

Original Article

Comparing Reproductive Performance Using P4 Vaginal Implant and eCG During Non-breeding Season in Cyclic and Anestrus Dairy Ewes



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ABSTRACT

Background: Estrus synchronization is widely used in ovulation induction in dairy ewes in the non-breeding season.

Objectives: This study aimed to investigate the differences in reproductive performance between cyclic and Anestrus Lacaune dairy ewes in synchronization protocol using a vaginal implant of progesterone and equine chorionic gonadotropin (eCG) in non-breeding season programming.

Methods: Eighty-four 15 to 20 months old Lacaune ewes were chosen, with one delivery, and 90 days had at least passed. Two blood samples were taken for progesterone (P4) evaluation 10 days apart. Plasma P4 concentrations were determined using an ELISA. Ewes with P4<1 ng/mL in two blood samples were considered acyclic and compared with cyclic ewes in estrus expression, pregnancy rate, lambing rate, litter size, fecundity, birth weight, and sex of lambs. The ewes were kept in a separate place about 5 km away from the rams for >2 months.

Results: Out of 84 ewes, 31 were cyclic (36.90%), and 53 were anestrus (63.10%). In anestrus ewes, mean P4 concentrations were 0.43±0.4 and 0.45±0.04 ng/mL in the first and second blood sampling, respectively. In cyclic ewes, mean P4 concentrations were 2.46±0.39 and 2.77±0.34 ng/mL in the first and second blood sampling, respectively. Expression of behavioral estrus did not differ between anestrus and cyclic ewes (P>0.10). The pregnancy and lambing rates tended to be greater in cyclic ewes than in anestrus ewes (odds ratio (OR)=3.35; 95% CI, 0.88%, 12.77%; P=0.08). Litter size, fecundity, birth weight of lambs, and offspring sex ratio were not different between anestrus and cyclic ewes (P>0.10).

Conclusion: The cyclic ewe using synchronization protocols outside the breeding season could cause high pregnancy and lambing rates compared to Anestrus ewe.

Keywords: Dairy ewe, Equine chorionic gonadotropin, Non-breeding season, Progesterone, Reproductive indices

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Introduction

Sheep and goats are seasonal polyestrous animals. The ovarian activity decreases in the seasons when the day length is longer—so-called out of the breeding or non-breeding season. Outside the breeding season, ewes enter the anestrus phase. Anestrus in ewes is characterized by the absence of estrus and ovulation due to a decrease in luteinizing hormone (LH) pulse frequency in response to increased sensitivity of the hypothalamus to the negative feedback effect of estradiol (Bedenbaugh et al., 2018). Various studies have shown that seasonal estrus in sheep results from changes in the hypothalamus-pituitary axis response to the ovarian estradiol hormone (Legan et al., 1977). Brain control centers must be re-sensitized to resume ovarian activity outside the breeding season (Grattan, 2015). For this purpose, progesterone (P4) priming is used. The source of P4 provided to the animal captures this artificial induction with a P4-producing corpus luteum. Before creating an active corpus luteum on the ovary, the dominant follicle or follicles must grow from the follicular wave and reach the dominance stage. Then ovulation occurs, leading to a P4-producing corpus luteum. Using an external P4 source, artificial induction occurs to re-sensitize brain centers to restart ovarian activity (Kuru et al., 2020). There are various methods to induce ovarian activity outside the breeding season of ewe, such as changing the amount of light exposure (Gómez et al., 2008), using melatonin implants (Mura et al., 2017), and employing hormones like natural and artificial P4.

Each of these techniques has a share of success and failure. The presence of male animals among female animals can play a significant role in releasing LH and follicle-stimulating hormone (FSH) and increasing ovulation and subsequent fertility and pregnancy (Ferdowsi et al., 2020). Using natural or artificial P4 in ewes to synchronize estrus has different forms (sponge, CIDR [controlled internal drug release], and oral and injectable forms). Each of these P4 sources releases a different amount of hormone into the blood (Gonzalez et al., 2020). When there is an intention to use P4 outside of the reproductive season, the equine chorionic gonadotropin (eCG) hormone can be used as a source of gonadotropins along with it to increase the success rate in inducing ovarian activity and also the number of lambs with the presence of a ram in each birth (Martinez et al., 2018).

