The use of prostaglandins and otxytocin for transcervical recovery of bovine fetuses at days 33–58 of gestation

M-C. Lavoir and K. J. Betteridge

Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

The study of bovine fetal germ cells of known developmental stage calls for alternatives to the recovery of fetuses by surgery or slaughter. Fetuses were therefore obtained during the second month of pregnancy by aborting 49 animals using a progressively modified treatment regimen of cloprostenol, prostaglandin E2 (PGE2) and oxytocin. The viability of fetuses was monitored by ultrasonography throughout treatment. Intracervical treatment with PGE2 led to cervical dilation in all treated animals. However, retrieval of the fetuses by subsequent flushing of the uterus was successful in only two of six animals. When i.m. injections of cloprostenol were given 20–40 h before PGE2 treatment, fetuses ≤ 40 days of gestation were expelled spontaneously, while the majority of fetuses ≥ 50 days of gestation were retained. When i.m. injections of oxytocin were given in relation to clinical signs of impending fetal expulsion after cloprostenol and PGE2 treatment, 20 of 22 fetuses were expelled 42–53 h after the cloprostenol injection. Of these 20 fetuses, 19 were expelled 0–7 h after the cessation of fetal heartbeat. The subsequent fertility of animals was not affected. Thus, the final protocol allowed bovine fetuses to be retrieved at predictable times, within a few hours of death, with little maternal trauma and without affecting subsequent fertility.

Introduction

A reliable method for recovering bovine fetuses of known ages without recourse to surgery or slaughter would have numerous experimental applications, including the study of bovine fetal germ cells (Lavoir et al., 1994). Although pregnant cows will abort their fetuses after luteolysis induced by administration of prostaglandin F2alpha (PGF2alpha) or its analogues, the intervals between treatment and fetal expulsion, as well as between fetal death and expulsion, are unpredictable and often unacceptably long (Lindell et al., 1981; Kastelic and Ginther, 1989; Lavoir and Betteridge, 1992). One approach to reducing these intervals and their variability is to induce cervical dilation in addition to luteolysis.

Prostaglandin E2 (PGE2) is known to affect the extensibility of the cervix (Conrad and Ueland, 1976, 1979; Hollingsworth et al., 1980; Ledger et al., 1983; Owiny and Fitzpatrick, 1990), and introduction of PGE2 into the vagina or cervix of women, ewes, mares and cows leads to a softening and dilation of the cervical tissue in vivo that is independent of pregnancy or the stage of pregnancy (Stys et al., 1981; Keirse, 1990; Volkman and De Cramer, 1991; Duchens et al., 1993). Here, we report the development of a reproducible method of treating cows with the PGF2alpha analogue, cloprostenol, PGE2 and oxytocin, to retrieve fetuses through the vagina within 7 h of the cessation of fetal heartbeat during the second month of pregnancy.

Materials and Methods

Animals

Canadian Holstein cattle were impregnated either by artificial insemination or by embryo transfer. They were used between days 31 and day 60 of pregnancy (oestrus = day 0). A total of 49 pregnant animals (48 heifers and one cow) were used in groups of various sizes on 15 occasions. Eight of the animals were used twice.

Ultrasoundography

Fetal heartbeat, fetal expulsion and maternal ovulation were monitored using a real-time B-mode ultrasound scanner (Tokyo Keiki LS-300, Sterne Equipment Company Ltd, Brampton, Ontario) equipped with a 5 MHz linear array transducer. Fetal death was assumed to have occurred between the last examination at which a heartbeat was detected, and the first examination at which it was absent. Thus the intervals between those examinations and the time at which the fetus was retrieved define the maximum and minimum death–expulsion intervals.

A fetus was considered retained if, after six treatments with PGE2 (see below), the fetus had been dead for at least 6 h and remained lodged anterior to the cervix, or if the fetus was dead and not expelled after seven treatments.

Drug treatment

Luteolysis was induced by i.m. injection of 500 pg cloprostenol (Estrumate, Coopers-Agropharm, Inc., Ajax, Ontario).
Prostaglandin E\textsubscript{2}, a gift from the Upjohn Company (Kalamazoo, MI), was provided in two different formulations: as suppositories, each containing 20 mg Prostin E\textsubscript{2} (dinoprostetone), and in powder form. Immediately before treatment, a suppository was partially thawed and pressed into a plastic AI sheath, from which it was expelled into the cervix by a silette. The PGE\textsubscript{2} powder was dissolved in absolute ethanol (100 mg in 80 ml) 1–2 days before treatment. This solution was mixed with KY lubricant (Johnson and Johnson Medical, Montreal) to provide a final PGE\textsubscript{2} concentration of 40 mg ml\textsuperscript{-1} for filling 0.5 ml AI straws (IMV, l'Aigle), which were frozen at −20°C until use. The gel was introduced into the cervix from the thawed straw using a standard AI gun. For control treatments, AI straws were filled with 0.5 ml KY jelly only.

