ABSTRACT

In vitro fertilisation (IVF) has become a useful breeding tool in most of the developed world. In this paper the success of bovine IVF and the birth of live calves under typical South African conditions is reported. Oocytes for IVF were collected from the ovaries of 6 slaughtered Bovelder beef cows. On average, 36.2 oocytes per donor were retrieved. From these oocytes, 43 blastocysts were produced from 5 of the donors by IVF with frozen Bovelder semen. The best 11 of these embryos were transferred into oestrous, synchronised Bovelder recipients in the same herd. As a result, 7 calves were born (a 64 % calving rate) from 4 of the original donors. The calves had a normal birth mass, but the mean gestation length of the male calves was significantly longer than the herd average (291.6 versus 285.2 days respectively). No calving difficulties were encountered. In summary, it was shown that IVF for bovine embryo production and transfer is possible on a commercial basis in South Africa.

Key words: blastocyst, Bovelder, bovine, embryo, IVF, oocyte.

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INTRODUCTION

The rescue of genetic material from valuable animals that die or need to be culled is a service that is currently available for certain species in many parts of the world6,8,11. In vitro fertilisation (IVF), a technology that uses eggs and sperm to produce embryos in a Petri dish, is particularly well-developed for cattle. Bovine IVF and embryo transfer have become standard procedures in which multiple offspring may be produced from a single dam. However, results are highly variable, with many donors yielding no offspring while others may yield many calves10. On average, 2–3 viable embryos can be produced from each donor, which, after transfer to a surrogate mother, will result in the birth of 1–2 live calves6,8. Although bovine IVF has been commercially available to farmers in many countries, this has occurred only recently in South Africa. This report describes the success of IVF and embryo transfer in a commercial herd of South African Bovelder beef cattle, with the birth of live calves.
CIDR synchronised with an EAZI-BREED Recipient management number of blastocysts produced.

One embryo was non-torty and transported to the farm at room temperature. One embryo was non-surgically transferred per recipient.

Additional blastocysts that appeared by Day 10 were also counted, to give the total number of blastocysts produced.

Recipient management

Thirteen Bovelder recipients were synchronised with an EAZI-BREED CIDR® (Solvay, AH) for 10 days (Days –12 to –2) in combination with an intramuscular injection of 500 µg cloprostenol (Estrumate®, Schering-Plough AH, South Africa) in the labora-

...tion.

DISCUSSION

The post-mortem production of offspring from an animal that dies suddenly or is culled is a valuable method for rescuing desirable genetic material. In the bovine, intensive research over the last 15 years has enabled the practical, commercial production of offspring by in vitro fertilisation (IVF)\(^1\). However, this technology is new in the domestic animal sector of South Africa, and in this study the success of these procedures under South African conditions using Bovelder donors and recipients was examined.

In general, embryo production and subsequent pregnancy rates from the Bovelder cows were on par with international rates. However, this technology is new in the domestic animal sector of South Africa, and in this study the success of these procedures under South African conditions using Bovelder donors and recipients was examined.

Table 1: Oocyte recovery and embryo production from 6 slaughtered Bovelder cows.

<table>
<thead>
<tr>
<th>Donor</th>
<th>Number of ovaries</th>
<th>Number of oocytes</th>
<th>Oocytes per ovary</th>
<th>Number cleaved</th>
<th>Number Day 7 blastocysts</th>
<th>Total number blastocysts (Day 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>88</td>
<td>44.0</td>
<td>36 (41 %)</td>
<td>7 (8 %)</td>
<td>13 (15 %)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>13</td>
<td>6.5</td>
<td>6 (46 %)</td>
<td>0</td>
<td>3 (23 %)</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>26</td>
<td>13.0</td>
<td>14 (54 %)</td>
<td>8 (31 %)</td>
<td>10 (38 %)</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>5</td>
<td>5.0</td>
<td>1 (20 %)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>22</td>
<td>11.0</td>
<td>2 (9 %)</td>
<td>6 (27 %)</td>
<td>11 (12 %)</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>45</td>
<td>22.5</td>
<td>26 (58 %)</td>
<td>10 (22 %)</td>
<td>11 (24 %)</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>199</td>
<td>18.1</td>
<td>93 (47 %)</td>
<td>27 (14 %)</td>
<td>43 (22 %)</td>
</tr>
</tbody>
</table>

The number of cleaved oocytes was counted approximately 44 hours after fertilisation and represents all embryos that had at least 2 cells. The number of blastocysts produced by Day 7 and Day 10 were also counted.

