



Review article

Nonsurgical embryo recovery and transfer in sheep and goats



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ARTICLE INFO

Article history:

Received 21 September 2015

Received in revised form 12 November 2015

Accepted 14 March 2016

Keywords:

Cervical dilation

Small ruminants

Transcervical embryo collection

ABSTRACT

The embryo transfer techniques used in small ruminants worldwide are based in surgical procedures. These actions are performed under general anesthesia which needs a combination of animal fasting and drugs for secure animal handling and surgery manipulations. Therefore, it involves risks to animal health and life. The major limiting sequels are adhesions formed by the abdominal surgery, in the ovaries, uterus, or between them. These occurrences can both compromise uterus accessing and oocyte capture and are responsible for decreasing success and limiting successive embryo collections. In contrast, nonsurgical embryo procedures can be performed in a relatively simplified way. Nonsurgical embryo recovery does not need animal prolonged starvation, drug retention is minimized, and donors can stay in a standing position. After the end of embryo recovery, donors are promptly restored to their routine housing and feeding. Furthermore, this technique does not need incisions and, therefore, can be used repetitively in superovulated or nonsuperovulated goats and sheep for embryo recovery—a similar procedure done in cattle. In Brazil, promising results are reported using nonsurgical embryo transfer in recipient goats, and studies are currently evaluating similar procedures in sheep. Therefore, this review aimed to present the current panorama of nonsurgical embryo transfer in sheep and goats.

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1. General introduction and a brief review of embryo collection and transfer in small ruminants

In vivo embryo production activities in small ruminants started as early as 1930s with the first successful report in 1934 [1]. Since then, the great majority of embryo recovery and transfer attempts were performed by surgery procedures. Basically, the embryo collection in small ruminants

can be performed using surgical [2–5], laparoscopic [6,7], or transcervical methods [7–14].

Laparotomy technique allows exact counting of the number of corpora lutea and evaluation of total structures recovery rate. However, disadvantages are the relative high cost of equipment, stress to the animal due the manipulation of the exteriorized reproductive tract that can cause adhesions, and progressive reduction in the success of embryo recovery rates [15,16]. The inherent adhesions of the reproductive tract could also hinder sperm transport leading to reduction in the fertilization rate [17]. For laparotomy to accomplish the success rate that can cover the cost inherent,

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repeated embryo collection from superior donors remains paramount. However, with this technique, collections per female are limited to two or three [5,17,18]. Laparoscopy is the second reported technique to recover embryos from goats and sheep. It leads to fewer adhesions and, therefore, a donor could be collected for more than seven times [7,19]. However, this method still requires special equipment and highly trained personnel. Therefore, regardless of good efficiency described [6,7,20], this technique did not become popular. The required refined ability to perform this technique associated with the relatively expensive equipment to perform embryo recovery can explain in part why laparotomy became more adopted worldwide. Both laparoscopic and laparotomy techniques are associated with prolonged donor fasting and general anesthesia.

The third way to recover embryos from sheep and goats is the nonsurgical embryo recovery (NSER) technique. It was first reported in goats [8,10,11] and sheep [21,22] during the 1980s. The anesthetic protocols for this technique are much simpler, and animals may remain in a standing position under sedation in combination with epidural block and local cervical anesthesia [14]. The reduced or nonadhesion formations are pointed as the main advantage of this technique [10,22,23] that can suggest that successive collections are more feasible in NSER than in laparotomy. Conversely, the introduction of a catheter through the cervix, mainly in sheep, and the incapability of rectal manipulation of the tract are the main difficulties for NSER procedures.

The last step of an *in vivo* embryo production program is the transfer of embryos from donors to recipients. Embryos can be transferred to the oviducts or uterine horns according to embryo stage, and the routine commercial chosen technique is also laparotomy. The semi-laparoscopy technique involves laparoscopy and the exteriorization of a little cranial portion of the uterine horn which is punctured to receive embryos by means of an instrument (tom cat catheter) that carries embryos. The total laparoscopic technique for embryo transfer follows the same principle of the semi-laparoscopy, except for not exteriorizing the uterine horn [20,24–26]. In the nonsurgical technique [27,28], corpora lutea are identified by means of ultrasound, cervix is clipped with Pozzi forceps that allow a traction, and a catheter inserted through the cervix to reach the uterine horn desired (ipsilateral to corpora lutea). Then, a device containing the embryo is coupled to the catheter and embryo is deposited. When comparing these techniques, nonsurgical embryo transfer reported pregnancies and births similar to laparoscopy in goats [7,28] and sheep [27]. Besides leading to results comparable with those of laparoscopy, the nonsurgical technique can be performed quickly and safely.

The current lack of expansion of embryo collection and transfer in small ruminants industry is mainly due to the need for surgical procedures which have inherent limitations that includes costs, animal health, and successive use. Therefore, it is hypothesized that the development of nonsurgical procedures can turn around this dilemma. Brazil is a good example to demonstrate that this scenario can be changed. In Brazil about to 100% of commercial goat embryos transferred to recipients in 2010 were recovered by transcervical via [29]. This review aimed to present the

current panorama of nonsurgical embryo collection and transfer in sheep and goats.