Oxytocin (Rogar/STB Inc., London, Ontario) was administered as an i.m. injection of 100 IU in relation to clinical signs of impending fetal expulsion after PGE\textsubscript{2} treatment (see below).

**Blood samples and progesterone assay**

Luteolysis was evaluated by withdrawing blood from the coccygeal vein or artery into heparinized blood collection tubes (10 ml; Sherwood Medical, St Louis, MO) immediately before cloprostenol treatment and at the start of the PGE\textsubscript{2} treatment. Samples were immediately centrifuged (at 1800 g for 10 min) and the plasma stored at −20°C until assayed for progesterone content by radioimmunoassay as described by Walton et al. (1987). The assay sensitivity was 0.125 ng ml\textsuperscript{-1}. Intra- and interassay coefficients of variation were 6% and <10%, respectively.

**Experimental design**

The study comprised a series of four experiments in which the hormone treatments were modified progressively in the light of results obtained previously. Limitations in space and animal availability necessitated the use of several different groups of animals at different times to make up each of the four experiments. These groups within an experiment are referred to as ‘replicates’.

**Experiment 1.** The effect of PGE\textsubscript{2} on cervical dilation was determined. Six animals (five heifers and one cow) were treated every 4 h for 24 h with 20 mg PGE\textsubscript{2} (three with suppositories, three with AI straws), while two control animals were treated with KY jelly. Fetal heartbeat was monitored every 4 h. At 28 h, fetal recovery was attempted by transcervical uterine flushing, using PBS plus 2% (v/v) fetal calf serum, with a catheter (14 mm o.d.) as used for horses (Sirois and Betteridge, 1988). After flushing, all animals were given a luteolytic dose of cloprostenol.

**Experiment 2.** The possibility that fetal recovery by flushing would be more efficient after the initiation of luteolysis was investigated. Six heifers were treated with cloprostenol 20 h before the start of PGE\textsubscript{2} treatments every 4 h, for which AI straws were used. Fetuses were examined by ultrasonography every 2 h, starting 20 h after the cloprostenol injection.

**Experiment 3.** Thirteen heifers were used. The interval between the cloprostenol and PGE\textsubscript{2} treatments was extended to 36 h (four animals) or 40 h (nine animals). In addition, the frequency of the PGE\textsubscript{2} treatments and fetal examination was increased to every 2 h.

**Experiment 4.** Twenty-two heifers were used to evaluate the use of oxytocin to aid the expulsion of fetuses by increasing uterine contractions. In an effort to improve luteolysis, two injections of cloprostenol were given 4 h apart to all but four heifers that received only one injection of cloprostenol 36 h before the first PGE\textsubscript{2} treatment. Oxytocin (100 IU, i.m.) was given when one or more of the following observations were made: (i) strong uterine contractions, palpable per rectum; (ii) protrusion of the fetal membranes from the vulva; (iii) movement of the fetus towards the cervix as detected by ultrasonography.

The animals were examined using ultrasonography before the first cloprostenol and PGE\textsubscript{2} treatments, every hour from 42 h after cloprostenol injection until expulsion of the fetus, and then daily until ovulation was detected.

**Fertility assessment after treatment**

Ten animals of Expts 2 and 3 were checked for behavioural oestrus for 1–2 oestrous cycles after the treatment. Thirteen of the 22 animals used in Expt 4 were inseminated at the oestrus associated with the induced abortion.

**Results**

There was no observable difference in cervical dilation obtained with the two different formulations of PGE\textsubscript{2} in Expt 1. However, inserting the suppository into the cervix was more difficult than treating the animals with a conventional AI gun. Consequently, treatment with the suppository was not continued and AI straws were used. The prostaglone concentration before cloprostenol injection in Expts 1–3 ranged from 3.1 ng ml\textsuperscript{-1} to 11.7 ng ml\textsuperscript{-1} (6.5 ± 2.3 ng ml\textsuperscript{-1}; mean ± sd). Three animals had to be excluded from the trial; two because of rectal trauma and one because of difficulty in penetrating the cervix.

