Journal of Animal and Veterinary Sciences
2014; 1(4): 25-29
Published online December 30, 2014 (http://www.opensciencedonline.com/journal/javs)

Effects of long-term fluorogestone acetate treatment combined with PMSG on oestrus synchronization and fertility in Guirra Ewes

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To cite this article

Francisco Marco-Jiménez, José Salvador Vicente, María Pilar Viudes-de-Castro. Effects of Long-Term Fluorogestone Acetate Treatment Combined with PMSG on Oestrus Synchronization and Fertility in Guirra Ewes. *Journal of Animal and Veterinary Sciences*. Vol. 1, No. 4, 2014, pp. 25-29.

Abstract

The first objective was to asses the fertility of Guirra sheep using a standard artificial insemination procedure. In a second step, arising from the first objective, were studies the residual levels of plasma progesterone after treatment with intra-vaginal sponges impregnated with fluorogestone acetate (FGA) and determine the effect upon estrus incidence, interval to estrus onset and pregnancy rate. Estrous synchronization was carried out with FGA sponges (30 mg) for 12 days plus 400 IU eCG administered i.m. at sponge withdrawal. Ewes were AI at 54±1 h using fresh semen deposited in the entrance of the cervix. Blood samples were collected at day 19 ewes after AI, for determination progesterone concentration (P4). Ewes with a concentration of more than 1 ng/ml P4 were considered to be pregnant. In a second experiment, 70 ewes were randomly divided into 2 groups (T1 and T2) and synchronized as previous, but ewes in the second group were synchronized 6 days later than T1. Twelve days after sponge withdrawal for T2, all ewes were divided into 7 lots. One ram was introduced to each lot. Ewes for the T1 were mated at next natural estrous cycle while T2 group ewes were mated at the synchronized estrous and the next natural estrous. During all period, the ewes and rams sexual behavior was recorded by video camera. The ewes were considered in estrous when they were directly observed to accept a ride from a ram (standing reflex). The fertility examined the AI was the 24.7%. Similar percentage of ewes exhibiting estrous were observed (for natural or synchronized) but onset of estrous during the natural estrous was much shorter. The conception rate was higher for T1 compared with T2 ($P<0.05$) and significant differences were obtained when type of estrous were compared ($P<0.05$). It was concluded that a detrimental effect of f FGA sponge used for estrous synchronization on fertility in Guirra breed sheep was observed.

Keywords

Ewes, Estrous Synchronization, Fertility, Progesterone, FGA

1. Introduction

The Levantina Red (Guirra) sheep is an autochthonous breed distributed in the southeast of Spain. The Guirra is an African ancestry breed, characterized by the reddish coat color, the high degree of wool greasiness and a convex frontonasal profile [1]. Nowadays, the total population size consists of 4850 ewes and 421 rams and the population trend is decreasing (DAD-IS, 2005; EAAP Animal Genetic Data Bank, 2005). The State of the World's Animal Genetic Resources for Food and Agriculture was presented in September 2007 in Interlaken, Switzerland and two main international meetings in 2008 have emphasized the negative consequences of the loss of animal genetic resources [2]. The report shows that 190 out of 7600 breeds on record have become extinct in the past 15 years, and 1500 are onsidered at risk of extinction. Around 60 breeds of cattle, goats, pigs, horses and poultry have been lost in the last five years [3]. Genetic resource banks are intended for use as repositories of germplasm, which is available for use when required in procedures such as artificial insemination (AI) or embryo transfer and as an interface between ex situ and in situ conservation programs [4]. There are several factors that affect the result of AI as ewe handling, farm system, environmental elements, health, physiological status of the ewes [5-9]. It is important to control these factors, taking into account their influence on the outcome of AI. To our best knowledge, no report in the literature described the use of AI in Guirra breed.