2. Panorama of NSER and transfer

2.1. Goats

The first study demonstrating the feasibility of nonsurgical embryo collection in goats was published in 1984 [8]. The authors administered *Laminaria japonica* tent into the cervical canal for 6 to 12 hours for cervical dilation and compared different catheters for embryo recovery. Although in one goat Foley catheter had perforated the uterus at the bifurcation; in general, results were satisfactory with 90% efficiency of fluid recovered when using certain devices. A total of 13 embryos were recovered from two donors.

Few years later, transcervical embryo collection resulted in successful recovery of 69 embryos from 19 Shiba goats by the use of a cervical expander. Embryo collection was successful in 15 of 26 attempts, yielding a recovery rate of 89.5%, and the average number of embryos collected from each female was 3.6 [9].

In superovulated Angora and cashmere goats, 296 nonsurgical and 40 surgical collections were performed, leading to a total number of 2785 harvested structures [10]. In Angora goats, catheters could be passed in over 90% of the attempts. Although the recovery rate was not estimated, mean numbers of ova collected per doe were high: 9.5, 7.3, and 11.3 for nonsurgical, laparoscopic, and surgical procedures, respectively. The authors concluded that nonsurgical technique was effective for embryo collection.

Pereira et al. [11] administered prostaglandin F_{2a} and oxytocin in Boer goats and described for the first time a technique for transcervical collection of embryos from superovulated goats maintained in a standing position with neither tranquilization nor anesthesia. These authors suggested that the recovery rate was comparable to that achieved by surgical collection. Later on, the same laboratory succeeded to simplify and accelerate the procedure of transcervical embryo collection in goats. They developed a new restraining device and used a wider-bore catheter in association with earlier induction of luteolysis (24 hours before embryo recovery). Authors concluded that these alterations were instrumental in saving labor and time, without impairing recovery rate or embryo yield [30].

Superovulated Saanen goats were subjected to transcervical technique, and the cervix was penetrable in 61% of goats. In the remaining goats, embryo recovery was surgically performed. The recovery rate was 53.2% (transcervical) and 36.9% (laparotomy) Embryo production yield and quality were similar in both techniques [31].

Currently, the most of embryo recovery in Brazil is performed by transcervical via [29]. Recent studies reported an average collection time of 35 minutes with approximately 97% of flushing media recovered. Although the embryo recovery rate was not described, a high number of total structures (~17) and viable embryos (~10) were obtained. It is noteworthy that no apparent lesions or sequels were noted in the reproductive tract [14]. Considering cloprostenol administration before

uterine flushing, it is common that donors show estrus behavior at the day after uterine flushing. One donor goat mated naturally after this estrus yielded three viable embryos recovered by nonsurgical uterine flushing 7 days later (two embryo recoveries in 8 days) [14]. Given the efficiency of uterine flushing by the cervical route and reduced anesthesia and surgical risks or sequels, we expect that surgical embryo recovery will be gradually restricted or even prohibited in near future.

Although researchers [32] have already obtained more than 40% of conception rate after nonsurgical embryo transfer two decades ago, its use is sporadic in goats. Nowadays with animal rights activists, it is even more essential to start focusing on transcervical embryo deposition in small ruminants. Recently, we have demonstrated the feasibility of efficient embryo deposition directly to the desired uterine horn, after corpus luteum (CL) location using transrectal ultrasonography [28]. These relatively non-traumatic methods for embryo collection and transfer produced comparable results with surgical methods in goats.

2.2. Sheep

In sheep, unlike goats, the alternative NSER and transfer techniques are limited by the anatomy of the ovine cervix. Moreover, the incapability of rectal manipulation of the tract generally makes the passage of the catheter/pipette through the uterus body more difficult [33]. Advances in the science of ovine cervical relaxation and its pharmacologic stimulation have led to improvements in physical penetration of the cervix which facilitated transcervical embryo recovery [34].

The first study demonstrating that sheep embryos could be recovered by NSER reported 42% (11/26) of success almost 30 years ago [21]. The authors informed that a digit was inserted into the rectum to allow cervical manipulation—the procedure that is performed to date. Few years later, the cervical penetration in virgin and adult ewes was achieved by “ripening” the cervix with prostaglandin E2 and estradiol, without detrimental effect to the embryos [22].

Currently, in Brazil, there are great efforts to adapt the NSER technique in sheep. The procedure was first tested in a native breed named Santa Inês. Females received cloprostenol, misoprostol, or no treatment to dilate the cervix. In animals that it was impossible to pass the catheter through the cervix, surgical recovery was performed. The transcervical recovery method was successful in ~61% of ewes receiving either cloprostenol or misoprostol, whereas no catheter passage was achieved in control-ewes. The authors concluded that the technique in pluriparous Santa Inês sheep was possible, but there was a great individual variation, even when using a pharmacologic cervical expander [12]. The same group of researchers assessed the effect of misoprostol on the cervical expansion in Dorper ewes. Animals receiving 200-mg misoprostol by vaginal route 5 hours before embryo recovery reached ~95% of cervical transposition, compared with 0% for control-ewes. On average, the technique was accomplished in 30 minutes and allowed a recovery of six embryos per ewe, demonstrating its feasibility for Dorper ewes [13]. All these data

suggest that the NSER in sheep can be possible and reliable, especially if is coupled with the induction of cervical dilation. Although we believe that the NSER in sheep is possible, the efficiency and repeatability in different breeds remain to be investigated.

