RPCV (2004) 99 (550) 109-115

REVISTA PORTUGUESA DE CIÊNCIAS VETERINÁRIAS

Twinning induction and its effects on embryo-foetal and calf survival, and on reproductive efficiency of Mertolengo cattle kept at pasture

Indução da gemelaridade e seus efeitos na sobrevivência embrio-fetal e perinatal e na eficiência reprodutiva de bovinos Mertolengos mantidos em pastoreio

Mário A. Quaresma[1], Luís Lopes da Costa[1]*, António E.M. Horta[2], José Robalo Silva[1]

[1] CIISA, Núcleo de Reprodução, Faculdade de Medicina Veterinária, Rua Prof. Cid dos Santos, Pólo Universitário, Alto da Ajuda, 1300-477 Lisboa
[2] Departamento de Reprodução Animal, Estação Zootécnica Nacional, Vale de Santarém, 2005-048 Vale de Santarém

Summary: The experiment was conducted during two years in a herd of Mertolengo cattle kept at pasture. In both years, cows (n = 141) were treated with a progestagen plus equine chorionic gonadotrophin (eCG) to synchronize the oestrous cycle. In year 1, cows were randomly allocated to three groups: i) artificial insemination (AI); ii) transfer of an embryo into the uterine horn contralateral to the *corpus luteum* seven days after AI (AI+ET); and iii) bilateral embryo transfer (2ET). Embryos were produced *in vitro* (IVP) and frozen in ethyleneglycol to allow the direct transfer after thawing. In year 2, cows were allocated to groups AI or AI+ET (only single ovulating cows in this group). In year 1, the calving and twinning rates were similar for the three groups (overall: 46.4 % and 11.5 %, respectively) and, in group AI+ET the survival of ET-derived embryos was negatively affected by the presence of double or multiple ovulations. In year 2, the calving and twinning rates of group AI+ET were 41.5 % and 52.9 %, respectively. In both years, the fertility rate of treated cows at the end of the breeding season was similar to that of the remaining untreated cows in the herd. Compared to single calving cows, twin calving cows had a significantly shorter gestation lenght, lower birthweight of calves, higher perinatal mortality of calves, and longer intercalving interval and decreased fertility in the subsequent breeding season. These results suggest that although Mertolengo cows can successfully carry and rear twins under the traditional management system, the economic efficiency of twinning is negatively affected by calf losses and by delayed rebreeding and reduced fertility of the cows. These negative effects may be overcome by management changes but the benefit of these changes needs to be evaluated.

Resumo: Este estudo foi realizado durante dois anos numa herdade de bovinos Mertolengos mantidos em pastoreio. Em ambos os anos, as vacas (n = 141) foram tratadas com um progestagéneo mais eCG (equine chorionic gonadotrophin) para sincronizar o ciclo éstrico. No ano 1, as vacas foram aleatoriamente distribuídas por três grupos: i) inseminação artificial (IA); ii) transferência de um embrião para o corno uterino contralateral à ovulação sete dias após a IA (IA+TE); e iii) transferência de 2 embriões bilateralmente (2TE). Os embriões foram produzidos *in vitro* (IVP) e congelados em etilenoglicol de forma a permitir a transferência directa após a descongelação. No ano 2, as vacas foram distribuídas pelos grupos IA e IA+TE (apenas vacas com ovulação unitária neste grupo). No ano 1, as taxas de parição e de partos gemelares foram similares para os três grupos (global: 46,4 % e 11,5 %, respectivamente) e no grupo IA+TE a sobrevivência dos embriões IVP foi negativamente afectada pela presença de ovulações duplas ou múltiplas. No ano 2, as taxas de parição e de partos gemelares foram de 41,5 % e de 52,9 %, respectivamente. A taxa de fertilidade global das fêmeas tratadas (proveniente dos tratamentos e da época de monta natural) foi similar à de fêmeas contemporâneas não tratadas. Em comparação com as fêmeas com partos singulares, as fêmeas com partos gemelares apresentaram uma duração da gestação mais curta, vitelos com menor peso vivo ao nascimento, maior mortalidade perinatal, alongamento do intervalo entre partos e diminuição da taxa de fertilidade na época reprodutiva de monta natural subsequente. Em conclusão, a raça bovina Mertolenga no sistema de produção tradicional tem potencial para produzir gémeos, mas a rentabilidade é condicionada pelo aumento da prevalência da mortalidade perinatal e do decréscimo da eficácia reprodutiva, factores que poderão ser controlados por alterações de maneio cuja análise custo-benefício requer verificação experimental.

