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Transcervical collection of bovine embryos up to Day 21: An 8-year overview

C. Richard [a,b,*], I. Hue [a,b], V. Gelin [c], A. Neveux [c], E. Campion [a], S.A. Degrelle [a,d,e], Y. Heyman [a], P. Chavatte-Palmer [a,b]

[a] INRA, UMR 1198 Biologie du Développement et Reproduction, Jouy-en-Josas, France
[b] ENVA, UMR 1198 Biologie du Développement et Reproduction, Maisons-Alfort, France
[c] INRA, UCEA Bressonvilliers, Leudeville, France
[d] INSERM, UMR-S1139767, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, France
[e] Université Paris Descartes, Sorbonne Paris Cité, Paris, France

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ABSTRACT

Transcervical embryo collection is used routinely in the bovine species throughout the world to collect Day 6 to Day 9 embryos (early embryos) for genetic selection. For research purposes, however, the collection of embryos at later stages of pregnancy, i.e., Days 12 to 21 (late embryos), is needed. So far, for the recovery of late embryos, females are euthanized and embryo collection is performed after recovery of the genital tract. To reduce the number of animals used and still provide valuable material for embryo research, we have therefore developed a transcervical technique to collect late embryos. The objective of this study was to compare embryo recovery results at early and late stages within our laboratory. Altogether, 232 cows were used for this study. One hundred forty-five flushes were performed to collect embryos from Days 6 to 9, and 251 flushes were performed to collect embryos from Days 12 to 21. For the early embryos, a classical three-way collection equipment was used. To collect the late embryos, the same equipment was used, but the extensible flexible catheter that goes inside the external rigid catheter was removed, so that larger embryos could be collected through the remaining larger hole (two-way collection). All females were submitted to ovum pick up to remove the dominant follicle and were subsequently superovulated with FSH. Luteolysis was induced 48 hours before artificial insemination. Two artificial inseminations were performed with frozen semen, 48 and 56 hours after PGF2α injection. Before embryo collection, cows were treated with an epidural injection of a local anesthetic drug. The presence of CL was checked, and they were counted by rectal palpation. For all collections, the cervix was prepared with the initial introduction of a dilator. Then, the catheter was introduced in one horn, and the cuff was inflated as low as possible. For the collection of late embryos, the flushing solution (30 mL) was injected slowly twice to suspend the embryos before flushing the horn with 500 mL, and the same operation was performed on the second horn. There was no significant difference in the number of embryos collected per flush in the early- and late-stage (758 embryos collected, 5.22 ± 6.02 per flush vs. 1238 embryos collected, 4.93 ± 5.07 per flush, respectively). The number of embryos collected per CL, however, was significantly lower in the early versus late group (0.39 ± 0.32% vs. 0.44 ± 0.34%, respectively). The late collection allowed the retrieval of full conceptuses (embryonic and extraembryonic tissues), even at very late stages such as Days 18 to 21. Careful collection is needed, however, so that conceptuses are not damaged or torn: the horn must be

* Corresponding author. Tel.: +33 1 34 65 24 19; fax: +33 1 34 65 23 64.
E-mail address: christophe.richard@jouy.inra.fr (C. Richard).

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massaged gently and the flush should be ideally recovered in one single flow. This technique is a powerful tool to collect the late-stage embryos for research purposes. Because it is not traumatic, animals can be used again for the same procedure.

1. Introduction

Fertility has decreased over the past 50 years in high-yielding dairy cows, but this decreased fertility has not been markedly associated with neither decreased embryo yield nor quality in embryo transfer (ET) practice [1,2]. Because high fertilization rates were maintained in the last 20 years, the early embryo loss is considered the primary cause of infertility in dairy cattle. The slaughter of cows at known times after artificial insemination (AI) and the recovery of oviductal/uterine contents and nonsurgical and surgical uterine flushes have been used to explore the timing and causes of embryo losses. Most embryo losses seem to occur between 8 and 18 days [3–6]. Most works, however, have focused on embryo losses before Days 7 to 8 because the collection of embryos at later stages of pregnancy can not, so far, be performed nonsurgically and requires either the slaughter of animals or the use of surgical approaches.

