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Using the Ram Effect as an Alternative to Ecg Before Artificial Insemination of Barbarine Ewes

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Abstract: The efficiency of the ram effect to synchronise Barbarine ewes prior to artificial insemination was examined in two trials conducted to (i) compare, in the first trial, conception rates after fixed-time artificial insemination of ewes synchronised with intravaginal sponges impregnated with progestagen followed by the introduction of rams or the administration of equine Chorionic Gonadotrophin (eCG) and (ii) establish, in the second trial, the level of lambing rate when ewes are inseminated 24 hours after oestrus, at the second ovulation following ram introduction and progesterone (20 mg i.m.) administration. Fresh, diluted semen at 400 x 10^6 sperm / ewe was used in both trials. The first trial involved 380 ewes in two flocks (A and B) and the second 155 ewes. In the first trial, the lambing rates obtained in ewes induced with eCG or the ram effect were respectively 58 and 55.5%. Flock had a significant effect ($P<0.05$) on the proportion of ewes conceiving to insemination after using the ram effect: 38 vs. 73% for A and B flocks respectively. In the second trial, 71 ewes (46%) exhibited oestrus between days 19-21 after ram introduction and progesterone injection and artificial insemination of these ewes yielded a lambing rate of 61%. It is concluded that under natural semi-arid conditions, substituting eCG by the ram effect when synchronising Barbarine ewes prior to artificial insemination could lead to satisfactory lambing rates.

Key words: Ram effect, progesterone, insemination and lambing rate

Introduction

Progestagen-impregnated intravaginal sponges and equine Chorionic Gonadotrophin (eCG) have an established role for synchronisation of oestrus in ewes when artificially inseminated or when induced to breed out of season. The cost of the exogenous hormones, the impairment of sperm transport through the cervix particularly in artificial insemination (Lightfoot and Salamon, 1970), the wide variation in litter size and the high incidence of embryo loss and infertility (Haresign, 1992) represent serious limits to the widespread application of this procedure.

The ram effect is a behavioural adaptation that modifies the breeding activity of sheep populations such that a greater synchrony of matings and therefore births occur. The technique has been extensively studied under different latitudes and production environments (Martin *et al.*, 1986; Rekik *et al.*, 1991; Thimonier *et al.*, 2000) and represents a major interest in Tunisia where most breeds have short seasonal anoestrus period Khaldi (1984). The synchronising ability of the ram effect can be improved at either the induced ovulation or the second ovulation by respectively treating the ewes with intravaginal sponges impregnated with synthetic progestagens (Oldham and Pearce, 1984) or a single injection of 20 mg progesterone (Cognié *et al.*, 1982). It is now well established that this improvement is associated to the beneficial effect of progesterone priming on the functioning of induced corpora lutea preventing these from regressing prematurely (Rekik, 1988; Lassoued *et al.*, 1997).

In Tunisia, the Barbarine breed which represents over 60% of the total sheep population, is the only breed for which a selection improvement programme is designed and artificial insemination is required for sire referencing schemes (Findlater *et al.*, 1991). Previous trials (Rekik and Ben Sassi, 1996) have demonstrated that a mean conception rate of nearly 49% was achieved when Barbarine ewes were synchronised with progestagen sponges + eCG and then inseminated. The trials described here investigated different strategies using the ram effect when Barbarine ewes are inseminated during their seasonal anoestrus (spring). In the first strategy, the ram effect is coupled to long progestagen treatment to synchronise both non cyclic and the spontaneously ovulating ewes at the ram-induced ovulation. In the second strategy, the ram effect is associated with a single injection of progesterone to synchronise only non cyclic ewes at the second ovulation

following rams introduction. The association between a progestagen derivative and the ram effect is hypothesised as an interesting perspective in artificial insemination programmes (Thimonier *et al.*, 2000) eliminating the drawbacks of eCG repeated use on subsequent fertility (Roy *et al.*, 1999).