Estrus synchronization has become the basis for successful AI and embryo transfer programs [10]. Among the hormonal treatments, intravaginal sponges with progesterone or its analogues (progestagens) are the most commonly used procedures. Main advantages are availability in the market and simplicity of application, either in breeding or non-breeding season; however, fertility yields are lowered in comparison to natural estrus [11-14]. Long term progestagen treatment has been showed to decrease fertility [15, 16]. Although many factors (type of intravaginal device, dose and timing of eCG injection, semen type, time and number of AI, breed and age of ewes, season and others) affect the fertility of ewes with synchronized estrus and ovulation, the effects on fertility at the first estrus after hormonal treatment are contradictory [17-19].

The aim of this study was to determine the effect of long-term progestagen treatment on fertility in Guirra ewe inseminated (i) artificially at a predetermined time and (ii) naturally after synchronized and natural oestrus.

2. Materials and Methods

2.1. Animals and Experimental Protocol

The experiment was carried out at the Polytechnic University of Valencia, Spain Farm, (40°38′N, 23°E) in Southeast of Spain under natural lighting. In this study, clinically healthy fat-tailed Guirra ewes (2 to 3 year old), 4 fertile Guirra rams for semen collection and 7 rams for oestrus detection and natural insemination. Ewes weighing between 50 to 60 kg with good body conditions (BCS: 3 to 4.0) were used. Oestrous synchronization was carried out with fluorogestone acetate sponges (30 mg FGA; Chronogest, Intervet, Boxmeer, Holand) for 12 days and 400 IU eCG (Sincrogest, Ovejero laboratories, León, Spain) was injected upon withdrawal of sponge.

2.2. Experiment 1: Fertility of Guirra Ewes Following Artificial Insemination at a Predetermined Time

Sixty-nine ewes were used during breeding season. Four rams with previously proven fertility were used for semen collection with the aid of artificial vagina in the presence of an ewe in oestrus. Each ejaculate was immediately evaluated for volume, motility and concentration. Only ejaculates with a volume of more than 0.5 ml, motility 75% and minimum 3×10^9 sperm cells/ml were used. The ejaculates from each ram were mixed, pooled and maintained at 30 °C. The semen was diluted to a sperm concentration of 1600×10^6 motile sperm cells/ml after being counted with the aid of of Thoma-Zeiss chamber. A one-step dilution was performed with the addition of Tes-Tris-Fructose-egg yolk containing 1000 IU sodium G penicillin and 1000 μg/ml dihydrostreptomycine sulphate. Ewes were vaginal inseminated at 54±1 h after sponge removal, with a 0.25 ml straw containing 400 million spermatozoa. Ten days later, all ewes were joined with fertile rams (n=7) during ten days.

Blood samples were collected at day 19 ewes after AI, for determination of plasma progesterone concentration (P4). Blood samples were obtained from the jugular vein into heparinised tubes and centrifuged immediately at 3000 rpm for 20 min. Plasma was pipetted and stored at −20 °C until assayed. P4 concentrations were measured using a commercial radioimmunoassay kit (Diagnostic System Laboratories, BeckMan Coulter, Madrid, Spain). The sensitivity of the assay was 0.11 ng/ml and the intra- and inter-assay coefficient of variation 9.2 and 10.1%, respectively. Ewes with a concentration of more than 1 ng/ml P4 were considered to be pregnant. Lambing rates (number of ewes lambed/number of ewes inseminated or mating) were recorded for the AI and natural services at 150±5 days and 165±5 days, respectively. Prolificacy (number of lambs born/number of ewes lambed) was also recorded.