The industry of ET in cattle is developing steadily throughout the world with more than 1 million embryos collected *in vivo* or produced by IVF since 2006 [7]. Nonsurgical embryo collection and transfer of Day-6 to -7 blastocysts (before hatching) was developed in the mid to late 1970s [8–10] to quickly become the technique used worldwide, enabling the widespread development of ET. Multiple ovulation and embryo transfer and IVF have been widely implemented in breeding schemes, specifically to target the exploitation of dam genetics. The difficulties initially encountered for the reliable induction of superovulation in donor animals have been overcome through the use of purified FSH and LH hormonal treatments and the manipulation of follicular waves by steroid treatments or follicular ablation [11].

The industry of IVF embryos is also rapidly increasing [7]. *In vitro* production (IVP) of embryos in ruminants has been initially associated with the occurrence of the large offspring syndrome at later stages of pregnancy [12–14], and transcriptomic signatures were shown to be different between Day-7 blastocysts produced by AI or IVF with or without *in vitro* maturation [15]. With the use of defined culture media, the occurrence of large offspring syndrome has been reduced but abnormalities related to IVP embryo development remain to be studied [16–18].

Finally, whether collected *in vivo* or produced *in vitro*, many embryos are frozen, sometimes after a biopsy has been performed, for transplantation into a recipient animal at a later stage. Indeed, the use of frozen embryos is increasing steadily with more than 58% of *in vivo* produced and near 10% of *in vitro*–produced embryos being transferred after having been stored frozen [7].

Thus, there is a need for the collection of bovine embryos for the fundamental study of embryonic development and for more applied research on the effects of environment or reproductive biotechnologies on post-hatching embryo development. The cost of collecting embryos at slaughter is prohibitive for most research groups. Moreover, because of ethical considerations, the use of surgical techniques to collect the late-stage embryos should be avoided. The aim of this study was to develop a nonsurgical method to collect embryos up to Day 21 of pregnancy, evaluate the quality of the collected embryos, and compare embryo yields at different stages of pregnancy.

2. Materials and methods

2.1. Animals

Animals used are described in Table 1. Briefly, 233 animals were used with a body condition score ranging from 3 to 4 on a 1 to 5 scale [19,20]. Breeds included Holstein (60%), Normande (26%), Holstein crossbred (8%), and Charolais (6%). There were 144 heifers (age, 2.97 ± 0.75 years), which were used for 280 embryo collections (mean, 2.00 ± 0.13 collection per heifer) and 89 cows totaling 116 embryo collections (mean, 1.30 ± 0.06 collection per cow; age, 5.35 ± 1.62 years, parity, 2.72 ± 1.72). The animals were randomly assigned to early or late embryo collection. The animals were housed in the same building and fed the same diet (grass silage, hay, straw, concentrates). Estrus was regularly detected and recorded five times daily by experienced herdsmen. All animals used were cycling before to start of the protocol.

Table 1
Number of embryo collections, corpora lutea, collected embryos and mean recovery rate for early and late collections.

	Early embryos stage Days 6 to 9; three-way collection equipment	Late embryos stage Days 12 to 21; two-way collection equipment	Total
Number of collections	145	251	396
Number of CL	1923	2796	4719
Mean ± SE	13.26 ± 7.11	11.14 ± 6.36	11.92 ± 6.71
Number of embryos	758	1238	1996
Mean ± SE	5.22 ± 6.02	4.93 ± 5.07	5.04 ± 5.43
Recovery rate (number of embryos/number of CL)	0.39 ± 0.32	0.44 ± 0.34	0.42 ± 0.43

Abbreviation: SE, standard error.