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SUPPLEMENT ARTICLE

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Current challenges in sheep artificial insemination: A particular insight

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Abstract

Ovine artificial insemination (OAI) is not commonly performed because of specific problems related to semen application techniques, leading to highly variable results. The ideal methodology (frozen-thawed semen/vaginal route) is unfeasible under field conditions due to the cervix morphology of the ewe, which prevents the process of intrauterine insemination necessary to obtain acceptable results. Currently, OAI commercial programmes use superficial cervical insemination, CAI (vaginal), with chilled semen (15°C) and intrauterine insemination, LAI (laparoscopic), with frozen-thawed semen. The ability to improve upon these contrasting techniques may be derived from examining certain poorly studied factors such as insemination time, productive state of females and alternatives of seminal preservation, some of which we reviewed in this work. This interim solution will remain in use until AI by the vaginal route with frozen-thawed semen is developed, but it poses new challenges in optimizing the freezing of the sperm and adapting the cervical (CAI) and/or transcervical intrauterine AI (TCAI). In this review, we address the current problems and evaluate their methodological (mechanical) and chemical (dilation) alternatives. Currently, TCAI is a methodologically complex technique with poor fertility results, so further studies are needed to improve the logistics of this procedure and the results of its application.

KEYWORDS
AI, cervix, cryopreservation, ovine, sperm, transcervical

1 | INTRODUCTION

Artificial insemination (AI) is a basic tool of breeding programmes. However, AI is not widespread across species, mainly due to the high variability of its fertility results and the specific problems that are presented by its application. Methodologically, the obstacle to developing ovine artificial insemination (OAI) resides in the route of application that is limited by the morphology of the ewe cervix (Kershaw et al., 2005). However, sperm preservation methods (cooling at 15°C and freezing) should also be considered, because in this species, they are closely linked to the route of sperm application that is used in the AI.

Since the optimal conditions for the application of OAI are through the vaginal application of frozen-thawed semen, two very important limitations of this procedure arise. The first is the difficulty of the frozen-thawed sperm from the ram to cross the cervix of the ewe and reach the uterus and the site of fertilization (Fair et al., 2005; Salamon & Maxwell, 1995). The second problem is the inability to vaginally deposit semen directly into the uterus because of the complex anatomy of the cervix (Kaabi et al., 2006). The low fertility rate of vaginal–cervical insemination with frozen-thawed semen (Naqvi, Joshi, Bag, Pareek, & Mittal, 1998; Sánchez-Partida, Windsor, Eppleston, Setchell, & Maxwell, 1999) is unacceptable in commercial breeding programmes, which dictate the use of

OAI with two alternatives that offer good fertility. Chilled semen (15°C) is commonly used and vaginally applied (CAI); in specific situations that require the use of frozen-thawed semen, the use of laparoscopic intrauterine insemination (LAI) is necessary (Anel et al., 2005; Hill, Thompson, & Perkins, 1998). Since these two models differ substantially from the ideal insemination procedure (frozen-thawed semen/AI via the vagina), it is necessary to develop strategies to improve the OAI procedures to increase the performance of these techniques and to develop protocols that enable the use of vaginally applied frozen-thawed semen. Thus, studies have been performed to overcome/cross the cervical barrier (Álvarez, Chamorro, et al., 2012; Falchi, Taema, La Clanche, & Scaramuzzi, 2012) or to optimize the sperm quality of the AI dose by improving the freezing and thawing methods (Allai, Benmoula, Marciane da Silva, Nasser, & El Amiri, 2018; Álvarez, Chamorro, et al., 2012; Anel et al., 2003) in order to ensure that the semen can colonize and migrate through the cervix.

Next, we conducted a review of some aspects of these three AI techniques in the ewe; two are in commercial use (vaginal–cervical and laparoscopic intrauterine), and another is in experimental development (transcervical intrauterine).

