# An investigation into the practicality of performing transcervical embryo collection from dairy goats under commercial conditions in British Columbia, Canada

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# Transcervical embryo recovery – Dairy goats 2013

**Abstract**

**An investigation into the practicality of performing non-surgical or transcervical embryo recovery from superovulated dairy goats in a commercial setting was undertaken during the fall and winter months of 2012-2013. The donor goats were multiparous and considered to be free of reproductive disorders at the beginning of the study but otherwise were not subject to specific selection criteria. Superovulation was performed using a decreasing dose of a proprietary preparation of pFSH administered every 12 hours over 4 days during treatment with CIDR-G devices. Prospective donors were mated while in estrus to bucks of proven fertility and underwent embryo recovery procedures 8 days after withdrawal of CIDR-G devices and 24h after administration of 100ug cloprostenol. Does in which transcervical embryo recovery could not be achieved underwent embryo recovery by laparotomy. Laparoscopy was performed to determine ovulation rates. Transcervical passage of an embryo collection catheter was possible in 17 of 28 (61%) attempts. Numerically (but not statistically significant) more embryos and ova (12, SD=6.32) were recovered from surgical than transcervical (5.94, SD=6.43) embryo collections. There was no significant difference between the number of IETS grade 1 and 2 embryos recovered surgically (6.67, SD=7.94) or transcervically (3.47, SD=5.79). Seven of 17 (41%) donors flushed transcervically yielded no grade 1 and 2 embryos. Five of 10 (50%) donors from which viable embryos were recovered yielded one to three grade 1 and 2 embryos. One doe responded with 23 grade 1 and 2 embryos. The response to superovulation (measured by CL counts) tended to be greater in April than in January or November although the efficiency of embryo recovery (total embryos and ova relative to CL count) was consistent through the trial period. Efficiency of this procedure could be enhanced by identifying females which have failed to respond to superovulation and not submitting them for embryo collection.**

## Introduction

Embryo transfer (ET) is a recognised and widely accepted reproductive modality in food-animal production. ET allows an increase in the contributions made by genetically superior females to breeding programs. Specific and documented procedures in the handling of embryos recovered from animals infected with chronic viral and bacterial infections allow those embryos to be transferred to healthy recipients with virtually no risk of disease transmission (1). Recovered embryos may be cryo-preserved for transfer at a future time or different location. The sale of embryos obtained from valuable donors represents an income stream for seed-stock producers, while allowing breeders to retain ownership of their valuable donors.

The size of small ruminants, and specific features of their reproductive tracts, has limited the routine commercial application of ET in goats to surgical embryo collection. Although surgical ET is successful when performed by skilled and experienced practitioners, several factors have discouraged its widespread adoption, especially in dairy goats. Surgical ET implies risks attendant with anesthesia and surgery and requires specific equipment, training and expertise. Repeated surgeries, and handling of the delicate tissues of the reproductive tract, can result in scar tissue which has a deleterious effect on future fertility. Many dairy goat breeders have not taken advantage of the benefits of ET due to their perception of these risks.

# Transcervical embryo recovery – Dairy goats 2013

Transcervical embryo recovery offers an alternative to conventional surgical embryo recovery. It should be repeatable several times during the breeding season, may be performed with only light (if any) sedation, is minimally invasive and humane and avoids the potential complications associated with anesthesia and surgery. It should confer upon producers many of the benefits anticipated with routine surgical embryo recovery with minimal risk.
Transcervical embryo collection under laboratory conditions is documented using super-ovulated Boer (2,3) and dairy goats (4,5) but clinical trials under commercial situations are not reported. Transcervical embryo collection from superovulated goats presents challenges even under laboratory conditions. Early reports (2) indicated the procedure was time consuming and therefore seemed impractical under commercial or field conditions. Another small trial, conducted with Saanen does in Brazil in which 35% of the donors could not be flushed transcervically, seemed to limit acceptance of the procedure due to technical limitations of introducing the flush catheter through the cervical canal (5).

