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Estrous synchronization treatments in sheep: Brief update

Atualização nos protocolos de sincronização do cio em ovinos

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Abstract

Fixed-time artificial insemination (FTAI) is the most effective method to increase the number of females inseminated in a single day, eliminating the necessity of estrus detection. Usually the treatments for FTAI in sheep are based on the use of progesterone-releasing devices and equine chorionic gonadotropin (eCG) administration at device removal, with an acceptable pregnancy rate. The current information about the ovarian physiology supports the idea of shortening the traditional progesterone exposure with intravaginal devices from 14 days to 5-7 days (Short-term protocols). These protocols ensure appropriate progesterone concentrations to induce follicular turnover and ovulation of a non-persistent follicle. One im dose of PGF2alpha is required at time of device removal with eCG administration, ovulation occurs around 60 h later and FTAI is performed at 48 or 54 h by cervical or intrauterine route, respectively. Several experiments have been conducted during last years to adjust this new protocol. In general, reported pregnancy rate is greater -or at least similar- than traditional 14 days protocols, and remaining progesterone in the silicone intravaginal devices may be enough for their reutilization with interesting results. Several improvements on follicular dynamics, time of ovulation, pharmacological associations, insemination time, sperm dose, among others, are summarized in this review in FTAI protocols facilitate a further adoption of insemination and genetic improvement in this species.

Keywords: ovine, ewe, ovulation, ovary, fertility.

Introduction

After the advent of transrectal ultrasonography for the study of ovarian physiology in small ruminants in the 1990s, it has been possible to unequivocally determine that follicular growth occurs in a wave like pattern, both in sheep (Ginther et al., 1995) as in goats (Ginther and Kot, 1994; de Castro et al., 1999). In sheep, follicular waves have been reported during the estrous cycle (Ginther et al., 1995; Rubianes et al., 1996), seasonal anestrus (Souza et al., 1996), transition period to reproductive season and during gestation (Bartleswki et al., 2000). Normally, the follicular wave pattern during the ovine estrous cycle consist of three or four waves, although there is an important variation in the number of waves by cycle (Evans, 2003). The main characteristics of follicular wave in sheep can be briefly summarized as follows (see review: Menchaca and Rubianes, 2004): 1) during a follicular wave at least one follicle reaches a diameter close to 5 mm or more; 2) the selected follicle grows during 5-7 days, with a growth rate of around 1 mm/day; 3) the maximum diameter of the largest follicle of a wave differs between waves, being larger in the wave 1 and in the ovulatory wave; 4) as the luteal phase progresses and the serum progesterone concentrations increase, follicular turnover is facilitated and the inter-wave intervals are shorter than during the early luteal phase; 5) during the mid-late luteal phase the follicles that do not grow beyond 4 mm seem not to be part of the wave phenomenon, and it has been suggested that they represent an underlying dynamic pool; 6) the follicle that finally reach the ovulation, usually is the largest follicle on the day of luteolysis; 7) in most double ovulatory cycles, the ovulatory follicles emerge as part of the same follicular wave; and 8) if ovulation happens from more than one follicle, usually its occurs in an interval within 12 hours.

Since follicular wave phenomenon was demonstrated, new insights for ovarian control and Fixed-time Artificial Insemination (FTAI) were proposed in sheep. Thereafter, novel protocols were developed to synchronize estrus and ovulation, and thus facilitate the adoption of insemination in small ruminants. This brief update introduces the most novel and relevant information produced by our Laboratory for estrous synchronization to perform FTAI in sheep. In addition, some information published by other colleagues is also included, deserving further review.

Progesterone and follicular dynamics

The use of intravaginal devices impregnated with progestogens (progesterone and its analogues) to synchronize estrus in sheep has a long history from the pioneering works of T.J. Robinson among others at the beginning of the 1960s. However, it was not until the 1990s where the first studies to determine the effect of progesterone on follicular dynamics were reported in sheep (Rubianes et al., 1996; Leyva et al., 1999; Viñoles et al., 1999). These studies demonstrated that serum progesterone concentrations have a negative relationship with the size of the dominant follicle, and that higher progesterone levels cause regression of the dominant follicle

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and accelerate the follicular turnover. On the contrary, low progesterone concentrations or subluteal levels induce an excessive growth of the dominant follicle inducing the ovulation of a persistent follicle.

This mechanism is due to the negative feedback that exerts the progesterone on the LH pulsatility that is required for the growth of the dominant follicle. In ruminants, subluteal progesterone levels promote higher frequency of LH pulses, increasing the size of the dominant follicle and turning it into a persistent follicle (Adams, 1999). This persistent follicle has lower fertility (Johnson et al., 1996) due to the early resumption of the meiosis by the oocyte that reaches Metaphase II before ovulation, and thus the quality is compromised due to the "aging" of the oocyte within the follicle. Most of these oocytes fail to fertilize or have a delay in the development of the zygotes with higher incidence of embryonic death at 2 to 16-cell stage (Ahmad et al., 1995).

