

Factors affecting the pregnancy rate in transfers of embryos in crossbred *Brown Swiss*

Abstract

In vivo embryo transfer was carried out in crossbred *Brown Swiss* cows from the influential provinces of National Toribio Rodríguez de Mendoza University (UNTRM) experimental stations, from the beginning of the autumn season of 2013 to the end of spring season of 2015, for which states of transfers. Transfers were used mainly in receptors crossbred *Brown Swiss* cows, with a population of 126 receptors cows aged 2 to 9 years with a number of births ranging from 0 to 7, of which 74 transfers were performed with embryos fresh and 52 with frozen embryos. After 28 to 35 days of transfer of the embryo, gestation was evaluated by ultrasonography. With the objective of obtaining a pregnancy rate by state and the totality of the transfers, they were evaluated with the ANDEVA technique with a statistical significance of 5%. Obtaining that the pregnancy rate presented a significant statistical variation due to factors like the station and the state (fresh or frozen). Likewise, the value of total embryo transfer was 46.0%, while embryo transfer in the fresh and frozen state was 54.1% and 34.6%, respectively, and it was considered successful.

Keywords: embryos, transfer, donors, receptors, superovulation

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Introduction

Embryo transfer is one of the techniques of reproductive biotechnology that helps us to take advantage of the genetic value in bovine production, allowing to raise the number of standards of quality and efficiency, since it increases especially unexcellent genetic potential, simultaneously shortening the generational interval. In addition, it facilitates different breeding activities such as progeny assessment and comparison between groups of animals.¹

Currently Peru along with other countries of tropical regions seek to have the need to increase *B indicus* in the complex phenomenon of reproduction, thus improving reproductive and genetic efficiency,² this is the highest degree that only represents the first step towards new biotechnologies in our region. While the high potential impact caused by climate changes in the livestock sector, have not been fully included or studied to determine the technical options in this sector.³ Climate change has direct effects on livestock production and indirect effects on changes in the availability, quantity and quality of different food sources.⁴

According to Kafi and McGowan classify the intrinsic and extrinsic factors. However, the climate patterns continue to change within a more intense cycle, causing factors that could affect the results of the pregnancy rates, which have different origins currently observed.

Economic viability is crucial to ensure the sustainability of livestock operations and the development of applications of new techniques, which can be altered by environmental factors such as hours of light, temperature and rainfall, giving rise to early warnings in breeding programs in some species.⁵ The population in general terms observes the problem of the national cattle ranching that is mainly linked to a low production and productivity that represents a low profitability of this activity. The main causes of these problems are the seasonality of production, changes in the availability of quantity and quality of pastures and forages, low reproductive rates, high mortality rates in replacement breeding, high production costs of intensive livestock,

shortages and high price of bellies, low milk and meat quality, and technological delay.

One of the main factors that determine economic success in a livestock operation for various purposes is reproductive efficiency; Ideally, one breeding per year of each breeding cow should be obtained, however, obtaining replacement females with high genetic quality in dairy farming is not easy, because almost all females are kept as possible future replacements. The specialized dairy producers would like to control the sex ratio of the offspring born in their farms, looking predominantly for females and, thus, increasing the population size from which to select.^{6,7}

In both natural and artificial insemination (AI), the proportion of sex in the offspring is 50% males and 50% females.^{8,9} However, when using superovulation and embryo transfer (TE), there is a higher proportion of males attributed to factors related to the degree of development and quality of the transferred embryos,^{7,9,10} as well as the male reproductive used in AI.¹¹ It should be noted that According to Ealy et al.¹² high temperatures during the summer delay embryonic development because embryos have a lower capacity to secrete interferon, which is the signal for maternal recognition of pregnancy. The objective of the present study was to determine the proportion of sex ness originating from embryo transfer and to identify factors that could modify this proportion.

Materials and methods

The study began in February 1996 and was completed in August 1997. We used 73 crossbred *Brown Swiss* cows from two to nine years of age (of one birth or more), of which 39 were of high body condition (CCA), between 5.5 and 7 ($X=6.4\pm 0.5$) and 34 of low body condition (BCC), from 3 to 4.5 ($X=4.1\pm 0.4$), on a scale of 1 (emaciated) and of 9 (extremely obese).

The body condition (CC) of the cows was evaluated and recorded in four moments: at parturition, when presenting the first corpus luteum (CL) postpartum, at estrus and at the beginning of the breeding.

