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USING GNRH FOR IMPROVING OVARIAN ACTIVITY AND FERTILITY OF EWES DURING BREEDING SEASON.

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ABSTRACT

The present study aimed to increase lamb production per ewe per year through increasing number of lambing per year by studying the effect of GnRH treatment on 12 days post-partum on ovarian activity and lambing rate (1st experiment) or 12 days post-mating on fertility of ewes (2nd experiment) during the breeding season. Ewes in both experiments were divided into two similar groups (10 ewes in each). The 1st group represented the control group (G1), which were allowed for natural mating without hormonal treatment and ewes in the 2nd one were i.m. injected on day 12 postpartum (1st experiment) or day 12 post-mating (2nd experiment) with 1 ml GnRH analogue (Receptal). Ram of proven fertility was introduced to ewes in both of control and treatment groups from 12 day postpartum up to the first estrus and mating. The pregnancy was diagnosed using ultrasonography after 73 and 88 d post mating. Results show that GnRH treatment of ewes on day 12 postpartum (1st experiment) markedly increased estrous rate to 100% and decreased postpartum period (1st estrus interval) to 22 days as compared to 90% and 30 days for the control group. Mating period reduced to 10.25 d in treated group (13/Nov. to 05/Dec.) vs. 15.33 d in the control group (20/Nov. to 21/Dec.) Based on total number of treated ewes, lambing rate increased to 100% as compared to 80% in the control group, respectively. Sex ratio was significantly ($P<0.05$) different between both groups. The control group yielded 70% male and 30% female lambs, versus 50% male and 50% female lambs in the treated group. Moreover, male or female lambs were heavier ($P<0.05$) in the control than in the treated group. In the 2nd experiment, GnRH treatment of ewes on day 12 post-first mating increased lambing rate to 80% and litter size ($P<0.05$) to 1.62 as compared to 70% and 1.14 in the control group, respectively.

In conclusion, the administration of exogenous GnRH 12 days postpartum or 12 days after mating could increase lambing rate and fertility of ewes during breeding season.

Keywords*:* Ewes, GnRH, postpartum, pregnancy rate.

INTRODUCTION

The postpartum (PP) period in the sheep is very important from a reproductive perspective. It is characterized by the involution of the uterus and return to the ovarian functions to prepare the animal for a new pregnancy period. The duration of postpartum acyclicity varies with body condition, health status, breed, management, season, dystocia and suckling (Rubianes *et al.*, 1996 and Hauser and Bostedt, 2002). Following parturition, the ewe undergoes a period of acyclicity which compromises reproductive efficiency and to obtain an optimum 6-month lambing interval, the ewe must conceive within 35 days of parturition (Peters and Lamming, 1990).

Hypothalamic gonadotropin-releasing hormone (GnRH) and its synthetic analogs cause a release of LH from the anterior pituitary in the

bovine animals. Possible suppressive effects of the involuting uterus may be present in ewes. Lewis and Bolt (1987) demonstrated that removal of the previously gravid uterus hastened the onset of ovarian activity in fall-lambing ewes.

Control of ovarian function and reproductive efficiency in cattle with GnRH has been studied intensively. The main clinical applications of GnRH treatments are post-partum and post-insemination treatments to improve fertility (Gordon, 1996). Treatment with GnRH may assist uterine involution and decrease the postpartum interval to first ovulation (Riek, 1982) and improve reproductive performance of dairy cows (Britt *et al.*, 1977) when it was administrated on 10-28 day- postpartum (Abdel-Khalek *et al.*, 2008). Furthermore, effect of GnRH administration on different days after estrus on reproductive performance was studied by several authors (Arnett *et al.*, 2002; Willard *et al.*, 2003 and Howard *et al.*, 2006)

The present study aimed to increase lamb production per ewe per year through increasing number of lambing per year by studying the effect of GnRH treatment on 12 days post-partum on ovarian activity and lambing rate or 12 days post-mating on fertility of ewes during breeding season.

MATERIALS AND METHODS

This study was carried out at Sakha Animal Production Research Station, belonging to Animal Production Research Institute, Ministry of Agriculture, in cooperation with Department of Animal Production, Faculty of Agriculture, Mansoura University, during the period from October, 2009 to May, 2010.

