Short communication

The effect of melatonin and season on in vivo embryo production of Dohne Merino ewes


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ABSTRACT

To determine the effect of melatonin on embryo production in superovulated donor Dohne Merino ewes, two experiments were carried out at Trelew, Argentina (43° 65′ S), during the breeding and the anestrous seasons. Twenty-two ewes were used in experiment 1 (breeding season). Animals were divided into two groups: group M (melatonin, n = 11) received a melatonin implant 48 days before superovulatory treatment, and C (control, n = 11), non-treated. Two intrauterine artificial inseminations were performed 33 and 48 h after sponge removal. Embryos were collected 7 days after the onset of oestrus (58 days after the onset of melatonin treatments). Twelve ewes of the same breed were used in experiment 2, which was carried out during the anestrous season, under the same experimental procedures (group M, n = 6; group C, n = 6). Treated ewes presented a significant delay in the onset of oestrus during the breeding season (M: 31.10 ± 1.04; C: 25.20 ± 0.98 h; p < 0.05). Melatonin induced a significant reduction in the number of degenerated embryos per ewe during seasonal anestrous (M: 0.60 ± 1.74; C: 3.00 ± 1.74; p < 0.05). No differences between groups and seasons were observed for ovulation, fertilization and viability rates, and the number of viable embryos per ewe. In conclusion, our results indicate that the use of melatonin to improve embryo production in MOET sheep programs in the Dohne Merino breed at 43° S does not improve fertility and embryo viability. Although, a reduction of the number of degenerated embryos during the anestrous season was observed in the treated group.

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1. Introduction

In order to accelerate genetic merit of sheep flocks, "Multiple Ovulation and Embryo Transfer" (MOET) technology was developed to increase the number of lambs born from the best animals. The anestrous season limits the number of animals that an embryo transfer team can manage per year. Extending the application of the embryo transfer programs to the non-breeding season would improve its efficiency.

Melatonin has been used to advance the sheep breeding season, and the number of lambs born per lambing during the seasonal anestrous (Abecia et al., 2007). Forcada et al. (2006) demonstrated that treatment with exogenous melatonin to donor ewes is able to improve viability of embryos collected from these animals during the anestrous, postulating that this could be a useful tool to extend the embryo recovery season. Zhang et al. (2013) reported that melatonin implantation improves the response to superovulation of donor ewes, providing high quality embryos.
Melatonin application also enhanced the ability of the recipients to support transgenic embryonic development.

The use of melatonin implants was initiated in Australia. However, its introduction to support MOET programs not only in this country but in the whole Southern hemisphere is scarce. As reviewed by Martin (1995), the commercial development of a melatonin implant has been ironically widely included in sheep husbandry in Europe and North America, since for Australian animal producers very few of their farmed animals are strongly photoperiodic. The use of exogenous melatonin in South Africa, Argentina, Uruguay or Chile, even for a general improvement of lamb production, has also been scarce.

The aim of this work was to study the effect of exogenous melatonin on embryo performance of donor ewes of the Dohne Merino breed at 43° 56’ W (Trelew, Argentina), both during the breeding season and the seasonal anestrus.

2. Materials and methods

The experiments were conducted at the Laboratory of Animal Reproduction of the INTA Research Station (Trelew, Chubut; 43° 56’ W), which has been approved by SENASA (National Health Service and Food of Argentina). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of experimental animals.

2.1. Animals and superovulatory treatments

Twenty-two adult Dohne Merino ewes, with a mean (±S.E.M.) liveweight (LW) of 62.4 ± 1.7 kg and body condition score (BCS) (on a scale of 0–5, where 0 = emaciated and 5 = obese; Russell et al., 1969) of 3.32 ± 0.04, were used in experiment 1 from February to April (breeding season). Ewes were assigned either to a group that received a subcutaneous melatonin implant (18 mg melatonin, Melovex®, CEVA Salud Animal, Barcelona, Spain) or to a non-implanted control group (group C, n = 11). In experiment 2 (September–November, seasonal anestrus), 12 Dohne Merino ewes (LW: 61.4 ± 0.5 kg; BCS: 3.10 ± 0.08) were divided into melatonin implant (group M, n = 6) or non-implanted control groups (group C, n = 6). In order to avoid interferences due to surgical adherences, animals used in experiment 1 were not used in experiment 2. Experimental ewes had been never exposed to any superovulatory treatment before.