2 | CERVICAL INSEMINATION: A CLASSIC TO IMPROVE

Cervical AI is the most commonly used technique due to its simplicity and satisfactory results. This technique presents two logistical problem in the dependence on a nearby AI centre, the preparation of doses on the day of insemination and the low performance of males (few doses/ejaculate). In ewes with induced oestrus, good results have been reported in Merino breeds with fresh semen -56.7%- (Fernandez-Abella, Preve, & Villegas, 2003), -65%- (Fernandez et al., 2019) or chilled semen at 15°C in the Lacaune breed 66.7% (Rocha et al., 2015). Under these conditions and in Spanish breeds, fertility is highly variable—Aragonesa: 45% (Abecia et al., 2016), Churra: 31% (Anel et al., 2005), Assaf: 39% (Álvarez, Chamorro, et al., 2012; Álvarez, Tamayo-Canul, et al. 2012) and Castellana: 45.5% (Kaabi et al., 2006). Usually, the maximum time of insemination for this kind of seminal samples is up to 6–8 hr from the sperm collection.

With frozen-thawed semen, cervical insemination rates are generally low and are obtained from unrepresentative experimental studies (few inseminations) under very diverse conditions, which makes it difficult to transfer this practice to a productive sector: 4% (Masoudi et al., 2017), 28% (Gil, Rodriguez-Irazoqui, Lundeheim, Söderquist, & Rodríguez-Martínez, 2003) and 13% (Byrne et al., 2000). Exceptionally, Paulenz et al. (2007) achieved good fertility using frozen-thawed semen in Scandinavian breeds under on-farm conditions. The success in Norway appears to be related to the ewe breed used there, and the migration of frozen-thawed spermatozoa through the cervix has been shown to be better in some ewe breeds than others (Fair, Meade, Reynaud, Druart, & de Graaf, 2019; Richardson et al., 2011).

Although classical cervical insemination provides acceptable results (cooled semen), there are possibilities for optimization in some aspects of the technique (time spent on AI, effect of the vaginal speculum, etc.) and in adjustment of factors related to the female (CAI handling, milk production) or the design of new seminal preservation strategies in the medium-term storage (72 hr, 5°C), among others.

2.1 | Time spent in insemination

Studies have reported that the stimulation time of the speculum in the vagina can produce a release of oxytocin that increases uterine contractibility and alters fertility (Raynal & Houdeau, 2004). In CAI, the AI execution times depend on the type of speculum used, the depth of insemination, female restraint and especially the experience of the technician, which directly influences fertility (Anel et al., 2005; Donovan, Hanrahan, Kummen, Duffy, & Boland, 2004). In preliminary results from our group (Mercedes Alvarez, Juan Carlos Boixo, Paulino de Paz, Luis Anel), we observed that when the cervical insemination time is less than 10 s, fertility is improved (Table 1). The short insemination times rarely occur since they require the ideal conditions for insemination (immobile ewe, accessibility of the cervix, etc.). On the other hand, the performance (inseminated ewe/hour) of the CAI method is much higher than those of the TCAI or LAI (85.5 vs. 11.4 vs. 56.8, respectively; Casali et al., 2017).

2.2 | Milk production

Many factors affecting the success of cervical OAI with chilled semen have been studied (Anel et al., 2005; Palacín et al., 2012). However, the effect on fertility of the productive state of females at the time of insemination is poorly understood and would be of great importance in determining the most appropriate time for AI. In a study conducted in the Assaf ewe (Mercedes Alvarez, Juan Carlos Boixo, Paulino de Paz, Luis Anel), we observed that in advanced stages of lactation (>500 kg produced), the fertility of cervical insemination is higher (Figure 1). When analysing the results of insemination in high-productivity breeds, we should consider the negative correlation between milk production and fertility after AI, as described in the Lacaune ewe (−0.23) (David et al., 2008), which is a correlation that has previously been described in dairy cattle (Wall, Brotherstone, Woolliams, Banos, & Coffey, 2003).

