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Superovulation and embryo quality with porcine follicle stimulation hormone (pFSH) in Katahdin hair sheep during breeding season

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Estrus synchronization of 21 ewes was carried out using intravaginal sponges (60 mg of medroxyprogesterone acetate, Sincrogest®-Sanfer, Mexico) for 14 days. 7 ewes were randomly assigned to one of the three treatments: T_1, 80 mg; T_2, 120 mg; T_3, 140 mg of porcine follicle stimulating (pFSH) (Folltropin-p®-Bioniche, Canada) administered on day 12 after sponge insertion. The dose was divided in decreasing doses; second and third day representing 50 to 75% and 25 to 33% of the first day dose, respectively. At the beginning of superovulation treatment, each ewe received 5 mg of dinoprost tromethamine (1 ml of Lutalyse® Pfizer, Mexico). An effect of pFSH level was observed on the interval from sponge removal and the onset of estrus (REI) ($P < 0.01$), with a difference of 13.5 h between ewes from treatment 2 and 3. The number of follicles (F), number of collected embryos (CE) and transferable embryos (TE) ($P < 0.05$) was also different among treatments, with highest values observed in T3 (3.0 ± 0.6 for CE and 2.3 ± 0.5 for TE). Embryo recovery rate was in average 66.5%, ($P > 0.05$) of CL observed. Further investigation is guaranteed to evaluate the effect of pFSH doses on estrus synchronization for selected breeding seasons.

Key words: Embryo transfer, hair sheep, porcine follicle-stimulating hormone (pFSH), breeding season.

INTRODUCTION

Sheep production in Mexico has increased during the last years due to high demand of ovine meat (AMCO, 2008). Currently, there is a sheep population of 8.5 million of heads, with a trend to reach 10 million animals in the next years, of which 33% is hair sheep (FAO, 2008). At present, sheep breeders are enforced to improve the genetic potential of their animals, and in this regard they also look for opportunities to spread the genetic material, using several reproductive techniques, like artificial insemination, as well as assisted reproductive techniques such as MOET (multiple ovulation and embryo transfer) integral program, where the aim is to speed up via mothers' genetic progress (Baldasarre, 2007). Since its beginning, this technique has had varied responses in

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Table 1. Procedure of experimental superovulation treatments applied in the present study to Katahdin hair sheep.

Treatment pFSH	Administration shift	Administration protocol (mg of pFSH per dose)		
		First day	Second day	Third day
1 (80 mg)	a.m.	20	15	5
	p.m.	20	15	5
2 (120 mg)	a.m.	30	20	10
	p.m.	30	20	10
3 (140 mg)	a.m.	40	20	10
	p.m.	40	20	10

goats and sheep in accordance with embryo recovery rate and depending on each of the protocols of superovulation that have been used on different breeds worldwide (Cognie et al., 2003; Gonzalez-Bulnes et al., 2004; Baldasarre, 2007; Paramio, 2010). Among the limitations recently found, one concerns optimal response of the different superovulation protocols that have been used, where dosage of the follicle-stimulating hormone, (FSH) may vary in accordance with body size and breed affecting follicular dynamic (Baldasarre, 2007; Veiga-Lopez et al., 2008; Menchaca et al., 2009, 2010; Paramio, 2010). Most of the studies have been done on wool sheep (Cognie et al., 2003). However, in hair sheep, great part of own features are due to their adaptation to diverse climatic conditions (Ortega-Abasolo, 2006), high prolificacy rates (Lopez-Junior et al., 2006; Sánchez et al., 2011) and too low seasonal anoestrus presence, as well as its resistance to parasites (Santos et al., 2007; Tabarez-Rojas et al., 2009; Ungerfeld and Sánchez, 2012); there is great interest in propagating hair breeds in Mexico, either from paternal or maternal side (embryo transfer). Since the year 2000, Katadhin breed started being introduced to the United States of America, because of its capacity for adaptation to diverse climates and for being a dual-purpose breed (AMCO, 2008). Currently, in Mexico, breeding programmes related with artificial insemination and embryo transfer have been developing; for which, in regard to Katadhin breed, there have been no reports of an embryo transfer programme. The aim of this study was to evaluate the effect of 3 doses of porcinefollicle-stimulating hormone (pFSH) on the response of embryo recovery and ovary structures in hair sheep, during the breeding season.

