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Superovulation of Mertolenga cows with two FSH preparations (FSH-P and FOLLTROPIN)

Superovulação de vacas Mertolengas com duas preparações de FSH (FSH-P e FOLLTROPIN)

M.A. Quaresma, L. Lopes da Costa*, J. Robalo Silva

CIISA, Núcleo de Reprodução, Faculdade de Medicina Veterinária, Rua Prof. Cid dos Santos, Polo Universitário, Alto da Ajuda, 1300-477 Lisboa

Summary: Response to superovulation, fertilization rate, production of viable embryos and fertility subsequent to superovulation were evaluated in Mertolenga cows treated with two follicle stimulating hormone (FSH) preparations of high (FSH-P, n=11) or low (FOLLTROPIN, n=10) LH (luteinizing hormone) activity. Fertilisation rate and production of viable embryos were significantly lower (P<0.001) in FSH-P than in FOLLTROPIN treated cows. Following transfer of fresh embryos to heifers of the same breed (n=25) a 50 % calving rate was obtained. Fertility of donors at the end of a six-month breeding period that followed superovulation was 95 %. These results show that commercial application of embryo transfer is feasible in Mertolenga cattle.

Resumo: Em vacas dadoras da raça Mertolenga foram comparadas duas gonadotrofinas com alta (FSH-P, n=11) ou baixa (FOLLTROPIN, n=10) actividade LH (luteinizing hormone), sobre a resposta superovulatória, a taxa de fertilização, a produção de embriões viáveis e a fertilidade da época de cobrição após o tratamento superovulatório. A FSH-P induziu uma diminuição significativa (P<0,001) da taxa de fertilização e da produção de embriões viáveis, quando comparada com a FOLLTROPIN. A taxa de parição obtida após transferência de embriões a fresco para novilhas receptoras (n=25) da mesma raça (50%) e a fertilidade das dadoras após a época de cobrição subsequente ao tratamento superovulatório (95%) perspectivam a eficiente aplicação prática da tecnologia de transferência de embriões na raça Mertolenga.

Introduction

Embryo transfer (ET) has been considered as a valuable tool for preservation of endangered species and breeds (Wildt *et al.*, 1992; Solti *et al.*, 2000). This technique includes superovulation of donor cows with gonadotrophins for increasing the number of ova released by the female and artificial insemination (AI) of donors at the superovulated oestrus followed by collection of embryos. These are then frozen and stored or transferred to recipients. However, the superovulatory response (SR) varies widely among individuals and treatments, affecting ET efficiency and limiting its practical use (Hahn, 1992). According to this author, superovulation of donors with identical physiologic characteristics and even superovulation of the same donor at different times can be ineffective in about 15% to 20% of the cases.

The variability of SR has been associated to several factors, including superovulatory (SOV) treatments and specific reproductive physiological characteristics of donors (Bindon *et al.*, 1986; Walton and Stubbings, 1986; Hahn, 1992; Kafi and McGowan, 1997). Gonadotrophin preparations obtained from hypophysial extracts have FSH (follicle stimulating hormone) and LH (luteinizing hormone) biological activity. The FSH:LH ratio affects the SR, and it has been shown that a high LH content negatively affects the SR, fertilisation rate and embryo quality (Donaldson and Ward, 1986; Donaldson *et al.*, 1986; Walton and Stubbings, 1986; Chupin *et al.*, 1987; Herrler *et al.*, 1991; Greve *et al.*, 1995; Kelly *et al.*, 1997). However, LH seems to be important for SR (Herrier *et al.*, 1991), although the optimal FSH:LH ratio varies with the breed of the donor (Chupin *et al.*, 1987).

Data obtained from two native breeds of cattle, Mertolenga (Lopes da Costa, 1995; Lopes da Costa *et al.*, 2001) and Alentejano (Lopes da Costa *et al.*, 1993; Lopes da Costa, 1995), showed that fertilisation rate and production of viable embryos were significantly lower after treatment with eCG (equine chorionic gonadotrophin; high LH content) than with FSH preparations with lower LH activity. Because purified hypophysial extracts with low LH content are commercially available, it was decided to determine its effect on fertilisation rate and production of viable embryos in Mertolenga cattle. In heifers, comparison of a purified commercial FSH preparation (FOLLTROPIN) with a preparation of FSH with high LH contamination (FSH-P) showed that the SR and production of viable embryos were greater in donors treated with the gonadotrophin with lower LH content (Mapletoft *et al.*, 1988).