In both experiments, melatonin implants were inserted 48 days before superovulatory treatment started. This consisted of a combination of prostagsten, eCG, PGF2α, analog, and pFSH. An intravaginal prostagsten sponge (60 mg medroxyprogesterone, Progestyn, Syntex, Buenos Aires, Argentina), was inserted for 14 days, 37 days after melatonin implants; 3.7 mg cloprostenol (Ciclase, Syntex, Buenos Aires, Argentina) injected 6 days after sponge insertion, when sponges were changed for new ones. Finally, 72 h before pessary withdrawal, the pFSH treatment (Folltropin, Bioniche, Armidale, Australia) was initiated, consisting of 256 mg pFSH in eight decreasing doses at 12 h intervals (48 h, 48, 36, 36, 24, 24, 20 and 20 mg pFSH, respectively). At pessary removal, 200 IU eCG (Novormon, Syntex, Buenos Aires, Argentina) were administered.

Oestrus was monitored by EBH (6 h from 12 h after sponge withdrawals); with unproven rams to avoid service. Rams were allocated in a separate sheepfold until the onset of oestrus detection procedures. Two intrauterine artificial inseminations (IUI) were performed 33 and 48 h after sponge removal by laparoscopy using a 0.25-ml straw (50 × 10⁶ sperm) injected half into the lumen of each uterine horn (25 × 10⁶ sperm/uterine horn). All inseminations were carried out under sedation induced by Xylazine (4–6 mg per ewe, i.m.; Rompun, Bayer AG, Leverkusen, Germany). Frozen-thawed semen from four proven rams was used. Semen straws were imported from Uruguay and Australia, and were distributed equally in both experimental groups to avoid any bias due to semen quality. Semen was collected and frozen in the reproductive season. From spermatozoa to embryo recoveries, animals were housed indoors and fed alfalfa hay and corn, to provide their maintenance requirements (AFRC, 1993), with unrestricted access to water.

2.2. Embryo recovery

Embryos were collected, through a mid-ventral laparotomy, 7 days after the onset of oestrus (58 days after the onset of melatonin treatments). For surgical procedures, ewes were anesthetized using an IM, administration of 2 mg/10 kg Xylazine 2% (Rompun, Bayer, Buenos Aires, Argentina), and 25 mg/10 kg Ketamine Chlorhydrate (Ketamina 50, Holliday Scott, Buenos Aires, Argentina). Ovaries were expose the and the number of corpora lutea (CL) recorded. Uterine horns were flushed using a Foley catheter (10 Fr), with a pre-warmed commercial flushing solution (Bovigro, Minitube, Verona, USA). To minimize the development of post-operative abdominal adhesions, the surface of the reproductive tracts were flushed with a 2.5% heparin solution in saline before closure. After surgery, ewes were treated with long-acting broad-spectrum antibiotics.

Ova and embryos were examined under a stereo microscope (20–40 magnification) and classified based on their stage of development and morphology (Wintenberger-Torres and Sevellec, 1987). Only compacted morulae and blastocysts without imperfections and with a spherical/symmetrical shape (Linder and Wright, 1983) in these two stages of development (hatched blastocysts excepted) deemed freezable.

For each ewe, the following variables were recorded: number of CL, number of recovered ova (oocytes + embryos), recovery rate (number of ova recovered/number of corpora lutea) number of oocytes, number of degenerated embryos and number of viable embryos. Fertilization rate was calculated as the number of embryos divided by the number of structures recovered. Viability rate was the number of viable embryos divided by the number of ova recovered. Ewes that did not provide recovered ova were excluded from the calculations of fertilization and viability rates.

2.3. Statistical analysis

The values expressed as percentages were compared between groups and among successive recoveries within each group using chi-squared or Fisher’s Exact tests, as appropriate. To integrate the percentages in the model to determine whether they were influenced by the considered effects, individual proportions were arcsine-transformed before being subjected to statistical analysis. Results were expressed as mean ± standard error of the mean (S.E.M.). The rest of the variables were subjected to a 2 × 2 analysis of variance using a mixed model that included the fixed effects of melatonin treatment (treated or not), season (breeding season or seasonal anestrus) and their interactions.

The probability level for statistical significance was set to p < 0.05 and trend to significance to p < 0.10. The Statistical Analysis System software (SAS Institute, Cary, NC, USA) was used.

3. Results

Results obtained for both experiments are shown in Table 1. Oestrus was observed in 86% and 100% of ewes in experiment 1 and 2, respectively, with no differences between groups or seasons. M ewes presented oestrus 6 h later than C ewes during the breeding season (p < 0.05) (Table 1). This difference was not evident during the seasonal anestrous experiment. No differences were observed for the percentage of ewes presenting CL.

