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Small Ruminant Research 71 (2007) 117–122
Small Ruminant Research
www.elsevier.com/locate/smallrumres

Reproductive performance and progesterone secretion in estrus-induced Manchega ewes treated with hCG at the time of AI

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Received 3 November 2005; received in revised form 7 April 2006; accepted 25 April 2006
Available online 30 June 2006

Abstract

Two experiments were conducted to determine the effects of supplementing hCG at insemination on the luteal function and reproductive performance in estrus-induced mature Manchega ewes. The first experiment was carried out under field conditions with 1560 ewes on 27 farms. After estrous synchronisation with progestagen sponges and eCG, cervical inseminations were performed between October and February (breeding season) on 665 ewes on 12 farms and between March and June (non-breeding season) on 895 ewes on 15 farms. In each period and within each farm, approximately half of the ewes received an i.m. injection of 500 IU hCG at the time of insemination. The other half remained untreated and served as controls. Neither fertility (hCG: 44.2%; control: 42.0%) nor prolificacy (hCG: 1.57; control: 1.54) were affected by hCG treatment. However, on farms where fertility in the control group was persistently low, fertility of the hCG treated ewes was increased by 7.6% relative to those not treated ($P < 0.1$). The second experiment was carried out on 64 ewes from an experimental flock. Estrus and ovulation were induced in all ewes as in experiment 1. Thirty-two ewes received a single i.m. injection of 500 IU hCG at the time of intrauterine insemination, while the other half were not treated. Plasma progesterone (P_4) concentrations was measured every 2 days over a 28 day period following insemination. Progesterone secretion in the inseminated pregnant ewes was not modified by the hCG administration. However, a tendency ($P < 0.1$) for higher P_4 concentrations from days 8 to 14 was observed in those ewes that had been treated with hCG, but that did not conceive at AI. Pregnancy (62.5% and 59.4%), fertility or number of ewes lambing/ewes inseminated (56.3% and 50.0%) and prolificacy (1.56 and 1.50) of the inseminated pregnant ewes did not differ between the hCG-treated and control ewes. In ewes that failed to conceive at AI and were mated at the next oestrus (return cycle), fertility was increased by 12.1% ($P < 0.1$) in those ewes that had previously been treated with hCG (58.3%), compared with those not treated (46.2%). Results indicate that hCG treatment did not improve reproductive performance in estrus-induced and AI'd Manchega ewes, but treatment may be beneficial in increasing fertility in ewes from farms with low fertility rates.

Keywords: Manchega ewes; hCG; Fertility; Prolificacy; Progesterone

1. Introduction

In general, the low fertility rates achieved in estrus-induced and artificially inseminated ewes is the primary factor limiting the advancement of AI programs

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0921-4488/$ – see front matter
doi:10.1016/j.smallrumres.2006.04.015

in sheep production systems (Evans, 1988). Although this reduced fertility has been related to many factors including breed, age, season, physiological and nutritional status of females, estrous synchronization treatment and time of insemination, insemination technique and semen quality, preimplantation embryonic loss has long been recognised as the major limiting factor for obtaining optimum reproductive performance in domesticated livestock (Windsor, 1995). In sheep, most fertilized eggs are lost between days 8 and 16 after AI (Nancarrow, 1994) and an inadequate luteal function is thought to be one of the critical factors affecting embryo survival (Wilmut et al., 1986; Ashworth et al., 1989).

Human chorionic gonadotropin (hCG), which is similar to LH in function, has been shown to increase luteal weight and endogenous synthesis of progesterone (P_4) from the corpus luteum in sheep (Farin et al., 1988; Nephew et al., 1994). Thus hCG has been administered to ewes at different times during the cycle after AI or breeding in an attempt to reduce embryonic mortality and improve reproductive performance—but the effectiveness of these treatments has not been consistent between studies. Kittot et al. (1983) reported increased plasma P_4 concentrations and enhanced pregnancy rates in synchronized lactating ewes following the treatment with multiple doses of hCG (100 IU) on days 11–13 after mating. In contrast, no improvement in pregnancy and lambing rates in estrus-induced and artificially inseminated ewes has been reported with multiple doses of hCG (100 IU) administered on days 3–5 after AI (Fukui et al., 2001), or with a single hCG (500 IU) injection given on days 4, 7 or 11 after AI (Gamboni et al., 1984; Nephew et al., 1994; Ishida et al., 1999), even though hCG treatment stimulated the corpus luteum (CL) and increased P_4 concentrations.