Using CIDR or sponge with eCG hormone outside the breeding season is a common and well-known method worldwide and has a high pregnancy rate (Pugh & Baird, 2012). The success rate of synchronization in sheep

without eCG administration outside the breeding season is low (Boscós et al., 2002). Increasing the dose of eCG improves the ovulation rate (Khan et al., 2022). The administration of eCG in sheep increases the ovulation rate by mobilizing small ovarian follicles, increasing the growth rate of dominant follicles, and changing the proportions of follicle size classes in estrus (Martemucci & Alessandro, 2011). eCG does not seem to rescue follicles from atresia in sheep (Barrett et al., 2004). eCG causes early oocyte activation (Hameed et al., 2021). Follicles exposed to eCG synthesize more P4 (Şen, 2020).

There are various methods to detect some degree of acyclicity in sheep. Laparoscopy, ultrasound, estrus monitoring, and plasma P4 measurements are the most accessible and accurate methods for detecting anestrus ewe. According to the amount of P4, it is possible to find out if the animal is non-cyclical. According to previous studies, the value of this number has been reported differently. From 0.2 ng/mL (Husein & Ababneh, 2008), 0.5 ng/mL (Talebkhani et al., 2012), and 1 ng/mL (Mirzaei et al., 2017) on two occasions and 9 to 10 days apart, they are considered as the basic amount of P4. If the amount of P4 is lower than this number, it is regarded as acyclic. We considered the highest value mentioned in the articles as the basic P4 level so that we can argue that if the P4 level of an ewe is higher than 1 ng/mL, it is a cyclic ewe.

This study investigated the difference between cyclic and non-cyclic dairy Lacaune ewes in the synchronization program outside the breeding season and compared the differences using P4 among the ewes regarding the reproductive performance, such as estrus expression, pregnancy rate, lambing rate, litter size, fecundity, birth weight, and gender.

Materials and Methods

Study location

This study was conducted in an industrial dairy sheep farm in Qazvin Province, Iran (longitude 50°18', and altitude 121 m). This study started in April 2022. The average temperature recorded at this time was 14°C.

Study animals

In total, 84 Lacaune dairy ewes were divided into cyclic and non-cyclic groups based on plasma P4 levels. These two groups were compared based on reproductive indices. The animals were kept in dense conditions. In short, these dairy ewes received 1 kg of corn silage, 420 g of corn flour, 420 g of barley flour, and 750 g of alfalfa.

To prevent ram effects (sight, smell, and sound), the ewes were kept in separate pens about 5 km from the rams for over 2 months. All rams had at least one mating season experience. The animals were fed a mix of alfalfa hay, barley, concentrate with trace elements, and wheat straw in the zone. They were treated by anthelmintics for external and internal parasites. The management of the ewes did not change throughout the entire period. All rams and ewes were fed properly for flushing 20 days before the start of this study. In the flushing, they got a higher amount of corn silage and flour with a higher amount of barley flour.

P4 assay

Two blood samples were taken from the jugular vein before the start of the synchronizing program and subsequent mating at an interval of 9 days to evaluate the amount of P4 in the ewes. Centrifugation was performed at 1000 rpm for 10 minutes to obtain plasma to determine P4 concentration. ELISA method and Monobind kit (made in the USA) were used to assess the plasma concentration of P4. If the amount of P4 in the plasma was less than 1 ng/mL, it was considered anestrus.

Protocol for synchronization of estrus

CIDR[®] (Eazi-Breed[™] Zoetis, Pfizer, New York, USA) containing 0.3 g was used for 14 days. In addition, 400 IU of eCG (Gonaser, Hipra, Spain) was injected intramuscularly on the day of CIDR removal.

Estrus detection

Estrus detection was performed by ram. The rams were introduced to the flock after implant removal. They were within the flock for 40 days. Sheep marking crayon or ewe harness crayon (Carmel[®]) was used. The back of the ewes was checked for coloring and the effect(s) of ram jumping that were recorded.

Management of mating and pregnancy diagnosis

Rams were introduced to ewes for natural mating in a ratio of one to ten. The rams were in the groups until 25 days after the start of the program. Transabdominal B-mode ultrasonography by WED 3100 (Well, D Schenzhen, China) with a 5 MHz convex array transabdominal transducer was performed for pregnancy diagnosis almost between days 30 and 45 of gestation. The second ultrasonic test was performed to confirm pregnancy in 80-90 days.

Reproductive performance

We calculated the reproductive performance indices, such as estrus expression, lambing rate, pregnancy rate, fecundity rate, prolificacy rate, or litter size rate (Table 1). Lamb genus and the average birth weight are shown in the Table 1.

Statistical analysis

Continuous data (litter size, fecundity, and birth weight of lambs) were analyzed using the GLM procedure. Binary data (estrus expression, pregnancy rate, lambing rate, and offspring sex ratio) were analyzed using the GENMOD procedure, including function link logit in the model. Multiple comparisons were performed using the LSMEANS statement. All analyses were conducted in SAS software, version 9.4. Differences at $P < 0.05$ and $0.05 \leq P < 0.10$ were considered significant and tended to be significant, respectively.