2.3. Experiment 2: Fertility of Guirra Ewes Following Natural Insemination after Synchronized or Natural Oestrous

Seventy ewes, in non-breeding-season, were randomly divided into 2 groups. Ewes in one group (Treatmen 1, T1) and in the second group (Treatmen 2, T2) were synchronized as previous, but ewes in the second group the synchronized start 6 days later than T1. Twelve days after sponge withdrawal for T2, all ewes were divided into 7 lots with equal treatments. One ram was introduced to the each lot (1 rams per 10 ewes maximun) where they remained for 25 days. Ewes for the T1 group not conceiving at the synchronized oestrous (first service), but they were mated at next natural oestrous cycle (second service). For the T2 group, ewes were mated at the synchronized oestrous (first service) and the next natural oestrous cycle (second service). During all period, the ewes and rams sexual behaviour was recorded by video cameras using a Digital Recorders DVR (Circontrol, Barcelona, Spain). Four video cameras, mounted on observation towers, were used to record in detail and continuously all movements of the animals. Each camera was dedicated to a two lots. The filming started as soon as rams were introduced. The recorded tapes were later analyzed in detail. In order to individually identify both rams and ewes at a distance were painted on their back and flanks, using distinctive trademarcks and colours for the different treatment groups. The ewes were considered in oestrous when they were directly observed to accept a ride from a ram (standing reflex). Lambing rates were recorded for the animals that conceived during the induced oestrous cycle (T2, first service) and for those, which returned to oestrous and conceived at the subsequent spontaneous oestrous cycle (T1, first service and T2, second service) at 150 ± 5 days and from 165 ± 5 days, respectively.

2.4. Statistical Analysis

A generic descriptive analysis was used for study the fertility in the experiment 1, considering ewes pregnant when concentration of P4 was than 1 ng/ml. In experiment 2, fertility was analyzed for each treatment (T1 and T2) and oestrous type (synchronized or natural) using a logistic regression. Statistical analyses were performed using a commercially available statistics package (Statgraphics Plus, Version 5.1, STSC Inc., Rockville, MD, USA).

3. Results

In experiment 1, on day 19 after AI, the ewes showed a P4 concentrations ⩾1.0 ng/ml was the 24.7%. Significant differences in lambing rates were obtained (21.7% and 46.1%, for artificial and natural insemination, respectively), but not for prolificacy (1.1 and 1.2, for artificial and natural insemination, respectively). In experiment 2, similar percentage of ewes exhibiting oestrous from ewes after natural or synchronized oestrous were observed (87.9 and 88.2 %, respectively). The interval from sponge withdrawal to onset of oestrous during the natural oestrous was much shorter when compared with the synchronized oestrous (32.1±17.3 vs 73.2±86.7 h, respectively. P=0.1352). The conception rate was higher for T1 (natural estrous, first service) compared with the T2 (synchronize, first service and natural estrous, second service) (54.3 vs 38.6 %, P<0.05. Fig1). Significant differences were obtained when type of oestrous were compared (53.7 vs 32.3 %, natural and synchronize estrous. P<0.05. Fig1).

4. Discussion

Oestrous response and fertility vary greatly when intra-vaginal sponges are applied, dependent on species, breed, co-treatment, management, and mating system [10]. The overall post-treatment conception rate with fresh diluted semen reported in this study was 24.7%. This is well below the range of 43.75 to 70.5% reported for ewes synchronized and inseminated [20]-[23]. Previously, in Churra breed using vaginal AI the fertility results was 31% [24].

The differences could explain, at least partially, the variability between breeds [24]. To our best knowledge, no repors in the literature study the AI in Guirra breed, but we considered the value obtained in this study too low. These findings could support the hypothesis that exits an effect of progestagen on fertility. The effect of progestagen dose on fertility at the synchronized oestrous has been discussed. Deweese et al. [25], reported a tendency towards a higher percentage of ewes conceiving at the first oestrous following treatment with sponges containing 40 mg of medroxyprogesterone acetate (MAP) in comparison with those impregnated with 60 mg MAP. Crosby et al. [26] postulated that the presence of a high level of progestagen followed by its rapid drop is a condition to achieve acceptable fertility. This hypothesis was studied by Freitas et al. [27] who showed that the increase of progestagen level at the end of treatment in goats did not improve oestrous synchronization and can decrease fertility. Recent work performed by Greyling et al. [21] indicated that fertility following treatment with halved pessaries was higher than that obtained with whole ones. However, Simonetti et al. [22] detected that pregnancy following insemination at the synchronized oestrous was similar among the three doses employed.

