

Residual levels on medroxyprogesterone acetate (MAP) impregnated sponges after estrus synchronization treatment in cyclic ewes

Níveis residuais em esponjas impregnadas com acetato de medroxiprogesterona (MAP) após o tratamento de sincronização do estro nas ovelhas em período reprodutivo

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SUMMARY

A pool of polyurethane sponges impregnated with medroxyprogesterone acetate (MAP) was prepared. Real level of progestagen on sponges was checked prior to sponge treatment insertion. During spring a total of 126 cyclic Merino ewes were intravaginally treated with MAP-impregnated pessaries for the synchronization of estrus. After 14 days treatment, sponges were removed. Estrus detection was performed with vasectomized rams. Ewes were inspected for the presence of marks twice a day. Ewes in estrus were artificially inseminated with fresh diluted semen containing a dose of 300×10^6 total sperm. Artificial insemination was performed 12 hours after estrus presentation. Residual levels of MAP (RMAP) on removed sponges were measured by spectrophotometry at 241 nM and examined in relation to their estrus response and fertility. Real dose of MAP was in average 54 mg. RMAP found on sponges following treatment were in average 25.00 ± 0.84 mg. Percentage of estrus synchronization was 92.86% and pregnancy rate was 50.43%. There were no significant differences for RMAP between ewes with (24.70 ± 0.86 mg) and without (28.89 ± 3.65 mg) estrus response ($p > 0.10$). There were also no significant differences for RMAP between pregnant (25.56 ± 1.25 mg) and non-pregnant (23.83 ± 1.18 mg) ewes ($p > 0.10$). It was concluded that the 60 mg MAP dose conventionally used for estrus synchronization in ewes is higher than the amount uptaken by the females.

UNITERMS: Ewes; Estrus synchronization; Medroxyprogesterone acetate; Fertility.

INTRODUCTION

The synchronization of estrus in sheep has been accomplished by means of intravaginal sponges impregnated with natural progesterone^{10,13} as well as with synthetic progesterone such as fluorogestone acetate (FGA; 9 α -fluoro-11 β -hydroxy-17 α -acetoxy-pregne-4-ene-3.20-dione)^{1,2,3,10,12,14,15,17} or medroxyprogesterone acetate (MAP; 6 α -methyl-17 α -acetoxy-pregne-4-ene-3.20-dione)^{1,3,4,5,6,10,11,15,16}.

The degree of success in synchronizing estrus varies among studies but the treatment is generally very effective. However, reduced fertility at synchronized estrus has been reported^{1,8,12}.

MAP-impregnated pessaries for estrus synchronization in small ruminants are commercially available as a unique presentation containing 60 mg of such hormone. In a preliminary study in goats (unpublished data), we found that residual levels on sponges remained after estrus synchronization treatment. This suggests that the amount of MAP effectively uptaken by these females is lower than the 60 mg dose presented on sponges.

The objectives of this study were: 1) to determine the effects of treatment with intravaginal sponges impregnated with MAP on estrus synchronization and fertility in ewes; 2) to determine residual and uptaken levels of MAP after estrus synchronization treatment; and 3) to analyze the relationship between residual and uptaken levels of MAP and the reproductive responses.

MATERIAL AND METHOD

Animals and management

The experiment was conducted during spring. A total of 136 cyclic Merino ewes were used in this study. The animals were kept under natural field conditions, having access to good quality mixture pasture and maintained in good health. They were managed under the same conditions on one farm.

These females were randomly assigned in two groups:

Group I (control; n = 10): ewes were treated with polyurethane vaginal sponges without MAP.

Group II (treated; n = 126): ewes were treated with polyurethane vaginal sponges impregnated with 60 mg MAP approximately.

Pessaries preparation and synchronization method

Polyurethane sponges were prepared by the method already reported by Robinson¹².

A total of 150 sponges were divided into four groups:

Group A (n = 10): sponges impregnated with MAP

dissolved in ethylic alcohol 96%, used to check the real dose of MAP on sponges prior to sponge treatment insertion.

Group B (n = 126): sponges impregnated with MAP dissolved in ethylic alcohol 96%, inserted deep into the vagina of each female of group II and left in place for 14 days.

Group C (n = 4): sponges impregnated with ethylic alcohol 96% without MAP, used as control of MAP dose of group A.

Group D (n = 10): sponges impregnated with ethylic alcohol 96% without MAP, inserted intravaginally in ewes of group I and left in place for 14 days.

