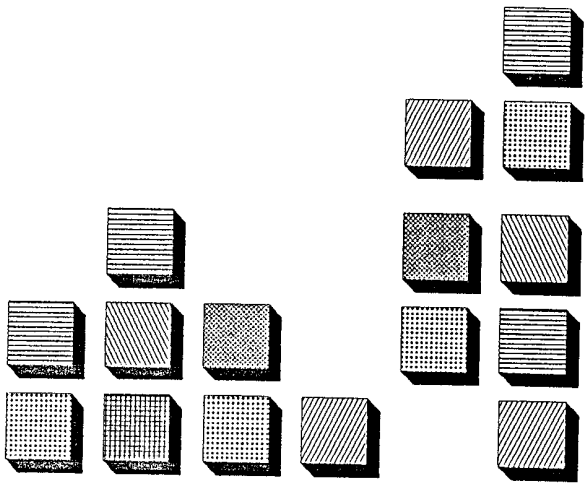
2- Factors affecting the ovarian functions (season, age and nutritional status) and superovulation regimens (PMSG or FSH) with hormonal profile (oestradiol-17B and progesterone) on superovulated Barki ewes.

PART I

THE OESTROUS CYCLE



INTRODUCTION

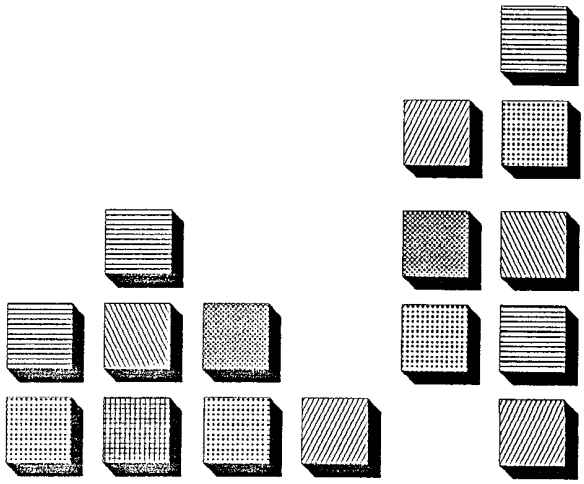


Introduction

The commercial utility of sheep in the form of meat, milk and wool depends largely on their adaptability, early maturation and good potential reproductive performance. Many aspects of the reproductive characteristics of Egyptian sheep are lacking and work in the fields of reproduction is much appreciated.

Studying the oestrous cycle of Barki ewes throughout the year, hormonal profile (oestradiol-17b and progesterone) and plasma minerals (calcium, inorganic phosphorus, iron, zinc) and proteins (total protein, Albumin, globulin) during the different phases of the oestrous cycle allover the year are important data needed for increasing reproductive efficiency.

REVIEW OF LITERATURE



Review of Literature

1. Oestrus:

1.1. Oestrus Behaviour:

Psychic oestrus is the outcome of the complex action or interaction of the gonadal steroids on the nervous system to bring about manifestation of heat (Pant, Hopkinsan and Fitzpatrick, 1972; Tomkins and Bryant, 1974; Cole and Cupps, 1977; McDonald, 1980).

Oestrous symptoms may not be easy to detect in the ewe in the absence of the ram (Gordon, 1983). The manifestation of oestrus takes a special appearance in ewes as they became restless, adopts a typical urintating posture, nudges the genetalia or head region of the ram, tail fanning and looks over her shoulders (Mounib, Ahmed and Hamada, 1956; Tomkins and Bryant, 1974; Elias, 1987). Moreover, ewes remain close to the

ram and stand to be mounted. It should be noticed that ewes display ram seeking activity when they are in oestrus. The oestrous ewe will move its tail vigorously as part of the display pattern when she is with the ram (Inkster, 1957; Lindsay and Fletchter, 1972). Ewes stand firm and the ram continues the love display and mate with the ewe (Tomkins and Bryant, 1974). Barki ewes in heat appear restless and spend whole time near the ram till mating occurs (Sabra, 1987). In addition, the external genitalia became red, the vulva swollen with a slimy clear vaginal discharge which turns to viscous and slightly yellowish. The signs disappear gradually from the 4^{th} to the 5^{th} day as the ewe refuses to accept the ram, then cheesy-like material was observed. In proestrus the ewes became restless and seeking the ram but does not permit mating. In oestrus the ewe may seekout the ram, and permits mounting and service which is the real evidence of oestrus (Hafez, 1979).

Furthermore, a copious clear mucus was noticed shortly after the start of oestrus and lasts till the mid of heat (Grant, 1933; Radford and Watson, 1955; Morrant and Dun, 1960; Restall, 1961). However, in both metoestrus and dioestrus the mucus becomes thick, heavy and cheesy formed of cornified squamous cells (Grant, 1933; Radford and Watson, 1955; Restall, 1961). In oestrus, Salisbury and Vandemark (1961) noticed that the vulva swells and becomes oedematous. The vestibulum becomes bright red, hypaereamic and the partio vaginalis starts to enlarge and release a serous discharge from the cervical and vaginal glands. Moreover, Schindler and Amir (1972) reported that, the appearance of a transparent or a cloudy fluid indicates that the ewes are still at least at the first half of the oestrous period, but when the fluid turns viscous creamy the ewe is approaching the end of oestrus and ovulation. Coincident with oestrus is the occasional enlargement of the vulva, liquification and flow of mucus from the cervix (Hafez, 1979). A

scanty, thick vaginal mucus appears at proestrus and early oestrus, which became serous, voluminous and transparent at the oestrous phase (McDonald, 1980).

1.2. Oestrous Duration:

Most authors recorded that the oestrous period in ewes averaged 35-35.5 hours (Hafez, 1952; Joubert and Lauw, 1964; Schindler, Amir and Eyal, 1969; Schindler and Amir, 1972; Mazzari, Fuenmayot and Chicco, 1977). The highest average duration of oestrus was 50 hours (McDonald, 1980). However 34.9-34.7 hours were recorded by Mounib et al. (1956) and Kassem, Owen and Fadel (1990), 31.5 hours (Badawy et al., 1973), 30 hours (Robertson, 1977; Thimonier, 1979), 29.3 hours (Hunter, 1964), 26.2-26.6 hours (Joubert and Lauw, 1964; Elfouly, Shafie, Abdel-Aziz and Kandeal, 1977; Gonzalez, Goychea and Forazo, 1981), 23 hours (Murrling, 1972), 22.2 hours (Elias, 1987).

1.3. Factors Influencing Oestrus:

1.3.1. Seasonal Variation:

Oestrus is often shorter near the beginning of the breeding season (Grant, 1934; Hafez, 1979; McDonald, 1980). Moreover, Mounib et al. (1956) and Aboul-Naga, Aboul-Ela and Hassan (1985) reported that there was no restricted season for oestrous activity in the Rahmany ewes. However, Aboul-Naga, Aboul-Ela, El-Nakhala and Mehrez (1987) reported that Rahmany ewes showed behavioural oestrus from August to January. The percentage of ewes in oestrus starts to decline during February - March and the decline was more pronounced during April - May with a minimum value in May. According to Robertson and Rakha (1965) the onset of oestrus at the

beginning of the breeding season (12^{th} Oct. - 28^{th} Nov.) was not uniformly distributed throughout the day, the period of highest activity appeared to be around the mean time of sunrise and sunset. Later in the breeding season (December - February), there was more uniform distribution of oestrus over the 24 hours. Ezzo (1989) reported that the oestrous signs were more pronounced during late autumn, winter and early spring. During summer and early autumn the oestrus signs were either slight or even absent.

1.3.2. Breed Variation:

There are some variations in oestrus associated with breed differences (Robertson, 1977; Hafez, 1979; Mcdonald, 1980). Hafez (1979) reported that the wool breeds may tend to have longer oestrous period than meat breeds. Joubert and Lauw (1964) detected the mean duration of oestrus as 35.1 hours (24-46 hours) in Dorper ewes and 26.6 hours (14-36 hours) in Merino ewes. Oestrus period averaged in Aussimi and Rahmani breeds as 26.2 hours and 31.5 hours respectively (Elfouly et al., 1977). In Barki ewes 16-38 hours and in Rahmani ewes 12-28 hours (Elias, 1987).

1.3.3. Age Variation:

Ewe lambs usually have the shortest oestrus periods, older ewes have the longest and the yearlings are intermediate (Mckenize and Terrill, 1937; Hafez, 1952; Mcdonald, 1980). Mounib et al. (1956) noticed that the mean durations of oestrus in Rahmany lambs, ewe and yearling were 8.75 $\pm$ 1.74, 34.9 $\pm$ 1.20 and 19.08 $\pm$ 2.44 hours respectively. Moreover, Davis and Allison (1976) found that young ewes of heavier weight showed longer duration of oestrus than those of lighter weights (10.7 vs 8.9 hours respectively), same authors observed in mature ewes, no significant variation could be detected although the average durations of their oestrus were longer than those of young ewes (12.7 vs. 12 hours respectively).

2. Types Of Oestrous Cycle Length:

The length of the oestrous cycle in sheep has been classified into normal, short, long and multiple cycle (Mounib et al., 1956; Elwishy, Elsawaff and Mikkawi, 1971; Aboul-Naga et al., 1985; Zaki, 1987).

The incidence of the oestrous cycle types varies among breeds. The incidence of short cycles (< 14 days), normal cycles (14-19 days), long cycles (20-26 days) and multiple cycle length (more than 27 days) in Osimi ewes averaged 0, 53.9, 7.7 and 38.4% respectively. Respective values in Awassi ewes were 25.4, 31.3, 7.4 and 35.9% (Elwishy et al., 1971). Moreover, incidence of normal cycles (15-17 days), short cycles (14 days or less), long cycles (more than 18 days) and multiple cycle (more than 27 days) were 54.03, 15.35, 19.16 and 11.47% respectively, in Rahmani ewes, 34.7, 32.91, 26.13 and 6.26% respectively in Barki ewes (Zaki, 1987).

The normal cycle ranges from 14 to 19 days in most studies (Elwishy et al., 1971; Lattoore and Cvitanic, 1977; Pant, Hopkinson and Fitzpatrick, 1977; Ateia, 1981; Ammer and Brudieus, 1982; Zaki, 1987; Ezzo, 1989).

The mean duration of normal cycle length was shown to be 16.0-17.9 days (Mckenzie and Terrill, 1937; Asdell, 1964; Schindler and Amir, 1972, El-fouly, et al., 1977; Smith, Jagusch, Brunswick and Mcgowon, 1980; Ateia, 1985; Kassem, Owen and Fadel, 1990). Moreover, other averages of normal oestrous cycle lengths were recorded as 14.65-15.00 days (Baihanov, 1965; Martemucci, Bellitti, Melodia, Manchisis and Sontore, 1983) and 18.24-18.70 days (Mikus and Longouer, 1961; Elias, 1987).

Shorter oestrous cycle averaged 12 days (Mounib et al., 1956, Mikus and Longauer, 1961; Baihanov, 1965). In this respect, short cycles were 14 days or less (Elwishy, et al., 1971; Lattoore and Cvitanic, 1977).

3. Factors Affecting The Oestrous Cycle:

3.1. Seasonal Variation:

Some breeds of ewes can bred allover the year, for example Egyptian fat - tailed sheep (Hafez, 1952), Barki (Badawy et al., 1973; El-Fouly, et al., 1977; Ateia, 1981, 1985), Rahmany (Mounib et al., 1956, Ateia, 1981), Awassi sheep (Amire and Volcani, 1965; Choueivi, Bair and Khalil, 1966; Ampy and Rottensten, 1969; Al-Wahab, Al-Murant and Al-Kass, 1982), Merino (Petcu, 1975; Fogarty, Dickrson and Young, 1984). In Osimi and Rahmani, the oestrous activity was significantly greater in summer (96.9%) and autumn (94.4%) as compared to winter (78.2%) and spring (68.3%) for both breeds (El-Fouly et al., 1977), and for Uda ewes (Gaillard, 1979), most of the low land mutton breeds and the fine wool breeds developed in warm climates and which are not subjected to extreme seasonal climatic changes have more prolonged breeding seasons (Cole and Cupps, 1977), Breeds of an origin around the Mediterranean Sea (Mcdonald, 1980) have no marked seasonality of oestrous cycle.

Autumn season is considered the breeding season in some breeds of ewes. In Rombouillet ewes the oestrous activity was greatest in autumn and lowest in spring (Wiggins, Barker and Miller, 1970), in Australia, the low-fecundity Merino ewes showed a decline in ovarian activity in mid summer, with an anoestrus that lasted from September - January, while, 66% of high fecundity ewes continued to ovulate throughout the summer with an

anoestrus which lasted only from November - January (Bindon and Piper, 1976). In fat-tailed ewes, the highest incidence of normal cycling ovaries was during September - November (81.5%) and lowest (57.5%) during March - May (Elwishy et al., 1976). In Perendle ewes, the oestrous activity was 86% in September - November (Smith et al., 1980).

Breeding season occurred in autumn and winter in some breeds of sheep. In Palestine, the customary breeding season of the Awassi ewe lasts from the end June to October (Amir , 1965). In Northern latitudes, the ovulatory season of the ewe occurs during autumn and early winter, the season extends from September to February for most of the lowland mutton breeds (Cole and Cupps, 1977).

Some authors found that autumn and spring were the breeding season in ewes. Juma and Dessouky (1969) reported that in Iraq the mating season is in May and June, so lambing coincides with early autumn. A second season is usually expected between November and December so that lambs may be obtained during spring. While, in West Africa, 52.6% of ewes came into oestrus in April-June and 33.5% in September - November (Gonzalz et al., 1981).

3.2. Breed Variation:

The length of the oestrous cycle in ewes varies among breeds (Williams, Carrigus, Norten and Walbandov, 1956). In Awassi breed the oestrous cycle length averaged 17.4-17.6 days (Elwishy et al., 1971; Saab and Hamade, 1984) and 21 days (Eloksh, Galal, Ghanem and Mabrouk, 1981). In Ausimi ewes, it averaged 17.9 days (Elwishy et al., 1971). In Barki ewes, it was 16.4 -16.6 days (El-oksh et al., 1981; Ateia, 1985) and 17.29 - 18.38 days (Badawy, et al., 1973; Elials, 1987). In both Perendal and Merino

ewes it was 17-17.2 days (Smith et al., 1980; El-oksh et al., 1981). While in Rahmany ewes the oestrous cycle length was 17.8-18.24 days on average (Aboul-Naga et al., 1985; Elias, 1987).

3.3. Influence of Feeding:

Nutritional factors are important as related to early growth and puberty and to the annual reproductive cycle of the adult (Coop, 1982). Many authors have shown that low body condition and live weight or severe under nutrition in the premating period may be associated with delayed onset or complete suppression of seasonal oestrus, lengthenning of the oestrous cycle and failure of ovulation or ovulation without oestrus (Doney, Gunn and Griffiths, 1973; Doney, Gunn and Smith, 1979; Rattrary, Jagusch, Smith, Winn and Maclean, 1981). While, the high plane of nutrition offered to ewe lambs may enhance the onset of first oestrus (Allen and Lamming, 1961). Furthermore, Smith (1961, 1964) reported that signs of oestrus and time of onset of breeding season are affected significantly by nutrition. Moreover, Rattary et al., (1981) noticed that the under nutrition restricts both growth rate and the development of endocrine function and it has both a short and a long-term effects on reproductive performance of ewes. Afiefy, Abul Fadle and Zaki (1971) reported that ovarian activity of farm animals in Egypt was affected by feeding Barseem during winter season. While, light foot and Worth (1974) pointed out that grazing oestrogenic pasture prior to and during mating reduced the proportion of ewes in oestrus. Furthermore, Omar and Shalash (1980) found that the prolificacy rate of Merino ewes was lost as the ewes were managed under desert condition. They attributed the reason to the poor pasture in the desert. Shalash, Omar and Tawfik (1980) added that parasitic infestation suppresses the ovarian activity. Furthermore, Mazzari et al., (1977) observed that the oestrous cycle length of tropical ewes was affected by the feeding

level, as the protein supplementation for 90 days prior to mating increases the average length of oestrous periods which reached 35.3 hours compared to 33.4 hours in non-supplemented ewes.

3.4. Influence Of Male:

The introduction of rams to seasonally anoestrous Merino ewes stimulated ovulation within 6 days without exhibiting oestrus behaviour. The oestrus behaviour was noticed after 3 weeks of ram introduction (Under wood, Shier and Davenport, 1944; Schinckel, 1954). Moreover, the presence of a ram may under certain conditions, stimulate sexual activity in the ewe outside the normal breeding season (Cole and Cupps, 1977). The ram stimulus was most probably a pheromone (Knight and lynch, 1980) and presumably influenced LH in the ewe through nerval pathways connecting the accessory olfactory bulbs and the anterior hypothalamus (Estes, 1972).

3.5. Age Variation:

Ewe lambs exhibited less regular cycles and tended to have slightly shorter ones than adults (Mounib et al., 1956). The same authors added that the mean oestrous cycle length in Rahmany ewes and ewe lambs was 17.39 and 16.70 days respectively. In West African breeds, 16.79 and 17.24 days respectively (Gonzalez et al., 1981), 17.4 and 16.4 days respectively (Kassem, et al., 1990).

4. Ovarian Hormones:

4.1. Oestradiol Values:

Moore Barrett, Brown, Irene, Schindler, Margery, Smith and Burbara Smith (1969) reported that oestrogen first appeared on day 14 and 40 hours before the onset of oestrus. Oestradiol levels reached a level of 100 ng/ 100 ml and remained about this level until 0-8 hours after the onset of oestrus, then rapidly declined within 24 hours after the onset of oestrus. Oestrogen concentrations fall to a very low non-detectable levels throughout the luteal phase of the cycle. Moreover, Scaramuzzi Coldwell and Moor (1970) estimated oestrogen in ovarian venous blood by Radioimmunoassay and recorded that the concentration started to increase by day 14 to reach its peak on day 3 and 8. These findings were confirmed by Cox, Mattner and Thorburn (1971). Australian workers (Obst, Seamark and Brown, 1971) using a competitive protein binding assay, recorded that oestradiol values ranging from 7 to 140 pg/ml. While, Goding, Baird, Cumming and McCracken (1971) noticed that the maximum value of oestradiol would be 12 pg/ml. Furthermore, Pant et al. (1972) noticed that the oestradiol-17B levels were basal at all times except for oestrus and they reached peak values of 21.1 $\pm$ 2.0 pg/ml and the timing of oestradiol peak ranged from 0 to 8 hour before the onset of oestrus. Reeves, Back and Nett (1974) concluded that the levels of oestrogen were very low during anoestrus and deviated around a mean level of 4.40 $\pm$ 0.01 pg/ml. However, oestradiol levels were recorded as 5.2 pg/ml for basal concentration and 13.3 pg/ml for peak concentration in oestrus (Yuthasastrakosol, Palmer and Howland, 1975). Moreover, Pant, Hopkinson and Fitzpatrick (1977) reported that the concentration of oestradiol begins to rise 12-14 hours before the onset of oestrus from values 11.2 $\pm$

0.36 pg/ml during the luteal phase to 21.1 ± 2.01 pg/ml between -8 to 0 hour (oestrus). Furthermore, Ezzo (1989) noticed that values of oestradiol-17B averaged 23.315 ± 11.56, 12.62 ± 9.16, 10.61 ± 4.26 and 25.45 ± 9.08 pg/ml during oestrus, metoestrus, dioestrus and proestrus respectively.

4.2. Progesterone Values:

Edgar and Ronaldson (1958) used the chemical assay of progesterone levels in the blood of ewes and found no progesterone level during the first 2 days of the oestrous cycle. The mean concentration of progesterone increased to 1.8 ug/ml from the 3rd to the 7th day, that level is maintained until the 16th day, with a sudden fall to < 0.15 ug/ml on the 17th day. Thorburn, Basset and Smith (1969) observed that progesterone level was low (0.12 ± 0.1 ng/ml) at oestrus (day 0), the concentration remained low during the first few days of the cycle (day 0-3), increased between day 4 and 9 to a mean of 1.7 ng/ml and remained at this level or gradually increased during the next 5 days before declining rapidly on days 14 and 15 to reach a low value on the day before oestrus. Moreover, Stabenfeldt, Holt and Ewing (1969) observed that plasma progesterone concentrations varied among individual ewes between 1.0-5.0 ng/ml during the luteal phase. Bjersing, Mary, Hay, Kann, Moor, Naftolin, Scaramuzzi, Short and Younglai (1972) reported that progesterone concentration in peripheral blood was high on day 13 (1.3-3.2 ng/ml). On day-15, there was an abrupt decline in the level (0.2-0.8 ng/ml), the peripheral levels were more than 0.2 ng/ml at 6-12 hours after the onset of oestrus. Allison and Mc-Natty (1972) noticed that progesterone concentrations were very low on the day of oestrus (0.05 ± 0.02 ng/ml) and remained low for the next 2 to 3 days. Then progesterone value increased during the luteal phase (1-2 ng/ml) and abrupt declined at 12 to 36 hours before the onset of oestrus. Cunningham, Symons and Soba (1975)

showed that the plasma progesterone levels increased progressively during the period of 15-9 days before oestrus to a mean level of about 2.5 ng/ml and the level was sustained for several days. However, 2 days before oestrus, the mean plasma progesterone concentration fell to 1.42 ng/ml and on the following day the level dropped to < 0.95 ng/ml and remained at this low level until after day 2 of the cycle, then showed a progressive rise. In this respect, Pant et al. (1977) observed that progesterone concentration was lowest during oestrus and 2 days after oestrus (0.25 ± 0.01 ng/ml). The level showed a marked rise on day-5 (1.6 ± 0.14 ng/ml) to a peak of 3.70 ± 28 ng/ml between days 7 and 13 followed by a decline over the 36 hours preceding the next oestrus. According to Botha and Morgenthal (1980) progesterone levels (0.06-0.65 ng/ml) during the day following oestrus day increased gradually to 1.0 ng/ml during the first 3 days following oestrus and to a peak (3.18 ± 1.5 to 5.34 ± 1.81 ng/ml) on day-12 of the oestrous cycle, followed by a rapid decrease between day 16 to 18 to reach a low point on the day of oestrus. Moreover, Zaki (1987) reported that plasma progesterone pattern showed an elevation from its basal level (0.292 ± 0.03 ng/ml for Barki ewes and 0.323 ± 0.26 ng/ml for Rahmany ewes) in day-0 to a level of 0.931 ± 0.104 ng/ml in Barki ewe and 1.481 ± 0.104 ng/ml in Rahmani ewes at days 3-5. On days 6-8 it was raised sharply to 1.735 ± 0.244 ng/ml in Barki ewe and 2.936 ± 0.173 ng/ml in Rahmani ewes. Peak levels were reached by 9-11 days (1.85 ± 0.254 ng/ml in Barki ewes and 3.54 ± 0.17 ng/ml in Rahmani ewes). Plasma samples on days 12-14 still showed high concentration of progesterone (1.458 ± 0.209 ng/ml in Barki ewes and 2.728 ± 0.178 ng/ml in Rahmani ewes). A sharp drop was identified after 12-14 days to reach nearly the basal level of 0.266 ± 0.03 ng/ml in Barki ewes and 0.33 ± 0.03 ng/ml in Rahmani ewes by 15-17 days of the oestrous cycle. Furthermore, Romero, Damian, Lueje and Morato

(1989) reported that mean progesterone levels were 0.3 ± 0.14 ng/ml. During the early part of the cycle, the levels remained low (0.5 ng/ml), but from day 6 concentrations increased reaching peak values (1.6 ± 0.66 ng/ml) by day 10. This level was maintained, with minor oscillations, until day-14. On day 15 there was a sharp drop in the levels. Mukasa-Mugerwa, Ezaz and Viviani (1990) reported that average progesterone concentration was significantly different between ewes (range 2.43 - 4.80 ng/ml) and days of the oestrous cycle (0.32 - 5.84 ng/ml). Progesterone concentration was < 1.0 ng/ml from 2 days before to 4 days after oestrus and rose steadily to a peak of 5.0-5.6 ng/ml on day 10-14. This was followed by a rapid decline to 3.0 ng/ml (53% of day-14 peak value) on day 15, 0.8 ng/ml (15% of peak value) on day-16 and 0.2 ng/ml (3.7%) on the day before oestrus. It was concluded that concentrations of < 1.0 ng/ml are indicative of anoestrus or the follicular and early luteal phases of the oestrous cycle.

4.3. Factors Influencing Ovarian Hormone Values:

Irregular fluctuations of oestrogens deviating from a level of 4.40 ± 0.1 pg/ml were observed during anoestrus. The mean level during the period from the first to the second oestrus was 5.2 ± 0.3 pg/ml (Yuthasastrakosol et al., 1975). Ezzo (1989) noticed that the highest oestradiol-17B concentration was during spring followed by autumn, while the lowest concentration was estimated during winter. In summer, oestradiol-17B averaged 13.85 ± 8.96 pg/ml plasma. This variation during the different season was not significant.

Thorburn et al. (1969) noted that progesterone values in Merino ewes were high (3.2 ng/ml) during March and low (0.5 ng/ml) during January. However, progesterone in Rombouillet ewes was higher in cyclic ewes during November where the temperature was lower than in July (Lammond, Goddy

and Kennedy, 1972) and in December than during October (Quirke, Hanrahn and Gosling, 1979) and in March (Breeding season) than October in South African Merino ewes (Botha and Morgenthal, 1980). Moreover, Martemucci et al. (1983) reported that Apulian and Altamura ewes had significant higher concentration in autumn (1.29 - 1.36 ng/ml) vs. in spring (1.07 - 1.19 ng/ml). In Egypt Sabra (1987) observed that the average progesterone concentration of oestrus Barki ewes did not vary significantly during autumn (0.76 $\pm$ 0.24 ng/ml) and winter (o.65 $\pm$ 0.25 ng/ml). Eliase (1987) recorded that the highest progesterone concentrations in Barki ewes were 5.094 ng/ml during autumn and 4.89 ng/ml in summer, while the lowest concentration was recorded in winter (3.83 ng/ml). Furthermore, Ezzo (1989) recorded that the highest progesterone concentration was recorded during autumn followed by spring, while, the lowest concentration was during winter. This variation among season was significant at $P < 0.01$.

5. Plasma Mineral Values:

5.1. Calcium:

The mean plasma concentration of calcium (mg/100ml plasma) was recorded as 9.2-9.65 (Hadleigh, Karl and Swingle, 1955; Wiener and Field, 1969; Lippmann and During, 1973), 10.15 (Wiener and Field, 1971; Healy and Falk, 1974; Idris, Tartour and Babiker, 1976), 11.3-11.55 (Nizar 1982; El-Dinary, 1987), 12.11-12.16 (Ibrahim, Serur, Gomaa, Farrag and Allam 1986; Hackett, Goylot and Bustad 1957) and 18.21 (Ghazy, 1987). Calcium values ranged between 9-12 mg (Underwood, 1966; Field, Weiner and Wood, 1969).

The concentration of calcium during oestrus and dioestrus was 12.95 $\pm$ 0.64 and 10.45 $\pm$ 0.13 mg % respectively (Bhagwan and Dutt, 1974).

Calcium levels averaged 9.24, 8.510, 8.87 and 8.482 mg/dl during oestrus, metoestrus, dioestrus and proestrus respectively (Ezzo, 1989). This variation was not significant. Higher levels of calcium were observed during oestrus than dioestrus and were due to increase metabolic activity under the oestrogen phase of the cycle (Singh and Dutt 1974; Hidiroglou, 1979).

5.2. Inorganic Phosphorus:

The mean concentration of inorganic phosphorus in sheep plasma (mg/100ml) was 4.3-4.5 (Hadleigh et al., 1955; Ibrahim et al., 1986), 5.21-5.50 (Hackett et al., 1957; Wiener and Field 1971; Healy and Falk, 1974), 6-6.38 (Idris et al., 1976; Nizar, 1982) and 7.4 (El-dinary 1987; Ghazy, 1987).

