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Profile Of Reproductive Hormones Secretion During Estrus And Pregnancy Associated With Different Estrus Synchronization Methods In Awassi Ewes In Non-Breeding Season

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Abstract

The study was conducted from March to August 2020 at the Al-Shatra Sheep and Goat Research Station in Thi-Qar Governorate, Iraq, to assess the estimation of the concentration of the reproductive hormones, luteinizing hormone (LH), Follicle-stimulating hormone (FSH), estrogen, and progesterone in Awassi ewes in various physiological states during the outbreeding season. Thirty-two mature, healthy Awassi ewes in the non-breeding season were used. Ewes were randomly divided into four equal groups (eight animals each) according to the following: Control; The animals were kept in a control group and were not given any hormones. First, treatment group (T1) (MAP+ equine chorionic gonadotropin (eCG); The animals received (intravaginal sponges saturated with 60 mg medroxyprogesterone acetate) (MAP) inserted in the ewes for 12 days. Second treatment group (T2); ewes were given double subcutaneous (s.c) melatonin ear implants for 35 days. Third treatment group (T3); The ewes were given s.c double melatonin ear implants for 35 days and on the 25th day from the beginning of melatonin implantation, the animals were treated with (MAP) for 12 days. All animals' groups received 400 IU of (eCG) at the end of the treatment. Rams were introduced to the flock directly after eCG injection of each group and all groups were visually monitored twice a day for signs of estrus. The results revealed that administration of exogenous progestogens (MAP)as intravaginal sponges and melatonin as ear implants alone or with MAP followed by eCG appear to be effective in synchronization of estrus, maintenance of pregnancy, and parturition in hormone-treated ewes in Non- breeding season confirmed by the levels of reproductive hormones (FSH LH, estradiol, and progesterone during estrus and pregnancy).

Keywords: Melatonin implants, vaginal sponges, eCG., FSH, LH, estrogen, progesterone, pregnancy, anestrus ewes.

1.Introduction

The reproductive technologies in animals now is regarded one of the most important techniques which is used to improve reproduction in many species of animals (Alsalim et al., 2021 and Alsalim et al., 2020). At the same time the reproductive disorder play a very critical point in decreasing the reproductive abilities in ewes (fahad et al., 2019). Estrus synchronization or estrus induction is a valuable tool for elevating pregnancy rates in ewes. Additionally, the use of estrus synchronization provides a timed breeding and lambing opportunities. During the last decades, various techniques have been developed to synchronize estrus in ewes (Sirjani et al., 2012; Abdalla et al., 2014 and Masoudi et al., 2016). Exogenous hormones can be used to promote the induction and synchronization of estrus in the anestrus cycle due to the seasonal aspect of the estrus cyclicity in ewes. (Carlson et al. 1989; Forcada et al., 1999; Chemineau et al., 2008 and Jackson et al., 2014). The pineal gland produces and releases melatonin, which is a neurohormone (Skotnicka and Heynczak, 2001). The pineal gland's secretion is influenced by lighting conditions in a cyclic fashion (Kowiak et al., 1999). This hormone, which is made up of the important of amino acid tryptophan, has a wide range of physiological roles. Malpaux et al., (2001) discovered that gonadotropin-releasing hormone (GnRH) released from the hypothalamus – pituitary – gonadal axis regulates serum levels of melatonin, which leads to regulation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion for regulation of estrus and the ovulation (Malpaux et al., 2001; Reiter et al., 2009). Intravaginal sponges impregnated with progesterone are one of the strategies for synchronizing estrus in sheep that can be used (Manvi, 2014). The estrus cycle can be controlled using melatonin implants, progesterone and analogues to sustain the luteal process (Blaschi et al., 2014). Therefore, the goal of this study was to investigate the effect of various methods of estrous induction on reproductive hormones during estrus and pregnancy in healthy, Awassi ewes during non-breeding season.

