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Reproductive Performance of Rahmani, Romanov and their Crosses Ewes in Reference with Progesterone Concentration

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ABSTRACT

This study was carried out to evaluate the relation among progesterone (P4) concentration, number and diameter of corpora lutea, with pregnancy in three ewe breeds. Total of 61 ewes (25 Rahmani, 11 Romanov and 25 crossbred, Rahmani x Romanov) were ultrasonographic examined after natural mating up to 21 days to determined number and diameter of corpora lutea. Blood samples were collected from 8 ewes per group (4 pregnant and 4 non-pregnant) prior to ultrasound scanning to assay P4 concentration. Results revealed that, average of CLs diameter and P4 concentration were the highest ($P<0.05$) in of Romonov (8.04 mm and 4.41 ng/ml), but were insignificantly higher in crossbred (7.48 mm and 3.5 ng/ml) than in Rahmani (5.52 mm and 3.43 ng/ml). Mean number of CL was not affected significantly by ewe breed. Mean number and diameter of CLs and P4 concentration were the higher ($P<0.01$) in pregnant than in non–pregnant ewes. The interaction between ewe breed and pregnancy status on all traits was not significant. A positive significant correlation was found between each of number and diameter (mm) of CLs and serum P4 concentrations was recorded. Conclusion, results of this study indicated that the differences in progesterone concentration among ewe breeds are in relation with pregnancy status, depending on number and diameter of corpora lutea.

Keywords: Ewes, progesterone, corpora lutea, pregnancy.

INTRODUCTION

Ovulation rate and embryo mortality as reproductive output in sheep are in relation with genotypic and nutritional factors (Lindsay *et al*. 1975). Australian Merino genotype show low fertility and prolificacy (Kleemann and Walker 2005) and achieve their genetic potentiality when they are maintained at appropriate body condition in season of breeding (Robinson *et al*. 2002).

Several factors are affecting embryo survival, of them luteal function is the highest factors (Wilmut *et al.,* 1986). In sheep, embryonic loss ranged between 20 and 30%. Prolificacy may be decreased if there is a marked partial loss of multiple ova in pregnant females. Embryo death includs fertilization failure, observable genetic damage, and idiopathic loss (Willingham *et al.,* 1986; Kleemann *et al.,* 2005).

Progesterone (P4) is an important hormone to maintain pregnancy (Foote *et al.,* 1989) and has functions of the blastocyst is to ensure counteraction of the uterine luteolytic mechanism (Bazer 1989). The normality in steroid balance induces synchrony between the uterus and embryo. Disturbance in this balance may result in alteration of growth rate of embryo relative to uterine timing (Lawson and Cahill, 1983; Lawson *et al.,* 1983). This could be viewed as a mechanism to re-establish synchrony, but generally asynchrony lead to embryonic loss (Wilmut *et al.,* 1986; Lawson *et al.,* 1983).

Luteal dysfunction, which culminates in inadequate synthesis and secretion of P4, is one of the main causes of embryonic death in mammals. The corpus luteum (i.e., adequate P4 levels) is indispensable for the recognition and maintenance of pregnancy in mammals. Its absence causes the interruption of pregnancy at any phase in goats (Meites *et al*., 1951).

Similarity in CL diameter and P4 concentration was indicated in the synchronized and subsequent estrus cycles. However, the mean CL diameter and P4 concentrations were significantly higher in the synch groups than in the control group (Bukar *et al.,* 2012). Many authors recorded a significant positive correlation between CL diameter and concentration of P4 in goats (Orita *et al.,* 2000; Chao *et al.,* 2008) and plasma P4 concentration was higher in goats with two CLs as compared to those having only one CL (Simoes *et al.,* 2007).

Therefore, aim of this study to evaluate the relationship among serum progesterone concentration and each of number and diameter of corpora lutea, and pregnancy in three ewe breeds under the Egyptian conditions.