Inorganic phosphorus values in sheep were 4.91 $\pm$ 0.09 and 4.30 $\pm$ 0.09 mg/100 ml, plasma at oestrus and dioestrus respectively (Bhagwan and Dutt, 1974). Ezzo (1989) recorded the average inorganic phosphorus value in Barki ewes at oestrus, metoestrus, dioestrus and proestrus as 7.033 $\pm$ 1.3, 6.56 $\pm$ 1.52, 6.605 $\pm$ 1.85 and 6.64 $\pm$ 1.56 mg/100 ml plasma respectively. This variation was highly significant ($p < 0.01$).

5.3. Ca/p Ratio:

Ca/p ratio in sheep was recorded as 1.5:1 or 2:1 (Underwood, 1966; Vipperman, Preston, Kintner and Pfander, 1969) and 2.55 (Ibrahim et al., 1986). The Ca/p ratio was higher during dioestrus (1.39 $\pm$ 0.41) than metoestrus (1.36 $\pm$ 0.34), proestrus (1.322 $\pm$ 0.338) and in oestrus (1.273 $\pm$ 0.31) (Ezzo, 1989).

A possible relationship exists between phosphorus intake or Ca/p ratio and fertility in apparently normal animals (Brochart, 1956; Ford, 1956, Munro 1957). Moreover, Moustgaard, (1959) states that infertility may be the first sign of phosphorus deficiency.

Phosphorus level was lower in ewes than lambs (Hackett et al., 1957). The concentrations of calcium declined greatly with the age of sheep (Gunn, 1969). These results were confirmed by Wiener and Field (1971) who reported that the lambs have substantially higher concentrations of phosphorus (7.63 ± 0.35 and 4.91 ± 0.61 mg/100 ml plasma) in lambs and ewes respectively and also significantly higher concentrations of calcium (11.56 ± 0.22 and 10.31 ± 0.64 mg/100 ml) in lamb and ewes respectively. This may not be attributed to age alone because the lambs and their mothers are actually different in genotype.

5.4. Iron Values:

Serum iron values in sheep averaged 300 and 141 ug/100ml respectively (Rasmay 1952; Ghosal, Dwarakanath and Jethar, 1976).

Ezzo (1989) showed higher concentration of iron in Barki ewes (1.873 ±0.516 mg/dl plasma) during metoestrus with slight range differences during oestrus (1.585 ± 0.516 mg/dl), dioestrus (1.535 ± 0.49 mg/dl) and proestrus (1.58 ± 0.48 mg/dl). This variation was not significant.

The value of serum iron in sheep did not show any significant inter sexual differences (Planas and Decastro, 1960).

5.5. Zinc Values:

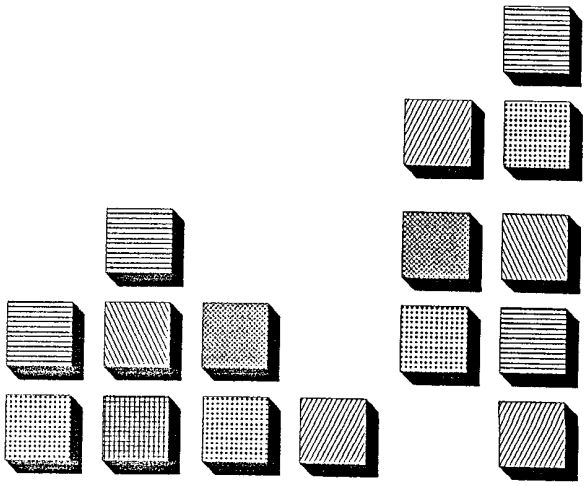
The normal level of serum zinc in sheep was at a range of 53-63 ug/100 ml at North Island and 74-83 ug/100 ml at South Island (Grace, 1972), 80-120 ug/100 ml in sheep and cattle (Miller, 1966) and was 117.9 ug/100 ml in Syria ewes (Ibrahim et al., 1986).

The concentration of zinc was higher (1.27 ± 0.30 ug/ml plasma) during metoestrus and lower (1.091 ± 0.337 ng/ml) during oestrus, with slight differences from 1.209 ± 0.41 to 1.20 ± 0.367 ug/100 ml plasma in dioestrus and proestrus respectively. This variation was not significant (Ezzo, 1989).

6. Plasma Proteins:

The mean concentrations of total protein in sheep were recorded as 5.7-5.8 (Contreras, 1967; Kronfeld and Medway, 1969; Coles, 1986), 7.0-7.25 (Braun, Rice, Benard, Thouvenol and Bonnefis, 1978; Fernandez, Mayer, Gomez, Casca, 1984; Ghazy, 1987) and 8.14-8.9 g/100 ml plasma (Keay, 1981; Hassaan, El-Seaaie and Amer, 1984). Total protein concentration in sheep ranged between 50-80 g/l (Cornelius, 1960). Mean albumin values in sheep were 2.56 (Contreras, 1967), 2.9 (Fernandez et al., 1984; Coles, 1986), 3.08-3.8 (Keay, 1981; Ghazy, 1987) and 6.01 g/100 ml (Hassaan et al., 1984). The mean values of globulin were 2.34 (Keay, 1981), 3.2-3.26 (Contreras, 1967; Ghasy, 1987), 3.45 (Coles, 1986), 3.98 (Hassan et al., 1984) and 4.35 g/100 ml (Fernandez et al., 1984).

MATERIALS AND METHODS



Materials and Methods

1. Experimental Animals:

The present work was carried out on 10 healthy non-pregnant Barki ewes, free from parasitic infestation. Their age ranged from 1 to 7 years old, the live body weight ranged 20 to 35 kg. A vasectomized ram was used to check oestrus. The animals were raised at the National Research Centre Experimental Farm at "Abou Rawash" Giza, Egypt.

This study was conducted allover the year where the animals were left loose in an open well ventilated pen. Throughout the course of the experiment, each animal received per day a ration composed of 500 gm concentrated balanced mixture and 500 gm green fodder "Barseem". During the dry season, animals were offered Barseem hay. Sufficient amounts of fresh water were supplied twice daily in the morning and afternoon.

At the beginning of the experiment, prostaglandin $F_{2\alpha}$ (Lutalyse, Upjhone, each ml contains 5 mg Dirprost Tromathamine) was given in two doses (10 mg each) 11 days apart to the 10 ewes to synchronize oestrus (Martemucci et al., 1983).

2. Oestrous Detection:

Ewes were penned adjacent to a vasectomized ram. The breast of the ram was painted with a coloured material every morning. Ewes were observed once daily for oestrus. Ewes in oestrus were known by marks on their rumps. External genitalia were examined and the vulvar secretions were noted. Furthermore, behavioural patterns, sexual receptivity and interest in the ram were recorded. Plasma oestradiol-17B and progesterone were determined throughout the cycle.

3. The Length of the Oestrous Cycle:

The length of the oestrous cycle was determined from the appearance of behaviour at oestrous to the reappearance of next oestrus.

Barki ewes oestrous cycles were classified according to Mounib et al. (1956), El-Wishy et al. (1971) and Aboul-Naga et al. (1985) into:

A. Normal cycles ranging between 15 to 19 days.

B. Short cycles 14 days or less.

C. Long cycles, 20 days or more.

D. Multiple cycles, more than 27 days.

The behavioural patterns of the ewe and characters of cervical mucus supported by the hormone profile of oestradiol-17B and progesterone throughout the cycle were used to determine the different phases of the oestrous cycle (Thorburn et al., 1969).

Oestrous cycles of the 10 Barki ewes were observed during the four seasons. autumn (21 September to 20 December), winter (21 December to 20 March), spring (21 March to 20 June) and summer (21 June to 20 September).

4. Blood Sampling:

Samples were taken from the external Jugular vein by direct vein puncture using hypodermic needles. Blood was collected into covered heparinized (20 unit/ml) test tubes, then transferred on ice bags to the laboratory. Plasma was separated by centrifugation at 3000 r.p.m for 30 minutes and stored at -20^{o}C until assayed. Blood samples were collected daily during oestrus (day 0) and metoestrus (day 1-2) then collection was carried out day after day during October to March (breeding season) and twice a week during Aprile to September (non-breeding season).

5. Hormone Assays:

Oestradiol-17B and progesterone values in plasma of Barki ewes were determined using the radioimmunoassay technique. Kits from Diagnostic Products Corporation (DPC), were used utilizing direct progesterone coated tubes, while oestradiol double antibody using uncoated tubes, oestradiol - I^{125} and progesterone I^{125} were used as a tracer (Siiteri and Febers, 1979; Anne Wentz, 1980 respectively).

6. Blood Chemistry:

6.1. Plasma Minerals:

6.1.1. Determination Of Calcium:

Total plasma calcium level was determined by Diamond Diagnostic kits. The principle of calcium determination was that calcium ions react with O-Cresolphthalein complexone to form a violet complex which is measured photometrically (Tietz, 1970). The standard and unknown samples were measured on Shimidzu graphicord spectrophotometer at 578nm against blank adjusted to zero.

6.1.2. Determination Of Inorganic Phosphorus:

Plasma inorganic phosphorus was determined by Bio-Analytic kits. This kit used O-phenylenediamine dihydrochloride as the reducing agent. The molybdinum blue complex that forms when inorganic phosphate reacts with the o-phenylenediamine dihydrochloride reagent is measured photometrically (Shimidizu graphicord spectrophotometer) at 660 nm against a blank adjusted to zero (Gomori, 1942).

6.1.3. Determination Of Iron:

The plasma iron was determined using Stanbio iron kits. The principle of the kits is that iron released from its combination with transferrin in acid medium, is reduced to ferrous form by hydroxylamine and reacted with ferrozine to form a violet coloured complex which is measured (Shimidzu graphicord spectrophotometer) at 560 nm against a blank adjusted to zero (Tietz, 1976).

6.1.4. Determination Of Zinc:

Plasma samples were diluted 1:5 with di-ionized water and measured for estimation of zinc using atomic absorption spectrophotometer (Perkin Elmer, 2380) according to Ali (1987).

6.2. Plasma Proteins:

6.2.1. Determination Of Total Plasma Proteins:

Total plasma proteins were determined using Bio-Anlytical kit (Doumas and Biggs, 1972). The procedure depends on a red chromophere which forms when cupric ions in an alkaline medium with the unsaturated electrons of the nitrogen and oxygen atoms of the protein peptide bonds, yielding a violet coloured complex with a maximum absorption at 540 nm using Shimidzu graphicord spectrophotometer.

6.2.2. Determination Of Albumin:

The procedure is based on the work of Doumas and Biggs (1972) which involves the dye binding properties of albumin, when albumin binds bromocresol green at Ph 4.2. The absorbance of the solution is measured at 630 nm and increases in direct proportion to albumin concentration.

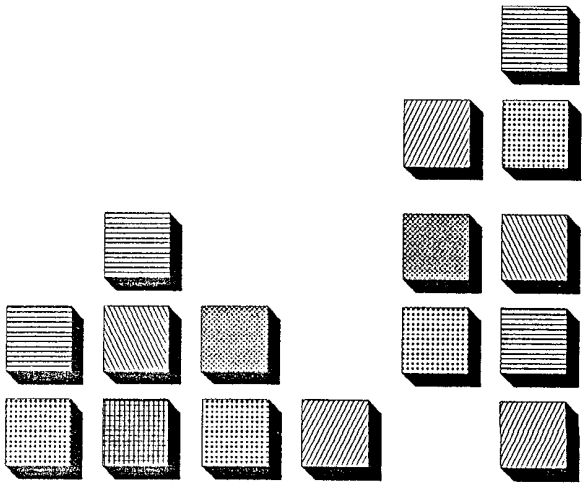
6.2.3. Determination Of Globulins:

Globulins were calculated by subtracting the amount of albumin from the total protein values of the same sample (El-Nouty, Hassan, Samark, Mekkawy and Salem, 1984).

7. Statistical Analysis:

The data were statistically computed for means and standard error. The student "t" test and analysis of variance, followed by least significant difference (LSD) in case of positive tests and the correlation coefficient were calculated as outlined by Snedecor and Cochran, 1976).

RESULTS



Results

1. Oestrous Behaviour In Barki Ewes:

Oestrus in Barki ewes was manifested by some changes in the ewe behaviour. On the marking day (day 0), the ewe is usually restless, adopts a typical urination posture, seeks the ram, looks over her shoulder and stands to be mounted. The ram Sniffs the vulva and then extends his neck with the head held high and the upper lip upcurled (Flehmen posture). The ewe stands firm and the ram continues courtship display until mount the ewe.

The vulva of the ewe in oestrus is oedematous and the mucous membrane of vestibulum becomes hypereamic and red in colour. Voluminous transparent mucus secretion are noticed shortly after the start of oestrus (day 0). Then, the mucus discharge is gradually replaced by a viscous creamy secretion which is still present during metoestrus and dioestrus. Proestrus ewes again become restless seek the ram but no mating occurs. The signs of oestrus are pronounced during autumn and winter, feeble during spring and summer.

2. Oestrous Cycle Length:

Data on oestrous cycle indicated in Table 1-1, Fig. 1-1 show that the majority (60.32%) of oestrous cycle lengths varies between 15 and 19 days and therefore, are considered normal cycles. While, 11.11% of the cycles are of short durations (10-14 days). The long (20 to 25 days) and multiple (ranging from 29 to 50 days), oestrous cycles have nearly the same incidence (14.29%, 14.297%).

The mean length of normal, short, long and multiple cycles averaged 16.83 $\pm$ 0.34, 12 $\pm$ 0.72, 21.56 $\pm$ 0.56 and 34.22 $\pm$ 2.16 days respectively.

3. Seasonal Variation In Oestrous Cycle:

Among seasons (Table 1-2) the highest incidence of normal cycles occurs during autumn (58.33%). A marked drop is observed in winter (22.22%), followed by summer (13.89%). The lowest incidence of normal cycles is noted during spring (5.56%). The higher incidence (57.14%) of short cycles occurs during winter, followed by autumn (28.57%), while the lowest incidence (14.28%) is observed during spring. Short cycles were not noticed during summer.

The highest incidence of long cycles observed during winter (44.44%) followed by spring (33.33%) then autumn (11.11%) and summer (11.11%).

The multiple cycles showed a similar incidence of 22.22% during winter, spring and summer. A higher incidence of 33.33% was noted during autumn.

Table 1-1: Types of the oestrous cycle in Barki Ewes:

Types of oestrous cycle	No. of cycles	Incidence %	Mean length (day) ± S.E.
Normal	38	60.32	16.83 ± 0.34 (15-19)
Short	7	11.11	12.00 ± 0.72 (10-14)
Long	9	14.29	21.56 ± 0.56 (20-25)
Multiple	9	14.29	34.22 ± 2.16 (29-50)

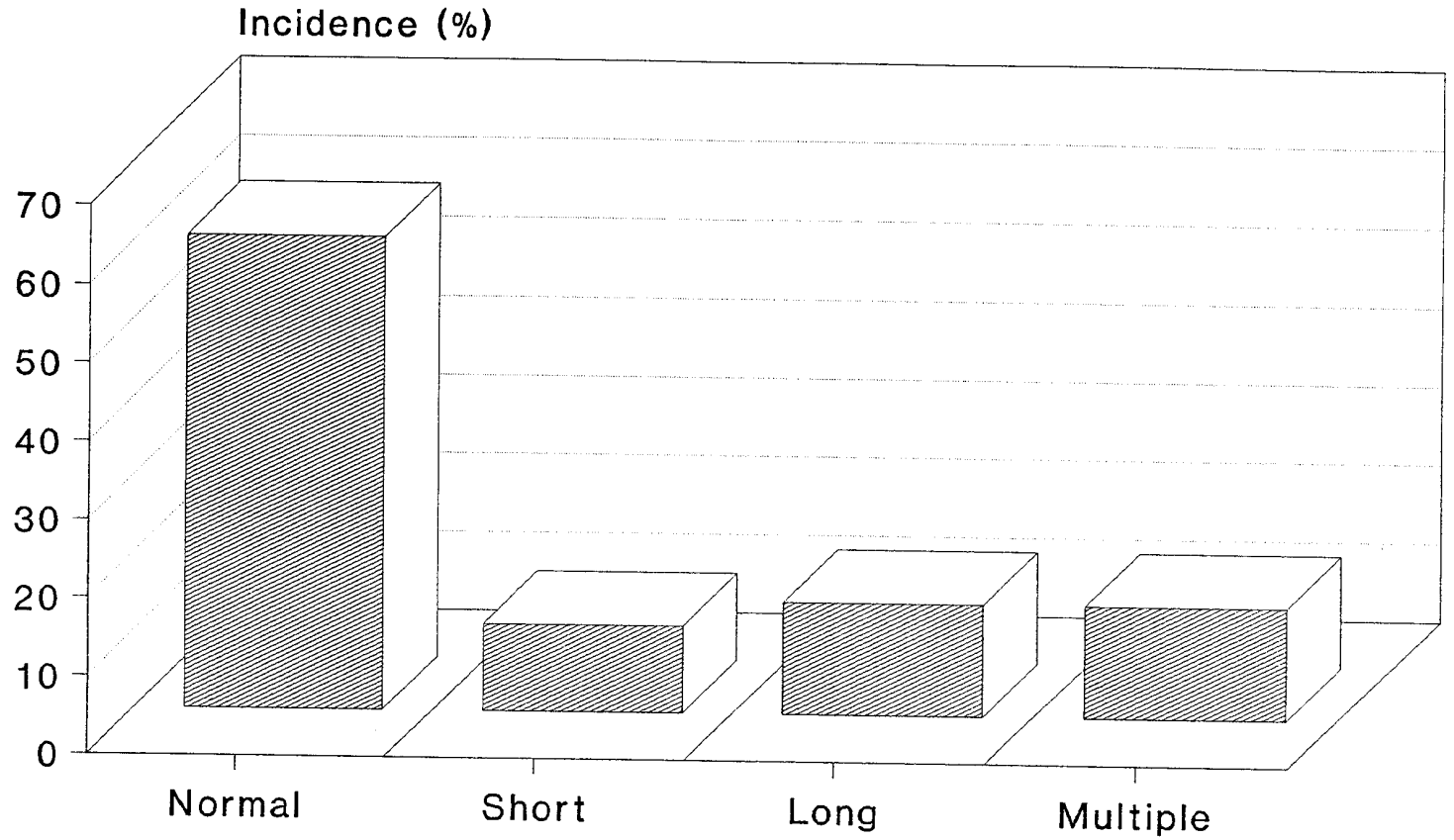


Fig. 1-1: Incidence of oestrous cycle types in Barki ewes

Table 1-2: Seasonal variation in types of oestrous cycle length and their incidence in Barki Ewes:

Type of cycle	autumn			winter			spring			autumn		
	Mean ± S.E. (d)	No.	%	Mean ± S.E. (d)	No.	%	Mean ± S.E. (d)	No.	%	Mean ± S.E. (d)	No.	%
Normal	16.81 ± 0.34	21	58.33	16.63 ± 0.60	8	22.22	17.50 ± 0.50	2	5.56	16.20 ± 0.37	5	13.89
Short	12.50 ± 1.50	2	28.57	12.00 ± 1.15	4	57.14	11.00	1	14.28	----	-	---
Long	21.00	1	11.11	22.25 ± 1.11	4	44.44	20.67 ± 0.67	3	33.33	22.00	1	11.11
Multiple	31.67 ± 1.20	3	33.33	32.00 ± 3.00	2	22.22	33.50 ± 3.50	2	22.22	41.00 ± 9.00	2	22.22

Table 1-3: Analysis of variance (F. test) of normal cycle among seasons in Barki Ewes:

Source of variance	D.F.	S.S.	M.S.	F
between seasons	3	3.48	1.160	0.517
error	32	71.74	2.242	
Total	35	75.22		

The mean length of the oestrous cycles (Table 1-2) during autumn, winter, spring and summer averaged 16.81 ± 0.34, 16.63 ± 0.6, 17.5 ± 0.5 and 16.2 ± 0.37 days respectively in normal cycles, 12.5 ± 1.5, 12.0 ± 1.5, 11 and 0 days respectively in short cycles, 21, 22.25 ± 1.11, 20.67 ± 0.67 and 22 days respectively in long cycles and 31.67 ± 1.2, 32.0 ± 3.0, 33.5 ± 3.5 and 41 ± 9 respectively in multiple cycles.

Statistical analysis revealed that the variation in the normal cycle length among seasons lacked any significance (Table 1-3).

4. Ovarian Hormone Profiles:

4.1. Oestradiol-17B Values:

On day-0 (oestrus) of the oestrous cycle, oestradiol-17B (Table 1-4, Fig 1-2) was at a maximum (14.376 ± 1.465 pg/ml plasma). Oestradiol value dropped to 1.349 ± 0.362 pg/ml plasma at day 1-2 and remained at such a plateau till day-8. A sudden increase in oestradiol-17B occurred on day-9 (9.353 ± 0.99 pg/ml plasma). Again, another drop was noted between day-10 to 15 (2.86-0.61 pg/ml). Later in the oestrous cycle (day 16-17) the value of oestradiol-17B was elevated to 4.015 ± 0.29 pg/ml plasma.

The results also showed that oestradiol-17B values during proestrus, oestrus, metoestrus and dioestrus averaged 4.015 ± 0.29, 14.376 ± 1.465, 1.349 ± 0.36 and 1.185 ± 0.29 pg/ml respectively (Table 1-6, Fig 1-3).

Statistical analysis revealed a significant difference in oestradiol-17B values ($P<0.01$) among the different phases of the oestrous cycle (Table 1-7). L.S.D value (Table 1-8) revealed a highly significant mean difference between oestrus and each of dioestrus, etoestrus and proestrus (L.S.D. = 3.930, 3.980 and 3.560 respectively).

4.2. Seasonal Variation In Oestradiol-17B Values:

The highest oestradiol-17B value was noted in winter (19.313 ± 3.88 pg/ml), followed by autumn (15.820 ± 1.945 pg/ml) and summer (15.047 ± 3.090 pg/ml), while the lowest value (7.41 ± 1.587 pg/ml) was observed in spring (Table 1-10). Such variations in oestradiol-17B value during oestrus were non-significant statistically among different seasons (Table 1-11).

Negative correlation coefficients were found between oestradiol-17B and each of progesterone, globulin, A/G ratio, zinc and iron. The positive correlation coefficients were found between oestradiol-17B and each of, Albumin, total protein Calcium, inorganic phosphorus and Ca/p ratio. The only significant association was noted between oestradiol and the value of progesterone ($r=-0.3$, $P < 0.01$), globulin ($r=-0.4$, $P<0.01$) and Ca/p ratio ($r=0.2$, $P<0.05$) (Table 1-12).

4.3. Progesterone Values:

The present results indicate that progesterone values (Table 1-5, Fig 1-2) during proestrus (day 16-17), oestrus (day-0) and metoestrus (day 1-2) were very low (0.167 ± 0.022, 0.124 ± 0.024 and 0.43 ± 0.053 ng/ml plasma respectively). A gradual increase in progesterone concentration at day 3-5 and 6-7 (1.768 ± 0.194 and 1.633 ± 0.192 ng/ml plasma respectively) was followed by a marked increase at day 8-9 (2.833 ± 0.25 ng/ml) reaching maximum values (3.554 ± 0.789 and 3.476 ± 0.69 ng/ml) at days 10-11 and day 12-13 respectively. Thereafter, the progesterone values decreased sharply (1.348 ± 0.235 ng/ml) at days 14-15. The Overall mean of progesterone values during dioestrus was 2.55 ± 0.18 ng/ml (Table 1-6). Variations in progesterone concentrations among the different phases of the oestrus cycle (Table 1-7) were found to be significant ($P<0.01$). L.S.D. test (Table 1-9) revealed a highly significant mean differences between dioestrus and each of oestrus, proestrus and Metoestrus (L.S.D. = 0.620, 0.748 and 0.503 respectively).

Table 1-4: Oestradiol-17ß values (pg/ml) during normal oestrous cycle days in Barki Ewes:

day of the cycle	Mean ± SE (pg/ml)
D0	14.376 ± 1.465
D1-2	1.349 ± 0.362
D3-5	0.190 ± 0.000
D6-8	1.729 ± 0.350
D9	9.353 ± 0.990
D10-11	2.863 ± 0.000
D12-13	1.464 ± 0.000
D14-15	0.610 ± 0.400
D16-17	4.015 ± 0.290

Table 1-5: Progesterene values (ng/ml) during normal oestrous cycle days in Barki Ewes:

day of the cycle	Mean ± SE (ng/ml)
D0	0.124 ± 0.024
D1-2	0.430 ± 0.053
D3-5	1.768 ± 0.194
D6-7	1.633 ± 0.192
D8-9	2.833 ± 0.250
D10-11	3.554 ± 0.789
D12-13	3.476 ± 0.690
D14-15	1.348 ± 0.235
D16-17	0.167 ± 0.022

Table 1-6: Oestradiol-17ß and progesterone values during oestrous cycle in Barki Ewes:

Oestrous cycle phases	Oestradiol-17ß (pg/ml)	Progesterone (ng/ml)
Proestrus	4.015 ± 0.290	0.167 ± 0.022
Oestrus	** 14.376 ± 1.465	0.124 ± 0.024
Metoestras	1.349 ± 0.362	0.430 ± 0.053
Dioestrus	1.185 ± 0.290	** 2.551 ± 0.180

