Egyptian J. Anim. Prod., 43, Suppl. Issue, Dec. (2006):129-140

ACCELERATING PUBERTY IN RAHMANI EWE LAMBS BORN IN AUTUMN AND WINTER BY GnRH INJECTION

Anas A. Salem[1], S. Fahmy[2] and A. Ali[3]

1- Department of Animal Production, Faculty of Agriculture, University of Assiut, Assiut, Egypt, 2- Department of Animal Production, Faculty of Agriculture, University of Al-Azhar, Assiut, Egypt, 3-Department of Theriogenology, Faculty of Veterinary Medicine, University of Assiut, Assiut, Egypt

SUMMARY

The experiment examined the effect of body weight, season of birth and GnRH injection i.v. (5 µg/kg body weight/animal 2-h intervals for 24 hours) on age at puberty and P_4 concentration in 10 Rhamni ewe lambs six months of age with mean body weight 19.88± 0.78 kg. This study started on June 20, 2001 and continued until the expected time of puberty in the Farm of Animal Production Department, Faculty of Agriculture, Al-Azhar University, Assiut. The ewe lambs were divided at random into two equal groups. The first group included three lambs born in autumn and two ewe lambs born in winter and this group was considered as control (Ctrl.). The second group also involved three ewe lambs born in autumn and two ewe lambs born in winter and this group was treated by GnRH i.v. injection and considered as treated ewe lambs (Tr.). After GnRH injection, two fertile rams were allowed to remain with each group for half an hour/day for estrus detection. All ewe lambs were fed the same ration with 11 % crude protein and 12% ash. Each animal was given 750 g dry matter/daily of the ration which consisted of yellow corn, wheat straw, wheat bran and decorticated cotton seed meal. Green fodder, water and mineral blocks were freely available throughout the experiment. Blood samples were collected from the ewe lambs starting from June 20, 2002 until the expected time of puberty. Blood (5 ml) was drawn from the jugular vein twice a week. Blood serum was separated and stored at –20 ^oC until assayed for P_4. When the P_4 concentration reached the peak for the first time over 1.0 ng/ml blood serum, this was taken as the occurrence of puberty.

Results indicated that the difference between body weights of ewe lambs treated with GnRH and the Ctrl. lacked significance. The overall mean of Rahmani body weight at puberty represented 38-42 % of the adult body weight. There was no significant difference between the two groups in age at puberty. .Most treated lambs displayed higher P_4 concentrations than control lambs. Season of birth had also a highly significant effect ($p<0.01$) on P_4 concentration. The first peak of serum P_4 (over 1.0 ng/ml) concentrations occurred 99 days after the beginning of GnRH treatment, while the second peak of serum P_4 (over 1.0 ng/ml) concentration occurred 90 days after the first peak of P_4. Treated lambs born in winter achieved the first peak of P_4 concentration much earlier than control lambs born in autumn. So, GnRH was more effective in lambs born in winter than those born in autumn in accelerating the time of puberty in Rahmani ewe lambs.

Kew words : *Puberty, Rahmani lambs, body weight, season of birth, GnRH and P_4.*

Issued by The Egyptian Society of Animal Production

INTRODUCTION

Puberty in the female sheep depends on body development, birth date, photoperiod length and management There is relatively little activity of the ovary prior to puberty. At the time of puberty, it begins the distinct functional rhythm known as the estrous cycle. There is evidence that month of birth will subsequently influence the age of puberty. The easiest image of puberty in the female sheep is the completion of the first follicular phase that successfully results in ovulation. Sheep breeds differ in occurrence of their initial reproductive cycle. For example, puberty in Rahmani sheep is between 12-17 mo compared to European breeds such as Rmbouillet, 277 days, Finnsheep, 258 days, Finn-Dorset 260 days, Dorset, 234 days, Suffolk; 245 days (Quirke *et al*., 1985). Age at first estrus is affected by both genetic and environmental factors. Application of exogenous treatments either by infusion, ingestion or injection is successfully used now in farm animals, because of the beneficial effects either in increasing the reproductive rate or in advancing the breeding season. Moreover, it reduces the unproductive period in the ewe's lifetime which extends from weaning to the first estrus (Dickerson, 1970). However, accelerating puberty in Egyptian sheep has received little attention, so the main purpose of this study was the estimation of age at puberty and investigation of the acceleration of the rate to reach puberty in Rahmani ewe lambs.