MATERIALS AND METHODS

The experimental work of this study was conducted at Experimental Station, Mehalet-Mosa, Kafrelsheikh governorate, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

Animals:

Total of 61 ewes (25 Rahmani, 11 Romanov and 25 Rahmani x Romanov) with an average age of 4.54±0.47, 4.67±0.59 and 3.35±0.13 years and live body weight of 50.36±1.72, 47.45±2.94 and 50.24±1.64 kg were used in this study, respectively. Ewes were fed concentrate feed mixture (CFM) containing 15% CP and 70% total digestible nutrients (TDN), plus 1.25 kg rice straw. Ingredients of the CFM included 45.3% corn grains, 11% undecorticated cotton seed, 12% soybean meal, 29% wheat bran, 1.8% limestone, 0.22% sodium bicarbonate, 0.4% common salt and 0.28% mineral mixture. Ewes had free access to water all the day.

Ultrasonography and blood sampling:

Ovaries of all ewes were examined by transrectal ultrasonography once weakly using a real-time B-mode ultrasound scanner (Aloka, 500 SSD, Japan), with a transrectal 7.5-MHz linear array probe (UST-660-7.5 model) after natural mating up to 21 days post-mating during the breeding season for determining pregnancy as well as number and diameter of corpora lutea (CLs) on days 7, 14 and 21 post-mating. On these days, blood samples (5 ml) were collected from 8 ewes in group (4 pregnant and 4 non-pregnant) prior to ultrasound scanning. Blood samples were taken via the jugular vein into vacutainer tubes, allow to coagulate and centrifuged at 3000 rpm for 15 min to isolate serum, which was stored at -20°C until assay of progesterone (P4).

The maximum CL diameter was calculated as the average of 3 successive daily recordings on days 7, 14 and 21 post-mating.

Progesterone assay:

Concentration of P4 in blood serum was determined by direct radioimmunoassay (RIA) technique using ready antibody coated tubes kit (Diagnosis Systems Laboratories, Texas, USA) according to the procedure outlined by the manufacturer.

The cross reaction of P4 antibody (at 50% binding), was 100% with P4, while was 6.00, 2.50, 1.20, 0.80, 0.48, and 0.10% with 5α-pregnone-3, 20-dione 11-Deoxycorticosterone, 17α-Hydroxyprogesterone, 5β-pregnone-3, 20-dione 11-Deoxycortisol, and 20α-Dihydroxyprogesterone, respectively and less than 0.1% with any of the other steroids, according to the manufacture's information,.

The standard curve of P4 ranged from 0.0 to 60.0 ng /ml. The sensitivity value was reported to be 0.12 ng /ml. The intra- and inter-assay coefficients of variation were 8.0 and 13.1%, respectively.

Statistical Analysis

The obtained results were statistically analyzed by ANOVA as a factorial design using program of SAS (2004). Mean significant differences were tested by Duncan's Multiple Range Test procedure (Duncan, 1955) at P<0.05 level.

RESULTS AND DISCUSSION

Pregnancy, parturition rate and Litter size

Results in Table 1 showed significant differences (P<0.05) in pregnancy and parturition rates as well as litter size among breed groupzs. Pregnancy rate was significantly (P<0.05) higher for Romonov (81.82%) than crossbred (65.38%) and Rahmani ewes (61.29%). Parturition rate was significantly (P<0.05) the highest for Rahmani (100%), followed by crossbred (64.71%) and the lowest for Romonov (44.44%). Meanwhile, litter size was significantly (P<0.05) the higher for Romonov (2.25 lambs/ewe) and crossbred (1.55 lambs/ewe) than for Rahmani (1.32 lambs/ewes).

Table 1. Pregnancy and parturition rates as well as litter size of Rahmani, Romonov and crossbred ewes.