The number of ova (oocytes + embryos) recovered was similar between groups, although due to the higher but not statistically different ovulation rate presented during experiment 2, recovery rate in experiment 1 was significantly higher (87%) than in experiment 2 (58%) (p < 0.05). No effect of either treatment or season was found for the mean number of oocytes and viable embryos, although melatonin induced a significant reduction in the number of degenerated embryos per ewe during seasonal anestru (p < 0.05). Fertilization and viability rates showed similar values for both studied groups and seasons.
4. Discussion

Results obtained from the anestrous experiment, in terms of ovulation rate and viable embryos per ewe, have been higher than those obtained by Forcada et al. (2006) at 41° N, who obtained 11 and 13 CL for the treated and control groups, and 3.6 and 4.2 embryos, respectively, with no differences between groups. The lack of differences in ovulation rate during the seasonal anestrus is similar to previous reports by McEvoy et al. (1998), suggesting that exogenous gonadotrophin treatment obliterates the seasonal effect on ovulation rate that occurs in spontaneously ovulating ewes. This is confirmed with data obtained by Samartzi et al. (1995), who observed similar ovulation and embryo rates in eCG superovulated ewes both in autumn and spring. The mechanism controlling ovulation rate at the ovarian level during the breeding season is still functional during seasonal anestrus (Webb et al., 1992), maintaining spontaneous ovulations and indicating that melatonin treatment can induce significant increases in ovulation rate (Haresign et al., 1990).

The high ovulation rate obtained in our study during the seasonal anestrus in both groups indicates a good response to the gonadotropin treatment of Dohne Merino ewes, although this was not reflected in the breeding season test. This is consistent with other observations, although using less seasonal sheep breeds and at the Northern hemisphere (Samartzi et al., 1995). Failure of exogenous melatonin to increase ovulation rate in our study suggests that FSH probably recruited independently every follicle of the mechanism of atresia decreased by melatonin (McEvoy et al., 1998). The absence of differences in ovulation rate between groups during the breeding season disagre with observations by Zhang et al. (2013) (15.1 vs 8.8 CL, for melatonin-treated ewes or not, respectively). Vázquez et al. (2009) observed that supplemental melatonin appeared to improve embryo quality only during the anestrous season. This could explain the poor results of the treatment during the breeding season, which could be due to an antagonistic effect of exogenous melatonin with the natural endogenous hormone.

The production of viable embryos in the control group was lower during the seasonal anestrus than in the breeding season. During seasonal anestrus there was an increase of the percentage of degenerated and delayed embryos in the control group; this was significantly reduced in the implanted animals. This fact was also reflected by Forcada et al. (2006), who demonstrated that the application of melatonin implants significantly reduces the number and rate of non-viable embryos (degenerate and delayed) in superovulated sheep during anestrous. Thus, the effect of melatonin occurred at medium term, 3 months after implantation, increasing the rates of blastocysts, viability and freezability of embryos because of a decreasing number and rate of non-viable embryos. Likewise, Mitchell et al. (2002) observed that during the anestrous season, compared to breeding season, there is an increased incidence of fertilization failure in superovulated ewes as a possible consequence of seasonal shifts in LH secretion and (or) associated effects on follicular function.

The mechanisms involved in improving embryo viability by melatonin are not fully known. They could be explained in part by the lutetotropic effect of the pineal hormone, observed both in vivo (Durotoye et al., 1997) and in vitro (Abecia et al., 2002). Moreover, its effects on the hypothalamic-pituitary axis have also been described (Malpaux et al., 1997), and its antioxidant and free radical scavenging capacity (Chetsawang et al., 2006). Likewise, the effect of the pineal hormone on embryo quality could be produced at the oocyte stage, since melatonin improves developmental oocyte competence in the seasonal anestrous period (Vázquez et al., 2010), and increased significantly the maturation rate of oocytes and tended to increase their cleavage rate (Casado et al., 2010).

The study of the timing of oestrous occurrence is useful to provide information to properly plan ahead the mating period or even the most appropriate time for AI. The results obtained in the breeding season experiment showed a significant difference in the presentation of oestrus, being later in the treated group. Considering that this protocol involved two fixed-time AI, 33 and 48 h after sponge removal, this delayed response in the expression of oestrus, and likely ovulation, in the treated group, could affect the
development of the embryos, although this was not the case of the present experiment.

In conclusion, our results indicate that the use of melatonin to improve embryo production in MOET sheep programs in the Dohne Merino breed at 43 °S does not improve fertility and embryo viability. Although, a reduction of the number of degenerated embryos during the anestrus season was observed in the treated group.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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