The failure of the sheep CL to function normally may also be caused by an inadequate gonadotrophin stimulation during the peri-ovulatory period and therefore, alternative research has concentrated on improving pregnancy rates by applying hCG at the time of AI—but results have also been variable. While some researchers observed both lambing percentage and litter size tended to be higher in ewe lambs supplemented with hCG on the day of mating (Khan et al., 2003), others have reported no improvement in reproductive efficiency in mature ewes (Zamiri and Hosseini, 1998).

The Manchega sheep breed is one of the most important Spanish dairy breeds, widely distributed in the central area of Spain. Their fertility following AI at an induced estrous cycle has been shown to range from a mean value of 40% with cervical inseminations, to a mean value of 60% following laparoscopic intra-uterine inseminations. However, fertility rates (number of ewes lambing/ewes inseminated) lower than 35% have also been recorded, depending on the sheep management and production systems (Montoro, 1995). Taking into account that increased fertility, under certain conditions of sheep husbandry, may be economical and profitable, this study was carried out to evaluate whether supplementing the preovulatory gonadotrohin surge with a single injection of 500 IU hCG, given at the time of AI, has any effect on serum progesterone concentrations and the reproductive performance in synchronized and AI'd Manchega ewes.

2. Material and methods

2.1. Experiment 1

This experiment was conducted across 27 farms distributed in the central areas of Spain (latitude 38°–40°N) and involved 1560 mature Manchega ewes in semi-intensive milking production flocks. Of these 1560 ewes, 665 ewes from 12 farms were evaluated between October and February (breeding season) and 895 ewes from 15 farms evaluated between March and June (non-breeding season).

Estrus and ovulation were induced by treating all ewes with an intravaginal sponge impregnated with synthetic progestagen (30 mg FGA, Intervet) for 12 days, followed by an i.m. injection of 500 IU eCG at the time of the sponge withdrawal. In both seasons and within each farm, ewes were inseminated cervically, approximately 55–56 h after eCG injection by a single operator, using semen provided by the CERSYRA (Regional Insemination Center, Castilla-La Mancha, Spain). Each insemination dose consisting of 0.25 ml cooled semen containing 400×10^6 sperm. Immediately after AI, ewes were randomly assigned to either a group receiving a single i.m. injection of 500 IU hCG (breeding season, $n = 331$; non-breeding season, $n = 450$) or to an untreated group that served as controls (breeding season, $n = 334$; non-breeding season, $n = 445$). Fertility (number of ewes lambed/number of ewes inseminated) and prolificacy (number of lambs born/number of ewes lambed) were recorded at lambing.

2.2. Experiment 2

This experiment was conducted in March using 64 mature Manchega ewes from an experimental flock kept at the facilities of the Animal Reproduction Department, INIA, Madrid (40°25′N). Estrus and ovulation were induced in all ewes as in experiment 1 with progestagen/eCG treatment. Ewes were subjected to intra-uterine AI using fresh semen from proven fertile rams, deposited in the uterine horns by laparoscopy 60 h after eCG injection (López Sebastián, 1992). Ewes were randomly allocated to two groups of 32 ewes each. Ewes in one group (hCG-treated group) received a single i.m. injection of 500 IU hCG immediately after AI, whereas, the other group (control group) was untreated. Fifteen days after AI, intact rams were introduced into the flock for 6 days to allow those ewes not conceiving at the intra-uterine insemination (first service), to be mated at the next natural estrous cycle (second service).

Blood samples were collected from all 64 ewes every 2 days from the insemination day (day 0), until day 28 after AI, for determination of plasma P_4 concentration. Ewes with a concentration of more than 1 ng/ml P_4 on day 18 after AI were considered to be pregnant. Lambing rates and the number of lambs born were recorded for the animals that conceived during the induced estrous (first) cycle and for those, which returned to estrus and conceived at the subsequent spontaneous estrous cycle (return cycle) at 150 ± 4 days and from 166 to 172 days, respectively. Overall reproductive performance was also calculated by combining the lambing performance in each cycle.