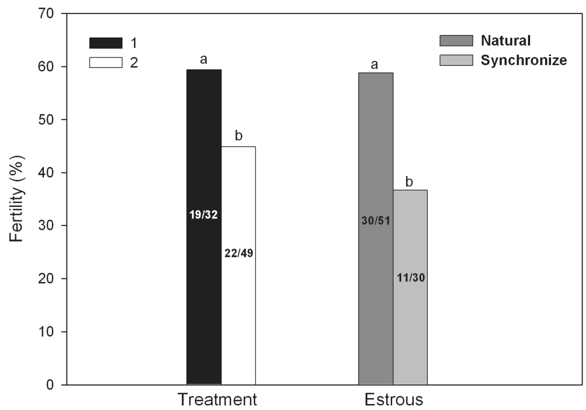

Fig 1. *Effect of natural or artificially synchronized estrus on fertility. Treatment 1 consisted in artificially synchronized estrus and the subsequent spontaneous estrus (first and second cycle). Treatment 2 consisted in a spontaneous estrus (second cycle). Letter a and b show statistically different values (P < 0.05).*

Progestagen concentrations in plasma increased 48 h after sponge insertion [28], [29], but decreased thereafter, and reached concentrations too low to simulate the activity of the corpus luteum at the end of the treatment [11]. Previously, in Manchega breed has been reported that P4 concentration increased starting 2 days after sponge removal, however the value detected at 2 days was 0.2±0.02 ng/ml [30]. The P4 concentration obtained in this study at 2 days was 1.31±0.16 ng/ml. Increase in P4 during these times has been implicated to play a role in induction of oestrous behavior [31] and assurance of normal luteal lifespan [32], [33]. In ewes, estrus synchronization with progestagen has been reported to lead to lower pregnancy rates due to factors such as alteration in patterns of LH release, quality of oocyte, sperm transport and survival in the female reproductive tract and quality of embryos [12], [13], [34]-[37]. Our results showed that the interval from the intra-vaginal sponge removal to the onset of oestrous was highest than natural estrus. Delayed time to oestrous for whole sponge has been reported, when whole and halved sponges were compared, as consequence to the residual progesterone available in the group treated with whole sponges [21]. However, in our study low progestangen sponge was used (30 mg).

The appropriate time of insemination after sponge removal was found by different workers to be between 48 and 65 h. Early 36 h or late 72-78 h insemination after sponge removal generally reduced the fertility [38]. For this reason, probably delay in the onset of oestrous for synchronized ewes could explained the low results obtained in this study when vaginal AI was used. However, in the third experiment, mating was used and the mean fertility rate in the synchronized group oestrous was only 34% versus 58% in the natural oestrous group. Previously results suggest that differences among initial doses of sponges correlate with differences among residual sponges remaining on sponges following treatment, but not with the absorbed levels [22]. Controversial results have been reported about the progestagen absorption. Mobarak et al. [39] found the level of progestagen absorbed to increase as the initial dose increased. Contrary to this finding, Greyling et al. [20] informed that the dose of progestagen in the sponge was superfluous. Long-term progestagen protocol did not suppress LH to the extent achieved during the luteal phase [40], leading to inadequate follicular development, with persistent large estrogenic follicles [36], [41]-[43]. In the absence of a corpus luteum, synchronisation resulted in the ovulation of a prolonged dominant follicle. Ewes that ovulate a prolonged dominant follicle will be less fertile than those ovulating short-duration follicles [15], [43]. This relationship between duration of dominance and fertility has been shown in cattle [44]. Also has been reported that administration of 500 IU eCG after long-term progestagen treatment has limited effects on the dynamics of ovarian follicular wave development [45].

5. Conclusion

In conclusion, detrimental effect of fluorogestone acetate sponge for synchronized oestrous in Guirra breed sheep on fertility was reported. This effect was observed as consequence of high plasma progesterone concentration observed after sponge removal. New methods must be proposed for synchronizing oestrous and applying artificial insemination in Guirra breed.

Acknowledgements

The authors received financial support for this work from the INIA (RZP2006-00004-00-00)

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