Drawing upon experience gained through surgical ET, and the literature on transcervical ET, this investigation reports upon the results of a field trial of transcervical embryo recovery from dairy goats of multiple breeds performed in the fall of 2012 and winter of 2013, under conditions typical of goat production in British Columbia, Canada.

## Materials and methods:

### *Donor animals*

Twelve mature does of the Lamancha, Saanen and Nubian dairy breeds, four year to eight years of age and weighing 52-65kg, were used as prospective embryo donors. The first group was flushed in November 2012 (first collection), the second group in January 2013 (second collection), and the third group in April 2013 (third collection). The does were managed under conditions typical of commercial goat production in British Columbia. The prospective donors were not lactating, were free from signs of infectious or contagious disease, were test negative (c-ELISA) for antibodies to caprine arthritis and encephalitis (CAE) virus and had been treated with a systemic parasiticide prior to the beginning of the study. Each prospective donor had a recent history of delivering normal kids and no recorded dystocia.

### *Superovulation*

The does were placed in groups of five, without regard for age or breed, and programmed so that two groups of five does underwent embryo collection within 10 days. Each donor was super-ovulated by intramuscular (i.m.) injection of a declining dose of pFSH (Folltropin, Bioniche Animal Health Canada Inc, Belleville, ON) every 12h beginning on day 10 of treatment with an intra-vaginal progesterone releasing device (CIDR-G, Pfizer Animal Health, Kirkland QC). Cloprostenol (100ug) (Estrumate, Intervet Canada Corp., Canada) was given by i.m. injection 24h prior to the removal of the CIDR-g device on day 13. Each doe was bred to a known buck of proven fertility every 12 h while she was in standing estrus. Donors were administered 100ug cloprostenol 24h prior to non-surgical embryo recovery which was performed 8 days after CIDR-G withdrawal.

# Transcervical embryo recovery – Dairy goats 2013

*Embryo recovery*

All embryo recovery procedures were performed under general anesthesia induced with xylazine 0.06mg/kg i.v. (Xylamax, Bimeda-MTC Animal Health, Cambridge ON) and ketamine 2mg/kg i.v. (Vetalar, Bioniche Animal Health Canada Inc. Belleville ON) and maintained with isoflurane (2-3%) (PPC of Canada inc., Richmond Hill, ON) delivered in oxygen. Does were placed in sternal recumbency on a laparoscopy table so that the hind end could be elevated. The perineum was prepared routinely with dry and 70% isopropyl alcohol-soaked gauze. A clean, lubricated, duck billed speculum (Cuscoe speculum, Vet-Surge Instruments, Maple Ridge, BC) was inserted into the vagina. The vagina was illuminated with a headlamp worn by the operator to visualise the dorsal rim of the external cervical os which was grasped with Aesculap (Bernhard BF 426, Tuttlingen, Germany) pointed tip forceps. The cervix was drawn caudally towards the vulva and immobilized.

A 55cm. silicone balloon tipped foley catheter (12 French) was stiffened with a stainless steel stylet. With the cervix stabilised by the grasping forceps, the catheter was gently manipulated through the cervix with a simultaneous gentle probing and twisting motion. Once the catheter tip was advanced through the cervix, the stylet was withdrawn and the balloon inflated with 3-6mL of air to secure the catheter rostral to the cranial cervical os. Aliquots of 20mL of proprietary embryo transfer flush medium (emP3, Partnar Animal Health, London, ON) were alternately introduced into, and withdrawn from, the uterus in 60mL catheter tipped syringes (Monoject, Tyco Healthcare Group LP, Mansfield, MA) which had been cleansed of lubricant and sterilised. The recovered fluid was collected in a search dish with a built in 64 um filter (EZ Way Filter, PETS, Canton, TX). This process was repeated until 500mL of flush medium had been introduced to the uterus. The volume of the fluid recovered was measured in a graduated beaker and recorded. The fluid retained in the search dish was examined for the presence of ova and embryos under magnification of 20-70x (Bausch and Lomb Stereo 7 Powerpod). Embryos were staged and graded according to the guidelines of the International Transfer Society (IETS).

The procedure was repeated with an additional 500mL of flush medium. The volume of flush medium and the number of ova and embryos recovered were recorded independently of the first 500mL.