Blood samples were obtained by jugular venipuncture into heparinized vacutainer tubes (5 ml). The plasma was immediately separated by centrifugation at $1500 \times g$ for 15 min and stored a $-20\,^{\circ}\mathrm{C}$ until analysed for progesterone concentration by RIA (López Sebastián et al., 1984). The detection limit was 0.12 ng/ml. Intra- and inter-assay coefficients of variation were 8.3% and 10.7%, respectively.

2.3. Statistical analysis

Fertility and prolificacy data in experiment 1 were analysed using an ANOVA (BMDP system, Statistical Software) for effects of treatment. Season and farm were also included in the model to identify factors interacting with treatment. Farms were classified prior to the analysis into three classes, according to the fertility rates of inseminated ewes: (i) low fertility farms (LFF) those, with fertility rates of 35% or less, (ii) normal fertility farms (NFF), being farms with fertility rates between 36% and 45%, and (iii) high fertility farms (HFF) being farms having fertility rates of above 46%.

In experiment 2, the progesterone data were analysed using an ANOVA for repeated measures. Analysis of variance included treatment, day, and the interaction. The effects of treatment on reproductive performance were analysed by one-way ANOVA (BMDP system, Statistical Software).

3. Results

3.1. Experiment 1

The data on reproductive performance of the estrus-induced and cervical inseminated ewes in the hCG-treated and control groups are set out in Table 1. No season by farm, treatment by season, or season by treatment by farm interactions were noted in any of the parameters measured. However, in both seasons, fertility in ewes from farms with low fertility (LFF), which received 500 IU hCG at the time of insemination tended to be greater, compared to the control ewes (Table 1). When

Table 1
Fertility and prolificacy of control and hCG (500 IU) treated Manchega ewes at the time of cervical insemination

Treatment group	Farms	No. of ewes	Fertility (%)	Prolificacy
Control				
Breeding-season	LFF ($n=4$)	110	27.3 a	1.63 a
	NFF ($n=4$)	122	45.1 a	1.55 a
	HFF ($n=4$)	102	53.9 a	1.69 a
Non-breeding season	LFF ($n=6$)	180	25.0 a	1.40 a
	NFF ($n=4$)	140	42.1 a	1.56 a
	HFF ($n=5$)	125	58.4 a	1.47 a
hCG-treated				
Breeding season	LFF ($n=4$)	108	34.3 a	1.65 a
	NFF ($n=4$)	127	44.1 ab	1.43 a
	HFF ($n=4$)	96	52.1 ab	1.68 a
Non-breeding season	LFF ($n=6$)	178	33.1 b	1.46 a
	NFF ($n=4$)	148	47.3 ab	1.60 a
	HFF ($n=5$)	124	54.0 ab	1.61 a

Values in the column with different letters indicate differences ($P<0.1$).

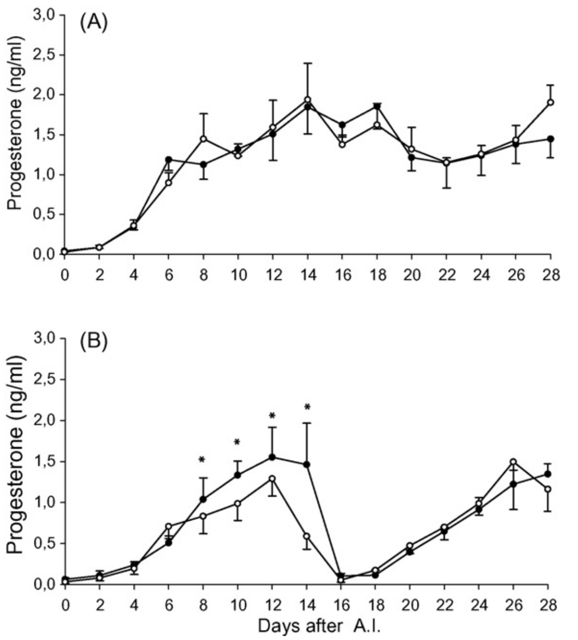

Fig. 1. Mean (±S.E.M.) concentrations of progesterone in pregnant (A) and non-pregnant (B) ewes in control (◯) and treated (●) Manchega ewes at the time of intrauterine insemination. Days marked with an asterisk (*) indicate a tendency to differ between groups ($P < 0.10$).

all data where pooled and further analyzed in relation to treatment, regardless of season and farm, fertility and prolificacy were not different between the hCG-treated (44.2% and 1.57) and control (42.0% and 1.54) ewes.