TABLE 1 Fertility (lambing %) according to the spent time in each cervical artificial insemination (CAI) in synchronized ewes using cooled sperm samples (15°C)

CAI spent time (sec)	Lambing (%)
<10 (n = 35)	51.4[a]
>10 (n = 107)	31.8[b]
Total (142)	35.6

Note: In the same column, different superscript letters indicate significant differences (p < 0.05).

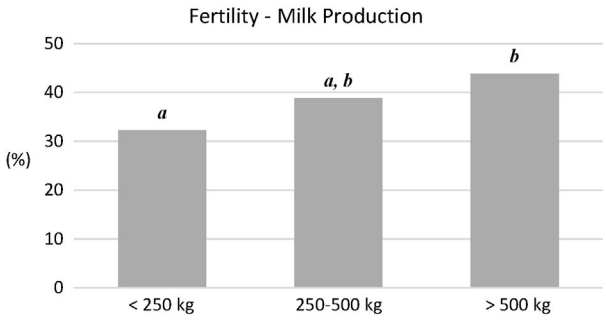

FIGURE 1 Fertility (cervical artificial insemination—CAI; syncronized ewes) according to the accumulated milk production (kg) in the current lactation (Assaf breed). The number of ewes used in this trial is as follows: <250 (n = 248), 250–500 (n = 458) and >500 (n = 286). Total (n = 992). Different letters show significant differences ($p < 0.05$)

2.3 | Seminal preservation

The diluent, storage time and temperature, and number of spermatozoa per dose are important factors that influence fertility in CAI. The preservation of sperm in a liquid medium (15 or 5°C) affects the sperm viability, synthesis of reactive oxygen species (ROS) and DNA integrity due to cold shock (Gibb & Aitken, 2016; Gürler et al., 2016). The membrane protectors classically used to preserve refrigerated sperm have been skim milk and egg yolk. For ovine semen stored for short periods (<12 hr – 15°C), milk: 66% (Paulenz, Söderquist, Ådnøy, Fossen, & Berg, 2003), egg yolk: 56% (Masoudi et al., 2017) or both together 70% (Donovan et al., 2004) offer good fertility results.

It is of special interest to use storage conditions at 5°C to prolong the storage time to above 24 hr. This extended refrigeration period would reduce the dependence of the AI centres, but the performance of the males would remain low (10–20 doses/ejaculate; 200–400 × 10^6 spz/dose). Although the effect of storage time has been evaluated in vitro (López-Sáez, Ortiz, Gallego, & Garde, 2000; Paulenz, Söderquist, Pérez-Pé, & Andersen Berg, 2002; de Paz et al., 2010), few studies have evaluated their fertility. In vitro tests have shown that refrigerated spermatozoa are able to maintain their viability, motility and mucus penetration capacity for days, but their fertility in the field decreases long before the deterioration of these parameters becomes apparent (O'Hara et al., 2010). After up to 24 hr of storage (5°C), good fertility has been observed with different extenders such as AndroMed®: 79.6% and BioXcell®: 70.8% (Khalifa, Lymberopoulos, & Theodosiadou, 2013), INRA 96®: 52.2% (O'Hara et al., 2010) or egg yolk that has been supplemented with seminal plasma -49.7%- (López-Pérez & Pérez-Clariget, 2012). However, none of these cases is significant, given the low number of inseminated ewes. Further studies will be needed to design a valid strategy for the preservation of liquid semen from the ram in the medium term (up to 72 hr as the first objective).

3 | LAPAROSCOPIC INTRAUTERINE INSEMINATION: THE SHORTEST ROAD FOR SPERMATOZOA

Laparoscopic intrauterine insemination (LAI) is the only technique that guarantees adequate fertility when good quality frozen-thawed semen is used. LAI is the technique of choice in particular situations such as the application of imported seminal doses, sperm samples of epididymal origin (Kaabi et al., 2003), doses of high genetic value and/or low quality, and experimental studies. The drawbacks of the technique that considerably limit its use are the need for highly qualified personnel and advanced equipment, which make it a much expensive application than the most common techniques. In addition, although LAI is a minimally invasive surgical technique, it may have implications for animal welfare.

