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Follicle Wave and Natural Estrus Synchronization Superovulation in Holstein Cows

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ABSTRACT

The effects of two superovulation protocols were compared (synchronization of the follicle wave SFW and natural estrus NE) in embryos collected from Holstein cows. Twenty cows were chosen as donors, body condition of 2.75 - 3.5; between 40 and 60 months, with normal cycles and no breeding problems. The cows were randomly assigned to SFW treatment (n=10), and the NE treatment (n=10). The SFW group was synchronized with intravaginal progesterone plus estradiol benzoate, on day 0, and increasing doses of FSH for 4 days, twice a day, from the 4th on. The implant was removed on the 6th day, and prostaglandin was applied. On the 8th day, insemination was made at 6 am and 6 pm. The NE group received increasing doses of FSH twice a day, during 4 days, from the 10th day on on. On the 12th day prostaglandin was administered, and insemination took place on the 14th day, at 6 am and 6 pm. The embryos were recovered from the two groups without surgery, 7 days after the first artificial insemination. The values of embryos for transference were 5.7 + 0.76 and 2.8 + 0.31 ($P < 0.05$) for SFW and NE, respectively.

Key words: *bovine, progesterone, estrus, prostaglandin*

INTRODUCTION

Understanding follicle dynamics and its self-regulated processes, is the bases of the new alternatives to control estrus cycle in cows. The generation of new knowledge about follicle dynamics in bovine ovaries helps reformulate the traditional methods of estrus control, generating new proposals of estrus and ovulation induction and synchronization.

Superovulation programs (SVP) have been developed in donor cows: based on natural estrus (NE), or by follicle wave synchronization (SFW) (String fellow and Seidel, 2000; Baruselli *et al*., 2006; Bó, Tríbulo and Mapletoft, 2011).

Superovulation includes dealing with endocrine processes during the estrous cycle, until ovulation of follicles that grew as a response to hormones occurs. It is triggered by follicle growth waves to produce ovocytes which can be fertilized, and seven days later the embryos can be recovered and classified according to their stage or quality, as established by the International Embryo Transference Society (IETS) (Bochesta and Nasser; 2005; Belacuba, 2007; Amaya, 2010; Luca and Castrillón, 2011).

The purpose of this paper was to compare the effects of two superovulation protocols (synchronization of the follicular wave, SFW, and natural estrus, NE) in the quality of embryos collected from Holstein cows.

MATERIALS AND METHODS

Research took place in the province of Azuay, Cuenca canton, parishes of Tarqui, Cumbe and Victoria del Portete, located on 20; 18 and 8 km from the city of Cuenca, respectively.

Twenty Holstein cows were chosen to make two 10-animal groups, based on the following parameters: body condition (2.75 - 3.5); 40 - 60 months old; normal estrous cycles, minimum of 60 open days, and no breeding problems, according to previous gynecological and ecographic assessments.

The following protocols were applied:

SFW group (n=10):

- Day 0: intravaginal implant (IV) of 1.38 g of progesterone (P4; CIDR®, Zoetis, Mexico), 2 mg of estradiol benzoate (EB; Gonadiol®, Syntex, Argentina) IM; and 100 mg of P4 (Gestavec® Vecol SA, Colombia) IM.
- Day 4: 40 mg of Folltropin® (Bioniche, Canada) IM twice a day (6 am and 6 pm).
- Day 5: 30 mg of FSH-p/IM twice a day(6 am and 6 pm).
- Day 6: 20 mg of FSH-p and 0,5 mg of D-chloprostenol (Estrumate® Intervet Schering-Plough, NJ, USA) IM twice a

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day(6 am and 6 pm); ó IV was removed at 6 pm.

- Day 7: 30 mg of FSH-p/IM twice a day(6 am and 6 pm).
- Day 8: artificial insemination(AI) at6 am and 6 pm, Gonadorelin (GnRH; Fertagyl®, Intervet Schering-Plough, NJ, USA) 500 ug/IM at 6 am.

NE group (n=10):

- Day 0: natural estrus.
- Day 10: observation of lutheal body and absence of the dominant follicle, through transrectal echographic scanning and 40 mg of FSH-p/IM twice a day (6 am and 6 pm).
- Day 11: 30 mg of FSH-p/IM twice a day(6 am and 6 pm).
- Day 12: 20 mg of FSH-p and 150 ug of D-cloprostenol/IM twice a day(6 am and 6 pm).
- Day 13: 30 mg of FSH-p/IM twice a day(6 am and 6 pm).
- Day 14: IA at 6 am and 6 pm, GnRH 500 ug/IM at 6 am.

