Journal of Animal Production, 30 (2), 54-58, 2018

Assessment of two Superovulation Protocols for Embryo Production in Holstein Cows

Manuel E. Soria Parra; Carlos A. Soria Parra; Silvana Méndez Álvarez; Daniel Argudo Garzón; Guillermo Serpa García; Carlos Torres Inga; Andrés Galarza Lucero

Faculty of Agricultural Sciences, School of Veterinary Medicine and Zootechnical Studies, University of Cuenca, Yanuncay Campus, Ecuador

manuel.soria@ucuenca.edu.ec

ABSTRACT

The aim of this paper was to compare the effects of two superovulation protocols (synchronization of the follicular wave (SFW), and natural estrus (NE) induction of embryos produced for transference in Holstein cows. Twenty cows were chosen as donors, with a body condition (BC) of 2.75-3.5; 40-60 months old; 1-2 previous gestation services, and without reproductive problems. Two superovulation protocols were applied: SFW and NE. SFW was not observed to produce more embryos than NE in the blastocysts and morula stages.

Key words: *embryos, blastocyst, morula, superovulation*

INTRODUCTION

The superovulation protocols imply risk and effort; they favor embryo transfer development for genetic improvement programs, with a significant increase in the production of transferable embryos. It has been demonstrated in several field investigations in dairy and double purpose cattle (Callejas *et al.*, 2008; Bo, Guerrero, and Adams, 2008).

Progestogens and estrogens are combined for SFW protocols, similar to the method based on natural estrus as reference (Carballo *et al.*, 2009; Baruselli *et al.*, 2015; Soria *et al.*, 2017). The SFW protocols used in combination with GnRH during artificial insemination (AI) enables ovulation as a way to get a larger number of transferable structures (Chankitisakul *et al.*, 2017).

Regardless of the estrus cycle moment, and fixed-time and artificial insemination (FTAI), superovulation has a positive effect on the commercial application of embryo transference strategies, since they facilitate work protocols. However, certain problems (individual condition or high temperatures) that lead to the absence of ovulating response are still taking place. The most commonly used method to synchronize follicular wave emergence for SOV is parenteral application of progesterone and estradiol (Barros *et al.*, 2008; Carballo *et al.*, 2009; Baruselli *et al.*, 2015).

The goal of embryo transfer (ET) is to increase the number of progenies with high genetic value, using superovulation (SO) schemes through synchronization of follicular waves (SFW), for which estrus is induced in the embryo donors and receptors (Callejas *et al.*, 2008; Bo and Mapletof, 2014), in order to produce fertile oocytes from the donors, which are then inseminated. The resulting embryos are then transferred to receptors or are preserved safely (Becaluba, 2007).

The aim of this paper was to compare the effects of two superovulation protocols (synchronization of the follicular wave (SFW), and natural estrus (NE) induction of embryos for transference produced in Holstein cows.

MATERIALS AND METHODS

This research took place in the province of Azuay, Cuenca canton, parishes of Tarqui, Cumbe and Victoria del Portete. Twenty Holstein cows were chosen and two groups of 10 animals each were made, according to their body conditions (2.75-3.5); 40-60 months old; normal estrus cycles, minimum of 60 open days, and no reproductive problems, based on previous gynecological and ecographic assessments.

Two superovulation (SO) protocols were carried out: synchronization of the follicular wave (SFW) and natural estrus (NE), in groups 1 and 2, respectively, according to Soria *et al.* (2017).

Seven days after the first AI, superovulation was verified in the two groups by ecographic monitoring of the ovaries. Embryo collection was made according to the established technique: washing of perineal area, disinfection in the lumbosacral area, and application of lidocaine chlorhydrate for application anesthetics in the low epidural area. The foley probe was introduced in the vagina through the cervix, and the anterior end of the probe was directed to the ipsilateral uterine horn, where most luteal bodies are. The anterior end was placed 5 cm facing cranial position of the uterine horn bifurcation, then the balloon was inflated depending on the size of the cervix.

One end of the probe was connected to the phosphate buffer solution (PBS) in "Y" position, and the other end was linked to the filter where the embryos were received. The filtered liquid was collected in a graduated container to measure the volume and make sure the output volume corresponded to the input volume. When the foley probe was in place, the mandrel was withdrawn, and it was connected to the free end of the tube in "Y" position. Washing was made with 50 mL of the collection medium (PBS) at a time (kept at 37.5 ºC), the inlet clamp was shut when 500 mL were in each horn.