* Correspondence: lcosta@fmv.utl.pt

Introduction

Natural occurrence of twin gestations is relatively rare in cattle, with a mean frequency of 1 to 5 % (Morris, 1984; Nielen *et al.*, 1989). Twinning may be of economical value in beef production where free-martinism does not represent a problem (Guerra-Martínez *et al.* 1990; De Rose and Wilton, 1991). However, the economical interest of twin induction in cattle is controversial because induced twinning rates can vary widely, and because twinning is often associated with increased perinatal mortality of calves and longer rebreeding intervals of cows. Such limitations seem to be related to loss of body condition of cows carrying twins due to increased energy requirements for foetal growth during the last phase of gestation and for milk production, to increased dystocia and placental retention, and to the twin suckling effect on delaying the onset of ovarian activity post partum (Marques *et al.* 1993a). It is known that these factors are usually associated with longer intervals to conception and decreased conception rates (Gregory *et al.*, 1990; Echternkamp and Gregory, 1999a; 1999b). According to Bosc (1978) and Gordon (1994) these negative effects may be controlled by adjusting energy requirements for twin pregnancies and by using closer observation at parturition.

Mertolengo is a small sized breed with mean fertility rate above 90 %, easy calving and good motherwood characteristics, frequently used in crosses with Charolais or Limousin bulls (Bettencourt *et al.*, 1987). Twin gestations could be of potential economical value in systems of meat production, through the use of an ovulation stimulatory treatment followed by AI and / or through the use of low cost IVP embryos of beef parentage. Additionaly, twinning could be of value for the production of replacement females by using bisected and sexed embryos. However, the biological and economical efficiency of these methods need experimental confirmation in the breed, mainly in the traditional management system at continuous pasture.

This study was conducted in Mertolengo cows maintained continuously at pasture with the objectives of evaluating: i) the efficiency of different methods of twin induction; ii) the ability of the breed to cope with twin pregnancies; and iii) the effect of twinning on perinatal calf mortality and on subsequent reproductive performance of the cows.

Materials and methods

The experimental work was conducted during the traditional breeding season (October to December) of 1995/96 (year 1) and 1997/98 (year 2) at an animal breeding centre in the southern of Portugal "Estação de Selecção e Reprodução Animal do Baixo Alentejo". From a population of 141 Mertolengo cows (80 in year 1 and 61 in year 2) with at least 50 days *postpartum*, those with positive response to oestrus synchronisation were selected for twin induction. All cows were maintained at pasture and were supplemented with *Avena sativa* X *Vicia villosa* silage during August to November when pasture is scarce. Following synchronisation of oestrus, the cows were taken into an open paddock for heat detection and AI, when they were fed with silage, wheat straw and water *ad libitum*. They returned to this paddock for embryo transfer, blood sampling and pregnancy diagnosis on the dates shown below.

In year 1 the cows were randomly allocated to one of three groups: Group AI (artificial insemination); Group AI+ET (AI plus transfer of an embryo); and Group 2ET (transfer of two embryos). The oestrous cycles were synchronised by the Crestar method (Intervet) with the ear implants maintained *in situ* for 9 days and including the im injection of 5 mg oestradiol valerate at the time of implant insertion. At implant removal all cows were injected im with equine chorionic gonadotrophin (eCG; Chronogest, Intervet) at a dose of 500 IU for Groups AI and 2ET or 250 IU for Group AI+ET. The lower eCG dose in AI+ET cows was to reduce the probability of multiple pregnancies at the time of embryo transfer, which is known to affect embryo survival (Echternkamp, 1992). All cows, except those from group 2ET, were inseminated with frozen-thawed semen from a Charolais bull. Although oestrus was detected and registered along three days by continuous observation during the daylight period, insemination was at fixed-time between 48 and 52 hours after implant removal. Seven days (Day 7) after oestrus (Day 0) cows allocated to Groups AI+ET and 2ET were palpated *per rectum* and those with, at least, one *corpus luteum* (CL) received *in vitro* produced embryos (IVP), produced according to procedures previously described (Lu *et al.*, 1988; Marques *et al.*, 1995). Belgian-Blue x Simental IVP embryos were frozen using ethyleneglycol as cryoprotectant to allow the direct transfer after thawing, following the method described by Voelkel and Xu (1992). Cows of Group AI+ET received a single embryo into the uterine horn contralateral to the CL, while cows from Group 2ET received one embryo into each uterine horn. Fifteen days after oestrus all treated cows were joined to the remaining of the herd and run with fertile bulls of the same breed for a 5-month breeding period. Data on calving date, number, sex and birthweight of calves were recorded.