Item	Rahmani	Romonov	Crossbred
Number of mated ewes	25	11	25
Number of pregnant ewes	19	9	17
Pregnancy rate	61.29[b]	81.82[a]	65.38[b]
Parturition rate (%)	100 (19/19)[a]	44.44 (4/9)[c]	64.71 (11/17)[b]
Litter size	1.32±0.11[b]	2.25±0.25[a]	1.55±0.21[a]
Number of ewes with embryonic loss	0	5	6
Rate of embryonic loss	0	56	35

Means denoted within the same row with different superscripts are significantly different at P<0.05.

These results indicated superiority of the foreign breed (Romanove) as compared to local Rahmani breed in most reproductive traits of sheep. On the other hand, crossing the local breed (female Rahmani) with (Romanve male) showed marked improvement in reproduction of their crosses. Generally, there is a wide variety of ewe breeds, each with different reproductive capabilities that have the potential to improve flock or band performance. Examples of reproductive traits include extended breeding season (Dorset, Polypay), early onset of puberty (Finnish Landrace, Polypay), increased ovulation rate (Finnish Landrace, Romanov), and environmental suitability (Scottish Blackface, Navajo Churro) (Buckrell, 1987). Ovulation rates differ widely among species and breeds within a given species: in sheep for example, Merinos d'Arles or Ile-de-France breeds have only one ovulation per cycle, whereas average rates from 2 to 4 ovulations/cycle in prolific breeds (Romanov or Finn) were reported by Land *et al.* (1993). In this respect, Bouix *et al.* (2002) suggested that crossbred ewes got the best maternal results from both adaptive genes (from hardy Romanov origin) and heterosis effect, confirming the advantage of F1 or Mule ewes being more productive than local breed ewes.

Although Romanov ewes showed the best reproductive performance, they showed the highest rate of embryonic loss (56%) versus 0% in Rahmani ewes. This finding may indicate that Romanov ewes had no adaptability in term of environmental or managerial factors under the Egyptian condition.

Characteristics of CLs and P4 profile:

In association with improving the reproductive performance, Romanov ewes showed the highest number and diameter of corpora lutea as compared to their crosses and Rahmani ewes, respectively. However, the differences were significant only for number of CLs. Such trend reflected significantly (P<0.05) higher P4 concentration in Romanov than in Rahmani and crossbred ewes. Also, the same trend was observed in pregnant as compared to non-pregnant ewes (Table 2).

The insignificant effect of interaction between breed and reproductive status on number and diameter of CLs as well as P4 concentration was reflected in higher values in pregnant than in non-pregnant ewes within each breed, being the highest in Romanov ewes (Figs. 1 and 2).

Progesterone is essential for the maintenance of pregnancy. Cahill *et al*. (1981) found that overall profile of P4 was a direct reflection of the differences in ovulation rate and cycle length between breeds, the highest concentration measured and the mean concentration on days from 4 to 13 were higher in the Romanov than in Ile-de-France ewes (P<0-05). If an animal does not produce sufficient P4 levels, the pregnancy will be lost. There are two major reasons for a lack of P4. The first category includes corpora lutea (CLs) that have a short lifespan. Thus, luteolysis occurs before the embryo has time to signal its presence through secreting bovine trophoblast protein-1 bTP-1 which alters the production of prostaglandins and prevents luteolysis from occurring. The second category includes those CLs that have a normal lifespan but secrete low P4 levels, which does not suppress the luteolytic affects of the prostaglandins (Robinson *et al.,* 1989).

Plasma P4 concentration reveled differences between breeds, Romonov breed were higher than in (Rahmani and Crossbred) breeds either pregnant or non-pregnant. These findings in agreement with (Fonseca and Torres, 2005), who found higher plasma P4 concentration

in Saanen than in Alpine nulliparous goats. In addition, the relationship between P4 level and CLs diameter was detected in goats. The average diameter of CLs and P4 level were significantly (P<0.05) higher in the synch than in control group (Bukarl *et al.,* 2012) and more double ovulations and the CLs were larger in the PGF2α, synch than in naturally cycling goats (Vazquez *et al*., 2010).