This technique offers acceptable and uniform fertility rates in the use of frozen-thawed sperm samples: 61% (Windsor et al., 1994); 62%, (Fair et al., 2005); 60% (Anel et al., 2006); and 64% (Masoudi et al., 2017). LAI was designed to be used with frozen-thawed sperm samples and oestrous synchronization (fixed-time insemination); however, it has also been used with refrigerated sperm samples and/or in natural oestrus, with results similar to those that are obtained with frozen-thawed samples (Aké-Villanueva et al., 2017).

Laparoscopic intrauterine insemination is a more "permissive" technique than CAI, as it mitigates many of the factors that may affect post-AI fertility (Anel et al., 2006). However, the influence of the year, breed, age and body condition on fertility has been demonstrated (Fukui et al., 2010; Hill et al., 1998), as well as the lambing-AI interval, season, technique (Anel et al., 2005), type of wool and insemination speed (Hill et al., 1998), among others.

The time of restraint on the surgical table can be a negative factor that depresses fertility. In this sense, Emsen, Gimenez-Diaz, Kutluca, and Koycegiz (2011) observed that when this time decreases, fertility increases (57% vs. 42% for 1–4 min or 4–8 min, respectively). Our group has verified (>2,700 LAIs; Mercedes Alvarez, Juan Carlos Boixo, Paulino de Paz, Luis Anel) that there are minimal and inconsistent variations that result as a function of female restraint time. However, it is uncommon to exceed 4 min/female. In fact, our data indicate a mean insemination time of 2.18–2.93 min (depending on the surgical table), which is similar to that described by Casali et al. (2017) (2.98 min).

The number of sperm that is needed for LAI is considerably reduced compared to CAI; with 25 × 10^6 sperm/dose, high fertility rates such as are obtained 60% (Anel et al., 2005). However, there are authors who use high doses of spz/ewe—100 × 10^6 (Santos-Neto et al., 2015) and 200–600 spz × 10^6 (Emsen et al., 2011), which decreases the performance of the ejaculates and does not improve fertility according to our results.

Seminal freezing is a key technique that influences the success of AI programmes. However, the LAI process is relatively undemanding with respect to frozen-thawed spermatozoa and its 'ability' to fertilize. Egg-based diluents and to a lesser extent milk–egg yolk

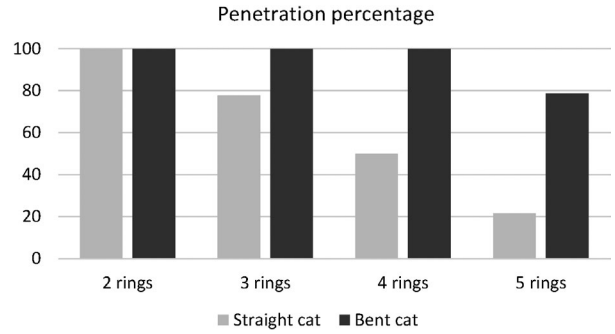

FIGURE 2 Relationship between the number of rings and the percentage of cervical penetration of straight and bent cat (catheter) over cervix (post-mortem) of Churra sheep (n = 64). Straight catheter: IMV®. Bent catheter: CAT06 rigid with a 7-cm-long needle and with a curved tip end 0.6 cm (Álvarez, Chamorro, et al., 2012; Álvarez, Tamayo-Canul, et al. 2012)

are the most commonly used diluents for ewe seminal freezing and show good results after the LAI (El-Alamy & Foote, 2001). In most studies, the freezing technique is tested by LAI, so few studies have attempted to develop a freezing model that is adapted to vaginal insemination (Valente et al., 2010). However, the doses that are destined for LAI are frozen under ideal conditions (100–200 × 10^6 spz/ml), since according to Álvarez, Tamayo-Canul, et al. (2012), freezing doses at high concentrations (necessary for vaginal insemination) results in worse fertility for LAI.