The objective of the present study was to compare

* Corresponding author: e-mail lcosta@fmv.utl.pt

the response of Mertolenga cows to superovulation with two commercial preparations of porcine hypophysial extracts with different LH activity.

Material and methods

Twenty-one Mertolenga parous cows, with at least 50 days *postpartum*, were randomly allocated to the following groups: FOLLTROPIN (n=10) and FSH-P (n=11). The cows, previously maintained at pasture, were separated from the herd and kept in an open paddock from synchronisation of the reference oestrus until embryo collection. Synchronisation of oestrus was performed by the Crestar® method, with the subcutaneous norgestomet implant maintained *in situ* during 10 days. At the time of implant removal, all cows were injected im with 500 IU of eCG (Chronogest) plus 15 mg of a PGF2α analogue (Prosolvin). The SOV treatment was started during the mid-luteal phase, 10 to 11 days after the reference oestrus. The FSH-P group received a total 36 mg Armour standard units (FSH-P, Schering Corporation, Kenilworth, NJ, USA) and group FOLLTROPIN was treated with 400 mg NIH-FSH-P1 (FOLLTROPIN-V, Vetrepharma, Canada). In both groups the total dose was divided in 8 equal doses, given at 12 hours interval for 4 days. At the time of the sixth and seventh doses all cows were treated with 15 mg of PGF2α (Prosolvin).

Artificial insemination was performed at 12 and 24 hours after the onset of oestrus with frozen-thawed semen previously collected by electroejaculation from a Mertolenga bull of proven fertility. Two straws ($20x10^6$ progressively motile spermatozoa/straw) per insemination were used. Microscopic observation of semen after thawing revealed that progressive motility was about 60%. On day seven (Day 7) after oestrus (Day 0), embryos were collected through uterine flushing by a standard non-surgical procedure (Lopes da Costa *et al.*, 2001). After recovery, the embryos were evaluated and classified for stage of development and quality according to criteria of the IETS (International Embryo Transfer Society, 1998). Embryos on stages 4 to 7 (compact morula to expanded blastocyst) and of quality 1-2 (excellent-good) were considered as viable. Fertilisation rate was calculated by the formula: fertilisation = total nº of fertilised ova / (total nº of oocytes + embryos recovered) x 100. Response to superovulation was considered to be positive when 3 or more *corpora lutea* (CL) were identified by rectal palpation at the time of embryo recovery. After embryo recovery all donors were injected im with 22.5 mg of PGF2α (Prosolvin) and, after joining to a Charolais bull, the traditional 6 month breeding period followed. Dates of calving and the phenotype of calves were recorded at parturition. These data were used for evaluation of fertility at the end of the breeding season (number of cows that calved / number of cows joined to the bull x 100) and for identification of paternity (Mertolenga from AI, *versus* Charolais Crosses from mating).

In vivo evaluation of embryo viability was done by nonsurgical transfer of one fresh embryo to the ipsilateral uterine horn of the CL-bearing ovary of two-year old recipient virgin heifers (n=25) of the Mertolenga breed (18 heifers received embryos from group FOLLTROPIN and 7 heifers received embryos from group FSH-P). Synchronisation of the recipient oestrus was done, like that of donors, by the Crestar® method combined to eCG (400 UI) and to PGF2α (7.5 mg). Embryos were transferred by a nonsurgical standard method (Lopes da Costa *et al.*, 2001), on Day 7 of the oestrus cycle (Day 0 = day of oestrus). Pregnancy of recipients was presumed when plasma P4<1.0 ng mL^{-1} on Day 0, ≥1.0 ng mL^{-1} on Day 7 and ≥2.0 ng mL^{-1} on Day 21. Pregnancy was confirmed by ultrasound scanning at 45 days of gestation and calving data were subsequently recorded. The same operator performed recovery and transfer of all embryos.