MATERIALS AND METHODS

Location

The present study was carried out during the natural breeding season (autumn) at the “Mary” sheep flock, located in Higueras, Nuevo Leon, at 25° 54’ North latitude and 99° 58’ West longitude, at an altitude of 451 masl. The weather is extreme, with an annual mean temperature of 25°C. Twenty one Katahdin ewes, (initial average age = 22 months, body weight = 47.5 ± 3.7 kg and average body condition (BC) of 2.75 were used. Embryo recovery was carried out at the Laboratorio de Reproducción Animal of the Departament of Agronomy of the Universidad Autónoma de Nuevo León, located at the km 17.5 of the Zuazua-Marin road in Marín, Nuevo León, México.

Selection and management of ewes

Ewes that had given birth during the last breeding season were selected, with a postpartum period of 3 months before initiating the superovulation treatment. Sanitary management was provided to the females, which consisted of administration of vitamins A, D and E at a rate of 1 ml/animal with a concentration of 500 000, 75 000 and 500 IU, respectively. Likewise, internal deworming was carried out using a combination of ivermectin (5 mg, ®Pfizer, Mexico) and closantel (125 mg, ®Bayer, Mexico) by subcutaneous via at a rate of 1 ml/25 kg of body weight. A month before hormonal treatment, nutritional supplementation was initiated; daily concentrate allowance was 0.81 kg, containing 18% of crude protein (CP) and 2.5 Mcal/kg of body weight. All ewes had access to green forage and were kept in confinement pens during the experimental period.

Synchronization and superovulation protocol

Estrus synchronization of the 21 ewes was carried out by the administration of intravaginal sponges, containing 60 mg of medroxyprogesterone acetate (MPA), for 14 days (Sincrogest®-Sanfer, Mexico). Superovulatory treatment consisted in intramuscular administration of porcine follicle-stimulating hormone (pFSH, Folltropin-p®-Bioniche, Canada). The administration of pFSH started on day 12 after sponge insertion. The pFSH dose was divided into 6 applications at intervals of 12 h (8 a.m. and 8 p.m., for 3 days) in decreasing doses (Table 1). Each ewe received 5 mg of dinoprost tromethamine (1 ml of Lutalyse® Pfizer, Mexico) at the moment superovulation treatment was initiated. 7 ewes were randomly assigned to each of the 3 treatments described in Table 1. Upon removing the sponges, estrus was monitored, twice a day during 3 days, with a fertile male ram. After estrus detection, ewes were fertilized by natural mating.

Embryo recovery

Embryo recovery was carried out via laparotomy technique at day 7 after vaginal sponge removal, using uterine washing enriched with PBS (phosphate buffered saline, Biolife, Agtech, KS, USA) (40 ml

Table 2. Responses to superovulatory treatments (mean ± SEM) in characteristics of observed follicles and collected embryos in Katahdin hair sheep.

pFSH dose (mg)	No. females	REI (h)REI	No. follicles/ ewe (F)	Corpus luteum/ ewe (CL)	Collected embryos/ewe (CE)	Transferable embryos/ewe(TE)
T1 = 80	7	20.1 ± 2.1[b]	0.1 ± 0.6[b]	1.4 ± 1.1[ns]	1.0 ± 0.6[b]	1.0 ± 0.5[b]
T2 = 120	7	14.9 ± 2.3[c]	1.8 ± 0.6[a]	4.5 ± 1.2[ns]	3.0 ± 0.7[a]	2.0 ± 0.6[a]
T3 = 140	7	28.4 ± 2.5[a]	2.1 ± 0.6[a]	4.6 ± 1.1[ns]	3.0 ± 0.6[a]	2.3 ± 0.5[a]

Values with different letters within each column are significantly different in each variation source ($P < 0.05$); [ns], non-significant.