Although the development of LAI has been a significant advance in ovine insemination in recent decades, this technique should be considered as an interim alternative until the development of an efficient TCAI.

4 | TRANSCERVICAL INTRAUTERINE INSEMINATION: A CHALLENGE MID-WAY

The transcervical intrauterine insemination route in sheep, which would allow the use of frozen-thawed sperm, is limited by the particular anatomy of the ovine cervix, which prevents access to the uterus with straight AI catheters. The cervix of the ewe has a variable number of funnel-like cervical rings that form a narrow lumen (Kaabi et al., 2006; Naqvi et al., 2005) that is eccentric of the 2nd ring (Anel et al., 2006). The cervical morphology is highly variable among individual ewe, making it difficult to design adapted instruments. Age, breed, season, phase of the cycle and prolificacy are factors that influence the morphology and cervical permeability up to the uterus (EL khalil et al., 2018; Kershaw et al., 2005).

The success of transcervical artificial insemination (TCAI) is highly dependent on the cervical anatomy and the moment of the oestrous cycle (Kershaw et al., 2005), which are both factors that determine the depth of AI. These are key factors because most previous studies observe a positive relationship between cervical insemination depth and fertility (Cameron, Tilbrook, Lindsay, Keogh, & Fairnie, 1986; Richardson et al., 2012). Eppleston, Salamon, Moore, and Evans (1994)observed that, for each cm of penetration, fertility improves by 7–12%. This fact is the basis for the development of deep or intrauterine cervical deposition procedures (transcervical insemination) that can apply two types of actions in the cervix: active or mechanical methods including cervical retraction (Guelph method) and the design of new catheters (Wulster-Radcliffe & Lewis, 2002), in addition to passive interventions such as cervical dilation with different drugs (Falchi et al., 2012).

4.1 | Mechanical methods: "Following the road"

The small size of the ewe prevents cervical manipulation through the rectum (AI method in cattle); this problem has been addressed with the cervical retraction (CR) towards the vulva, with which it is possible to manipulate the cervix while inserting the catheter. The CR combined with maintaining the ewe in a supine decubitus position constitutes the Guelph method (Guelph TCAI system) that was designed by Halbert, Dobson, Walton, Sharpe, and Buckrelll (1990) and has been used by several researchers. An experienced technician can access the uterus in >75% of ewes. The fertility (frozen-thawed semen) is variable: 45% in 38 ewes (Halbert et al., 1990); 26% in 264 ewes (Windsor et al., 1994); and 32.5% in 1,809 ewes (Buckrell et al., 1994). The main problem lies in the percentage of ewes in which the cervix is not crossed, as well as the time that is invested in the penetration 2 min (Windsor et al., 1994); 3.6 min (Buckrell et al., 1994); 4.7 min (Husein et al., 1998); and 5.2 min (Casali et al., 2017). When the TCAI time exceeds two min, the technique stops being attractive, and the LAI becomes more favourable because it is faster and has better results.

However, to try to pass the cervix more efficiently, different models of curved tip catheters, both semi-flexible (Wulster-Radcliffe & Lewis, 2002) and rigid (Álvarez, Chamorro, et al., 2012; Naqvi et al., 1998), have been successfully used. The previous work by our research group has characterized the ovine cervix (Álvarez, Chamorro, et al., 2012) and designing curved catheters adapted to the morphometry of the cervical canal of different breeds. In a post-mortem study, we observed that transcervical uterine penetration is always more efficient with adapted catheters and depends on the number of rings of the cervix (Mercedes Alvarez, Juan Carlos Boixo, Paulino de Paz, Luis Anel) (Figure 2).