Animals:

Total of 40 Rahmani ewes during early postpartum (45-61 kg LBW, 3-4 years old and 2-3 Parities) from the flock of Sakha Experimental Station were used in two experiments in this study, 20 in each experiment. The experimental ewes were housed in semi-open sheds in treatment groups. They were fed concentrate feed mixture and roughages according to NRC (2001). The daily feed offered per ewe composed of 1.250 kg concentrate feed mixture (14% CP) and 5 kg Egyptian clover during winter feeding or 1.5 kg clover hay during summer feeding with free access to trace mineralized salt lick blocks and drinking water all time.

Experiment 1:

Ewes in this experiment were divided into two similar treatment groups (10 ewes in each) according to age, body weight and physiological condition as follows. Ewes in the first group represented the control group (G1), which were allowed for natural mating without hormonal treatment. In the second group (G2), ewes were i.m. injected on day 12 postpartum with 1 ml GnRH analogue (Receptal, Intervet International B.V. Boxmeer-Holland). Each ml of Receptal contained 0.0042 mg Buserelin acetate equivalent to 0.004 mg Buserelin. Ram of proven fertility was introduced to ewes in both of

control and treatment groups from 12 day postpartum up to the first estrus and mating.

Experiment 2:

Ewes in this experiment were divided into two similar experimental groups (10 ewes each) according to age, body weight and physiological condition. All ewes were allowed for natural mating and were divided into two groups; (G1) the first group served as control group, in which ewes were allowed for natural mating without hormonal treatment. However, ewes in the second group (G2) were i.m. injected on day 12 post-mating) with 1 ml GnRH analogue (Receptal, Intervet International B.V. Boxmeer-Holland). Each ml of Receptal contained 0.0042 mg Buserelin acetate equivalent to 0.004 mg Buserelin.

Ultrasonography examination:

The ewes were not withheld from food and water before ultrasonography examination. During the examination each ewe was placed in dorsal recumbence. A portable Scanner (480 Vet, Pie Medical, Maastricht, The Netherlands) with a 5 MHz rectal transducer was used. The probe was fitted into rectal rod (3 x 64 cm) and contact gel was applied to the surface of the transducer and rod to provide better contact and lubrication. The rod was inserted into the rectum, 15 or 20 cm deep, to be able to scan the uterine horns. The surface of the transducer was first towards the right ileum to scan the bladder and then it was rotated 120° to 180° clockwise or counterclockwise across the uterine horns to scan the entire pelvic region in each group. In the 1st experiment, the pregnancy was diagnosed using ultrasonography on day 73 and 88 post-mating.

Blood sampling:

Blood samples were taken via the jugular vein from all ewes at 8.30. a.m. into evacuated tubes (10 ml). In the 1st experiment, sampling started twice weekly from 12 days after parturition up to first estrus and mating.

In the 2nd experiment, sampling started twice weekly after mating up to 35 days. Just after sampling, the blood samples were separated to obtain serum by centrifugation of blood at 2500 rpm for 15 min. Serum was packed in labeled plastic tubes and stored at -20^{o}C until assayed later for progesterone concentration.

Progesterone assay:

Progesterone concentration was determined by Radioimmunoassay procedure in samples of selected 5 animals (3 does kidded + 2 does of which did not kid) of each treatment group in each experiment.

Statistical analysis:

Data were analyzed by using Chi-squares for estrus rate and lambing rate to test the significant differences between both groups in the 1st and 2nd experiment.

REULTS AND DISCUSSION

Effect of GnRH treatment on day 12 postpartum: (Experiment 1)

Results presented in Table (1) show that GnRH treatment of ewes on day 12 postpartum markedly increased estrous rate to 100% and decreased postpartum 1st estrus interval to 22 days as compared to 90% and 30 days in the control group, respectively.

The observed decrease in postpartum 1st estrus interval in treated group was associated with reducing mating period to 10.25 days (13/Nov. to 05/Dec.) versus 15.33 days in the control group (20/Nov. to 21/Dec., Table 1).

Table (1): Effect of GnRH treatment on day 12 postpartum during breeding season on estrous rate (%) and postpartum 1st estrus interval of ewes.

Group	N	Ewes in heat		Postpartum 1st estrus interval		Mating period
		n	%	Date	Mean (day)	
G1(Control)	10	9	90	20/Nov.-21/Dec.	30±0.86	15.33±2.28
G2 (treated)	10	10	100	13/Nov.-05/Dec.	22±1.56	10.25±3.14

N: Total number of ewes

Also, GnRH treatment of ewes on day 12 postpartum increased conception rate to 100% based on total number of treated ewes or number of mated ewes (came in heat) as compared to 80% and 88.9% in the control group, respectively (Table 2).