Following transcervical flushing procedures, the anesthetised does were placed in dorsal recumbency and the ventral abdomen routinely and aseptically prepared for laparoscopic visualisation of the ovaries. The number and type of ovarian structures were recorded for each ovary.

*Surgical embryo recovery*

In cases where transcervical penetration was not possible, and the anesthetic condition of the donor was stable, the procedure was converted to a surgical embryo recovery. Following routine aseptic preparation of the ventral abdominal wall, a mid-line incision was made to exteriorise the uterine horns. Flush medium in two 20mL aliquots was instilled at the utero-tubal junction under gentle pressure to direct fluid and embryos to the uterine horn . Embryo flush medium flowed through a cuffed 55cm silicone foley catheter occluding the middle one third of the uterine horn and into a combination search dish and embryo filter. The procedures were repeated for the contra-lateral uterine horn. Closure of the incisions was routine. In the absence of cervico-vaginal adhesions, the donor was returned to her group for processing for non-surgical embryo recovery at a later date.

# Transcervical embryo recovery – Dairy goats 2013

All donors were administered a post-operative analgesic (Flunazine 1mg/kg bw, Bimeda-MTC Animal Health Inc., Cambridge ON) by i.m. Injection and long acting penicillin (Duplocillin 5mL/45 kg b.w., Intervet Canada Ltd., Whitby, ON) by s.c. injection and monitored for signs of lethargy, inappetance and pain for seven days post collection.

## Results

Transcervical passage of a flush catheter was possible in 17 of 28 donor attempts (61%). Six donors were attempted 3 times, 4 donors were attempted twice, and two donors attempted once each (Table 1). One donor had extensive adhesions of her cervix to the mucosal lining of her vagina which precluded visualisation and non-surgical catheterisation which resulted in her withdrawal from the program. One donor was programmed three times without successful inroduction of a transcervical catheter on any attempt.

One hundred and one total ova and embryos (TOE) were recovered through 17 transcervical embryo recoveries. Six donors whose cervix could not be penetrated underwent surgical embryo collection and 72 TOE were recovered.

**Table 1. Relative difficulty in passing a transcervical catheter by collection attempt (1 = very easy to 5 = not possible).**

| Donor | 1st collection | 2nd collection | 3rd collection |
|---|---|---|---|
| Karisma | 2 | 1 | 1 |
| Windsong | 2 | 4 | WD |
| Wanda | 3 | 3 | 2 |
| Extreme | 5 | WD | WD |
| Wanita | 5 | 5 | 5 |
| Wesla | NE | 3 | 4 |
| Victoria | 5 | 3 | WD |
| Tiara | 5 | 3 | 3 |
| Dotty | 2 | 3 | 3 |
| Wind | 5 | 3 | 5 |
| Brown Sugar | 5 | 4 | WD |
| Vanquish | NE | NE | 5 |
| **Median value** | **5** | **3** | **3.5** |
| **No. attempted** | **10** | **10** | **8** |
| **No.passed** | **4** | **9** | **5** |
| **Fraction entered** | **0.4** | **0.9** | **0.63** |

NE – New entrant to study
WD – Withdrawn from study.

The mean TOE recovered transcervically (X=5.94, SD=6.43) did not differ significantly from the mean TOE recovered surgically (X =12, SD=6.32). There was no difference between the number of transferable embryos (IETS grade 1 and 2) recovered non-surgically (X=3.47, SD=5.79) or surgically (X=6.67, SD=7.94) (Figure 1).

Four donors which could not be flushed transcervically at the first collection attempt were successfully flushed transcervically and produced viable embryos at subsequent collection attempts in January and April. The donor Wesla entered the study to participate in the second (January) embryo recovery program but had a profuse hemorrhagic and purulent discharge through the cervix and was not flushed. She was treated and became the most prolific embryo producer of the entire non-surgical embryo recovery program when flushed in April. Where donors had an obvious medical concern (vaginal adhesions, respiratory disease) that precluded non-surgical embryo collection, they were removed from the study.

