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# Determination of Plasma Progesterone in Awassi ewes using Radioimmunoassay*

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ABSTRACT. The Level of Progesterone on sera samples collected from pregnant and nonpregnant Awassi ewes was determined (16-20) days after breeding using radioimmunoassay. Progesterone concentration was higher (1.89 ng/ml) in pregnant ewes than that of non pregnant ewes (0.34 ng/ml). Pregnancy diagnosis results, based on the levels of progesterone in peripheral serum were, confirmed by lambing when 1 ng/ml or more progesterone on the specified period was used as an indicative of pregnancy. The overall accuracy of this diagnosis was more than 90%. The concentration of progesterone in the 60 months old ewes was higher (2.65 ng/ml) than that of 24 months old ewes (1.66 ng/ml), while there was no significant difference in the serum progesterone between the different weight groups ewes. The quantitative analysis of serum progesterone in (16-20) days after breeding of Awassi ewes might be used for early pregnancy detection.

The levels of serum progesterone during the oestrus cycle of cow, and sow (Robertson and Sarda 1971), mares (Mantri Sardeshpande and Mantri 1985) and cow and ewes (Schemesh, Ayalon and Lindner 1973) have been reported and results based upon plasma progesterone concentration were used as an early pregnancy testing in these animals. In all these studies significant difference in serum progesteron levels between pregnant and non-pregnant females was always observed. Various techniques have been designed for quantitative determination of progesterone in serum or plasma samples with and without preliminary treatment of the samples.

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Radioimmunoassay determination of plasma progesterone concentration is the most widely used early pregnancy detection method because of its simplicity, rapidity and sensitivity (Mantri, Stellflug and Lowry 1983, Burfening and Beradinelli 1986). A noteworthy method developed recently to determine the accuracy of detection of pregnancy in sheep using pregnancy - specific protein B has been shown to be a reliable and accurate test for pregnancy in sheep (Ruder *et al.* 1988).

Awassi sheep a common domestic breed, widely distributed in Mediterranean countries are of great interest for meat and milk production in these countries. Their breeding season is restricted to a few months in each year and failure to breed them during this short period will render them infertile for the rest of the year. In an effort to establish the concurrent changes in the level of serum progesterone during the period between 16-20 days posmating of Awassi ewes and to correlate if possible these with an early pregnancy testing, we have undertaken the present investigation on the quantitative determination of Awassi ewes serum progesterone using radioimmunoassay.

## Materials and Methods

*Ewes:* eighty ewes of the local Awassi breed were used in present study. The ewes assigned to the different experimental groups according to their age, body weight and date of their mating and lambing. The ewes of different groups were kept under semi - intensive care at Al-Khanaser agriculture station (Latitude 32° 30°N). Behavioral measures were taken to verify day of breeding after synchronization.

*Blood sampling and progesterone determination:* jugular blood samples (5 ml) were collected (16-20) days after breeding onto heparin in vacuum tubes, cooled immediately and centrifuged within one hour of collection at 4 C° using desk centrifuge.

Of the resulting plasma 0.5 ml aliquots were separately extracted with 5 ml of petroleum ether by shaking for 10 minutes in stoppered glass tubes. The tubes were then placed in deep freeze untill freezing of the plasma and the supernatant was decanted to glass tubes and evaporated to dryness under nitrogen at 40-50 C°. Three control sera containing known amount of progesterone were also subjected to the same extraction procedure in each set of assays to assess extraction yields. The residues resulting after evaporation were suspended in 0.5 ml of 0.1 M sodium Phosphate buffer pH 7.2 and subjected to radioimmunoassay using reagents from Biodata Laboratories (Italy) essentially as described (Chang and Estergreen 1983). The Sensitivity of the assay was about 0.3 ng/ml with an intra-assay coefficient of variation about 14.6% and intra-assay coefficient of variation about

6.6%. The recovery of progesterone from serum samples following petroleum ether extraction was greater than 85%.

## Result and Discussion

The plasma progesterone concentration in blood samples obtained from thirty seven non pregnant Awassi ewes range between 0.10-0.66 ng/ml. It can be seen that the mean plasma progesterone concentration in non pregnant ewes was lower (0.34 ng/ml) than that of pregnant ewes (1.89 ng/ml) (Table 1). The plasma progesterone values for pregnant Awassi ewes on days (16-20) after breeding was similar to values (2-3 ng/ml) reported by Basset *et al.* (1969) using Cross-bred ewes and those reported by kittok, stellflug and Lowry (1983) and Shemesh, Ayalon and Lindner (1973).

In the present study ewes with plasma progesterone concentration of 1 ng/ml or more were classified as pregnant while those with plasma progesterone concentration less than 1 ng/ml were classified as nonpregnant. Based upon this criterion the over all accuracy of diagnosis as compared with the actual lambing was greater than 90% (Tables, 1,2,3,4). Our accuracy measurements were similar to those reported by Robertson and Sarda (1971) and Shemesh, Ayalon and Lindner (1973). A more accuracy measurements for pregnancy detection in sheep have been obtained recently utilizing radioimmunoassy for sheep pregnancy-specific protein B were shown to detect pregnancy earlier and more accurately than in pregmatic 3 ultrasonic device (Ruder *et al.* 1988).

