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Comparative Study between Melatonin and Progesterone -PMSG on Onset of Ovarian Activity in an Estrous EWES

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Abstract--- *A study was conducted on 30 adult Iraqi ewes and 6 fertile rams. Ewes were divided into 3 groups 10 animals each, first group treated with melatonin implants (18 mg / animal) for 30 days, second group was treated with intra-vaginal sponges for 10 days followed by injection of PMSG (500 IU / IM) at the time of sponges withdrawal, third group was injected with distilled water (1 ml / IM) and considered as control group. Blood samples were collected from all ewes for estimation of progesterone concentrations at 2 hours before treatments, 7 days after treatments, at 0, 15, 30, 45, 60, 75 and 90 days of gestation. Duration of estrous phase in group 2 (21.9 ± 1.48) was appeared significantly (P≤0.05) higher as compared with group one and group three (18.8 ± 0.43, 17.7 ± 0.88) respectively. Single pregnancy rates (82.40%) were significantly (P≤0.01) increased as compared with twining rates (17.60%). The conception rates in group 2 (80%) significantly (P≤0.01) increased as compared with group 1 and 3 (60 and 30 %) respectively. The lambing and fertility rates in group 2 (125%) appeared significantly (P≤0.01) higher as compared with group 1 and 3 (116.7% and 100%) respectively. In nature of parturition there was no significant difference among treated groups, while in total percentage sex of lambs, male newborn was (65%) significantly (P≤0.01) higher as compared with female newborn (35%). In conclusion the administration of progesterone to ewes by intra-vaginal sponges gave a good results in estrus synchronization, pregnancy rate, lambing rate particularly when it was used with PMSG.*

Keywords--- *Estrus, Pregnancy, Melatonin, Ovarian Activity, Progesterone, Vaginal Sponges.*

I. INTRODUCTION

The ewe is a seasonally polyestrous animal. Its sexual activity starts during the autumn months when day length shortens and heat decreases (Pineda and Dooley, 2003; Kirkwood et al., 2012). Post-partum morphological changes and their delay in the post-partum uterus and ovaries of farm animals act as a limiting factor for the reproductive performance following parturition (Hussain et al., 2016). The importance of day length in the seasonal control of reproductive activity in the ewe has long been recognized. Decreasing day lengths were thought to stimulate the endocrine changes associated with cyclicity. Melatonin, a pineal hormone, mediates the response to changes in the photoperiod in sheep. Melatonin levels are high during dark periods and low during light periods; probably this difference in the pattern of melatonin secretion acts as a signal indicating day length to the neuroendocrine axis (Jainudeen and Hafez, 2000, Younis, 2017). Although sheep lamb once a year, ovulation can be induced during anestrus and their reproduction frequency can be increased (Hafez, 2000, Noakes et al., 2018). For many years the standard method of inducing estrus and ovulation during anestrus in ewes has been the use of progesterone sponges combined with pregnant mare's serum gonadotropin (PMSG) treatment when the sponge is withdrawn. Recently,

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the importance of the pineal gland and its hormone melatonin in the control of the breeding season in the ewe has been established and it has been demonstrated that melatonin given by injection, oral administration, or vaginal or subcutaneous implantation can advance the breeding season in ewes (Dunston et al., 1989; Pal and Dar, 2020). Because melatonin administration mimics the naturally occurring increase in melatonin production during the shortened day lengths in autumn, it might be expected to give more reliable and more uniform ovulation and conception than the less natural progesterone—PMSG treatment. Melatonin overcomes the effects of seasonality (Jainudeen and Hafez, 2000). Combined treatment gives significantly more lambs born per ewe (Dunston et al., 1989).

It is reported that the ovarian activities of sheep can be induced during seasonal anestrus by various treatments to increase the lambing frequency and lambs per ewe (Haresign, 1992; Floris et al., 1994). The most effective treatment for this purpose is reported to be slow releasing subcutaneous implants containing melatonin or melatonin combined with other hormones (Williams et al., 1992; Noakes, 2009). Fernando and Rombauts (2014) have reported that melatonin treatment could start ovarian activities in 5 weeks and achieves estrus and ovulation. However, others have said that this period could extend to 12-15 weeks. The aims of this study to investigate the efficiency of intra-vaginal sponges of progesterone and PMSG with subcutaneous implants of melatonin administration and its effects on estrous induction and reproductive performance.