3.2. Experiment 2

The mean plasma P_4 profiles of the pregnant and open ewes in the hCG-treated and control groups are depicted in Fig. 1. In the pregnant ewes resulting from the intra-uterine insemination, the mean plasma P_4 concentrations increased ($P < 0.001$) in both groups from day 2 onwards. There were no differences between the hCG-treated and controls at any time during the sampling period. In the non-pregnant ewes, no significant differences were recorded in plasma P_4 concentration between the hCG-treated and control ewes. Mean plasma P_4 concentrations on day 8, 10, 12 and 14 were slightly higher ($P < 0.1$) in the hCG-treated, than the control ewes. The estrous cycle length was similar in the two groups.

There were no differences between the hCG-treated and control ewes in any of the parameters measured following AI at the first induced estrous cycle (Table 2). However, fertility at the second spontaneous estrus (58.3% and 46.2%) and after two service periods (78.1% and 68.8%) tended to increase in ewes that had been treated with hCG at the first induced estrous cycle. Prolificacy was similar in hCG-treated and control ewes (Table 2).

Table 2
Reproductive performance of control and hCG (500 IU) treated Manchega ewes at the time of intra-uterine insemination

	Treatment group	
	Control	hCG-treated
No. of ewes	32	32
First service (AI)		
Pregnant ewes (%)	59.41 a	62.52 a
Fertility (%)	50.02 a	56.33 a
Prolificacy	1.50 a	1.56 a
Second service		
Fertility (%)	46.23 a	58.32 b
Prolificacy	1.33 a	1.43 a
Total (two services)		
Fertility (%)	68.83 a	78.14 b
Prolificacy	1.45 a	1.52 a

Values in the same row with different letters indicate differences ($P < 0.10$).

4. Discussion

In the present study, results from experiment 1 as well as those from experiment 2 showed that in synchronized and artificially inseminated Manchega ewes, the injection of 500 IU hCG at the time of cervical or laparoscopic AI did not improve overall fertility or prolificacy. These results are similar to that previously reported on the same breed by Montoro et al. (1993).

These results are in agreement with earlier studies in cattle (Swanson and Young, 1990) and sheep (Zamiri and Hosseini, 1998), in which treatment of hCG on the day of AI or mating was not effective in improving fertility. These results are in contrast with reports by Khan et al. (2003), in which hCG given on day of mating increased the pregnancy rate and litter size of ewe lambs.

Despite the current findings, the tendency for higher fertility in ewes receiving hCG treatment on farms with low fertility in the control group of experiment 1, or in ewes that failed to conceive at AI and were mated at the subsequent spontaneous estrus (return cycle) in experiment 2, cannot be overlooked. Interestingly, in the farms (LFF) in which mean fertility rate in the control group was only 26%, hCG given at the time of AI improved the fertility in the hCG-treated ewes by 7.6%—reaching a mean fertility rate of 33.6%. In contrast, this effect was not apparent neither for the hCG-treated ewes on farms with normal or relatively high fertility in the control group of experiment 1 (Table 1) and experiment 2 (Table 2). A trend toward higher fertility rates using GnRH or hCG treatment at AI has also been observed in cows on farms where reproductive efficiency was low (Nakao et al., 1983), as well as in heat-stressed dairy cows (Willard et al., 2003). These results suggest that the use of additional hormonal treatment, e.g. hCG or GnRH at the time or after AI in estrus-induced ewes, could be beneficial in farms with persistently low fertility rates. In this study, an improvement of almost 8% in fertility in ewes on such farms could have an economic benefit to the producers.