Characteristics of the field of study

The present investigation was carried out in the bovine cattle of the provinces of Bongará, Chachapoyas, Luya and Rodríguez de Mendoza in the Amazonas region, zones of influence of the Experimental Stations of the National University Toribio Rodríguez de Mendoza, in which results are reported from the beginning from the autumn season of 2013 until the end of spring of 2015, managed by personnel specialized in embryo transfer using the latest generation technology (Figure 1).

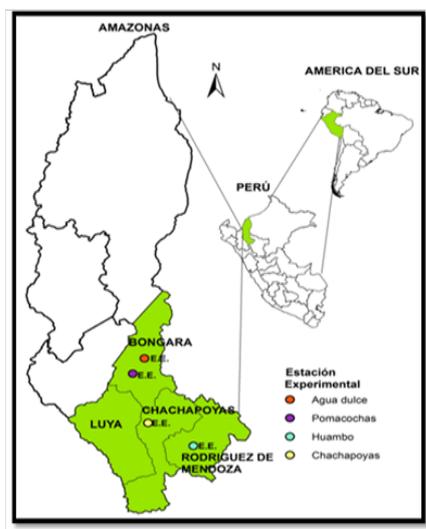


Figure 1 Map of the zones of influence of the experimental stations of the National University Toribio Rodríguez de Mendoza - Amazonas.

Evaluation and selection of recipients

In the present study, 86 recipients of the *Brown Swiss* breed, aged 3 to 7 years, from 0 to 7 calving numbers, with a body condition of 2.5 to 3.5 were selected. Two transfer states were evaluated. Embryos in cows of the crossed *Brown Swiss* race, reporting pregnancy rates.

The body condition was categorized as 3.25 or greater than 3.25, in order to analyze its relationship with the diameter of the major follicle, the degree of body condition of the cows at birth represents the most important factor affecting the interval of labor to estrus and the pregnancy rate in multiparous cows. Likewise, biostimulated cows (exposed to bulls) initiate estrus cycles earlier than non-biostimulated cows, thus the effect of biostimulation with bulls in the postpartum is more marked in cows with lower body condition than in cows with greater body condition.

Implementation of the heat synchronization protocol

In the protocol, the following days were worked on: day (0) placing progesterone (P4) estradiol benzoate (BE), prostaglandin (PG) and equine gonadotropin (eCG) as follows: P4 0.8 mL BE 2.5 mL PG foaming ovule, day (5) eCG 2MI, day (8) withdrawal of P4 2.5 PG, day (9) 0.4 mL BE, day (10) visual observation of heat, day (16) evaluation by rectal palpation of ovaries and uterus of recipients (presence of functional corpus luteum in ovaries and flaccid uterus) and day (17) embryo transfer.

Embryo collection and its classification

The hygiene of the working areas of the cow, the ovarian evaluation (right, left), application of the anesthesia, installation of the collection

means, dilation of the cervix, fixation of the Foley catheter, embryo collection, filter washing, search and classification of embryos.

Embryo transfer

The embryo transfer was performed with the evaluation of the ovarian response (corpus luteum > 16 mm).

Pregnancy diagnosis

The pregnancy evaluation was carried out 28 to 35 days after the embryo was transferred, by means of ultrasonography (Portable Ultrasound).

Results and discussion

According to the results obtained, it can be deduced that the pregnancy rate corresponding to the embryo transfer of each station and by state presented a significant difference ($p > 0.05$) (Table 1).

Table 1 Pregnancy rate according to seasons

Seasons	% of pregnancy	
	State	
	Frozen	Cool
Spring	100,0	50,0
Summer	42,1	0,0
Autumn	25,0	59,1
Winter	0,0	63,2

% of Pregnancy, percentage of pregnancy

Table 2 shows that there are a greater number of transfers in the summer season with a total of 19 transfers, 8 in autumn and 1 in winter and spring respectively. Also the best pregnancy rate was observed in the spring season with 100% (1/1), followed by summer 42.1% (8/19), autumn 25.0% (2/8) and finally, winter with 0.0% (0/1) respectively.

Table 2 Pregnancy rate of the frozen state for each season

Seasons	No. transfer	Reproductive state		% of pregnancy
		Pregnant	Empty	
Spring	1	1	0	100,0
Summer	19	8	11	42,1
Autumn	8	2	6	25,0
Winter	1	0	1	0,0

% of Pregnancy, percentage of pregnancy

Table 3 shows that the largest number of transfers was made in the fall season with a total of 22 transfers, 19 in winter, 16 in spring and 0 in summer respectively. Also the best pregnancy rate was obtained in the winter season 63.2% (12/19), autumn with 59.1% (13/22), spring with 50.0% (8/16) and last season summer 0.0% (0/0) respectively.