Table (2): Effect of GnRH treatment on day 12 postpartum on estrus and conception rates (%) of ewes.

Group	N	Ewes came in estrus		Conception		
		n	%	n	%(1)	%(2)
Control	10	9	90	8	80[b]	88.9
Treatment	10	10	100	10	100[a]	100

a and b: Means within the same column with different superscripts are significantly different at 5% level. N: Total number of ewes. (1): Based on total number of ewes in each group. (2): Based on number of ewes came in estrus in each group

Moreover, GnRH treatment of ewes on day 12 postpartum increased lambing rate to 100% and litter size to 1.6 in treated group as compared to 80% and 1.25 in the control group, respectively. The differences were significant only in litter size. It is of interest to note that sex ratio was significantly ($P<0.05$) different between both groups. The control group yielded 70% male and 30% female lambs, versus 50% male and 50% female lambs in the treated group. Moreover, male or female lambs were significantly heavier in the control than in the treated group (Table 3).

Table (3): Effect of GnRH treatment on day 12 postpartum on lambing rate (%) and litter size and lambed body weight of ewes.

Group	Lambing		Litter size		Sex ratio (%)		LBW(kg) of lambs	
	n	%	Total	Mean	♂	♀	♂	♀
Control	8	80	10	1.25[b]	70	30	3.75±0.12[a]	3.21±0.14[a]
Treatment	10	100	16	1.60[a]	50	50	3.02±0.16[b]	2.85±0.11[b]

a and b: Means within the same column with different superscripts are significantly different at 5% level.

Such trend in estrous, conception and lambing rates as well as in litter size of treated ewes indicated benefits of GnRH treatment on day 12 postpartum on estrous activity and fertility of ewes during breeding season.

Progesterone profile in blood serum of control ewes indicated elevating P4 level after estrus and service, thereafter this increase continued to prove pregnancy in pregnant ewes (Fig. 1 A). However, P4 level showed stable level behind 1 ng/ml for one month at least in non-pregnant ewes (Fig. 1 B).

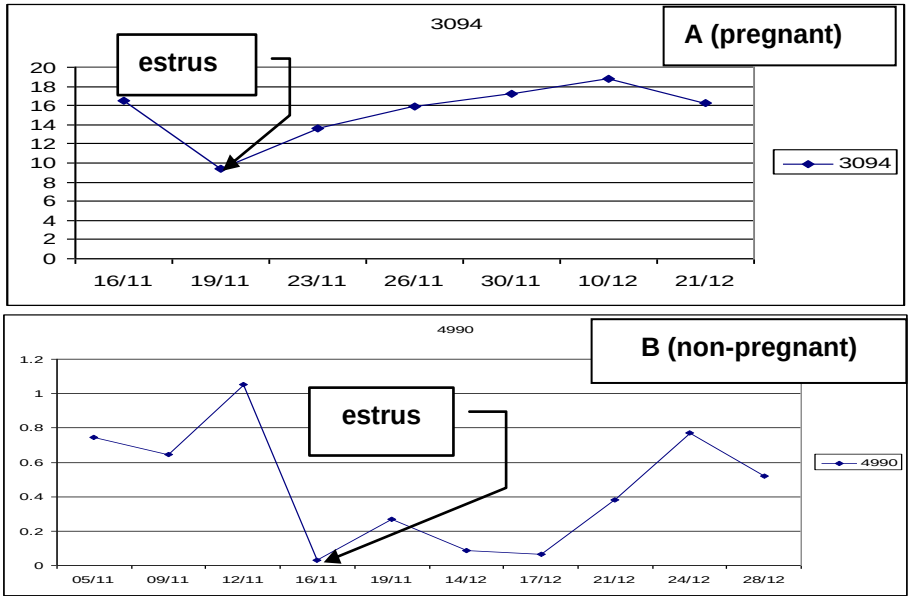

Fig. (1): Progesterone profile in control ewes.

Progesterone profile in blood serum of treated ewes showed marked reduction in P4 concentration at estrus and service, being less than 0.5 ng/ml, then showed marked increase up to 2 weeks, being almost above 1 ng/ml in all pregnant ewes (Fig. 2).