The embryos were located using a diascopic light stereoscope, and isolated in Holding maintenance medium, (Vigro™, Bioniche, Pullman, WA, USA). Quality assessment was made according to the embryo classification parameters of the International Embryo Transfer Society.

The descriptive statistics were obtained for the variables studied with 95% confidence interval, and the normality tests devised by Shapiro Wilk to determine data normality. The Student T test was used to compare the treatment means for independent samples, with or without homogeneous variances (Levine test).

RESULTS AND DISCUSSION

The three variables studied were distributed normally, according to the Shapiro-Wilk test for small samples; in all cases the values were ($P < 0.05$) (within 0.114-0.952).

Comprehension of the function of follicular dynamics and its self-regulation mechanisms is fundamental to understand the rationale of new alternatives for estrus cycle control in female bovines to ensure the best possible application of follicular wave synchronization, and be successful in bovine embryo production and transference (Baruselli *et al.*, 2006; Small, Colazo, Kastelic, Mapletoft, 2009; Amaya, 2010; Bo and Mapletof, 2014; and Hasler, 2014).

The protocol used for SFW includes monitoring of the follicular wave from the onset (emergence), to recruit follicles and prevent atresia with E2 and P4, and simultaneous growth of several follicles with FSHp to produce more embryos. A comparison of SOV, including SFW and NE for the number of embryos recovered, showed no significant difference in the means of four embryos in favor of SFW. A comparison of the highest values in the treatments showed that SFW produced six embryos more than NE (Table 1).

These results coincided with the reports of Bo, Guerrero, and Adams (2008), and Steel and Hasler (2009). In that study, no significant differences were observed between the total embryo means for SFW and NE.

Several superovulation methods exist nowadays to synchronize and control follicular wave, in order to perform fixed-time inseminations (Bravo, 2017). They have been tested in production Holstein cows with acceptable results in terms of number and quality of embryos achieved, even with the possibility to combine them with sexed semen (Soares *et al.*, 2011), thus allowing specialists to choose the insemination hours based on the expected ovulation time (Argudo *et al.*, 2016). It corroborated these results, since the control of the follicular wave potential during ovulation and embryo collection were evidenced.

Singh, Domínguez, Jaiswal and Adams (2004), and Vieira *et al.* (2014) observed that the number of follicles at the beginning of a follicular wave is positively correlated to the superovulating response, and top quality embryos. The stages of cytological evolution in which the embryos are harvested, also play a part in this, coinciding with this research, where the highest quality and quantity of embryos were obtained using the SFW protocol.

No significant differences between the means were observed, regarding the number of blastocyst embryos in the treatment means. During the maximum production of blastocysts, the SFW cows were more than two-fold higher than blastocyst production in NE cows, which demonstrated the variability of individual conditions (Table 2).

The control of follicular wave emergence through ablation or dominant follicles, using various treatments to achieve a new follicular wave is the most commonly used method of SOV, according to reports (Bo, Guerrero, and Adams, 2008; Amaya, 2010; and Baruselli *et al.*, 2015). Bo and Mapletof (2014) also reported higher efficiency resulting from total embryo transference to receptors with blastocyst embryos in SFW, compared to NE.

Pérez (2011) found amounts of transferable embryos using experimental SOV. He pointed that the largest proportion of these embryos were first quality, which confirms the results of this study, regarding the total quantity of viable structures collected, and their quality.

Spencer and Bazer (2004) said that morula is an early embryo stage consisting of a compact mass of blastomeres with populations of inner and outer cells. The former develop wall units that enable intercellular communication, and allow them to stay, developing cell-cell adhesion.

No significant statistical difference was observed for treatments in Group 1 SFW and Group 2 NE in the morula stage, which demonstrated that the treatments were not influenced by this stage (Table 3).

López *et al.* (2008) and Baruselli *et al.* (2015) suggest transferring intact morulae made of interacting cells, thus allowing them to continue embryo development. In this study, morulae were generated by transferable SFW and NE likely to be implanted.

In this research, the cows that received the SFW treatment produced more quality blastocysts and morulae; therefore, they showed a better response to treatment, which is positively correlated with the quantity and quality of structures produced. These results coincided with Baruselli *et al.* (2006).