II. MATERIALS AND METHODS

This study was carried out in a state board for Agricultural Researches, Ruminant Researches Station – Ministry of Agriculture 25 Km north west of Baghdad (Agurgof). Thirty local breed ewes were aged (2-4 years) with an average body weight of (50-60 kg) with a history of one lambing at least and six fertile rams were aged (3-5 years) were used for detecting estrus and mating.

They were housed in semi opened shade regarding the nutritional regime. Before starting the experiment all ewes were submitted to trans-rectal and trans-abdominal ultrasonography and vaginal inspection by vaginal speculum to be sure that the ewes were empty and free from any infection or abnormalities. All ewes were signed by different numbers and colors then the females were isolated from the males to avoid sight, smell and hearing. The ewes were divided to three groups randomly, group 1 was treated with melatonin hormone, group 2 was treated with vaginal sponges and group 3 was the control group.

Group 1: Ten ewes were treated with ear implant melatonin hormone containing 18 mg (Melovine®, Ceva Santè Animale, France) for 30 days. All EWES were mated (hand mating) naturally twice a day with fertile rams at standing heat, mating day was considered as Day 0 for gestational period calculation. Group 2: Ten ewes were treated by insertion of intra-vaginal sponges having 60 mg Medroxy progesterone acetate (ESPONJAVER®, Girona - Spain) for ten days and injection of eCG (500 IU / I.M, ÇÖZÜCÜ, Girona, Spain) at the time of sponges removal.

All EWES were mated (hand mating) naturally two times per day with fertile rams at standing heat. The mating was began at 24h after sponge removal and injection of eCG, mating day was considered as Day 0 for gestational

period calculation. Group 3: Ten ewes injected with distilled water (1ml / IM) and considered as control group. Estrus detection of ewes was monitored in the experimental animals at various time after ram induction, the estrus signs that recorded were: Female seeking out a ram and standing to be mounted by ram, the vulva is redder than usual and swollen, female showing a special status when urinating (Squatting) and sniffing and licking urine and show a special mode (Flehmen). All ewes were inseminated by different rams using hand mating twice daily.

Pregnancy, lambing, and fertility rates were calculated as follows (Zeleke et al., 2005):

$$\text{Pregnancy rate} = \frac{ews\ lambing}{ewes\ inseminated} \times 100$$

$$\text{Lambing rate} = \frac{Lambs\ born}{ewes\ inseminated} \times 100$$

$$\text{Fertility rate} = \frac{Lambs\ born}{ewes\ lambing} \times 100$$

Statistical Analysis

The software Statistical Analysis Method- SAS (2012) was used to detect the effect of difference factors in parameters of the study. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare among means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) CI in this study.

III. RESULTS

Estrous Response and its Duration/ Hours

The results in current study revealed that 6 (33.33%), 9 (50.00%) and 3 (16.67%) ewes in group 1, 2 and 3 respectively were expressed estrous signs after estrous induction regimes, with higher significant difference (P≤0.01) between group two as compared with group one and group three, while group one appear significantly higher (P≤0.01) as compared with group three. In non-estrous ewes, group two 1 (8.33%) showed significantly (P≤0.01) lower as compared with group one 4 (33.33%) and group three 7 (58.34%). Duration of estrous phase/ hours in group two appeared significantly (P≤0.05) higher as compared with group one and group three. The number of follicles and corpus luteum in estrous ewes were 23 and 17 in group 2 as compared with group 1 (14 and 9), (5 and 3) in group 3 respectively. The details presented in table (1).