3. Key limiting points for successful nonsurgical embryo transfer in goats and sheep

The NSER in sheep and goats is reported to be performed with animals in sternal [8] or dorsal recumbency [10,21,22,35], or in a standing position [11,14]. Regardless of the position, NSER involves a series of steps that includes animal preparation and ovary evaluation (laparoscopy or ultrasonography); physical and chemical immobilization (anesthesia); cervical immobilization, traction, and transposing; uterine flushing; and transcervical embryo transfer. These key points require different skills and attention from the technician and are related to the overall efficiency of the technique. The main steps are revised in the following section.

3.1. Initial animal handling and preparation

Either superovulated or nonsuperovulated animals [14,36] can be subjected to NSER. Embryos are normally recovered from 5 to 7 days of estrous cycle [10,11,14,21,22]. There is no need for food or water restriction when NSER is done with donor in the standing position [37]. When dorsal recumbency is preferred [10,21,22,35], animals need fasting as in surgery embryo recovery.

At the day of uterine flushing, the perineal region must be washed with clean water and detergent. No alcohol-based solution should be used in this area. Special attention should be paid to removal of any residual fecal material on anus or vulva [14]. The trichotomy of areas such sacrococcygeal or lumbosacral region is needed for epidural anesthesia. In some donors, tail hair must be shaved 1 day before uterine flushing. At the same day, CL can be well characterized [38,39] and evaluated by ultrasound to determine ovarian superovulatory donor response. The first uterine horn to be flushed is the one ipsilateral to the ovary with better response. In recipient females, CL localization and evaluation can be performed for choosing the uterine horn to receive embryos [28].

3.2. Physical and chemical restraint

Physical contention is required, and its rigor depends on the position at which the animal is maintained during the NSER procedure. It is generally done under chemical restraint. This step must give maximal comfort to both, donor and technician.

Sedation and local anesthesia must be applied to allow an efficient embryo collection. Besides improving animal welfare, this technique makes the procedure easier and safer [40]. Reproductive tract manipulation for NSER in small ruminants, such as cervical clamping and traction, induces pain and requires relaxation for its best performance. Furthermore, animals should be ready to be reintroduced in the flock as soon as possible after the end

of the procedure, minimizing stress, and its undesired consequences [41]. Therefore, anesthetic protocols for NSER should consider a mild sedation and the relaxation and analgesia of vulva and vagina. The xylazine is extensively used in ruminants due to its sedative, analgesic, and muscle relaxing effects despite its unwanted effects such as excessive salivation, bradycardia, hypotension, depressed respiratory rate, and decreased ruminal motility [42]. When used at 0.1-mg/kg dose, it may keep goats sedated for approximately 75 minutes [43] which is a long period considering the mean duration of the NSER procedure (~30 minutes). In order to reduce its adverse effects, lower doses such as 0.05 mg/kg i.m. could be used to reduce the sedative length. The benzodiazepines as diazepam or midazolam have mild sedative, muscle relaxant, but not analgesic effects [40] and, thus, should be combined with analgesics or local anesthetic techniques. Acepromazine and other phenothiazines are not commonly used in ruminants due to their limited sedative effects; however, when combined with other drugs they seem to reduce required doses [40]. In our laboratory, for sheep, we commonly use the association of 0.1 mg/kg of acepromazine with 0.2 mg/kg of diazepam IV; females show moderate sedation lasting approximately for 45 minutes and being able to be reintroduced to the flock soon after the end of the procedure. Despite providing adequate relaxation and sedation, this combination provides no analgesia. Epidural anesthesia is recommended either with 1 mL/7 kg body weight (BW) of 2% lidocaine hydrochloride [44] or with 2.5-mg/kg BW ketamine [45]. When using ketamine (10%), total volume of injection should be 1 mL/7.5 kg BW and completed with saline solution for cranial migration of the drug. Epidural injections may be performed both in sacrococcygeal and lumbosacral space. The advantage of performing sacrococcygeal epidural is that motor blockade of fibers innervating pelvic limbs is limited, allowing the animal to stand without support. On the other hand, analgesia of cervix and uterus is not completely accomplished with this volume. The option of using ketamine (2.0 mg/kg) in lumbosacral space promotes cervical analgesia with less motor blockade and return to standing position in 45 minutes (unpublished results).

In our NSER routine for both sheep and goats, we use combined lidocaine-acepromazine [14]. Females receive acepromazine injection of 0.1 mg/kg BW i.m 10 minutes before cervical clipping. Before vaginal speculum introduction, females also receive 1 to 2 mL of 2% lidocaine hydrochloride administered in the sacrococcygeal space or between C1 and C2 coccygeal vertebrae. Then, a speculum is introduced into the vagina, the cervix is clipped, and sterile gauze soaked with 3 mL 2% lidocaine is pushed under the cervical opening [14].