** $P < 0.01$

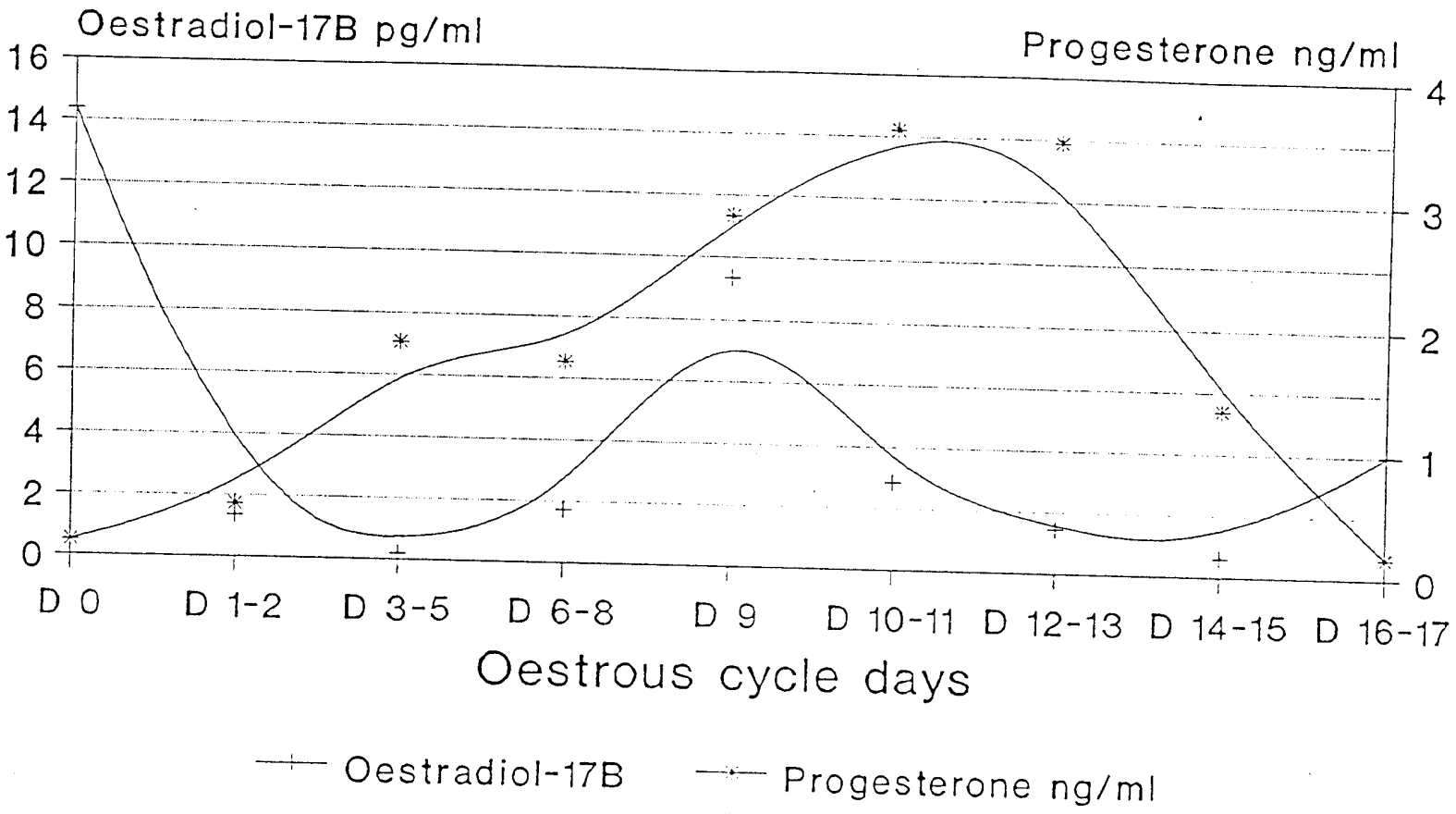


Fig. 1-2: Oestradiol-17B and progesterone values during oestrous cycle days

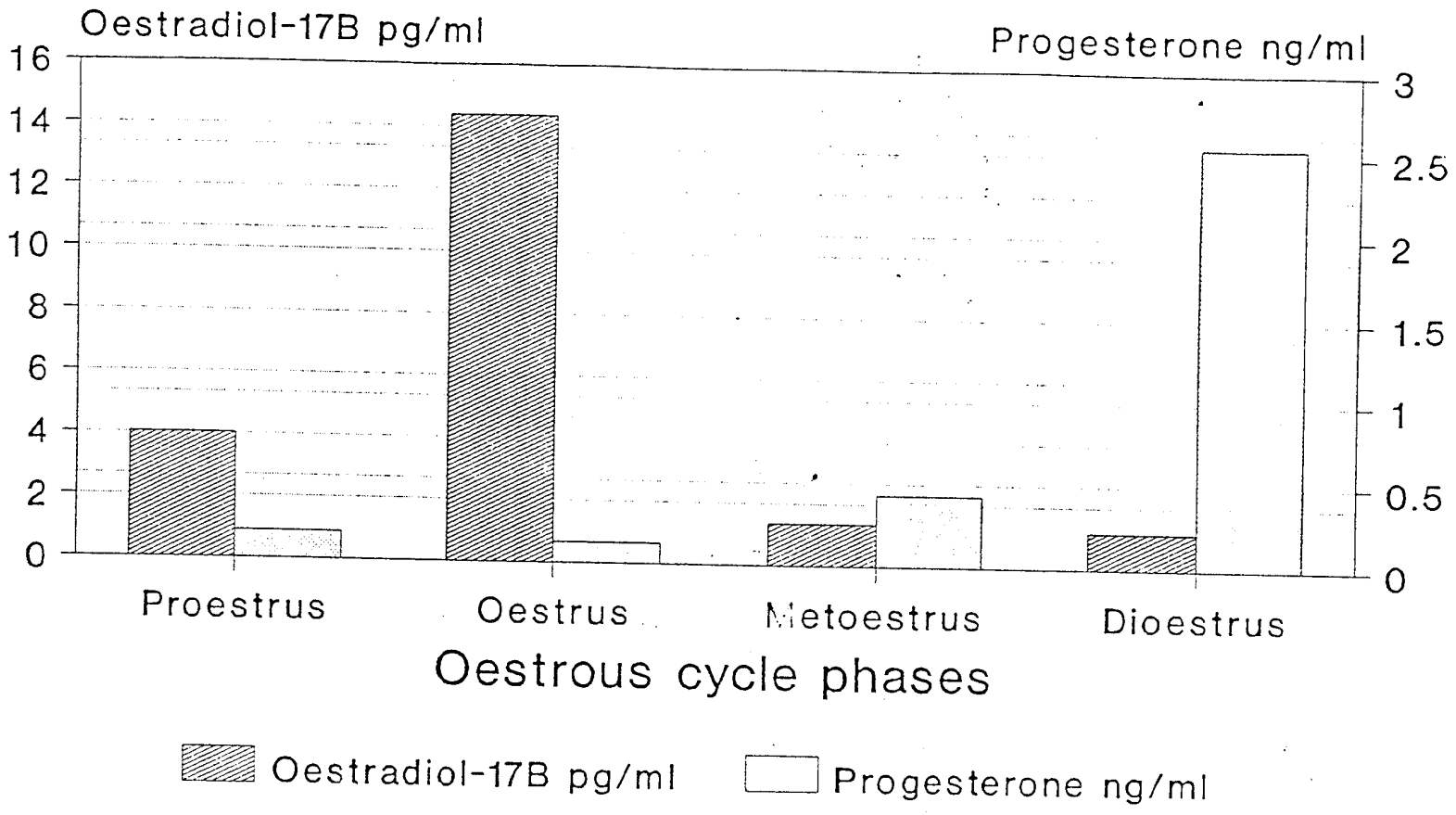


Fig. 1-3: Oestradiol-17B and progesterone values during oestrous cycle phases

Table 1-7: Analysis of variance for estradiol-17ß and progerterone during oestrous cycle phases in Barki Ewes:

Item	Source of variance	D.F.	S.S.	M.S.	F
Estradiol-17ß	Between treatment	3	1978.695	659.565	** 33.380
	Error	52	1027.501	19.760	
Progesterone	Between treatment	3	110.610	36.870	** 51.200
	Error	82	59.180	0.720	

** P < 0.01

Table 1-8: The least significant difference of estradiol-17ß during oestrous cycle phases in Barki Ewes:

L.S.D.	Mean Substraction	Periods of substraction
4.590	> 0.164	Metoestrus - Dioestrus
4.230	> 2.830	Prostrus - Dioestrus
3.930	< 13.191	Oestrus - Dioestrus
4.230	> 2.666	Proestrus - Metoestrus
3.980	< 13.027	** Oestrus - Metoestrus
3.560	< 10.361	** Oestrus - Proestrus

** P < 0.01

Table 1-9: The least significant difference of progesterone during oestrous cycle phases in Barki Ewes:

L.S.D.	Mean Substraction	Periods of substraction
0.871	> 0.040	Proestrus - Oestrus
0.670	> 0.300	Metoestrus - Oestrus
0.620	< 2.420	** Dioestrus - Oestrus
0.350	> 0.260	Metoestrus - Proestrus
0.748	< 2.380	** Dioestrus - Proestrus
0.503	< 2.120	** Dioestrus - Metoestrus

** P < 0.01

Table 1-10: Oestradiol-17ß and progesterone values (Mean ± S.E.) during the different seasons in Barki Ewes:

Seasons	Oestradiol-17ß (pg/ml)	Progesterone (ng/ml)			
	during oestrus	Oestrus	Metoestrus	Dioestrus	Proestrus
Autumm	15.820 ± 1.945	0.102 ± 0.014	0.482 ± 0.052	2.710 ± 0.210	0.145 ± 0.024
winter	19.313 ± 3.880	0.090 ± 0.033	0.284 ± 0.094	1.597 ± 0.173	0.126 ± 0.012
spring	7.410 ± 1.587	0.068 ± 0.001	0.449 ± 0.074	1.888 ± 0.380	0.164 ± 0.028
summer	15.047 ± 3.090	0.159 ± 0.029	0.189 ± 0.038	2.191 ± 0.467	0.188 ± 0.020

Table 1-11: Analysis of variance (F. test) of oestradiol-17ß and progesterone among seasons in Barki Ewes:

Item	Source of variance	D.F.	S.S.	M.S.	F
Oestradiol-17ß	Between treatment	3	310.500	103.500	2.89
	Error	17	638.313	35.783	
Progesterone during proestrus	Between treatment	3	0.006	0.002	0.590
	Error	10	0.034	0.003	
Oestrus	Between treatment	320	0.012	0.004	0.067
	Error	20	0.124	0.006	
Metoestrus	Between Treatment	3	0.351	0.117	2.170
	Error	32	1.715	0.054	
Dioestrus	Between Treatment	3	6.210	2.070	1.640
	Error	41	51.508	1.260	

Table 1-12: Correlation cocfficient of minerals and plasma proteins with oestradiol-17ß and progesterone in Barki Ewes:

Items	Oestradiol-17ß	Progesterone
Progesterone	- 0.300 **	--------
Oestradiol-17ß	-------	- 0.300 **
Calcium	0.100	0.200 *
Inorganic phesphorus	0.100	0.020
Ca/p ratio	0.200 *	0.100
Iron	- 0.200	- 0.100
Zinc	- 0.100	- 0.100
Total protein	0.100	- 0.100
Albumin	0.100	0.300 **
Globulin	- 0.400 **	- 0.100
A/G ratio	- 0.200	- 0.200 *

* P < 0.05 ** P < 0.01

4.4. Seasonal Variation In Progesterone Values:

Among seasons the highest progesterone values (ng/ml plasma) were noted during summer (0.188 ± 0.020, 0.159 ± 0.029, 0.189 ± 0.038 and 2.191 ± 0.467) and autumn (0.145 ± 0.024, 0.102 ± 0.014, 0.482 ± 0.052 and 2.71 ± 0.21) for the proestrus, oestrus, metoestrus and dioestrus phases respectively. Respective values were lowest during winter (0.126 ± 0.012, 0.090 ± 0.033, 0.284 ± 0.094 and 1.597 ± 0.173) and spring (0.164 ± 0.028, 0.068 ± 0.001, 0.449 ± 0.074 and 1.888 ± 0.380) same phases in the same order. The above mentioned differences in progesterone values among oestrous cycles and during seasons of the year lacked significance (Table 1-11) Plasma progesterone values were significantly correlated with plasma oestradiol-17B ($r=-0.3$, $P<0.01$), albumin ($r=0.3$, $P<0.01$), Calcium ($r=0.2$, $P<0.05$) and A/G ratio ($r=-0.2$, $P<0.05$).

5. Plasma Minerals:

5.1. Plasma Mineral Values During Oestrous Cycle:

In Barki ewes (Table 1-13) calcium, inorganic phosphorus and Ca/p ratio averaged 10.81 ± 0.29, 6.22 ± 0.21 mg/dl plasma and 1.78 ± 0.070 respectively. The mean values for plasma iron and zinc in Barki ewes were 2.08 ± 0.250 mg/dl and 0.100 ± 0.010 ppm respectively.

Throughout the oestrus cycle (Table 1-14), calcium values were high during metoestrus and dioestrus (11.10 ± 0.52 and 11.1 ± 0.43 mg/dl plasma respectively). While, high inorganic phosphorus concentrations were obtained during proestrus (6.46 ± 0.63 mg/dl) and oestrus (6.67 ± 0.48 mg/dl), Ca/p ratio was high at metoestrus (1.8 ± 0.12) and dioestrus (1.90

± 0.11). Lowest values of calcium, (mg/dl) were noted during oestrus (10.17 + 0.55) and proestrus (10.72 + 0.88). For inorganic phosphorus lowest values were during (mg/dl) metoestrus (6.08 ± 0.34) and dioestrus (5.97 ± 0.28). The Ca/p ratio was lowest in proestrus (1.54 ± 0.14) and oestrus (1.60 ± 0.13).

Iron values were lower (1.033 ± 0.55 mg/dl) during metoestrus than proestrus, oestrus and dioestrus (2.61 ± 1.00, 2.581 ± 0.76 and 2.33 ± 0.60 mg/dl respectively), zinc values were higher during proestrus and oestrus (0.094 ± 0.01 and 0.090 ± 0.01 ppm respectively) than during metoestrus and dioestrus (0.089 ± 0.01 and 0.084 ± 0.003 ppm respectively) statistical analysis (Table 1-15) revealed that the differences in calcium, inorganic phosphorus, Ca/p ratio, iron and zinc values were not significant among the different phases of the oestrus cycle.

5.2. Seasonal Variation In Plasma Minerals:

Among seasons plasma calcium, inorganic phosphorus values and Ca/p ratio were lowest (9.48 ± 2.31, 4.67 ± 0.27 mg/dl and 1.52 ± 0.60 respectively) during summer as compared to other seasons of the year. Higher values for there criteria were noted during spring (10.59 ± 0.9 mg/dl) and winter (5.84 ± 0.39 mg/dl and 1.80 ± 0.12) respectively. While zinc values were higher (0.010 ± 0.010 ppm) during summer than other seasons (ranging between 0.08 ± 0.004 to 0.090 ± 0.077 ppm).

Statistical analysis (Table 1-17) revealed that the differences for calcium, inorganic phosphorus, Ca/p ratio and zinc were not significant among the four seasons.

6. Plasma Proteins:

6.1. Plasma Proteins During Oestrous Cycle:

In Barki ewes the total protein, albumin, globulin, (A/G) ratio averaged 6.65 ± 0.130, 3.21 ± 0.070, 3.760 ± 0.090 g/dl and 1.030 ± 0.050 respectively (Table 1-13).

Among the different phases of the oestrus cycle total protein, albumin values and A/G ratio were high during dioestrus (6.85 ± 0.18, 3.24 ± 0.12 g/dl and 1.06 ± 0.07 respectively). Higher globulin values were noted during proestrus (3.98 ± 0.26 g/dl). Lower total protein, were found during metoestrus (5.98 ± 0.30 g/dl), lower values for albumin were during proestrus (3.05 ± 0.19 g/dl, but lower values for globulin were during oestrus and dioestrus (3.70 ± 0.20 and 3.70 ± 0.12 g/dl respectively). The lowest values for the A/G ratio were oestrus (0.96 ± 0.09) (Table 1-14).

Such variations were not significant among oestrus cycle phases (Table 1-15).

6.2. Seasonal Variation In Plasma Proteins:

Table 1-16, reveals that high values of total protein, were noted during autumn, winter and spring (6.89 ± 0.150, 6.89 ± 0.21 and 6.54 ± 0.37 g/dl respectively) and was lower in summer (5.70 ± 0.28 g/dl), for albumin high values came in spring, autumn and summer (3.47 ± 0.15, 3.32 ± 0.09 and 3.13 ± 0.18 g/dl respectively) winter come last (2.93 ± 0.12 g/dl), for globulin the order was winter, autumn and spring than summer (3.81 ± 0.16, 3.79 ± 0.12, 3.63 ± 0.21 and 2.44 ± 0.23 g/dl respectively) The A/G ratio was highest in winter (1.18 ± 0.09 and lowest values observed during

autumn, spring and summer (0.89 $\pm$ 0.04, 0.83 $\pm$ 0.06 and 0.70 $\pm$ 0.04 respectively).

Statistical analysis (Table 1-17) reveals that variations in plasma albumin values and A/G ratio were significant ($P<0.05$ and $P<0.01$ respectively) among four seasons. L.S.D. value showed that the most significant ($P<0.05$ and $P<0.01$ respectively) among the four seasons. L.S.D. value showed that the most significant high mean differences in albumin was observed between spring and winter (L.S.D. = 0.46) (Table 1-18). In A/G ratio L.S.D. value with high significant mean difference was between spring and summer (L.S.D. = 0.11) and winter and summer (L.S.D. = 0.41), in table 1-19.

Table 1-13: Mean values of plasma minerals and proteins in Barki Ewes:

Items	Mean ± S.E.	Range
Calcium mg/dL	10.810 ± 0.290	6.460 - 15.750
Inorganic phosphorus mg/dL	6.220 ± 0.210	3.740 - 10.020
Ca/p ratio	1.780 ± 0.070	0.750 - 2.790
Iron mg/dL	2.080 ± 0.250	0.500 - 5.750
Zinc ppm	0.100 ± 0.010	0.045 - 0.150
Total protein g/dL	6.650 ± 0.130	4.210 - 12.240
Albumin g/dL	3.210 ± 0.070	2.910 - 4.680
Globulin g/dL	3.760 ± 0.090	1.900 - 5.800
A/G ratio	1.030 ± 0.050	0.530 - 2.970

Table 1-14: Mean values of plasma minerals and proteins during oestrus cycle phases in Barki Ewes:

Items	Proestrus	Oestrus	Metoestrus	Dioestrus
Calcium mg/dL	10.72 ± 0.88	10.17 ± 0.55	11.18 ± 0.52	11.10 ± 0.43
Inorganic phosphorus mg/dL	6.46 ± 0.63	6.67 ± 0.48	6.08 ± 0.34	5.97 ± 0.28
Ca/p ratio	1.54 ± 0.14	1.60 ± 0.13	1.80 ± 0.12	1.90 ± 0.11
Iron mg/dL	2.61 ± 1.00	2.581± 0.76	1.033± 0.55	2.33 ± 0.60
Zinc ppm	0.094± 0.01	0.090± 0.01	0.089± 0.01	0.084± 0.003
Total protein g/dL	6.82 ± 0.54	6.69 ± 0.25	5.98 ± 0.30	6.85 ± 0.18
Albumin g/dL	3.05 ± 0.19	3.13 ± 0.10	3.20 ± 0.09	3.24 ± 0.12
Globulin g/dL	3.98 ± 0.26	3.70 ± 0.20	3.80 ± 0.18	3.70 ± 0.12
A/G ratio	0.98 ± 0.15	0.96 ± 0.09	1.05 ± 0.11	1.06 ± 0.07

Table 1-15: Analysis of variance (F. test) of plasma minerals and proteins during oestrous cycle phases in Barki Ewes:

Item	Source of variance	D.F.	S.S.	M.S.	F
Calcium	Between treatment	3	16.900	5.560	0.750
	Error	97	723.360	7.46	
Inorganic phesohorus	Between treatment	3	11.210	3.740	0.750
	Error	128	639.480	4.996	
Ca/p ratio	Between treatment	3	1.760	0.590	1.430
	Error	95	38.890	0.410	
Iron	Between Treatment	3	9.640	3.210	0.620
	Error	32	165.820	5.810	
Zinc	Between Treatment	3	0.001	0.0003	0.330
	Error	144	0.181	0.001	
Total protein	Between treatment	3	17.390	5.800	2.390
	Error	131	317.950	2.430	
Albumin	Between treatment	3	0.560	0.190	0.33
	Error	162	94.640	0.580	
Globulin	Between Treatment	3	1.100	0.370	0.410
	Error	109	91.77	0.840	
A/G ratio	Between Treatment	3	0.26	0.09	0.28
	Error	125	38.45	0.31	

Table 1-16: Mean values of plasma minerals and proteins during seasons in Barki Ewes:

Items	Calcium mg/dL	Inorganic phosphorus mg/dL	Ca/p ratio	Zinc ppm	Total protein g/dL	Albumin g/dL	Globulin g/dL	A/G ratio
Autumm	10.49 ± 0.37	5.74 ± 0.17	1.67 ± 0.09	0.090 ± 0.007	6.89 ± 0.150	3.32 ± 0.09	3.79 ± 0.12	0.89 ± 0.04
winter	10.41 ± 0.57	5.84 ± 0.39	1.80 ± 0.12	0.090 ± 0.007	6.89 ± 0.21	2.93 ± 0.12	3.81 ± 0.16	* 1.18 ± 0.09
spring	10.59 ± 0.90	5.67 ± 0.23	1.63 ± 0.18	0.080 ± 0.004	6.54 ± 0.37	* 3.47 ± 0.15	3.63 ± 0.21	0.83 ± 0.06
summer	9.48 ± 2.31	4.67 ± 0.27	1.52 ± 0.60	0.100 ± 0.010	5.70 ± 0.28	3.13 ± 0.18	2.74 ± 0.23	0.70 ± 0.04

* $P < 0.05$

Table 1-17: Analysis of variance of plasma minerals and protein during seasons in Barki Ewes:

Items	Source of variance	D.F	E.S.	M.S	
Calcium	Between T.	3	3.98	1.33	0.17
	Error	88	696.46	7.91	
Inorganic phosphorus	Between T.	3	7.59	2.53	1.71
	Error	95	140.92	1.48	
Ca/p ratio	Between T.	3	0.37	0.12	0.26
	Error	84	37.84	0.45	
Zinc	Between T.	3	0.01	0.003	1.67
	Error	131	0.3	0.002	
Total protein	Between T.	3	14.43	4.81	2.61
	Error	142	260.59	1.84	
Albumin	Between T.	3	4.18	1.39	2.73*
	Error	127	65.15	0.51	
Globulin	Between T.	3	5.39	1.8	2.27
	Error	106	84.03	0.79	
A/G ratio	Between T.	3	1.08	0.36	4.5**
	Error	101	8.49	0.08	

* $P < 0.05$

** $P < 0.01$

Table 1-18: Least significant difference of albumin during seasons in Barki Ewes:

L.S.D.	Mean Substraction	Seasons substraction
0.56	> 0.20	summer - winter
0.36	> 0.39	autumn - winter
0.46	< 0.54	* spring - winter
0.49	> 0.19	autumn - summer
0.56	> 0.34	spring - summer
0.40	> 0.15	spring - autumn

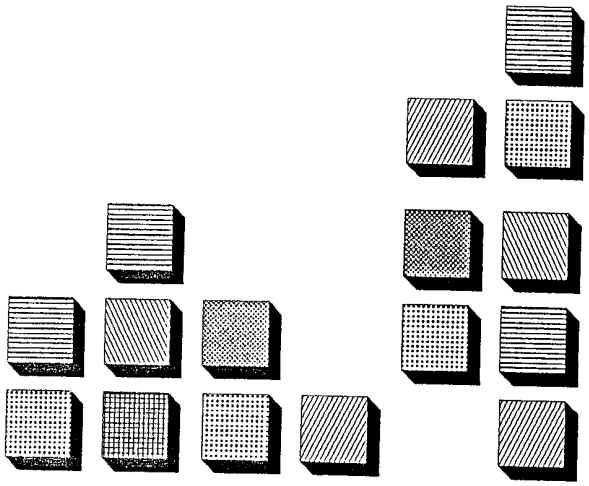
* P < 0.05

Table 1-19: Least significant difference of A/G ratio during seasons in Barki Ewes:

L.S.D.	Mean Substraction	Periods of substraction
0.11	< 0.13	** spring - summer
0.21	> 0.19	autumn - summer
0.41	< 0.48	** winter - summer
0.21	> 0.06	autumn - spring
0.41	> 0.35	summer - spring
0.38	> 0.29	summer - autumn

** P < 0.01

DISCUSSION



Discussion

1. Oestrus:

In the present work the characteristic behaviour of Barki ewes during the oestrous cycle is in general agreement with that observed in other breeds of sheep (Tomkins and Bryant, 1974 ; Sabra, 1987). Oedema of the vulva and hyperamia of vestibulum are among the prominent oestrous signs. (Salisbury and Van demark, 1961 ; Hafez, 1979). Mucus secretion observed shortly after the start of oestrus as a serous, voluminous and transparent fluid is in agreement with that reported by Morrant and Dun (1960) and Restal (1961). Then mucus discharge was gradually replaced by a viscous creamy secretion which is still present during metoestrus and dioestrus (Schindler and Amir, 1972; Sabra, 1987). These changes in the physical characters of the vaginal mucus are dependent on the interaction between oestrogen and progesterone hormones (Robinson and Moore, 1955).

Among seasons, signs of oestrus of Barki ewes were pronounced during autumn and winter and feeble during spring and summer. Similar findings were reported by Sabra (1987) in Barki ewes. In Rahmany ewes, Aboul-Naga et al. (1987) noticed behavioural oestrus from August to January, the percentage of ewes in oestrus started to decline during February, March and the decline was more pronounced during April-May with a minimum value in May. Ezzo (1989) observed that oestrous signs were more pronounced during autumn, winter and early spring. During summer and early autumn the oestrous signs were either slight or absent. The differences observed in signs of oestrus in ewes among seasons may be due to that the breeding season activity is associated with the genetic evolution of breed types, latitude of their habitat, climatic environment and the nutritional status (Cole and Cupps, 1977; Coop, 1982). El-Wishy et al. (1976) showed that in fat tailed ewes the length of photoperiod was the main factor affecting oestrus and ovulation. In this respect, unobserved heats were recorded at summer and at the beginning and end of the breeding season when the day lengths was long as reported by the same authors.

2. Oestrous Cycle Length:

Due to the wide variation in the length of the oestrouscycle of Barki ewes. The cycles were classified as normal, short, long and multiple. In the present study, the incidence of normal oestrous cycles (60-32%) is higher (53.9% and 34.7%) than those reported by El-Wishy et al. (1971) and Zaki (1987) respectively, but incidences of 76%, 88.7% and 73.89% were noticed by Hunter (1970), Lattore and Cvitanic (1977) and Elias (1987) respectively. The percentage of short cycles in the present work averaged 11.11% which is lower than that (25.4% and 15.35%) reported by El-Wishy et al. (1971)

and Zaki (1987) as respectively. The incidence of long cycles (14.29%) is higher than 7.7% reported by El-Wishy et al. (1971) and lower than 26.13% reported by Zaki (1987). The incidence of multiple cycles (14.29%) investigated in the present work is lower than 38.4% of El-Wishy et al. (1971) and higher than 6.26% of Zaki (1987).

The mean length of normal oestrous cycles (16.83 days) in the present study is within the range (16-17.9 days) given by Mounib et al. (1956), El-Fouly (1977), Smith et al. (1980), Ateia (1985) and Kassem, Owen and Fadel (1990), but higher than that stated by Baihanov (1965), Martemucci et al. (1983) as 14.65-15 days and lower than figures given by Mikus and Longouer (1961), Ponzoni and Azzarini (1968) and Elias (1987) as 18.24-19.6 days. Such variation in the type and length of the oestrus cycle could be due to breed, age, season, climatic environment and nutritional status (Mounib et al., 1956; Williams et al., 1956; Cole and Cupps, 1977; Coop, 1982). Nutritional factors are important in relation to early growth and puberty and to the reproductive cycle of the adult in general (Coop, 1982). Ewe lambs exhibit less regular cycles and tend to have slightly shorter cycle than adult (Hafez, 1952).

The mean length of short cycles (12 day) observed herein are similar to that reported by Mounib et al. (1956), Mikus and Longouer (1961) and Baihanov (1965). The occurrence of short oestrous cycle seems to be a normal phenomenon in ewes which could be attributed to several factors. In this respect, Knight, Tervitt and Fairclough (1981), suggested that short cycles are associated with premature C.L. regression. Moreover, Camp, Wildt, Howard, Stuart and Chakraborty (1983) reported that an episodic LH release was accompanied with short cycles. Some times luteinization of follicles occurs as a response to inadequate gonadotrophin stimulation during the process of follicle maturation and ovulation (Walton, Mc-Neilly, McNeilly and

Cunningham, 1977), progesterone from luteinized follicles was detected in animals with short cycles. But this is doubtful for it may originate from CLs with a short life span. Moreover, Hafez (1979) noticed that the length of the oestrous cycle tends to be shorter at the peak of the breeding season, also meat breeds may have slightly shorter cycles than wool breeds.

The length of long oestrous cycle in the present study ranged between 20 to 25 days (av. 21.56 days). This finding is in agreement with the observations of 20 to 26 days noticed by El-Wishy et al. (1971), Zaki, (1987) and Elias (1987). The multiple type of oestrous cycles herein ranged between 29-50 days (av. 34.22 days). This result is partially in agreement with the length of multiple cycles (more than 27 days) reported by El-Wishy et al. (1971), Aboul-Naga et al. (1985) and Elias (1987), this may be due to the occurrence of ovulation without oestrous manifestations. Multiple cycles are more common in some mountain breeds (Hafez, 1979). Other causes of abnormal cycle length include failure of ovulation and luteinization, early regression of the corpus luteum and early prenatal death of the embryo. Abnormal cycle lengths are more common early and late in the breeding season. The length of the oestrous cycle is longer on a low plan of nutrition as compared to a high one but breed differences do not manifest clear cut variations (Hafez, 1979).

3. Seasonal Variation:

The higher incidence (58.33%) of normal oestrous cycles in Barki ewes reported herein during autumn than during winter, spring and summer is in agreement with the observations of El-Wishy et al. (1976), who noticed that the highest incidence of normal cycling ovaries in fat tailed ewes was during

September to November (81.5%). In Perendle ewes, oestrous activity was 86% in September to November (Smith et al., 1980). Badawy, Habib and Zaki (1978) found that the incidence of anoestrus was higher in summer (50.31%) than autumn, winter or spring (17.65, 30.13 or 29.7% respectively). Moreover, Al-Wahab et al. (1982) noticed that Awassi ewes mating 100% in all periods except July-August (90%) and May-June (95%). In Ramboiullet ewes the oestrous activity was greatest in autumn and lowest in spring (Wiggins, Barker and Miller, 1970). The causes of such variations in the incidence of oestrous cycle among seasons are due to the lack of preovulatory rise of oestradiol-17B and LH at the end of the breeding season leading to a failure of the hypothalamic pituitary system to respond to withdrawal of luteal progesterone by secretion of FSH and LH in amount sufficient for maturation of Graafian follicles or causing a delay in follicular growth (Legan, Karsch and Foster, 1977; Wheeler and Land, 1977; Quirke, Stabenfeldt and Bradford, 1985).

