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Is the Production of Embryos in Small-Scale Farming an Economically Feasible Enterprise?

Z Sánchez¹, MA Lammoglia², MA Alarcón², JJ Romero³ and CS Galina¹

¹Departamento de Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico;

²Facultad de Ciencias Biológicas y Agropecuarias, Universidad Veracruzana, Tuxpan, México; ³Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica

Content

The present assay attempts to evaluate the feasibility of using embryo transfer in small community farmers by *in vivo* study and by modelling the results obtained. From the total of 59 donor cows, 62.7% responded to treatment, with a significant difference ($p = 0.002$) in the percentage of the response between breeds, being 90.5% (19/21) in Holstein and 47.4% (18/38) in Brahman. A total of 283 embryos were graded as transferable, while 141 as non-transferable, without difference in the percentage of transferable embryo by breed ($p = 0.18$). The mean of transferable embryos graded as class I and II was not different between Holstein and Brahman ($p = 0.96$ and $p = 0.92$, respectively); besides, no differences were observed in the other grades (non-transferable). The highest difference in costs, regardless of its quality by breed, was seen in the lower levels of probable fertility of the embryo transferred, even reaching several hundred dollars. When modelling the expected costs for embryo produced and transferred, values can reach nearly \$2000.00 when the probable fertility is only 10%. However, when the probable fertility was 60%, embryo cost was close to \$300.00. This technology seems to be viable on average or high-scale systems, having a superovulatory response between 60 and 80% with 4–6 transferrable embryos. Yet, in small-scale farming, due to the reduced number of donors and/or recipients, the costs surpass the economical feasibility of the technique.

Introduction

The industry of embryo transfer (ET) has grown considerably in the last 20 years. In a survey carried out by Perry (2013), it was shown that the use of ET has emerged as an important tool for the improvement of cattle. However, in a review, Hasler (2003) stated that nearly all of the 500 000 embryos produced worldwide from superovulated cows are from animals raised in temperate zones and not in the tropics. Nonetheless, several reports have been published to favour the use of this technique particularly from Brazil and Argentina where the cattle industry is an important source of revenues and has grown considerably in the last few years (Baruselli et al. 2006; Baruselli et al. 2010; Nasser et al. 2004). Recent reviews with a considerable number of animals being exposed to the ET procedure (Baruselli et al. 2010; Nasser et al. 2004) claimed that the technique is probably affordable only within large producers as with them, the popularity of ET in the region seems to be on the raise.

The opposite appears to be the case in others with scarce infrastructure for development. In effect in a recent study undertaken in Mexico, it was calculated

that the final cost of a replacement F1 heifer (*Bos taurus* x *Bos indicus*) using embryo transfer soared to almost 3000 dollars (Alarcón et al. 2010). This observation is in agreement with Hasler's review (2003) where he indicates that the current success level of superovulation represents a significant obstacle, even in industrialized countries to the future growth of the ET industry. According to the author as long as mean embryo production remains at less than six, with a range of (0 to >60), with 20% of donors producing zero embryos, this technology will remain an expensive, inefficient procedure.

Several researchers have provided sufficient evidence to sustain that the best cross-breeding programme to produce milk in the tropics is the direct cross between *Bos taurus* and *Bos indicus* (F1). The problem arises when the farmer faces the challenge to breed this cross-bred animal. If the choice is to cross with *Bos taurus*, the resulting product is quite vulnerable to the harsh environmental condition in the tropics. If, on the other hand, the selection is to sire with *Bos indicus*, then the offspring will be deficient in milk production (Madalena 1993). Another alternative is to transfer F1 embryos to F1 dams, hence avoiding the hazards of cross-breeding (Cunningham 1989).

The purpose of this study is to calculate the costs related to the production of F1 embryos and by modelling assess the value of this technique in cattle raised in undersized scale farming to discern the feasibility of the procedure as an alternative tool for small farmers to improve their genetic stock maintaining the hybrid vigour of the cross-bred *Bos taurus* x *Bos indicus*.