Based on the results of year 1, in year 2 the following changes were made to the protocol: for the oestrous cycle synchronisation the ear implants were maintained *in situ* for 11 days and all cows received an eCG dose of 250 IU and were fixed time inseminated at 48 and 72 hours after implant removal; on Day 7 all cows were palpated *per rectum* and only those with a single CL were transferred a pure-breed Charolais frozen-thawed IVP embryo (Group AI+ET), while those with double or multiple CLs received no further treatment (Group AI); pregnancy was confirmed by ultrasonography (Ultra-Scan II, Alliance Medical Inc., Montreal, Canada, 5 MHz linear probe) at Day 60 when foetal number, location and viability (heart beat) were evaluated.

Blood samples for progesterone (P4) determinations were collected from the coccygeal vessels into heparinised tubes, centrifuged within 30 minutes and the plasma was stored frozen at – 20 ºC until assay. Progesterone was measured by a solid phase radioimmunoassay previously described (Lopes da Costa *et al.*, 2001). Samples were assayed in duplicate and the intra- and inter-assay coefficients of variation of sessions run at the time of the study were 8.9 % and 9.6 %, respectively. Sampling days were: eight days prior to implant insertion, days of implant insertion and removal, Day 0, Day 7 and Day 22. On Day 7, a plasma aliquot was used for field P4 determination by a semi-quantitative enzyme immunoassay (Premate, Rhone Merieux, France) in order to confirm normal function of the palpated CL and to support the decision of ET.

For statistical analysis were only included cows with synchronised oestrus and P4 < 1.0 ng mL^{-1} on Day 0 and P4 > 1.0 ng mL^{-1} on Day 7, in order to eliminate cows with no conditions to get pregnant. Pregnancy on Day 22 was presumed if P4 < 0.5 ng mL^{-1} on Day 0, ≥ 1.0 ng mL^{-1} on Day 7 and ≥ 2.0 ng mL^{-1} on Day 22. Data were analysed by means of a statistical package (STATISTICA 5.0, StatSoft Inc., 1995, Tulsa, OK, USA), using chi-square tests in contingency tables (Fisher exact test) and the t-test for unpaired data, where appropriate. Significance was tested at the 5 % probability level ($P < 0.05$) and a P value of $0.1 > P > 0.05$ was considered as tendency to significance.

Results

In year 1, fifty-six out of 80 cows (70.0 %) had a positive response to synchronisation and from the 24 unresponsive cows, 21 (87.5 %) presented luteal plasma concentrations of P4 at AI time and the other three cows remained anoestric. Therefore in year 2 the progestagen was kept *in situ* two additional days (11 instead of 9) to avoid the use of a luteolytic agent. This induced a positive response to synchronisation in 56 out of 61 cows (91.8 %) and only one out of five unresponsive cows presented luteal plasma concentrations of P4 at AI time (three others remained anoestric and one presented ovarian cysts on Day 7). Table 1 presents the number of synchronised cows, the presumed pregnancy rates on Day 22, the calving and twinning rates for the three groups in year 1. Table 2 presents the prevalence of double and multiple ovulations (3-4 CLs) of cows of Groups AI+ET and 2ET and, the calving outcomes of cows with different ovulation numbers. In cows of Group AI+ET with double or multiple ovulations, the embryo was transferred to the side ipsilateral to the ovary presenting the least number of CLs. As shown in Table 2, the proportion of cows displaying double and multiple ovulations was similar in the two eCG doses (250 IU in Group AI+ET and 500 IU in Group 2 ET), which lead to the use of the lower eCG dose in all cows of year 2. Both in Groups AI+ET and 2ET, the probability of pregnancy establishment was higher ($P < 0.1$) for cows with double/multiple ovulations than for single ovulating cows. In the AI+ET group only one ET-derived calf was born (single calving). Therefore, it was hypothesised that the chance of survival of the IVP embryo was reduced in the presence of native AI-derived embryos and, because of that, in year 2 only single ovulating cows were allocated to the AI+ET group.

Because in year 1 pregnancy losses after Day 22 were heavy (overall 32.2 %), in year 2 cows were ultrasound scanned on Day 60 of gestation to evaluate the prevalence of twin gestations and the prevalence of embryo-foetal losses from Day 60 onwards. Table 3 presents data from year 2. Overall, 5 out of 14 single pregnancies (35.7 %) and 4 out of 15 twin pregnancies (26.7 %) were lost between Day 60 and calving. From the ten ET-derived calves born in Group AI+ET, nine were co-twins of an AI-derived calve and one was singleton.