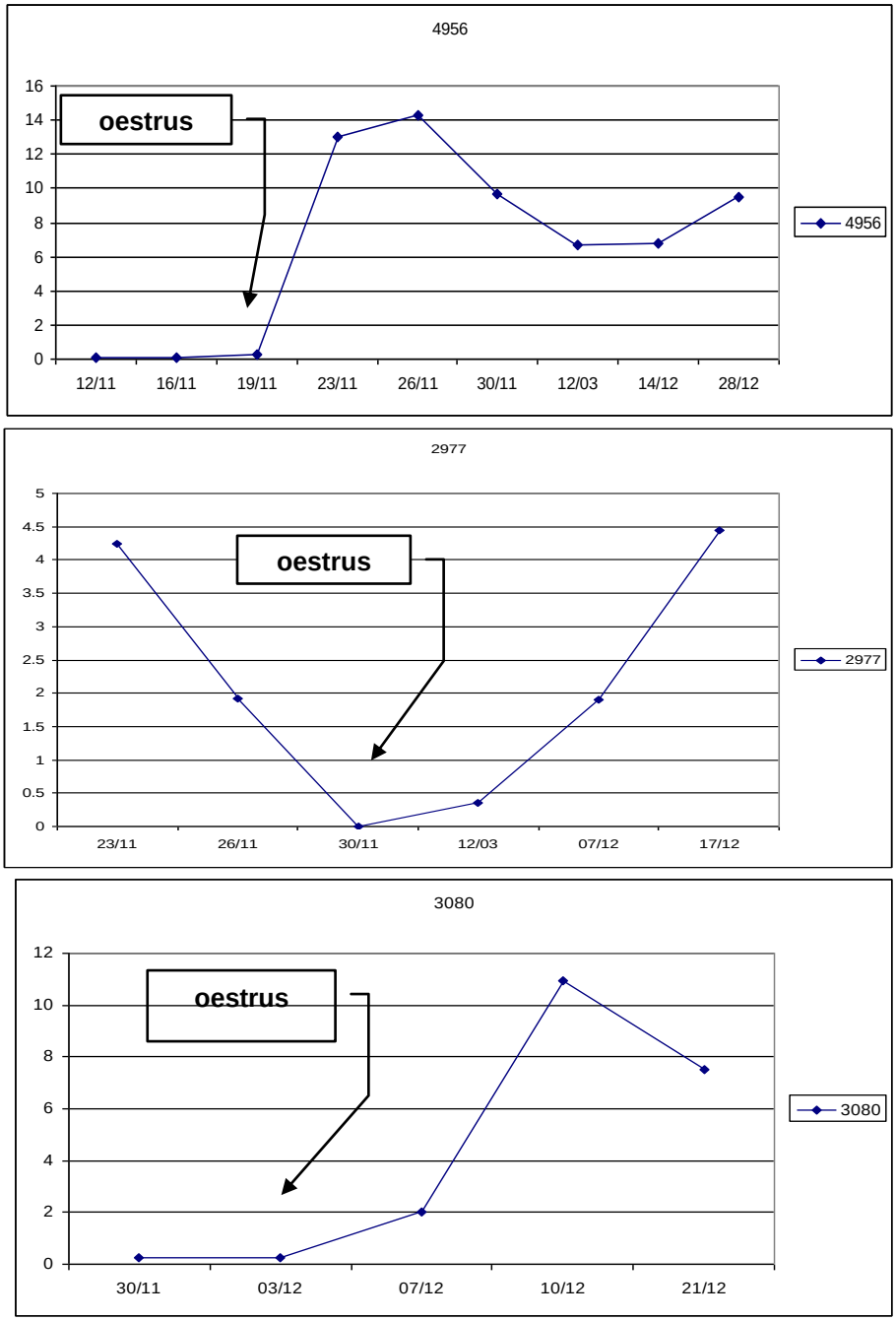

Fig. (2): Progesterone profile in pregnant ewes treated with GnRH on day 12 postpartum.

Ultrasonography examination:

Regarding the ultrasonography examination for pregnancy diagnosis of mated ewes on day 73 or 88 post-mating, the present results in Table (4) revealed the highest accuracy rate on day 88 as compared to day 73 post-mating as indicating by lambing rate in both groups.