Amaya (2010) claimed that a female donor can produce structures with preserved pellucid areas during the morula, compact morula, or blastocyst stages, after 6.5 or 7 days of growth. In the current study, morulae were generated through SFW and NE, with no significant differences after seven days.

CONCLUSIONS

SFW was not observed to produce more embryos than NE in the blastocyst and morula stages.

REFERENCES

AMAYA, A. (2010). *Efecto del diámetro del folículo ovulatorio, tamaño del cuerpo lúteo y perfiles de progesterona sobre la tasa de preñez en la hembra receptora de embriones bovinos*. Retrieved on April 12, 2012, from http://www.bdigital.unal.edu.co/3775/1/780174.2010.pdf.

ARGUDO, D. E.; SORIA, C. A.; DURÁN, J. C.; BRAVO, S. E., ALVARADO, J. P. y ALBERIO, R. H. (2017). Setting Time of Artificial Insemination of Superovulated Lactating Holstein Cows with Sex-Sorted Sperm. *Animal Reproduction*, *14* (1), 286-286.

BARROS, C. M.; BARCELOS, A. C.; GOUVEA, L. M.; MENEGHEL, M.; BARCELOS, D. S.; BARCELOS, L. N. *et al.* (2008). Improvement of a Superovulatory Protocol in Nelore Cows: Replacing the Last Two Doses of pFSH by eCG. *Reprod. Fertil. Dev.*, *20* (1), 152-155.

BARUSELLI, P. S.; SÁ FHILO, M.; MATINS, C. M.; NASER, L. F.; NOGUEIRA, M. E., BARROS, C. M. *et al.* (2006). Superovulation and Embryo Transfer in *Bos indicus* Cattle. *Theriogenology*, *65* (1), 77-88.

BARUSELLI, P. S.; MARQUES, M. O.; VIEIRA, L. M.; KONRAD, J. L.; CRUDELI, G. A. (2015). Aplicación de biotecnologías para una mayor producción de terneros, *Rev vet*, *26* (2), 154-159.

BECALUBA, F. (2007). *Factores que afectan la superovulación en bovino*. Retrieved on May 13, 2011, from http://www.produccion-animal.com.ar/informacion_tecnica/transplante_embrionario/17-superovulacion.pdf.

BÓ, G.A.; GUERRERO, D. y ADAMS, G. P. (2008). Alternative Approaches to Setting up Donor Cows for Superstimulation. *Theriogenology*, *69* (1), 81-87.

BO, G y MAPLETOF, R. (2014). Nuevos protocolos de superovulación para programas de transferencia de embriones en bovinos. *Spermova*, *1* (1), 26-33.

BRAVO, S. (2017). *Efecto de la bST aplicada al inicio de un programa superovulatorio con FSHp y al momento de la inseminación artificial sobre la respuesta ovárica y la producción de embriones transferibles en vacas mestizas*. Tesis de maestría, Universidad de Cuenca, Azuay, Ecuador.

CALLEJAS, S.; CABODEVILA, J.; PALMA, G.; ALBERIO, R. H.; TORQUATI, S.; BUTLER, H. *et al.* (2008). Evaluación de los Embriones Bovinos. En *Memorias del curso de superovulacion y transferencia de embriones bovinos*. Mar del Plata, Argentina: INTA Balcarce

CARBALLO, D.; TRÍBULO, A.; TRÍBULO R.; TRÍBULO, H. y BÓ G.A. (2009). Superovulatory response in beef cattle treated during the first follicular wave

following synchronization of ovulation with a progestin device and GnRH. *Reprod. Fertil. Dev*, *21* (1), 242-243.

CHANKITISAKUL, V.; PITCHAYAPIPATKUL, J.; CHUAWONGBOON, P.; RAKWONGRIT, D.; SAKHONG, D.; BOONKUM, W. *et al.* (2017). Comparison of three Superovulation Protocols with or without GnRH Treatment at the Time of Artificial Insemination on Ovarian Response and Embryo Quality in Thai Native Heifers. *Tropical animal health and production*, *49* (3), 633-639.

HASLER, J. F. (2014). Forty Years of Embryo Transfer in Cattle: A Review Focusing on the Journal the Growth of the Industry in North America, and Personal Reminisces. *Theriogenology*, *81* (1), 152-169.

LÓPEZ, A. P.; GÓMEZ, L. F.; RUIZ, Z. T.; OLIVERA, M. y GIRALDO, C. A. (2008). Reconocimiento materno de la preñez e implantación del embrión: modelo bovino. *Analecta Veterinaria*, *28* (1), 1-6.