The use of curved catheters (with/without cervical retraction) has several limitations: first, the percentage of penetration in the uterus is variable and unpredictable: 85% (Buckrell et al., 1994); 43–76% (Windsor et al., 1994); and 44% (Naqvi et al., 1998). Second, the fertility rate after deep cervical AI or TCAI with frozen-thawed semen is low: 32.5% (Buckrell et al., 1994); 19% (Windsor et al., 1994); 22.7% (Naqvi et al., 1998); and 4% (Wulster-Radcliffe, Wang, & Lewis, 2004), which compromises its extrapolation to field conditions. The poor and highly variable response to TCAI suggests that the procedure could have deleterious effects on fertility.

What repercussions do cervical manipulation strategies have? Alvares, Cruz, Romano, and Brandão (2016) confirmed that the Guelph TCAI system does not induce an inflammatory reaction that could compromise the pregnancy. However, repeated attempts

to pass the narrow cervical canal (TCAI) with adapted catheters can damage the delicate areas of the cervix, whose cranial segment (3–4 cm) would be very sensitive to manipulations (Álvarez, Chamorro, et al., 2012). Although the sperm sample is deposited laparoscopically inside the uterus, fertility is affected after experimental manipulations in the cervix. Wulster-Radcliffe et al. (2004) observed that deep cervical manipulation (sensitive segment) did not affect sperm transport or fertility until day 3 but that it did affect the viability of gestation. The cervical damages could cause a discharge of prostaglandin F2α, which promotes neutrophil migration and changes in the uterine immune environment, thus causing the failure of pregnancy (Wulster-Radcliffe et al., 2004), which could justify the poor results of the TCAI method. Cervical lesions and consequent modification of the uterine environment are spontaneously resolved, and the fertility is not affected in the subsequent cycles (Anel et al., 2006).

4.2 | Cervical dilatation: 'Widening the road'

The partial dilation of the ovine cervix before manipulation would facilitate the passage of the modified catheters and decrease the possible cervical damage. For the dilation of the ovine cervix, oxytocin (OT), parenterally (Khalifa, Sayre, & Lewis, 1992), and locally (Falchi et al., 2012), interleukin-α (Croy et al., 1999), carazolol (Gündüz et al., 2010), prostaglandin E_2 (Candappa, Bainbridge, Price, Hourigan, & Bartlewski, 2009) and hyalurone (Perry, Haresign, Wathes, & Khalid, 2010) have been used as well. Almost all of these substances are related to the maturation/softening of the cervix during birth.

Prostaglandin E2 (PGE_2) induces the production of glycosaminoglycans, which act by separating the collagen fibres, culminating in the relaxation of the cervix (Kershaw, Scaramuzzi, McGowan, Wheeler-Jones, & Khalid, 2007). PGE_2 receptors are widely distributed throughout the cervix (Schmitz, Levine, & Nathanielsz, 2006), and their expression is regulated by oestrogens. However, the expression of oestrogen and OT receptors varies according to the phase of the oestrous cycle (Falchi & Scaramuzzi, 2013) and the area of the cervix. Oestrogen and PGE_2 receptors are more present in the medial and caudal zone, whereas OT receptors are more common in the cranial zone (Rodríguez-Piñón et al., 2014). During the periovulatory period, the cervix of the ewe is slightly dilated due to the effect of hormones such as PGE_2 and OT in response to the increase in oestrogen (Falchi & Scaramuzzi, 2015). Cervical penetration is improved in the reproductive season and is related to an oestrogen 'dilator' effect in consecutive oestrus (Windsor, 1995).

To dilate the cervix prior to TCAI, PGE analogues (E2-dinoprostone-Cervidil®; E_1 Misoprostol®) have been used in various presentations (suppositories, gel, liquid perfusion), and they have generally been shown to have positive results in the depth of cervical penetration. Cervical dilatation/penetration with PGE_2 is related to breed, mucus impedance, the timing of PGE_2 application, the timing of insemination and even with the presence of males (Bartlewski & Candappa, 2015; Falchi et al., 2012). PGE_2 appears to have a double effect, since in addition to promoting dilation, it also increases the amount of hyaluronate in the mucus of the caudal region of the cervix, which could be related to the spermatozoa colonization capacity (Leethongdee, Kershaw-Young, Scaramuzzi, & Khalid, 2010). In this sense, the cervical penetration depth increased from 1.22 cm to 3.66 cm with the local application of hyaluronan (Perry et al., 2010).