Ovaries from 6 mature Bovelder cows were recovered within 30 minutes of slaughter. One of the oocytes from cow number 4 was not recovered, thus the results reflect the use of only 1 of her ovaries, or 11 ovaries in total. Using a razor blade to lightly slice the ovarian surface, 199 reasonable quality oocytes were obtained. This corresponds to an average of 18.1 oocytes per ovary (range: 5–44) or 36.2 per cow.

The overall percentage of initial cleavage to at least 2 cells was 47 % (93/199), with a range of 20–58 %. By early Day 7, 27 blastocysts had been produced from the 11 ovaries. Of these, 24 were of transferable quality\(^2\). The best 11, all Grade 1, were chosen for embryo transfer (results described below). No blastocysts were produced by Day 7 from 2 of the donors, numbers 2 and 4. For all donors, a mean of 4.5 ± 1.8 blastocysts had developed by Day 7 (range0–10). By Day 10, a total of 43 blastocysts (7.8 per donor) had been produced from 199 oocytes (22 %).

Embryo transfer

The results of the embryo transfers are summarised in Table 2 and the details of the calves born in Table 3. A single embryo was transferred into each of 11 synchronised, 1st-calver Bovelder recipients. Seven of the 11 transferred embryos resulted in pregnancy on Day 42, a pregnancy rate of 64 % (Table 2). All 7 cows diagnosed as pregnant produced a live calf (2 heifers and 5 bull calves). No assistance was necessary for the calvings. The average mass of the male and female calves in this study were 38.8 ± 6.2 and 34.0 ± 7.1 kg respectively and the males were not significantly different from the herd average of 36.76 kg (P > 0.05). The male and female calves were born at mean gestation periods of 291.6 ± 3.7 and 293.5 ± 7.8 days respectively, the males having a significantly longer gestation period than the breed average of 285.2 days (P < 0.05).

Because there were only 2 female calves, the power of the statistical tests was too low to provide valid tests of differences of the calves in the present study from herd averages. 32.84 kg birth mass and 283.8 days gestation.

DISCUSSION

The post-mortem production of offspring from an animal that dies suddenly or is culled is a valuable method for rescuing desirable genetic material. In the bovine, intensive research over the last 15 years has enabled the practical, commercial production of offspring by in vitro fertilisation (IVF)\(^1\). However, this technology is new in the domestic animal sector of South Africa, and in this study the success of these procedures under South African conditions using Bovelder donors and recipients was examined.

In general, embryo production and subsequent pregnancy rates from the Bovelder cows were on par with interna-
Table 3: Sex, gestation length and birth mass of 7 Bovelder calves produced by IVF.

<table>
<thead>
<tr>
<th>Calf ID</th>
<th>Donor</th>
<th>Sex</th>
<th>Gestation length (days)</th>
<th>Mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>M</td>
<td>294</td>
<td>28</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>M</td>
<td>293</td>
<td>39</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>F</td>
<td>288</td>
<td>29</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>M</td>
<td>285</td>
<td>43</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>M</td>
<td>293</td>
<td>42</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>M</td>
<td>293</td>
<td>42</td>
</tr>
<tr>
<td>G</td>
<td>6</td>
<td>F</td>
<td>299</td>
<td>39</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>M</td>
<td>291.6 ± 3.7</td>
<td>38.8 ± 6.2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>F</td>
<td>293.5 ± 7.8</td>
<td>34.0 ± 7.1</td>
</tr>
</tbody>
</table>

One concern regarding IVF production of embryos is that foetuses produced might be oversized, causing calving problems\(^{2,3}\). In this study, the average birth mass of the male calves was not significantly different from the herd average and no calving problems were observed. Among calves born as a result of over 1000 bovine embryo transfers, no problems due to large calves occurred, but increased incidence of hydroallantois was reported\(^{20}\). No hydroallantois was observed during the pregnancies of the cows that gave birth to the 7 calves in the present study. However, the gestation mass of the male calves (291.6 ± 3.7) was significantly longer than the breed average of 285.2 days \(P < 0.05\). Because the average gestation length of calves from the sire used in this study is not available, it cannot be determined whether the prolonged gestation period is a sire, IVF or other effect.