Table 2. Number and diameter (mm) of corpora lutea, and serum progesterone concentration (ng/ml) as affected by ewe breed, reproductive status and hier interaction.

Item	P4 concentration (ng/ml)	Corpora lutea number	Corpora lutea Diameter (mm)
Breed:			
Rahmani (Rah)	3.42±0.59^{b}	1.25±0.16	5.52±0.59^{b}
Romonov (Rom)	4.41±0.78^{a}	1.75±0.25	8.04±1.08^{a}
Crossbred (Rah x Rom)	3.50±0.59^{b}	1.75±0.25	7.48±1.04^{ab}
Reproductive status :			
Pregnant	5.36±0.33^{a}	1.83±0.21^{a}	8.36±0.78^{a}
Non-pregnant	2.19±0.14^{b}	1.33±0.14^{b}	5.67±0.62^{b}
Interaction between breed and reproductive status:			
Rah x pregnant	4.85±0.43	1.5±0.29	6.33± 0.93
Rah x Non- pregnant	1.99±0.14	1.0±0.0	4.71±0.58
Ro x pregnant	6.24±0.74	1.75±0.48	8.75±1.69
Ro x Non- pregnant	2.58±0.25	1.75±0.25	7.33±1.51
Cro x pregnant	4.99±0.26	2.25±0.25	10.0±0.78
Cro x Non- pregnant	2.01±0.21	1.25±0.25	4.96±0.44

Means denoted within the same column with different superscripts are significantly different at P<0.05

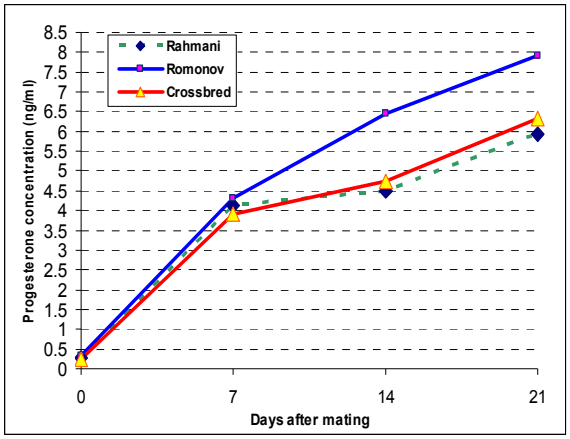

Fig. 1. Plasma progesterone concentration (ng/ml) in pregnant of three ewe breeds.

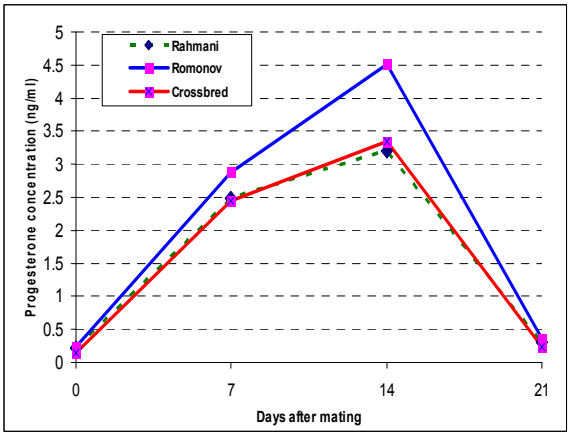

Fig. 2. Plasma progesterone concentration (ng/ml) in non-pregnant of three ewe breeds.

According to our results, higher P4 level was a result of active embryos that promote recognition and establishing of pregnancy and maintenance of corpus luteum function. As previously reported by Engeland *et al.* (1999), the normal P4 level in goats with foetal loss indicated that the function of the corpus luteum was not disturbed and the rapid decline in P4 level associated with foetal loss may therefore be a result, rather than the cause, of foetal death (Kalender *et al.,* 2014). It was suggested that CLs, large luteal cells contain 1.8-fold more mitochondria per unit volume of cytoplasm in mature sheep. This may explain why small luteal cells produce less P4 than large luteal cells (Kenny *et al.,* 1989).