Results

P4 concentration

Out of 84 ewes evaluated for P4 concentration by two consecutive blood samples 10 days apart, 31 were cyclic (36.90%), and 53 were anestrus (63.10%). In anestrus ewes, P4 mean concentrations were 0.43 ± 0.4 and 0.45 ± 0.04 ng/mL in the first and second blood sampling, respectively (Figure 1). In cyclic ewes, P4 mean concentrations were 2.46 ± 0.39 and 2.77 ± 0.34 ng/mL in the first and second blood sampling, respectively (Figure 1).

Expression of behavioral estrus did not differ between anestrus and cyclic ewes ($P > 0.10$; Table 1). Pregnancy and lambing rates tended to be greater in cyclic ewes than anestrus ewes (odds ratio (OR)=3.35; 95% CI, 0.88%, 12.77%; $P = 0.08$; Table 1). Litter size, fecundity, birth weight of lambs, and offspring sex ratio were not different between anestrus and cyclic ewes ($P > 0.10$; Table 1).

Discussion

The findings of this study showed that the rate of pregnancy and lambing in cyclic ewes was higher compared to anestrus ewes in the synchronization program during out of the breeding season using a synchronization protocol consisting of CIDR and eCG (Table 1). The lack of comparison between cyclic and non-cyclic ewes in synchronization protocols out of the breeding season and the only citation being the off-season at this period, it is

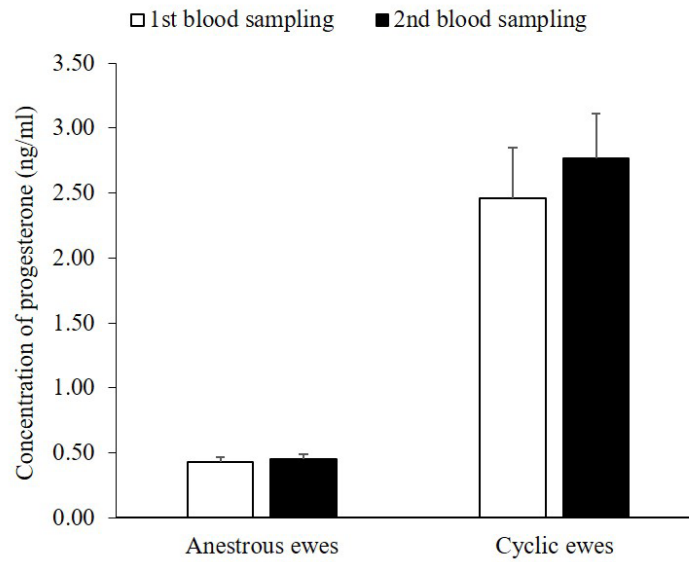


Figure 1. The concentration of P4 in Anestrus and cyclic ewes in the first and the second blood sampling in non-breeding season programming

clear that the percentage of ewes in the off-season is also cyclic, it has been tried to study the response difference between these two groups of ewes in the common synchronization protocol of using CIDR and eCG hormone. Naturally, if the amount of P4 before the synchronization program is extracted, the proper program for each group of ewes can be presented separately. Reyna et al. (2007) showed that the synchronization and response to eCG hormone is better outside the breeding season than inside. Ewes are cyclic within the breeding season, and this study showed that the pregnancy rate was reduced in cyclic ewes in dairy ewes outside of the breeding season. The main reason for this topic is the insemination method. In the study of Reyna et al., the insemination

was done at a fixed time and laparoscopically, which indicates that it is more suitable to create synchronization with CIDR and then inject eCG hormone in the off-season (Reyna et al., 2007). In cyclic ewes, the response to CIDR may be completely different. From luteinizing follicles to preventing ovulation, etc. In the case of anestrous ewes, the only purpose of using CIDR is P4 priming. It is important to mention that the results of our study were obtained by natural mating, and artificial insemination was not performed in these groups. This finding suggests that cyclic ewes may only affect the timing of estrus symptoms. The results of this study also showed that the estrus expression is not lower in cyclic ewes compared to non-cyclic ones, whereas in terms of

Table 1. Reproductive performance of anestrous and cyclic dairy ewes in out of breeding season program

Variables	Mean±SEM/No. (%)	
	Anestrous Ewes (n=53)	Cyclic Ewes (n=31)
Estrus expression (%)	75.47(40.53)	90.32(28.31)
Pregnancy rate (%)	73.58(39.53) ^a	90.32(28.31) ^b
Lambing rate (%)	73.58(39.53) ^a	90.32(28.31) ^b
Litter size	1.44±0.09	1.39±0.11
Fecundity (%)	105.66±10.37	125.81±13.56
Birth weight of lambs (kg)	3.86±0.12	3.96±0.14
Sex ratio of offspring (%)	57.14(32.56)	64.10(25.39)