For this reason, with the aim to promote the follicular turnover and thus allow the ovulation of a young follicle and a healthy oocyte with good fertility, the treatments for estrus synchronization should avoid the exposure to low levels of progesterone during an excessive period.

Short-term progesterone treatments

Several hormonal treatments have been used to synchronize estrus associated with artificial insemination in small ruminants. During recent years the interest for insemination without estrous detection or FTAI has been increased, mainly in South American countries. With this in mind, the interest on estrous behavior was now moved to a second place and the priority is focused on the control of ovulation. Thus, our studies are conducted to synchronize follicular dynamics and ovulation, mainly assisted by ultrasonography.

The most popular protocols for FTAI in ruminants are based in progesterone administration, which has been the default method for several years. In sheep, the administration of this hormone is performed by intravaginal route by using silicone devices (like CIDR) or polyurethane devices (sponges) historically placed during 12-14 days associated with a dose of eCG at the end of the treatment. The technique is based on the inhibitory role of progestogens on the hypothalamus-pituitary axis, acting like an exogenous corpus luteum. However, the current information published in the last years about follicular dynamics, endocrinology and control of the corpus luteum opens new questions about the use of these traditional long treatments. In sheep and goats, the insertion of an intravaginal device containing 0.3 g of progesterone results in a rapid increase in blood concentrations of this hormone (>5 ng/mL) during approximately 4-5 days of treatment (Menchaca and Rubianes, 2004), similar to those levels observed during medium-late luteal phase. However, after 6 or 7 days of treatment, blood progesterone concentrations decrease to subluteal levels (<2 ng/mL), enough for blocking ovulation but predisposing to a persistent growth of the dominant follicle. If the intravaginal device is maintained during 12 or 14 days, the detrimental conditions of low progesterone levels described above are present during an excessive period, and then fertility is affected.

In order to avoid this situation, some years ago we put into practice the "Short-term protocols" for FTAI, which consist of only 5-7 days of exposure to progesterone (see review: Menchaca and Rubianes, 2004). This treatment is associated with one dose of 200 to 400 IU of eCG to induce ovulation and one luteolytic dose of PGF2α at device removal. During many years in several sequential studies, plenty of information has been published in sheep and goats using this protocol, during the breeding and non-breeding season, using different intravaginal devices, evaluating different PGF2alpha analogues and doses, associated to natural mating and insemination after estrous detection, and of course, also evaluated in large scale FTAI programs.

Period of progesterone exposure

Studies in the late 1990s showed that long progestogen exposure (i.e. 12 days) is not necessary to induce estrus in sheep during non-breeding season. Ungerfeld and Rubianes (1999) demonstrated that 3 days of medroxiprogesterone acetate (MPA) treatment was as effective as 6 or 12 days to induce estrus in anestrous sheep, and better than 1 and 2 days of treatment. This study was associated with natural mating and pregnancy rate was similar between 3, 6 and 12 days of treatment. During various studies we evaluated different protocols for FTAI and most of this information has been previously reported (see review: Menchaca and Rubianes, 2004). Recently, we produced new information comparing the Short-term protocol (6 days) versus the traditional long treatment (14 days) using progesterone in silicone intravaginal devices, throughout different experiments in large-scale FTAI programs (unpublished results). In one of these trials on 1,750 multiparous ewes receiving intrauterine insemination by laparoscopy, pregnancy rate was significantly greater with the Short-term protocol than the long traditional protocol (43.5% vs. 37.8%, respectively; $P < 0.05$). In a following experiment with 922 ewes with FTAI using fresh semen by cervical route in which the females were treated during 6 vs. 14 days with intravaginal devices of second use (in both cases previously used for 6 days), pregnancy rate was also greater with the Short-term protocol (41.2% vs. 29.1%, respectively; $P < 0.05$). These results confirm previous studies obtained with the Short-term protocol associated with FTAI in sheep and goats (Menchaca and Rubianes, 2004), and overall this information add more evidence to the concept that as progesterone levels decrease by using intravaginal devices during long periods, negative conditions that predispose to lower fertility are promoted.

Moment of ovulation and FTAI

The moment of ovulation after the Short-term protocol (i.e. CIDR by 5-7 days *plus* PGF2alpha and 250-300 IU eCG at device removal) in average occurs approximately 60 hours from intravaginal device removal in sheep (Vilariño et al., 2010; 2013) and goats (Menchaca and Rubianes, 2004; Menchaca et al., 2007; Vilariño et al., 2011), suggesting that insemination should be performed some hours earlier. In order to determine the optimal time of FTAI after this protocol, we evaluated individual data of 3,893 ewes that received insemination from 46 to 56 h from device removal (unpublished results). When new devices containing 0.3 g of progesterone were used during 6 days associated with intrauterine FTAI, greater pregnancy rate was obtained when insemination was performed on the afternoon of Day 8 rather than in the morning (i.e. Day 8 PM; ~52-56 h after device removal). On the other hand, when insemination was performed by cervical route with fresh semen, greater pregnancy rate was obtained with FTAI in the morning on Day 8 (~46-50 h) rather than in the afternoon. This outcome was modified when the progesterone devices were reused (i.e. used two or three times by 6 days), and in this case no differences were found between FTAI performed in the morning or in the afternoon of Day 8 (i.e. from 46 to 56 h), with no interaction with the insemination by cervical or intrauterine route.