Table 4 presents data of years 1 and 2 regarding the calving rate and calf production of treated cows. Fertility rates of treated cows, including calvings from treatments and from natural service (85.0 % in year 1 and 75.4 % in year 2), were similar to those of non-treated contemporary cows in the herd (62/79 = 78.5 % in year 1; 88/100 = 88.0 % in year 2). Data on length of gestation, on calving and on survival of calves until weaning are presented in Tables 5 and 6. Two cows with twins (Group AI+ET) and one cow with a singleton (Group 2ET) required assistance at calving, due to foetal postural defects (unilateral shoulder flexion) and to foetal oversize, respectively. In year 2, one cow had a set of triplets originated by natural service during the breeding season subsequent to treatment, but none of the calves survived. As shown in Table 6, reproductive performance in the subsequent breeding season was lower in twin bearing cows than in those calving singletons. Only one cow presented retention of the foetal

Table 1 - Pregnancy, calving and twinning rates after treatments for twin induction in Mertolengo cows (year 1).

Group	n	Pregnant Day 22 n (%)	Calving n (%)	Twin calvings n (%)
AI	18	15 (83.3)	12 (66.7)	1 (8.3)
AI+ET	17	15 (88.2)	5 (29.4)	1 (20.0)
2ET	21	14 (66.7)	9 (42.9)	1 (11.1)
Total	56	44 (78.6)	26 (46.4)	3 (11.5)

Table 2 - Calving outcome of cows with different number of ovulations (year 1).

Group *	Ovulation number					
	Single		Double		Multiple (3-4 CLs)	
	n	calving	n	calving	n	Calving
AI+ET	11	1 (9.1)	3	3 (100.0)	3	1 (33.3)
2ET	13	4 (30.8)	5	3 (60.0)	3	2 (66.7)
Total	24	5 (20.8)	8	6 (80.0)	6	3 (50.0)

* Group AI+ET received 250 IU of eCG at the end of the progestagen treatment for oestrous synchronisation, while Group 2ET received 500 IU.

membranes after calving (group AI+ET, twin calving with two stillborn calves and a gestation length of 267 days). Overall, gestation length was significantly correlated with calf birthweight (r = 0.48, P < 0.001).

Discussion

The results obtained in this study show that twin pregnancies and calvings can be induced in Mertolengo cows at pasture. The 500 IU and 250 IU eCG doses induced similar rates of double and multiple ovulations. Previously Lopes da Costa *et al.* (1994) reported that, in Charolais cows at pasture, the rate of multiple pregnancies was higher in cows stimulated with 750 IU than with 500 IU of eCG. However, the number of calves born was identical in both groups due to heavier losses along gestation in cows treated with 750 IU. In year 1, where 35 % of double or multiple ovulations were detected in cows of Group AI+ET, only one ET-derived singleton was born and, in year 2, where only single ovulating cows were allocated to this group, the rates of twinning and of survival of ET-derived embryos were similar to those reported in other cattle breeds (Takada *et al*., 1991; Reichenbach *et al*., 1992; Horta *et al*., 1993; McEvoy *et al*., 1995; Penny *et al*., 1995). These results suggest that whenever more than one native AI-derived embryo is present in the uterus, the IVP embryo has less chance of survival, probably due to a lower intrinsic viability. However, double or multiple ovulating cows seem to have a higher chance of conception when compared to single ovulating cows.

Pregnancy losses between Days 22 to 60 were relevant, namely in AI+ET treatment, a feature also reported in other studies where IVP embryos were used (Izaike *et al*., 1991; Reichenbach *et al*., 1992; Horta *et al*., 1993; Penny *et al*., 1995; Sakakibara *et al*., 1996). Penny *et al*. (1995) reported that the calving rate of cows that received one IVP embryo after AI was higher than that of cows receiving two IVP embryos. These authors associated the increase of embryo-foetal survival in cows that received an embryo after previous AI to the beneficial effect of the native embryo (AI produced) upon the transferred IVP embryo. This would have resulted from luteotrophic and anti-luteolytic factors that help to provide conditions within the uterus making it easier for the transferred embryo to survive. Lopes da Costa *et al*. (1997), on the other hand, suggested that the death of the IVP embryo might affect negatively the fate of the native embryo, and this effect may still be responsible for twin losses beyond Day 60.