2.Materials and Methods

The study was carried out in Al-Shatra Sheep and Goat Research Station, Thi- Qar Governorate, Iraq, during the period extending from March 2020 to August 2020. In this study, 32 mature clinically healthy Awassi ewes (aged 2.5- 3 years and weighed 35-38 kg) in non-breeding season were used. Before onset of the study, 4 rams with well-known fertility according to station records were separated from the flock until re-introduce after estrus induction. They were numbered by plastic ear-marking and fed according to the Research Station Program. In the morning they were feed concentrated feed supplemented with vitamins, minerals, and water, in the evening, green feed is presented to them. Ewes were treated previously by prophylactic doses of external and internal parasites. The ewes (n=32) were randomly divided into four equal groups (eight animals per each) according to the following protocol, Control group (CON); The animals were kept as a control group and received no hormonal treatment. First treatment group (T_1) (MAP+ eCG); The animals of this group received Progespon (intra-vaginal sponges impregnated with 60 mg medroxyprogesterone acetate) (MAP) were inserted in the ewes for 12 days followed by intramuscular injection (i.m) of 400 IU eCG. Second group (T_2) (MEL+eCG); The animals were given double subcutaneous (sc) melatonin (Melovine®) ear implants (18 mg ×2) for 35 days, followed by an i.m with 400 IU of eCG. Third group (T_3) (MEL+ MAP+eCG,); The animals were given sc double melatonin ear implants (18mg ×2) on the 11th of March for 35 days and on the 25th day from the beginning of melatonin implantation, the animals were treated with Progespon (intravaginal sponges impregnated with 60 mg) (MAP) for 12 days followed by i.m of 400 IU of eCG.

Rams were introduced to the groups at the end of each treatment period, and they were all visually monitored twice a day (morning and evening) for signs of estrus, which included uneasiness, increased

occurrence of repeated tail wagging, frequent urination, an abnormal amount of bleating, reddish and swollen vulva, and mucus under the tail. Ultrasound was used to confirm the pregnancy.

All animals had five ml of blood drawn from their jugular vein five times: before treatment (0 day), after hormonal treatment, at estrus, early pregnancy on day (28) and late pregnancy on day (130). Blood samples were separated by centrifugation at 3000 rpm for 15 minutes before being stored at – 20°C until analysis.

Hormonal Assay: Sheep FSH, LH, estrogen, and progesterone levels were determined by using the Enzyme-Linked Immune Sorbent Assay (Sheep ELISA) technique (Shanghai Yingxin; China) according to (Alwan and Al-Saeed, 2021).

Statistical Analysis:

The obtained data were analyzed statistically using the SPSS (2012) program was utilized to see how different factors affected research parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between periods and groups .

3.Results

Follicle Stimulating Hormone (FSH) Level (mIU/ml)

It is noted from the current results that the level of the FSH in the treatment and estrus increased significantly (P≤0.05) in T_1, T_2 and T_3, respectively compared to the control group (Table, 1). In early pregnancy, FSH level in T2 was significant increased compared to T_1, T_3 and the control group. While no a significant differences (p ≥0.05) were observed in FSH level between T_3 and T_1 groups. However, FSH level increased significantly(P≤0.05) in T_3 group compared with the control group. On the other hand, during late pregnancy FSH level decreased significantly (P≤0.05) in T_1 and T_3 compared to control group, while no significant differences (p ≥0.05) were observed in FSH between T_2 and control group.

The study also included the effect of the periods on the hormone level within each group. It was noted from the results that the level of FSH in first treatment during estrus was the highest value compared to the other periods. A significant increase (P≤0.05) in serum FSH level was recorded at the estrus of the T_1 group compared with the 0 days, early and late pregnancy. While the low significant (P≤0.05) FSH value was recorded in 0 day and late pregnancy. In the second treatment(T2), a significant increase (P≤0.05) in FSH were observed (P≤0.05) in treatment, estrus and late pregnancy periods compared with 0 day and late pregnancy periods, while low significant (P≤0.05) FSH value was recorded in the 0 day. Moreover, in T_3 , a significant increase (P≤0.05) in FSH was recorded at estrus period compared with all other periods, while low significant (P≤0.05) FSH values were recorded in 0 day and late pregnancy period.

Table (1): FSH (mIU/ml) Levels Between and Within Hormonal Treatments and Control Groups

Follicle Stimulation Hormone Mean ± SD						
Period / Group	0 Day	Treatment	Estrus	Early pregnancy	Late pregnancy	LSD
Control	1.57 ± 0.25 A/a	1.85 ±0.56 A/b	1.68 ± 0.33 A/b	1.88 ± 0.49 A/c	2.09 ± 0.33 A/a	**NS**

T_1	1.56 ± 0.32 C/a	2.49 ± 0.41 AB/a	2.86 ± 0.48 A/a	2.28 ± 0.35 B/bc	1.44 ± 0.25 C/b	**0.42**
T_2	1.53 ± 0.29 C/ a	2.74 ± 0.44 A/a	3.26 ± 0.57 A/ a	2.96 ± 0.51 A/a	1.96 ± 0.61 B/ab	**0.59**
T_3	1.39 ± 0.29 C/a	2.55 ± 0.39 AB/a	2.94 ± 0.60 A/a	2.37 ± 0.36 B/b	1.56 ± 0.25 C/b	**0.49**
LSD	**NS**	**0.59**	**0.60**	**0.52**	**0.47**	

* A significant difference (P≤0.05) between treated groups is indicated by a different small letter inside a column. Within a column, different capital letters indicate a significant difference within the group.