Table 3. Responses to superovulatory treatments (mean ± SEM) in characteristics of collected and transferable embryos in Katahdin hair sheep.

pFSH dose (mg pFSH)	% Embryo recovery	% Transferable embryos
T_1 = 80	1/1.4 (67.4)[ns]	1.0/1.0 (100)[a]
T_2 =120	3/4.5 (66.6)[ns]	2.0/3.0 (66.6)[c]
T_3 = 140	3/4.6 (65.6)[ns]	2.3/3.0 (76.3)[b]

Values with different letters within each column are different ($P < 0.05$); [ns], non-significant.

for each uterine horn). The technique described by Baril et al. (1995), where ewes were subjected to a nutritional and liquid diet of 24 h before surgery, was used. Once the washed uterine liquid was collected, identification of ovary structures was carried out, as well as search and classification of embryos based on the description of Robertson and Nelson (1999). Embryos showing a symmetric and spherical mass, with individual and uniform blastomeres in regard to size, color and density, were considered able to be transfered. Parallel with evaluation of collected embryos, at the moment uterine flushing, the number of follicles and corpus lutea were visually counted, without considering type and size of these 2 ovarian structures.

Statistical analysis

The evaluated variables were: estrus percentage, interval from sponge removal to the onset of estrus (REI), follicle number (F), corpus lutea number (CL), collected embryo number (CE), and transferable embryo number (TE). The influence of treatment was analyzed using a lineal model after normalizing the data through the square root transformation, according to Snedecor and Cochran (1980). When significant differences occurred, the minimum significant difference (MSD) was used for post-hoc comparisons. Data were analyzed using SPSS 17 (2008) and are presented as mean ± SEM.

RESULTS

In the 3 treatments, 100% of estrus was observed in the treated ewes. A significant effect ($P < 0.05$) of pFSH level on REI, as well as on the number of follicles (F), number of collected embryos (CE) and transferable embryos (TE) was detected. There was no effect of treatments on the number of corpus luteum (CL).

Table 2 shows the results for every pFSH dose used. Overall REI average was 21.1 ± 2.1 h in average for all ewes. REI was longest for T3, shortest for T2 and intermediate for T1 ($P < 0.05$). Ewes from T3 and T2 showed the highest values for F, CE and TE ($P < 0.05$); the lowest response with regard to the evaluated variables was shown in ewes receiving 80 mg of pFSH (T1) ($P < 0.05$).

There were no differences among treatments ($P > 0.05$) in the percentages of embryo recovery (Table 3). The lowest ($P < 0.05$) values of transferable embryos (66.0%) were found in ewes receiving 120 mg of pFSH (T2), followed by T3 (76.3%) and T1 (100%), respectively.

DISCUSSION

In the present study, estrus percentage was higher than the one reported by Lopes-Junior et al. (2006) in Morada Nova native ewes, from the north of Brazil, where they obtained 88.9 and 90.9% presence of estrus in young and adult females, respectively. This may be due to good body condition of sheep, as well as having been carried out during the breeding season, which guarantees a better response than if conducted outside their reproductive time. Likewise, the sponge used contained 60 mg of medroxyprogesterone, which ensures high progesterone concentration at the moment of device removal and therefore, estrus rate is higher, since there are sponges containing 30 and 40 mg medroxyprogesterone available in the market.