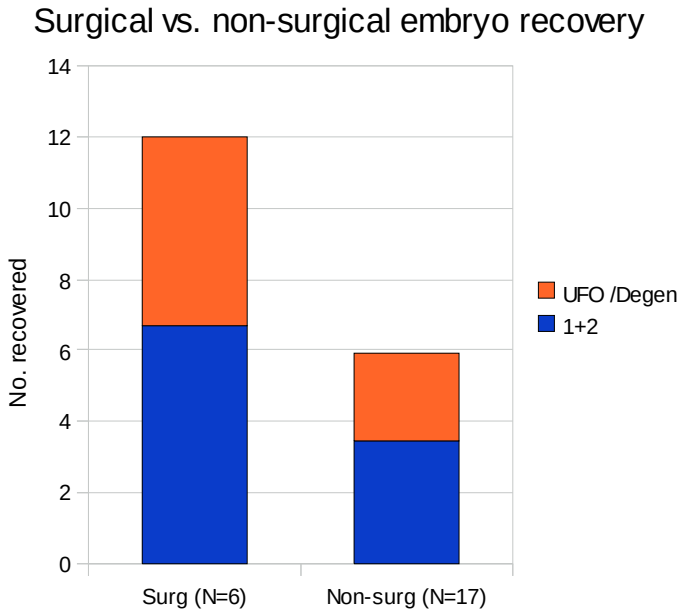


Figure 1. Pooled data showing the mean numbers of unfertilised ova and degenerated embryos and IETS grade 1+2 embryos collected by surgical and non-surgical embryo recovery from super-ovulated dairy goats from November 2012 to April 2013.

The proportion of IETS grade 1 and 2 embryos relative to the TOE recovered was not different for non-surgical or surgical embryo recovery (Figure 2).

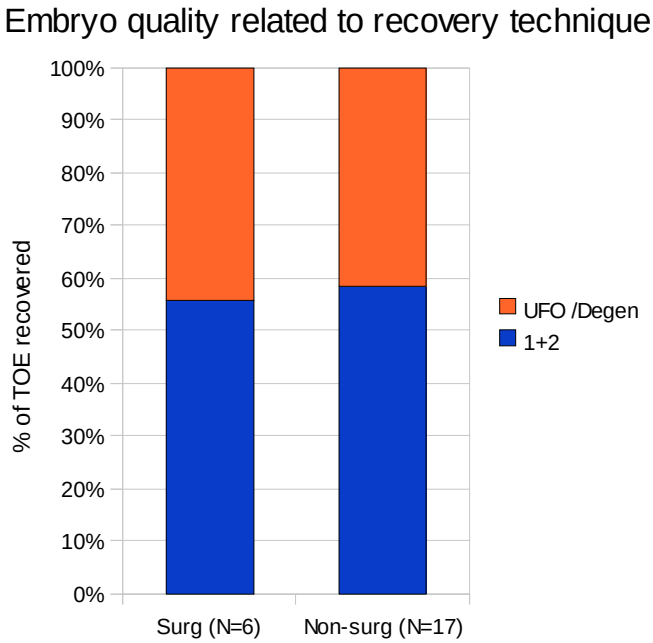


Figure 2. Pooled data from surgical and non-surgical embryo recoveries from super-ovulated dairy goats indicating the relative proportions of embryo grade quality.

The TOE recovered transcervically did not differ significantly between the first (November) and second (January) attempts (p=0.599), but tended towards a significant difference (p=0.0795) when the second and third (April) attempts were compared (Table 2). The number of corpora lutea (CL) observed at laparoscopy of the donors at the time of the third collection (X=16.8, SD=10.18) was significantly greater (p=0.0386) than the number of CLs observed at the time of the first (X=7, SD=6.22) and second (x=7, SD=3.65) collections. The efficiency of transcervical embryo recovery (the ratio of TOE recovered relative to the number of Cls counted at laparoscopy) remained consistent throughout the study (Table 2).