Materials and Methods

Experiment 1: The experiment was carried out in the sylvo-agro-pastoral pilot centre of Jebibina (Office de l'Elevage et des Pâturages) under semi-arid conditions (330 mm average annual rainfall; 35° N). A total of 380 mature parous Barbarine ewes belonging to two flocks (A: 198 ewes; B: 182 ewes) and weighing 40.7±4.42 kg at the time of sponges insertion were used. The ewes of both flocks were maintained on natural rangeland throughout. However, ewes in flock B were transferred into the farm where the trial took place about 8 weeks before inseminations in a poor body condition and they were, in addition, fed a daily concentrate (14% crude protein in average) supplementary feeding (approximately 500 g / ewe / day). Ewes in both flocks remained in isolation from rams for at least two months before the date of insemination. Within each flock, ewes were randomly allocated at the time of sponge insertion to two treatment groups balanced for live weight. In mid-April, intravaginal pessaries impregnated with 30 mg fluorogestone acetate (FGA) were inserted in all ewes and were left *in-situ* for 14 days. At sponge withdrawal, ewes in the first treatment group received 400 i.u. of eCG and remained isolated from rams (FGA + eCG) while those in the second treatment group were joined to 10% of Barbarine aproned rams (FGA + ram effect). Ewes in both treatment groups were inseminated with fresh semen approximately 55 hours after sponge removal without a prior detection of oestrus. Semen was collected by artificial vagina from 8 Barbarine rams. Only ejaculates presenting a minimum concentration of 1.2×10^9 sperm per ml, determined by spectrophotometry, and an individual motility of at least 3 (determined on a scale of 5) were selected for use. Immediately after collection, the semen was diluted with a skimmed-milk diluent and ewes were inseminated cervically with 0.2 ml of diluted semen containing in average 400×10^6 sperm. Semen from different ejaculates was at random attributed to ewes from different flocks and different treatment groups. In order to permit mating of ewes which failed to conceive to artificial insemination and returned to service, fertile rams of the Barbarine breed were introduced to the flocks 10 days after insemination and were left with the ewes for the next 30 days.

Experiment 2: This experiment was carried out in the sheep research station of Weslatia (Institut National de la Recherche Agronomique de Tunisie) in semi-arid, central Tunisia (370 mm average annual rainfall but highly variable between years). In early May, a total of 155 adult ewes maintained in complete isolation from rams for at least the previous two months and weighing 47.1±4.7 kg in average were chosen. Immediately prior to the introduction of 13 Barbarine aproned rams, each ewe was injected intramuscularly with 20 mg progesterone (Sigma Ltd.) in 2 ml oil (day 0). Oestrus was detected twice daily and ewes detected in oestrus between days 19 and 21 after ram introduction were drafted from the flock and inseminated 24 hours after oestrous detection using fresh semen as described in experiment 1. Ewes detected in oestrus until day 18 and those not detected in oestrus up to day 21 p.m. were put to fertile rams for natural mating. Fertile rams were introduced 10 days after artificial insemination to allow mating of ewes which failed to conceive to insemination.

Data collection and statistical analyses: The date of lambing and the number of lambs born per ewe were recorded in both experiments. These previous results, along with the oestrus non-return rates, permitted the number of ewes conceiving to artificial insemination in the various treatment groups to be determined. In the first experiment, the effects of flock and the method of synchronisation on the proportions of ewes returning to oestrus or conceiving to artificial insemination was subjected to an analysis of deviance involving logit transformation. Proportions were then compared by χ^2 analysis. Litter size differences were compared by contingency χ^2 analysis of the proportions of ewes producing one or two lambs using the CATMOD procedure in SAS (Statistical Analysis System, 1991).

Results

Experiment 1: Out of the original number of ewes included from the two flocks, 7 ewes were not inseminated mainly because of clear signs of infection of the vaginal tract and 12 others aborted and the data related to these 19 animals were discarded (Table 1). Of the remaining 361 ewes, 34 (24 and 10 in respectively FGA + ram effect and FGA + eCG) did

Table 1: The effect of method of synchronisation on reproductive parameters of artificially inseminated (AI) Barbarine ewes