Correlation coefficients:

Results of correlation coefficients (Table 3) revealed significantly a positive correlation between number and diameter of the corpora lutea and blood P4 concentration. These correlations were more pronounced in Rahmani and crossbred ewes than in Romanov due to higher embryonic loss Also, there was significant correlation between diameter and number of CLs within each breed.

Table 3. Correlation coefficients in different ewe breeds between progesterone and each of number and diameter of corpora lutea.

Breed	Item	P4 concentration(ng/ml)	CLnumber
Rahmani	CL number	0.789*	-
	CL Diameter	0.677	0.850**
Romonov	CL number	0.365	-
	CL Diameter	0.547	0.819*
Crossbred (Rah x Ro)	CL number	0.802*	-
	CL Diameter	0.982**	0.856**
All breeds	CL number	0.588**	-
	CL Diameter	0.699**	0.858**

*** Significant at P<0.05. ** Significant at P<0.01.**

The present correlations are in agreement with previous studies, showing that P4 concentration significantly and positively correlated with CLs diameter in goats (Orita *et al.,* 2000; Chao *et al.,* 2008). In this way, Simoes *et al.* (2007) reported that P4 concentration in blood plasma of goats was higher in animals having 2 CLs (6.4 ng/ml) than in those with one CL (5.4 ng/ml) only. Also, Lamming and Darwash (1998) found a positive association between blood P4 concentration and pregnancy.

The obtained results of this study indicated that the differences in progesterone concentration among ewe breeds are in relation with pregnancy status, depending on number and diameter of corpora lutea.

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الكفاءة التناسلية لنعاج الرحماني والرومانوف وخلطانها فيما يتعلق بتركيز البروجسترون

على دغيدى ، عادل عبد العزيز البدوى ، محمود المغربى ، شريف شامية ، محمد عبد الحافظ ، امجد ابو العلا و السيد ابو فندود

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تمت هذه التجربة بتقييم العلاقة ما بين تركيز البروجسترون، عدد وقطر الأجسام الصفراء مع الحمل في ثلاث سلالات من النعاج. استخدمت في هذه الدراسة 61 نعجة: 25 رحماني ، 11 رومانوف ، 25 خليط (رحماني×رومانوف) كانت تفحص بالسونار بعد التلقيح الطبيعي حتى اليوم 21 لتحديد عدد وقطر الأجسام الصفراء على المبايض. تم أخذ عينات دم من 8 نعاج في كل مجموعة (4عشار و4 غير عشار) قبل الفحص بالسونار؛ وذلك لتقدير مستوى البروجسترون. أوضحت النتائج أن متوسط قطر الأجسام الصفراء وتركيز البروجسترون كانت الأعلى معنوياً في سلالة الرومانوف (8.04 ملليمتر، 4.41 نانوجرام/مل)، عن السلالات الأخرى. كانت مرتفعة بدرجة غير معنوية في الخلطان (7.48ملليمتر، 3.50 نانوجرام/مل) عن سلالة الرحماني (5.52l ملليمتر، 3.43 نانوجرام/مل). لم يتأثر متوسط عدد الأجسام الصفراء معنوياً بالسلالة. أيضاً كان عدد وقطر الأجسام الصفراء وتركيز هرمون البروجسترون أعلى معنوياً في العشار عن غير العشار. كان تأثير التداخل ما بين السلالة والحالة التناسلية على كل الخصائص غير معنوي. كان معامل الارتباط بين كل من عدد وقطر الأجسام الصفراء مع تركيز البروجسترون إيجابي ومعنوي. أكدت نتائج هذه الدراسة أن الاختلافات ما بين تركيز البروجسترون وبين سلالات النعاج يكون على علاقة بالحالة التناسلية، وذلك بالاعتماد على عدد وقطر الأجسام الصفراء.