**Experiment 1**

At 28 h after initiation of PGE\textsubscript{2} treatments, the fetuses of both control, and five out of six treated heifers, were still alive. The cervix was not dilated in either control animal and the catheter could not be passed. For this reason, control treatments were not repeated in subsequent experiments. In all the treated animals, the cervix was sufficiently dilated to allow passage of the catheter. However, in only two of the six could the fetuses be flushed out of the uterus (Table 1). This suggested that cervical relaxation in the presence of a functional corpus luteum was unlikely to be adequate for retrieving fetuses. Consequently, the protocol was modified for Expt 2 by inducing luteolysis before PGE\textsubscript{2} treatment.
Table 1. Effects of seven intracervical treatments of pregnant cattle with 20 mg PGE₂ at intervals of 4 h (Expt 1)

<table>
<thead>
<tr>
<th>Replicate number</th>
<th>Day of pregnancy at flushing</th>
<th>Plasma progesterone (ng ml⁻¹) at start of PGE₂ treatment</th>
<th>Treatment</th>
<th>Heartbeat at time of flushing</th>
<th>Cervical dilation at time of flushing</th>
<th>Fetus retrieved</th>
<th>Duration of subsequent oestrous cycle(s) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>3.9</td>
<td>Control</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>23, 21</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>5.6</td>
<td>Control</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>33 (induced)</td>
</tr>
<tr>
<td>1</td>
<td>34</td>
<td>6.5</td>
<td>PGE₂</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>18, 19</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>3.7</td>
<td>PGE₂</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>21</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>4.6</td>
<td>PGE₂</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>25, 19</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>5.2</td>
<td>PGE₂</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>23, 22</td>
</tr>
<tr>
<td>1</td>
<td>43</td>
<td>4.3</td>
<td>PGE₂</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>23, 20</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>3.1</td>
<td>PGE₂</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>17</td>
</tr>
</tbody>
</table>

Fig. 1. Conceptus retrieved at day 54 of pregnancy after treatment of a heifer with cloprostenol and PGE₂ in Expt 2. Crown-rump length = 51 mm; scale bar represents 1 cm.

Experiment 2

In the first two animals treated, the fetuses had disappeared from the uterus within 30 h of the luteolytic cloprostenol injection (after four treatments with PGE₂) but were not found. In the four remaining animals, the fetuses were spontaneously expelled 12–16 h after the start of the PGE₂ treatments (Fig. 1: Table 2).

In four of the six animals the progesterone concentrations exceeded 1 ng ml⁻¹ at the time PGE₂ treatment began (Table 2). It was considered that this persisting concentration of progesterone may have delayed expulsion. In addition, since PGE₂ treatments were initiated at 08:00 h, expulsions occurred during the evening. For these reasons, the treatment protocol was modified in Expt 3 with a view to allowing more time for completion of luteolysis before PGE₂ treatment and to hasten the expulsion of the fetuses so that they could be retrieved during the day.

Experiment 3

In seven of the 11 animals in which luteolysis was achieved, the fetus was recovered after 1–5 treatments with PGE₂ (2–9 h after the start of PGE₂ treatment) and within 5 h of the cessation of heartbeat (Table 3). In the remaining four cases, the fetal membranes were expelled after 4, 3, 6 and 1 PGE₂ treatments, respectively, but the fetus itself was retained. These four fetuses died between 1 h and 9 h after the first PGE₂ treatment and were still detectable by ultrasonography in the uterus until 2 days before (two animals) or 1 day before (two animals) the cloprostenol-induced ovulation, as detected by ultrasound. All four were of more than 50 days gestation when the PGE₂ treatments began.

In two of the 13 animals, luteolysis did not occur. In both of these animals, the fetuses were alive and not expelled by the end of the PGE₂ treatments; expulsion and ovulation occurred only after an additional injection of cloprostenol.

These results, and those of Expt 2, indicated that fetuses of ≤40 days could be retrieved within a reasonable time after death but that older fetuses seemed to require more vigorous uterine contractions to complete their expulsion. For Expt 4, therefore, oxytocin was added to the treatment regimen.