Table 1: Mean of the Duration of Estrus Phase/ Hours in Different Groups of Treated EWES

Group NO.	**NO. of ewes**	**NO. of estrus ewes%**	**NO. of non-estrus ewes%**	**No. of follicles in estrous ewes**	**No. of C.L in estrous ewes**	**Duration of estrus phase / hours (Mean± SE)**
Group1(melatonin implant 18 mg/ s/c)	10	6 (33.33)	4 (33.33)	14	9	18.8 ± 0.43 b
Group2(Intra-vaginal sponges 40 mg + PMSG 500 IU)	10	9 (50.00)	1 (8.33)	23	17	21.9 ± 1.48 a
Group 3 (Control)	10	3 (16.67)	7 (58.34)	5	3	17.7 ± 0.88 b

Total	30	18 (100)	12 (100)	42	29	---
---	---	**x^2 Value** = 9.03 **	**x^2 Value** = 10.52 **	---	---	**LSD value** = 2.085 *
Means with different letters in the column are significantly different**.** * (P≤0.05), ** (P≤0.01).						

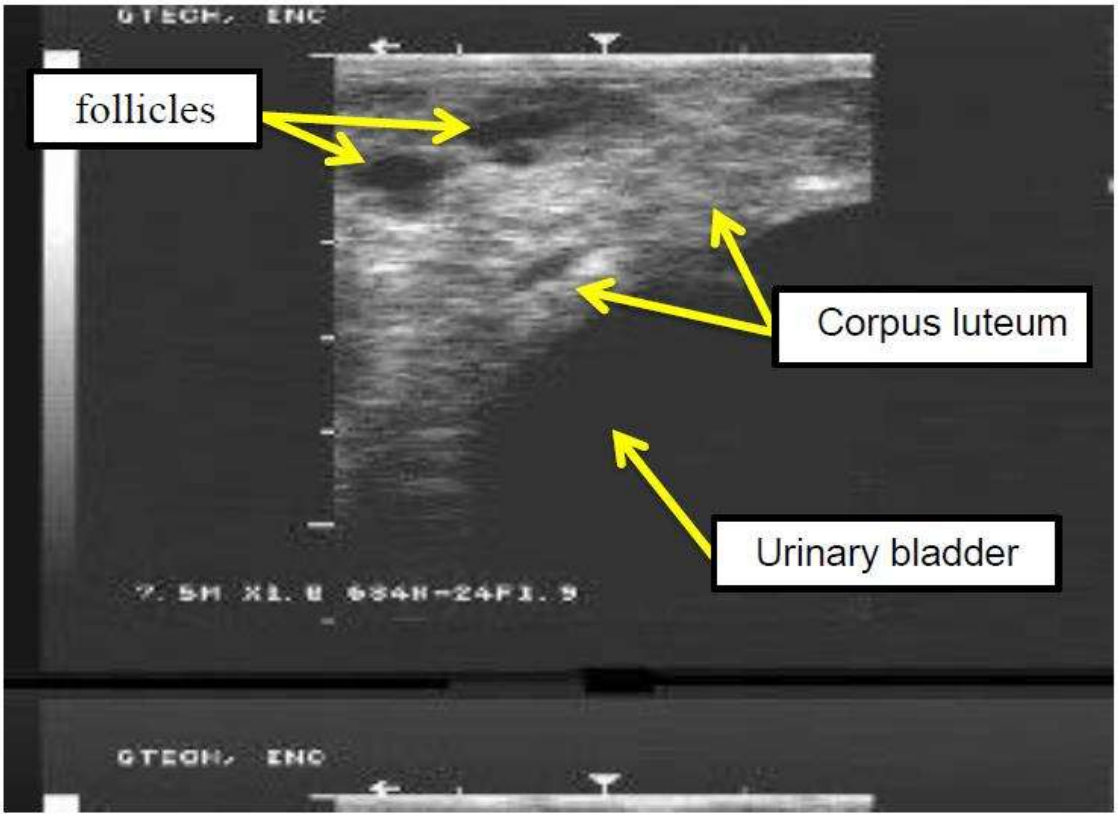

Figure 1: Ultrasono Graphic Image Showed Ovarian Activity in Treated EWE

Effect of Melatonin and Vaginal Sponges on Reproductive Performance in EWES

The current study showed that the number of pregnant ewes in group two 8 (47.06%) appeared significantly (P≤0.01) higher as compared with group one 6 (35.29%) and group three 3 (17.65%), while in group one 6 (35.29%) appeared significantly (P≤0.01) higher compared with group three 3 (17.65%).