4. Ovarian Hormones:

4.1. Oestradiol-17B:

In Barki ewes oestradiol-17B values reached their maximum concentration (14.376 pg/ml) during oestrus (day 0). This value is higher than 12 and 13.3 pg/ml given by Goding et al. (1971) and Yuthasastrakosol et al. (1975) respectively, and lower than 21.1 pg/ml reported by Pant et al. (1972), (1977). In agreement with the finding of Moore et al. (1969), oestradiol levels rapidly declined 24 hours after the onset of oestrus (1.34 pg/ml) and fell to very low or non-detectable levels (0.19) observed throughout the luteal phase of the cycle.

Sudden increase in oestradiol-17B occurred at day-9 (9.353 pg/ml plasma). This could be due to the wave of follicular development during luteal phase (Baird, Swanston and Scaramuzzi, 1976; Schallenberger, Schondorfer and Walters, 1985). The rapid decline in oestradiol-17B values (ranged 0.61 to 2.86 pg/ml) during days 10-15 of the oestrous cycle agrees with the observation of Yuthasastrakosol et al., (1975) and Pant et al. (1977). However the increase in oestradiol-17B (4.015 pg/ml) means that Graafian follicles develop quickly during proestrus and oestrus. In this respect Pant et al. (1977) recorded that oestradiol-17B values for the last 2 days of the oestrous cycle are related to the subsequent oestrus.

The present results revealed that the oestradiol-17B values varied significantly among seasons (19.31, 15,82, 15.05 and 7.41 pg/ml) during winter, autumn, summer and spring respectively. This result disagree with Ezzo (1989), who recorded that the highest oestradiol-17B concentration was 32.02 pg/ml during spring, followed by autumn (20.60 pg/ml) and lowest during summer and winter (19.255 and 13.85 pg/ml respectively).

4.2. Progesterone:

Progesterone pattern during oestrous cycle in the present work was similar to that reported by Thorburn et al. (1969), Cunningham et al. (1975), Pant et al. (1977), Botha and Morgenthal (1980), Ezzo (1989) and Mukasa-Mugerwa et al. (1990).

The plateau of progesterone concentration during oestrus and metoestrus was very low (0.124 and 0.43 ng/ml respectively). Similar values were reported by Thorburn et al. (1969), Pant et al. (1977) and Botha and Morgenthal (1980). The present value is higher than 0.04-0.09 ng/ml (Martimucci et al., 1983) and lower than 0.47-0.92 ng/ml (Ezzo, 1989). The

present results revealed a gradual increase in progesterone values (1.768 and 1.633 ng/ml) at days 3-5 and 6-7 respectively. These values are in agreement with those reported by Thorburn et al. (1969), Pant et al. (1977), Zaki (1987) and lower (2.938 ng/ml) than that recorded by Ezzo (1989). In the present work, progesterone values increased (2.833 ng/ml) at day 8-9, followed by a peak value 3.554-3.476 ng/ml at day 10-11 and day 12-13 respectively. Similar results were reported by Pant et al. (1977), Botha and Morgenthal (1980). Moreover, a higher values for peaks of progesterone (5.14 ng/ml) were noticed by Sabra (1987), Ezzo (1989) and Mukasa-Mugerwa et al. (1990). However, lower peaks were recorded as 1.07 to 2.5 ng/ml (Stabenfeldt et al., 1969; Cunningham et al., 1975; Martemucci et al., 1983; Zaki, 1987; Romero et al. 1989). Progesterone values sharply decreased to 0.167 ng/ml at day 16-17. This sudden withdrawal of progesterone possibly influences the occurrence of the next heat and ovulation (Edgar and Ronaldson, 1958). Concentrations less than 1.0ng/ml are indicative of anoestrus, the follicular and early luteal phases of the oestrous cycle (Mukasa-Mugerawa et al., 1990). The difference in steroid values between the present work and other studies may be due to the difference in season and nutrition (Lamond et al. 1972), as well as breed and ovulation rate (Bindon, Blanc, Pelletier, Terqui and Thimonier, 1975; Quirke and Gosling, 1976), which have an influence on the maximum concentration of steroids. Thorburn et al. (1969) noticed that variations occurring in progesterone values may be attributed to differences in techniques of assay.

The temporal variation in the prepheral oestradiol-17B and progesterone values in the present work is similar to that noticed by stabenfeld, Edqvist, Kindahl, Gustaffsson and Bane (1978). Follicles grow rapidly during the period of luteal regression due to gonadotropin stimulation and progesterone withdrawal, follicles secrete increasing amounts of oestrogen as they develop.

The increased amounts of oestrogens are important for the onset of sexual respectively as well as initiation of the surge release of gonadotropins that is essential for the ovulatory process. Oestrogens initiate the release of preovulatory luteinizing hormone (LH) and (FSH) follicular stimulating hormone (Stabenfeldt et al., 1978). The release of FSH and LH hormones may be attributed to the action of oestrus-inducing a peak of oestradiol-17B. In so far as the initiation of luteal function is concerned, this preovulatory rise in plasma gonadotropins, luteinization and secretion of progesterone are initiated when LH stimulates adenylate, luteinization of the granulosa cells, is accompanied by the synthesis and secretion of progesterone. From the basal concentration of 0.2 ng/ml (at day-4) till reaching a plateau of 2 to 4 ng/ml by day-7, then rapidly declining (day-15) about 36 hours before the onset of the next oestrus (Cole and Cupps, 1977). The first point of interest is that there is some follicular growth during the luteal phase of the large domestic animals in spite of the general inhibitory nature of progesterone (Stabenfeld et al., 1978).

The decrease in peripheral progesterone concentration during the latter part of the oestrous cycle follows a pattern which is more consistent with the cytological changes in the regressing corpus luteum, which was marked on day 15 (Deane, Hay, Moor, Rowson and Short, 1966).

The present result reveals that the maximum progesterone values during autumn (2.71 ng/ml) are higher than that observed during summer, winter and spring (2.191, 1.888 and 1.597 ng/ml) respectively. This result is in agreement with that found in Ramouillet ewes (Lamonde et al., 1972). Apulian and Altamura ewe (Martemucci et al., 1983) and in Barki ewes (Eliase, 1987, Ezzo, 1989). While Zaki (1987) noticed that progesterone values in Barki ewes were higher during summer (2.24 ng/ml) than during autumn, winter and spring (1.869, 0.981 and 1.115 ng/ml respectively). In this respect,

the effect of temperature on progesterone concentration might be mediated by prolactin which is influenced by a variety of stress stimuli (Krulich, Hefco, Iuner and Read, 1974), one of which is the increased environmental temperature (Hooley, Findlay and Staphenson, 1979). The concentration of prolactin was found to be inversely proportional to the peripheral progesterone concentration (Rhind, Robinson, Chesworth and Philipo, 1978).

5. Plasma Minerals:

The present findings indicated that plasma calcium values averaged 10.81 mg/ml in Barki ewes, this is similar to the amounts reported by Underwood (1966); Weiner and Field (1971), Healy and Falk (1974) and Idris et al. (1976) (10.15 mg/ml). The present value is higher than (9.2 mg/ml) that recorded by Heleigh et al. (1955) and lower than 11.3-11.55 mg/ml which is found by Nizar 1982; Eldinary (1987), but Ibrahim et al. (1986) found values of (12.11-12.16mg/ml), while Ghazy (1987) found a value of 18.21 mg/100ml.

The value of inorganic phosphorus in Barki ewes averaged 6.22 mg/dl plasma, which is similar to that reported by Idris et al. (1976) and Nizar (1982) and higher than those reported by Haleigh et al. (1955) and Ibrahim et al. (1986) (4.3-4.5 mg/ml) and Hockett et al., (1957), Wiener and Field (1971), Healy and Falk (1974) as (5.2-5.5 mg/100ml). Moreover, it is lower than those reported by El-dinary (1987) and Ghazy (1987) as (7.4 mg/dl).

Ca/p ratio (1.78) in Barki ewes in the present study is nearly similar to that recorded by Underwood (1966) and Vipperman et al. (1969) (1.5 to 2.1) and lower than (2.55) that noticed by Ibrahim et al. (1986).

The available literature lacks data concerning variations in calcium and inorganic phosphorus during the oestrous cycle of ewes. The present investigation indicated that calcium values were higher during luteal phase than follicular phase. The present study revealed a significance ($r = 0.2$, $P < 0.05$) correlation between progesterone and plasma calcium levels during oestrous cycle. This result is similar to that observed in normal cows (Agarwal, Tripath and Saxena, 1982).

Inorganic phosphorus values in Barki ewes are higher during oestrus (6.67 mg/ml) than during dioestrus (5.97mg/dl). This result is in agreement with that observed by Bhagwan and Dutt (1974) and Ezzo (1989). Osman, El-Naggar, Allam and Farrage (1979) In cattle and Sharaway (1980) found a significant increase of inorganic phosphorus in buffaloes during the follicular phase than during the luteal phase, this could be attributed to the greater mobilization of inorganic phosphorus due to increase in metabolic activity during the oestrogenic phase of the cycle (Bhagwan and Dutt, 1974; Osman et al., 1979). Moreover, the present study indicates a week positive correlation coefficient ($r = 0.1$) between inorganic phosphorus and oestradiol-17B besides the result is no correlation with progesterone ($r = 0.02$) was found.

The present work shows that Ca/p ratio is higher during dioestrus and metoestrus (1.9 and 1.8 respectively) than (1.6 and 1.54) during oestrus and proestrus respectively. This result is in agreement with Ezzo (1989).

The present result in Barki ewes reveals that the values of calcium, inorganic phosphorus and Ca/p ratio were lower during summer than other seasons. This result is in agreement with cycling heifers showing calcium values obtained during the green season were higher than the values obtained during the dry season (Osman et al., 1979). Moreover, in goats, the serum calcium fluctuated but did not show any particular trend between

months (Upadhyay and Rao, 1985). They added that the correlation coefficient analysis of inorganic phosphorus with atmospheric temperature showed a negative correlation (non-significant) indicating that high ambient temperature decreases inorganic phosphorus levels in goats.

The present work shows that the average of plasma iron values is 2.08 mg/dl (ranged from 0.5 to 5.75 mg/dl plasma). This result is higher than (141 ug/100ml) that recorded by Ghosal et al. (1976), and 300 ug/100ml) that noticed by Rasamy (1952).

The total iron content in the animal body varies with age, sex, nutrition, state of health and species (Granick, 1958). The present result reveals that iron values in Barki ewes are lower during metoestrus (1.033 ng/dl) than during other phases (from 2.33 to 2.6 mg/dl). This result differs from that reported by Ezzo (1989) who noticed that higher concentration of irons in Barki ewes occurred during met-oestrus with slight difference during other phases. The present study indicates that variations in iron values among the oestrous cycle phases were not significant. This is in agreement with Planas and Decastro (1960) who reported that the value of serum iron in sheep did not show any significant inter sexual differences.

The present work shows that plasma zinc concentration ranges from 0.045 to 0.15 ppm (av. 0.100 ppm). This result is in agreement with the range of normal levels of serum zinc in sheep at North Island (53.63 ug/100ml) and at South Island (74.83 ug/100ml) (Grace, 1972), and in sheep and cattle (Miller, 1966) 80-120 ug/100ml and in Egypt (1.09-1.3 ug/ml) recorded by Ezzo (1989).

The present study reveals that plasma zinc values are higher during oestrus and proestrus (0.09 and 0.094 ppm respectively) than at during metoestrus and dioestrus at (0.089 and 0.084 ppm) respectively. This result

disagrees with Ezzo (1989) who observed that the concentration of zinc was higher during metoestrus than during oestrus, dioestrus and proestrus. Statistical analysis indicates that the differences in the values of iron and zinc in Barki ewes herein during oestrous phases are not significant. This result is in agreement with Ezzo (1989).

The present result reveals notes that the seasonal variation has no significant effect on zinc levels. This result agrees with Ezzo (1989).

6. Plasma Proteins:

The total plasma protein level in Barki ewes in the present study ranged between 4.21 to 12.24 g/dl. This is higher than that found by cornelius (1960) (50-80g/l). Total plasma protein values in the present work averages 6.65 g/dl, this figure is higher than 5.7-5.8 g/100ml recorded by other investigations (Contreras, 1967; Kronfeld and Medway, 1969 and Coles, 1986), values in the present results are lower than 7.0-7.25 reported by Braun et al. (1978), Fernandez et al. (1984) and Ghazy (1987) and 8.14-8.9 g/100ml noticed by Keay (1981) and Hassaan et al. (1984).

Albumin value in Barki ewes averaged 3.21 g/dl (ranged from 2.91 to 4.68 g/dl). This average is similar to that reported by Keay, 1981 (3.08 g/dl), and higher than 2.56 g/dl that noticed by Contreras (1967) and Fernandez et al. (1984) and Coles (1986) 2.9 g/dl. Moreover, the present result is lower than that estimates as (Ghazy, 1987) 3.8 g/dl.

Golbulin values in Barki ewes averaged 3.76 g/dl (ranged between 1.9 to 5.8 g/dl). This average is nearly similar to that noticed by Hassan et al. (1984) (3.98 g/dl), and higher than that of Keay (1981) as 2.34, Contreras,

(1967) and Ghazy (1987) 3.2-3.26 and Coles (1986) 3.45 g/dl. Moreover, the present figure is lower than that of Fernandez et al. (1984) 4.35 g/100ml.

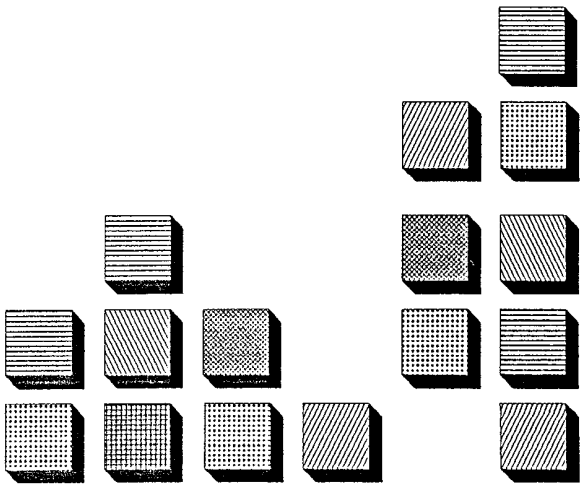
These variations between the present work and other studies may be due to differences in age, breed, season, physiological status (Lactating or dry), climatic conditions or nutrition.

The present result reveals that the difference in plasma values of total protein, albumin, globulin and A/G ratio were not significant and have no clear variations during the different oestrous cycle phases. Albumin values have significant ($r=0.3$, 1%) correlation with progesterone. While, globulin values show a highly significant ($r=-0.4$, 1%) negative correlation with oestradiol-17B.

The variation in albumin levels of the Barki ewes were significant ($P<0.05$) during spring (3.47 g/dl) than other seasons (ranging from 2.93-3.3 g/dl). Moreover the A/G ratios were highly significant ($P<0.01$) during winter (1.18) and spring (0.83) than during autumn (0.89) and summer (0.7). Upadhyay and Rao (1984) observed that serum protein levels in goats were highest during cooler months (January) and added that the variation in serum protein levels change with age and that winter conditions cause an elevation in the protein level.

The present study reveals that total protein and globulin values are not significant and have no definite alteration during the different seasons.

SUMMARY



Summary

Studying the physiological changes during the oestrous cycle are needed to formulate the basis for any program to increase the reproductive efficiency of Barki ewes.

The investigations were carried out on 10 non-pregnant Barki ewes, with an age range from 1 to 7 years. Ewes were kept with a vasectomized ram throughout the year. The animals were raised at the National research Center Experimental Farm "Abou-Rawash".

Prostaglandin f_{2o} was used for synchronization of the oestrous cycle. Detection of oestrus was carried out by monitoring ewes once daily and was confirmed by hormonal values. Blood samples were collected at day of oestrus and day after day during the breeding season or twice a week during the non-breeding season. Harvested plasma was analysed for progesterone and oestradiol-17B hormones (RIA), Calcium, Inorganic phosphorus, Iron, Zinc, total proteins albumin and globulin (Spectrophotometry).

Oestrus in Barki ewes was manifested by some changes in the ewe behaviour. Ewes seek the ram following him with characteristic looked over the shoulder and stand to be mounted. The vulva was oedematus and the mucous membrane hyperamic. Mucous discharges were voluminous and transparent at oestrus then became viscous and creamy during metoestrus and dioestrus. Oestrous signs were more pronounced at autumn and winter but, feeble during spring and summer.

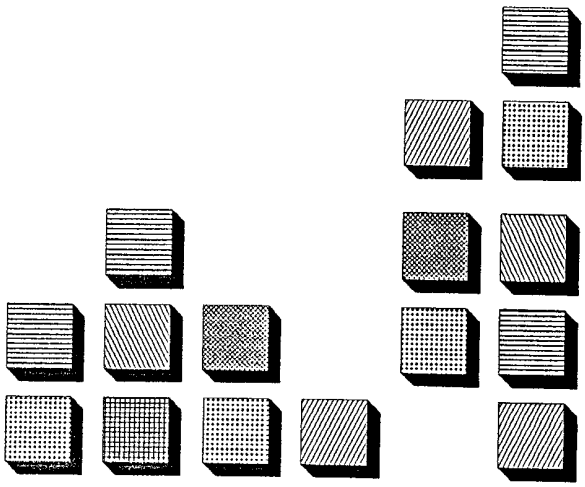
The incidence of various types of oestrous cycle in barki ewes was 60.32% in normal cycle (av. 16.83 $\pm$ 0.34 days), 11.11% in short cycle (av. 12.0 $\pm$ 0.72 days), and 14.29% in both long cycle (av. 21.56 $\pm$ 0.56 days) and multiple cycles (av. 34.22 $\pm$ 2.16 days). The incidence of normal cycles was high in autumn (58.33%) and low during winter (22.22%), summer (13.89%) and spring (5.56%).

The values of oestradiol-17B and progesterone in Barki ewes showed highly significant changes during the oestrous cycle phases ($P<0.01$). Oestradiol-17B values were 4.02 $\pm$ 0.29, 14.38 $\pm$ 1.47, 1.35 $\pm$ 0.36 and 1.19 $\pm$ 0.29 pg/ml during proestrus, oestrus, metoestrus and dioestrus respectively. The respective values of progesterone were 0.17 $\pm$ 0.02, 0.12 $\pm$ 0.02, 0.43 $\pm$ 0.05 and 2.55 $\pm$ 0.18 ng/ml. Seasonal variation in oestradiol-17B and progesterone values were non significant, Estradiol values showed a significant negative correlation with progesterone ($r=-0.3$, $P<0.01$), globulin ($r=-0.4$, $P<0.01$) and positively correlated with Ca/p ratio ($r=0.2$, $P<0.05$). Progesterone values were significantly correlated with albumin ($r=0.3$, $P<0.01$), calcium ($r=0.2$, $P<0.05$) and negatively correlated with A/G ratio ($r=-0.2$, $P<0.05$).

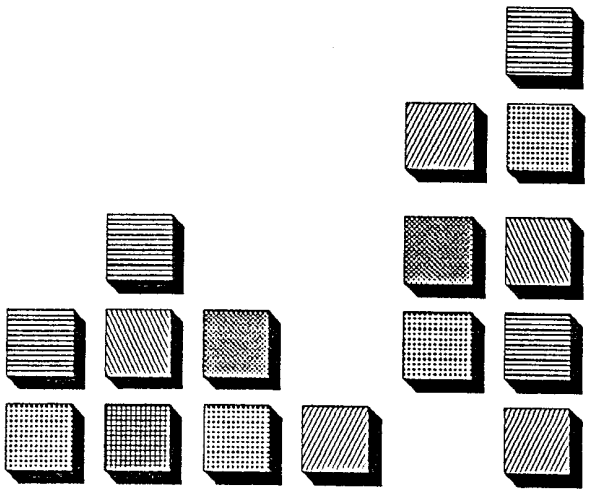
Plasma mineral values (Calcium, inorganic phosphorus, Ca/p ratio, iron and zinc) and plasma proteins (total protein, albumin, globulin and A/G ratio) showed non-significant changes among the different phases of the oestrous cycle. There were significant variations in albumin value ($P<0.05$) and A/G ratios ($P<0.01$) among seasons.

PART II

SUPEROVULATION AND FACTORS AFFECTING OVARIAN FUNCTIONS



INTRODUCTION

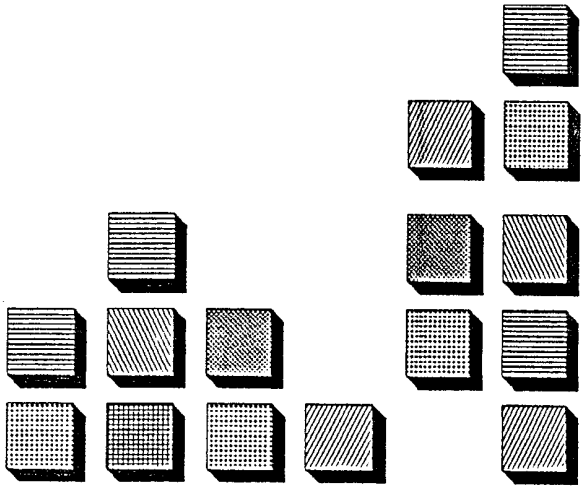


Introduction

Sheep was the first species of domestic farm animals used in embryo transfer experiments. Superovulatory treatments are now widely used in embryo and ova from animals of superior genetic merits. The number of lambs reared per ewe mated has a marked effect on the biological and economic efficiency of sheep production. Hence, superovulation could prove a useful aid in preferential selection for fecundity in animals within populations and a large number of lambs can be obtained from selected ewes.

So, in this study strict attention was given to the factors affecting the tremendous variations in ovarian functions (Season, age, nutritional status and side of ovary), superovulation regimens (PMSG or FSH) and hormonal profile (Oestradiol-17B) and progesterone) on superovulated Barki ewes.

REVIEW OF LITERATURE



Review of Literature

1. Factors Affecting Ovarian Functions In Ewes:

The main factors influencing ovulation rate in ewes are, breed and the level of nutrition during premating and mating periods; but seasonal and age-related factors are also important (Haresign, 1985). Furthermore, genetic factors; heterosis and season of lambing have some effects on ovulation rate (Treijo and Gonzalz, 1986).

1.1. Season:

There is an ample evidence from several breeds of sheep that ovulation rate increases after the commencement of the breeding season and then falls away towards the end (Fletcher and Geytonbeck, 1970; Wheeler and Land, 1977; Lees, 1978; Gunn, Doney and Smith, 1979). The mean ovulation rate of the Black face and Merinos was as high around the shortest day as

at any other time, whereas that of the Finn ewes, reached a maximum soon after the onset of cyclic ovulation and then declined gradually till the end of the season (Land, Pelletier, Thimonier and Mauleon, 1973). Furthermore, El-Wishy et al. (1976) mentioned that the ovulatory activity indicated by the average number of metoestrus and dioestrus corpora lutea in the ovaries of the ewe studied was highest in the autumn (1.86) and lowest in the spring (1.33), ovulation rate during summer and winter were 1.61 and 1.59 respectively. According to the last mentioned authors, variation in ovulation activity due to season of the year was not statistically significant. Ovulation rate in the Finn breed was 3.5 in November and 2.6 in March (Wheeler and Land, 1977). Pulu and Fletcher (1984) reported that the mean ovulation rates in February, May, August and November were, 1.8, 1.9, 1.8 and 1.9 respectively. Armstrong and Evans (1984) mentioned that, superovulation was performed at three times: early (September-October), late (December-January) and after (May-June). Ewes were injected with 22.5 mg FSH-P S.C. twice daily over 4 days period. Progestogen sponges were removed at the time of the 5^{th} or 6^{th} FSH injection after being in place for 12-14 days. Ovulation rate by Laparoscopic procedures was 9.8 ± 1.4, 12.8 ± 1.9 and 15.8 ± 3.2 respectively. In addition, Ile-de-France ewes were synchronized with a vaginal sponge of FGA (40mg) given for 14 days, 16 mg FSH injected at 2 days before sponge removal, every 12 hours in decreasing doses over 3 days. Ovulation rate was 18.6 ± 5.8 in breeding season and 10.9 ± 4.2 in non-breeding season (Congie, Chupin and Saumande, 1986). During seasonal anoestrus, the mean ovulation rate in superovulated ewes using 16 mg FSH at the end of progestin treatment decreased from 11.2 to 8.4; 40.6% of the embryos recovered were of transferrable quality vs. 74.5% during the normal breeding season (Torres, Cognie and Coles, 1987).

1.2. Age:

Young ewes have lower ovulation rates and litter size than mature ewes of the same live weight (Jones and Rouse, 1967; Brien, Boxter, Findlay and Cumming, 1976). The overall mean ovulation rates were 3.37, 1.09 and 1.36 for older Finns, Merinos and Black face ewes respectively and 2.53, 1.0 and 1.15 respectively for younger (1.5 years) ones (Wheeler and Land, 1977). In anoestrus Caucasian sheep, aged 3-6 years, it was found that the number of new and old corpora lutea in the ovaries averaged 0.67 and 1.17 respectively, the number of follicles > 3 mm and 1-2 mm in diameter were 0.67 and 17.2 respectively (Stepanov, Dmitriev, Gerasimova, Donskaya, Zeltobryukh and Kunizhev, 1981). The response to superovulatory treatment (16 mg FSH) in groups of ewes as indicated by ovulation rate was 8.8 $\leq$ 3 yr and 12.0 in those aged $\geq$ 6 yr (Torres, et al., 1987).

1.3. Nutrition:

It is known that there can be certain components of ewe nutrition which can have a marked effect on ovulation rate with little change in live weight (Knight, Oldham and Lindsay, 1975; Smith, Jagusch, Brunswick and Kelly, 1979). The use of hormones to induce ovulation seldom gives a satisfactory results if females were undernourished (Hafez, 1987).

Ducker and Boyd (1974) reported that the high planes of nutritions (0.9 kg hay and 0.9-1.1 kg of molassed sugar beet pulpnut containing urea, 17% crude protein, per head per day) had non-significant effect on both oestrus and ovulatory activity in ewe. The only statistically significant effect of the plane of nutrition were observed within the low plane control ewes, occurred in August for the percentage of ewes ovulating in low (42%) and high planes (7%). The mean ovulation rate was 1.4 in low and 1.0 in high

plane for all ewes. The values for the low plane ewes were higher ($p<0.05$). Furthermore, when Panama ewes were given feed levels (65, 85 and 105% of N.R.C.), there was an apparent but non-significant increase in ovulation rates (1.86, 2.02 and 2.05 respectively) due to the increase in feed level between September and October. Feed levels near the peak of the breeding season (November to December) had very little effect on ovulation rates (1.93, 1.95 and 1.98 respectively). However, following a seasonal decline in ovulation rate, feed levels between January and February had a significant effect on the ovulation rate (1.78, 1.81 and 1.50 CL/ewe) on 105, 85 and 65% N.R.C., respectively (Hulet, Price and Foote, 1974).

The feeding of high protein supplements, such as lupine grains (Knight et al., 1975) and soyabean meal (Davis and Cumming, 1976) has resulted in a significant increase in ovulation rate. It seems that the responses can vary according to environment and season (Lightfoot and Marshall, 1974; Rizzoli, Reeve, Boxter and Cumming, 1976). Also, Dahmen, Hulet, Price and Everson (1976) found that in ewes flushed for 14 days in pasture, there was a high significant effect of flushing on the ovulation rate in mature ewes. On the other hand, the ovulation rate of the ewes which had been in the high levels of nutrition did not differ significantly from that of ewes which had been on the low levels of nutrition (Gunn, 1977). Coopworth ewes offered a high feed allowance (1.0 kg dry matter/day) for 3 weeks showed a 25% increase in the ovulation rate (Smith, Jagusch and Farquhav, 1983). In addition, mature Merino ewes, fed on a 9 days basal rations (controls) before the estimation time of ovulation or on the basal ration plus 750 g lupine seed per day or on the basal ration plus 525.1 mM glucose per day intravenously showed ovulation rates averaging 0.39 and 0.32 higher in the treated groups respectively than in controls (Teleni, Rowe and Croker, 1984).