Experiment 4

Luteolysis was achieved in all 22 animals. In 19 of these, the fetus was expelled and retrieved after 1–3 oxytocin injections, within 7 h of cessation of heartbeat (Table 4). In one heifer, the fetus died between 24 h and 40 h after the cloprostenol injection. In two cases, the fetus was retained despite expulsion of the fetal membranes, even after two injections of oxytocin. However, both were presumably expelled, unobserved, overnight because they could not be detected by ultrasound in the uterus or vagina the next day.

Effect of PGE₂ on subsequent reproductive activity

Of the 49 animals used in these experiments, 47 ovulated within 7 days after the PGF₂α treatment, while two animals
developed cystic ovaries. The durations of the oestrous cycle after the PGE\(_2\) treatments varied between 18 days and 25 days (Tables 1 and 2). Of the 13 animals inseminated at the cloprostenol-induced heat in Expt 4, eight were pregnant at 6 weeks, as determined by ultrasound.

\section*{Discussion}

These results show that at between day 33 and day 58 of pregnancy in cows, a luteolytic dose of cloprostenol, followed by intracervical treatments with PGE\(_2\) can induce expulsion of the entire conceptus. The four fetuses that were retained despite such treatment were all of more than 50 days of gestation. However, when oxytocin was given in relation to clinical signs of impending expulsion after PGE\(_2\) treatment, the age of the fetus did not influence expulsion up to day 58 of pregnancy, and all but two of the 22 fetuses were expelled. Fetuses of all ages were often expelled with the amnion intact, while the younger fetuses (<40 days) were regularly retrieved within their complete placental membranes. After expulsion of the fetuses, no retention of fetal membranes could be detected by ultrasonography. It is unclear why fetal retention occurred in two cases in replicate 4 of Expt 4.

Intracervical treatments with PGE\(_2\) dilated the cervix irrespective of the plasma concentration of progesterone. However, PGE\(_2\) did not bring about expulsion of the conceptus when maternal progesterone concentrations were high. These findings are in agreement with the results of Duchens \textit{et al.}
(1993), who did not report any interruption of pregnancy after intracervical PGE\(_2\) treatments, although much lower doses were used. However, in the experiments of Zerobin et al. (1973), the one heifer injected i.v. with PGE\(_2\) at 60 days of gestation did abort 5 days later. Because a luteolytic dose of cloprostenol was given after the PGE\(_2\) treatments in Expt 1 and in two animals of Expt 3, we do not know whether pregnancy would continue if intracervical PGE\(_2\) treatments fail to induce abortion.

In contrast to its relaxing effect on the cervix, the effect of PGE\(_2\) on the uterus was to cause contractions, once the inhibitory influence of progesterone had been removed by luteolysis. In humans, PGE\(_2\) and PGF\(_{2\alpha}\) are known to stimulate uterine contractions at all stages of pregnancy; prostaglandins have been used clinically since 1968 for the induction of labour and, more recently, for termination of pregnancy (Karim et al., 1968; Karim and Filshie, 1970; Roth-Brandel et al., 1970). However, in contrast to the case in humans, progesterone-dominated uterine muscle is refractory to the stimulatory effects of oxytocin and prostaglandins in many species (Csapo, 1956; Lye and Porter, 1978) and, except for one animal in the experiments of Zerobin et al. (1973), we are unaware of reports of using PGE\(_2\) to terminate early pregnancy in species other than humans.

In cattle, when only a luteolytic dose of PGF\(_{2\alpha}\) is given at about day 40, fetuses are expelled, on average, 3.3 days later (Kastelic and Ginther, 1989). The present study showed that intracervical PGE\(_2\) treatments after luteolysis reduces this interval to 42–53 h. It remains to be determined whether this is the optimum protocol to retrieve bovine fetuses in the second month of pregnancy; it is possible that the doses of PGE\(_2\) and oxytocin and their route of administration could be modified to improve the method further. Although PGE\(_2\) was deposited intracervically, some may have leaked into the uterus and influenced uterine contractions locally. Differences in the extent of such leakage may explain the variation between animals. It is possible that intruterine application of PGE\(_2\), perhaps at lower doses, could shorten the death-expulsion interval and reduce its variability.

Reproductive performance was not affected adversely by treatment with PGE\(_2\); a very high proportion of animals ovulated within 2–7 days of the preceding cloprostenol injection, oestrous cycle durations were within the normal range, and high conception rates resulted after insemination at the oestrus associated with the abortion.