***T1(intravaginal sponges soaked with 60 mg medroxyprogesterone acetate +400 IU eCG), T2 (Melovine®) ear implants (18 mg ×2) +400 IU), T3 Melovine®) ear implants (18 mg ×2) + intravaginal sponges saturated with 60 mg medroxyprogesterone acetate +400 IU eCG..

Luteinizing Hormone (LH) Level (mIU/ml)

After hormonal treatment, blood LH levels in the T_1 and T_3 groups were significantly lower (P≤0.05) than in the T_2 and control groups (Table, 2). While no significant differences (p≥0.05) were recorded in LH between T_2 and control. On the other hand, a significant increase (P≤0.05) in LH were observed in all treatment groups during estrus compared with the control group and the high LH value was recorded in T_2 group. During early and late pregnancy, significant increase (P≤0.05) in serum LH level was observed in T_2 group compared with control and other treated groups.

Within groups significant increase (P≤0.05) in LH levels were recorded in the after treatment, estrus and early pregnancy periods compared with the 0 day and late pregnancy in first treatment (T_1) group. In T_2 a significant increase(P≤0.05) in serum LH level was recorded in estrus period compared with all treatment periods. While low significant (P≤0.05) level of LH was recorded in 0 day. Moreover, significant increase(P≤0.05) in LH level was recorded in estrus period compared to other treatment periods.

Table (2): LH (mIU/ml) Levels Between and Within Hormonal Treatments and control Groups

Luteinizing Hormone Mean ± SD						
Period / **Group**	**Day 0**	**Treatment**	**Estrus**	**Early pregnancy**	**Late pregnancy**	**LSD**
Control	5.63 ± 0.74 A/a	6.29 ± 0.87 A/a	5.31 ± 1.23 A/c	6.11 ± 1.13 A/b	5.73 ± 0.88 A/b	**NS**
T1	5.56 ± 0.85 B/a	6.69 ± 0.1.40 A/b	7.62 ± 0.98 A/b	7.03 ± 1.16 A/b	5.46 ± 1.00 B/b	**1.3**
T2	5.50 ± 0.74 D/a	9.38 ± 1.41 B/a	11.7 ± 1.86 A/a	9.26 ± 1.15 B/a	7.60 ± 0.70 C/a	**1.4**
T3	5.52 ± 0.91 C/a	6.07 ± 0.85 BC/b	8.33 ± 1.00 A/b	6.80 ± 1.55 B/b	4.85 ± 1.10 C/b	**1.3**

LSD	**NS**	**1.4**	**1.5**	**1.5**	**1.1**	

* A significant difference (P≤0.05) between treated groups is indicated by a different small letter inside a column. Within a column, different capital letters indicate a significant difference within the group.

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Estradiol (E_2) Level (pg/ml)

The results of the present study (Table, 3) revealed that serum estrogen levels after the treatment period increased significantly (P≤0.05) in all treatment groups compared with control group. On the other hand, serum estrogen was significantly increased (P≤0.05) in the T_1 compared to T_2, while no a significant (p≥0.05) difference when compared to T_3. During estrus and early pregnancy periods, serum estrogen levels increased significantly (P≤0.05) in all treatments compared to control group. Furthermore, in late pregnancy period a significant increase(P≤0.05) in serum estrogen were recorded in all treatment compared to control group, while estrogen level increased significantly in the T_1 group compared with the control and T_2 groups. Within groups estrogen levels increased significantly (P≤0.05) during estrus period in all treatment groups compared with other treatment periods.