3.3. Cervical access, immobilization, and traction

Cervical access for both embryo recovery or transfer can be done with animals in dorsal recumbent position [21] or in standing position [14,28]. To access cervix, it is necessary to use a light source, a vaginal speculum, and lubricant gel. Different specula can be used as duck-billed

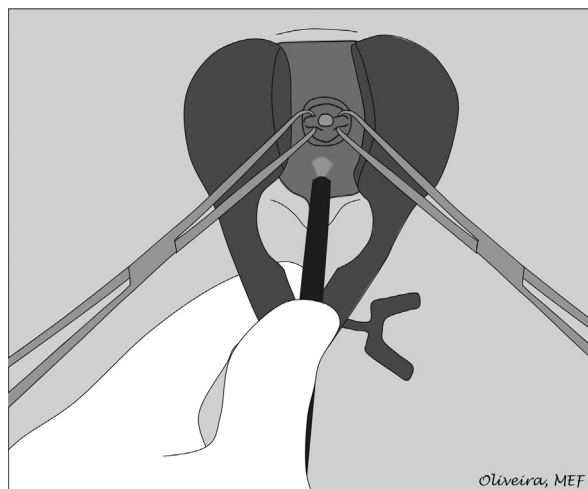


Fig. 1. Collin speculum inserted into goat vagina. Observe a light source and central projection of cervical os. Two 26 cm Pozzi forceps are used to clip cervix laterally.

[11], human Collin [14,21], and elongated human Collin (15–20 cm). When using duck-billed speculum, the cervix is clipped outside (laterally) the speculum, whereas with Collin speculum, the cervix is clipped inside the speculum. The number of human Collin specula to be used depends on individual vaginal dilation. Normally, No.1 speculum is recommended for nulliparous and primiparous donors or recipients, and No. 2 and No. 3 are used for pluriparous females [46]. The use of Collin speculum allows cervix projection through the speculum center. When using duck-billed or elongated human Collin specula, there is more vaginal distention and increased distance to see and clip the cervix. Technicians are encouraged to try both possibilities and choose the easier form to immobilize the cervix. Good cervical immobilization and traction are key points determining the success of nonsurgical procedures followed by the facility or difficulty of cervical handling for transposing, and finally, the uterine flushing efficiency.

After speculum introduction, a light source aids to maintain the speculum in a vaginal central position; cervical exposition when the vagina is opened and projection of the cervix into the speculum center [14,46]. Three types of forceps can be used for immobilization and traction of the cervix. When using duck-billed speculum, a non-traumatic forceps can be inserted into and above the cervical opening. A little traction can help the use of two 26 cm Pozzi [12–14] or two Allis forceps [11] that are clipped 0.5 to 1 cm laterally to the cervical opening. With the use of Collin speculum, cervical projection gives optimal choice to

Table 1
Embrapa's protocol for cervical relaxation and uterine flushing by cervical route in sheep.

Drug	Dose per ewe	Time relative to uterine flushing	Route of drug administration
Estradiol benzoate	1.0 mg	18 h	Intramuscular
Cloprostenol	37.5 µg	18 h	Laterovulvar
Oxytocin	50.0 IU	20 min	Intravenous

Table 2
Efficiency of uterine flushing through cervical route in goats.

Reference	Number of donors	Success collections (%)	Media infused per time (mL)	Total flushing media infused (mL)	Success media recovered (%)	Ova-embryo/CL counted (%)	Average ova-embryo recovered
BonDurant et al., 1984 [8]	6	4	—	—	Minimal to 90	—	2.2
Nagashima et al., 1987 [9]	37	51.4	—	—	—	89.5	3.6
Bessoudo et al., 1988 [10]	296	—	5–10	75–150	—	—	9.47
Flores-Foxworth et al., 1992 [7]	18	—	5–10	75–150	—	36.9	—
Pereira et al., 1998 [11]	7	100.0	20	480	97.0	91.0	11.7
Andrioli et al., 1999 [23]	30	73.3	—	40	64.3	57.1	0.2
Suyadi et al., 2000 [30]	9	100.0	40	1200	—	79.0	8.4
Suyadi et al., 2000 [30] ^a	8	100.0	40	1200	—	43.0	4.4
Androukovitch et al., 2002 [50]	18	83.3	20	220	94.3	81.2	13.2
Lima-Verde et al., 2003 [31]	8	61.5	20	500	—	53.2	6.3
Amorim et al., 2011 [47]	18	100.0	15–20	400	—	—	5.5–7.2
Fonseca et al., 2013 [14]	10	100.0	15–20	360	96–97	—	15–18
Moura et al., 2014 [51]	2	100.0	15–20	400	—	73–87	8–13

^a Without drugs for cervical dilation.

clip and tract the cervix, and; therefore, auxiliary non-traumatic forceps is not necessary. The use of Collin speculum and Pozzi forceps are showed in Figure 1. After adequate immobilization, the cervix is tracked back slowly and positioned to enable good manipulation. The middle finger is inserted in the rectum, whereas the thumb finger is inserted into the vagina. This procedure can keep the cervix between both fingers, which may facilitate cervical handling and transposing.

3.4. Cervical dilation and transposing

The cervical transposing can be related to many factors including species, breed, parturition rate, lactation status, technician ability, drugs, and time of drug administration related to embryo recovery. Although there are no studies comparing NSER technique in sheep and goats, it is presumed that goat cervix is easier to transpose than sheep cervix. More recent, studies had shown 100% cervical transposing in donor goats [14,47]. In both sheep and goats, drugs or their equivalents that mimic physiological conditions for natural cervix relaxation are used. In goats, the use of PGF2 α analogues alone is sufficient to promote adequate cervix relaxation [11,14,47]. In sheep, PGF2 α analogues are also used and showed relative success in nonsurgical uterine flushing in Santa Inês females [12,36]. With the addition

of another drug such as misoprostol, 95% uterine flushing in Dorper sheep has been reported [13]. Our research team tested the combination of cloprostenol, estradiol, and oxytocin in Brazilian sheep breeds [36,37,48,49] with good cervical transposing and transcervical uterine flushing. The so-called Embrapas's protocol for cervical relaxation and uterine flushing by cervical route in sheep is presented in Table 1.