This approach to therapeutic abortion has many advantages over the alternative of surgical recovery of fetuses. The treatments involved are no more traumatic than those associated with artificial insemination, and several animals can be treated simultaneously by one person for fetal recovery at predictable times. The protocol evolved during this study is proving very satisfactory for collecting fetal germ cells of defined ages. Fetal germ cells obtained in this way by Lavoir et al. (1995) were fused to enucleated oocytes and produced

---

### Table 4. Effects of intracervical treatment of pregnant cattle with PGE\(_2\) (20 mg per treatment) at intervals of 2 h from 40 h after a luteolytic injection of cloprostenol with additional oxytocin injections (i.m.) (Expt 4)

<table>
<thead>
<tr>
<th>Replicate number</th>
<th>Cloprostenol treatment</th>
<th>Fetal expulsion</th>
<th>Plasma progesterone (ng ml (^{-1})) at start of PGE(_2) treatment</th>
<th>Number of PGE(_2) treatments</th>
<th>Number of oxytocin injections</th>
<th>Time after last oxytocin injection (h)</th>
<th>Time after first PGE(_2) (h)</th>
<th>Time after death (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>35</td>
<td>—</td>
<td>0.4</td>
<td>6</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>Retained</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>38</td>
<td>0.1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>2–3</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>—</td>
<td>0.5</td>
<td>6</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>Retained</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>42</td>
<td>0.2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1–2</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>42</td>
<td>0.3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1–2</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>42</td>
<td>0.2</td>
<td>1</td>
<td>0</td>
<td>—</td>
<td>2</td>
<td>0–2</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>42</td>
<td>0.6</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>2–3</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>44</td>
<td>0.2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1–2</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>45</td>
<td>0.6</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>12</td>
<td>2–4</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>47</td>
<td>0.9</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>0–1</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>48</td>
<td>0.7</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1–3</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>48</td>
<td>0.3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1–2</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>49</td>
<td>0.2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1–2</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>50</td>
<td>0.4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>4–5</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>50</td>
<td>0.4</td>
<td>5</td>
<td>2</td>
<td>9</td>
<td>5</td>
<td>2–3</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>52</td>
<td>0.2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>2–4</td>
</tr>
<tr>
<td>8</td>
<td>52</td>
<td>54</td>
<td>0.5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>2–3</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>55</td>
<td>0.5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>0–1</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>55</td>
<td>0.7</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>5–7</td>
</tr>
<tr>
<td>9</td>
<td>56</td>
<td>58</td>
<td>0.5</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1–3</td>
</tr>
</tbody>
</table>

*Not applicable. Fetus died between 24 h and 40 h after cloprostenol injection.*
blastocysts. Furthermore, the protocol then allowed a conceptus resulting from the transfer of such a blastocyst to be retrieved at day 43 for DNA analysis. In addition, fetal tissue obtained in this way from other nuclear-transfer embryos has been used successfully for cytogenetic analysis after culture, indicating that the short delay between death and expulsion is not severely detrimental to its viability. Thus, this abortion protocol should facilitate the study of conceptuses derived from various forms of micromanipulated embryos. Although not investigated in this series, there is no reason to doubt that the regimen would be equally effective for recovering fetuses at <33 days of gestation.

In summary, local application of PGE_2 has been shown to dilate the bovine cervix, irrespective of progesterone concentrations, and to hasten the expulsion of the conceptus when used after induced luteolysis. This abortifacient action is enhanced by judicious oxytocin treatment, resulting in an atrumatic method of recovering bovine fetuses predictably within a short time of death, at least during the second month of pregnancy.

The generous collaboration of the Upjohn Company is gratefully acknowledged. The authors thank J. S. Walton and D. Rieger for their stimulating and helpful discussions; J. S. Walton and C. Veres for performing the progesterone assays; numerous colleagues for their willing participation in long vigils; barn staff at the Elora Dairy Research Centre and the OVC teaching hospital for their help with the animals; MRC Canada for the senior author’s fellowship; NSERC, Semex Canada and OMAFRA for other financial support.

References

Duchens M, Frederiksson G, Kindahl H and Aiumlamai S (1993) Effect of intracervical administration of a prostaglandin E_2 gel in pregnant and non-pregnant heifers Veterinary Record 133 546–549

Zeebin K, Jochle W and Steingruber Ch (1973) Termination of pregnancy with prostaglandin E_2 (PGE_2) and F_2α (PGF_2α) in cattle Prostaglandins 4 891–901