However, up until 90% of estrous can be achieved in hair sheep, by using new CIDR, comparing with 80.5% achieved by using intravaginal sponges, or 61.8% by previously using CIDR (Godfrey et al., 1999; Kohno et al., 2005; Ortega-Abasolo, 2006). According to González-Bulnes et al. (2004), an additional alternative for increasing estrus percentage can be the use of a second

sponge inserted 7 days apart from the first one. With this protocol it is possible to record up to 95% estrus percentage, which represents 18% improvement in results, compared with the figures obtained by using a single sponge over 14 days. This improvement supposes application of a prostaglandin analogue at the beginning of the superovulation (González-Bulnes et al., 2004).

Lopes-Junior et al. (2006) reported a greater variation in the onset of estrus when progesterone concentration is low at the time of removing the intravaginal device. New short synchronization protocols (5 to 7 days) are being used in order to obtain higher progesterone concentrations at the moment of removing the intravaginal device (Menchaca et al., 2009). With both alternatives, it is possible to achieve a maximum concentration of serum progesterone, 48 h after insertion of the intravaginal device (Lopez, 2004) and to avoid decreasing progesterone concentrations afterwards, which can turn insufficient to stimulate the corpus luteum activity at the end of the long synchronization protocol (Menchaca et al., 2010). Acording to Lopez (2004), this issue may be responsible for the great variability in estrus appearance as a result of conventional long term synchronization protocols.

In this study, body condition score of the ewes was 2.75, and hence better than the average of 2.5 in a scale of 0 to 5 (Mendizabal et al., 2011). Thus it can be assumed that females used in the present experiment were in positive energy balance, this influencing positively blood estrogen levels and consequently the estrous rate (Scaramuzzi et al., 2006; Sosa et al., 2009). Moreover, the rate of device losses was zero (Wildeus, 2000; Avendaño-Reyes et al., 2007). Also, it must be considered that the present study was carried out during the breeding season, when better rate of estrus in contrast to anoestrus season has been reported (Wildeus, 2000). Regarding the interval from sponge removal to the onset of estrus (REI), the results were lower than those reported by Godfrey et al. (1999), Lida et al. (2004) and Kohno et al. (2005) in ewes synchronized with CIDR (26.5 ± 2.3, 23 ±1.8 and 36.3 ± 15.7 h, respectively). Differences in REI can be due to genetics, as well as to great variability of the onset of estrus, and its relation to ovulation onset (Lopes et al., 2006; Bartlewski et al., 2011). Lopez (2004) stated that once the intravaginal device is applied, the maximum progesterone concentrations are reached within the following 48 h, decreasing thereafter to very low concentrations, which are insufficient for stimulating corpus luteum activity at the end of the treatment. In the case of this study, REI was shorter than the one reported by Lopez (2004); however, it was similar to that reported by Chagas e Silva et al. (2003) in native Saloia de Portugal ewes (25.3 ± 0.5 h). Hair sheep have a shorter REI than wool breed sheep and this effect is even stronger if superovulation treatment is performed during the normal breeding season (Bartlewski et al., 2011). The majority of the studies in sponge and CIDR report CIDR values between 28 and 35 h, considering that the device administration, in the present study, works in Katadhin sheep, according to the conditions in which it is exploited in northeast of Mexico.