Parameter	Treatment group				
	FGA+eCG		FGA + Ram effect		
	Flock A	Flock B	Flock A	Flock B	Overall
Number of ewes	99	91	99	91	380
Ewes not inseminated or aborting	4	2	9	4	19
Live weight ± s.e. (kg)	41.1±3.55	40.3±3.55	41.6±4.32	39.8±3.84	40.7±4.42
Ewes returning to service (%)	44(46.3)^{a}	40 (44.9)^{a}	53 (58.8)^{a}	24 (27.6)^{b}	161 (44.6)
Barren ewes	8	2	12	13	34
Ewes conceiving to AI (%)	52 (59.7)^{ac}	49 (56.3)^{a}	30 (38.4)^{b}	55 (73.3)^{c}	186 (56.9)
Mean litter size per ewe conceiving to AI ± S. E.	1.09±0.297^{cd}	1.24±0.431^{a}	1.13±0.346^{ac}	1.03±0.346^{ac}	1.12±0.323

^{a,b,c,d} Values on the same line with different superscirpts are significantly different at $P<0.05$

Table 2: Conception rate and litter size of artifically inseminated (AI) Barbarine ewes synchronised with the ram effect coupled to 20mg progesterone

	Number	Ewes lambing of Ewes mated (%)	Lambs born per ewe lambing
Ewes in oestrus between days 19 and 21 after ram introduction (AI)	71	43 (61)	1.28±0.45
Other ewes * (joined to rams)	112	93 (83.0)	1.14±0.35

*Including ewes not conceiving to artificial insemination

not lamb after both artificial insemination and the period of natural mating that ensued and these barren ewes were not included when calculating conception rates at insemination (Table 1). Table 1 reports the number (proportion) of ewes displaying oestrus after insemination, those conceiving to artificial insemination and the corresponding litter sizes. Overall, 161 (44.6%) displayed oestrus after insemination with only the method of synchronisation ($P<0.05$) and the interaction between flock and the method of synchronisation ($P<0.01$) having significant effects. When treatment groups are compared, a significantly ($P<0.05$) much lower proportion of ewes in flock B synchronised with FGA + ram effect were detected in oestrus after insemination.

When all factors (flock, method of synchronisation) are confounded, the average conception rate reached 56.9%. Both main effects (flock and method of synchronisation) as well as their interaction were significant in the model. The highest conception rate (73.3%) was recorded in ewes of flock B introduced to the rams and this was significantly ($P<0.05$) higher than conception rates in both ewes of the same flock treated with eCG and ewes of flock A introduced to the rams, and tended to be higher than in ewes of flock A treated with eCG.

Average litter size of ewes conceiving to artificial insemination reached 1.12±0.323. Flock and the interaction between flock and the method of synchronisation had significant effects ($P<0.05$). The highest litter size was recorded in ewes of flock B treated with eCG while ewes of the same flock stimulated by the rams yielded the lowest litter size (Table 1). There were no differences in litter sizes between ewes of flock A whether treated with eCG or stimulated by the ram effect.

Experiment 2: The distribution of ewes detected in oestrus up to day 21 after the injection of progesterone and ram introduction is shown in Fig. 1. A total of 71 ewes were drafted in oestrus between days 19 and 21 representing 61.2% of the sheep detected in oestrus up to day 21 after ram introduction and 45.8% of the initial number of sheep treated with progesterone. In inseminated ewes, lambing rate and litter size per ewe lambing respectively averaged 61% and 1.28±0.45 (Table 2). The ewes in oestrus between days 0 and 18 (females spontaneously cycling before rams introduction), those not in oestrus up to day 21 (females that did not respond to the ram effect) as well as those

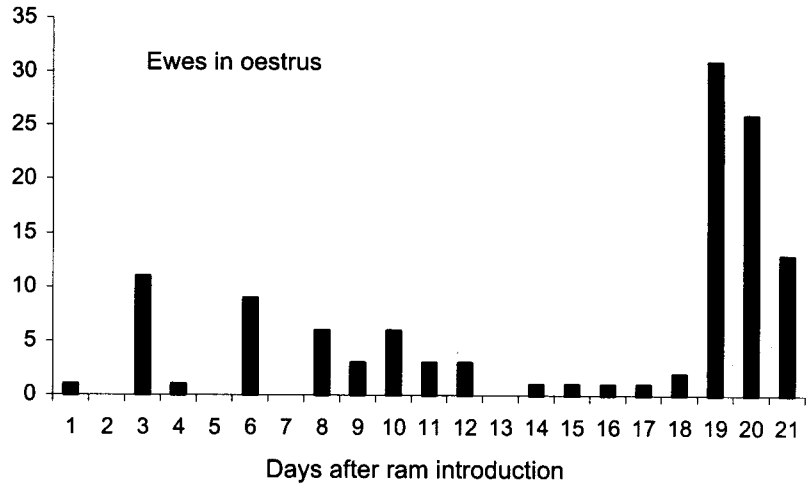

Fig. 1: Oestrous distribution of Barbarine ewes synchronised with the ram effect coupled to 20mg progesterone

failing to conceive at insemination were naturally mated and their conception rate and mean litter size were respectively 83.0% and 1.14±0.35 (Table 2).