1.4 Breed:

Differences in ovulation rate between breeds and between strains within breeds have been well documented, and there is a good evidence suggesting that ovulatory responses to exogenous gonadotrophin reflects these differences (Moore, 1982). The vast majority of domesticated sheep breeds has a mean ovulation rate between 1 and 2, there were several "prolific" breeds in which the mean ovulation rate approaches or even exceeds a value of three (3.31 ± 0.17 and 3.11 ± 0.18) in Finns and Hyyang breeds (Bradford, Quirke and Hart, 1971; Guo, Ding, Shi, Zong, Jiang, Wang, Xu and Zhu, 1981). The ovulation rate varied among breeds: 2.99 in Finn, 1.08 Merino and 1.3 in Black face ewes (Wheeler and Land, 1977), only 15 of 205 Merino ewes ovulations observed were multiples (double), whereas only 3 of 128 Finn ewes ovulations observed were singles. Finn and Romanov sheep have a high ovulation rate than pure bred Welsh or Black face ewes (Land, 1979).

Prolific sheep were generally more sensitive to PMSG treatments than sheep of other breeds, but this may not apply to exogenous FSH (Bindon, Piper, Cahill, Driancourt and O'Shea, 1986). Merinos selected for multiple births show an increase in ovulation rate and their ovulatory response to graded doses of PMSG (750, 1500 i.u.) was almost twice (3.8 and 7.1 respectively) as reported by Bindon, Chang and Turner (1971) and Trounson and Moore (1972). In superovulation response with 1000 to 1500 i.u. PMSG per donor, the total number of ovulations from 34 Merino X Polwarth, 34 Merino X Chokla and 19 Gopal Merino ewes assessed by corpora lutea counts were 282 (average 11.33), 236 (average 6.94) and 126 (average 6.63) respectively. The total number of large follicles were 167 (average 4.91), 44 (average 1.29), 89 (average 4.68) respectively (Zanwar and Deshpande, 1984). At the beginning of the mating season in mid-April, 56 Romney Marsh

(RM) and 47 Corridale ewes were injected with 1000 i.u. PMSG at the time of implant withdrawal. Ewes exhibited oestrus within 48 h were 61.5% in RM ewes vs 21.4% in Corridales ($P<0.01$). The number of ovulations averaged 2.4 and 3.4 for RM ewes and in Corridales respectively (Salomon and Alberio, 1984). Despite the differences in the genetic basis of their high prolificacy, the pattern of response to PMSG over the range of dosages used was similar in Finnish Landrace, D'Man and Booroola Merino X Romney (FT) ewes, and all breeds had a means of about 10 ovulations in response to 1500 i.u. PMSG (Quirke, Meyer, Lahlou-Kassi, Hanrahan, Bradford, Stabenfeldt, 1987). Same authors noticed that amongst the non-prolific breeds, the Timhadite was the most responsive to PMSG treatment, although it had the lowest natural ovulation rate. Moreover, Gootwine, Bor and Brawta1(1989) reported that, 400 in PMSG at 20 weeks of age induced ovulation in 82% of 22 Finn Awassi, 79% of 34 Assof and 61% of 28 Awassi ewe lambs. Ovulation rate averaged 1.78, 1.41 and 1.23 CL for the 3 breeds respectively.

The injection of 16 mg FSH pituitary extracts at the end of progestin treatment resulted in an average of 9.0, 12.0 and 19.5 corpora lutea per ewe in the Prealpsrdusud, Lacaune and Romanov X Prealpes breeds respectively (Torres et al., 1987) 23 Suffolk and 24 Dorset ewes, 2 to 4 years of age were superovulated by 3 daily intramuscular injections of 10 mg follicular stimulating hormone (total dose, 30mg) starting on day 12 after insertion of vaginal pessary. At the third FSH injections each donor ewe received 50 ug estrumate i.m. and the pessary was removed, breeds did not differ in the number of corpora lutea, 6.6 and 6.5 respectively (Maurer, 1988).

1.5. Side Of Ovary:

The total follicular population in the sheep's ovary consists of a large reserve of primordial and small follicles and a much smaller number of larger vesicular follicles in the growth phase, a direct relationship exists between the number of follicles in the growth phase and the ovulation rate (Cahill, Mariana and Mauleon, 1979).

The activity of the ovaries indicated by the average number of metoestrus and dioestrus corpora lutea was always higher for the right than for the left ovaries (El-Wishy et al., 1976). The same authors observed that the highest activity of the right ovaries was observed in winter (57.4%), whereas the lowest activity was noted in spring (51.4%). Analysis of variance revealed non-significant differences between sides and between seasons.

2. Superovulation:

The differences in ovulation rate were primarly due to differences in ovarian sensitivity to gonadotrophin, thus it seems likely that animals characterized by high ovulation rates will produce larger number of eggs in response to exogenous gonadotrophins than those which normally released few ova (Bindon, Blance, Pelletier, Terqui and Thimmier, 1979; Cahill et al., 1979).

2.1. PMSG:

Superovulation has been attempted using a variety of gonadotrophins, particularly PMSG (Hunter, Adams and Rowson, 1955; Averill, 1958; Moore, Rowson and Short, 1960; Hancock and Hovell, 1961; Cumming and

McDonald, 1967; Lawson, Adams and Rowson, 1972). Ovulation rate (endoscopy) in Atamura ewes was 2.14 in response to the injection of 400 i.u. PMSG at the time of sponge removal as compared to 1.86 in untreated control group (Toteda, Martemucci, Gamacorta, Manchisi and Bellitti, 1986). Moreover, when Tsigai ewes in the breeding season and the non-breeding season were received 600 i.u. PMSG and compared to control groups, ovulation rates shown in the breeding season were 0.6 and 0.7 respectively, while in non-breeding season they were 2.2 and 0.0 respectively (Stancic, 1986). The same dose of PMSG (600 i.u.) gave an average ovulation rate of 8.4 in Romanov ewes (Driancourt, 1987). Although, in Zackel ewes the ovulation rate averaged 2.1 when treated with 750 i.u. PMSG (Kremer, 1981). While, the ovulations rate was 3.4 per ewe when the same dose was used in Suffolk ewes (Armstrong and Evans, 1984). When ewes were treated with 1000 i.u. PMSG at the time of implant removal, the percentage of ewes exhibiting oestrus was 75.7 (Stancic, Krajinovic, Vitorouic and Kolaric, 1983). In Merino-Land Schaf ewes received 1000 i.u. PMSG, oestrus occurred on averaged 41.4 hours after treatment and lasted for 28.4 hours (Meineche, Tillmann, Evers, Meinecke and Gips, 1988). Same author's observed that in cross bred ewes ovulations rate and the number of large follicles averaged 7.33 and 1.54 respectively. PMSG in a dose of 1200 to 1300 i.u. injected in Merino ewes produced ovulation rates averaging 10-12 per ewe (Moore, 1982).

The response to the same dose of PMSG observed in different breed of ewes as, in Karakul ewes, the number of ovarian follicles and corpora lutea averaged 11 and 9 respectively (Abdul-Tairov, 1983), Finnish-landrace ewes showed 95.8% ovulated follicles (Sergeev, Mazepkin, Smyslova, Shikhov and Bukarova, 1983), the ovulation rate was 7.3 per donor (Dawinov, Sartaev and Toishibekov, 1986), in non-lactating Dorper ewes, the number of corpora

lutea and non-ovulated follicles averaged 18.23 and 3.00 respectively (Zyle, Stein, Niekerk, Coertze and Groene wald, 1987). Injection of 0, 250, 500, 750 and 1000 i.u. PMSG (at sponge removal) in Booroola Merino ewes produced an ovulation rate of 2.83, 3.59, 4.65, 5.16 and 6.65 respectively for heterozygous Booroola gene vs. 1.86, 2.39, 2.21, 3.0 and 7.06 for ewes had not Booroola gene (Kelly and Owens, 1983), In Yankasa ewes, ovulation rates were 1.0, 1.3, 2.0, 5.5 and 7.0 as response to the previous doses respectively (Oyedipe, Pathiraja, Gyang and Edovist, 1989). Furthermore, Vintila, Padeanu, Hodus, Popa, Anghelescea, Suta, Harezean, Colgea and Barbat (1986) reported that the ovulation rate of ewes treated with 800 to 1000 or 1200 to 1400 i.u. PMSG averaged 5.8 (2-12) and 11.3 (2.20) respectively. Smith, Cruickshank, McGowan, Parr and Mortimer (1988) noticed that in Coop worth ewes, the increase in PMSG dose levels (0, 400 or 800 i.u.) at CIDR removal is directly proportional to the ovulation rate (1.18, 2.11 and 2.88 respectively).

2.1.1. GnRH:

Findlay and Cumming (1976) reported that an analogue of GnRH was used to bring about the release of gonadotrophin towards the end of sheep's oestrous cycle. Fukui, Takenaka, Domeki and Ona (1984) observed that Suffolk ewes treated with 750 i.u. PMSG at sponge removal during anoestrus season and injected with 150 ug GnRH, showed a lambing percentage of 57.9. Furthermore, Fukui, Kobayashi, Kojima and Ona (1985) noticed that in anoestrus ewes injected with 600 i.u. PMSG at sponge removal and a single injection of 100 ug synthetic GnRH 36 hours after sponge removal, the percentage of ewes showing oestrus and the lambing rate averaged 68.2 and 26.7 respectively.

2.1.2. HCG:

Moore (1982) showed that in ewes given 750 i.u. Folligon and 400 to 800 i.u. HCG, increased the proportion of follicles which ruptured. In this respect, ewes receiving 1500-2000 i.u. PMSG at day 9-13 of the oestrous cycle and 1000 i.u. HCG at oestrus showed an ovulation rate averaged 3.2-3.3 (Donskaya, Kunizhev and Radchenko, 1985; 1986). At 15 October to 15 November the percentage of ovulation and the ovulation rate were 65.00 and 9.30 (range 2-32) respectively in KF ewes (Toishibekov, Daminov, Sartaev and Umirzhanov, 1986). Moreover, Hunter and Southee (1989) found that in anoestrus ewes, the number of corpora lutea per ewe ranged from 2 to 12 in ewes received 1000 i.u. PMSG and 1000 i.u. HCG.

2.2. FSH:

Bondioli, Allen and Wright (1982) noticed that ovulation rate was recorded as 13.9 by mid ventral laparotomy in ewes treated with 24 mg FSH /ewe in 6 injections at 12 hours intervals (5.5, 4.4, 3.3 mg). The first dose was given at a day before pessary withdrawal (day 11 of pessary insertion). A dose of 25 mg LH was given to each ewe within 8 hours after onset of behavioural oestrus. In Dorper ewes treated by the same dose of FSH, ovulation rate was 6.7 (Zyl et al., 1987). Moreover, Armstrong and Evans (1984) reported that the ovulation rate was 5.5 in Suffolk ewes during anoestrum, as a response to 15 mg FSH-P given in a decreasing dose order (8, 4, 2 and 1 mg) twice for 4 days. The first injection was given 2 days before sponge removal. Cross bred Torghee ewes (Smith, 1984) treated with 12, 17, 19.5, 22 and 30 mg FSH twice a day for 3.5 days, the initial dose was given on day 7 of the oestrous cycle of the ewes. At the 3rd day of FSH injection, each ewe received 20 mg PGf_2o to induce oestrus. The ovulation rate averaged 6.67, 8.40, 12.06, 14.39 and 10.73 respectively with

a significant variation ($P < 0.05$). In this respect, the ovulation rate was 10.8 (97.8%) in ewes treated with 30 mg FSH at a rate of 10 mg FSH once daily, the first injection started 2 day before pessary withdrawal and each ewe received 50 ug estrumate (Maurer, 1988). Moreover, Lopez Sebastian, Cognie, Cocero, Fuente and Poulin (1990) injected Manchega ewes with 16 mg FSH on two regimens either on 3 days injections (4.4, 3.3, 1.1 mg) or 2 days injections (5.5, 3.3 mg). The first injection as given 48 hours before sponge removal. At last two injections in both groups 100 ug of LH/ewe were administered. The ovulation rate was high on 3 day injection (8.7) than 2 day injection regimen (5.8). Furthermore, Thompson, Simpson, James and Tervit (1990) injected ewes with FSH in two doses (12 mg and 18 mg) in six injections with 12 hours intervals, on decreasing dose regimen of 3.3, 2.2 and 1.1 mg FSH and 4.4, 3.3, and 2.2 mg FSH respectively. The first injection was given with 800 i.u. PMSG at a day before CIDR devices removal. Ovulation rate did not differ significantly in both doses (7.1 and 7.4 respectively).

2.3. Repeated Superovulation:

Moore and Shelton (1962), Palsson (1962) and Charardi and Martin (1978) induced superovulation at intervals of one year in sheep without finding any significant decrease in the ovulatory response. In Ireland, studies on PMSG (Lynch, 1968) and HAP (Boland, 1973) showed that sheep could be superovulated on three occasions in the course of a 6-9 month period, this included treatments during the ewes anoestrum as well as in the breeding season. Donskaya, et al. (1984) reported that 69 Caucasian ewes were used repeatedly for ova collection, the interval between successive collections being at least 3 oestrous cycles.

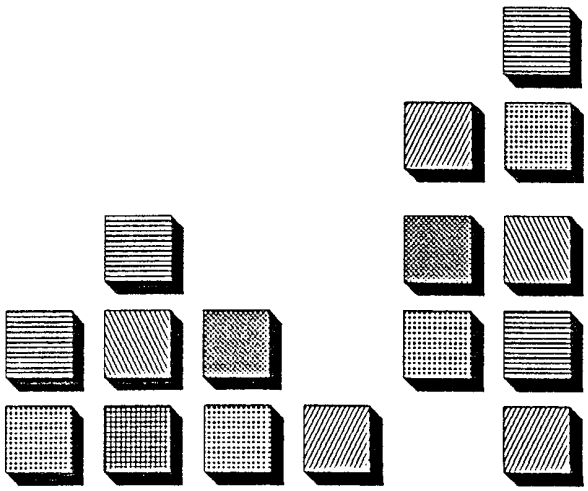
Hafez (1987) recorded that response to subsequent treatment, becomes lower in some individuals, probably due to production of antibodies against exogenous gonadotrophins. This may be avoided to some extent by increasing the interval between successive hormone treatments. Antibody production may be minimized by using gonadotrophins derived from the same species of the treated animals. A 23 sexually mature Han ewes were treated on several occasions with FSH and LH (Zhu, Jiang, Guo, Lo, Zhu and Feng, 1986). A superovulatory response occurred in all ewes at the 1st treatment, in 14 ewes at the 2nd, in 6 ewes at the 3rd and in one ewe at the 4th treatment. The average numbers of ovulations per ewe at the 2nd, 3rd and 4th treatments were 3.3, 7.0 and 4.0 respectively, the differences between treatments were not significant. On the other hand, 18 ewes were superovulated twice with similar FSH-P regimens, the intervals between successive superovulations were broken down into 3 sub-groups of 2 months, 4 months and 6-8 months. The ovulation rate in < 2 months interval was 12.0 in first and 23.3 in second treatment. In the 4 months interval, the ovulation rate averaged 5.4 and 10.0 in both treatments respectively. While in the 6-8 month interval the ovulation rate was 12.6 in first and 17.7 in the second treatment. The increase in ovulation rate at the second superovulation was not significant (Armstrong and Evans, 1984). Furthermore, A 18 Prealpes du Sud ewes were superovulated repeatedly using a series of injection of FSH-P on 3 occasions at intervals of 45-55 days during the same breeding season following mating. The 1st and 3rd repeated superovulation treatments gave averages of 5.2 and 4.5 corpora lutea. The 2nd one yielded 3.4 corpora lutea, which was a significantly lower than in treatments 1 and 3, but adhesions did not permit a perfect view of the whole surface of each of the ovaries (Torres and Sevellec, 1987).

2.4. Effect Of Superovulation Regimens On Ovarian Hormone Values:

PMSG treatment raises plasma progesterone concentration almost immediately (Bondurant, 1986). The concentration of PMSG in blood serum 24-48 hours after treatment was > 57 i.u./ml for all sheep with > 4 corpora lutea, and progesterone concentration was $\geq$ 3.5-4.0 nmol/litre on day 2 and $\geq$ 17-18 nmol/litre on day 4 and ewes that responded to treatment had a plasma oestradiol-17B concentration of $\geq$ 23 pmol/litre. Evers (1988) noticed that ovarian response was significantly greater in heavier than in lighter ewes. In addition, Hunter and Southee (1989) noticed that anoestrus ewes ovulation was induced by using progestogen priming followed by injections of PMSG (1000 i.u.) and HCG (1000 i.u.). The plasma progesterone concentrations on the day of slaughter (day 2, 4, 6, 8, 10, 12 and 15) were correlated with time ($P<0.05$), total weight of luteal tissue ($P<0.001$) and the number of corpora lutea ($P<0.05$). There was much variation between individual corpora lutea particularly in terms of weight and progesterone content, although both traits were correlated ($P<0.001$) with day of recovery until day 10. The number of corpora lutea induced by superovulation in anoestrus ewes was extremely variable and suggests that ovulation may have continued during the luteal phase.

Oyedipe et al. (1989) observed that, in 30 Yankasa ewes, oestrus was synchronized using progestogen sponges. At sponge withdrawal, the ewes were given 0, 250, 500, 750 or 1000 i.u. PMSG. Plasma progesterone concentrations averaged 15.3 $\pm$ 2.5, 36.6 $\pm$ 16.9, 56.1 $\pm$ 7.7, 82.2 $\pm$ 5.6 and 125.7 $\pm$ 9.0 ng/ml in all the groups respectively.

MATERIALS AND METHODS



Materials and Methods

1. Experimental Animals:

The present work was carried out on 25 healthy non-pregnant Barki ewes, free from parasitic infestation. Their age ranged from 1 to 7 years old and live body weight from 20-35 kg. A vasectomized ram was used to help in oestrus detection. The animals were raised in the National Research Center Experimental Farm at "Abou-Rawash" Giza, Egypt.

Animals were left loose in an open well ventilated pen. Throughout the course of the experiment, each animal received per day, a basal ration composed of 0.5 kg concentrate balanced ration and 0.5 kg green fodder (Barseem). During the dry season animals were offered Barseem hay (This ration contains 10% crude protein). Sufficient amounts of fresh water were supplied twice daily on the morning and the afternoon.

2. Hormones Used During Experiments:

2.1. Synchro-Mate B (SMB):

SMB is a part of a system suitable to synchronize oestrus in ewes. SMB consists of a silastic ear implant (17-0-acetoxy-11B-methy-4-norpregan-4-en-3,20 dione) and 2ml SMB solution for injection (3mg Norgestomet and 5mg oestradiol valerate) from Intervet Int., Holland.

2.2. Pregnant Mare's Serum Gonadotrophin (Folligon, PMSG):

Folligon (Intervet) batch number 29149 is presented as a white-dried crystalline plug, containing 6000 i.u. PMSG per vial together with a sterile solvent.

2.3. Follicle Stimulating Hormon (FSH):

FSH  Japan) each vial contains 10 mg highly purified FSH together with 5 ml sterile solvent for each vial.

2.4. Receptal (GnRH):

Receptal (Hoechest-GFR) is the injectable solution of a highly active peptide hormone which is chemically analogue to the releasing hormone (RH) of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Each 1 ml Receptal contains 0.0042 mg bureselin acetate equivalent to 0.004 mg bureselin.

2.5. Pregnel (HCG):

Pregnel "Nile Co. Egypt" prepared in the form of white freeze dried crystalline plug containing 5000 i.u. of HCG per vial together with 1 ml sterile solvent.

3. Experimental Procedures:

3.1. Oestrous Cycle Synchronization:

Barki ewes were synchronized using SMB ear implant for 13 days subcutaneously in the outer aspect of the ear (using applicator) and 2 ml intramuscular injection of SMB solution (3 mg Norgestomet + 5mg oestradiol valerate). Implants were removed on day-13 according to Salomon and Alberio (1984). Oestrus was detected using vasectomized ram within few hours of implant removal.

3.2. Laparotomy:

Mid ventral laparotomy was performed on day 5 or 6 of oestrous cycle (oestrus = day-0) according to Hunter, Adams and Rowson (1955) and Vaughan (1980).

Barki ewes were fasted for 24 hours before surgery then placed on its back on an operating table.

The site of incision was immediately infront of the udder (pre-oparative preparation: The site of incision was clipped and shaved then washed by soap and water and disinfected using tincture iodine). Animals were secured using tranquillizer (0.1 ml Rompun 2%, Byer leverkuseni, Germany, intramuscular injection). Local anesthesia infiltrated in the line of incision (2%

procain hydrochloride, El-Nasr Company for Medicinal Chemicals). An incision was made immediately infront of the udder along the mid line in skin, subcutenous fascia and muscles (Bleeding points can be dealt with hemostat procedures). Exteriorization of the uterus and grasping the enlarged ovary was carried out.

Ovarian finding were carried out by determine the number of corpora lutea and Graafian follicles (mature $\geq$ 8 mm and growing $<$ 8 mm) reported by Thompson et al. (1990). Ovarian response in Barki ewes was observed in both right and left ovary either in superovulated ewes or controlled ewes.

The peritonium and muscles were sutured by mattress suture using chromic cat gut number 3 (Ethicon) through a round needle. Dose of antibiotic was placed intraperitoneum before complete the closure of abdominal wall. Skin was sutured by mattress suture using silk (pearsalls sutures braided silk) by sharp pointed needle. Then ewes were injected with dose of long acting antibiotic Uvomycin (Hoechst).

After laparotomy, detection of subsequent oestrus was detected by vasectomised ram to estimate the interval between laparotomy and the occurrence of oestrus.

3.3. Blood Sampling:

Samples were taken from the external Jugular vein by direct vein-puncture using hypodermic needles. Blood was collected in heparinized (20 units/ml) covered test tubes which were, transferred to the laboratory on ice. The plasma was separated by centrifugation at 3,000 r.p.m. for 30 minutes and stored at-20^{o}C until assayed for oestradiol-17B and progesterone hormone.

Blood samples were collected on day of gonadotrophin (FSH or PMSG) injection, day of oestrus (days-0), 3rd day of oestrous cycle and at day of laparotomy.

4. Factors Affecting Ovarian Functions:

The Barki ewes used in the following experiments (Experiments 1 to 4) were synchronized at the beginning of this study (section 3.1). Ovarian findings were evaluated by means of midventral laparotomy on days 5-6 of the ewes oestrous cycle (section 3.2). Both right and left ovaries were examined for number of corpora lutea, mature Graafian follicles ($\geq$8mm) and growing ones (<8mm) (Thompson et al., 1990).

Exp. 4.1. Effect Of Season:

The Barki ewes followed up throughout the whole year (in part I), were used to figure out the incidence of normal oestrous cycle during the breeding season (autumn and winter) versus the non-breeding season (spring and summer). To confirm the seasonal variations in ovarian functions, laparotomy was performed on other 6 ewes in summer and 4 ewes in autumn.

Exp. 4.2. Effect Of Age:

In this experiment, the ewes were allotted into 2 groups according to their age. The ovarian functions were evaluated by laparotomy, in 7 mature ewes 2-5 years old compared to 4 older ewes $\geq$ 6 years of age.

Exp. 4.3. Effect Of Nutrition:

Barki ewes (13 ewes) were classified to two nutritional groups. Group A (n=10 ewes) received a basal ration (10% crude protein) while ewes in group B (n=3 ewes) were fed approximately 1 kg barseem and 0.5 kg concentrate mixture (21% soyabean meal of 44% crude protein and 9% cotton seed cake of 14% crude protein) per head daily. This mixture had 17.9% protein (Duker and Boyd, 1974). These regimens were offered for 28 days before the day of laparotomy.

Exp. 4.4. Side Of Ovary:

During the course of those experiments, the corpora lutea were counted in each of the right and left ovaries of control and superovulated ewes.

Exp. 4.5. Synchronization Regimens:

Two groups of Barki ewes, 6 ewes synchronized with SBM ear implant only control group and experimental one of 3 ewes synchronized with SBM ear implant plus 750 i.u. PMSG injected at implant removal (Kasztetan, 1985).

5. Superovulations:

5.1. Superovulation Procedure:

Multiple ovulation was induced in Barki ewes through gonadotrophin therapy. pregnant mare serum gonadotrophin (PMSG) or follicle-stimulating hormone (FSH) was used to stimulate additional follicular growth. To

overcome ovulation problems, Human chorionic gonadotrophin (HCG) or gonadotrophin - releasing hormone (GnRH) was also administered several days later to induce ovulation of the follicles.

At the beginning of superovulation experiments, all ewes were synchronized for oestrus with SMB ear implant for 13 days (sect. 3.1). According to gonadotrophin used for follicular growth, PMSG was given as a single dose intramuscularly (i.m.) on day of implant removal (day 13) whereas FSH was administered twice daily in a decreasing doses for 3 days starting on day 11 of ear implant insertion. This treatment was followed by administration of gonadotrophins for ovulation: HCG or GnRh at the onset of oestrus.

The response to the superovulatory treatment, in terms of ovulation rate, was determined via laparotomy (section 3.2) to all treated and control ewes on day 5 or 6 of the oestrous cycle (oestrus = day-0).

5.2. Methods of Superovulation:

Exp. 5.2.1. Gonadotrophins for Follicular Growth (FSH vs. PMSG):

In order to investigate the effect of FSH compared to PMSG in inducing multiple ovulations, ewes were assigned into 3 groups. all treatments were carried out during the same season (summer).

Gp. 1: Five ewes were twice daily (12-hr interval) treated (i.m.) with a decreasing doses of FSH (5.5, 3.3, 2.2mg) for 3 days (20 mg FSH/ewe). The treatment was initiated 2 days before implant withdrawal (day 11 of ear implant insertion). Then, each ewe received 1000 i.u. HCG (i.m.) on day of oestrus.

Gp. 2: Each ewe in this group (6 ewes) received 2000 i.u. PMSG "Folligon" (i.m.) on day of heat.

Gp. 3: Six ewes in this group received no hormone therapy considered as controls.

Exp. 5.2.2. Pregnant Mare Serum Gonadotrophin (1000 vs. 2000 i.u.):

Ewes in this experiment were allotted into 3 groups in order to determine the effect of different doses of PMSG on superovulations. Three ewes received 1000 i.u. PMSG while other 3 ewes were given 2000 i.u. PMSG (i.m.) on day of implant removal (day 13). Each ewe was injected (i.m.) 1000 i.u. HCG "pregnel" on day of oestrus. Ewes in the control group (4) received no hormone treatments. This study was conducted during autumn.

Exp. 5.2.3. Gonadotrophins For Ovulation (GnRH vs. HCG):

Treated ewes (9) in this experiment received 2000 i.u. PMSG (Folligon) on day of implant removal (day 13). At oestrus, six ewes treated with 1000 i.u. HCG (pregnel) (Exp. 5.2.1) were compared to those (3 ewes) receiving 2.5 ml Receptal (10 ug GnRH/ewe) intramuscular. Control group, comprised of 9 ewes, received no hormone therapy. This study was conducted in summer.

5.3. Factors Affecting Responses to Superovulation Regimens:

Exp. 5.3.1. Nutrition (10 vs. 17.9% protein):

Ewes (12) in this study were allotted into 2 nutritional regimes (Exp. 4.3). A groups of 6 ewes received basal ration of 10% protein, while other

group of 6 ewes also offered improved ration of 17.9% protein. Ewes in both groups were superovulated using the same regimen: 2000 i.u. PMSG (Folligon) on day of implant removal (day 13) and (1000 i.u.) HCG (pregnel) on day of oestrus.