Plasma progesterone (P4) was measured using a commercial solid phase radioimmunoassay (RIA) system (Coat-A-Count, Diagnostic Product Corporation, Los Angeles, CA, USA), and samples were run in duplicate. Blood samples were collected from a tail vessel into heparinised tubes at the following times: donors – day of reference oestrus, days of gonadotrophin injections, Day 0 and Day 7; recipients – Days 0, 7 and 21. Immediately after collection, blood samples were centrifuged and plasma was separated and stored at –20 ºC till P4 analysis. The intra-assay and inter-assay coefficients of variation were 8.9% and 9.6%, respectively.

Data were analysed using a statistical package (STATISTICA 5.0, StatSoft Inc., 1995, Tulsa, OK, USA) by Chi-square tests in contingency tables (Fisher exact test), and the non-parametric Mann-Whitney U test where appropriate. Significance was tested at the 5% level ($P < 0.05$). A P value of $0.1 > P > 0.05$ was considered a tendency to significance.

Results

As shown in Table 1, the number of donors with SR and the mean number of embryos recovered were similar in both groups. However, donors from group FOLLTROPIN yielded a significantly higher mean number of viable embryos and a significantly lower mean number of unfertilised oocytes than donors from group FSH-P did. These results are presented in Table 2 as percentages. This table also shows that fertilisation rate was significantly higher in the group FOLLTROPIN than in the group FSH-P. In donors with SR, the mean plasma P4 level on Day 7 was significantly higher in those treated with FOLLTROPIN than in those treated with FSH-P (mean ± standard error of the mean: 29.8 ± 5.4 ng mL^{-1} *versus* 15.3 ± 3.7 ng mL^{-1}; $P < 0.05$).

Figure 1 illustrates the stage of embryo development.

Although there were no significant differences on embryo stage between groups, the number of embryos in the blastocyst stage tended to be significantly greater ($P < 0.06$) in group FOLLTROPIN than in group FSH-P. Three recipients that received embryos from group FOLLTROPIN had P4 concentrations on Day 7 lower than 1.0 ng mL^{-1} (0.6; 0.1; 0.1), although a CL was identified by rectal palpation in all of them. None of the embryos transferred to these heifers survived. Excluding these recipients, pregnancy rate was 73.3% (11/15) and 85.7% (6/7) in groups FOLLTROPIN and FSH-P, respectively. However, calving rate was only 50% (11/22; 5 from group FOLLTROPIN and 6 from group FSH-P). At the end of the traditional six-month breeding period calving rate of donors was 95% (all but one donor from group FSH-P calved). The calves born were all Charolais crosses, except two (one from each group) that were pure Mertolenga, as shown by their phenotype.

Discussion

It is presumed that the two gonadotrophins used in this study varied widely in LH activity, although the exact LH content of the gonadotrophins was not determined. In fact, it is known that FSH-P is a relatively crude porcine pituitary extract with high LH content, although it varies from batch to batch (Donaldson, 1986; Lindsell *et al.*, 1986). FOLLTROPIN is a purified preparation of lower LH activity because of removal of about 80 % of its original LH content (Wu *et al.*, 1988). It is claimed that LH content of gonadotrophins affect response to superovulation, and that fertilisation rate and embryo quality are favoured by treatments with gonadotrophins of low LH activity (Donaldson and Ward, 1986; Donaldson *et al.*, 1986; Herrler *et al.*, 1991), although the correct FSH:LH ratio is breed dependent (Chupin *et al.*, 1987).

The results obtained in this study show that the mean total number of embryos recovered was similar to that obtained in other breeds of cattle also submitted to superovulation with identical FSH preparations (Donaldson and Ward, 1986; Mapletoft *et al.*, 1988; Kelly *et al.*, 1997; Chagas e Silva *et al.*, 2002a). Results suggest that LH activity of the gonadotrophin had a significant influence on fertilisation rate and on embryo quality. Results obtained previously, also in Mertolenga, (Lopes da Costa *et al.*, 2001) showed that eCG, a preparation with high LH activity, had a negative effect upon embryo quality when compared to FSH-P. The negative effects of non-purified gonadotrophin preparations upon fertilisation and embryo quality may be due to problems with transport of the gametes to the fertilisation site or to oocyte maturation problems (Greve *et al.*, 1995).