Discussion

The Barbarine breed is reported to have a shallow seasonal anoestrus with a variable proportion of ewes continuing to cycle throughout the year (Lassoued *et al.*, 1995). This physiological feature of the breed explains that in the case of the second trial, 32% of ewes came in oestrus up to day 18 after both ram introduction and the injection of progesterone. These ewes were spontaneously cycling prior to ram introduction and the good synchrony of oestrus in the other ewes starting day 19 is consistent with the results of Cognié *et al.* (1982) using anoestrous Merino d'Arles ewes. However, the response is shortly delayed in comparison to previous data from Lassoued *et al.* (1995) using the same protocol in Barbarine breed, who reported a distribution of oestrus between days 17 and 20 after ram introduction and the injection of 20 mg progesterone. Therefore, the problem of accurately controlling oestrus by this method would limit the use of fixed-time artificial insemination and satisfactory conception rates are achieved only when ewes are inseminated upon oestrous detection. Under these conditions and even though direct comparison with the method using progestagens and eCG is not possible, lambing rate using the ram effect coupled to a single injection of progesterone was as good as, and in many cases higher than, those reported previously with the first method (Rekik and Ben Sassi, 1996).

In this study, the average conception rate was also acceptable when ewes in the first trial are primed for 14 days with progestagens followed by ram introduction or eCG injection. Both procedures could be regarded as reliable in artificial insemination inducing ovulation and oestrus in anoestrous ewes and synchronising ewes already cycling in the flock. When ewes are synchronised with FGA + eCG, the overall lambing rates achieved in this study are similar to those obtained following large scale routine inseminations of the Barbarine ewes (Rekik, unpublished data). Furthermore, they are similar of those encountered following similar protocols in northern countries (Meat and Livestock Commission, 1982; Corteel and Paquignon, 1984).

In experiment 1, the different levels of fertility between flocks, suggest that the effectiveness of FGA + ram effect is inconsistent. The good result obtained in flock B is at contrast with those reported by Oldham and Pearce (1984) in which the ram effect associated to short (6 days) or long priming periods (14 days) of progestagen did not give a synchrony of oestrus that could justify inseminating at a fixed time. In the

present study, the degree of variability between flocks is difficult to explain in the absence of data on the degree of synchronisation of oestrus in these 2 flocks. The supplementary feeding of ewes in flock B could have improved their ovarian activity and their ovulatory response to the ram effect (Thimonier *et al.*, 2000). This nutritional stimulus may well provide the explanation for the high conception rate obtained in ewes of flock B stimulated by the rams, illustrate the degree of variation likely to be encountered if this technique is to be applied under field conditions and point out that interactions between reproduction and nutrition in dry, hot countries have yet to be adequately explained. Across the two trials, litter sizes recorded are low indicative of the genetically-poor prolificacy of the Barbarine breed. Nevertheless, the dose of 400 i.u. eCG increased litter size in ewes of flock B and not in those of flock A. Differences between flocks are, perhaps, due, once again, to differential feeding. Supplementary feeding has a well documented effect on ovulation rate and litter size (O'Callaghan and Boland, 1999) but little is known on its interaction with eCG. There is, nevertheless, indication that in both the mouse and sheep (Lamond and Bindon, 1969), a reduced plane of nutrition followed by refeeding results in a greater ovulatory response to exogenous gonadotrophins.

In conclusion, conception rates after fixed-time artificial insemination of ewes at the ram-induced ovulation are variable between flocks. Presumably, other factors like the body condition or the nutritional background of the ewes are important factors that have to be considered. Conception rate reached a satisfactory level at the post-induced ovulation when the ram effect is coupled with a single injection of progesterone but oestrous spreading needs to be narrowed for artificial insemination to be performed more practically.

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