The number of corpus lutea, was smaller than that reported by Lopes-Junior et al. (2006) in young (10.2 ± 1.2) and adult females (5 ± 0.8) Morada Nova ewes, using a dose of 200 IU of pFSH, distributed in 6 applications. Chagas e Silva et al. (2003), found CL values of 8.3 ± 0.8 in summer and 9.3 ± 1.1 in autumn with no differences between the 2 seasons ($P > 0.05$). Variations in corpus luteum are due, among other factors, to the age of the ewe (Lopes-Junior et al., 2006), breed prolificity (Gonzalez-Bulnes et al., 2004) and nutritional status of the ewe donors (Sosa et al., 2009). In the present study pFSH-doses were lower than those used by others. Considering that there are no pFSH doses reported in Katadhin sheep, lower doses to evaluate the response under environmental and management exploitation in Mexico were applied, for instance, Herrera-Camacho et al. (2008) applied a total dose of 200 mg pFSH and obtained a greater number of CL per superovulated Pelibuey ewe, when they were supplemented with high energetic ingredients (corn oil) (14.7 ± 1.9) in contrast to unsupplemented control group (10.7 ± 1.4). A main aspect of the region is the high environmental temperature that could have affected the ovulatory response, since according to Tabarez-Rojas et al. (2009), exposure of superovulated ewes to heat stress may have an influence on incidence of premature CL regression, which should be considered in MOET programs, and with greater caution in hair sheep that are constantly exploited in warm and semi-desert regions, with high environmental temperatures. In this study, the number of follicles (F) was smaller than that reported by Chagas e Silva et al. (2003) (average of 2.7 follicles larger than 5 mm in Saloia de Portugal ewes), who evaluated 2 hormone sources in superovulated ewes, and found that animals treated with equine chorionic gonadotropin (eCG) showed a greater number of follicles (4.7 ± 0.9) compared with those receiving FSH in decreasing doses (1.3 ± 0.2 follicles); thus pFSH-dose used in the present study may be increased in future evaluations. Menchaca et al. (2010) reported that the administration of eCG causes high peak estradiol levels compared to FSH, which may interfere with fimbria ovum capture or with oviductal ovum transport. In this study, the presence of more follicles at the end of Treatment 1 was due to lower concentration of pFSH. Compared to eCG, the administration of decreasing doses of FSH may have a better effect on the number of developing follicles (Gonzalez-Bulnes et al., 2004; Bartlewski et al., 2008), based on a better emulation of naturally observed FSH decreasing concentrations after luteolysis, primarily due to the inhibitory effect of estradiol and inhibin released by preovulatory follicles.

In this study, the number of collected transferable embryos was smaller than those reported by several groups of researchers. Gonzalez-Bulnes et al., (2004), found values up to 11.9 of CE and 6.1 of TE. Administration of 160 mg of FSH in decreasing doses promoted better CE (5.8 ± 0.8 collected embryos/ewe), and TE response (4.0 ± 0.7 transferable embryos/ewe), in contrast to ewes which received 1500 IU of eCG, (CE = 3.6 ± 0.6 collected embryos/ewe, and TE = 2.6 ± 0.5 transferable embryos/ewe) (Chagas e Silva et al., 2003). It is necessary to consider the lower body weight of the animals used in this study, than in former ones. Rate of transferable embryos can improve, when young hair sheep are used, with values up to 5.6 ± 1.1/ewe (Lopes-Junior et al., 2006). With pFSH doses used in this work, quality and quantity of embryos in hair sheep are not compromised, considering that higher doses can be used to achieve better embryo response. The percentage of collected and transferable embryos is within the range obtained by several researchers which have been working in MOET programs (Bari et al., 2000; Cordeiro et al., 2003; Gonzalez-Bulnes et al., 2004; Lopes- Junior et al., 2006). However, doses can be increased considering other factors for the administration of pFSH, as the use of different progestogen sources, presence of a dominant follicle, as well as CL, which leads to the adequate starting point of the ovary to initiate the superovulation treatment (Gonzalez-Bulnes et al., 2004; Baldasarre, 2007). Hair sheep of Katahdin breed are being used as males in intensive meat producing systems. In Mexico, the use of this technique depends on the evaluation of different factors, studied by other researchers, and on the adaptation of this information to the prevailing local conditions related to environment, health, and nutrition. Considering that recent interest in embryo transfer for animal production systems is growing, this technique assists the genetic improvement of animals as pursued by genomic selection. For this reason, efforts to improve this biotechnology will make it easier for farmers to have access to animals with higher genetic merit.

Conclusion

The results show that the doses of pFSH used in this work may be modified to obtain greater embryo recovery rate without compromising their quality, for which it is suggested that higher doses should be evaluated, as well as out of breeding season, to be able to implement a MOET program, under breeding conditions of hair sheep in Mexico.

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