Table 2 . Efficiency of non-surgical embryo recovery based on the ratio of total ova and embryos (TOE) and the number of corpora lutea (CL) observed at laparoscopy

| | 1st collection | | 2nd collection | | 3rd collection | | Pooled | |
|---|---|---|---|---|---|---|---|---|
| | TOE | CL | TOE | CL | TOE | CL | TOE | CL |
| **Totals** | 19 | 28 | 29 | 49 | 53 | 84 | 101 | 161 |
| **Donors** | 4 | 4 | 7 | 7 | 5 | 5 | 17 | 17 |
| **Mean** | **4.75** | **7** | **4.14** | **7** | **10.6** | **16.8** | **5.94** | |
| **SD** | 4.99 | 6.22 | 2.38A | 3.65B | 9.86A | 10.18B | | |
| **Efficiency (TOE/CL)** | **0.68** | | **0.59** | | **0.63** | | **0.63** | |

A and A – differences are significant at 90%
B and B – differences are significant at 95%

Fifteen of 17 transcervical flushes yielded at least one ova or embryo, although 7 of those produced only unfertilised ova or degenerated embryos and no grade 1 or 2 embryos. Fifty percent of the donors that produced viable embryos yielded between one and three grade 1 and 2 embryos. One outlier produced 23 viable embryos; another produced 19 TOE of which 9 were grades 1 and 2.

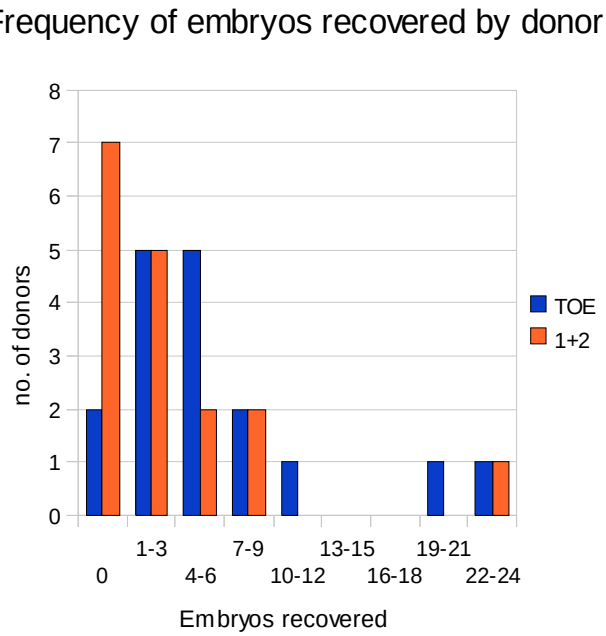


Figure 3. Frequency distribution of TOE (total embryos and ova) and IETS quality grade 1 and 2 embryos by the number of donors flushed transcervically.

Embryos and ova were found distributed in approximately equal numbers in the first and second 500mL volumes of embryo flush medium (Figure 4).

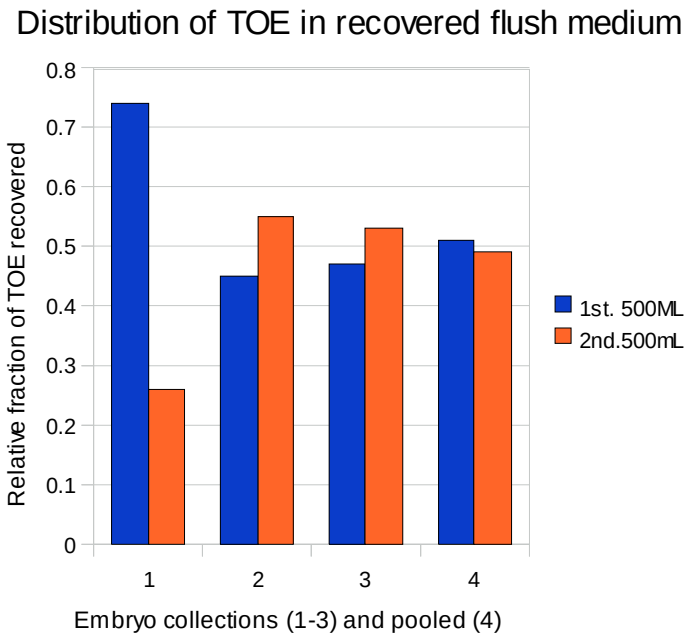


Figure 4. The proportion of the TOE recovered in the first and second 500mL volumes of flush medium used for transcervical embryo recovery.