The NSER is reported using different types of catheter with [8,11] or without balloon [12,14]. Some reports include the use of Foley [21], urethral [47,50], and recently, a special catheter developed by our team for uterine flushing in goats and sheep [14]. All catheters need a mandrel to reduce flexibility and help cervical transposing. In our opinion, a great attention should be paid for adequate manipulation of the catheter, which will lead to efficient uterine horn draining, and eventually, efficient embryo recovery. Therefore, a catheter that cannot be moved is not recommended.

3.5. Uterine flushing and embryo recovery

Following cervical transposing, the next step is uterine flushing for embryo recovery. Efficiency of uterine flushing through cervical route in goats (Table 2) and sheep (Table 3) are presented. There are variable amounts of flushing media used. Little quantities can compromise embryo

Table 3
Efficiency of uterine flushing through cervical route in sheep.

Reference	Number of donors	Success collections (%)	Media infused per time (mL)	Total flushing media infused (mL)	Success media recovered (%)	Ova-embryo/CL counted (%)	Average ova-embryo recovered
Coonrod et al., 1986 [21]	26	42.0	5–10	75–150	—	—	5.5
Barry et al., 1990 [22]	10	100.0	—	—	84.0	65.0	2.2
Gusmão et al., 2007 [12] ^a	13	0.0	—	—	—	—	—
Gusmão et al., 2007 [12]	17	59.8	20	480	95.6	—	6.5
Gusmão et al., 2007 [12]	19	63.1	20	480	95.7	—	6.5
Gusmão et al., 2009 [13]	10	0.0	—	—	—	—	—
Gusmão et al., 2009 [13]	58	94.8	20	480	95.7	—	6.0
Zambrini et al., 2014 [36] ^{a,b}	24	25–33	10–15	400	94.0	—	1.1
Fonseca et al., 2015 [48] ^b	4	100.0	10–15	80	96.9	—	—
Fonseca et al., 2015 [49] ^c	23	80–91	10–15	400	90.1	—	1–1.4
Zambrini et al., 2015 [37]	16	78–86	10–15	400	96.2	—	6.4–7.4

^a Without drugs for cervical dilation.

^b Estrous-induced ewes.

^c Estrous-synchronized ewes.

Table 4

Reproductive efficiency parameters of transcervical embryo transfer in goats.

Reference	Number of recipients	Embryos transferred per recipient	Pregnancy rate (%)	Embryo survival rate (%)	Parturition rate (%)	Fetuses born
Otsuki and Soma, 1964 [53]	7	1–3	14.3	7.1	14.3	1
Lin et al., 1979 [52]	8	—	62.5	54.5	—	—
Agrawal and Bhattacharyya, 1982 [32]	7	2–3	42.9	11.8	14.3	2
Flores-Foxworth et al., 1992 [7]	18	2–3	38.9	—	—	7
Fonseca et al., 2014 [28]	6	1–2	50.0	50.0	50.0	3

recovery rate. Our team uses and recommends 400 mL. The first 20 mL is used to humidify the system throughout the filter. The last 20 mL is used to wash the circuit through which the filter is accessed. The intermediate 360 mL is used to wash uterine horns (180 mL per uterine horn). If a 15-mL volume is injected and recovered per cycle, it enables 12 washes per uterine horn.

A good embryo recovery rate can be reached through integrated conjunct of good contention (including sedation and anesthesia); efficient cervical dilation, clipping, and transposing; the use of adequate catheter; and adequate uterine horn positioning and change of the catheter. These processes and equipment used will directly determine the duration of embryo recovery from few minutes (20 minutes) to hours. Through our routine procedures that take an average of 30 minutes, more than 90% flushing efficiency has been obtained.

3.6. Embryo transfer by cervical route

There are relatively few studies regarding nonsurgical embryo transfer in goats (Table 4) and sheep. The first successful newborn was reported by Otsuki and Soma [53] in goats and Fonseca [27] in sheep. Recently, in sheep treated with dinoprostone (PGE₂), Candappa and Bartlewski [54] described a 55% (6/11) success of cervical transposing and embryo transfer. Although a 33% pregnancy rate had been observed at 25 days of pregnancy, no fetuses were detected 30 days later (55 days of pregnancy).

Several reports in the context of transcervical embryo transfer in goat are listed in Table 4. With actual accuracy of CL localization and evaluation by transrectal ultrasonography, laparoscopy is not essential to determine which uterine horn will receive embryos. As recently described [28], embryo transfer through cervical route can be performed in similar procedures carried for AI. Positive aspects of this technique are diverse, including less time-consuming; safe and noninvasive procedure; no need for use of complex anesthesia or food and water restriction. Throughout the entire procedure of embryo transfer, recipient goats do not present behavioral signs of pain, vocalization, and postural discomfort. In general, their behavior is similar to that observed during other routine activities, such as traditional AI [28]. The procedure resulted in an efficient embryo deposition into the ipsilateral horn. Further studies may confirm the use of transcervical embryo transfer in large-scale operations [28] demonstrating the potential of this technique.