In total single and multiple pregnancy rate where 14 (82.40%) and 3 (17.60%) respectively. In conception rate, ewes in group two 8 (80.00%) appeared significantly (P≤0.01) higher as compared with group one 6 (60.00%) and group three 3 (30.00%), while in group one 6 (60.00%) appeared significantly (P≤0.01) higher compared with group three 3 (30.00%). The details in table (2).

Table 2: Effects of Melatonin and Intra-Vaginal Sponges on Reproductive Performance in EWES

Group NO.	NO. of ewes	NO. of pregnant ewes%	NO. of non-pregnant ewes%	Single pregnancy%	Twining pregnancy%	Conception rate %
Group 1	10	6 (35.29)	4 (30.77)	5 (83.30)	1 (16.70)	6 (60.00)
Group 2	10	8 (47.06)	2 (15.38)	6 (75.00)	2 (25.00)	8 (80.00)

Group 3	10	3 (17.65)	7 (53.85)	3 (100)	0 (0.00)	3 (30.00)
Total	30	17 (100)	13 (100)	14 (82.40)	3 (17.60)	17 (56.70)
x^2 **Value**	---	9.84 **	9.61 **	8.42 **	8.42 **	12.05 **
** (P≤0.01).						

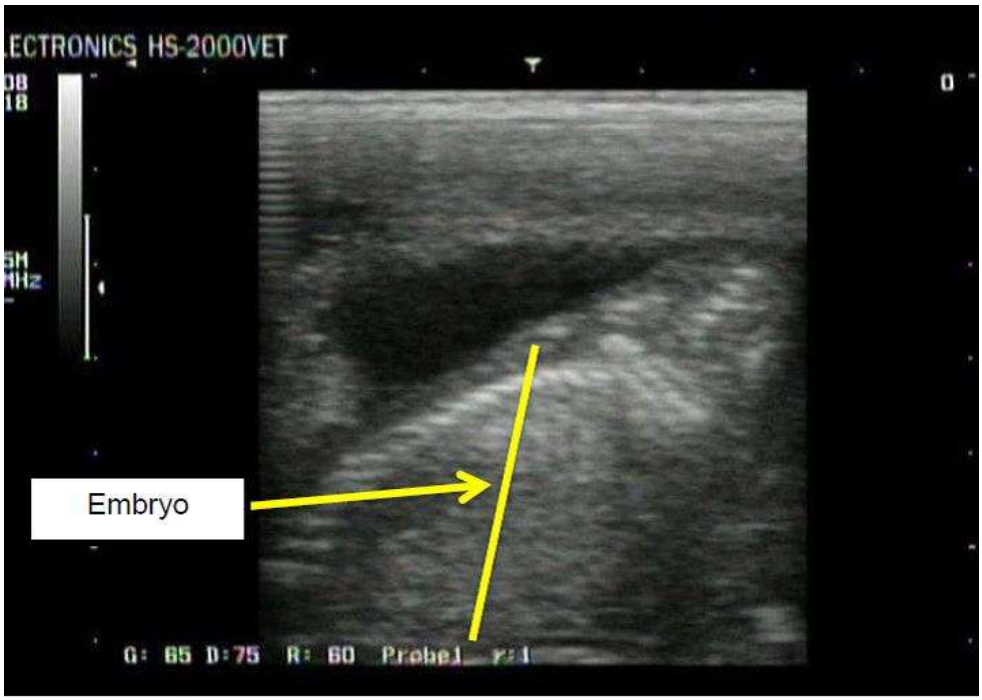

Figure 2: Ultrasono Graphic Image of Pregnant EWE 56 Days Post Mating

Nature of Parturition, Fertility Rate, Lambing Rate and Sex of Offspring

This results showed statistically significant difference in the total sex ratio of offspring in which male lambs increased significantly 13 (65.00%) compared with female lambs 7 (35.00%) at (P≤0.01). The number of lambs in group 1,2 and 3 were (7, 10 and 3) respectively. Total lambing and fertility rates were 117.6%, the lambing and fertility rates in group 2 (125%) were statistically significant at (P≤0.01) as compared with group 1 and 3 (116.7% and 100%) respectively. Total percentage of normal birth was 100% (17/17), there was no significant difference in the nature of parturition between all groups as in table (3).