MATERIALS AND METHODS

A total of 10 autumn and winter-born Rahmani ewe lambs six months of age (with mean body weight 19.88±0.78 kg) were divided at random into two equal groups. The first group included three lambs born in autumn and two ewe lambs born in winter and this group was considered as control lambs (Ctrl.). The second group also involved three ewe lambs born in autumn and two ewe lambs born in winter and this group was treated by GnRH i.v. injection and considered as treated ewe lambs (Tr.). Birth dates and dates of puberty of all ewe lambs are presented in Table (1). Each group was housed in 20 m^2 semi-open pen under the natural daylight length.

Table 1. Dates of birth and puberty ewes

Animal number	Group	Birth date of ewe lamb	Date of puberty
1	Ctrl.	Sept. 26, 2001	Nov. 10, 2002
2	Ctrl .	Dec. 1, 2001	Sept. 30, 2002
3	Ctrl.	Nov. 27, 2001	Oct. 13, 2002
4	Ctrl.	Jan. 15,2002	Estrus was not detected by the ram
5	Ctrl.	Oct. 24, 2001	Died on Nov. 10, 2002
1	Tr.	Nov. 24, 2001	Estrus was not detected by the ram
2	Tr.	Jan. 3, 2002	Sept. 30, 2002
3	Tr.	Oct. 26, 2001	Nov. 17, 2002
4	Tr.	Sept. 1, 2001	Oct. 19, 2002
5	Tr.	Jan. 21, 2002	Nov. 17, 2002

All nutritional and climatic conditions were considered random or free from bias. Management of the ewe lambs was conducted in accordance with recommended commercial practices in the Farm of Animal Production Department, Faculty of

Agriculture, Al-Azhar University, Assiut. Lambs fed the same ration containing 11 % crude protein and 12% ash. Each animal was given 750 g dry matter/day (3% dry matter of its body weight) of the ration which consisted of yellow corn, wheat straw, wheat bran and decorticated cotton seed meal. Green fodder, water and mineral blocks were freely available throughout the experiment. All ewe lambs were weighed in kg once a month for five months.

GnRH was injected intra venous at the rate of 5 µg/kg body weight/animal 2-h intervals for 24 hours by means of an indwelling jugular catheter to treated ewe lambs (Tr.) (Racabarren *et al*., 2000), while the Ctrl group did not receive any treatment. After GnRH injection, two fertile rams were allowed to remain with each group early in the morning at 8:00 a.m. for half an hour for estrus detection. The rams were rotated biweekly between the two groups to avoid the ram effect.

Blood samples were collected from all ewe lambs starting from June 20, 2002 until the expected time of puberty. Blood (5 ml) was drawn from the jugular vein twice a week. Blood samples were kept overnight in the refrigerator at 4 ^{o} C , then blood serum was separated and stored at –20 ^{o}C until assayed for P_4 so as to determine the time of puberty. It was considered that puberty occurred if the P_4 concentration rose to the peak for the first time over 1.0 ng/ml blood serum (Suttie *et al*., 1991 and Al-Mauly *et al*., 1991).

Progesterone (P_4) assay

P_4 was assayed by enzyme immunoassay (ELISA) described by DSL (2000).

Data were analyzed using analysis of variance with SPSS for MS Window Release 6.0. Body weight, age at puberty and P_4 profile are dependent variables , while the main effects in the model are GnRH injection, the interval from start of GnRH injection to the expected time of puberty and season of birth. Significance was taken at ($p<0.05$ and $p<0.01$).

RESULTS AND DISCUSSION

Body weight:

Estimates of mean body weight of all ewe lambs throughout the experiment are presented in Table (2). The difference between body weights of ewe lambs treated and the Ctrl. lacked significance.

Table 2. Overall mean of monthly body weight development throughout the course of study.

Group	Monthly body weight in kg				
	Jun.	**July**	**Aug.**	**Sept.**	**Nov.**
Ctrl.	20.88±0.86 (n=5)	20.88±1.07 (n=5)	22.0±1.41 (n=5)	24.75±2.08 (n=5)	24.75±3.06 (n=5)
Tr.	18.88±0.69 (n=5)	20.25±0.76 (n=5)	20.75±0.76 (n=5)	22.50±1.06 (n=5)	25.75±1.55 (n=4)

n=number of animals.

