A NOVEL SUPEROVULATORY PROTOCOL FOR ENHANCEMENT OF BOVINE *IN VIVO* EMBRYO YIELD

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Superovulation protocols are named according to the time from the first PGF2α treatment to the time of progesterone (P4) source removal, which occurs before the induction of ovulation to avoid the deleterious effect of high P4 concentration on embryo quality during the ovulation period. Therefore, when the PGF2 α is given on Day 6 AM and the P4 device is removed on Day 7 AM, the treatment is called "P-24" (i.e., a 24-h interval between PGF2α and P4 device removal). The purpose of this study was to evaluate embryo production and embryonic quality of cross bred cows, using two different protocols with different duration of progesterone exposure.

Six cross bred cows were gynaecologically examined by rectal palpation and ultrasonography before the start of the experiment, in order to determine their cyclicity and absence of diseases or abnormalities in their reproductive tract. The animals were divided into two groups; Group I: Treatments P12 (P12, n=3) and Group II: Treatments P24 (P24, n=3) in an experimental design.

The donor animals were selected with random stages of the estrous cycle (Day 0) and a progesterone device (TRIU-B® -Virbac Animal Health, São Paulo-SP) was inserted. The Superstimulatory treatment was initiated in D7 with the application of FSHp (Folltropin V ® - Bioniche Animal Health, Belleville, Ontario, Canada) in eight decreasing doses, administered at every 12 hours. Along with the fifth dose of FSHp, 500µg Cloprostenol (Pragma™, Intas Pharmaceuticals, India) was administered. The progesterone device was removed 12h (Group I) and 24 h (Group II) after the first injection of Cloprostenol. Twelve hours after the eighth dose of FSHp (D11) 25µg of Buserelin was administered (GnRH – Gynarich, Intas Pharmaceuticals, India) and the inseminations was done 12 and 24 hours later, using semen of bulls with known fertility. Embryos were collected in the forenoon of D18 of the schedule using nonsurgical method of embryo transfer. For the evaluation of the superovulatory responses ultrasound examinations were made for counting follicles and corpus luteum. An ultrasound scanner B Aloka SSD 500Vet (Aloka CO., LTD. - Tokyo,

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Japan) were used with a transrectal linear transducer 7.5 MHz: at D12 for counting superstimulated follicles (follicles ≥ 8 mm); at D15, for counting non-ovulated follicles (follicles > 8 mm) and at D20, for counting the number of corpus luteum before embryo collection. The embryos were collected by a non-surgical transcervical method with the aid of a Foley catheter. The uterine flush was performed with 1 liter of modified saline phosphate solution (ViGRO complete flush, Bioniche). The recovered embryos were evaluated using a stereoscopic microscope (Nikon SMZ645, Nikon ®, Tokyo, Japan) and classified according to the standards of the International Embryo Transfer Society (IETS, 1998), according to the stage of development (morulae, compact morulae, initial blastocyst, blastocyst, expanded blastocyst, hatching blastocyst and hatched blastocyst) and the quality (Grades 1, 2, 3, degenerated and nonfertilized ova). It was assumed that viable embryos would be those classified as grade 1, 2 and 3; freezable structures: grades 1 and 2; and unviable structures classified as degenerated or non-fertilized ova. All transferable embryos were vitrified using open pulled straw method for further use. The student's t test was used to compare the two different protocols with different duration of progesterone exposure.

The number of follicles after superovulation were 7.00 ± 0.58 and 17.33 ± 6.44 for P12 and P24 treatments, respectively suggested that the superstimulatory response could be greater with a possible adjustment of the doses used, as described by Nasser (2006) in *Bos indicus* (18.4 ± 3.4 and 23.0 ± 3.7, respectively) and Bó *et al*. (2002) in *Bos taurus* (18.6 ± 2.5). In general, *Bos taurus* and *Bos indicus* have different number of follicles (Baruselli *et al*., 2006) and *Bos indicus* recruit a larger number of follicles than *Bos taurus* (Carvalho *et al*., 2008) probably because they had higher plasma IGF-I and lower concentrations of FSH (Bo *et al., 2003*). No significant differences ($P > 0.05$) were found between treatments on the number of follicles after superovulation. This might be due to the different exposures to progesterone did not have significant effect on the number of follicles. The amount of non-ovulated follicles were different between P12 and P24 treatments(2.67 ± 0.67 vs 0.66 ± 0.33) possibly due to the injection of the same doses of FSH and to the ovulation induction with GnRH analogue, which has been performed at the same time in all experimental groups.

The number of corpus luteum at the time of embryo collection observed in this study in crossbred cows was 4.33 ± 1.2 and 16.33 ± 6.89 for P12 and P24 treatments, respectively. The donors of this breed have a great potential for embryo production with an adequacy of superovulation protocols. Carvalho *et al*. (2008) found that 22.4 ± 0.5 and 16.6 ± 3.4 corpus luteum for Nellore and Angus x Hereford, respectively. The differences found might indicate that future adjustments in superovulation protocols and selection of cross bred cows as donor animals may increase the superovulatory response.

The total number of structures collected was higher ($n=45$, $P > 0.05$) in cows superovulated with protocol P24 than in cows superovulated with the P12 ($n=11$) while the number of viable embryos was higher (41vs 6, $P > 0.05$) in P24 than

in the Control. There were no significant differences (P > 0.05) on the recovery rate and on the number of freezable and unviable structures between treatments. Silva *et al*. (2002) showed the positive effect of the higher plasma progesterone level on the quantity and quality of embryos in *Bos taurus*. The increase in progesterone due to the presence of the implant could be the reason for cows superovulated with P24 has a better embryo yield than cows superovulated with P12 treatment. Moreover, Carvalho *et al*. (2008) studied the effect of progesterone in a fixed-time artificial insemination (based on progesterone device) in *Bos taurus, Bos indicus* and *Bos taurus x Bos indicus* and observed that *Bos indicus* presented greater progesterone serum concentration than *Bos taurus* and crossbred animals. They argued that *Bos indicus* have lower speed to metabolize progesterone. Therefore, the more suitable protocol for *Bos indicus* would be P24, as this group keep higher progesterone levels for longer period, according to their metabolism, due to the anticipation in the removal of the progesterone device in these animals allowing that pulses of LH remain frequent and do not hinder the development of follicles (Baruselli *et al*., 2006). Thus, keeping the progesterone device longer, the influence of progesterone on the modulation of LH pulses was sufficient to prevent ovulation from occurring before the full maturation of follicle/oocyte (Carvalho *et al*., 2008). P24 presented better embryo yield than the P12 treatment, showing that these protocols are able to produce greater quantity of embryos with higher quality in crossbred cows. Embryo recovery rate were 65.56 ± 8.68 % and 89.77± 5.37% for P12 and P24 treatments, respectively (P > 0.05). The low recovery rate of the P12 treatment has resulted in fewer total and viable structures on the animals of this group. Furthermore, the smallest amount of viable structures presented by the P12 may be related to the concentration of progesterone supported only by corpus luteum formed in the pre-synchronization unlike the P24. Further trials will be conducted to facilitate the application of embryo transfer program to enhance the embryo yield.

The present study was conducted to evaluate embryo production and embryonic quality of cross bred cows, using two different protocols (P12 and P24) with different duration of progesterone exposure. P24 presented better results than the P12 treatment showing that these protocols are able to produce greater quantity of embryos with higher quality in crossbred cows.

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