Exp. 5.3.2. Season (breeding vs. non-breeding):

All ewes in this experiment (9 ewes) received the same superovulated regimen consisting of 2000 i.u. PMSG on day of implant removal plus 1000 i.u. HCG (pregnel) at heat detection. Six ewes treated during the non-breeding season (summer) were compared to those (3) treated in the breeding season (autumn).

6. Hormonal Assay:

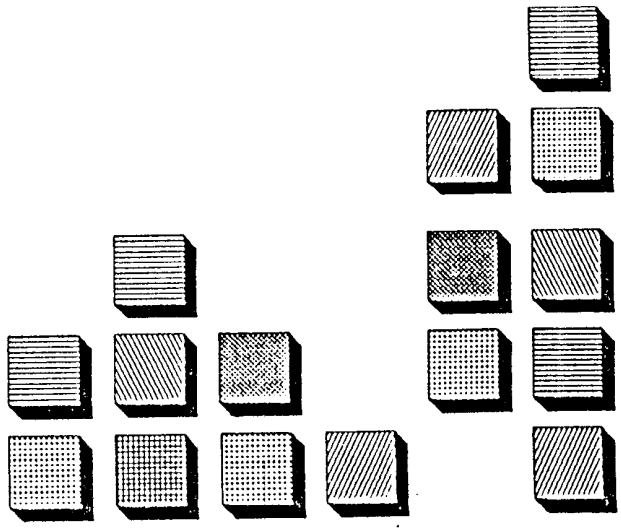
Oestradiol-17B and progesterone values in the blood plasma of superovulated Barki ewes were determined by using radioimmunoassay technique kits from Diagnostic Products corporation, were used utilizing direct progesterone coated tubes, while oestradiol double antibody comprised uncoated tubes. Oestradiol I^{125} and progesterone I^{125} were used as a tracer (Siiteri and Febres, 1979; Anne ., 1980 respectively).

Details described in materials and methods of part I.

7. Statistical Analysis:

The data obtained in this study was statistically analysed for the different variables according to the methods described by Snedecor and Cochran (1976).

RESULTS



Results

1. Factors Affecting Ovarian Functions:

1.1. Season:

The incidence of normal oestrous cycle in Barki ewes was found to be 80.55% during the breeding season (autumn and winter) compared to 22.45% in the non-breeding season (spring and summer).

In order to confirm these aforementioned seasonal variations in ovarian functions, laparotomy was performed on other 6 ewes in summer and 4 ewes in autumn. Only one ewe out of 6 (16.67%) ovulated in the non-breeding season whereas 3 out of 4 (75%) ewes ovulated during the breeding season (Table 2-1, Fig. 2-1). Respective ovulation rates were 0.17 $\pm$ 0.17 and 0.75 $\pm$ 0.25.

The results demonstrated a significant ($P < 0.05$) seasonal variation in the mean ovulation rate in Barki ewes.

1.2. Age:

Laparotomy was performed on mature (2-5 years old) and aged ($\geq$ 6 years old) ewes to evaluate their ovarian functions. Three of 7 mature ewes (42.85%) showed ovulations whereas only 1 of 4 aged ewes (25%) ovulated (Table 2-2). Respective ovulation rates were 0.57 $\pm$ 0.30 and 0.25 $\pm$ 0.25. Such variations were found to be non-significant statistically.

1.3. Nutrition:

In this experiment, ewes were assigned into two nutritional groups, basal ration (10% crude protein) and improved one (17.4% crude proteins). Laparotomy showed that 4 of 10 ewes (40%), receiving basal ration, ovulated versus 2 of 3 (66.7%) ewes fed improved ration. Respective ovulation rates were 0.40 $\pm$ 0.16 and 0.67 $\pm$ 0.33. However, this variation between the two regimens (10% and 17.9% crude protein) of nutrition were not significant (Table 2-3).

1.4. Side of Ovary:

During the course of all experiments the incidence of ovulations (Table 2-4) from the right ovaries was 89.47% and 25% in superovulated and control ewes which was higher than ovulations from the left ovary 84.21% and 8.33% in both superovulated and control ewes respectively.

The ovulation rate in the right and left ovaries averaged 3.32 $\pm$ 0.53 (range 0 to 8) and 3.11 $\pm$ 0.62 (range 0 to 9) respectively in superovulated Barki ewes while, it was recorded as 0.33 $\pm$ 0.19 (range 0 to 2) and 0.08 $\pm$ 0.08 (range 0 to 1) in right and left ovaries for the controls. Statistical analysis reveals that there is no significant difference between right and left ovary either in superovulated or control Barki ewes.

1.5. Synchronization Regime:

The incidence of ovulated ewes was 16.67% in control group (SMB implant only) versus 66.67% in experimental group (SMB implant + 750 i.u. PMSG). The respective mean ovulation rate (Table 2-5) in both groups was 0.17 $\pm$ 0.17 (range 0 to 1) and 0.67 $\pm$ 0.33 (range 0 to 1). Statistical analysis showed a non-significant effect of synchronization on the ovulation rate.

Table 2-1: Effect of season on ovulation rate:

Items	Seasonal variation	
	Breeding	Non-breeding
* No of ewes	4	6
Incidence of normal oestrous cycle	80.55 %	22.45 %
Incidence of ovulated ewes	75.00 %	16.67 %
Ovulation rate	* 0.75 ± 0.25 (0-1)	0.17 ± 0.17 (0-1)

* $P < 0.05$

Table 2-2: Effect of age on ovulation rate:

Items	Age (years)	
	2-5	≥ 6 years
No of ewes	7	4
Ewes ovulated	3 (42.85%)	1 (25%)
Ovulation rate M ± SE	0.57 ± 0.30 (0-2)	0.25 ± 0.25 (0-1)

Table 2-3: Effect of nutrition on ovulation rate:

Items	Nutrition (Protein)	
	10%	17.9
No of ewes	10	3
Ewes ovulated	4 (40%)	2 (66.67)
Ovulation rate M ± SE	0.40 ± 0.16 (0-1)	0.67 ± 0.33 (0-1)

Fig. 2.1- Effect of season on ovulation rate.

Fig. 2.1.a:
Ewe's ovary with a C.L during the breeding season.

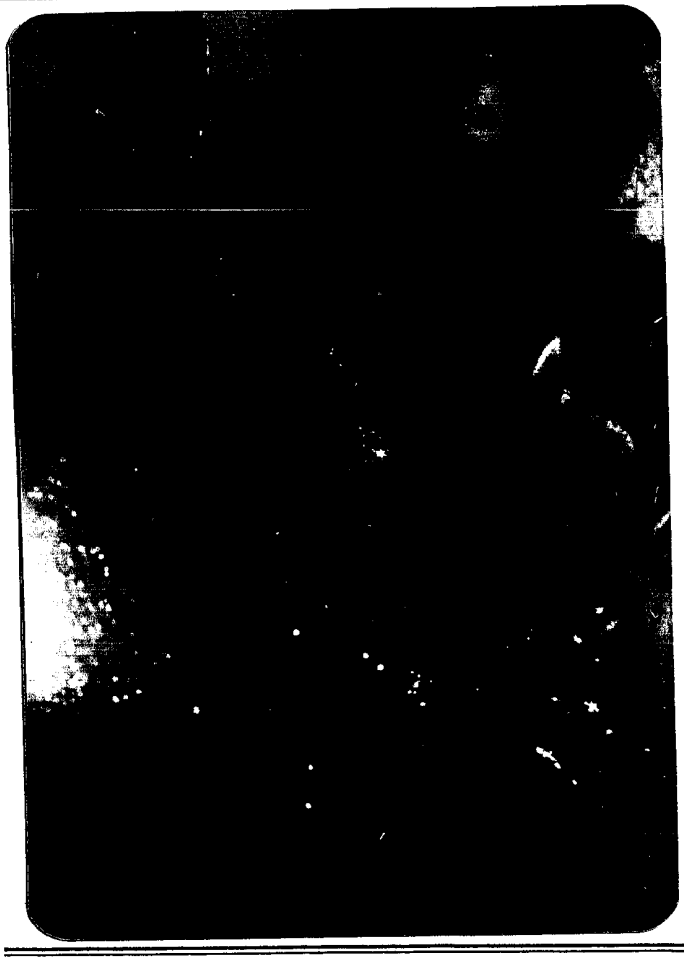


Fig. 2.1.b:
Inactive ovaries of ewes in the non-breeding season.

Table 2-4: Effect of ovarian side on ovulation rate:

Items	Experiment ewes		control ewes	
	R	L	R	L
No of ewes	19		12	
Ewes ovulated	17(89.47%)	16(84.21%)	3 (25%)	1 (8.33%)
Ovulation rate M ± SE	3.32 ± 0.53 (0-8)	3.11 ± 0.62 (0-9)	0.33 ± 0.19 (0-2)	0.08 ± 0.08 (0-1)

Table 2-5: Effect of Synchronization regime on ovulation rate:

Items	Regime	
	SMB Ear implant	SMB ear implant + 750 iu PMSG
No of ewes	6	3
Ewes ovulated	1 (16.67%)	2 (66.67%)
Ovulation rate M ± SE	0.17 ± 0.17 (0-1)	0.67 ± 0.33 (0-1)

2. Superovulation:

2.1. Superovulation Regimens:

2.1.1. Gonadotrophins For Follicular Growth (FSH vs. PMSG):

In the FSH group, 2 out5 ewes 40% showed oestrus and ovulated (laparotomy). Ovulation rate (No. of cl) averaged 5.6 $\pm$ 3 (range 0 to 15). No graafian follicles were noted while the number of growing follicles was 1.5 $\pm$ 0.5 (Table 2-6, Fig. 2-2).

All ewes (6 ewes) in the PMSG-group showed oestrus and ovulated (100%). Ovulation rate (No. cl) averaged 6.5 $\pm$ 1.61 (range 2 to 11). Mature unovulated graafian follicles averaged 9.5 $\pm$ 2.91. There is no significant variation in ovulation rate between ewes receiving FSH and PMSG.

Only 1 of 6 ewes (16.67%) in the control group manifested oestrus and ovulated. Ovulation rate was 0.17 $\pm$ 0.17 only (range 0 to 1). There was significant variation in ovulation rate between each of FSH group ($P<0.05$) and PMSG group ($P<0.01$) as compared with control group.

2.1.2. Pregnant Mare Serum Gonadotrophin (1000 vs. 2000 i.u.):

All ewes (3 ewes) receiving 1000 i.u. PMSG showed oestrus and ovulated (100%). Ovulation rate (No. cl) averaged 3.67 $\pm$ 0.88 (range 2 to 5). Mature unovulated follicles averaged 3.67 $\pm$ 2.03 (range 0 to 7) while, no growing follicles were noted (Table 2-7, Fig. 2-3).

The incidence of ewes showing oestrus and ovulation was 100% in ewes receiving 2000 i.u. PMSG (in breeding season). Ovulation rate (No. cl) averaged 7.33 $\pm$ 1.2 (range 5 to 9). Mature unovulated follicles and growing follicles averaged 4.33 $\pm$ 1.33 and 1.33 $\pm$ 0.67 respectively. There is a

Table 2-6: Ovarian response to Gonadotrophins for follicular growth (FSH PMSG):

Items	Treatment		
	FSH (20 mg)	PMSG (2000 iu)	Control
No of ewes	5	6	6
Oestrus ewes	2 (40%)	6 (100%)	1 (16.67%)
Ovulated ewes	2 (40%)	6 (100%)	1 (16.67%)
Ovulation rate M ± SE	* 5.6 ± 3 (0-15)	** 6.5 ± 1.61 (2-11)	0.17 ± 0.17 (0-1)
Graafian follicles	-----	9.5 ± 2.91 (1-17)	------
Growing follicles	1.5 ± 0.5 (1-2)	5.2 ± 0.44 (4-10)	2 (0-2)
% of follicles ovulated	100%	39.8%	100%

* P < 0.05 ** P < 0.01

Fig. 2.2- Ovarian response to gonadotrophins for follicular growth.

Fig. 2.2.a:
Ovarian response to PMSG treatment.

Fig. 2.2b:
Ovarian response to FSH treatment.

significant ($P<0.05$) variation in ovulation rate between ewes received 2000 i.u. and 1000 i.u. PMSG.

In the control group, 3 out 4 ewes (75%) showed oestrus and ovulation (laparotomy). Ovulation rate averaged 0.75 $\pm$ 0.25 (range 0 to 1). No mature unovulated follicles or growing follicles were observed.

Ovulation rate means were highly significant in both ewe groups receiving 1000 i.u. PMSG ($P<0.05$) and 2000 i.u. PMSG ($P<0.01$) as compared with control group.

2.1.3. Gonadotrophins For Ovulation (GnRH vs. HCG):

The incidence of ewes showing oestrus and ovulation (Table 2-8, Fig. 2-4) was 100% and 66.66% respectively in GnRH groups, 100% and 100% respectively in HCG group and 11.11% and 11.11% respectively in control groups. Ovulation rate mean in HCG group (6.5 $\pm$ 1.61 ranged from 2 to 11 cl) was significantly higher ($P<0.01$) than that in both GnRH groups (0.67 $\pm$ 0.33 ranged from 0 to 1 cl) and control group (0.11 $\pm$ 0.11 ranged from 0 to 1 cl). The mean number of mature graafian follicles was 11.33 $\pm$ 4.4 (5 to 20) and 9.5 $\pm$ 2.91 (1 to 17) in GnRH and HCG groups respectively while, in control group no mature graafian follicles was observed.

2.2. Factors Affecting Responses To Superovulation Regimens:

2.2.1. Nutrition (10% vs 17.9% protein):

All ewes receiving basal ration (10% protein) manifested oestrus (100%) and ovulated (100%). respective values were 71.43% and 66.67% in ewes fed on higher protein ration (17.9%).

Table 2-7: Ovarian response to various doses of Pregnant mare serum gonadotrophin:

Items	Treatment		
	PMSG (1000 iu)	PMSG (2000 iu)	Control
No of ewes	3	3	4
Oestrus ewes	3 (100%)	3 (100%)	3 (75 %)
Ovulated ewes	3 (100%)	3 (100%)	3 (75 %)
Ovulation rate M ± SE	*3.67 ± 0.88 (2-5)	**7.33 ± 1.2 (5-9)	0.75 ± 0.25 (0-1)
Mature G.F. M ± SE	3.67 ± 2.03 (0-7)	4.33 ± 1.33 (3-7)	------
Growing follicles M ± SE	------	1.33 ± 0.67 (0-3)	------
% of follicles ovulated	50%	59.46%	100%

* P < 0.05 ** P < 0.01

Table 2-8: Ovarian response to Gonadotrophins for ovulation (GnRH v.s. HCG):

Items	Treatment		
	GnRH	HCG	Control
No of ewes	3	6	9
Oestrus ewes	3 (100%)	6 (100%)	1 (11.11%)
Ovulated ewes	2 (66.66%)	6 (100%)	1 (11.11%)
Ovulation rate M ± SE	0.67 ± 0.33 (0-1)	**6.5 ± 1.61 (2-11)	0.11 ± 0.11 (0-1)
Mature G.F. (0.8 -2 cm) M ± SE	11.33 ± 4.4 (5-20)	9.5 ± 2.91 (1-17)	------
Growing G.F. (< 0.8 cm) M ± SE	0.67 ± 0.33 (0-1)	5.2 ± 0.44 (4-10)	2 (0-2)
% of follicles ovulated	5.26 %	39.8%	100%

** P < 0.01

Fig. 2.3- Ovarian response to various doses of pregnant mare serum gonadotrophin.

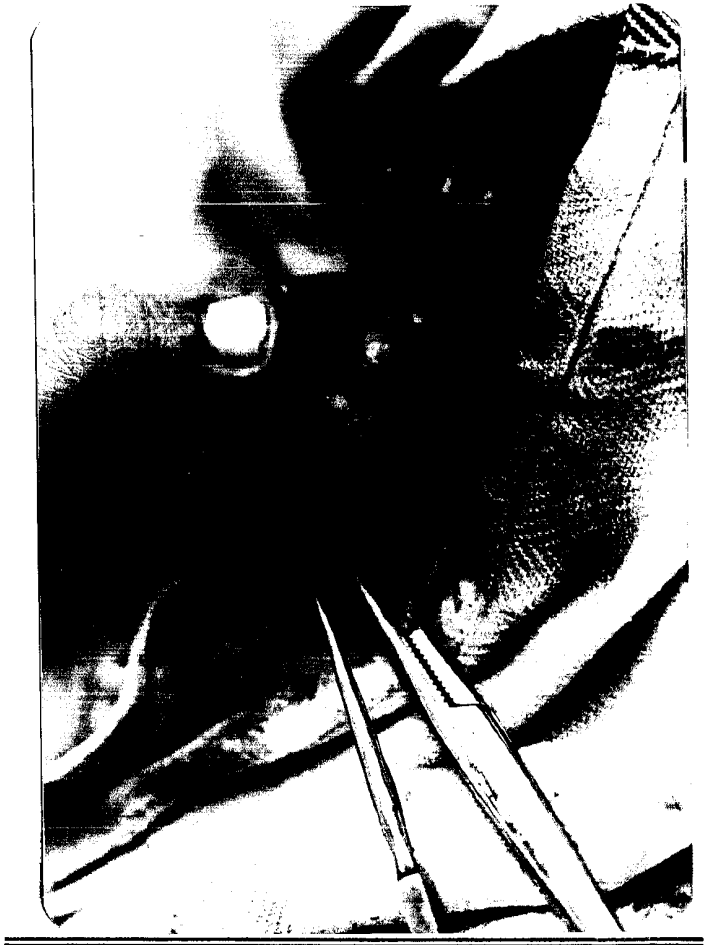


Fig. 2.3.a:
Ovarian response to 1000 i.u PMSG.

Fig. 2.3.b:
Ovarian response to 2000 i.u. PMSG.

Fig. 2.4- Ovarian response to gonadotrophins for ovulation.

Fig. 2.4.a:
Ovarian response to GnRH.

Fig. 2.4.b:
Ovarian response to HCG.

Ovulation rate averaged 6.5 ± 1.61 (ranged from 2 to 11 cl) and 5.2 ± 2.22 (ranged from 0 to 11 cl) in group of 10% protein and 17.9% protein respectively (Table 2-9, Fig. 2.5). Statistical analysis revealed that ovulation rate differences were not significant between the two nutritional regimens. The mean number of mature graafian follicles was 9.5 ± 2.91 (1 to 17) and 3.8 ± 1.66 (0 to 9) in group of 10% protein and 17.9% protein respectively.

2.2.2. Seasons (Breeding vs. non-breeding):

All ewes responded will to the superovulated regimens in both breeding and non-breeding season. The incidence of ewes showing oestrus and ovulation was 100% (Table 2-10, Fig. 2-6). Ovulation rate mean was non-signifcantly higher (7.33 ± 1.2 ranged from 5 to 9 cl) in breeding season than (6.5 ± 1.61 ranged from 2 to 11 cl) in non-breeding season.

Mature graafian follicles means were 9.5 ± 2.91 (1 to 17) and 4.33 ± 1.33 (3 to 7) in non-breeding season and breeding season respectively.

In all previous superovulation regimens oestrus occurred after 48 hours after implant withdrawal.

Table 2-9: Nutrition as a factor affecting superovulation regimen:

Items	Nutritional variation	
	10% protein	17.9% protein
No of ewes	6	6
Oestrus ewes	6 (100%)	5 (83.33%)
Ewes ovulated	6 (100%)	4 (66.67%)
Ovulation rate M ± SE	6.5 ± 1.61 (2-11)	5.2 ± 2.22 (0-11)
Mature G.F. (0.8 - 2 cm) M ± SE	9.5 ± 2.91 (1-17)	3.8 ± 1.66 (0-9)
Growing follicles (< 0.8 cm)	5.2 ± 0.44 (4-10)	2.4 ± 1.44 (0-8)
% of follicles ovulated	39.8%	57.78%

Table 2-10: Season as a factor affecting superovulation regimen:

Items	Seasonal variation	
	Breeding	Non-breeding
No of ewes	3	6
Oestrus ewes	3 (100%)	6 (100%)
Ewes ovulated	3 (100%)	6 (100%)
Ovulation rate M ± SE	7.33 ± 1.2 (5-9)	6.5 ± 1.61 (2-11)
Mature G.F. (0.8-2 cm) M ± SE	4.33 ± 1.33 (3-7)	9.5 ± 2.91 (1-17)
Growing follicle (< 0.8 cm) M ± SE	1.33 ± 0.67 (0-3)	5.2 ± 0.44 (4-10)
% of follicles ovulated	59.46 %	39.8%

Fig. 2.5- Nutrition as a factor affecting superovulation regimen.

Fig. 2.5.a:
Ovarian response to 2000 i.u PMSG plus 10% protein.

Fig. 2.5.b:
Ovarian response to 2000 i.u PMSG plus 17.9% protein.

Fig. 2.6- Season as a factor affecting superovulation regimen.

Fig. 2.6.a:
Ovarian response to 2000 i.u PMSG during breeding season.

Fig. 2.6.b:
Ovarian response to 2000 i.u PMSG during non-breeding season.

2.3. Hormone Profiles In Superovulated Ewes:

2.3.1. Oestradiol-17B:

Oestradiol-17B values (pg/ml) means at day of implant removal ranged from 2.53 ± 1.19 to 4.06 ± 0.13 among superovulation regimens. Oestradiol-17B values in control ewes averaged 4.02 ± 0.29 pg/ml at proestrus (Table 2-11).

Oestradiol-17B values on day of oestrus averaged 130.09 ± 36.31 pg/ml in FSH group, and ranged from 5.32 ± 0.32 to 17.02 ± 4.61 pg/ml in PMSG regimens at day of oestrus. In control groups, oestradiol-17B value at oestrus averaged 17.57 ± 1.75 pg/ml during breeding season and 11.23 ± 3.82 pg/ml during non-breeding season.

Statistical analysis (Table 2-12) reveals that there is a highly significant ($P<0.01$) difference at day of oestrus between oestradiol-17B values in FSH group and PMSG regimens. LSD values (Table 2-13) were highly significant in mean difference between FSH group and each group of 2000 iu PMSG (summer), 2000 i.u. PMSG (autumn), 1000 i.u. PMSG and PMSG + 17.9% protein (L.S.D. = 31.32, 36.171, 32.95 and 30.26 respectively). While, Oestradiol-17B values were non-significant difference between superovulation regimens at day of implant withdrawal.

2.3.2. Progesterone:

Progesterone values at day of implant removal and day of oestrus ranged from 0.02 ± 0.00 to 0.54 ± 0.27 ng/ml and 0.024 ± 0.01 to 0.22 ± 0.02 ng/ml respectively among superovulation regimens. Respective progesterone values in control group averaged 0.14 ± 0.01 and 0.095 ± 0.01 ng/ml during breeding season and 0.18 ± 0.02 and 0.12 ± 0.05 ng/ml during non-breeding season (Table 2-14, Fig. 2-1). Progesterone value at

Table 2-11: Effect of various superovulation regimens on the oestradiol-17 value (pg/ml) in Barki ewes at day of ear implant removal and oestrus:

Drug	Dose	Oestradiol-17B (pg/ml) M ± SE		Season
		implant removal	oestrus (day 0)	
FSH	20 mg	4.06 ± 0.13	*130.09 ± 36.31	summer
PMSG	2000 iu	3.97 ± 1.65	17.02 ± 4.61	summer
PMSG	2000 iu	2.53 ± 1.19	5.32 ± 0.32	autumn
PMSG	1000 iu	2.93 ± 0.68	11.77 ± 0.64	autumn
PMSG + protein	2000 iu + 17.9%	3.97 ± 1.54	8.26 ± 1.99	spring
Control	-------	4.02 ± 0.29	17.57 ± 1.75	breeding
	-------		11.23 ± 3.82	Non-breeding

** P<0.01

Table 2-12: Analysis of variance (F. test) for oestradiol-17B among superovulation treatments in Barki Ewes:

Item	Source of variance	D.F	S.S.	M.S.	F.
At implant Withdrawal	Bet. treatment	4	59.18	14.795	2.88
	Error	11	56.52	5.14	
During Oestrus	Bet. treatment	4	25029.097	6257.27	23.148**
	Error	11	2973.454	270.314	

** P < 0.01

Table 2-13: The least significant difference of oestradiol-17B during superovulation treatments in Barki ewes:

L.S.D.	Mean difference	Period of substraction
30.26	> 2.940	PMSG (2000 iu) + protein (17.9%) vs. PMSG (2000 iu) autumn
32.95	> 6.447	PMSG (1000 iu) autumn vs. PMSG (2000 iu) autumn
31.32	> 11.695	PMSG (2000 iu) summer vs. PMSG (2000 iu) autumn
36.171	< 124.770**	FSH (20 mg) vs. PMSG (2000 iu) autumn
26.333	> 3.507	PMSG (1000 iu) vs. PMSG (2000 iu) + protein (17.9%)
24.26	> 8.755	PMSG (2000 iu) summer vs. PMSG (2000 iu) + protein (17.9%)
30.26	< 121.830**	FSH (20 mg) vs. PMSG (2000 iu) + protein (17.9%)
27.547	> 5.248	PMSG (2000 iu) + protein (17.9%) vs. PMSG (1000 iu) autumn
32.95	< 118.323**	FSH (20 mg) vs. PMSG (1000 iu) autumn
31.32	< 113.075**	FSH (20 mg) vs. PMSG (2000 iu) summer

$P < 0.01$

Table 2-14: Effect of various superovulation treatments on the perioestrous progesterone pattern in Barki Ewes:

Drug	Dose	Progesterone (ng/ml)				Season
		Implant removal	Oestrus (day 0)	Metoestrus (day 3) **	Laparotomy day 5-6) **	
FSH	20 mg	0.03 ± 0.00	0.024 ± 0.01	**7.66 ± 1.99	**19.09 ± 0.62	summer
PMSG	2000 iu	0.14 ± 0.02	0.22 ± 0.07	1.28 ± 0.33	9.28 ± 1.61	summer
PMSG	2000 iu	0.04 ± 0.01	0.098 ± 0.02	1.35 ± 0.48	7.54 ± 0.93	autumn
PMSG	1000 iu	0.02 ± 0.00	0.038 ± 0.01	2.66 ± 0.72	3.68 ± 0.08	autumn
PMSG + protein	2000 iu + 17.9%	0.54 ± 0.27	0.097 ± 0.04	1.89 ± 0.35	8.46 ± 0.16	spring
Control	-------	0.14 ± 0.01	0.095± 0.01	0.38 ± 0.1	2.16 ± 0.56	Breeding season
	-------	0.18 ± 0.02	0.12 ± 0.05	0.295± 0.16	2.04 ± 0.15	non-breeding season