Table (3): Estradiol (pg / ml) Levels Between and Within Hormonal Treatments and Control Group

Estradiol Hormone Mean ± SD						
Period / Group	**Day 0**	**Treatment**	**Estrus**	**Early pregnancy**	**Late pregnancy**	**LSD**
Control	122.7 ± 8.63 A/a	114.2 ± 7.52 A/c	124.9 ± 12.6 A/b	128.4 ± 8.98 A/b	138.8 ± 17.4 A/c	**NS**
T_1	131.7 ± 8.45 C/a	169.9 ± 27.8 BC/a	256.6 ± 56.8 A/a	189.6 ± 40.3 B/a	187.0 ± 21.2 B/a	**41.7**
T_2	128.7 ± 13.8 C/a	145.2 ± 15.4 BC/b	240.9 ± 29.8 A/a	164.8 ± 16.9 B/a	161.0 ± 14.7 B/b	**22.7**
T_3	123.4 ± 8.55 D/a	160.3 ± 13.7 C/ab	266.8 ± 26.1 A/a	181.8 ± 20.6 B/a	175.2 ± 14.3 BC/ab	**21.1**
LSD	**NS**	**21.3**	**42.4**	**32.0**	**20.6**	

* A significant difference (P≤0.05) between treated groups is indicated by a different small letter inside a column. Within a column, different capital letters indicate a significant difference within the group.

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Progesterone (P4) Level (ng/ml)

The results of the current study (Table, 4) showed that serum progesterone levels increased significantly(P≤0.05) in group T_2 during treatment, estrus, and early pregnancy compared with the control and other treatment groups. While progesterone levels during late pregnancy increased (P≤0.05) in all treatment compared to control group.

Within the groups progesterone levels increased significantly (P≤0.05) in early and late pregnancy periods compared to 0-day, treatment and estrus periods in all treatment groups as shown in Table (4).

Table (4): Progesterone (ng / ml) Levels Between and Within Hormonal Treatments and Control Groups (Mean ± SD)

Progesterone Hormone Mean ± SD						
Period / Group	**Day 0**	**Treatment**	**Estrus**	**Early pregnancy**	**Late pregnancy**	**LSD**
Control	7.57 ± 0.92 A/a	6.68 ± 1.07 A/b	7.26 ± 1.39 A/b	7.88 ± 1.21 A/c	8.48 ± 1.57 A/b	**NS**
T_1	7.27 ± 0.94 B/a	7.47 ± 0.68 B/b	8.65 ± 2.17 B/b	13.0 ± 0.84 A/b	14.1 ± 1.26 A/a	**1.8**
T_2	7.59 ± 0.77 E/a	9.62 ±1.05 D/a	11.9 ± 2.20 C/a	17.9 ± 1.83 A/a	13.9 ± 2.07 B/ a	**2.0**
T_3	7.43 ± 1.04 CD/a	6.76 ± 0.79 D/b	9.16 ± 1.95 C/b	12.5 ± 3.91 B/b	14.5 ± 2.41 A/a	**1.8**
LSD	**NS**	**1.1**	**2.3**	**1.8**	**2.2**	

* A significant difference (P≤0.05) between treated groups is indicated by a different small letter inside a column. Within a column, different capital letters indicate a significant difference within the group.

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4. Discussion

The results of the current study revealed that the hormonal treatments used changed the level of FSH hormone during the experiment period, also the physiological state had an effect on the level of the hormone, it was found that the effect of the estrus and treatment period was clear on the level of the hormone and this is due to the interference of the hormones used to stimulate the development of follicles. In general, as the effect of the period on the level of the hormone level, During the treatment period, the FSH level in the T3 group increased, which could be attributed to melatonin, which promotes FSH and LH synthesis and secretion directly by the hypothalamus or by -adrenergic receptors in the ovaries (Diaz et al., 2000). This result agreed with Sayel et al., (2020) who showed that melatonin led to an increase in the number of ovarian follicles and the corpus luteum as well as an increase in the level of

FSH. The elevation in FSH level during the estrus period may be related to the development of ovarian follicles which may occur due to the effect of melatonin and association with eCG. This result also agreed with Sayel et al., (2020) Who reported that the level of the hormone increased during estrus period when melatonin was given. The presence of melatonin receptors in ovarian follicular cells (Soares et al., 2003) and high levels of melatonin in follicular fluids (Rönnberg et al., 1990) indicate that it plays a direct role in folliculogenesis . The rate of follicle growth is dependent on FSH concentration. eCG acts as an additional endogenous FSH function that supports follicle growth. It has been observed that eCG injection is required to stimulate follicular growth, leading to tighter synchrony of ovulation in both anestrous and cycling of sheep (Greyling et al., 1991; Cline et al., 2001). This indicated that eCG stimulates the development of follicles producing estrogen resulted by the proliferation of granulosa cells, thus leads to the sign of estrus. Low FSH levels in late pregnancy compared to other periods may be due to a gradual decrease in estradiol concentration as a result of increasing progesterone concentration, causing a negative feedback effect on the hypothalamus-pituitary glands, preventing the synthesis and secretion of follicle-stimulating hormone (FSH), preventing the growth of ovarian follicles and/or estradiol production (Pineda,2003; Ku´znicka et al.,2016).