In donors with SR, mean plasma P4 concentrations on Day 7 were significantly higher in group FOLLTROPIN than in group FSH-P, which might be explained by the higher mean number of ova recovered observed in the former group when compared to the latter group. The fact that the stage of development of embryos produced from FOLLTROPIN-treated cows was more advanced than that of embryos produced by donors treated with FSH-P may be related to differences in P4 concentrations on Day 7. In fact, it has been reported that, in the cow, development of the embryo is accelerated when there is a faster increase of post-ovulatory P4 concentration (Mann and Lamming, 2001).

The occurrence of abnormal luteal function following oestrus synchronisation of Mertolenga recipient heifers has been reported earlier (Lopes da Costa *et al.*, 1992). These authors have reported that below a minimum P4 level (1.0 ng mL^{-1} on Day 7), embryo survival is significantly reduced or null, an observation that was confirmed in a more recent study (Chagas e Silva *et al.*, 2002b). Foetal losses observed in this study were attributed to severe management changes dependent on adverse climatic and nutritional conditions, which negatively affected fertility of the whole group of heifers in the herd. However, the reason why only pregnancies originated from embryos from group FOLLTROPIN were interrupted could not be explained.

The donors conception rate subsequent to superovulation was similar to that reported in studies that used equivalent treatments (90 %) (Lopes da Costa *et al.*, 2001) and to that of non-treated cows of the same breed submitted to the same traditional mating system (92 %) (Bettencourt *et al.*, 1987). The fact that 2 pregnancies were not interrupted after injection of the cows with a luteolytic agent, on day of embryo recovery, may suggest a luteotrophic/antiluteolytic effect of the

Table 1 - Effect of gonadotrophins on superovulatory response (SR) in Mertolenga cattle.

Parameter	Gonadotrophin		P Value
	FOLLTROPIN	FSH-P	
Donors – n	10	11	-
positive SR	9 (90.0%)	8 (72.7%)	1.00
Total ova recovered	10.0 ± 2.2	8.5 ± 2.4	0.64
Viable embryos	6.4 ± 1.2	1.6 ± 0,7	0.005
Degenerated embryos	2.8 ± 0.9	1.1 ± 0.4	0.10
Non-fertilised oocytes	0.8 ± 0.3	5.8 ± 0.9	0.05

Values for recovery of embryos are means ± standard error of the mean, per cow with positive SR

Table 2 - Effect of gonadotrophins on fertilisation rate and on embryo production in Mertolenga cows.

Parameter	Group		P value
	FOLLTROPIN	FSH-P	
Total ova recovered – n	90	68	0.77
Viable embryos – n (%)	58 (64.4%)	13 (19.1%)	0.0003
Degenerated embryos – n (%)	25 (27.8%)	9 (13.2%)	0.07
Non-fertilised oocytes – n (%)	7 (7.7%)	46 (67.6%)	0.0001
Fertilisation rate - n (%)	83 (92.2%)	22 (32.3%)	0.0002

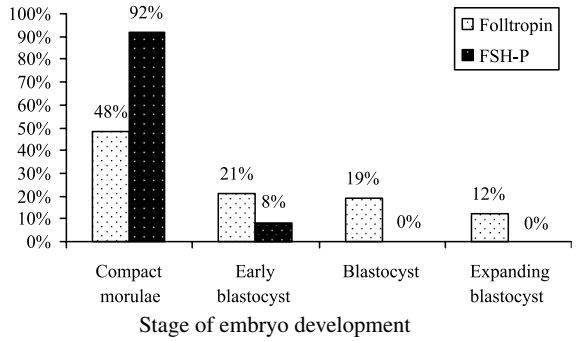

Figure 1 - Effect of gonadotrophins on stage of embryo development.

gonadotrophin and/or of the retained embryo.

In conclusion, gonadotrophins with high LH content seem to have negative effects on fertilisation rate and production of viable embryos in Mertolenga cattle. The reasonable calving rate obtained after transfer of fresh embryos to heifers, and the fact that fertility of donors subsequent to superovulation was similar to that observed in non-treated cows kept under the same management system, suggests that ET has potential practical application in this breed.

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