** P < 0.01

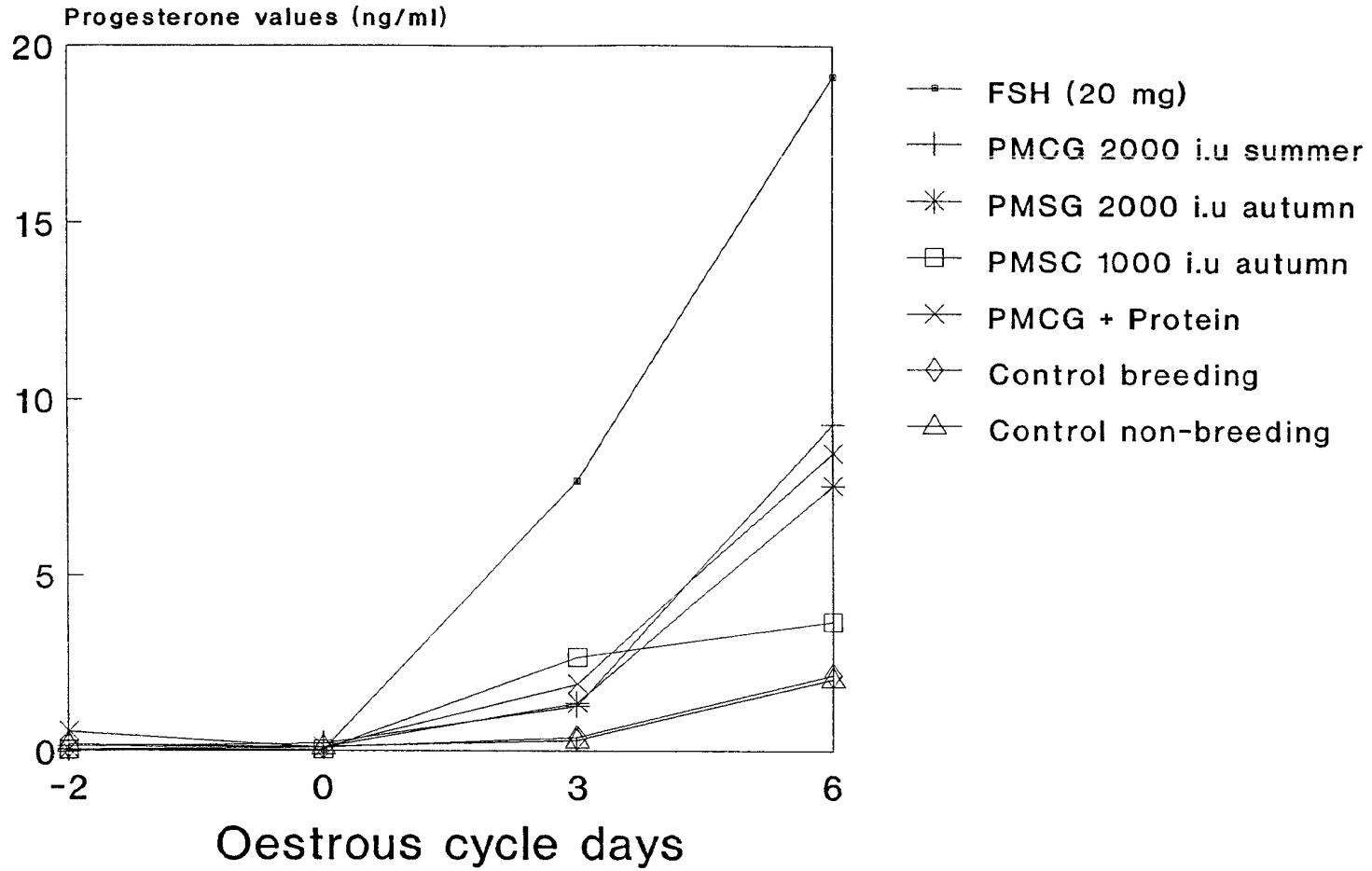


Fig 2-1: Perioestrous progesterone pattern in superovulated Barki Ewes

Tabe 2-15: Analysis of variance (F test) for progesterone during superovulation treatments in Barki Ewes:

Item	Source of Variance	D.F	S.S.	M.S.	F
Implant Withdrawal	Bet. Exp. Error	4 11	0.581 0.44	0.145 0.044	3.295
Oestrus	Bet. Exp. Error	4 12	0.105 0.162	0.026 0.014	1.875
day 3 of Oestrous cycle	Bet. Exp. Error	4 11	66.14 14.01	16.54 1.27	** 13.02
Laparotomy	Bet. Exp. Error	4 10	266.12 58.45	66.53 5.845	** 11.38

** $P < 0.01$

Table 2-16: Least significant difference for progesterone during superovulation treatments (day 3):

L.S.D.	M.D.	Experiments
2.55	> 1.376	PMSG (1000) autumn vs. PMSG (2000 iu) summer
2.55	> 0.07	PMSG (2000 iu) autumn vs. PMSG (2000 iu) summer
2.35	> 0.61	PMSG (2000 iu) + protein (17.9%) vs. PMSG (2000 iu) summer
2.93	< 6.38 **	FSH (20 mg) vs. PMSG (2000 iu) summer
2.67	> 0.54	PMSG (2000 iu) + protein (17.9%) vs. PMSG (2000 iu) autumn
3.19	> 1.305	PMSG (1000 iu) autumn vs. PMSG (2000 iu) autumn
3.19	< 6.31 **	FSH (20 mg) vs. PMSG (2000 iu) autumn
3.04	< 5.77 **	FSH (20 mg) vs. PMSG (2000 iu) + protein (17.9%)
3.04	> 0.765	PMSG (2000 iu) autumn vs. PMSG (2000 iu) + protein (17.9%)
3.5	< 5.01 **	FSH (20 mg) vs. PMSG (1000 iu) autumn

** P < 0.01

Table 2-17: Least significant difference for progesterone during superovulation treatments (day of laparotomy):

L.S.D.	M.D.	Experiments
6.99	> 3.86	PMSG (2000 iu) autumn vs. PMSG (1000 iu) autumn
6.99	> 4.78	PMSG (2000 iu) + protein (17.9%) vs. PMSG (1000 iu) autumn
6.41	> 5.6	PMSG (2000 iu) summer vs. PMSG (1000 iu) autumn
7.67	< 15.41 **	FSH (20 mg) vs. PMSG (1000 iu) autumn
6.28	> 0.92	PMSG (2000 iu) + protein (17.9%) vs. PMSG (2000 iu) autumn
5.58	> 1.74	PMSG (2000 iu) summer vs. PMSG (2000 iu) autumn
6.99	< 11.55 **	FSH (20 mg) vs. PMSG (2000 iu) autumn
5.58	> 0.82	PMSG (2000 iu) summer vs. PMSG (2000 iu) + protein (17.9%)
6.99	< 10.63 **	FSH (20 mg) vs. PMSG (2000 iu) + protein (17.9%)
6.41	< 9.81 **	FSH (20 mg) vs. PMSG (2000 iu) summer

** P < 0.01

metoestrus (day-3) and day of laparotomy averaged 7.66 ± 1.99 and 19.09 ± 0.62 ng/ml respectively in ewes receiving FSH. Respective progesterone values ranged from 1.28 ± 0.33 to 2.66 ± 0.72 ng/ml and 3.68 ± 0.08 to 9.28 ± 1.61 ng/ml among different PMSG regimens. In control group, progesterone value means during metoestrus and day 5-6 of oestrous cycle averaged from 0.38 ± 0.1 and 2.16 ± 0.56 ng/ml respectively at breeding season and 0.295 ± 0.16 and 2.04 ± 0.15 ng/ml at non-breeding season.

Statistical analysis (Table 2-15) reveals that progesterone values are highly significant ($P<0.01$) in FSH group at metoestrus and day of laparotomy than that observed in PMSG groups and control group. L.S.D. values (Table 2-16, 2-17) were highly significant in FSH group (at day of metoestrus and day of laparotomy) and each of PMSG groups receiving 2000 i.u. (summer), 2000 i.u. (autumn), 1000 i.u. and PMSG + 17.4% protein (Respective LSD values 2.93, 3.5, 3.19 and 3.04 in metoestrus and LSD = 6.41, 7.67, 6.99 and 6.99 in day of laparotomy).

2.3.3. Correlation Between Progesterone Values And Number Of Corpora Lutea:

Progesterone values (Table 2-18) increased according to the number of corpora lutea in superovulated Barki ewes. Higher progesterone values observed in Barki ewes were linked to the number of 15, 13, 10, 9 and 11 corpora lutea (19.71, 18.47, 17.47 ± 4.51, 13.45 and 8.01 ng/ml respectively) followed by 7.79 ± 4.19, 5.71, 5.44 ± 3.29 and 5.04 ± 1.94 ng/ml in ewes having 4, 8, 3, 2 corpora lutea respectively. Progesterone values were lower in Barki ewes having 1 and 5 corpora lutea (1.86 and 3.76 ng/ml respectively). There is a strong and a highly significant ($P<0.01$) positive correlation ($r = +0.86$) between the number of corpora lutea and progesterone values.

Table 2-18: Progesterone level in relation to the number of corpora lutea in superovulated Barki Ewes:

No of ewes	Number of C.L.	Mean of progesterone ng/ml
1	1	1.855
3	2	5.04 ± 1.94
3	3	5.44 ± 3.286
2	4	7.793 ± 4.194
1	5	3.758
1	8	5.708
1	9	13.451
2	10	17.47 ± 4.51
1	11	8.008
1	13	18.468
1	15	19.705

$P < 0.01$

2.4. Ewes Cyclicity Following Superovulation:

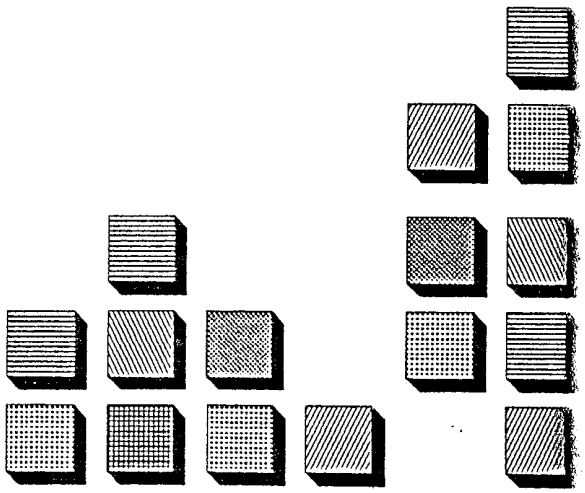
Days between end of treatment and onset of oestrus in superovulated ewes (Table 2-19) ranged from 18 to 34 as observed in 10 out of 19 ewes (52.63%), while, 21.05% of ewes showed oestrus after 35 to 51 days. Oestrus was observed in 26.32% of ewes after 52 to 110 days (averaged 75.4 $\pm$ 10.04 days). In control ewes 2 out of 12 ewes (16.67%) showed oestrus at $\leq$ 17 days. However, 8 out of 12 ewes (66.67%) came in oestrus within 18 to 34 days. Oestrus was observed in 16.67% of ewes within 56 to 72 days (averaged 64 $\pm$ 8 days).

A problem observed after laparotomy is the adhesions between ovarian bursa and the uterine body.

Table 2-19: Oestrous cyclicity following superovulation treatment in Barki Ewes:

Items	Days between end of treatment and onset of oestrus			
	≤ 17	18-34	35-51	≥ 52
Superovulated ewes	-	10 (52.63%)	4 (21.05%)	5 (26.32%) av. 75.4 ± 0.04 (range 52-110 day)
Control ewes	2 (16.67%)	8 (66.67%)		2 (16.67%) av. 64 ± 8 (range 56-72 day)

DISCUSSION



Discussion

Factors Affecting Ovarian Functions:

SEASON: In the present study, 75% of the Barki ewe ovulated during the autumn compared to 16.67% during the summer season. In accordance, higher incidence of ovarian cyclic changes (81.5%) was noted in fat tailed ewes during autumn, in Egypt (El-Wishy et al., 1976). Wheeler and Land (1977), reported that the ovulatory season for a breed is defined as the period during which more than 50% of the ewes ovulated. Therefore, it could be assumed that, autumn is the breeding season for Barki ewes in Egypt.

The prsent finding indicates that ovulation rate in Barki ewes is higher (0.75 ± 0.25) during the breeding season than in non-breeding season (0.17 ± 0.17). This is in agreement with El-Wishy et al. (1976) where the maximum number of ovulations per fat tailed ewe (1.86) were attained in the

autumn season with the decrease in day light periods. These present findings are lower than that noticed by Wheeler and Land (1977), where ovulation rate is 3.5 in November and 2.6 in March in Finnish Landrace ewes. However, Land et al. (1973) noticed that the ovulation rate of the Black faces and Merinos was as high around the shortest day as at any other time. Kammland, Welch, Nalbandov and Noiton (1952) reported that seasonal variation is not only an all or none difference between the breeding and non-breeding seasons, as follicular growth occurs during both seasons.

The present significant ($P < 0.05$) seasonal variation in the ovulation rate of Barki ewes, disagrees with El-Wishy et al. (1976) where variation in the ovulation rate due to season of the year was not statistically significant. The reduction in ovulation rate with advancing season may be the result of stress, particularly weather stress, since the later stages of the season tend to include periods of harsh weather (Griffiths, Gunn and Doney, 1970; Doney et al., 1976).

AGE: In the present study, the ovulation rate averaged 0.57 ± 0.30 (42.85%) and 0.25 ± 0.25 (25%) in Barki ewes aging 2 to 5 and $\geq$ 6 years respectively. This result is in agreement with Caucasian sheep aged 3 to 6 years where ovulation rate averaged 0.67 ± 0.37 (Stepanov et al., 1981). However, Wheeler and Land (1977) noted higher ovulation rates of 1.09 and 1.00 for the older ($\geq$ 2.5 years) and younger (1.5 year) in Merino ewes. Moreover, Bindon et al. (1986) recorded that the mean ovulation rate was 1.19 and 1.65 in ewes age 1.5 years and 2.5 to 7.5 years respectively.

Age variations in ovulation rate in the present study are not significant. So variations between different studies may be due to genetic differences (Bindon et al., 1986), differential early growth patterns (Gunn, 1977), change in management (Dahman et al., 1976) or breed difference (Maurer, 1988).

NUTRITION: In this study, ovulation rate of Barki ewes was 0.67±0.33 (66.67%) for ewes receiving 17.9% crude protein while in ewes receiving 10% crude protein it was not significant 0.40 ± 0.16 (40% ovulated ewes). Mofiamed (1985) recorded that the response to flushing is due to the increased intake of nutrients particularly protein which increase the levels of hepatic SME (hepatic steroid metabolizing enzymes). A high level of hepatic SME associated with an increased clearance rate of steroids and a decrease in steroids is associated with an increase in gonadotrophins and thus increased ovulation. Moreover, Knight et al. (1975) noticed that feeding of high protein supplements such as lupin grains and soyabean meal (Davis and Cumming, 1976) has resulted in a significant increase in the ovulation rate. In this respect, Brien et al. (1976) reported that ewes fed on supplements of high protein had higher plasma FSH values than control groups. Moreover, the maintenance of higher FSH values (Haresign, 1985) in prolific ewes may be important in providing a larger pool of antral follicles with the appropriate complement of LH receptors which can respond to the rising titre of tonic LH at this time and be stimulated to undergo the final stages of maturation and ovulation. However, Bindon et al. (1975) observed that attempts to correlate the level of FSH with the ovulation rate shown by adult ewes was not successful.

SIDE OF OVARY: The right ovary in both superovulated and control groups of Barki ewes has higher but non-significant ovulation rates (3.32 ± 0.53 and 0.33 ± 0.19 respectively) than the left ovary (3.11 ± 0.62 and 0.08 ± 0.08 respectively). El-Wishy et al. (1976) reported also higher but not significant activity of right ovaries. However, Ezzo (1985) found that the left ovary has higher but not significant number of primordial follicles count than right ovary in Barki ewes (age 1 to 2 years). The same author observed that a decline in the number of primordial follicles is associated with an

increase in the number of growing follicles, mature of graafian follicles as well as atretic follicles. Chaill et al. (1979) reported that a direct relationship has been found to exist between the number of follicles in the growth phase and ovulation.

SYNCHORNIZATION REGIMENS: Ovulation rate of Barki ewes in the present work averaged 0.67±0.33 (0 to 1) and 0.17±0.17 (0 to 1) in ewes synchronized by SMB implant with injection of 750 i.u. PMSG at implant withdrawal and ewes synchronized by SMB ear implant only respectively. The present ovulation rate values were lower than ovulation rate observed in other ewes receiving the same dose of PMSG, in Zackel ewes the ovulation rate averaged 2.10 (Kremer, 1981), 3.40 in Suffolk ewes (Armstrong and Evans, 1984) and 5.50 in Yankasa ewes (Oyedip et al., 1989). This variation may be due to sensitivity of the ovary to stimulation by gonadotrophins, breeds noted for fecundity, age of the donor, nutritional status and the particular drug used and perhaps even the particular lot of both of that drug (Bondurant, 1986).

TIME OF OESTRUS: Occurrence after implant removal: Oestrus in Barki ewes synchronized herein with synchromete implant and in all superovulated regimens occurs 48 hours after implant withdrawal. Torres et al. (1987) noticed that oestrus of the donor ewes always began 24 hours after withdrawal of the sponges, several exceptions were at 36 and 48 hours. Furthermore, Lopez Sebastian et al. (1990) reported that all ewes in seasons showed oestrus between 24 and 32 hours and remained in oestrus 48 hours after sponge removal.

Superovulation:

FSH vs. PMSG: The ovulation rate in Barki ewe treated with PMSG (6.5±1.61) is not-significantly higher than those treated with FSH (5.6±3.00) in summer season. In accordance, Armstrong and Evans (1983) found that ewes treated with PMSG have a greater ovulation rate than those treated with FSH. However, Walkar, Smith and Seamark (1986) observed that ovulation rate was higher ($P<0.05$) in Merino ewes treated with FSH than those treated with PMSG (8.4 ± 0.81 vs. 7.3 ± 0.21). According to Armstrong and Evans (1984), PMSG may lead to ovarian hyperstimulation, accompanied by endocrine abnormalities which is unfavourable to normal fertilization and embryo development. While, the more rapid metabolic clearance of porcine FSH decreases the incidence of magnitude of ovarian hyperstimulation.

The present results indicate high numbers of mature unovulated graafian follicles observed in ewes receiving PMSG. While, in ewes receiving FSH all follicles were ovulated. This result is in agreement with the prolonged effect of PMSG, which is due to its long half-life often causes extensive unovulated follicular developments (Moore and Shelton, 1964; Mc-Intoch, Moor and Allen, 1975; Armstrong and Evans, 1983). Driancourt (1987) noticed a non-significant correlation between the number of healthy follicles (0.8-2 mm) and PMSG-induced ovulation rate $r=0.62$. However this close correlation suggests that these follicles are those recruited by PMSG. Moreover, Turnbull, Braden and Mattner (1977) observed that mature graafian follicles (0.8 to 2.0 mm) manage to catch up through the limited increase in the mitotic index of the granulosa cells induced by PMSG and ovulated with an adequate number of granulosa cells. Saumande, Chiupin, Mariana, Ortavant and Mauleon (1978) recorded that the response to PMSG was related to the

features of the surface follicular population, ovaries devoid of large follicles (>2.0 mm) and rich in small ones (0.8-2.0 mm) had the highest response.

Bindon, Blanc, Pelletier, Terqui and Thimonier (1979) and Cahill, et al. (1979) concluded that the differences in ovulation rates were primarily due to differences in ovarian sensitivity to gonadotrophins. Thus, it seems likely that animals characterized by higher ovulation rates will produce larger numbers of eggs in response to exogenous gonadotrophins, than those which normally shed few ova. Moreover, Moore (1982) reported that the differences in ovulation rate between breeds and between strains within breeds have been well documented. Furthermore, there are tremendous individual variations in response to standard doses of gonadotrophin (Moore, 1982; Zanwar and Deshpande, 1984). Similar variability in ovarian response is often observed in the same animals treated at different times the quality of gonadotrophin preparations used in superovulation and the genetic effect on ovarian function may be responsible (Bindon, Piper, Cahill, Driancourt and Shea, 1986). High doses of gonadotrophins can depress ovulatory response due to failure of high proportion of follicles to ovulate (Moore, 1982) and in practice it would seem appropriate to administer a dose of little below that estimated to give a maximum ovulatory response in order to minimize the chances of excessive follicular response in individual animals.

FOLLICLE STIMULATING HORMONE: The present finding indicates that the ovulation rate in Barki ewes averaged 5.6±3.0 in response to injection of 20 mg FSH, is partially in agreement to ovulation rate of 6.0±4.4 as a response to 24 mg FSH (Bondioli et al., 1982) and 5.5±1.6 as a response to 15 mg FSH (Armstrong and Evans, 1984). The ovulation rate in the present experiment is lower than 7.5±1.3 obtained as a response to 32 mg FSH (Armstrong and Evans, 1984), 10.73, 14.39, 12.06, 8.40, 6.67 and 6.67 obtained as a response to 30, 22, 19.5, 17, 14.5 and 12 mg FSH

respectively (Smith, 1984), 10.8±0.8 and 6.6±1.6 in ewes receiving 30 and 17.5 mg FSH respectively (Maurer, 1988), 7.4±1.1 and 7.1 ± 0.9 as response to 18 and 12 mg FSH respectively (Thompson et al., 1990), 7 ± 4.8 due to injection of 16 mg FSH (Lopezsebastian et al., 1990) and 6.7 as response to 24 mg FSH (Zyl et al., 1987).

The variability observed in the ovarian response to FSH due to multiple interactions between the physiological status of the ovaries at the time of hormone treatment, nature of hormone, preparations used and its batch (Armstrong and Evans, 1984), Breed of ewes (Thompson et al., 1990) and variability in the FSH/LH ratio of the commercial FSH (Lindsell, Rajkumar, Manning, Emery, Mapletoft and Murphy, 1986). Moreover, Armstrong and Evans (1983) noticed that the amount of LH in the preparation plays an important role in superovulatory response.

PREGNANT MARE SERUM GONADOTROPHINS: The ovulation rate was lower ($P<0.05$) in ewes receiving 1000 i.u. PMSG than those injected by 2000 i.u. PMSG (3.67±0.88 vs. 7.33±1.2 respectively) in autumn. The ovulation rate (3.67±0.88) in Barki ewes injected with 1000 i.u. PMSG in the present study was lower than ovulation rate in ewes receiving the same dose of PMSG as 5.8 (Vintila et al., 1986), 6.65 in Mooroola Merino ewes (Kelly and Owens, 1983), 8.5±4.9 in Merino-Landschaf (Meinecke et al., 1988) and 7.0±1.2 in Yankasa ewes (Oyedipe et al., 1989). On the other hand, Ovulation rate (7.33±1.2) in Barki ewes receiving 2000 i.u. PMSG (in autumn season) in the present result was higher than that observed during breeding season as 5.13 in Cross breed ewes receiving the same dose of PMSG (Toishibekov et al., 1986), and lower than 9.0 noted in ewes injected with 2000 i.u. PMSG (Donskaya et al., 1986). The ovulation rate mean (6.5±1.61) in Barki ewes receiving PMSG (2000 i.u.) in summer is in agreement with

Donskaya et al. (1986) in non-breeding season where as 2000 i.u. PMSG gave an average ovulation rate of 6.5.

Gordon (1983) recorded that 2000 i.u. PMSG would be regarded as the highest permissible dose level therefore most authors studied the effect of PMSG on ovulation rate using doses less than 2000 i.u. PMSG. The response of 1500 i.u. PMSG in cross bred ewes had 7.33 as ovulation rate mean (Zanwar and Deshpande, 1984), in prolific ewes had means of about 10 ovulations (Quirk et al., 1987). Ovulation rate recorded for 1200 to 1450 i.u. PMSG was 10 to 12 corpora lutea in Merino ewes (Moore, 1982), 9 in Karakul ewes (Abdul-tairov, 1983), 7.3 (Dawinov et al., 1986), 11.3 (Vintila et al., 1986) and 18.23 in Droper ewes (Zyl et al., 1987). The ovulation rate mean as a response to 1000 i.u. PMSG during non-breeding season was recorded as 6.65 in Booroola Merino with heterozygous genes (Kelly and Owens, 1983), 6.50±4.9 in Merino Landschaf ewes (Meinecke et al., 1988) and 7.0±1.2 in Yankasa ewes (Oyedipe et al., 1989).

GONADOTROPHINS FOR OVULATION (GnRH vs. HCG): Ovulation rate of Barki ewes receiving 2000 i.u. PMSG plus 1000 i.u. HCG was significantly ($P<0.01$) higher (6.5±1.61) than 0.67±0.33 in ewes receiving 2000 i.u. PMSG plus 10 ug GnRH. The lower ovulation rate in GnRH ewes was also associated with higher number of unovulated follicles (average 11.33±4.48). In support, Walker et al. (1986) found that ovulation rates were reduced by GnRH treatment but the reason for this reduction is not known. Nancarrow, Murray, Boland, Sutton and Hazelton (1984) reported an increase in ovulation rates and embryo yields from 1.9 to 4.2 per ewes with the use of GnRH. Wheaton, Hamra, Sabeur and Capstick (1984) observed that multiple GnRH injections of 0.25 ug increased diameter of the largest follicle and resulted in a corpus luteum per ewe. Moreover, Hafez (1987) reported in treatment regimens that employment of pituitary or placental

gonadotrophins is possible to induce ovulation with GnRH. A single large dose will cause a mature follicle to ovulate through the release of endogenous LH and FSH.

There had been some attempts to confine the occurrence of ovulation within narrow-limits more directly, this has involved administering an ovulating hormone preparation (HCG) at a certain time (e.g. 28-32 hours) after progestagen withdrawal (Dziuk, Ellicott, Webel and O'Reilly, 1970; Roche and Crawley, 1971). Gonadotrophin treated ewes given HCG indicate an increase in the proportion of follicles ovulating (Killeen and Moore, 1970).

Mcleod, Haresign and Lamming (1982) reported that the decrease in ovulation rate during seasonal anoestrus apparently may be due to inadequate pattern of episodic LH secretion.

NUTRITIONAL INFLUENCE ON RESPONSES TO SUPEROVULATION TREATMENTS: Using the same superovulation regimen, the ovulation rate was not-significantly higher (6.5$\pm$1.61) in ewes receiving 10% protein in their ration than ewes receiving 17.9% protein 5.2$\pm$2.22). This finding is in agreement with panama ewes given 3 levels (65, 85 and 105% of NRC) of feeding, where there was an apparent but non-significant increase in the ovulation rate averaging 1.86, 2.02 and 2.05 respectively (Hulet et al., 1974). Moreover, the ovulation rate of the ewes having high level of nutrition (2.13) did not differ significantly from that of ewes subjected to low levels (1.98) of nutrition (Gunn, 1977). Moreover, Ducker and Boyd (1974) reported that neither plans of nutrition nor low planes of nutrions had a significant effect on ovulatory activity. On the other hand, Dahman et al. (1976) found that in ewes flushed for 14 days in pasture, there was a high significant effect of flushing on the ovulation rate in mature ewes. Moreover, light foot and Marshall (1974) and Rizzol et al. (1976) recorded that these responses can

vary according to environment and season. However, as the seasonal stimulus for ovulation declines toward the end of the breeding season the ovulation rate can be stimulated by a higher level of nutrition (Hulet et al., 1974).

SEASONAL INFLUENCE ON RESPONSES TO SUPEROVULATION TREATMENT: Using the same superovulatory regimen, the ovulation rate was not-significantly higher during breeding season (7.33±1.2) than during the non-breeding season (6.5±1.61) in Barki ewes. This observation is in agreement with Donskaya et al. (1986) who reported that the ovulation rate mean was higher in breeding season (11.0) than during the non-breeding season (6.5). However, Lopez Sebastian et al. (1990) noticed that there was no significant differences in the ovulation rate between the breeding and non-breeding season.

The present finding indicates that the superovulation regimen induced ovulation in all ewes during the breeding (100%) and non-breeding season (100%), disagrees with Donskay et al. (1986) who reported that the incidence of ewes ovulated was 100% and 75% in breeding and non-breeding seasons respectively. Moreover, the respective percentage was 65% and 14.63% (Toishibekov et al., 1986).

OESTRADIOL AND PROGESTERONE PROFILES IN SUPEROVULATED EWES: Oestradiol-17B values in Barki ewes in the present work are in a range 2.53±1.19 to 4.06±0.13 pg/ml at the time of implant removal among the superovulation regimens. This is nearly similar to oestradiol-17B value (4.02±0.29) observed in proestrus of normal oestrous cycle. At oestrus, oestradiol-17B averaged 130.094±36.31 pg/ml in FSH treatment which was much higher ($P<0.01$) than any PMSG treatments (range 5.32 to 17.02 pg/ml). Oestradiol values in Barki ewes during normal

oestrous cycles was nearly similar to those noticed during PMSG superovulation treatments than during the FSH treatment. Evers (1988) noticed that in Merino ewes had > 4 corpora lutea after PMSG treatment, the oestradiol-17B values were $\geq$ 23 pmol/litre.

Progesterone values in Barki ewes in the present study at the time of implant withdrawal and at oestrus ranged from 0.02 to 0.54 and 0.02 to 0.22 ng/ml respectively among superovulation treatment. These values were nearly similar (0.13-0.19 and 0.07 to 0.16 ng/ml) to that observed in proestrus and oestrus of normal oestrus cycle. While, progesterone values were higher ($P<0.01$) in FSH treatment at day-3 and day of laparotomy (7.66 and 19.09 ng/ml respectively) than PMSG treatments (range 3.68 to 9.28 and 3.68 to 9.28 ng/ml respectively).