The results of the current study Table (2) are revealed. When comparing the hormonal treatments used with control, it was found that T_2 (MEL+eCG) had a clear effect on the concentration of the luteinizing hormone, and the reason for this was the direct role of melatonin in stimulating the hypothalamus cells release GnRH and causes an effect on gonadal function or increases the RNA of GnRH in the hypothalamus (Wheaton et al., 1990 and Bernard et al., 1999). Melatonin affects gonadal hormones via increasing the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus-pituitary-gonadal axis, which controls the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to govern estrus and ovulation (Malpaux et al., 2001; Reiter et al., 2009; Goodman et al., 2010). Furthermore, melatonin therapy and LH levels in the blood had a substantial positive association. (Wang et al., 2014). These observations are consistent with previous studies which indicate that melatonin treatment increases serum LH level and progesterone levels in sheep (Abecia et al.,2002). Also, when compare the mean level between periods for each group, the mean serum LH level was found in a higher level during estrus in hormonal treated groups (T_1, T_2 and $T_{3)}$. Ewes exhibited estrus from 24 to 84 hours following sponge removal. These results agree with those of (Lunstra and Christenson, 1981). In present study, Progesterone and eCG were found to be effective in speeding up the follicular growth process, allowing estrus to occur. The use of eCG minimizes the variation in the time between implant removal and estrus and ovulation (Cline et al., 2001). P4 levels in the blood may rise to the point where the number of estradiol receptors in the mid–basal hypothalamus increases, increasing sensitivity to estradiol (Arroyo-Ledezma et al., 2006). (Abecia et al.,2012). As a result, such a scenario is most likely caused by follicle growth, which produces more estradiol, as well as positive feedback at the hypothalamic level, where increased GnRH release appears to have generated the LH surge, enhancing the formation of antral preovulatory follicles, promoting estrus activity, and eventually ovulation. (McNeilly et al., 1991;Bartlewski et al.,2016).

In the present study, administration of a combination of exogenous melatonin with MAP followed by eCG was effective to induce estrus in Awassi ewes. Melatonin treatment reduces estradiol negative feedback, allowing LH pulsatility and follicular development and estradiol secretion to continue (Wheaton et al., 1990). Furthermore, Forcada et al., (2007) found that melatonin implants increase the pituitary gland's responsiveness to GnRH stimulation. According to Kridli et al., (2006b), melatonin treatment combined with medroxyprogesterone acetate sponges and eCG can successfully induce

estrus in sheep. During estrus, the concentration of estrogen was high as mentioned above, but this concentration decreased during pregnancy because the concentration of progesterone increased. The changes in progesterone and estrogens before parturition follow a pattern similar to that during the cycle. LH levels were positively correlated with estradiol levels before the onset of estrus and during the estrous period, implying that estradiol not only has the ability to induce a pre-ovulatory LH surge (Karsch et al., 1983), but also that progesterone priming has the ability to regulate the timing of the LH surge via estradiol-dependent mechanisms. Furthermore, changes in brain sensitivity to progesterone or estradiol could be to responsible for the LH surge. (Harris et al., 1999; Skinner et al., 2000).

The gradual decline in LH concentration along the gestational period occurs as a result of the gradual elevation of progesterone concentration affecting on hypothalamus-pituitary glands by negative feedback action causing prevention in synthesis and secretion of FSH and LH leading to inhibition growth of ovarian follicles and/or estradiol production (Pineda,2003; Ku´znicka et al.,2016). The current study's estradiol concentrations were expected to reflect estradiol release by the ovarian follicles after the progesterone device was removed. Estradiol release by developing follicles is stimulated by a drop in progesterone levels. This could be because eCG stimulates ovarian follicular growth, resulting in a high estrogen level. (Salih et al., 2018; pineda and Dooley, 2003). The results of our study agree with Barrett et al., (2004) and Simonetti et al., (2008) who reported that administration of eCG following 12 days of P4 treatment increased serum concentration of estradiol and resulted in synchronized estrus and ovulation in anestrous ewes. Also, agree with Abdalla et al., (2014), who reported that eCG administration reduced the interval from progestagen device withdrawal to estrus induction and improved the efficiency of synchronization of estrus and ovulation during and outside the breeding season. Additionally, Forcada et al., (2007) have shown that melatonin implants improve the responsiveness of the pituitary gland to GnRH stimulation. Previous studies have shown that exogenous melatonin (Rajkumar et al., 1989) and progestins (Safranski et al.,1992) promote ovarian activity and follicular development in the anestrous ewe.