The mean gestation length of cows bearing twins was eight days shorter than that of those bearing singles, and at birth, twin calves were 9 kg lighter than singles, confirming observations made by others (Anderson *et al*., 1979; Guerra-Martínez *et al*., 1990; Horta *et al.*, 1992; Penny *et al*., 1995; Vasques *et al.*, 1995; Schmidt *et al*., 1996). The percentage of calvings that required assistance was lower than that reported in the literature (Gregory *et al*., 1990; Penny *et al*., 1995). Although the genotype used for AI and ET resulted in calves significantly heavier than the native calves, dystocia due to foetal oversize was very low, confirming the low predisposition to oversize dystocia of this native cattle breed (Bettencourt *et al*., 1987). On the other hand, the rate of perinatal mortality was higher than that reported by others (Schmidt *et al*., 1996; Van Wagtendonk *et al*., 1998), although similar to that reported in Alentejano cattle (Horta *et al*., 1992). This may have resulted from lack of assistance to the neonates, once calvings occurred at pasture and were unobserved. It is likely that the

Table 3 - Ovulation, pregnancy, calving and twining rates of Mertolengo cows (year 2).

Group	n	Double Ovulations*	Pregnant n (%)		Calving n (%)	Twin calvings n (%)
			Day 22	Day 60		
AI	15	9 (60.0)**	11 (73.3)	7 (46.7)	3 (20.0)	2 (66.7)
AI+ET	41	-	34 (82.9)	22 (53.7)	17 (41.5)	9 (52.9)

* All cows received 250 IU of eCG at the end of the progestagen treatment for oestrous synchronisation, but only single ovulating cows received an embryo (Group AI+ET); ** 2 multiple ovulations (3-4 CLs).

Table 4 - Fertility and calf productivity of Mertolengo cows after twinning induction treatments (years 1 and 2).

Parameter - n (%)	Year 1	Year 2
Calving rate from treatments	26 / 56 (46.4)	20 / 56 (35.7)
Calving rate from natural service	25 / 56 (44.6)	23 / 56 (41.1)
Calving rate from synchronised cows	51 / 56 (91.1)	43 / 56 (76.8)
Total calving rate *	68 / 80 (85.0)	46 / 61 (75.4)
Calves born	71	59 **
Calves born per treated cow	71 / 80 (88.8)	59 / 61 (96.7)
Calves born per calved cow	71 / 68 (104.4)	59 / 46 (128.3)
Calves weaned	68	48
Calves weaned per treated cow	68 / 80 (85.0)	48 / 61 (78.7)
Calves weaned per calved cow	68 / 68 (100.0)	48 / 46 (104.3)

* including calvings from non-synchronised cows; ** one spontaneous triplet after natural service.

Table 5 - Descriptive table of characteristics of calvings of Mertolengo cows according to calf number and origin (AI or ET-derived).

Calving	n	Gestation length (days)	Calves born (n)	Calf birthweight (kg)	Perinatal mortality n (%)	Assisted calvings n (%)
Single AI	22	284 ± 3	22	34 ± 4	0	0
Single ET	10	284 ± 4	10	40 ± 6	2 (20.0)	1 (10.0)
Twin AI	4	276 ± 4	8	25 ± 4	1 (12.5)	0
Twin ET	1	261	2	26	1 (50.0)	0
Twin AI+ET	9	278 ± 4	18	31 ± 7	6 (33.3)	2 (22.2)
Total	46	-	60	-	10 (16.7)	3 (6.5)

Values for gestation length and birthweight of calves are means ± standard deviation.

Table 6 - Effect of twinning on gestation length, calving assistance, birth weight and survival of calves till weaning.

	Single calvings	Twin calvings
n	32	14
Gestation length (days)	284 ± 3 [a]	276 ± 6 [b]
Calves born	32	28
Perinatal mortality (calving - 8 days)	2 (6.3 %) [c]	8 (28.6 %) [d]
Calves weaned	30 (93.8 %)	20 (71.4 %)
Weaning rate / calved cow	93.8 %	142.9 %
Birthweight of calves (kg)	36 ± 6 [a]	27 ± 6 [b]
Assisted calvings	1 (3.1 %)	2 (14.3 %)
Retention of foetal membranes	0	1 (7.1 %)
Fertility in subsequent breeding season (%)	85.2 [e]	71.4 [f]
Intercalving interval (days)	374 ± 34 [c]	422 ± 46 [d]