4. Associated sanitary risks with nonsurgical embryo transfer in sheep and goats

Recent data pointed that the nonsurgical uterine flushing seems to have an important role in the caprine arthritis encephalitis virus (CAEV) control programs [55]. Many studies revealed the presence of the virus in uterine flushing media after surgical technique [56–61]. However, no positive samples for CAEV were reported from flushing media and embryos recovered by nonsurgical procedure in the first centrifugation. The virus was only identified after viral concentration with PEG 40%. The authors suggested that the lack of CAEV cases may be attributed to the greater volume of liquid used in NSER (more than 300 mL vs. 40 mL for surgical technique) which may apparently dilute the virus, and due to less manipulation of the uterus in NSER than surgical techniques [55]. Although there are no data in sheep regarding this concern, it is reasonable to propose that Maedi-Visna virus transmission can also be diminished with nonsurgical embryo procedures.

5. Conclusion

The sheep and goat embryo transfer world industry is supported by *in vivo* embryo production. These embryos are recovered by surgical procedures in majority of countries, despite many reports that pointed the viability of nonsurgical recovery of embryos and a few others that indicate the possibility of transcervical embryo transfer. Considering the increasing concern and restrictions to handling with regard to animal welfare, Brazilian researchers have given special attention to enable complete nonsurgical procedures to recover and transfer embryos. Both embryo recovery and transfer show acceptable and encouraging success in goats and sheep. We believe that increased number of studies and consequent progress in nonsurgical embryo procedures in these species will allow massive application of these procedures as has happened in the bovine species. If this proposal is followed, it is possible that in a short time, embryo surgical procedures in small ruminants, especially in goats, will be unacceptable—a similar scenario to current procedures in cattle.

Acknowledgments

The authors thank Brazilian Agricultural Research Corporation (EMBRAPA; Projects 01.07.01.002.05 and 03.12.01.031.00) and “Fundação de Amparo à Pesquisa de Minas Gerais” (FAPEMIG; Project CVZ-APQ 01367–9 and PPM 00042–14) for the financial support of recent studies in the context on nonsurgical embryo transfer in sheep

and goats. J.F.F. and F.Z.B. are CNPq fellows, and J.M.G.S.-F. is supported by CAPES.

Competing Interests

The authors do not have any conflict of interest to declare.