Besides aiding stock farmers, the development of in vitro fertilisation and embryo transfer technology in South Africa may contribute to the preservation of genetic diversity of endangered or threatened wildlife species by salvaging the oocytes and sperm from culled animals, or animals that have died unexpectedly. Such embryos can then be frozen indefinitely and transferred into a recipient at a convenient time and place anywhere in the world.

ACKNOWLEDGEMENTS

We thank the Johannesburg Metropolitan Council for making animals and staff available for the trial. This work was supported by CryoLogic Pty Ltd, Australia (www.cryologic.com).

REFERENCES


Book review — Boekresensie

Comprehensive reports on technical items presented to the International Committee or to Regional Commissions


The 2000 edition of comprehensive reports made to various meetings of the OIE comprises papers on the control and eradication of a wide variety of important diseases, ranging from screwworm and bovine tuberculosis to aquatic animal diseases. The first section consists of 2 reports presented at the 68th General Session of the International Committee in May 2000. The first deals with principles of prevention and control of diseases of aquatic animals. This is an expanding field and breaks new ground for most veterinarians. The paper gives an excellent overview of the major notifiable diseases, procedures for inspection and control, import regulations, quarantine measures, procedures for introduction of new species, transport regulations and movement restrictions, disinfection procedures, contingency plans, training of personnel, and disease control by water treatment, vaccination, therapy and hygiene. It provides a legislative framework that is helpful in formulating control measures. The second report summarises recent progress in the diagnosis, control and eradication of bovine tuberculosis in domestic and wild animals, a subject that is extremely pertinent in South Africa at the moment. Important aspects that are emphasised are vaccination, and population management where wildlife reservoirs occur.

The second section consists of 5 papers presented at the 15th Conference of the OIE Regional Commission for the Americas in March 2000. Three of these concern eradication of screwworm, mainly by creating biological barriers using the sterile fly technique. This technique has been used successfully in Africa to control fruit flies and tsetse fly, and should perhaps be considered also in the control of flystrike. The other 2 papers concern surveillance, diagnosis and monitoring systems for vesicular stomatitis, which fortunately does not occur in South Africa, and prospects for diagnosis and control of brucellosis using new vaccines and/or new diagnostic tests. Awareness of diseases that do not occur in South Africa is essential in view of globalisation of trade, so that general papers such as the report on vesicular stomatitis offer an easy means for South African veterinarians to become familiar with such diseases. The paper on brucellosis concentrates on the problem of distinguishing immune reactions to vaccination from those provoked by natural infection. This can be achieved either by using vaccines such as the RB51 strain that elicit a different response from natural infection, or by using serological tests that can distinguish the antibodies provoked by pathogenic Brucella from vaccine-induced and cross-reacting antibodies. The last section consists of 2 papers presented at the 19th Conference of the OIE Regional Commission for Europe in September 2000. The first is a comprehensive overview of swine vesicular disease, which can cause severe production losses, more in terms of the control measures applied than the actual effect of the disease. Its main importance lies in the fact that it is clinically impossible to distinguish from foot-and-mouth disease. Controversy exists as to whether this disease should be retained as a List A disease, but in general countries have been in favour of risking the losses incurred by eradication procedures rather than risking confusion and consequent delayed diagnosis of foot-and-mouth disease. The last paper concentrates on how to limit erosive diseases that are not OIE-listed diseases but merit control owing to their devastating effects on productivity. The approach is valid and should be taken seriously, even if the outbreaks of foot and mouth disease that have occurred subsequent to the publication of these reports have demonstrated again why List A diseases should never be ignored simply because they may only appear at long intervals! This publication is of considerable value to all veterinarians.

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