Progesterone values at normal oestrus cycles during metoestrus and dioestrus (0.43 and 2.55 ng/ml respectively) are very low than that noticed among superovulation treatments.

However the available literature lacks any data concerning the variations of oestradiol-17B and progesterone values among superovulation treatments.

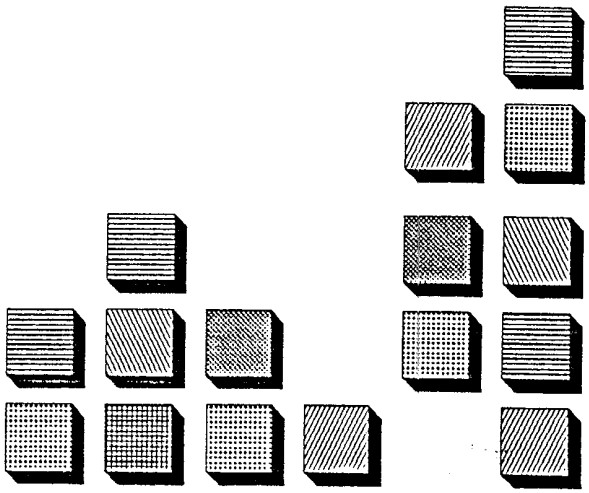
The progesterone values in the present results were higher than progesterone values > 1 ng for 9 consecutive days in ewes receiving 0.25 ug GnRH (Wheaton et al., 1984), Merino ewes had > 4 corpora lutea after PMSG treatment, the progesterone concentration was $\geq$ 3.5-4.0 nmol/litre on day 2 and $\geq$ 17-18 nmol/litre on day 4 (Evers, 1988). Oyedipe et al. (1989) noticed that progesterone values of 125.4 ± 9.0 ng/ml in Yankasa ewes receiving 1000 i.u. PMSG which is higher than progesterone values in Barki ewes of the present work. Furthermore, Moor, Osborn and Crosby (1985) reported that development of steroidogenesis was abnormal in most of the stimulated follicles after treatment of ewes with 1250 i.u. PMSG, but not when

they were treated with the pituitary extract. Bondurant (1986) observed that PMSG treatment raises plasma progesterone concentration almost.

THE CORRELATION BETWEEN PROGESTERONE VALUES AND CORPORA LUTEA NUMBER: The present data reveals a strong and highly significant ($P<0.01$) positive correlation ($r=0.86$) between the number of corpora lutea and progesterone value. This result is in agreement with Hunter and Southee (1989) where, the plasma progesterone concentrations on the day of slaughter (day 2, 4, 6, 8, 10, 12 and 15) were correlated with time $P<0.05$), total weight of luteal tissue ($P<0.001$) and number of corpora lutea ($P<0.05$). Edgar and Ronaldson (1958) noticed that the concentration of progesterone was similar in blood from ewes with ovaries containing one or two corpora lutea. This seems of interest as individual corpora lutea tend to be smaller when more than one CL is present in the ovary. Thorburn et al., (1969) noticed that the considerable variation in the mean progesterone concentration during the luteal phase of different cycles was partially related to the number of corpora lutea present.

EWES CYCLICITY FOLLOWING SUPEROVULATION: Onset of oestrus occurrence in Barki ewes after 18 to 34 days after treatment were observed in 8 out of 12 ewes (66.67%) in control group, while, it was noticed in 10 out of 19 ewes (52.63%) in superovulated ewes. Superovulated ewes showed oestrus after $\geq$ 52 days in high incidence (26.32%) than control ewes (16.67%). In this respect, Crister, Rowe, Delcampo and Ginther (1980) found a longer oestrous cycle in superovulation associated between the number of CL and length of the oestrous cycle.

SUMMARY



Summary

The present investigation was carried out on 25 Barki ewes, 1 to 7 years old. Each ewe received per day basal ration (10% protein) composed of 0.5 Kg concentrate (cotton seed cake) plus 0.5 kg Barseem during the breeding season or 0.5 kg hay during the non-breeding season. Ewes were synchronized at the beginning of the experiments using synchromet implants for 13 days. Following implant removal detection of oestrus was carried out by the help of vasectomized ram. On day 5 or 6 of the oestrous cycle (oestrus = day 0), midventral laparotomy was performed for determination of the ovarian response through counting the number of corpora lutea and graafian follicles in both ovaries. Several experiments were conducted inorder to investigate the factors affecting the ovarian response in Barki ewes such as season (breeding v.s. non-breeding season), age (2.5 years v.s. $\geq$ 6 years), Nutrition (10% v.s. 17.9% protein), ovarian side (right v.s. left ovaries) and effect of synchronization (SBM implant v.s. SBM + 750 iu PMSG). Superovulation response studied. Another group of experiments were carried

out to determine the superovulation responses using gonadotrophins for follicular growth (FSH v.s. PMSG), pregnant mare serum gonadotrophin (1000 v.s. 2000 iu) and gonadotrophins for ovulation (GnRH v.s. HCG). Factors affecting responses to superovulation regimens were also investigated, e.g. effects of nutrition (10 v.s. 17.9% protein) and season (breeding v.s. non-breeding season) on ovulation rate. Moreover, plasma oestradiol-17B and progesterone profiles were determined and evaluated among the various superovulation regimens used in this study.

Oestrus occurred 48 hours after implant removal. Season is an important factor that affect ovarian functions in Barki ewes. Ovulation rate was significantly ($P < 0.05$) higher during the breeding season (autumn) (0.75 ± 0.25) than non-breeding season (summer) (0.17 ± 0.17). There were non-significant variations in the ovulation rate among ages (2-5 years $\geq$ 6 years) or nutritional regimens (10% v.s. 17.9% protein). The ovulation rate was higher but without significance in the right ovary than in the left one. There were also non-significant effect of synchronization treatment on the ovulation rate.

Several superovulation regimens were carried out to identify the best one in the Barki ewes. Ovulation rate was non-significantly higher (6.5 ± 1.61) in PMSG treatment than that (5.6 ± 3.00) found in FSH treatment. Among doses, ovulation rate was significantly ($P<0.05$) higher (7.33 ± 1.2) with 2000 iu PMSG than that (3.67 ± 0.88) noticed in 1000 iu PMSG treatment. Among gonadotrophins for ovulation, the HCG showed a significantly ($P<0.01$) higher ovulation rate (6.5 ± 1.61) than that (0.67 ± 0.33) found in GnRH treatment.

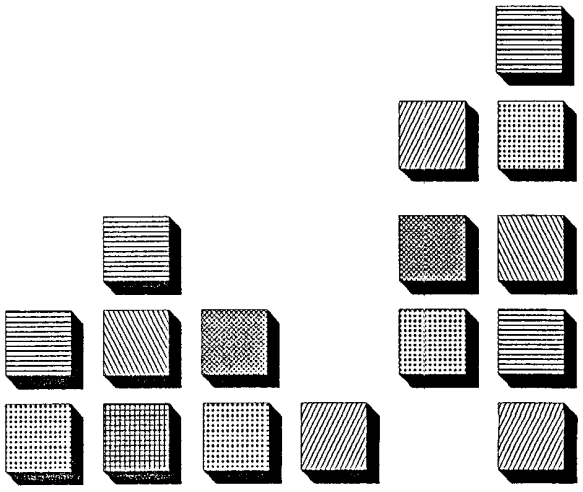
Throughout the superovulation treatment, the ovulation rate was not significantly higher during the breeding season (7.33 ± 1.2) than that (6.5

± 1.61) observed during the non-breeding season. There was non-significant variation in ovulation rate (6.5 ± 1.61) in ewes received PMSG + 10% protein compared to that (5.2 ± 2.22) found in ewes treated with PMSG + 17.9% protein.

Following superovulation treatments, oestrous cyclicty was observed after 18-34 days in 52.6% of the ewes experimental versus 66.7% in the control ewes and respective values after ≥ 52 days were 26.3% and 16.7%.

Oestradiol-17B and progesterone values were significantly ($P<0.01$) higher with FSH treatment (130.09 ± 36.31 pg/ml and 19.09 ± 0.62 ng/ml respectively) than other treatments where the respective values were 5.32 to 11.77 pg/ml and 3.68 to 9.28 ng/ml. Progesterone values were a significantly correlated ($r=0.86$, $P<0.01$) with the number of the corpora lutea.

GENERAL SUMMARY AND CONCLUSION



General Summary and Conclusion

Studying the physiological changes during the oestrous cycle, superovulation and factors affecting ovarian response are useful aid in preferential selection for fecundity in animals within populations. The present study on Barki ewes was undertaken:

a) To study the oestrous cycle with the associated hormonal and blood chemistry during the different seasons of the year.

b) To study the factors affecting ovarian functions (season, age, nutrition) and trials for various superovulation regimens.

I- Oestrous Cycle In Barki Ewes:

Ten Barki ewes reared in the National Research Center Experimental Farm in Cairo, were followed throughout the whole year in order to determine

the type and length of the oestrous cycle, signs of oestrus, hormone profile and plasma minerals and proteins.

The length of normal, short, long and multiple averaged 16.83 $\pm$ 0.34, 12.0 $\pm$ 0.72, 21.56 $\pm$ 0.56 and 34.22 $\pm$ 2.16 days respectively. Normal cycle occurred in higher incidence (58.33%) during autumn than other seasons. Oestrous signs are pronounced at autumn and winter and, feeble during spring and summer. The values of oestradiol-17B and progesterone in Barki ewes showed highly significant ($P<0.01$) changes during oestrous cycle phases. The peak values of oestradiol-17B averaged 14.38 $\pm$ 1.47 during oestrus. Progesterone peak observed during dioestrus (2.55 $\pm$ 0.18 ng/ml). There is no significant seasonal variation in oestradiol-17B and progesterone values. Plasma minerals and proteins values were not in a significant manner among oestrous cycle phases. However, there were significant variation in albumin value ($P<0.05$) and A/G ratio ($P<0.01$) among seasons.

II- Factors Affecting Ovarian Functions And Superovulation:

This study was carried out on 25 Barki ewes. Ewes were synchronized (SMB implants) for 13 days. Gonadotrophin treatment was administered at the day of implant removal. Laparotomy was performed at day 5 or 6 of oestrous cycle to determine ovarian response in both ovaries.

The present investigations showed that oestrus occurred after 48 hours of implant removal. Seasons have a significant ($P<0.05$) effect on ovulation rate (0.75 $\pm$ 0.25 vs 0.17 $\pm$ 0.19) during breeding and non-breeding season. However age (2-5 years vs. $\geq$ 6 years), nutrition (10% vs. 17.9% protein), ovarian side (right vs. left) or synchronization (SMB vs. SMB + 750 i.u PMSG) were showed non-significant variation on ovulation rate.

In superovulation regimens, PMSG treatment has a higher ovulation rate (6.5 ± 1.61) without a significance than FSH treatment (ovulation rate av. 5.6 ± 3.00). Ovulation rate was significantly higher ($p<0.05$) in ewes received 2000 i.u PMSG (7.33 ± 1.2) than ovulation rate (3.67 ± 0.88) in ewes received 1000 i.u PMSG. Ewes treated with HCG have a significantly ($P<0.01$) higher ovulation rate (6.5 ± 1.61) than ovulation rate (0.67 ± 0.33) observed in ewes treated with GnRH. Also ovulation rate observed in breeding season (7.33 ± 1.2) was higher without a significance than ovulation rate (6.5 ± 1.61) noticed in non-breeding season. There is non-significant variation in ovulation rate of the two nutrional groups (10% protein vs. 17.9% protein).

Oestradiol-17B and progesterone values were significantly ($P<0.01$) higher at FSH than PMSG treatments.

There is a significant ($P<0.01$) correlation ($r=0.86$) between progesterone values and numbers of corpora lutea recorded at the day of laparotomy.

In conclusion, Barki ewes showed distinct breeding activities with the breeding season during autumn in which most ewes (58%) display normal cycle length (16.81 day), the rest showed various types of oestrous cycle (short, long and multiple). Such findings lie within the range given by other breeds. The oestradiol-17B and progesterone hormone profiles as well as plasma minerals and proteins values throughout the oestrous cycle phases, all over the year round emphasis that autumn is the best season for breeding in Barki ewes.

Such data are utilized in the second part of this study for determine the factors affecting the ovarian functions in Barki ewes (season, age and nutrition). Various regimens of superovulation were tried (PMSG, FSH, HCG, GnRH). Ewes synchronized with synchromet-B for 13 days, followed by injection of PMSG (2000 i.u) and HCG (1000 i.u) showed the best response (ovulation rate).

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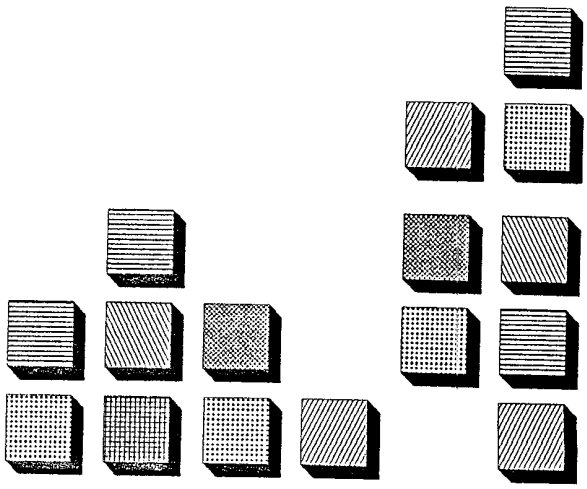

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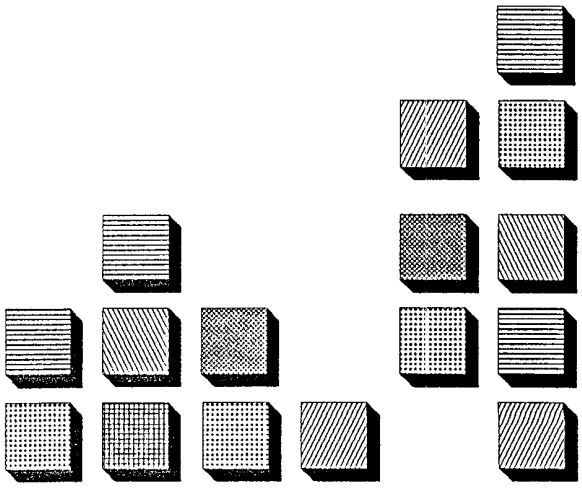
ARABIC SUMMARY



جـــامعة القاهـــرة

كلـــية الطـــب البيطـــرى

قسم التوليد والتناسل والتلقيح الصناعى

تعدد التبويض والعوامل المؤثرة على وظائف المبيض فى نعاج البرقى

رسالة مقدمة من

ط.ب/ اميمة محمد توفيق قنديل

ماجستير فى العلوم الطبية البيطرية

التوليد والتناسل والتلقيح الصناعى (١٩٨٨)

مقدمة للحصول على

درجة دكتوراه الفلسفة فى العلوم الطبية البيطرية

التوليد والتناسل والتلقيح الصناعى

تحت إشراف

أ.د/ نبيل عبد المنعم حميدة

قسم التوليد والتناسل والتلقيح الصناعى

كلية الطب البيطرى - جامعة القاهرة

أ.د/ محمد رفعت شلش

أستاذ متفرغ بقسم التكاثر الحيوانى

والتلقيح الصناعى - المركز القومى للبحوث

أ.د/ عقيل عبد الشافى سلامة

رئيس قسم التكاثر الحيوانى والتلقيح الصناعى

المركز القومى للبحوث

١٩٩٢

بسم الله الرحمن الرحيم

جـــامعـــة القـــاهرة

كلـــية الطـــب البيطرى

قسم التوليد والتناسل والتلقيح الصناعى

قرار لجنة الحكم والمناقشة

قررت لجنة الحكم والمناقشة ترشيح السيده ط.ب/ اميمة محمد توفيق قنديل للحصول على درجة الدكتوراه الفلسفة فى العلوم الطبية البيطرية فى مادة التوليد والتناسل والتلقيح الصناعى.

اللجـــنة

الأستاذ الدكتور / العزب على العزب

أستاذ ورئيس قسم التوليد

كلية الطب البيطرى - جامعة الزقازيق

الأستاذ الدكتور / ابو بكر عبد القادر الويشى

أستاذ ورئيس قسم التوليد

كلية الطب البيطرى - جامعة القاهرة

الأستاذ الدكتور / نبيل عبد المنعم حميدة

أستاذ التوليد والتناسل

كلية الطب البيطرى - جامعة القاهرة

والمشرف على الرسالة

بتاريخ: ١٣-٢-١٩٩٢

الملخص العربى

تعتبر دراسة التغيرات الفسيولوجية أثناء دورة الشبق واحداث تعدد التبويض وكذلك العوامل المؤثرة على استجابة المبيض من العوامل المؤثرة والمساعدة على الأختيار الأفضل للانتاجية بالنسبة لقطيع من الحيوانات. والدراسة الحالية على آناث الاغنام البرقى استهدفت شقين: أولا، دراسة دورة الشبق مع التغييرات الهرمونية وكيمياء الدم على مدار العام. ثانيا، دراسة العوامل المؤثرة على وظيفة المبيض (فصول السنة، العمر، التغذية) وكذلك استخدام طرق مختلفة لاحداث تعدد التبويض.

أولا: دورة الشبق فى آناث أغنام البرقى:

أجريت هذه الدراسة على ١٠ من نعاج البرقى يتراوح أعمارهم من ١-٧ سنوات وتمت الملاحظة لمدة عام بأستخدام علاقات الشبق وبمساعدة كبش مقطوع الوعاء الناقل للحيوانات المنوية. وقد تم تجميع عينات الدم على مدار العام وتم تحليل العينات لقياس هرمونات البروجستيرون والاستراديول (بأستخدام الطرق المناعية الاشعاعية). وقياس كل من الكالسيوم، الفوسفور والحديد والزنك والبروتين والالبيومين (بأستخدام الجواهر الكاشفة والمقياس الضوئى).

يصحب الشبق فى نعاج البرقى بعض التغيرات فى السلوك. تبحث النعاج عن الذكر وتتبعه مع النظر فوق الكتف وتقف حتى حدوث التلقيح مع ملاحظة تورم واحتقان الغشاء المخاطى للمهبل إثناء الشبق، الافرازات المخاطية كثيرة وشفافة ولكنها تصبح سميكة وكريمى بعد ذلك. علامات الشبق واضحة أثناء الخريف والشتاء ولكنها ضعيفة أثناء الربيع والصيف.

دورة الشبق تحدث بنسبة ٦٠,٣٢٪ فى دورة الشبق الطبيعية (متوسطها ١٦,٨٣ + ٠,٣٤ يوم) و١١,١١٪ الدورة القصيرة (متوسطها ١٢ + ٠,٧٢ يوم) و١٤,٢٩٪ فى كل من الدورة الطويلة (متوسطها ٢١,٠٦ + ٠,٥٦ يوم) والدورة المضاعفة (متوسطها ٣٤,٢٢ + ٢,١٦ يوم). نسبة حدوث دورة الشبق الطبيعية عالية أثناء الخريف (٥٨,٣٣٪) ولكنها قليلة أثناء الشتاء (٢٢,٢٢٪) والصيف (١٣,٨٩٪) والربيع (٥,٥٦٪).

تتفاوت قيم الاستراديول والبروجستيرون بوضوح فى نعاج البرقى خلال الفترات المختلفة لدورة الشبق وقيم الاستراديول كانت كما يلى ٤،٠٢ ± ٠،٢٩، ١٤،٣٨ ± ٦،٤٧، ١،٣٥ ± ٠،٣٦، ١،١٩ ± ٠،٢٩ بيكوجرام/ملى بلازما خلال فترة ما قبل الشبق والشبق وما بعد الشبق وفترة وجود الجسم الاصفر على التوالى وايضا قيم البروجستيرون المتوالية تكون كما يلى ٠،١٧ ± ٠،٠٢، ٠،١٢ ± ٠،٠٢، ٠،٤٣ ± ٠،٠٥، ٢،٥٥ ± ٠،١٨ نانوجرام/مل بلازما. قيم الاستراديول والبروجستيرون أثناء الفصول المختلفة ليس لها تغير واضح. التغير فى قيم الاستراديول له علاقة عكسية واضحة مع البروجستيرون (ر=-٠،٣، أح « ٠،٠١) والجلوبيولين (ر = -٠،٤، أح « ٠،٠١) وله علاقة طردية مع نسبة الكالسيوم الى الفوسفور (ر=٠،٢، أح « ٠،٠٥) قيم البروجستيرون لها علاقة واضحة مع قيم الالبيومين (ر=٥،٣، أح « ٠،٠١)، الكالسيوم (ر=٠،٠٢، أح « ٠،٠٥) وعلاقة عكسية مع نسبة الالبيومين الى الجلوبيولين (ر=-٠،٢، أح « ٠،٠٥).

قيم الاملاح المعدنية (كالسيوم، فوسفور، النسبة بينهم، الحديد، الزنك) والبورتينات (البروتين الكلى، الالبيومين، الجلوبيولين والنسبة بينهم). ليس لها تغيرات واضحة أثناء الفترات المختلفة لدورة الشبق. قيم الالبيومين (أح « ٠،٠٥) والنسبة بين الالبومين والجلوبيومين (أح « ٠،٠١) لهم تغير واضح أثناء الفصول المختلفة.

ثانيا: دراسة العوامل المؤثرة على وظيفة المبيض وتعدد التبويض:

اجريت هذه البحوث على ٢٥ من إناث البرقى الذى يتراوح عمرهم بين سنه وسبع سنوات لدراسة العوامل المؤثرة على استجابة المبيض فى نعاج البرقى. ومن أمثله ذلك: فصول السنه (موسم الاخصاب بالمقارنة مع موسم غير مخصب) والعمر (٢-٥ سنوات بالمقارنة مع ٦ سنوات أو أكثر) والتغذيه (١٠٪ بروتين بالمقارنة مع ١٧،٩ بروتين) وجهة المبيض (يمين بالمقارنة مع اليسار) وأيضاً تأثير وسيله تنظيم دورة الشبق (استخدام السينكرومات بالمقارنة مع استخدام السينكرومات مع حقن ٧٥٠ وحده دولية من الجونادوتروفين المستخلص من دم الفرس أثناء الحمل).

وقد اجريت تجارب آخرى لمعرفه الاستجابة لزيادة التبويض بأستخدام الجونادوتروفين FSH بالمقارنة مع PMSG وتمت المقارنة بين جرعات الـ PMSG

المختلفة (١٠٠٠ بالمقارنة مع ٢٠٠٠ وحده دوليه) وأيضاً تم استخدام الـ GnRH بالمقارنة مع الجونادوتروفين المشيمى (HCG) وكذلك التغذية (١٠٪ بروتين بالمقارنة مع ١٧.٩٪ بروتين) وفصول السنه (موسم الاخصاب بالمقارنة مع موسم غير مخصب). بالاضافة إلى ذلك تم تعين مستوى الاستراديول والبروجستيرون فى بلازما الدم اثناء الطرق المختلفة لتعدد التبويض المستخدم فى هذه الدراسة.

تحدث دورة الشبق دائماً بعد يومين من إزالة السينكرومات. من أهم العوامل التى تؤثر على وظائف المبيض فى نعاج البرقى هى فصول السنه. معدل التبويض أعلى ومعنوى (٠.٠٥) أثناء موسم الاخصاب (الخريف) (٠.٧٥ ± ٠.٢٥) عن الموسم الغير مخصب (٠.١٧ ± ٠.١٧). التغيرات الناتجة فى معدل التبويض فى الاعمار المختلفة (٢-٥ سنين) بالمقارنة مع ٦ سنوات أو أكثر وأيضاً نظام التغذية (١٠٪ بالمقارنة مع ١٧.٩٪ بروتين) غير معنويه. المبيض الايمن له معدل تبويض أعلى ولكنه غير معنوى عن الايسر. التغير فى طرق تنظيم دوره الشبق ليس له تأثير معنوى على معدل التبويض.

اجريت عدد من الطرق لزياده التبويض لمعرفة أفضل طريقة تتناسب مع إناث الاغنام البرقى. وجد أن اختلاف نوع الجونادوتروفين ليس له تغير معنوى فى معدل التبويض. معدل التبويض (٦.٥ ± ١.٦١) بعد استخدام الجونادوتيروفين (PMSG) اعلى من معدل التبويض (٥.٦ ± ٣) الناتج من استخدام (FSH) بالنسبه للجرعات المستخدمه من الجونادوتروفين (PMSG) فهو له تأثير معنوى (٠.٠٥) على معدل التبويض فهو أعلى (٧.٣٣ ± ١.٢) عند استخدام ٢٠٠٠ وحده عنه (٣.٦٧ ± ٠.٨٨) عند استخدام ١٠٠٠ وحده من الهرمون. بالنسبه للجونادوتروفين الخاصه بالتبويض لها تأثير معنوى على معدل التبويض. معدل التبويض أعلى (٦.٥ ± ١.٦١) عند استخدام الجونادوتروفين المشيمى (HCG) من معدل التبويض الملاحظ عند استخدام (GnRH) (٠.٦٧ ± ٠.٣٣).

من خلال الطرق المختلفة لزيادة التبويض: وجد أن فصول السنه ليس لها تغير معنوى فى معدل التبويض. معدل التبويض ٧.٣٣ ± ١.٢ أثناء موسم الاخصاب ولكنه ٦.٥ ± ١.٦١ أثناء الموسم الغير مخصب. وأيضاً التغير فى نظام الغذاء ليس له تأثير

معنوى فى معدل التبويض. معدل التبويض ٦,٥ ± ١,٦١ عند النعاج التى تناولت ٢٠٠٠ وحده من الجونادوتروفين (PMSG) مع ١٠٪ بروتين بالمقارنة مع معدل التبويض ٥,٢ ± ٢,٢٢ عن النعاج التى اخذت نفس جرعة الجونادوتروفين مع ١٧,٩٪ بروتين.

بعد المعالجات المختلفة لزيادة التبويض جاءت دوره الشبق بعد ١٨-٣٤ يوم فى ٥٢,٧٪ من النعاج المستخدمه فى التجارب بالمقارنة مع ٦٦,٧٪ من النعاج التى لايتم معالجتها بالهرمونات. وظهرت دوره الشبق بعد ٥٢ يوم أو أكثر فى ٦٢,٣٪ و ١٦,٧٪ بالتتابع للمجموعتين السابقتين.

قيم الاستراديول والبروجستيرون أعلى ولها تغير معنوى عند استخدام FSH (١٣٠,٩ ± ٣٦,٣١ بيكوجرام/مل ، ١٩,٠٩ ± ٠,٦٢ نانوجرام/مل بالتتابع) عن المعالجات الاخرى ونفس القيم بالتتابع تتراوح بين ٥,٣٢ إلى ١١,٧٧ بيكوجرام/مل و ٣,٦٨ إلى ٩,٢٨ نانوجرام/مل. مستوى البروجتيرون فى بلازما الدم له علاقة قوية ومعنوية مع عدد الاجسام الصفراء الموجودة فى المبيض.

ومن خلاصة النتائج تبين أن أغنام البرقى تظهر نشاط جنسى محدد مع حدوث اعلى معدلات الاخصاب خلال فصل الخريف من السنة حيث تزيد نسبة دورة الشبق الطبيعية (٥٨٪) وقد اظهرت تحاليل هرمونات الاستراديول والبروجستيرون وكذلك نسب الاملاح والمعادن والبروتين فى الدم ان فصل الخريف هو الفصل القياسى لزيادة النشاط الجنسى فى أغنام البرقى.

وقد استحدثت النتائج السابقة لدراسة العوامل المؤثرة على وظائف المبيض فى الأغنام البرقى (فصول السنة، العمر، التغذية، ونوع الجونادوترفين وجرعاته المختلفة) وقد تبين ان استخدام الـ PMSG بجرعة ٢٠٠٠ وحدة دولية وكذلك ١٠٠٠ وحدة دولية من HCG عند الشبق هو أفضل البرامج المستخدمة لزيادة معدلات التبويض فى آناث أغنام البرقى.