References

- Warwick BL, Berry RO, Horlacher WR. Results of mating rams to Angora female goats. In: Proceedings of the American Society of Animal Production 1934. p. 225–7.
- Armstrong DT, Evans G. Factors influencing success of embryo transfer in sheep and goats. *Theriogenology* 1983;19:31–42.
- Nowshari MA, Holtz W. In vitro culture of goat morulae to blastocysts before freezing. *Theriogenology* 1995;44:983–8.
- Lehloenyha KC, Greyling JPC. Effect of route of superovulatory gonadotrophin administration on the embryo recovery rate of Boer goat does. *Small Rumin Res* 2009;87:39–44.
- Bruno-Galarraga MM, Cueto M, Gibbons AE, Pereyra-Bonnet F, Catalano R, Gonzales-Bulnes A. Repeatability of superovulatory response to successive FSH treatment in Merino sheep. *Small Rumin Res* 2014;120:84–9.
- McKelvey WAC, Robinson JJ, Aitken RP, Robertson IS. Repeated recoveries of embryos from ewes by laparoscopy. *Theriogenology* 1986;25:855–65.
- Flores-Foxworth G, McBride BM, Kraemer DC, Nuti LC. A comparison between laparoscopic and transcervical embryo collection and transfer in goats. *Theriogenology* 1992;37:213.
- BonDurant RH, Skirrow IS, Anderson GB, Hanson F, Rogers WH. Nonsurgical collection of blastocysts from dairy goats. *Theriogenology* 1984;22:423–31.
- Nagashima H, Matsui K, Sawasaki T, Kano Y. Nonsurgical collection of embryos in Shiba goats. *Jikken Dobutsu* 1987;36:51–6.
- Bessoudo E, Davies L, Coonrod S, Gamez J, Kraemer DC. Non-surgical collection of caprine embryos under commercial quarantine conditions. *Theriogenology* 1988;2:221.
- Pereira RJTA, Sohnrey B, Holtz W. Nonsurgical embryo collection in goats treated with prostaglandin F₂-alpha and oxytocin. *J Anim Sci* 1998;76:360–3.
- Gusmão AL, Silva JC, Quintela A, Moura JCA, Resende J, Gordiano H, et al. Colheita transcervical de embriões ovinos da raça Santa Inês no semi-árido nordestino. *Rev Bras Saúde Prod Anim* 2007;8:1–10.
- Gusmão AL, Silva JC, Bittencourt TCC, Martins LEP, Gordiano HD, Barbosa LP. Coleta transcervical de embriões em ovinos da raça Dorper no semiárido do nordeste Brasileiro. *Arq Bras Med Vet Zootec* 2009;61:313–8.
- Fonseca JF, Zambrini FN, Alvim GP, Peixoto MGCD, Verneque RS, Viana JHM. Embryo production and recovery in goats by non-surgical transcervical technique. *Small Rumin Res* 2013;111:96–9.
- Torres S, Sevellec C. Repeated superovulation and surgical recovery of embryos in the ewe. *Reprod Nutr Dev* 1987;27:859–63.
- Forcada F, Ait Amer-Meziane B, Abecia JA, Maurel MC, Cebrián-Pérez JA, Muiño-Blanco T, et al. Repeated superovulation using a simplified FSH/eCG treatment for in vivo embryo production in sheep. *Theriogenology* 2011;75:769–76.
- Lehloenyha KC, Greyling JPC, Grobler S. Can repeated superovulation and embryo transfer in Boer goats limit donor participation in a MOET programme? *S Afr J Anim Sci* 2009;39(Suppl 1):193–7.
- Nellenschulte E, Niemann H. Collection and transfer of ovine embryos by laparoscopy. *Anim Reprod Sci* 1992;27:293–304.
- Baril B, Casamitjana P, Perrin J, Vallet JC. Embryo production, freezing and transfer in Angora, Alpine and Saanen goats. *Zuchthyg* 1989;24:101–5.
- Schiewe MC, Bush M, Stuart LS, Wildt DE. Laparoscopic embryo transfer in domestic sheep: a preliminary study. *Theriogenology* 1984;22:675–82.
- Coonrod SA, Coren BR, McBride BL, Bowen MJ, Kraemer DC. Successful non-surgical collection of ovine embryos. *Theriogenology* 1986;2:149.
- Barry DM, Van Niekerk C-H, Rust J, Van der Walt T. Cervical embryo collection in sheep after “ripening” of the cervix with prostaglandin E₂ and estradiol. *Theriogenology* 1990;33:190.
- Andrioli A, Simplício AA, Soares AT, Visintin JA. Eficiência da recuperação de embriões e os efeitos de consecutivas colheitas sobre o aparelho reprodutor de doadoras da espécie caprina. *Braz J Vet Res Anim Sci* 1999;36:136–43.
- Besenfelder U, Zinovieva N, Dietrich E, Sohnrey B, Holtz W, Brem G. Tubal transfer of goat embryos using endoscopy. *Vet Rec* 1994;135:480–1.
- Shin ST, Jang SK, Yang HS, Lee OK, Shim YH, Choi WI, et al. Laparoscopy vs. laparotomy for embryo transfer to produce transgenic goats (*Capra hircus*). *J Vet Sci* 2008;9:103–7.
- Lehloenyha KC, Greyling JPC. Embryo transfer using cryopreserved Boer goat blastocysts. *S Afr J Anim Sci* 2010;40(Suppl 1):446–50.
- Fonseca JF. Biotecnologias da reprodução em ovinos e caprinos. Sobral, CE: Embrapa Caprinos; 2006. (Documentos, n. 64).
- Fonseca JF, Esteves LV, Zambrini FN, Brandão FZ, Peixoto MGCD, Verneque RS, et al. Viable offspring after successful non-surgical embryo transfer in goats. *Arq Bras Med Vet Zootec* 2014;66:613–6.
- Fonseca JF, Souza JMG, Camargo LSA. Produção de oócitos e embriões de pequenos ruminantes: passado, presente e futuro. *Acta Sci Vet* 2010;38:337–69.
- Suyadi B, Sohnrey W, Holtz. Transcervical embryo collection in Boer goats. *Small Rumin Res* 2000;36:195–200.
- Lima-Verde JB, Lopes Jr ES, Teixeira DIA, Paula NRO, Medeiros AA, Rondina D, et al. Transcervical embryo recovery in Saanen goats. *S Afr J Anim Sci* 2003;33:127–31.
- Agrawal KP, Bhattacharyya NK. Non-surgical transplantation of embryo in goats. In: Proc. 3rd Inter. Conf. on goat prod. and disease (abstr.). Jan 10–15, Tucson, AZ, U.S.A. Scottsdale, AZ: Dairy Goat Journal Publishing Co; 1982. p. 340.
- Kershaw CM, Khalid M, McGowan MR, Ingram K, Leethongdee S, Wax G, et al. The anatomy of the sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen. *Theriogenology* 2005;64:1225–35.
- Robinson JJ, McKelvey WAC, King ME, Mitchell SE, Mylne MJA, McEvoy TG, et al. Traversing the ovine cervix—a challenge for cryopreserved semen and creative science. *Animal* 2011;5:1791–804.
- Melican D, Gavin W. Repeat superovulation, non-surgical embryo recovery, and surgical embryo transfer in transgenic dairy goat. *Theriogenology* 2008;69:197–203.
- Zambrini FN, Guimaraes JD, Esteves LV, Castro ACR, Fonseca JF. Cervix dilation and transcervical embryo recovery in cervical Santa Inês sheep. *Anim Reprod* 2014;11:419.
- Zambrini FN, Guimaraes JD, Prates JF, Esteves LV, Souza-Fabjan JMG, Brandao FZ, et al. Superovulation and non-surgical embryo recovery in Santa Inês ewes. *Anim Reprod* 2015;12:720.
- Arashiro EKN, Viana JHM, Fonseca JF, Camargo LSA, Fernandes CAC, Brandão FZ. Luteal dynamics in goats: morphological and endocrine features. *R Bras Zootec* 2010b;39:1937–42.
- Figueira LM, Fonseca JF, Arashiro EKN, Souza-Fabjan JMG, Ribeiro ACS, Oba E, et al. Colour Doppler ultrasonography as a tool to assess luteal function in Santa Inês ewes. *Reprod Domest Anim* 2015;50:643–50.
- Galatos AD. Anesthesia and analgesia in sheep and goats. *Vet Clin North Am Food Anim Pract* 2011;27:47–59.
- Fitzpatrick J, Scott M, Nolan A. Assessment of pain and welfare in sheep. *Small Rumin Res* 2006;62:55–61.
- Shah Z, Ding MX, Hu ML. A review on the current use of alpha 2 agonists in small ruminants. *Kafkas Univ Vet Fak Derg* 2014;20:633–9.
- Shah Z, Kalthore AB, Kachiwal AB, Ahmad I, Sattar H, Khan MA. Comparative studies on sedative and analgesic effects of xylazine and detomidine in goats. *J Anim Plant Sci* 2013;21:1019–23.
- Habibian S, Bigham AS, Aali E. Comparison of lidocaine, tramadol, and lidocaine-tramadol for epidural analgesia in lambs. *Res Vet Sci* 2011;91:434–8.
- DeRossi R, Pompermeyer CTD, Silva-Neto AB, Barros ALC, Jardim PHA, Frazílio FO. Lumbosacral epidural magnesium prolongs ketamine analgesia in conscious sheep. *Acta Cir Bras* 2012; 27:137–43.
- Fonseca JF, Oliveira ME, Viana JH. Uso de procedimentos não cirúrgicos para a produção, recuperação e inativação de embriões em pequenos ruminantes. *Rev Bras Reprod Anim* 2011;35:113–7.
- Amorim LS, Torres CAA, Siqueira LGB, Fonseca JF, Guimarães JD, Carvalho GR, et al. Embryo development and follicular status of Toggenburg does fed urea diet. *R Bras Zootec* 2011;40:277–85.
- Fonseca JF, Zambrini FN, Guimarães JD, Pereira VSA, Souza-Fabjan JMG, Ribeiro C, et al. Successful transcervical uterine flushing in Morada Nova sheep. In: Anais do XXI Congresso Brasileiro de Reprodução Animal. Belo Horizonte: CBRA; 2015. p. 180.