Table 3: Effect of Melatonin and Vaginal Sponges on Nature of Parturition, Fertility Rate, Lambing Rate and Sex of Offspring

Group	Ewes	Lambed ewes	Lambs	Sex of lambs		Lambing rate%	Fertility rate%	Nature of parturition	
				M %	F %			N %	D %
Group 1	10	6 (35.29)	7 (35.00)	5 (71.40)	2 (28.60)	116.7	116.7	6 (100.00)	0 (0.00)
Group 2	10	8 (47.06)	10 (50.00)	6 (60.00)	4 (40.00)	125	125	8 (100.00)	0 (0.00)

Control	10	3 (17.65)	3 (15.00)	2 (66.70)	1 (33.30)	100	100	3 (100.00)	0 (0.00)
Total	30	17 (100)	20 (100)	13 (65.00)	7 (35.00)	117.6	117.6	17 (100)	0 (0.00)
x^2 Value				8.019 **		10.683 **	10.683 **	NS	
** (P≤0.01), NS: Non-significant.									

IV. DISCUSSION

Induction of Estrous

The results in current study revealed that 6 (33.33%), 9 (50.00%) and 3 (16.67%) ewes in group 1, 2 and 3 respectively, were expressed estrus sings after estrus synchronization, with higher significant difference (P≤0.01) between group 2 as compared with group 1 and group 3, this result is similar to a previous study of AL- Mansoury, (2013) and disagreed with Manvi, (2014) and Kumar et al. (2016) who obtained 100% (30/30) and 85% (17/20) estrus synchronization percent, respectively. This variation in percentage due to administration of higher amount of PMSG (750) I.U and its effect on the development of more number of follicles (Hafez, 2008) (Table 1).

Estrus Response and Estrus Duration (Hours)

This study showed that groups 2 (vaginal sponges + PMSG) had highly significant effect on percentage of estrus response (P≤0.01) than group 1 (melatonin) and group 3 (control). In this study, we determined that the estrus response in group 2 was (9/10), this result is in accordance with Rekik et al. (2002) who found that, the estrus response in ewe lambs (aged 9 months) was 93%, when using intra-vaginal progesterone (40 mg MAP) for 14 days followed by intramuscular injection of 400 IU PMSG after sponges withdrawal. Similar result was found by Zeleke et al. (2005), they reported that, the estrus response in adult ewes was 94.00% and 98.3%, respectively; when they used intra-vaginal sponge impregnated 60 mg MAP and injected 500 IU PMSG after sponge removal. Also; Hashemi et al. (2006), found that the estrus response was 100%, when used 60 mg MAP and injected 500 IU eCG at sponge withdrawal. However this result was higher than that recorded by Simonetti et al. (2000) and Ali (2007), they stated that the estrus response were 60, 80% and 83%, respectively when using different dose of MAP and PMSG. These differences may be due to the difference in dose of MAP and PMSG. Administration of progesterone prevents the ewe from returning to estrus and ovulating and, therefore, supplementing the ewe with progesterone for a period equal to the duration of the life of corpus luteum (CL) and then withdrawing it will synchronize the release of gonadotropins causing estrus and ovulation in groups of ewe (Knights et al., 2002).

Estrus duration of treated groups increased combined with that of control one, particularly that of group 2 (vaginal sponges + PMSG) which had the most obvious increment in estrus duration than that of control and melatonin treated group, such increment was significant (P≤0.01)(table 4-1), this result is in accordance with Zeleke et al. (2005) and Hashemi et al. (2006), they recorded that, the estrus duration in adult ewes ranged between 18.7 and 22.11 hours, when they used intra-vaginal sponge impregnated with 60 mg medroxyprogesterone acetate (MAP) for 14 days and injected with 300 - 500 IU PMSG on the day of sponge removal. However, this result was lower than that recorded by Sefidbakht et al. (1978), they reported that, the estrus period was 35.2 hours.