Seven days after the first AI in the two groups, the embryos were collected without surgery, using saline buffer solution (PBS).

The embryos were spotted and isolated in a Holding maintenance medium, using a diascopic light stereoscope (Vigro™, Bioniche, Pullman, WA, USA). Quality assessment was made according to the embryo classification parameters (IETS, 2000). The variables studied were total recovered structures (ovocytes and embryos), transferable embryos (quality embryos 1 and 2), and non-transferable embryos (quality embryos 3, degenerated embryos and non-fertilized ovocytes). The basic statigraphs were estimated, and the Student test was made to independent samples; $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The current protocols include estrogens (E2) and P4, which control the appearance of the follicle wave (Bó, Tríbulo and Mapletoft, 2011), and the moment of ovulation (Baruselli *et al*., 2006) have made possible the application of superovulation treatments (SVT) in donor cow groups, at any stage of estrus. Comparison of the SVT (which included NE), and SFW in terms of structures recovered, resulted in 6.5 + 2.02 and 8.7 + 1.86, respectively, with $P > 0.05$ (see table). It corroborated the study made by Bó *et al*. (1996), where no significant differences were found concerning the amount of ovocytes and embryos recovered. It compared a superovulation treatment with progesterone + beta estradiol to synchronize the wave, to the control group that took estrus as reference. Similar results were found by Goulding *et al*. (1990), with 8.5 + 0.9 ovocytes and embryos in SVT, and initiated treatment with FSH on the 10th day of estrus.

Several studies (Bo *et al.*, 1995; Mapletoft, Bennett Steward and Adams, 2002) coincided in that starting a superovulation treatment requires the absence of a dominant follicle. It can be achieved with E2 + P4 to synchronize follicle wave, or initiate treatment with FSH-P between days 10 and 12 during the cycle. In both cases, emergence follicles with similar superovulation response were achieved; additionally, a similar amount of ovocytes and recovered embryos were generated, using the proper gonadotrophin dose were achieved.

Concerning embryo quality, a significant difference was observed between the transferable structures achieved by SFW, when the SOV treatment was made, taken NE 5.7 + 0.76 and 2.8 + 0.31 as reference ($P < 0.05$), respectively (see table). These results match the parameters of the International Embryo Transfer Society (IETS, 2000), in relation to the fact that the structures recovered must be 40 - 50% transferable. Exogenous P4 applied to the SFW treatment could guarantee the generation of transferable structures. P4 is known to play a role in ovocyte quality. Salhab *et al*. (2011) claimed that P4 has an important effect on ovocyte quality because the cummulus cell apoptosis frequency decreases during maturation (Aller *et al*., 2015).

Rivera *et al.* (2011) described the existence of a positive relationship between high blood P4 levels during follicle growth and embryo quality, in dairy cows. However, other authors, like Fair and Lonergan (2012), indicated that such effect has not been confirmed, but it might be related to a decline in ovocyte exposure to LH, thus preventing premature maturation (Cerr *et al*., 2011). Synchronization of the follicle wave dependos on GnRH to facilitate development of a wave of follicles by the action of FSH and P4, which allow for maturation of ovocytes. Also, an increase in LH pulse frequency triggers ovulation.

Regarding non-transferable structures, the results were 3.0 + 0.86 and 3.67 + 1.45 for SFW and NE, respectively (see table). Jiménez (2009) said that there are external factors that affect superovulation response: species, breed, age, physiological state, fertility, characteristics of follicle waves, individuality, and others. It would determine that regardless of the superovulation treatment applied, there will always be non-transferable structures.

CONCLUSIONS

The superovulation treatment based on SFW produces a greater amount of transferable embryos in comparison to the treatment based on NE. The SFW protocol has the advantage of better follicle wave due to ablation of the dominant follicle. It also permits synchronization of follicle wave. Besides, exogenous P4 contributed to more high quality ovocytes. The addition of GnRH during insemination provided synchronization at ovulation, producing higher quality and quantity of transferable embryos.

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Table. Results from embryos in treatments with SFW and NE

Structures *Estructuras*	SFW group (n=10)	NE group (n=10)	P value
Recovered (ovocytes and embryos)	8.7 ± 1.86	6.5 ± 2.02	NS
Transferable embryos (Quality 1 and 2)	5.7 ± 0.76	2.8 ± 0.31	*P < 0.05
Non transferable (Quality 3, degenerated and non-fertilized ovocytes)	3.0 ± 0.86	3.67 ± 1.45	NS

NS indicate insignificant differences SFW: synchronization of follicle wave
NE: natural estrus