Table (4): Pregnancy diagnosis by ultrasonography examination and actual lambing rate of ewes in treated and control group

Group	N	Ultrasonography examination		Actual lambing rate
		73 d post-mating	88 d post-mating	
Control	10	7 (70%)	8 (80%)	8 (80%)
Treatment	10	10 (100%)	10 (100%)	10 (100%)

a and b: Means within the same column with different superscripts are significantly different at 5% level. N: Total number of treated ewes.

The pregnancy was diagnosed, using one or more of following criteria: luminised and fluid filled uterine horns or body of the fetus, fetal heart beats depending on the status of pregnancy (Fig. 3).

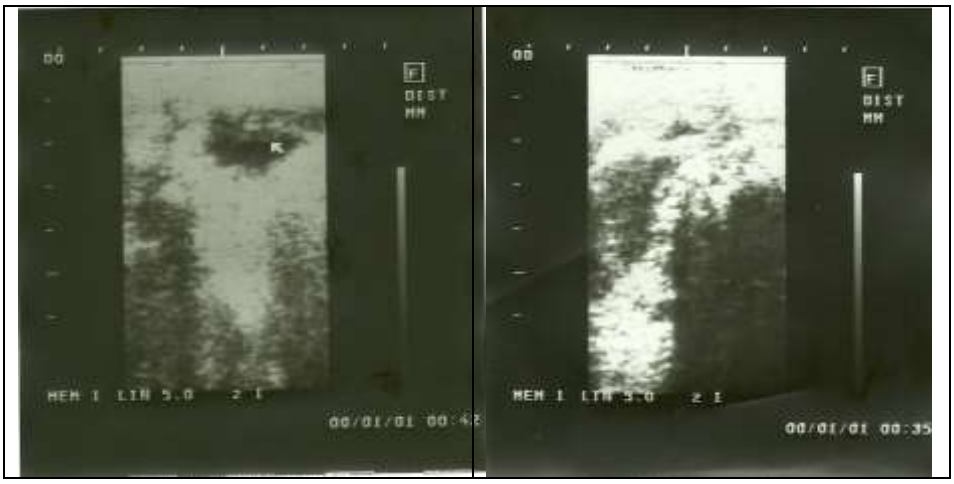

Fig. (3): Ultrasonography examination of mated ewes on day 88 post-mating in treated (A) and control group (B).

Effect of GnRH treatment on day 12 post mating: (Experiment 2)

Results presented in Table (5) show that GnRH treatment of ewes on day 12 post-mating increased lambing rate to 80% and litter size to 1.62 as compared to 70% and 1.14 in the control group, respectively. The differences were significant ($P<0.05$) only in litter size. Sex ratio was nearly similar in both groups, being 37.5: 62.5 in control group and 46.2: 53.8 in treated groups for male : female lambs. However, male and female lambs were significantly ($P<0.05$) heavier in the control group than in the treated group.

Table (5): Effect of GnRH treatment on day 12 post-mating on lambing rate and litter size of ewes as well as sex ratio and live body weight (LBW) of lambs.

Group	N	Lambing		Litter size		Sex ratio (%)		LBW (kg) of lambs	
		n	%	Total	Mean	♂	♀	♂	♀
Control	10	7	70	8	1.14[b]	37.5	62.5	2.70±0.17[a]	3.0±0.12[a]
Treatment	10	8	80	13	1.62[a]	46.2	53.8	2.25±0.17[b]	2.5±0.20[b]

a and b: Means within the same column with different superscripts are significantly different at 5% level. N: Total number of ewes.

The progesterone assay of pregnant ewes in both treated and control group revealed inconsistent trend of change in P4 concentration in control ewes and even in treated ewes.

In the control group, the rise in P4 concentration started on day of estrus/mating up to 11 day post-mating, and began to be stable above 10 ng/ml thereafter (Fig. 4 A). In another case, the rise in P4 concentration started on day of estrus/mating up to 34 day post-mating, and began to be stable above 14 ng/ml thereafter (Fig. 4 B). The differences in P4 concentration between both cases was mainly related to number of CL and was associated with litter size.