Table 3 Pregnancy index of the fresh state for each season

Seasons	No. transfer	Reproductive state		% of pregnancy
		Pregnant	Empty	
Spring	16	8	8	50,0
Summer	0	0	0	0,0
Autumn	22	13	9	59,1
Winter	19	12	7	63,2

% of Pregnancy, percentage of pregnancy

In Table 4, it can be inferred that of the total transfers made in this study, the best season of the year to carry out this technique was the winter season because it represented 60.0% pregnancy rate, spring as the second season, autumn and summer as the last two seasons.

Table 4 Pregnancy rate of total transfers per station

Seasons	No. transfer	Reproductive state		% of pregnancy
		Pregnant	Empty	
Spring	17	9	8	53,0
Summer	19	8	11	42,1
Autumn	30	15	15	50,0
Winter	20	12	8	60,0

% of Pregnancy, percentage of pregnancy

Hasler¹³ conducted a study on the factors that affect pregnancy by embryo transfer in a conventional manner, indicating that he did not find differences ($p > 0.05$) in pregnancy rates for the different seasons, spring 68.8%, summer 67.7%, autumn 67.8% and winter 68.5%, while, in Table 4, statistical differences were found in the percentages of pregnancy in the different seasons, spring 53.0%, summer 42.1%, autumn 50.0 % and winter 60.0%.

When evaluating the success of the transfer, individually from the variables contributed by the embryo, we can see in Table 1, that the highest pregnancy rate, presents the state of fresh embryo transfer, which could determine, not only the capacity to gestate the transferred embryo, but also to be able to raise it more efficiently. However, in other studies, significant differences were found in pregnancy rates between states,¹⁴ while in other studies they did not find significant differences when using heifers or cows¹⁵ that our obtained result could be valid for multiparous cows.

In Table 2, it can be said that embryos in fresh and frozen represent 54.1% and 34.6% pregnancy rate respectively, but according to Spell et al.¹⁶ report that they have worked with Angus females, of which pregnancy rates with fresh and frozen embryos were 83% and 69% respectively, higher than the rates reported in the present study. This could be due to the fact that in our study we have worked with the *Brown Swiss* breed, which is a dual purpose breed while the Angus breed is for a single purpose.

In Colombia, results have been reported using female recipients of embryos of different racial types and crosses, so Rodríguez et al.¹⁷ in a study conducted in the middle and lower Colombian tropics report pregnancy rates with fresh embryos of 31% and, frozen at 46%. The results according to this author and related to our results, are only higher in the pregnancy rate 54.1% in fresh state and frozen state is lower with 34.6%, which could be due to the conditions of *in vitro* embryos transferred in some proportion or in the referential study, among others, and to a lesser degree to the worked racial typologies, since according to what was previously mentioned, different pregnancy rates have been obtained using racial typologies.¹⁸⁻²⁰

In cattle *Bos Indicus*, without embryo transfer programs or hormonal treatments, Aguirre et al.²¹ reported 45% of pregnancy rate, however in Table 3 it is detailed that the pregnancy rate of this investigation was 46.0%, stating that there was no significant difference, however, Cutini et al.²² reported that the success rate is, when the pregnancy technique after embryo transfer, is close to 60%,

including embryo transfer in the frozen state; In addition, Lohouis et al.²³ described that the pregnancy rate is successful when the averages vary from 40 to 70%, even depending on the use of embryos whether fresh or frozen; of which our result was 46.0% which is located within the range according to the author, and concluding that our result was a pregnancy success rate.

Due to the similar percentage found in several other studies, such as Fuentes and De la Fuente, which used Holstein heifers, which were treated hormonally (1000 IU ECG on day 5 with reference to the start of treatment), reported indices of pregnancy with embryos frozen in glycerol of 54%, in ethylene glycol of 48% and fresh 52% (without statistical differences). However, in our study cows of crossed *Brown Swiss* breed were used, reporting pregnancy rates with frozen embryos of 34.6% and fresh 54.1% with a high percentage of difference, which could be dependent on the season of the year in which the study.²⁴⁻²⁷

Conclusion

- The total pregnancy rate of embryo transfers was 46.0%.
- The pregnancy rates of embryo transfer in fresh and frozen state were 54.1% and 34.6% respectively.
- In the winter season (60.0%) the best pregnancy rates were obtained, followed by the spring, autumn and summer seasons respectively.

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Conflict of interest

The authors declare no conflict of interest.

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