^{a, b}A tendency to the statistical difference between two categories of ewes (0.05<P<0.10).

percentage, 15% more than in estrus expression shown in cyclic ewes. Another important issue is the difference in ovulation time. In cyclic ewes and the breeding season, if the synchronization program is used, the time of ovulation in these groups is very different, and this issue also causes a low pregnancy rate in insemination at a fixed time (Santos et al., 2020). This finding does not mean that the response to the synchronization program is low in these groups; however, in cyclic animals, the response to the hormone should be stronger than in non-cyclic animals (Barrett et al., 2004). Cyclic cows can be easily induced with two injections of prostaglandin, and there is no need to use CIDR and inject hormones. However, sheep are very different from cows. If P4 is measured outside the breeding season, usually 50% of the herd is non-cyclic based on P4 levels, and it is expected that in the case of natural mating without any intervention, approximately 50% of the herd should become pregnant. Whereas only 10% to 15% of the cyclic animals became pregnant, remnants needed to perform the synchronization program (Arjmandi et al., 2021). This is the reason why it is not recommended to inject prostaglandin into any herd of sheep and goats. In another study, a transient peak of FSH was observed in both reproductive and non-reproductive seasons in ewes before the appearance of follicular waves. Thus, eCG administration is expected to affect follicular wave dynamics (Barrett et al., 2004; Viñoles et al., 2001). Synchronization success rate in sheep without eCG administration outside the breeding season is low (Martinez et al., 2015). The administration of eCG in sheep increases the ovulation rate by mobilizing small ovarian follicles, increasing the growth rate of dominant follicles, and changing the proportions of follicle classes in estrus (Uslu et al., 2012). eCG does not appear to rescue follicles from atresia in ewes (Marte-mucci et al., 2011). eCG induces early oocyte activation (Dogan et al., 2018). Follicles exposed to eCG can produce more P4 (Garoussi et al., 2019; Ghasemzadeh-nava et al., 2017). It was expected that in cyclic ewes, litter size, and fecundity increased in the flock and were higher than in anestrus ewes. In our study, no significant difference was observed between the two groups (Table 1). This possibility was proposed because the response to the hormone in cyclic ewes should have shown itself better. The studies showed that the ratio of multiple ovulations in the first ovulation is higher outside the breeding season than in the second ovulation (Acar et al., 2013). Perhaps the most likely reason is that the ovulation rate in non-cyclic animals is high during the first ovulation. Another possible reason is the ram effect, which increases the amount of secreted LH and causes an increase in ovulation and subsequent multiple births in non-cyclic

ewes. In the studies carried out, it has been shown that the mating of a male animal and the direct contact of the ewe with a ram lead to an increase in the secretion of LH, and an increase in the growth of follicles and the production of estradiol, and subsequently, estrus, LH surge, and ovulation (Scaramuzzi & Downing, 1997; Berean et al., 2019; Mirzaei et al., 2017; Ayaseh et al., 2021). Ewes constantly accompanied by rams become accustomed to their presence and have the same level of estrus as ewes without rams. About 65% of Romney ewes ovulate within 65 hours after the ram enters (Nakafeero et al., 2020), and 50% of Merino ewes ovulate within 41 hours after the ram enters. When the ram enters at the same time as the sponge is removed, ovulation and estrus symptoms occur (Ungerfeld et al., 2020). If the ram enters with a delay of 48 to 52 hours, ovulation occurs, but the ewes do not show signs of estrus (Mahmoud et al., 2019). The lambing rate in our study was higher than the pregnancy rate in cyclic ewes than in non-cyclic ewes, and that was due to the absence of abortion in the studied ewes. Many studies have been done to replace gonadotropin-releasing hormone (GnRH) with eCG (Reyna et al., 2007; Santos et al., 2020). The main reason for these studies is to lower the price and comply with the ethical principles of animal treatment because the eCG hormone is extracted from pregnant horses. However, in most studies, GnRH outside the breeding season and in non-cyclic ewes showed a much lower pregnancy percentage than eCG, which is not recommended. However, GnRH has shown a very appropriate and similar response to eCG within the reproductive season or in cyclic ewes (Reyna et al., 2007). In addition, the use of eCG with GnRH on the day of insemination increased reproductive performance (Hosseinzadeh et al., 2016). Another study showed that injecting 300 IU of hCG hormone after insemination improves the pregnancy rate in ewes (Didarkhah & Vatandoost, 2022). So, if you know that the ewe is cyclical outside the breeding season, it can be suggested that instead of eCG hormone, GnRH can be used instead. Perhaps the importance of this study lies in the fact that until now, outside of the reproductive season, a comparison between cyclic and non-cyclic ewes included in the synchronization protocol has not been made in terms of reproductive indices.