There is a gradual increase in the mean body weight in the interval from June to November (near the time of puberty). At the time of puberty, treated lambs had an advantage of 1.0 kg over those in the Ctrl. This increase may be due to the difference in birth weight or to the effect of GnRH dose. Yamasaki *et al*. (2001) indicated that GnRH increased the percentage of body fat and the trunk-leg fat ratio, but decreased

lean mass deposit with no change in body weight. The reason why GnRH treatment induces body fat distribution is the complete suppression of ovarian steroids. Estrogen deficiency by GnRH may influence gluteofemoral adiposity with the pubertal onset of ovarian function (Rebuffe-Scribe *et al*., 1986). The present results indicated that the average body weight at puberty of treated and control ewe lambs represented 38.0-42.0 % of the adult body weight (on basis of adult body weight in Rahmani female is between 60.0 and 65.0 kg). Results obtained by Hafez (1952) support these findings. He indicated that attainment of puberty is more closely related to body weight than to age, Romney ewes reached puberty when the body weight is 40% of the adult weight.

Age and time at puberty

Age at first estrus was relatively lower in the treated lambs (340.5±20.07 days) compared to those in the Ctrl. (341.5±34.19 days) (Table 3). There was no significant difference between the two groups in age at puberty, but season of birth had a significant effect ($p<0.05$) on age of puberty. Ewe lambs born in winter resulted in earlier puberty (287.3±13.1.days) than those born in autumn (381.00±22.5.days).

Table 3. Age at puberty (days) in Ctrl. and Tr. ewe lambs born in autumn and winter

	Age at puberty in days		
	Ctrl.	Tr.	**Overall mean**
Season of birth	341±34.2	340±20.1	341±26.1
Overall	(n=3)	(n=4)	(n=7)
Autumn	362±38.1	400±28.4	381±22.5
	(n=2)	(n=2)	(n=4)
Winter*	299±	282±16.0	287±13.1
	(n=1)	(n=2)	(n=3)

n = number of animals in the experiment

Papachristoforou *et al*. (2000) indicated that the age and live weight at puberty in autumn-born Chios ewe lambs were higher ($P<0.01$) than in February-born ones, the respective values being 33 weeks and 51.1 kg versus 29.9 weeks and 42.0 kg. Furthermore, in Damascus she-kids born in autumn or in February, onset of puberty was at the same time in the following autumn (Papchristoforou *et al*., 2000). Foster and Ryan (1979) reported that in lambs born in late winter and spring, puberty occurred in autumn between 25 and 35 weeks of age. Hafez and Scott (1962) indicated that ewe lambs differed in their ages at puberty between 6-16 months and this variation was attributed to climate and season of birth. The present results indicated that all the control and treated lambs born in winter, puberty occurred in the next autumn, whereas those born in autumn , puberty occurred in the next autumn (Table 1). These results are consonant with that reported by Geithovel *et al*. (1985) who indicated that pulsatile GnRH application can induce ovarian hyperstimulation. Pirl and Adams (1987) indicated also that Circhoral administration (250 ng/h i.v.) of GnRH was effective in inducing precocious puberty in ewe lambs. Foster *et al*., (1983) indicated that a deficit in GnRH production may delimit the prepubertal

development in primates. Laws *et al*. (2003) observed that i.v. injection of GnRH restored the estrogen-induced secretion of LH in the pubertal development of female Wister rats. Therefore, exogenous administration of GnRH has a potential effect for induction of precocious puberty especially in ewe lambs born in winter.