**Discussion**

This trial demonstrated the practicality of commercialising non-surgical or transcervical embryo recovery in dairy goats from November to April, in British Columbia. Due to concerns for the appropriate restraint of the donor animals during the technical aspects of the procedure, and to minimise the potential for pain, discomfort, or distress arising from this trial, embryo collections were performed with the donors under general anesthesia. Other researchers have devised hammock-like devices through which the donor animals legs are placed (3) or administered epidural analgesia (5). Placing the donors under general anesthesia in this study facilitated converting the procedure to a surgical embryo recovery if necessary, or to proceed to laparoscopy for an assessment of ovarian response.

Transcervical embryo recovery in dairy goats is an alternative to the more typical surgical approach. It may offer several potential advantages including: minimising operative or post-operative pain, anesthetic risk, operative and post-operative surgical complications including infection or adhesions, and, in some cases, compromised reproductive health.

# Transcervical embryo recovery – Dairy goats 2013

In more than 60% of super-ovulated non-lactating dairy goats, it was possible to place a transcervical catheter and recover embryos or ova. Although higher rates of cervical penetration are reported with Boer goats (2), our results are consistent with work from Brazil in Saanen dairy goats (5). This may reflect breed specific anatomic differences between Boer and dairy goats, or it may reflect increased operator experience gained by flushing a larger number of Boer goats.

Although a numerically greater TOE (12) and grade 1 and 2 (6.67) embryos were recovered surgically, these numbers were not statistically different from the TOE (5.94) and grade 1 and 2 embryos (3.47) recovered non-surgically. This is likely attributable to the relatively small sample sizes in this trial.

Suyadi et al. (3) recovered 8.4 embryos per donor from 9 Boer does by transcervical flushing while Periera et al. (1998) recovered 11.7 embryos per donor from 7 Boer does. Lima-Verde et al. (5) recovered 6.3 embryos per Saanen donor, comparable to 5.94 ova and embryos recovered from dairy goats in this trial. These Brazilian workers reported that 80% of the embryos they recovered transcervically were grades 1 and 2. A lower proportion (58%) of the embryos recovered transcervically in this trial were graded 1 and 2, but this is consistent with other studies (2) where 55-70% of transcervically recovered embryos were classified as transferable quality.

The number and quality of embryos recovered per donor may reflect the nutritional status of the donors, effects arising from the seasonally polyestrus reproductive cycle of goats, breed and individual variation in response to superovulatory treatments, or variation in technique and interpretation of embryo grading criteria. The contribution of each of these factors is difficult to interpret, given the geographically diverse locales involved, the differences in breeds, and the relatively small numbers of subject animals involved (2-5).

Transcervical embryo collection had no effect on the proportion of grade 1 and 2 embryos recovered relative to the TOE. Surgical (56% grade 1 and 2 embryos) and non-surgical recovery (58% grade 1 and 2) yielded similar proportions of transferable embryos, consistent with other reports (5)

Embryo transfer efficiency implies that embryos present in the reproductive tract of the donor are removed at the time of embryo recovery. Ideally, all follicles recruited through superovulation ovulate with synchrony (or at least within a within a few hours) and multiple ova are then available for fertilisation. The ovulation sites should undergo luteinisation to form corpora lutea, many of which are visible on the surface of the ovary. The TOE anticipated at the time of embryo recovery may be estimated by counting CIs, either by ultrasound or by visualising the ovaries laparoscopically.

Following transcervical embryo recovery procedures, and while still anesthetised, donors underwent laparoscopic examination of their ovaries. One study (5) performed laparoscopy 24h prior to attempting transcervical embryo recovery and eliminated donors from the trial if fewer than 5 ovulation sites or CIs were counted. In doing so, they would have biased their outcome to increasing TOE, and probably the number of grade 1 and 2 embryos, per donor. Prospective donors with below average or modest responses to superovulation, and therefore fewer TOE, were removed from the trial and did not contribute to the data set.