Values for gestation length, birthweight of calves and intercalving intervals are means ± standard deviation; rows with different superscripts differ significantly, [ab] $P < 0.0001$; [c,d] $P < 0.05$ or tend for significance [e,f] $P < 0.1$.

calves that did not survive may have been submitted to a long expulsive phase which is known to influence survivability of calves at birth (Berger *et al.,* 1992) but that could not be confirmed because calvings were not observed. That might have been particularly relevant in single calvings originated from IVP embryos. The culture system for IVP embryo production used in this study has been shown to induce large calves at birth (Horta *et al.*, 1992). In the case of twin births, where the individual weight of calves was low compared to that of singletons, perinatal mortality might have been due to lack of experience of the cows to deal with twins. In this breed the natural occurrence of twins is very rare and the presence of twins might promote abnormal maternal behaviour. Horta (unpublished data) observed that Alentejano cows with twins can walk away from the place of foetal expulsion with one of the calves, leaving behind the other calf that had fallen asleep after the first suckling. Cows can walk long distances (several kilometers per day), leaving calves exposed to hypothermia and to predators (some of the dead calves were found with signs of attacks by predators).

The prevalence of placental retention was also much lower than that reported in the literature for twin calvings (Guerra-Martínez *et al.*, 1990; Marques *et al.*, 1993b; Penny *et al.*, 1995; Echternkamp and Gregory, 1999a; 1999b). This may have been due to the lack of calving assistance and to the fact that calvings occurred at pasture, once it is known that retention of foetal membranes is more frequent in housed cows than in cows at pasture and in manipulated than in non-assisted cows at calving (Joosteen *et al.*, 1987; Horta, 1994). Nevertheless such low prevalence of placental retention is rather surprising when compared with that reported for Alentejano cows with twin calvings under identical management conditions (Marques *et al.*, 1993b).

Twinning is associated with subsequent decreased fertility and longer intercalving intervals. This may be due to delayed resumption of *postpartum* cyclic ovarian activity, which is known to occur in cows nursing twins. The negative effect of suckling on *postpartum* ovarian function is mediated both by intensity and frequency of suckling (Giménez *et al.*, 1980; Horta *et al.*, 1987; Marques *et al.*, 1993a; Sinclair *et al.*, 1994a). In addition, the loss of body weight during the last phase of gestation is usually heavier in cows carrying twins due to increased energy requirements for foetal growth and to reduction of intake dependent on abdominal crowding (Guerra-Martínez *et al.*, 1990). This greatly affects the duration of the *postpartum* acyclic period, shown to be mainly dependent on body condition at calving (Horta *et al.*, 1990; Jolly *et al.*, 1995; Viscarra *et al.*, 1998; Robalo Silva, 1999). However, in Alentejano cattle, also maintained continuously at pasture, the carrying of twin foetuses did not cause any loss in maternal body weight (Marques *et al.,* 1993a). This may be related to the quality of forage or to the ability of the breed to adjust the capacity of food intake to the nutritional needs.

In conclusion, results of the present study demonstrate the ability of Mertolengo cows to produce and rear twin calves under the traditional management system at pasture. However, twin calvings were associated to increased perinatal mortality of calves and to subsequent reduced reproductive performance of cows, problems that need to be alleviated. Early diagnosis of twin pregnancies, appropriate nutrition of twin-bearing cows and calving assistance may improve calf survival. Further studies are needed to evaluate the economical benefit of these management changes.

Bibliography

Anderson, G.B., Cupps, P.T., Drost, M. (1979). Induction of twins with bilateral and unilateral embryo transfer. Journal of Animal Science, 49, 1037-1042.

Berger, P.J., Cubas, A.C., Koehler, K.J., Healey, M.H. (1992). Factors affecting dystocia and early calf mortality in Angus cows and heifers. Journal of Animal Science, 70, 1775-1786.

Bettencourt, A.J., Vaz, I.M., Cláudio, D., Robalo Silva, J., Sieuve Monteiro, L. (1987). Productivity and reproductive performance of Mertolengo cattle: comparisons between purebred and crossbred calves. Proc. 38th Meeting of the European Association for Animal Production, Lisboa, Portugal, pp. 702 (*abstract*).

Bosc, M.J. (1978). Problèmes soulevés para la gémelléité au moment de la parturition chez les bovins. Ann. Méd. Vét., 122, 5-27.

De Rose, E.P., Wilton, J.W. (1991). Productivity and profitability of twin births in beef cattle. Journal of Animal Science, 69, 3085-3093.