Progesterone profiles

Results in Table (4) indicate that there are highly significant differences ($P<0.01$) among individuals within each group in P_4 concentrations. Treated lambs displayed higher P_4 concentrations than control lambs (Figure 1). During the interval from start of GnRH treatment to the expected time of puberty, P_4 concentration was less than 1.0 ng/ml blood serum in all the lambs except one lamb of the treated group which showed P_4 concentration over 1.0 ng/ml (Figure 1). The overall mean of P_4 concentrations in control and treated lambs and in the ewe lambs born in autumn and winter are shown in Figure (2). Season of birth had also highly significant effect ($p<0.01$) on P_4 concentration (Table 5). This table reveals that there are two peaks of P_4 concentration (over 1.0 ng/ ml blood serum) that occurred in all the lambs. The first peak of P_4 concentrations occurred 99 days after beginning of GnRH treatment (from June 20, 2002 to September 28, 2002), while the second peak of P_4 concentration occurred 90 days after the first peak of P_4 concentration (from September 28, 2002 to December 27, 2002) (Table 5). Furthermore, magnitudes of P_4 peak concentration in treated lambs (1.34±0.37 ng/ml and 1.77±0.57.ng/ml) were greater than control lambs (1.09 ±0.20 ng/ml and 1.0±0.32 ng/ml). This increase of P_4 concentration can reflect its early effect on the ovarian activity and CL formation. These findings are similar to those reported by Suttie *et al*. (1991) who indicated that puberty was defined as the date when plasma of P_4 concentration first exceeded 1.0 ng/ml. McNatty *et al*. (1998) indicated that pubertal development was defined in ewe lambs from behavioral estrus and from P_4 concentration. Season of birth has a strong significant effect on P_4 concentration, especially with ewe lambs treated with GnRH injection (Figure 3). Furthermore, treated lambs born in winter achieved earlier first peak of serum P_4 concentration in August, 2002 than control lambs born in autumn which produced the first peak of P_4 concentration in September, 2002 (Table 6). So, GnRH was more effective in lambs born in winter than those born in autumn. In this study, puberty was attained at a significantly earlier age in the treated ewe lambs born in winter compared to the control lambs born in autumn. P_4 concentration may have a physiological role in the endocrine mechanism governing transitions from acyclic to cyclic states and that a transient increase in P_4 concentration ensures LH surge to initiate an estrous cycle in anestrous ewe (Legan *et al*., 1985). McLeod *et al*., (1991) indicated that administration of GnRH given either intermittently i.v. (2.0 µg/injection at 2-h intervals) for 4 days or continuously s.c. infusion (1.0 µg/h) for 14 days induced ovulation in a proportion of seasonally anoestrous Pere David's deer.

Table 4. Mean ± SEM of blood serum P_4 concentration in individuals within each group of Ctrl. and Tr. ewe lambs

# Animal	Group of animals	Number of observations	P_4 concentration (ng/ml) blood serum
1	Ctrl**.	26	0.78±0.13
2	Ctrl**.	26	0.62±0.09
3	Ctrl**.	17	0.84±0.25
4	Ctrl**.	17	0.38±0.16
5	Ctrl.**	Not detected	
Total	Ctrl.	**86**	**0.66±0.8**
1	Tr.**	24	0.57±0.11
2	Tr.**	18	0.81±0.21
3	Tr.**	23	0.65±0.11
4	Tr.**	17	1.26±0.31
5	Tr.**	No detected	.
Total	Tr.	**82**	**0.82±0.09**

** significance among individuals within each group was high ($p<0.01$)

Table 5. Level of P_4 , ng/ml blood serum, in the interval from start of GnRH treatment (June, 2002) until the expected time of puberty (December, 2002) in Ctrl. and Tr. ewe lambs

Group of animals	Month of blood collection						
	June	July	August	September	October	November	December
Control	0.30±0.02 (n =13)	0.40±0.07 (n=25)	0.91±0.37 (n=9)	1.09±0.20 (n=15)	0.58±0.06 (n=9)	0.86±0.24 (n=12)	1.0±0.32 (n=3)
Treatment	0.61±0.21 (n=14)	0.38±0.04 (n=22)	0.86±0.25 (n=11)	1.34±0.37 (n=16)	0.81±0.31 (n=7)	0.50±0.18 (n=8)	1.77±0.57 (n=4)

n=number of observations.

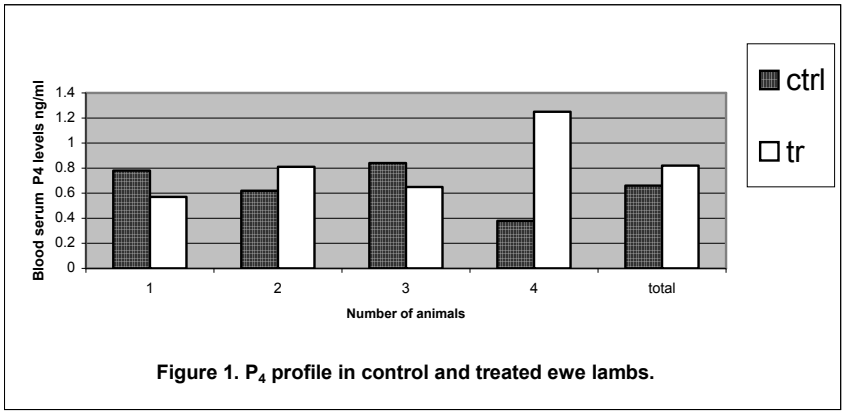

Figure 1. P_4 profile in control and treated ewe lambs.