Conclusion

When using synchronization protocols outside the breeding season, the cyclic ewe can cause high pregnancy and lambing rates compared to the Anestrus ewe.

Ethical Considerations

Compliance with ethical guidelines

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by [Faculty of Veterinary Medicine, University of Tehran, Iran](#).

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Authors' contributions

Methodology and data curation: Navid Jahan Roshan and Vahid Akbarinejad; Investigation: Navid Jahan Roshan and Sareh Azarmi; Formal analysis: Vahid Akbarinejad; Writing the original draft: Navid Jahan Roshan; Conceptualization, resources, supervision, review and editing: Massoud Talebkhani Garoussi and Vahid Akbarinejad.

Conflict of interest

The authors declared no conflict of interest

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مطالعه پژوهشی

مقایسه عملکرد تولیدمثلی با استفاده از کاشت واژینال پروژسترون و eCG در خارج از فصل تولید مثل در میش‌های شیری سیکلیک و آنستروس

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چکیده

زمینه مطالعه: هم‌زمان‌سازی فحلی برای القای تخمک‌گذاری در خارج از فصل تولیدمثل در گوسفند شیری به‌صورت گسترده استفاده می‌شود.

هدف: این مطالعه باهدف بررسی تفاوت عملکرد تولیدمثلی بین میش‌های شیری سیکلیک و آنستروس نژاد لاکون در برنامه هم‌زمان‌سازی با استفاده از کاشت واژینال پروژسترون CIDR و eCG در خارج از فصل تولیدمثل انجام شد.

روش کار: ۸۴ رأس میش لاکون در محدوده سنی ۱۵ تا ۲۰ ماهه انتخاب شدند که یک بار زایمان داشتند و حداقل ۹۰ روز از زمان زایش آن‌ها گذشته بود. ۲ نمونه خون برای ارزیابی پروژسترون P4 (به فاصله ۱۰ روز) گرفته شد. غلظت P4 پلازما با استفاده از ELISA تعیین شد. میش‌هایی با کمتر از P4 گرم/میلی‌گرم در دو نمونه خون غیرسیکلیک در نظر گرفته شدند و از نظر فحلی، میزان آبستنی، میزان بره‌زایی، تعداد بره، باروری، وزن تولد و جنسیت بره‌ها با میش‌های سیکلیک مقایسه شدند. میش‌ها به‌مدت بیش از ۲ ماه در محل جداگانه‌ای با فاصله حدود ۵ کیلومتری قوچ‌ها نگهداری شدند.

نتایج: از ۸۴ رأس میش، ۳۱ میش سیکلیک (۳۶/۹۰ درصد) و ۵۳ میش غیرسیکلیک (۶۳/۱۰ درصد) بودند. در میش‌های غیرسیکلیک، غلظت پروژسترون به‌ترتیب ۰/۴۳±۰/۴ و ۰/۴۳±۰/۴۵ در خون‌گیری اول و دوم بود. در میش‌های سیکلیک، غلظت پروژسترون در خون‌گیری اول و دوم به‌ترتیب ۲/۴۶±۰/۳۹ و ۲/۷۷±۰/۳۴ نانوگرم بر میلی‌لیتر بود. بیان رفتار فحلی بین میش‌های سیکلیک و غیرسیکلیک تفاوتی نداشت (P>۰/۱۰). نرخ آبستنی و نرخ بره‌زایی در میش‌های سیکلیک در مقایسه با میش‌های غیرسیکلیک بیشتر بود (نسبت شانس=۳/۲۵، ۹۵٪ فاصله اطمینان=۰/۸۸-۱/۲۷۷، P=۰/۰۸). جنسیت بره‌ها و جنسیت بره‌ها بین میش‌های غیرسیکلیک و سیکلیک تفاوتی نداشت (P>۰/۱۰).

نتیجه‌گیری نهایی: سیکلیک بودن میش در هنگام استفاده از پروتکل‌های هم‌زمانی در خارج از فصل تولیدمثل می‌تواند سبب بالا بودن نرخ آبستنی و بره‌زایی نسبت به میش‌های غیرسیکلیک گردد.

کلیدواژه‌ها: میش شیری، eCG، خارج فصل تولیدمثل، پروژسترون، شاخص‌های تولید مثلی

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