PÉREZ, L. (2011). *Evaluación de la respuesta superovulatoria utilizando la ablacion folicular como alternativa para la induccion del crecimiento de una nueva onda folicular en donadoras Brahman*. Retrieved on May 10, 2013, from http://iracbiogen.com.ar/admin/biblioteca/documentos/TESIS%20IRAC%20-%20PEREZ%20SANDOVAL.pdf.

SINGH, J.; DOMÍNGUEZ, M.; JAISWAL, R. y ADAMS, G. P. (2004). A Simple Ultrasound Test to Predict the Superstimulatory Response in Cattle. *Theriogenology*, *62* (1), 227-243.

SMALL, J. A.; COLAZO, M. G.; KASTELIC, J. P. y MAPLETOFT, R. J. (2009). Effects of Progesterone Presynchronization and eCG on Pregnancy Rates to GnRH-based, Timed-AI in Beef Cattle. *Theriogenology*, *71* (1), 698-706.

SORIA, M. E.; SORIA, C. A.; ARGUDO, D.; SERPA, G.; MÉNDEZ, S.; TORRES, C. *et al.* (2017). Superovulación con sincronización de la onda folicular y con celo natural en vacas Holstein. *Revista de Producción Animal*, *29* (1), 40-43.

SOARES, J. G.; MARTINS, C. M.; CARVALHO, A. T.; NICACIO, A. C.; ABREU-SILVA, A. L.; CAMPOS FILHO, E. P. *et al.* (2011). Timing of insemination using sex-sorted sperm in embryo production with Bos indicus and Bos taurus superovulated donors. *Animal Reproduction Science*, *127* (3-4), 148-153.

SPENCER, T. E. y BAZER, F. W. (2004). Conceptus signals for establishment and maintenance of pregnancy. *Reproductive Biology and Endocrinology*, *2* (1), 49.

STEEL, R.G., HASLER, J. F. (2009). Comparison of three Different Protocols for Superstimulation of Dairy Cattle. *Reprod Fertil Dev*, *21* (1), 246-246.

VIEIRA, L. M.; RODRIGUES, C. A.; MENDANHA, M. F.; Sá FILHO, M. F.; SALES, J. N. S.; SOUZA, A. H. *et al.* (2014). Donor Category and Seasonal Climate Associated with Embryo Production and Survival in Multiple Ovulation and Embryo Transfer Programs in Holstein Cattle. *Theriogenology*, *82* (2), 204-212.

.

Received: 1-10-2018
Accepted: 1-16-2018

Table 1 Embryos achieved through synchronization of follicular wave (SFW) and natural estrus (NE)

	Treatment														Sig.
	SFW					CI (95%)		NE					CI (95%)		
	Mean	SE±	Med	Min	Max	IL	SL	Mean	SE±	Med	Min	Max	IL	SL	
Total embryos (units)	12	1.8	12	4	20	7.6	15.6	8	0.9	8	4	14	5.9	10.1	N/S

N/S = no significant differences according to T-Student test (P = 0.095)
SE = standard error; Med = median; Min = minimum value; Max = maximum value; CI = confidence interval

Table 2 Number of embryos in the blastocyst stage through SFW, in relation to NE

	Treatment														Sig.
	SFW					CI (95%)		NE					CI (95%)		
	Mean	SE±	Med	Min	Max	IL	SL	Mean	SE±	Med	Min	Max	IL	SL	
Blastocysts (units)	8	1.6	7	2	15	3.9	11.6	4	0.6	5	1	6	2.9	5.5	N/S

N/S = no significant differences according to T-Student test (P = 0.07)
SE = standard error; Med = median; Min = minimum value; Max = maximum value; CI = confidence interval

Table 3 Effect of SFW treatment versus NE in the production of embryos in the morula stage

	Treatment													Sig.
	SFW					CI (95%)		NE				CI (95%)		
	Mean	SE±	Med	Min	Max	IL	SL	Mean	SE±	Med	Min	Max	IL	SL	
Morulae (units)	4	1.2	3	1	11	0.7	6.3	3	0.4	3	1	4	1.8	4.4	N/S

N/S = no significant differences according to T-Student test (P = 0.57)
SE = standard error; Med = median; Min = minimum value; Max = maximum value; CI = confidence interval