# Trans-vaginal oocyte retrieval and subsequent *in vitro* production of embryos from a cow involuntarily culled

D G Shaw[a], C M Bowles[a], K Raja[a] and A W Lishman[a]

**ABSTRACT**

A Holstein cow of high genetic merit, in late lactation (205 days) and diagnosed with salpingitis (after 4 infertile services and veterinary consultation), was subjected to 1 trans-vaginal oocyte collection attempt, prior to slaughter. Of an estimated 10 follicles punctured, a total of 4 cumulus-oocyte complexes were retrieved. These were matured *in vitro* in a maturation medium for 24 hours. After 24 hours maturation, the oocytes were fertilised *in vitro* with Percoll-processed frozen/thawed imported semen, of the owner's choice. Fertilisation was achieved in a modified Tyrode's medium. At 18 hours post-insemination, the presumptive zygotes were transferred into culture *in vitro* in Charles Rosenkran's amino-acid medium and supplemented on Day 4 post-insemination with 10 % foetal calf serum. All *in vitro* procedures were conducted in 50 $\mu\ell$ medium droplets, under oil, in a humidified incubator at 38.5 °C in 5 % $CO_2$ in air. Three of the potential zygotes cleaved and, by Day 7 of culture, these had developed to the morula stage. The embryos were frozen in 1.5 M ethylene glycol and later transferred non-surgically to synchronised Holstein recipient heifers. One morula resulted in the only pregnancy and subsequent birth of a healthy heifer calf. An independent commercial company confirmed parentage through standard blood-typing assays. The genetic salvage of oocytes, for *in vitro* production of embryos, has potential benefits to the producer.

**Key words**: embryo production, embryo transfer, *in vitro,* oocyte recovery, ultrasound.

Shaw D G, Bowles C M, Raja K, Lishman A W **Trans-vaginal oocyte retrieval and subsequent *in vitro* production of embryos from a cow involuntarily culled**. *Journal of the South African Veterinary Association* (1999) 70(3): 130–131 (En.). School of Agricultural Sciences and Agribusiness, University of Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209 South Africa.

## INTRODUCTION

Cows with chronic reproductive problems are frequently presented for veterinary treatment. There are many factors that may cause a cow to become a 'repeat breeder'. Salpingitis, inflammation of one or both of the oviducts, is one of the causes of reproductive problems and may result in sterility in the cow[3]. Reproductive problems of this nature frustrate the farmer in his efforts to produce numerous offspring from cows of high genetic merit. The conventional technique of super-ovulation and non-surgical embryo recovery cannot be used, as the infection usually affects the uterine horns and oviducts. Since the ovaries and oocytes are normally not affected, the technique of transvaginal ultrasound-guided oocyte retrieval, coupled with *in vitro* maturation (IVM), fertilisation (IVF) and culture, can produce embryos where natural systems fail. It is also possible to produce multiple offspring from a single donor animal through the simultaneous production of a larger number of embryos than would result under natural breeding conditions.

The objective of this study was to collect oocytes from a cow's ovaries without using surgical techniques. These oocytes would then undergo *in vitro* culture and produce embryos to be transferred to recipient cows and thus produce offspring that would not naturally have been possible.

## CASE HISTORY

A Holstein cow in late lactation (205 days), diagnosed with salpingitis and consequent infertility after 4 failed artificial inseminations and veterinary consultation, was submitted for a single oocyte collection procedure before slaughter. The donor animal was under continuous observation to determine the occurrence, date and time of oestrous activity. The procedure was performed 4 days after natural oestrus (day of standing heat = Day 0), when the maximum number of tertiary follicles (>2 mm in diameter) are present[2] and no super-ovulatory treatment is necessary before attempting collection.

### *Oocyte recovery*

Before commencement of the procedure, an epidural anaesthetic (5 m$\ell$ Lignocaine 2 %, Centaur Labs) was administered to prevent abdominal straining and to facilitate manipulation of the ovaries for follicle puncture. The animal was restrained in the standing position. The rectum, vulva and surrounding areas were thoroughly cleaned and disinfected.

An ultrasound Scanner 200 (Pie Medical Equipment, The Netherlands) and accompanying sector probe (dual frequency 5/7.5 MHz Annular Array Sector Endovaginal Transducer) were used to guide the transvaginal oocyte retrievals. An 18-gauge needle (Terumo Corporation, Japan), fixed to the end of the needle guide, was used to pass through the vaginal wall and puncture the follicles. The monitor is equipped with a biopsy puncture line to assist the operator in estimating where the needle is in relation to the follicle. The aspirate, comprising follicular fluid and cumulus-oocyte complex (COC), was drawn up by negative pressure from a 10 m$\ell$ syringe, through a Teflon tube, collected in the syringe and rinsed into a 90 mm Petri dish. The Petri dish contained Hepes Medium199, supplemented with 1 mg/m$\ell$ bovine serum albumen (Fraction V), 0.2 mM pyruvate, 25 $\mu$g/m$\ell$ gentamicin and 5 $\mu$g/m$\ell$ heparin (Sigma Chemical Co., USA). All COCs were identified under a microscope, washed in M199-Hepes and then transported in this medium to the *in vitro* laboratory.