When the mean serum estradiol concentration was compared between periods for each group, the mean serum estradiol concentration was shown to be greater during estrus in T_1, T_2, and T_3. The presence of estrus phase in animals was confirmed by these high levels of estradiol, which was caused by follicular growth. These findings could be due to an increase in estrogen levels generated by PMSG effect on the follicles, as PMSG has been shown to enhance not just the number of follicles, but also the rate at which big follicles grow. (Shelton et al.,1967). Estradiol was regulating estrus cycle, development of female genital tissues and the udder, it is also involved in ovulation, preparation of implantation of oocytes in the uterus, pregnancy, and parturition in combination with progesterone (Faye et al., 2018).

The results of the current study showed that the level of progesterone was high in T_2 during the treatment period, and the reason for this may be attributed to the role of melatonin in stimulating the pituitary gland during the period of anestrus, and this study agreed with Cevik et al (2017) which indicated that administration of melatonin to ewes during the period of anestrus led to the activation of the hypothalamic-pituitary-gonadal axis. This study agreed with Durotoye et al., (1997) and Uslu et al., (2012) who indicate that melatonin affects the corpus luteum resulting in an increase in the level of the hormone progesterone, but it differs with Kassim et al., (2016) who found that treatment with melatonin has no effect on progesterone level. Also differed from Sasa et al., (2016) which found that the treatment of a Suffolk sheep and a Romney sheep Romney Marsh with melatonin implants did not affect the level of progesterone until after the introduction of rams. The non-significant difference in

progesterone hormone concentrations in T_1 and T_3 during treatment could be attributed to insufficient time for follicular maturation and corpus luteum development. As a result, progesterone levels in the blood do not fluctuate significantly.

During estrus period, it was found the concentration of progesterone was high in the T_2, and the reason for this is due to the importance of the link between melatonin and the eCG. Melatonin administration during development of the ovulatory follicle, prior to formation of the corpus luteum, can lead to beneficial changes in corpus luteum function (morphological or biochemical) that last throughout the corpus luteum's life span, resulting in a prolonged elevation of circulating progesterone concentrations. The ewes' fertility improves as progesterone levels rise as a result of eCG therapy (Wildeus, 2000 and Kor et al., 2012). Melatonin given to a mare or sheep during the follicular phase of the cycle can boost progesterone levels and stimulate uterine endometrial glandular production, improving the uterine environment for pregnancy establishment and maintenance, as well as embryo development (Singh et al.,1983). The drop in progesterone at T_1(MAP+ eCG) could be due to the fact that progesterone levels drop shortly after the sponges are removed. This result agreed with Greyling et al., (1994) who indicated that the initial release of progesterone from intravaginal sponges increase during the first 48 h. of treatment and reached maximum values, but decreases with time. Also agreed with Husein and Kridli, (2002); Husein and Haddad, (2006); Kridli and Al-Khetib, (2006).Whose found that in day of estrous, plasma progesterone values fall to very low levels.

Generally, it was noted that the level of progesterone was significantly affected during the early and late pregnancy in all treatments, the increase in the concentration of progesterone hormone is due to the development of the placenta as well survival of the corpus luteum. The secretion of placental progesterone is one of the most important functions for maintaining pregnancy after the corpus luteum retraction on the surface of the ovary (Abu Nasar et al.,2006).

5. Conclusion

From the results of this study, it can be concluded that administration of exogenous progestogens (MAP)as intravaginal sponges and melatonin as ear implants alone or with MAP followed by eCG appear to be effective in synchronization of estrus, maintenance of pregnancy, and parturition in hormone-treated ewes out of the breeding season which proved by the levels of reproductive hormones (FSH, LH, estradiol, and progesterone during estrus and pregnancy).

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