**Table 1.** Plasma progesterone concentrations (ng/ml[a]) in pregnant and nonpregnant Awassi ewes (16-20) days after breeding

| Laboratory diagnosis | No. of ewes | Plasma Progesterone ng/ml: mean ± S.D. | Accuracy based upon lambing |
|---|---|---|---|
| Pregnant | 34 | 1.89 ± 0.12 | 90 |
| Nonpregnant | 37 | 0.34 ± 0.06 | 100 |

a: means ± S.D.

**Table 2.** Effect of age on the level of plasma progesterone (16-20) days after breeding of Awassi ewes

| Age (month) | No. of ewes | Progesterone (ng/ml) ± S.D. | Accuracy % |
|---|---|---|---|
| 24 | 6 | 1.66 ± 0.11 | 91 |
| 36 | 21 | 1.97 ± 0.12 | 92 |
| 48 | 14 | 2.24 ± 0.12 | 89 |
| 60 | 4 | 2.65 ± 0.16 | 93 |

Age and weight have been shown to be important factors that influence the occurrence of puberty in ewes and progesterone treatments have been shown to induce puberty in various animals. (Burfening *et al.* 1976). The levels of plasma progesterone in age-groups Awassi ewes range between 24-60 months were measured. As shown in Table (2), the level of progesterone in pregnant ewes increased with increasing the age of ewes. This could be attributed to the increase production of progesterone in older ewes as compared with younger ewes or due to individual traits. However, whether the observed increase in serum progesterone in older ewes was direct or indirect cannot be resolved from the available data. Likewise there was no observed correlation between the serum progesterone level and the weight of the ewes (Table 3). A slight correlation was observed between blood serum progesterone level and twining. The mean progesterone level for ten ewes that gave birth to twins was higher (2.37 ± 0.14 ng/ml) than that of ewes gave birth to singles (1.73 ± 0.1 ng/ml) and one ewe gave birth to triplet had higher level (3.00 ng/ml) (Table 4). Robertson and Sarda (1971) and Shemesh, Ayalon and Lindner (1973) did observed similar differences in plasma progesterone levels between ewes gave birth to single and those gave birth to twins. From the results of the present study it seens that quantitative determination of plasma progesterone in Awassi ewes (16-20) days after breeding had a beneficial application as an early pregnancy detection method with more than 90% accuracy

**Table 3.** Effect of ewes weight on the level of plasma progesterone[a] in Awassi ewes 16-20 days after breeding

| Weight (kg) | No. of ewes | Progesterone ng/ml ± S.D. | Accuracy % |
|---|---|---|---|
| 45 - 50 | 12 | 1.76 ± 0.14 | 90 |
| 51 - 55 | 11 | 1.73 ± 0.1 | 91 |
| 56 - 62 | 20 | 1.75 ± 0.1 | 91 |

a: means ± S.D.

**Table 4.** The relationship between number of fetuses and plasma progesterone[a] level in Awassi ewes 16-20 days after breeding

| Fetuses | No. of ewes | Progesterone ng/ml ± S.E. | Accuracy % |
|---|---|---|---|
| Single | 32 | 1.73 ± 0.10 | 89 |
| Twins | 10 | 2.37 ± 0.14 | 95 |
| Triplet | 1 | 3.00 | 100 |

provided that day of estrus is known. The high level of blood progesterone during the normal luteal phase of the estrus cycle certainly will reduce the accuracy of this method at this stage and therefore it is essential to know the stage of estrus to increase the accuracy of this method.

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# تقدير مستوى هرمون البرجستيرون في أغنام العواسي بواسطة الطريقة المناعية الإشعاعية

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لقد أجريت دراسة لقياس مستوى هرمون البروجستيرون في عينات من أمصال نعاج العواسي الحوامل وغير الحوامل في الفترة ما بين ١٦ - ٢٠ يوماً بعد التلقيح وقد استعملت الطريقة المناعية الإشعاعية لهذه الغاية، وتبين من هذه الدراسة ان مستوى هرمون البروجستيرون أعلى (١,٨٩ نغم/ملم) في نعاج العواسي الحوامل منه في نعاج العواسي غير الحوامل (٠,٣٤ نغم/ملم). وقد استعمل تركيز البروجستيرون الذي يعادل ١ نغم/ملم أو أكثر كدليل للحمل. وقد تراوحت دقة هذا الدليل مقارنة بالنسبة الحقيقية للولادة ما يعادل ٩٠٪ من الحالات المفحوصة، وبينت النتائج أن مستوى هرمون البرجستيرون في النعاج الحوامل التي عمرها ٦٠ شهراً أعلى (١,٦٦ نغم/ملم) منه في النعاج التي عمرها ٢٤ شهراً (١,٦٦ نغم/ملم) بينما لم يلاحظ أي فروق في مستوى هرمون البروجستيرون في النعاج ذات الأوزان المختلفة. وقد أثبتت النتائج إمكانية استعمال تراكيز البرجستيرون في أمصال نعاج العواسي في الفترة ما بين ١٦ - ٢٠ يوماً بعد التلقيح كدليل لتميز الحمل المبكر.