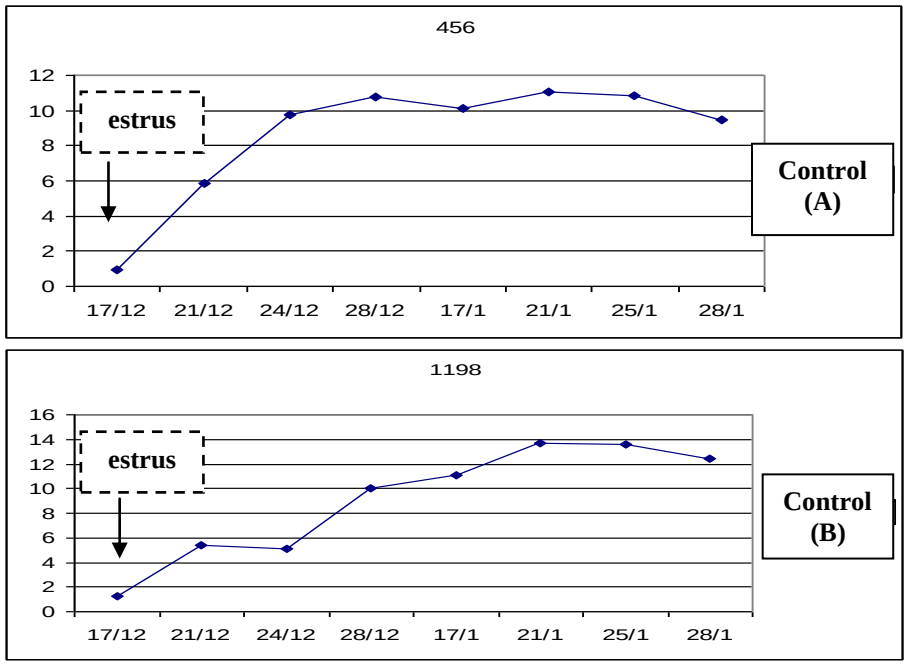

Fig. (4): Progesterone profile in pregnant ewes in the control group during post-mating days.

In the treated group, nearly similar trend of change occurred in P4 concentration up to 12 day post-mating (pre-GnRH treatment) in the control group. However, post-GnRH treatment P4 concentration showed fluctuated trend in one case (Fig. 5 A) and maintained its elevation more than 14 ng/ml in another case (Fig. 5 B). It is of interest to observe that the pronounced increase in P4 concentration in treated ewes was associated almost with higher litter size as compared to the control ewes.

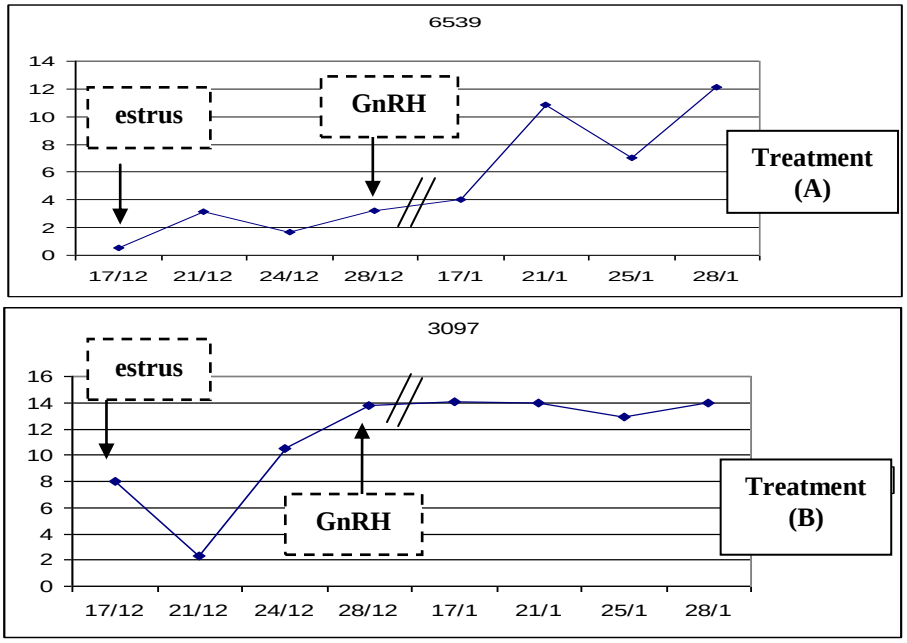

Fig. (5): Progesterone profile in pregnant ewes treated with GnRH on day 12 postpartum.