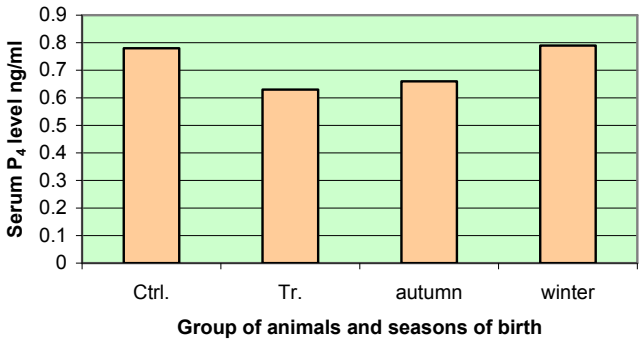

Figure 2. The overall mean of P_4 level in all lambs and ewe lambs born in autumn and winter.

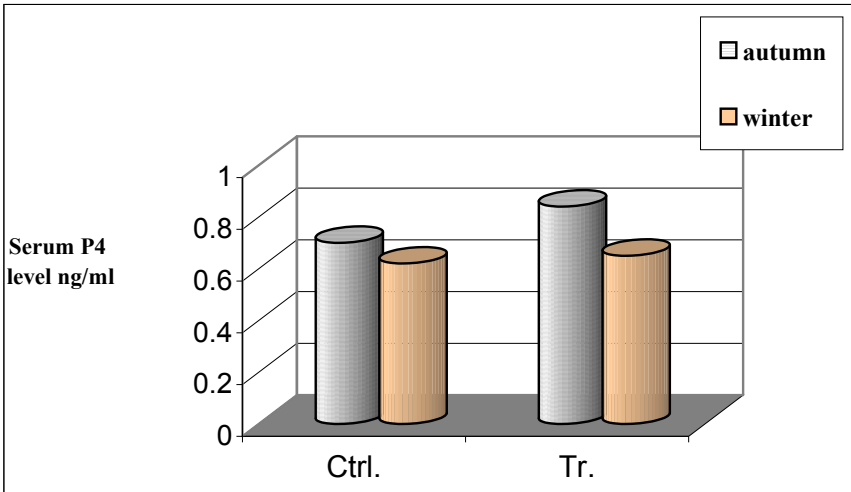

Figure 3. Effect of season of birth on the overall mean of P_4 concentrations in control and treated ewe lambs

Table 6. Effect of season of birth on the average blood serum P_4 concentration in the interval from start of GnRH treatment (June, 2002) until the expected time of puberty (December, 2002) in Ctrl. and Tr. ewe lambs

Season of birth	Months of blood sera collection													
	Jun.		Jul.		Aug.		Sept.		Oct.		Nov.		Dec.	
	Ctrl.	Tr.	Ctrl..	Tr.	Ctrl..	Tr.	Ctr.l.	Tr.	Ctrl..	Tr.	Ctrl..	Tr.	Ctr.l.	Tr.
Autumn	0.30 (n=7)	0.68 (n=10)	0.52 (n=12)	0.38 (n=15)	0.93 (n=4)	0.74 (n=7)	1.19 (n=8)	1.51 (n=12)	0.38 (n=5)	0.88 (n=6)	0.90 (n=6)	0.50 (n=6)	1.50 (n=1)	1..85 (n=3)
Winter	0.30 (n=6)	0.45 (n=4)	0.30 (n=13)	0.36 (n=7)	0.90 (n=5)	1.08 (n=4)	0.97 (n=7)	0.85 (n=4)	0.83 (n=4)	0.40 (n=1)	0.83 (n=6)	0.50 (n=2)	0.75 (n=1)	1.50 (n=2)

n= number of observations

It could be concluded of the study that there was no significant difference between body weights of ewe lambs treated with GnRH and the Ctrl.. Ewe lambs born in winter in both groups exhibited earlier puberty than those born in autumn . Furthermore, most treated lambs displayed higher P_4 concentrations than control lambs. Treated lambs born in winter achieved the first peak of P_4 concentration much earlier than control lambs born in autumn. So, GnRH was more effective in lambs born in winter than those born in autumn in the acceleration of puberty in Rahmani ewe lambs.