The intrauterine cervical penetration with PGE_2 and the time that this process takes vary greatly between authors and experiments, mainly because, it is almost always combined with the Guelph system and curved catheter (Bartlewski & Candappa, 2015). The studies generally report slight improvements in penetration 1.60 vs. 2.84 cm (Falchi et al., 2012) and low penetration times (1–2 min) in ewes, in which it is possible to pass to the uterus, but since they use combined systems, the real effect of PGE_2 is not clear. Published fertility tests are scarce. Barbas et al. (2013) observed an increase (not significant) in the lambing rate in the group that was treated with PGE_2 with frozen-thawed semen (23.3% vs. 32.5%). The discrete effect on cervical dilation may be due to the difficulty in the intracervical application of the products, which in many cases are deposited in the vagina, and its action on the cervix is uncertain.

Oxytocin has been studied for years for its ability to induce cervical dilation in AIO (Khalifa et al., 1992; Sayre & Lewis, 1996). Increased oestradiol during the oestrous cycle stimulates OT receptors and promotes the release of prostaglandins (Kershaw et al., 2007). In transcervical insemination, intravenous OT application produces strong contractions of a dose-dependent duration throughout the genital tract (Sayre & Lewis, 1996), but the dilation that occurs is debatable. Reports on the results of cervical penetration with OT are highly variable. The authors who pioneered the use of OT published cervical penetration rates up to the uterus in 76% (Khalifa et al., 1992) and in 100% of ewes (Sayre & Lewis, 1996). Other studies have not achieved complete penetration in any ewes (Falchi et al., 2012; King et al., 2004). We have found that 200 IU OT (iv) administered 30 min before CAI does not result in any improvement in cervical penetration and have in fact observed it to have a negative effect on fertility (Anel et al., 2006).

For cervical or transcervical insemination in oxytocin treated ewes, a reduction in fertility can be partially attributed to OT, since when it is administered (without cervical manipulation) in females that are inseminated by laparoscopy, fertility decreases 63% vs. 49%, (Stellflug, Hatfield, Wulster-Radcliffe, & Walker, 2001); 69% vs. 58% (King et al., 2004), but this affect also applies to cervical manipulations because the decrease is even more pronounced in cervically inseminated ewes (42% vs. 10%, control and OT, respectively (King et al., 2004).

The use of OT in TCAI would produce an insufficient level of cervical dilation, possibly due to failures in the dosage and/or time of application (Falchi et al., 2012) and the fertility-suppressing effect of the intracervical manipulations (TCAI catheters) with sperm transport or with the inhibition of fertilization or embryonic development, although these possibilities remain controversial (Rigby et al., 1999; Sayre & Lewis, 1996).

In summary, fertility with TCAI, either with the use of modified catheters, dilating substances or both combined methods, is low and

inconsistent. Many of the published studies are conducted under experimental conditions with a low number of animals, and therefore, their conclusions are difficult to extrapolate to field conditions. The combined protocols (modified catheters plus dilator substances) could be the beginning of the solution for transcervical insemination, but the complexity of the technique, the time spent in cervical penetration and the side effects that it produces are key factors for the success and dissemination of the TCAI and should be optimized to achieve an efficient procedure.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Every single of the authors have contributed substantially to this manuscript: M Alvarez and L Anel designed the review and wrote it. L Anel, M Alvarez and P Paz got the founding. JC Boixo, C Chamorro, M Neila, R Montes and L Anel-Lopez carried out the bibliography revision and the data assessment and organization. L Anel, M Alvarez, P Paz and L Anel-Lopez carried out the revision of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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