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Are cattle capable of producing litters of calves?

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ABSTRACT

Early embryo survival was examined in recipient heifers following the transfer of either 5 or 15 (Expt 1, N=19), or either 2 or 10 (Expt 2, N=46) in vitro produced embryos transferred into the same uterine horn. In Expt 1, the mean \pm s.e. number of conceptuses per pregnant recipient was 2.5 ± 0.6 and 10.8 ± 0.8 ($P < 0.001$) in recipients receiving either 5 or 15 embryos. Overall, $63 \pm 4\%$ of embryos survived (mean \pm s.d.), with no difference among recipients receiving 5 embryos and examined on Day 15, or receiving 15 embryos and slaughtered on either Day 15 or Day 22 ($56 \pm 10\%$, $77 \pm 5\%$ and $68 \pm 5\%$, respectively, $\chi^2 = 3.67$, NS). Survival was inexplicably lower on Day 22 in recipients receiving 5 embryos ($24 \pm 9\%$ vs. $69 \pm 4\%$, $\chi^2 = 19.15$, $P < 0.001$). Pregnancy rate was similar in heifers receiving either 5 or 15 embryos (9/9 vs. 8/10, $\chi^2 = 2.01$, NS), and the mean \pm se number of conceptuses per pregnant recipient was 2.5 ± 0.6 vs. 10.8 ± 0.8 , ($P < 0.001$). In Expt 2, embryo survival was $80 \pm 5\%$ and $73 \pm 2\%$ (NS), and the mean \pm s.e. number of conceptuses per pregnant recipient 1.6 ± 0.2 and 7.3 ± 0.3 ($P < 0.001$) in recipients receiving either 2 or 10 embryos. These preliminary results demonstrate that recipient cattle are apparently capable of supporting large numbers of viable conceptuses (5-15) up until at least the fourth week of pregnancy. Whether these can be maintained to term is yet to be demonstrated, although published evidence suggests that the biological limit may be 5 calves per cow at term.

Keyword: cattle reproduction; litter size; embryo transfer; multiple births.

INTRODUCTION

The life cycle efficiency of beef production is closely related to reproductive rate (Davis *et al.*, 1983, 1984). It follows that an increased frequency of twin calving should increase the efficiency of beef production. Simulation studies have indicated a 20-30% increase in both the biological as well as economic efficiency of beef production systems incorporating twin calving cows (Guerra-Martinez *et al.*, 1987; Herd *et al.*, 1993; Morris *et al.*, 1994).

While natural ovulation rates of three or more can result in triple or quadruplet births, the incidence of these higher order ovulations is very low (e.g., 9 in 10,000 for triples and 2 in 10,000 for quadruple ovulations, Scanlon *et al.*, 1973). Several studies have reported reproductive outcomes where triple or higher order calving has resulted after using either embryo transfer (e.g., Izaik *et al.*, 1991; Kennedy *et al.*, 1984.) or stimulation with gonadotrophin (e.g., Johnson *et al.*, 1973; Davis and Bishop, 1992; Echternkamp, 1992).

In vitro embryo production technologies enable the routine generation of large numbers of embryos for transfer. Although in vitro cattle embryos are normally transferred singly into recipients, many laboratories have been attempting to induce twin pregnancies using twin transfers (McMillan, 1996). Recipient pregnancy rate to term is about 15% higher following twin rather than single embryo transfer. However, there appears to be only one published report where large numbers (>10) of embryos have been transferred into each recipient (Eyestone *et al.*, 1995). If cattle are able to support higher order litters to term following the bulk transfer of in vitro produced embryos, the potential exists for developing new systems for intensifying breeding cow production.

The purpose of this paper is to report on progress in

a study examining the ability of heifer recipients to support high numbers of conceptuses in early pregnancy. The normality of the conceptuses was assessed using a number of developmental criteria.

MATERIALS AND METHODS

Recipient oestrous synchrony and embryo transfer

Heifers at approximately 18 months of age were used as recipients following treatment with the GENEMATE[®] oestrous synchrony protocol (Cliff *et al.*, 1995). On Day 7 (day of oestrus = Day 0), each heifer received embryos by standard non-surgical transfer into the uterine horn adjacent to the corpus luteum-bearing ovary (unilateral transfers).