Echternkamp, S.E. (1992). Fetal development in cattle with multiple ovulations. Journal of Animal Science, 70, 2309-2321.

Echternkamp, S.E., Gregory, K.E. (1999a). Effects of twinning on postpartum reproductive performance in cattle selected for twin births. Journal of Animal Science, 77, 48-60.

Echternkamp, S.E., Gregory, K.E. (1999b). Effects of twinning on gestation length, retained placenta and dystocia. Journal of Animal Science, 77, 39-47.

Giménez, T., Henricks, D.M., Ellicott, A.R., Chang, C.H., Rone, J.D., Grimes, L.W. (1980). Prolactin and luteinizing hormone (LH) release throughout the postpartum period in suckled first-calf beef cow. Theriogenology, 14, 139-149.

Gordon, I. (1994). Laboratory production of cattle embryos. CAB International, Wallingford, UK.

Gregory, K.E., Echternkamp, E., Dickerson, G.E., Cundiff, L.Y., Koch, R.M., Van Vleck, L.D. (1990). Twinning in cattle III. Effects of twinning on dystocia, reproductive traits, calf survival, calf growth and cow productivity. Journal of Animal Science, 68, 3133-3144.

Guerra-Martínez, P., Dickerson, G.E., Anderson, G.B., Green, R.D. (1990). Embryo-transfer twinning and performance efficiency in beef production. Journal of Animal Science, 68, 4039-4050.

Horta, A.E.M. (1994). Etiopatogenia e terapêutica da retenção placentária nos bovinos. Proc. 7as Jornadas Internacionales de Reproducción Animal, Murcia, Espanha, pp. 181-192.

Horta, A.E.M., Vasques, M.J., Leitão, R.M. (1987). The effect of calf removal on the resumption of postpartum ovarian activity in alentejano cows during spring or autumn. Proc. 38th Annual Meeting of the EAAP, Lisboa. Vol. II, p. 730.

Horta, A.E.M., Vasques, M.J., Leitão, R.M., Robalo Silva, J. (1990). Studies on post-partum anoestrus in Alentejano beef cows. In "Studies on the reproductive efficiency of cattle using radioimunoassay techniques", International Atomic Energy Agency, Vienna, pp. 9-19.

Horta, A.E.M., Marques, C.M.F., Vasques, M.J., Leitão R.M. (1992). Effect of inducing calvings on calf birth weight. Proc. 12th International Congress on Animal Reproduction, The Hague, Vol.2, pp. 895-897.

Horta, A.E.M., Marques, C.M., Vasques, M.J., Leitão, R.M., Vaz Portugal, A. (1993). Inducing twinning in beef cows by the transfer of *in vitro* produced embryos. Proc. 5th International Symposium on Animal Reproduction, Luso, Portugal, Vol. II, pp. 163-172.

Izaike, Y., Suzuki, O., Shimada, K., Takenouchi, N., Takanashi, M. (1991). Observation by ultrasonography of embryonic loss following the transfer of two or three embryos in beef cows. Theriogenology, 36, 939-947.

Jolly, P.D., McDougall, S., Fitzpatrick, L.A., Macmillan, K.L., Entwistle, K.W. (1995). Physiological effects of undernutrition on postpartum anestrus in cows. Journal of Reproduction and Fertility, Suppl. 49, 477-492.

Joosteen, I., van Eldik, P., Elving, L., van der Uey, G.J.W. (1987). Factors related to the etiology of retained placenta in dairy cattle. Animal Reproduction Science, 14, 251-262.

Lopes da Costa, L., Lemos Costa, C., Robalo Silva, J. (1994). Efeito da dose de PMSG sobre a fertilidade e a gemelaridade em vacas Charolesas após sincronização do estro com progestagéneos. Proc. 7as Jornadas Internacionales de Reproducción Animal, Murcia, Espanha, pp. 217 (*abstract*).

Lopes da Costa, L., Horta, A.E.M., Robalo Silva, J. (1997). Efeito da transferência contralateral de um embrião produzido *in vivo* ou *in vitro* sobre a manutenção da gestação em vacas aleitantes inseminadas. Proc. 1º Congresso Ibérico de Reprodução Animal, Estoril, Portugal, pp. 334-339.

Lopes da Costa, L.F., Chagas e Silva, J., Robalo Silva, J. (2001). Superovulatory response, embryo quality and fertility after treatment with different gonadotrophins in native cattle. Theriogenology, 56, 65-77.