To improve the fertility, hCG would have to increase the fertilization rate, reduce the embryonic death rate, or both. Previous studies have suggested that hCG supplementation could improve the pregnancy rate by initiating endogenous increases in progesterone via modulation of ovarian follicular populations and the promotion of accessory corpus luteum formation (Schmit et al., 1996). Although the design of experiment 1 did not allow to determine the means by which hCG given at AI increased fertility in low fertility farms, it is unlikely that enhanced luteal function was responsible for the beneficial effects of hCG. In experiment 2, peripheral concentration of progesterone were unaffected by hCG administration on the day of AI. The explanation for the increase in fertility in ewes treated with hCG on LFF farms probably involves mechanisms for controlling ovum fertilization. Thus, the improvement in fertility in ewes from those farms was probably the result of ovulation and luteinisation occurring at the appropriate time relative to insemination as has been speculated to occur in cows treated with GnRH or hCG on the day of insemination (Peters et al., 1992).

As inadequate levels of progesterone during the early and mid-luteal phases of the estrous cycle has been related with decreased fertility due to abnormal development of embryos and early embryonic death (Wilmut et al., 1985; Ashworth et al., 1987), the main purpose of experiment 2 was to determine whether a single injection of hCG given to estrus-induced ewes at the time of insemination could increase the secretion of P_4 and thus, subsequent fertility. It has been reported that hCG treatment in the middle of the luteal phase increased plasma P_4 concentrations in ewes and enhanced pregnancy or lambing rate (Kittot et al., 1983; Nephew et al., 1994). To the contrary, Ishida et al. (1999) and Fukui et al. (2001) reported hCG treatment given at the early luteal phase also to increase the plasma P_4 levels in hCG-treated ewes, but this was not reflected in the pregnancy and lambing rates of the inseminated ewes. In the present study, data from experiment 2 showed that hCG (500 IU) given at the time of insemination, did not modify the plasma P_4 concentrations, or pregnancy rate or fertility in ewes that became pregnant following AI. These results are consistent with an earlier study in cattle (Sianangama and Rajamahendran, 1992), revealing that hCG treatment on the day of insemination did not enhance plasma P_4 concentrations during early pregnancy or, fertility in hCG-treated animals, compared to those not treated. These findings suggest the ability of hCG to either promote synthesis of P_4 by the corpus luteum or increase fertility, varies with the stage of the estrous cycle, like in cows (Breuel et al., 1989). Alternatively, it is also possible that a positive effect of hCG treatment is obtainable only when fertility in the control group is comparatively low, as in LFF farms from experiment 1.

When evaluating changes in P_4 concentrations in ewes that failed to conceive at AI, it should be noted that the corpus luteum regressed between days 14 and 16 in the two groups and plasma P_4 levels on days 8, 10, 12, and 14 were slightly higher in the hCG-treated ewes. Furthermore, the fertility at natural mating at the second spontaneous estrus and overall fertility after combining the lambing rates in each cycle, increased by 12.1% and 9.3%, respectively, for ewes receiving hCG at the time AI at the first induced estrous cycle (Table 2). These findings are in agreement to those of Helmer and Brit (1986), who reported cows, which require additional services to have higher fertility rates if treated previously with hCG. In contrast, in those reported by Khan et al. (2003), any beneficial effect of hCG treatment was not carried to the next cycle as animals returning to service did nor perform better than the controls. Although results observed in the inseminated ewes that failed to conceive at the treatment cycle, appear to indicate the existence of a possible relationship between the level of P_4 during the hCG treated cycle. The subsequent reproductive performance at natural mating, the frequency of blood sampling of this study and the limited number of animals involved does not allow making a definitive conclusion.

In the study, no differences in prolificacy between hCG-treated and control ewes were found. These results disagree with previous studies in ewes (Radford et al., 1984; Zamiri and Hosseini, 1998; Khan et al., 2003), in which hCG treatment given at time of insemination increased the number of lambs born per ewe lambing as a result of an increase in ovulation rate. These differences could possibly be due to the different protocols used, but it is also probable that other factors such as breed, management systems, nutritional and physiological status, could also have affected the response.

Based on the results of experiments 1 and 2, it can be concluded that the treatment of Manchega ewes with hCG (500 IU) given at the time of AI did not improve the general fertility or prolificacy, but it may be beneficial in enhancing fertility when administered to ewes on farms with persistently low fertility rates or in ewes that require a repeat service.

Acknowledgements

Dr. Butcher from the West Virginia University is thanked for the progesterone antiserum used and CERSYRA from Valdepeñas (Ciudad Real, Castilla-La Mancha, España) for providing semen used in experiment 1 and the Manchega farmers who supplied the animals.

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