- [49] Fonseca JF, Zambrini FN, Prates JF, Martins TLT, Castro ACR, Esteves LV, et al. Estradiol route and non-surgical embryo recovery in synchronized Santa Inês ewes. *Anim Reprod* 2015;12:734.
- [50] Androukovitch JL, Kozicki LE, Kozemjakim DA, Abreu RM, Nunez C. Coleta de embriões caprinos com sonda uretral humana. *Arch Vet Sci* 2002;7:37–41.
- [51] Moura RR, Souza-Fabjan JMG, Fonseca JF, Melo CHS, Sanchez DJD, Vieira MP, et al. Reproductive parameters and the use of MOET in transgenic founder goat carrying the human granulocyte colony-stimulating factor (hG-CSF) gene. *Anim Reprod* 2014;11:37–43.
- [52] Lin A, Lee K, Chang S, Lee P. Non-surgical embryo transfer in goats. *Memoir Coll Agr* 1979;19:25–33.
- [53] Otsuki K, Soma T. Transfer of fertilized ova through the cervix in goats. *Bull Nat Inst Anim Ind* 1964;6:27–33.
- [54] Candappa IBR, Bartlewski PM. Induction of cervical dilation for transcervical embryo transfer in ewes. *Reprod Biol Endocrinol* 2014;12:1–9.
- [55] Nascimento-Penido PMP, Santos GB, Penido AO, Fonseca JF, Leite RC. Identification of arthritis encephalitis caprine virus (CAEV) in flushing media and embryos from naturally infected dairy goats. *Anim Reprod* 2015;12:703.
- [56] Fieni F, Rowe J, Van Hoosear K, Buruca C, Oppenheim S, Anderson G, et al. Presence of caprine arthritis encephalitis virus (CAEV) infected cells in flushing media following oviductal-stage embryo collection. *Theriogenology* 2001;57:931–40.
- [57] Lamara A, Fieni F, Mselli-Lakhal L, Chatagnon G, Bruyas JF, Tainturier D, et al. Early embryonic cells from in vivo-produced goat embryos transmit the caprine arthritis-encephalitis virus (CAEV). *Theriogenology* 2002;58:1153–63.
- [58] Andrioli A, Gouveia AMG, Andrade JS, Andrade JS, Pinheiro RR, Yorinori EH, et al. Diagnostic of the caprine arthritis encephalitis virus in uterine fluid and embryos of goats by virus isolation in cell culture and PCR nested. *Theriogenology* 2002;57:567.
- [59] Ali Al Almad MZ, Fieni F, Guiguen F, Larrat M, Pellerin JL, Roux C, et al. Culture early goat embryos and cells are susceptible to infection with caprine encephalitis virus. *Virology* 2006;353:307–15.
- [60] Fieni F, Rowe J, Van Hoosear K, Buruca C, Oppenheim S, Anderson G, et al. Presence of caprine arthritis encephalitis virus (CAEV) proviral DNA in genital tract tissues of superovulated dairy goat does. *Theriogenology* 2003;59:1515–23.
- [61] Cavalcante FRA, Andrioli A, Pinheiro RR, Souza KC, Veras AKA, Lopes TA, et al. Detecção do vírus da artrite encefalite caprina por nested PCR e nested RT-PCR em ovócitos e fluido uterino. *Arq Inst Biol* 2013;80:381–6.