Intravaginal devices and progesterone analogues

During the last years we evaluated different devices and progestogen types. The new silicone device DICO (*Dispositivo Intravaginal Caprino y Ovino*, Syntex) induces very similar results to those obtained with the traditional CIDR-G (Zoetis). Both devices contain 0.3 g of progesterone in a silicone matrix, and no differences were found neither in serum progesterone levels, follicular dynamics, time of ovulation, nor in pregnancy rate (Vilariño et al., 2010; dos Santos Neto et al., 2015). When these intravaginal devices containing progesterone were compared with intravaginal sponges containing MPA in the Short-term protocol, pregnancy rate after FTAI was lower with the synthetic analogue MPA, both by cervical and intrauterine FTAI (dos Santos Neto et al., 2015). Rather than the type of drug and its different pharmacokinetic (e.g. extended half-life of MPA), the type of the device (silicone vs. sponge) also may affect fertility. Manes et al. (2014) found that the insertion of an intravaginal sponge with placebo (i.e. without MPA) during 13 days before spontaneous estrus, reduced conception rate in comparison with those ewes that did not receive a sponge. Thus, the local effect of the sponge in this case negatively affected fertility (at least for the long treatment). In addition, the use of intravaginal sponges was associated with an increase in a CFU mL^{-1} and vaginitis at time of device removal when compared with CIDR-G (Martins et al., 2010) suggesting a negative effect on vaginal environments and subsequent fertility.

In another study (Blaschi et al., 2014) using a different implant and other progesterone analogue (subcutaneous ear implants containing norgestomet; Crestar, Intervet/MSD), estrus was delayed and conception rate was lower with 5 days treatment (48%; *n*=23) in comparison with 14 days treatment (83%; *n*=24). In this report, the Crestar implant designed for cows containing 3 mg of norgestomet was cut in half and used in cyclic ewes, probably inducing high serum progestogen levels (exogenous plus endogenous) and excessive blockage of LH and follicular growth, requiring in this case a longer period to return to appropriate progesterone levels. Thus, this information all together suggests that the type of the device, the route of administration, the pharmacokinetics and metabolism of the active compound, and the amount of hormone contained -but mainly released- by the device, probably interacts with the period of progestogen exposure, affecting fertility.

Reuse of intravaginal devices

The fact of shortening the treatment with intravaginal devices containing 0.3 g of progesterone (e.g. CIDR-G® and DICO®) that were designed to use during 14 days, leads to the question whether it is possible to reuse these silicone devices. With this in mind, we evaluated the use of these devices two or three times in a series of experiments conducted in sheep (Vilariño et al., 2010; 2013; dos Santos Neto et al., 2015). Briefly, the proportion of sheep showing estrus and ovulation seems not to be affected by the devices used one, two or three times. However, pregnancy rate fell substantially with three times used devices in comparison with new ones, and was intermediate with second use devices. Similar results have been obtained in goats after exposure to progesterone intravaginal devices with this short treatment (Vilariño et al., 2011; Souza et al., 2011). Interestingly, in both species the insertion of new devices induce follicular turnover in all females, while with reused devices the treatment failed to induce a new follicular wave in about 20% of the females in which ovulation occurred from an older large follicle (Vilariño et al., 2011; 2013). This could explain the lower pregnancy rate in these females treated with reused devices. We suggest that with the short treatment of 6 days, even though pregnancy rate may be slightly reduced with reused devices, the decision to reuse the intravaginal devices require a case by case deep cost-benefit analysis.

Final remarks

Further understanding of the follicular dynamics in sheep and goats have revealed that 12 to 14 days long treatments using progesterone intravaginal devices, do not seem to be appropriate to ensure high fertility. For this reason, some years ago we proposed the shortening of the progesterone treatments to 5 - 7 days to be applied in FTAI programs. This Short-term protocol includes a dose of PGF2alfa and eCG at device removal. The FTAI is performed 46 to 56 hours depending on the insemination route and the amount of progesterone containing in the device. We suggest that these short treatments result in a series of benefits like a better control of follicular response and ovulation, acceptable fertility rates (no lower than conventional progesterone treatments), shorter period for implementation of large scale FTAI programs, and in some cases allowing the reutilization of intravaginal devices. In general, these advantages have important implications for the widespread adoption of insemination in sheep.

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