# Transcervical embryo recovery – Dairy goats 2013

This trial was intended to simulate embryo recovery programs conducted under commercial conditions and performed 'on-farm'. Typically, in such a situation, the response to superovulation would not be assessed 24h in advance of an embryo recovery procedure. As a result, some donors that failed to respond (or responded modestly) to superovulatory treatment in this trial were subjected to transcervical embryo recovery procedures. Of 17 donors that were subjected to transcervical embryo recovery, four (24%) were found at laparoscopy to have fewer than four CLs. Those does contributed to lowering the mean TOE and grade 1 and 2 embryos recovered.

This finding suggests there is merit in identifying and applying a practical, non-invasive means of classifying donors which have failed to respond to super-ovulation, prior to investing time and resources in attempting to flush them, either transcervically or surgically.

Despite not removing from the study donors which failed to respond to superovulatory treatment, the TOE recovered (5.94) and the overall recovery efficiency (TOE / Total CL count) of 0.63 compared favourably with results where such pre-selection had occurred (5).

Interestingly, donors given superovulatory treatment responded with a statistically significant ($p<0.05$) greater number of CLs in April, which is generally considered outside the normal breeding season for goats at this latitude, than they did in the preceding months of either January or November. This contrasts with findings that more transferable embryos were recovered from transgenic dairy goats in the months September to December than January to May (4).

Without knowing the extent to which prospective donors have responded to superovulatory treatment it is not possible to predict if one has recovered some, most or all of the available embryos, how long to continue flushing or the total volume of fluid that should be instilled or recovered. Earlier workers (2) intermittently infused 20mL aliquots of flush medium, 12 times over approximately 45 minutes. They allowed a 2h pause and repeated the process, resulting in a transcervical flushing procedure almost 4h in duration. Although apparently effective, this was considered impractical under commercial conditions where a maximum of 60 minutes was deemed appropriate. Lima-Verde et al (5) instilled and removed 20mL volumes of flush medium by syringe, and repeated the process 25 times to flush Saanen does transcervically. As the commercially prepared flush medium used in this trial is prepared in 1000mL bags, it was convenient to use 20mL aliquots of flush fluid to a total of 500mL, pause to search for embryos and repeated the process with a second 500mL. To allow for possible uterine distension proposed elewhere (2,3,5), we increased the aliquots to 25-30mL for the second 500mL. While some embryos were consistently located in the first 500mL of fluid, an almost equal number was located in the second 500mL (Figure 4). Based on our observations, an interval of 2h between flush attempts is not indicated, nor practical, although the use of 1000mL of flush medium is recommended. While additional flush (e.g. more than 1000mL) medium could be used, this is probably not necessary given that the efficiency of recovery reported here is consistent with that of other studies (2,5).

**Conclusions**

This limited trial demonstrated that transcervical embryo recovery is possible under farm conditions in British Columbia. Dairy goats responded to superovulatory treatments administered from October to April. There is possibly a reduction in the number of total embryos and ova that may be recovered, relative to surgical techniques, but the relative proportion of grade 1 and 2 embryos is unaltered. Fifty percent of dairy goat donors in this trial from which embryos were recovered transcervically yielded one to three grade 1 and 2 embryos although a recovery of 23 grade 1 and 2 embryos occurred.

Transcervical embryo recovery is repeatable. It may be conducted on dairy goats that were either successfully flushed transcervically or not penetrable on previous collection attempts. It can be effective on dairy goats that have previously undergone surgical embryo recovery. In situations where transcervical embryo recovery is not possible, donors may be flushed surgically to prevent the loss of embryos that otherwise could not be recovered.

This study suggests that with the flushing technique described, approximately 1000mL of flush medium may be necessary to recover most embryos.

Failure to respond to superovulation can reduce the overall success of programs if those non-responding animals are not identified prior to attempting embryo recovery. Investigating a non-invasive means, for example ultrasonographic assessment of the ovaries, to identify non-responding donors is warranted.

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Transcervical embryo recovery – Dairy goats 2013

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