DISCUSSION

Resumption of cyclic ovarian activity and reestablishment of a proper uterine environment must occur to ensure successful conception postpartum. Postpartum acyclicity in general is characterized by reduced pituitary LH secretion. Early restoration of normal ovarian function may be limited by deficiencies in hypothalamic or pituitary function resulting in failure of release of pituitary LH, which can be successfully interrupted following a low dose GnRH infusion given via the intravenous or subcutaneous routes (McNatty *et al.*, 1988). Several investigators indicated that the time required for complete uterine involution in the sheep varies between 17 and 40 days (Rubianes *et al.*, 1996 and Hauser and Bostedt, 2002) or averaged 33 days (Ainsworth *et al.,* 1982). It is difficult to judge the time of uterine involution in the sheep, because the uterus cannot be examined by rectal or abdominal palpation.

Therefore, treatment with GnRH may reduce the time of postpartum uterine involution in ewes and resumption of ovarian and estrous activity by about 10 days to obtain an optimum 6-month lambing interval (Peters and Lamming, 1990).

Hypothalamic GnRH and its synthetic analogs cause a release of LH from the anterior pituitary in the animal. In agreement with the reducing postpartum 1st estrus interval to 22 d and mating period to 10.25 d in ewes treated with GnRH on day of estrus/mating, Riek (1982) and Gordon (1996) mentioned that GnRH treatment assisted uterine involution and decreased the postpartum interval to first ovulation in cows. Such finding confirmed the ability of exogenous GnRH to promote the expression of estrous behavior in treated ewes. However, ewes in the control group exhibited a more variable pituitary response to the endogenous GnRH during early postpartum period. Within both groups of ewes, plasma P4 concentrations were different in pregnant ewes, but was almost at a normal P4 profile. Normal P4 profiles in ewes induced to ovulate 35 d postpartum was observed by Wallace *et al.* (1992). Corpora lutea (CLs) secrete P4 later with respect to the LH surge and at a lower rate than CLs formed after subsequent ovulations (Schirar *et al.*, 1989). It would also appear that basal and LH stimulated P4 secretion is suppressed in luteal cells collected following the first postpartum ovulation in the ewe (Braden *et al.*, 1989). Therefore, the different plasma P4 levels recorded in the control ewes may be explained by the reduced ovulation rate recorded in these animals and/or the formation of deficient luteal structures in those postpartum ewes which ovulated following GnRH therapy. In addition, it has previously been suggested that low P4 during early embryonic development may cause pregnancy failure and thereby reduce the pregnancy rate (Lucy, 2001). This finding may explained the decreased litter size of the control ewes as compared to those in treated group. Increasing peripheral P4 concentrations at an early stage (day 5) and late stage (day 15) of the estrous cycle may help reduce embryonic death in cattle. High P4 after insemination may enhance embryo development and may suppress luteolysis, ultimately resulting in reduced embryonic loss (Peters *et al.*, 1992 and Mann *et al.*, 1999). In this respect, Abdel-Khalek *et al.* (2008) found increased conception rate of cows administrated with GnRH on day of estrus and insemination.

It was postulated that exogenous GnRH administered after artificial insemination (AI) may cause ovulation and (or) luteinization of an antral follicle(s) resulting in the formation of an accessory CL, thereby increasing blood P4 concentrations, and improving fertility (Howard *et al.*, 2006)

Data from the 2nd experiment regard to GnRH treatment on day 12 post-mating are in accordance with the results of Peters *et al.* (2000), who reported a significant improvement of pregnancy when GnRH was administered 11–14 days after AI. Similarly, Willard *et al.* (2003) observed increased serum P4 concentrations between days 9 and 19 when heat-stressed dairy cows were administered GnRH 5 days after insemination. Also, Abdel-Khalek *et al.* (2008) found significant improvement in pregnancy rate of cows treated with GnRH on day 14 postpartum.

The present results are consistent with the hypothesis that GnRH given on day 5 of the estrous cycle induced ovulation resulting in formation of an accessory CL thereby increasing endogenous P4 concentrations on day 13. This is in agreement with previous report of Howard *et al.* (2006), who found that all cows in the GnRH group developed an additional CL and had greater P4 concentrations by day 13 after AI compared with cows in the saline group. Moreover, Schmitt *et al.* (1996) observed increased P4 concentrations between days 11 and 16 in Holstein heifers administered GnRH 5 days after estrus.