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تسريع البلوغ الجنسى فى حملان إناث الرحمانى المولودة فى الخريف والشتاء بواسطة الحقن بالجونادوتروبين

أنس أحمد العربى سالم[١] ، سالم فهمى[٢] ، أحمد مصطفى[٣]

[١] قسم الانتاج الحيوانى –كلية الزراعة–جامعة أسيوط
[٢] قسم الانتاج الحيوانى–كلية الزراعة–جامعة الأزهر–فرع أسيوط
[٣] قسم الولادة وأمراضها–كلية الطب البيطرى–جامعة أسيوط

قحصت التجربة تأثير وزن الجسم وموسم الولادة وحقن الجونادوتروبين فى الوريد بمعدل (٥ ميكروجرام/كجم وزن جسم/حيوان/ساعتين لمدة ٢٤ ساعة) على البلوغ الجنسى وتقدير تركيز هورمون البروجيستيرون فى ١٠ حملان إناث رحمانى عند عمر ٦ شهور بمتوسط وزن الجسم ١٩.٨٨ ± ٠.٧٨ كجم. بدأت الدراسة فى ٢٠ يونيو ٢٠٠١ واستمرت التجربة لغاية الوقت المتوقع لحدوث البلوغ الجنسى وقد أجريت هذه التجربة فى مزرعة قسم الانتاج الحيوانى–كلية الزراعة–جامعة الأزهر–أسيوط. قسمت الحيوانات عشوائيا إلى مجموعتين متساويتين فى العدد فاشتملت المجموعة الأولى على ثلاثة حملان ولدت فى الخريف ، واثنتين ولدتا فى الشتاء واعتبرت هذه المجموعة الكونترول، أيضاً تضمنت المجموعة الثانية على ثلاثة حملان ولدت فى الخريف ، واثنتين ولدتا فى الشتاء واعتبرت هذه المجموعة المعاملة بالجونادوتروبين. و بعد الحقن بالجونادوتروبين سمح بدخول كبش خصب على كل مجموعة لمدة نصف ساعة يومياً لاكتشاف الشياع. غذيت الحملان على عليقة غذائية واحدة بها ١١% بروتين ، ١٢% رماد وقد أعطى كل حيوان ٧٥٠ جرام مادة جافة يومياً من هذه العليقة المكونة من ذرة صفراء ، تبن قمح ، ورده ، ومسحوق بذرة القطن الغير مقشور. بالإضافة إلى إتاحية العلف الأخضر والماء وقوالب الأملاح المعدنية التى وضعت أمام الحيوانات طول فترة التجربة. بعد ذلك تم أخذ عينات الدم من جميع الحيوانات من بداية التجربة لغاية الوقت المتوقع لحدوث البلوغ الجنسى. وقد سحب من كل حيوان ٥ ملى دم عن طريق وخز الوريد الودجى مرتين/أسبوع ثم فصلت العينات بواسطة الطرد المركزى للحصول على مصل الدم. ثم حفظت العينات بعد ذلك تحت –٢٠ درجة مئوية لحين تقدير تركيز هورمون البروجستيرون. وقد أخذ من هذا التقدير قاعدة أى عندما يرتفع تركيز البروجستيرون لأول مرة إلى أكثر من ١.٠ نانوجرام/ملى مصل دم دل ذلك على حدوث البلوغ الجنسى.

بينت النتائج أنه لا يوجد هناك فرق معنوى بين المجموعتين فى وزن الجسم وأن متوسط وزن الجسم عند البلوغ الجنسى كان يمثل ٣٨–٤٢% من وزن الجسم الناضج. كذلك لا يوجد فرق معنوى بين المجموعتين فى العمر عند البلوغ الجنسى. وجد أيضاً أن هناك فروقا معنوية كبيرة بين الأفراد داخل كل مجموعة فى تركيز البروجستيرون. وقد اتضح من التقدير أيضاً أن معظم الحملان المعاملة بالجونادوتروبين كان لها تركيزات عالية فى البروجيستيرون مقارنة بالكونترول. وأن موسم الولادة كان له تأثير معنوى ($p<0.01$) على تركيز البروجيستيرون. وقد لوحظ أن أعلى تركيز للبروجستيرون لأول مرة حدث بعد بداية الحقن بالجونادوتروبين بـ ٩٩ يوم وثانى ارتفاع له كان بعد ٩٠ يوم من إرتفاعه فى المرة الأولى. بينت التجربة أيضاً أن الحملان المولودة

فى الشتاء حققت تركيوات عالية فى البروجيستيرون عند ارتفاعة لأول مرة بالمقارنة بحملان الكونترول المولودة فى الخريف. مما سبق يتضح أن المعاملة بالجونادوتروبين له تأثيره الفعال فى الحملان المولودة فى الشتاء عن المولودة فى الخريف فى إسراع أو تبكير البلوغ الجنسى فى حملان إناث الرحمانى.