The ethylic alcohol 96% utilized during this study came from a pool.

Quantification of real and residual MAP

At withdrawal, each pessary from groups B and D was placed into a sterile flask until assay in order to determine the residue (remnant MAP on removed sponges used for estrus synchronization).

Real and residual levels of MAP were measured by spectrophotometry.

Absorbance values for known concentrations of MAP (0 to 0.0035%) were determined at the wavelength of 241 nM to construct a standard curve.

Sponges were washed with 100 ml of ethylic alcohol 96% to extract the MAP contained. Each solution was filtered utilizing SS blue ribbon filter paper. 0.5 ml of the filtered solution was taken and completed up to 10 ml with ethylic alcohol 96%.

Absorbance value of each sample was measured by spectrophotometry and its MAP concentration was obtained from the standard curve.

Real and residual levels of the synthetic progestagen were determined as follows:

$$a = b \times d$$

where

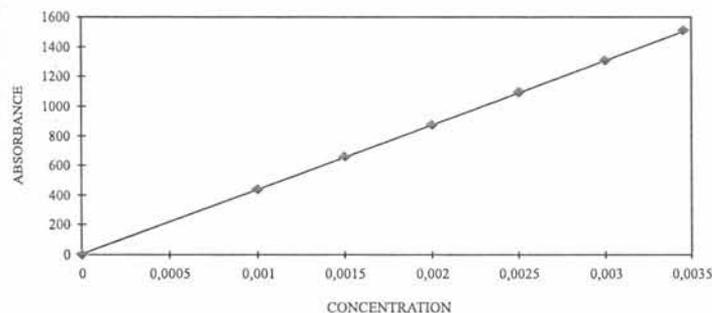


Figure 1

Standard curve for MAP at 241 nM. Absorbance X 0.001

Table 1

Amount of hormone (MAP) remaining and uptaken, estrus response and fertility of 126 cyclic ewes treated with MAP-impregnated intravaginal sponges for 14 days. Buenos Aires, Argentina, 1995.

Reproductive response	Number of ewes (%)	Residual MAP (mg:mean ± SE)	Uptaken MAP (mg:mean ± SE)
-estrus	117 (92.86)	24.70 ± 0.86 ^a	29.30 ± 0.86 ^a
-not estrus	9 (7.14)	28.89 ± 3.65 ^a	25.11 ± 3.65 ^a
-pregnant	59 (50.43)	25.56 ± 1.25 ^a	28.44 ± 1.25 ^a
-not pregnant	58 (49.57)	23.83 ± 1.18 ^a	30.17 ± 1.18 ^a

^a = Same letters within a column do not differ significantly (p>0.10).

a = mg MAP.

b = MAP sample concentration (measured by spectrophotometry).

d = 1:20 dilution factor.

Estrus detection

The onset of estrus was carried out by the use of vasectomized rams in a ratio of 5%. Rams were painted with a vegetable dye mixed in a vaseline base so that mounted ewes could be identified. The males were introduced in the flock after pessary removal and for a total period of 96 hours. Ewes were inspected for the presence of marks at 12-hours intervals.

Artificial insemination

Semen from a ram with probed fertility was collected using an artificial vagina.

Having determined seminal characteristics, it was diluted with egg yolk-tris-fructose extender.

Ewes from group B that had exhibited estrus were artificially inseminated with fresh diluted semen containing a dose of 300 millions total sperm. Cervical artificial insemination was performed 12 hours after estrus detection.

Pregnancy detection

Ewes with non-return to estrus were assumed to be pregnant. After 60 days artificial insemination, pregnancy was confirmed by ultrasound.

Evaluation of results

Statistical differences for residual and uptaken MAP with respect to estrus response and fertility of the synchronized estrus were determined by Student's t-test.

RESULTS

Fig. 1 illustrates the absorbance values of the standard curve for known concentrations of MAP as determined by spectrophotometry at 241 nM.

Tab. 1 summarizes estrus synchronization rate, pregnancy rate and residual and uptaken MAP levels in relation to the reproductive performance.

DISCUSSION

Mean real MAP level found on sponges from Group A was 54 mg.

Mean residual MAP level found on removed sponges from all treated ewes following treatment during 14 days was 25.00 ± 0.84 mg, being the uptaken level 29.00 ± 0.84 mg (Tab. 1). The 46.29% of the MAP dose remained on pessaries following 14 days treatment. This result is similar to the MAP

dose remained on pessaries from treated goats according to our preliminary study (unpublished data). In the same way, Mac Donnell⁹ detected a 36% of the 500 mg progesterone dose used in sponges to synchronize estrus in ewes, after a 17 days treatment.

