Birth of live calves by *in vitro* embryo production of slaughtered cows in a commercial herd in South Africa

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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 world[6,11]. *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 several[10]. 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 calves[6,7]. 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.

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MATERIALS AND METHODS

Oocyte recovery and embryo production

Six Bovelder cows due for slaughter with no special prior treatment were selected for the IVF trial. The ovaries were collected at an abattoir within 30 minutes of slaughter and were delivered to the laboratory within 2 hours in phosphate buffered saline at approximately 30 °C. The ovaries were collected on Day 1 relative to IVF (Fig. 1).

Oocytes were retrieved by superficially slicing the cortex of the ovaries with a razor blade into tissue culture medium (TCM) 199 (all chemicals obtained from Sigma unless otherwise indicated) containing 0.3 % bovine serum albumin and 25 µg/mℓ gentamicin. Oocytes were located under a stereo microscope and washed in TCM199 to remove ovarian debris. Those with at least one complete layer of compact granulosa cells were selected for further culture[3]. Selected oocytes were matured for 22 h under mineral oil in 50 µℓdroplets of TCM199 supplemented with 25 µg/mℓ gentamicin, 100 µM 2-mercaptoethylamine, 25 mM HEPES and 10 % steer serum[12,19]. The steer serum was collected from a steer housed at the Faculty of Veterinary Science, University of Pretoria. All incubations occurred at 39 °C in 5 % carbon dioxide in air with 100 % humidity.

After maturation oocytes were fertilised with 2 $\times 10^6$ percoll-separated sperm per mℓ from a Bovelder bull (Day 0, Fig. 1). The frozen semen was thawed at 35 °C for 1 minute followed by separation on a discontinuous percoll gradient[15]. The live sperm pellet was washed and re-suspended in a modified TALP (Tyrode-albumin-lactate-pyruvate) medium including 25 µg/mℓ gentamicin, 0.6 % essentially fatty acid-free BSA, 30 µg/mℓ heparin and PHE (2.0 mM penicillamine, 1.0 mM hypotaurine and 25 µM epinephrine)[1,16,17]. After determination of sperm concentration, approximately 50 000 sperm and 10 eggs were co-incubated in 50 µℓ of modified TALP droplets under mineral oil.

Sperm and cumulus cells were washed from the presumptive zygotes approximately 20 hours after the addition of sperm (Day 1, Fig. 1). For the embryo culture period, embryos were co-cultured with granulosa cells derived from the Bovelder follicles in 50 µℓ droplets of TCM199 supplemented with 10 % steer serum and 25 µg/mℓ gentamicin under oil[8]. The number of zygotes cleaved to at least 2 cells was counted on Day 2 of culture (44 h after the addition of sperm)

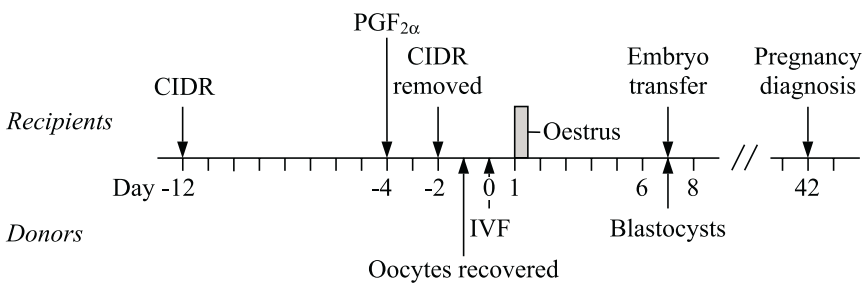

Fig. 1: **Diagram of the recipient management programme and IVF schedule.**

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

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

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.

as an indication of the success of fertilisation.

On Day 7 after fertilisation (Fig. 1), all blastocysts were counted and graded. The best 11 embryos, all grade one[22], were selected for fresh embryo transfer. Embryos were loaded into 0.25 mℓ straws (IMV, Taurus, South Africa) in the laboratory 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) on Day –4 (Fig. 1). Once the CIDR® devices were removed, field staff observed the recipients for signs of oestrus at 12-hourly intervals. All 13 cows were in heat on Day 1 of the IVF programme. The embryos were transferred on Day 7 of the programme (6 days after oestrus). Of the 13 synchronised cows (all first calvers), 11 were chosen as recipients. All the selected recipients had a corpus luteum, which was, judged by transrectal palpation, fully developed. Pregnancy was diagnosed 42 days after fertilisation (or 35 days after embryo transfer) by transrectal ultrasonography. At birth the date, sex and mass of each calf was recorded.

Statistical analysis

t-tests were performed on the gestation lengths and birth masss of calves born. Variation is reported as ± one standard deviation.

RESULTS

Embryo production

Table 1 summarises the results of the oocyte recovery and embryo production. Ovaries from 6 mature Bovelder cows were recovered within 30 minutes of slaughter. One of the ovaries 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[22]. 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 (range 0–10). 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 ($P > 0.05$). 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)[4,7]. 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 2: **Pregnancy and parturition data resulting from the transfer of 11 Bovelder IVF embryos into 11 recipients.**

Donor	Number of embryos transferred	Number of recipients pregnant	Number of calves born
1	5	2 (40 %)	2 (40 %)
2	0		
3	2	2 (100 %)	2 (100 %)
4	0		
5	3	2 (67 %)	2 (67 %)
6	1	1 (100 %)	1 (100 %)
Total	11	7 (64 %)	7 (64 %)

Table 3: **Sex, gestation length and birth mass of 7 Bovelder calves produced by IVF.**

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

tional standards. As expected, the number of good-quality oocytes retrieved per donor was variable, but the average of approximately 36 was within normal expectations[5,13]. The initial cleavage to 2 cells of 47 % was lower than the 70 % that is normally expected[16], but less stringent oocyte quality selection would at least partially account for this decrease. Although not typically cultured by research laboratories, oocytes with only a single layer of cumulus cells were used in the present study in order to maximise the potential for blastocyst production per donor, as some of these oocytes are capable of forming blastocysts[3]. The overall percentage of blastocysts (22 %) was comparable to results published internationally despite the selection of poorer quality oocytes[13,23]. Again, there was considerable variation among donors (0–38 % of total oocytes cultured), as has been reported in other studies[2].

The number of transferable blastocysts per donor, 4.4, is equal to that reported in other studies. Mylne *et al.*[14] produced 3 blastocysts per cow, while Stringfellow *et al.*[18] reported 5 per cow.

The pregnancy rate of 64 % after embryo transfer corresponds with international standards. With good-quality embryos and recipients, pregnancy rates of 60–70 % can be obtained[7,9]. In this study, calves were produced from 4 of the 6 donors, again indicating the variability that can be seen in this type of system. Three of these had 2 offspring each, while the 4th had only 1. Because of the limited number of recipients available, not all good-quality Day 7 embryos were transferred, therefore on a per donor basis more calves could have been obtained with additional recipients. The lack of recipients would not have affected the 2 cows that did not produce blastocysts by Day 7. Similar to results obtained here, an Italian study reported 1–3 calves produced per donor[6].

One concern regarding IVF production of embryos is that foetuses produced might be oversized, causing calving problems[20,21]. 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[10]. 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.

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Book review — Boekresensie

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

2000. Office International des Épizooties, Paris. 301 pp., soft cover. €25. ISBN 92 9044 523 8

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 legislatory 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 contains 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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