Embryo production

Embryos were produced using standard in vitro procedures and using oocytes from ovaries sourced from local abattoirs.

Experiment 1

Recipients received either 5 or 15 embryos and were slaughtered on either Day 15 or Day 22 of pregnancy in a 2x2 factorial design (N=19).

Experiment 2

Recipients received either 2 or 10 embryos and were slaughtered on either Day 15, Day 22 or Day 55 of pregnancy in a 2x3 factorial design (N=46).

Post-mortem examinations

Following slaughter, uterine contents were either flushed from the reproductive tract using physiological saline (Day 14 or 15), or were carefully dissected out (Day 22 and Day 55). The length and width of elongated trophoblasts were measured (Day 14 or 15). On Day 22, developmental stage was assessed using: the length of the

embryo proper; the number of somite pairs; vascularity of the embryo; presence of an allantois; status of the neural tube and vascularity of the yolk sac. On Day 55, the status of the allantois and embryo was described, and each embryo was weighed.

RESULTS

Experiment 1

Overall, $63 \pm 4\%$ of the 185 embryos which were transferred survived. There was no effect of 5 vs. 15 embryos at transfer on survival rate to Day 15 (56 ± 10 vs. 77 ± 6 , $\chi^2 = 3.63$, NS). However, survival was inexplicably lower to Day 22 in the group receiving 5 embryos (24 ± 9 vs. 68 ± 5 , $\chi^2 = 14.81$, $P < 0.001$). Overall, $89 \pm 7\%$ of the recipients were pregnant, with no difference amongst those with 5 vs. 15 embryos at transfer (80 ± 13 vs. 100% , $\chi^2 = 2.01$, NS). Pregnancy rate was the same at Day 15 and Day 22 ($89 \pm 11\%$). Amongst pregnant recipients receiving either 5 or 15 embryos, mean litter size (embryos/pregnancy) was 2.5 ± 0.6 and 10.8 ± 0.8 ($P < 0.001$). Embryo survival rates were $50 \pm 8\%$ and $72 \pm 4\%$, respectively ($\chi^2 = 6.65$, $P < 0.01$).

Mean length and width of each conceptus on Day 15 was similar in the two groups (length: 38.7 ± 10.0 vs. 30.2 ± 4.2 mm, NS; width: 1.0 ± 0.1 vs. 1.2 ± 0.1 mm, NS). The length of the embryo proper on Day 22 was similar (2.8 ± 0.6 vs. 2.9 ± 0.2 mm, NS), as was the mean number of somites (6.0 ± 2.9 vs. 7.4 ± 1.1 pairs, NS). No embryos were vascularised, and an allantois was present on Day 22 in a similar proportion of embryos (33 ± 19 vs. $26 \pm 6\%$, $\chi^2 = 0.17$, NS). The neural tube was open in a similar proportion of embryos (75 ± 22 vs. $39 \pm 10\%$, $\chi^2 = 1.78$, NS) and vascularisation was apparent in a similar proportion of yolk sacs (50 ± 25 vs. $68 \pm 9\%$, $\chi^2 = 0.50$, NS).

Experiment 2

Amongst pregnant recipients receiving either 2 or 10 embryos, litter size was 1.6 ± 0.2 vs. 7.3 ± 0.2 ($P < 0.001$). Embryo survival in these recipients was $80 \pm 5\%$ vs. 73 ± 2 (NS). Embryo length on Day 14 appeared to be longer in recipients which received 2 embryos (59 ± 23 vs. 22 ± 6 mm, NS), although embryo widths were similar (1.3 ± 0.3 vs. 1.3 ± 0.1 mm, NS). Embryo length on Day 22 was similar in the two groups (4.9 ± 0.3 vs. 4.6 ± 0.2 mm (NS), and the number of somites was also similar (10 ± 1.6 vs. 9.3 ± 0.9 , NS). The allantois had commenced development at a similar rate ($88 \pm 13\%$ vs. $65 \pm 8\%$, NS), and the neural tube was open in a similar number of conceptuses (86 ± 13 vs. $70 \pm 7\%$, NS).