Lu, K.H., Gordon, I., Chen, H.B., Gallagher, M., McGovern, H. (1988). Birth of twins after transfer of cattle embryos produced by in vitro techniques. Veterinary Record, 122, 539-540.

Marques, C.C., Vasques, M.I., Horta, A.E.M. (1993a). Efeito da amamentação gemelar e retenção placentária no inicio da actividade ovárica pós-parto em vacas Alentejanas. Proc. 5º Simpósio Internacional de Reprodução Animal, Luso, Portugal, Vol. 2, pp. 8-14.

Marques, C.C., Vasques, M.I., Horta, A.E.M. (1993b). Efeito da gemelaridade e da indução dos partos na incidência de retenção placentária: sua prevenção através da administração de PGF2 alfa. Proc. 5º Simpósio Internacional de Reprodução Animal, Luso, Portugal, Vol. 2, pp. 112-117.

Marques, C.C., Baptista, M.C., Pereira, R.M., Vasques, M.I., Lopes da Costa, L.F., Horta, A.E.M. (1995). Influência do sémen de diferentes touros sobre as taxas de fertilização in vitro e de desenvolvimento de embriões em co-cultura. Revista Portuguesa de Zootecnia, 2, 103-110.

McEvoy, J.D., Mayne, C.S., McCaughey, W.J. (1995). Production of twin calves with *in vitro* fertilised embryos: effects on the reproductive performance of dairy cows. Veterinary Record, 136, 627-632.

Morris, C.A. (1974). A review of the genetics and reproductive physiology of dizygotic twinning in cattle. Animal Breeding Abstracts, 52, 803-819.

Nielen, M., Schukken, Y.H., Scholl, D.T., Wilbrink, H.J., Brand, A. (1989). Twinning in dairy cattle: a study of risk factors and effects. Theriogenology, 32, 845-862.

Penny, C.D., Lowman, B.G., Scott, N.A., Scott, P.R., Voelkel, S., Davies, D.A. (1995). Management aspects of induced twinning in beef suckler cows. Veterinary Record, 136, 506-510.

Reichenbach, H.D., Liebrich, J., Berg, U., Brem, G. (1992). Pregnancy rates and births after unilateral or bilateral transfer of bovine embryos produced in vitro. Journal of Reproduction and Fertility, 95: 363-370.

Robalo Silva, J. (1999). Estratégias de preservação da eficácia reprodutiva e da produtividade em bovinos autóctones de carne mantidos em pastoreio em zonas áridas mediterrânicas. Proc. II Congresso Ibérico de Reprodução Animal, Lugo, Espanha, pp. 558-567.

Sakakibara, H., Kudo, H., Boediono, A., Suzuki, T. (1996). Induction of twinning in holstein and japanese black cows by ipsilateral frozen embryos transfer. Animal Reproduction Science, 44, 203-210.

Schmidt, M., Greve, T., Avery, B., Beckers, J.F., Sulon, J., Hansen, H.B. (1996). Pregnancies, calves and calf viability after transfer of *in vitro* produced bovine embryos. Theriogenology, 46, 527-539.

Sinclair, K.D., Broadbent, P.J., Hutchison, S.M. (1994a). The effect of pre and post partum energy and protein supply on the blood metabolites and reproductive performance of single and twin suckling beef cows. Animal Production, 59, 391-400.

Takada, N., Ohisa, N., Numabe, T., Ishikawa, Y. (1991). Production of twin calves by transfer of embryos produced *in vitro*. Veterinary Record, 128, 307

van Wagtendonk, de Leeuw, A.M., Aerts, B.J.G., den Daas, J.H.G. (1998). Abnormal offspring following *in vitro* production of bovine preimplantation embryos: a field study. Theriogenology, 49, 883-894.

Vasques, M.I., Horta, A.E.M., Marques, C.C., Sasser, R.G., Humblot, P. (1995). Levels of bPSPB throughout single and twin pregnancies after AI or transfer of IVM/IVF cattle embryos. Animal Reproduction Science, 38, 279-289.

Vizcarra, J.A., Wettemann, R.P., Spitzer, J.C., Morrison, D.G. (1998). Body condition at parturition and postpartum weight gain influence luteal activity and concentrations of glucose, insulin and non-esterified fatty acids in plasma of primiparous beef cows. Journal of Animal Science, 76, 927-936.

Voelkel, S.A., Xu, Y.X. (1992). Direct transfer of frozen-thawed embryos. Theriogenology, 37, 23-37.