Hulet *et al.* (1956) estimated that embryonic mortality occurring within the first 2 wk in ewes bred by natural service constitutes about two-thirds of total embryo loss. Embryo survival to d 12 is necessary to prolong luteal function, maintain elevated serum progesterone levels and delay a return to estrus (Matral *et al.*, 1979). Loss of pregnancy after the first 2 wk was estimated at 24% for ewe lambs and 9% for adult ewes, based on the difference between the number of ewes pregnant 18 d after AI (as determined by serum P4 concentration) and the number of ewes lambing. The high loss of pregnancy associated with ewe lambs agrees with reports that embryonic loss in lambs contributes substantially to their subfertility (Qurirke, 1979).

In accordance with the present results, Arnett *et al.* (2002) found that administration of GnRH on days 5 or 6 after estrus in beef heifers increased systemic P4 resulting in a 45% greater pregnancy rate in the GnRH-treated group than the control group. Also, Willard *et al.* (2003) indicated that administration of GnRH 5 or 11 days post-insemination tended to improve conception rates of dairy cattle under heat stress conditions. Schmitt *et al.* (1996) observed increased P4 concentrations between days 11 and 16 in Holstein heifers administered GnRH 5 days after estrus.

Conclusion

The administration of exogenous GnRH 12 days postpartum or 12 days after insemination resulted in greater conception rates. Supplemental GnRH during early postpartum or after insemination may be beneficial in improving pregnancy rate during normal breeding season.

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استخدام المعاملة بهرمون الجونادوتروفين لتحسين النشاط المبيضى والخصوبة فى النعاج خلال موسم التلقيح

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تهدف هذة الدراسة الى زيادة محصول المواليد لكل نعجة لكل عام من خلال زيادة عدد الولادات /عام بدراسة استخدام هرمون الجونادوتروفين فى اليوم 12 بعد الولادة على النشاط المبيضى ومعدل الولادات (التجربة لاولى) ، وكذا المعاملة باستخدام هرمون الجونادوتروفين فى اليوم 12 بعد التلقيح فى النعاج (التجربة الثانية) خلال موسم التلقيح. النعاج فى كلا التجربتين قسمت الى مجموعتان متماثلتان (10 نعجة بكل منها). المجموعة الاولى مجموعة ضابطة (ج1) والتى تركت مع الكبش للتلقيح الطبيعى بدون معاملات هرمونية ، والمجموعة الثانية (ج2) تم حقنها فى اليوم 12 بعد الولادة (التجربة الاولى) أو اليوم 12 بعد التلقيح (التجربة الثانية) بـ 1 مل جونادوتروفين (رسبتال) . تم تقديم الكبش لتلقيح النعاج المعاملة فى كلا المجموعتين الضابطة والمعاملة بدءاً من اليوم 12 بعد الولادة حتى ظهور او شبق والتلقيح . تم عمل اختبار كشف الحمل باستخدام جهاز السونار بعد 73 ، 88 يوم من التلقيح فى التجربة الاولى .

اوضحت النتائج ان المعاملة بالجونادوتروفين للنعاج فى اليوم 12 بعد الولادة (التجربة الاولى) زادت من ظهور الشبق بنسبة 100% وقللت فترة ما بعد الولادة (فترة ظهور اول شبق) الى 22 يوم مقارنة بنسبة 90% و 30 يوم فى المجموعة الضابطة. انخفض متوسط الفترة اللازمة للتلقيح الى 10.25 يوم (13 نوفمبر اى 5 ديسمبر) مقارنة بـ 15.33 يوم للمجموعة الضابطة (20 نوفمبر الى 21 ديسمبر). ايضا أدت المعاملة بهرمون الجونادوتروفين فى النعاج فى اليوم 12 بعد الولادة الى زيادة معدل الولادات بنسبة 100% محسوبة على اساس جملة النعاج او النعاج التى جاءت فى الشبق مقارنة بنسبة 80% و88.9% للمجموعة الضابطة على الترتيب. فى التجربة الثانية ادت المعاملة بالجونادوتروفين فى انعاج فى اليوم 12 بعد أول تلقيحة الى زيادة نسبة الخصوبة الى 70% مقارنة بـ 50% فى المجموعة الضابطة.

توصى نتائج الدراسة الى ان حقن النعاج بالجونادوتروفين فى اليوم 12 بعد الولادة أو بعد التلقيح تزيد من معدلات الولادات والخصوبة فى النعاج.

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