Percentage of estrus response after hormonal treatment was 92.86% (117/126) (Tab. 1). The high degree of synchrony achieved with MAP has been reported by other workers^{6,10,11}.

Mean residual MAP level for ewes that exhibited estrus was 24.70 ± 0.86 mg, being the uptaken level 29.30 ± 0.86 mg (Tab. 1). Mean residual MAP level for ewes without estrus response was 28.89 ± 3.65 mg, whereas the uptaken level was 25.11 ± 3.65 mg (Tab. 1).

Conception rate based on non-return to estrus and ultrasound-proof was 50.43% (59/117) (Tab. 1).

Levels of residual MAP for pregnant ewes were in average 25.56 ± 1.25 mg, being the amount absorbed 28.44 ± 1.25 mg (Tab. 1). Non-pregnant ewes had a mean residual MAP level of 23.83 ± 1.18 mg, having uptaken 30.17 ± 1.18 mg (Tab.1).

There were no statistical differences for residual and uptaken MAP with respect to estrus response and fertility of the synchronized estrus.

In the comprehensive study of synthetic progestagens impregnated in sponges, it was suggested¹³ that the progestagen dose required to inhibit ovulation during treatment is lower than that required to synchronize estrus. Also the dose required for such conditioning is lower than the one required to obtain a fertile response. This conclusion differs from that of Lamond⁷ who postulated that optimal fertility in synchronized sheep was associated with minimal doses of progesterone.

Deweese *et al.*⁶ found that 40 mg MAP-impregnated sponges were as effective as the 60 mg dose in synchronizing estrus. This lower dose resulted in a higher number of ewes conceiving at the first estrus following treatment.

Our results suggest that the dose of 60 mg MAP is not entirely utilized by ewes. In addition, the dose required for estrus synchronization is similar to that needed to obtain a fertile response.

CONCLUSION

1- In this study it was possible to determine the existence of a residue of MAP on sponges removed from ewes' vaginas after 14 days of permanence. Therefore, the 60 mg MAP dose conventionally used for estrus synchronization in small ruminants by means of intravaginal pessaries, is higher than the amount uptaken by these females;

2- There is no relationship between residual levels of MAP and estrus response and fertility of the synchronized estrus in ewes;

3- There are individual differences in the amount of residual MAP.

RESUMO

Preparou-se um conjunto de esponjas de poliuretano impregnadas com acetato de medroxiprogesterona (MAP). O nível real do progestágeno nas esponjas foi checado com anterioridade à inserção das esponjas do tratamento. Um total de 126 ovelhas Merino em período reprodutivo (primavera) foram tratadas com esponjas intravaginais impregnadas com MAP para sincronização do estro. As esponjas foram retiradas após 14 dias de tratamento. Utilizaram-se carneiros vasectomizados para detecção do cio. As ovelhas foram observadas para a presença das marcas duas vezes ao dia. As ovelhas que apresentaram cio foram inseminadas artificialmente com sêmen fresco diluído, empregando-se uma dose de 300×10^6 espermatozoides totais. A inseminação artificial foi praticada 12 horas depois da apresentação do cio. Os níveis residuais de MAP (RMAP) nas esponjas retiradas foram medidos por espectrofotometria a 241 nM e examinados em relação à resposta estral e à fertilidade. A dose real de MAP foi como média 54 mg. RMAP encontrados em esponjas após o tratamento foram como média $25,00 \pm 0,84$ mg. A porcentagem de sincronização dos cios foi 92,86% e a taxa de prenhez, 50,43%. Não foram encontradas diferenças significativas entre RMAP das ovelhas com ($24,70 \pm 0,86$ mg) e sem ($28,89 \pm 3,65$ mg) resposta estral ($p > 0,10$). Também não foram encontradas diferenças significativas entre RMAP das ovelhas prenhes ($25,56 \pm 1,25$ mg) e não-prenhes ($23,83 \pm 1,18$ mg) ($p > 0,10$). Conclui-se que a dose de 60 mg MAP utilizada convencionalmente para sincronização do estro em ovelhas é superior à quantidade utilizada pelas fêmeas.

UNITERMOS: Ovelhas; Sincronização do estro; Acetato de medroxiprogesterona; Fertilidade.

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