Of the recipients that previously received 2 embryos and were slaughtered on Day 55, half of those that were pregnant had two viable foetuses present. A single recipient in the group receiving 10 embryos was pregnant on Day 55 (2 viable foetuses), although some recipients had up to 5 non-viable foetuses. These non-viable foetuses appeared to have developed normally to about Day 35, but lack of normal placental development appeared to prohibit subsequent development.

DISCUSSION

The main finding from this study is that cattle are capable of nourishing and supporting very high numbers of conceptuses during early pregnancy. The range of indices used in this study to assess developmental competence indicates that large litter size does not lead to abnormal development, at least over the first month or so of early pregnancy. However, it appears that after some time during the second month of pregnancy the number of viable conceptuses is dramatically reduced. In this study, the result was that no more than two viable foetuses were present at about Day 60.

The ability of cattle recipients to support large numbers of apparently normal conceptuses during early pregnancy is consistent with a previous report using bulk embryo transfer (Eyestone *et al.*, 1995). There do not appear to be other reports that have continued to assess foetal development beyond the first month of pregnancy after bulk embryo transfer. It is therefore difficult to assess whether the large reduction in foetal viability noted in this study is a more general outcome when using in vitro-produced embryos. The abnormal placental development that affected higher order litters in an all-or-none manner does not appear to have been documented elsewhere. Nonetheless, 11% of calving recipients that previously received three in vivo produced embryos produced triplets at term (Izaike *et al.*, 1991), indicating that embryo transfer can be used to produce higher order litters at term.

Higher order litters have been produced following ovarian stimulation with gonadotrophins (Echternkamp, 1992). These authors showed that up to five foetuses were capable of developing to about Day 50 of pregnancy. Furthermore, individual foetal weight, length, and width, as well as placental weight and amniotic fluid volume was no different in single, twin, triplet, quadruplet and quintuplet pregnancies. Other data indicates no difference in foetal weight amongst pregnancies (1-4 foetuses/cow) on Day 95 of pregnancy, although by Day 180 foetal weight was about 15% lower in multiple pregnancies compared with singles (Bellows *et al.*, 1990). Collectively, these data indicate that maternal restriction on foetal development does not occur until the second trimester of pregnancy.

In studies aimed at increasing litter size at calving using gonadotrophin stimulation, no more than 5 calves have been produced by individual cows (review by Bellows *et al.*, 1990; Echternkamp, 1992). These findings lead to the postulation that litter size in cattle peaks at either 3 calves per uterine horn or a total of 5 calves per pregnancy (Echternkamp, 1992). Furthermore, according to Echternkamp, this potential appears to be set by Day 35 of pregnancy. Our data appear to support this finding in that no more than 5 conceptuses (non-viable in our case) were present at Day 55.

Practical problems associated with higher order litters at term include calf birth weight and calf losses. Results from two studies confirm the negative relationship between litter size and birth weight (Turman *et al.*, 1971; Echternkamp *et al.*, 1992). Quadruplet and quintuplet calves

are about 40-50% of the weight of contemporary single calves. In one study, calf losses for triplet and higher order litters were of 50-60% compared with fewer than 5% in singles and twins (Turman *et al.*, 1970). In another study, calf loss rates for cows with from 1-5 calves born were 2, 7, 16, 36 and 0%. The nil loss rate for quintuplets in this study indicates that higher order births need not be synonymous with high calf losses.

In summary, we have confirmed earlier work that showed cattle recipients are capable of producing at least 15 early pregnancy conceptuses. We have extended this work to show that early conceptus development is apparently normal during the first 3 weeks. The bulk embryo transfer technique described in this study provides a more convenient and less expensive means of generating large numbers of early conceptuses in order to investigate the aetiology of early pregnancy failure in recipient cattle. Our data support the notion that no more than 5 calves can develop beyond about Day 35. We report for the first time the intriguing finding of an all-or-none phenomenon of foetal survival associated with abnormal pregnancy membrane development. Until the reasons for this are understood and overcome, the production of litters of calves in cattle from in vitro-produced embryos is unlikely.

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