### *Laboratory procedure*

In the laboratory, the COCs were washed in maturation medium (TCM199, Sigma Chemical Co., USA) supplemented with 10 % FCS (Highveld Biological), 0.2 mM pyruvate, 25 $\mu$g/m$\ell$ gentamicin, 2.5 $\mu$g/m$\ell$ LH (Sigma), 20 $\mu$g/m$\ell$ FSH-p (Sigma) and 1 $\mu$g/m$\ell$ oestradiol-17β (Sigma). COCs were allocated to a 50 $\mu\ell$ drop of TCM199 under mineral oil (maximum of 10 COCs per drop) and incubated at 38.5 °C in a humidified incubator, under 5 % $CO_2$ in air, for 24 h. After 24 h in the

[a]School of Agricultural Sciences and Agribusiness, University of Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209 South Africa.
E-mail: cbowles@cedara2.agric.za

Received: March 1998. Accepted August 1999.

maturation medium, COCs were transferred to fertilisation medium (modified Tyrode's-lactate medium supplemented with 0.2 mM pyruvate, 10 mM lactate, 6 mg/mℓ BSA-fatty acid free (Sigma) and 25 μg/mℓ gentamicin) and fertilised with Percoll-processed, frozen/thawed, imported bovine semen ($1 \times 10^6$/mℓ, >75 % motile).

At 18 hours post-insemination, the presumptive zygotes were placed in TCM199-Hepes, vortexed for 2.5 min to remove all cumulus cells and then washed in Charles Rosenkran's amino-acid (CR1aa) culture medium. Finally, the presumptive zygotes were placed in a 50 μℓ drop of CR1aa under mineral oil and incubated. On Day 4 of incubation, 5 μℓ of FCS was added to the drop of culture medium. On Day 7, assessment was made of the stage of development of the presumptive zygotes. Those that had reached the morula or blastocyst stage were frozen in 1.5 M ethylene glycol. The frozen embryos were stored for later transfer.

Six Holstein recipient heifers were synchronised using Crestar (Intervet, Netherlands) implants which were removed 11 days after insertion. Three recipients each received 1 embryo by non-surgical transfer approximately 6.5 –7.0 days after the middle of standing oestrus. Pregnancy diagnosis was carried out 6 weeks later by ultra-sound scanning.

Two of the recipients were confirmed pregnant, and 1 recipient carried the pregnancy to term. Of an estimated 10 follicles punctured, a total of 4 COCs were retrieved. Three of the potential zygotes cleaved and, by Day 7 of culture, 3 morula-stage embryos had developed. One morula-stage embryo resulted in the only full-term pregnancy and subsequent birth of a healthy heifer calf. An independent commercial company confirmed parentage through standard blood typing assays.

## DISCUSSION

By allowing visualisation and manipulation of the genital tract, ultrasound-guided ovum pick-up techniques can help to overcome some infertility problems. Gordon[1] reported that ultrasound-guided oocyte recoveries could result in an annual yield of 130 oocytes per animal and an output of 30 embryos. The use of IVM/IVF techniques and oocytes collected twice a week from the same live animal over a period of several months may be a useful alternative to the use of superovulation and embryo recovery through flushing, as a means of producing multiple offspring from a single animal. Repeated aspirations can be performed at least twice a week for several months without any need for hormonal stimulation[1]. The technique described above can be used to accelerate genetic progress by producing multiple offspring from a single donor of high genetic merit. The technique is also a useful tool for salvaging genetic material from clinically infertile cows of high genetic merit. The genetic salvage of oocytes, for *in vitro* production of embryos, has potential benefits for the producer.

## ACKNOWLEDGEMENTS

We thank Mr Viv Turner and his family, of Hlogoma Holsteins (Underberg, KwaZulu-Natal, South Africa) for his continuing support of, and enthusiastic and progressive approach towards, this project.

## REFERENCES

1. Gordon I 1994 *Laboratory production of cattle embryos.* University Press, Cambridge
2. Pieterse M C, Vos P M, Kruip T H, Wurth Y A, Van Beneden T H, Williemse A H Taverne M A M 1991 Transvaginal ultrasound guided follicular aspiration of bovine oocytes. *Theriogenology* 35: 19–24
3. Van Rensburg S W J 1973 *Breeding problems and artificial insemination.* Van Schaik, Pretoria