The result of this study was lower than that recorded by Bekyurek (1993), who found that, the estrus duration was 42 to 61 hours treated with MAP for 14 day and injected with 500 IU PMSG in ewes. Such differences may be attributed to the route of drug PMSG administration (S/C or I/M), variation in the dose and concentration, and age of ewes (Cline et al., 2001) (Table 1).

Effect of Melatonin and Vaginal Sponges on Reproductive Performance in EWES

The conception rate in this study revealed that group 2 (vaginal sponges + PMSG) had significantly higher values ($P \leq 0.01$) than that of group 1 (melatonin) and group 3 (control) (80%, 60% and 30%), respectively (Table 2). This result was higher than that recorded by Zeleke et al., (2005) they found that, the conception rate in adult ewes was from 60 to 78%, when they used intra-vaginal sponge impregnated with 60 mg MAP and injected with 500 IU PMSG after sponge removal. Also, higher than that reported by Martemucci and D'Alessendro, (2011); Elsherry et al. (2012) and Kaya et al. (2013) they recorded the conception rates were 64%; 30-44%; 45-73% and 66.6%, respectively. Good conception rates indicated the importance of administering PMSG, shortly after intra-vaginal progestagens sponge withdrawal to obtain a more predictable and compact estrus or ovulation (Cline et al., 2001), as well as due to the hand mating breeding method twice a day in breeding season which help in breeding of high number of ewes. Higher percentage of total single and multiple pregnancy in treated groups were 82.40 and 17.60% respectively (Table 2). This results were disagreed with Al-Hamedawi and Tamer (2013) who recorded 28.6% to 50% twinning rate from the treated groups compared with 12.5% in the control group. These variations are depending on various factors such as breed, age, time and dose of PMSG administration (Dogan and Nur, 2006). Salehi et al. (2010) reported that the different sheep breeds have been identified as a major source of variation in the super ovulatory response.

Nature of Parturition, Fertility Rate, Lambing Rate and Sex of off Spring

Results of table (3) showed statistically significant differences in the sex of the off spring in all groups were recorded 65% which represented male and 35 % female with significant differences ($P<0.01$) related to male, these results were similar to those by Zarkawi (2001) and Al-Hamedawi et al. (2003) who showed significant differences in there ratio and disagreed with finding of Olfati and Moghaddam, (2013) whom obtained non-significant variation in lamb sex ratio. Results of current study showed that there was non-significant difference in eutocia and dystocia percentage among the experimental groups, this result was agreed with Mohammad (2019) who recorded non-significant difference in eutocia and dystocia percentage, and disagreed with AL-Hamedawi and Tamer, (2013) whom obtained 83.3% normal birth and 16.67% dystocia, this indicating that the hormonal treatment (P4, eCG and melatonin) not effect on the nature of lambing of ewes. Lambing and fertility rates was statistically significant ($P \leq 0.01$) in (Vaginal sponges + PMSG) treated group and recorded 125%, as compared with the melatonin and control groups 116.7 and 100% respectively (Table 3). These results were higher than that recorded by Zeleke et al. (2005), they recorded that the lambing and fertility rates were 73% and 70%, when they used intra-vaginal sponge impregnated with 60 mg MAP and injected with 300 IU PMSG before sponge removal, and 300 IU PMSG after sponge removal, respectively. Also, higher than that the reported with Niar et al. (2001); and Kridli et al. (2003),

they recorded that the lambing rates were from 43 to 84% and from 20 to 60%, respectively. However, higher than that the reported with Olfati and Moghaddam, (2013), whom record lambing rate 123.3, 100 and 73.3% respectively and their results of fertility rate record 154.2, 130.4 and 110 respectively in GnRH treated groups as compare with the control. This may be due to the difference in the rate of absorption and metabolism of PMSG between ewes administered PMSG, or difference in dose of MAP and PMSG. Besides the more administration of PMSG, the time of administration relative to intra-vaginal progesterone withdrawal and the routes of administration were observed to be essential to improve the reproductive performance and indicate the importance of administering PMSG to achieve better fertility (Zeleke et al., 2005). As well as, this study was conducted during breeding season.

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