Material and Methods

Location

Bos indicus embryos were obtained from two commercial farms located in the State of Veracruz, Mexico, at 17°03' de latitude north and 93°36' and 98°38' longitude west (INEGI, 2012¹). Annual mean precipitation averages 1500 mm³, temperature ranges between 11 and 32°C, and altitude below 300 metres above sea level.

¹http://www.inegi.org.mx/prod_serv/contenidos/espanol/bvinegi/productos/integracion/estd_perspect/ver/Pers-ver.pdf

Embryos from *Bos taurus* were obtained in the State of Querétaro situated at 20°35' and 20°37' longitude north and at 100°19' and 100°21' longitude west. Ambient temperature ranges between 18 and 24°C with an average rainfall between 400 and 500 mm³ (INEGI, 2012²). The three farms had a total population averaging 200 cows. Embryos were obtained during the summer of 2013 by two of the authors (Alarcón and Lammoglia) which have considerable experience in the technique. After flushing and grading, suitable embryos were frozen according to standard procedures.

Animals

A total of 59 cows were used, 38 Brahman and 21 Holstein. Animals selected were at least 90 days post-partum and had a mean of five calvings without gross pathological conditions. The animals were cycling at the onset of the superovulatory treatment and had a body condition score of 3–4 on a scale of 1–5 (Pullan 1978).

Preparation for embryo retrieval

Donor animals received two doses of 100 µg of GnRH intramuscularly (Zoetis, México) within a 7-day interval. After the second GnRH application, an injection of 25 mg of dinoprost (P_gF₂α, Lutalyse, Zoetis México) followed. Twenty four hours later, oestrous detection began continuously for 72 h. Cows were declared in oestrus if they received three or more mounts. Ten days after the onset of oestrus, a rectal examination was performed to verify the presence of a corpus luteum (CL). One day after CL identification, cows received FSH treatment, and the total dose of FSH-P for *B. indicus* cows was 280 and 400 mg for *B. taurus*. The reason for this disparity is that *B. indicus* cows are more susceptible to the gonadotrophin treatment as there is a pronounced effect on the follicular milieu and more immature follicles are recruited following treatment (Baruselli et al. 2006). The total dose was divided into eight applications with decreasing dosages 12 h apart starting at 50, 40, 30 and 20 mg. In the third day of the FSH treatment, an injection of 25 mg of prostaglandin P_gF₂α was administered. Animals were AI 12 and 24 h after the onset of oestrus. Cows forming more than 2 CL's were chosen suitable for embryo collection 7 days after the onset of oestrus.

Embryo grading

Embryos were immediately evaluated by a qualified technician and classified as grade 1 (I) excellent and good, grade 2 (II) fair, grade 3 (III) poor and grade 4 (IV) dead or degenerating according to the criteria described by Bó and Mapletoft (2013).

Statistical analysis

Descriptive statistics

The global percentage of responders as well as the specific percentage by breed were calculated and compared using a chi-square test. Means of embryos obtained from responders, by grade and by breed, were compared using Student's *t*-test. In both chi-square and Student's *t*-test, the α value was set in 0.05.

Economical assessment

In order to determine the cost of embryos produced from those selected to be transferable, the percentage of cows responding to superovulation, plus the average of embryos, was considered viable to be transferred (categories I and II). Embryos classified as poor or degenerate were not considered in the calculations. Also, the model included the possible rate of success (0–100%). Using this approach, values were adjusted taking into account the percentage of cows not responding.

On the other hand, the preparation of a donor cow, including hormonal therapy, material to collect embryos, semen, veterinary services, labour and feeding costs, was 512.75 (US dollars). Also, the cost to prepare recipient animals was estimated in 106.21 (US dollars) which include hormonal therapy, material for transferring embryos of superovulated cows to each recipient, feeding, labour and veterinary services.

Though a deterministic model, a simulating accounting for breed of the animal (Holstein and Brahman) was compared with three different sources of variation (a) the response to superovulation, (b) the number of embryos produced and (c) the probable fertility of embryos according to their quality (grade I 75%, grade II 50%). This analysis was carried out in three phases (i) modelling the possible costs based in the superovulatory response and the average of transferrable embryos obtained in the survey with ranges from (0–100%) indistinctively of the embryo grading, (ii) modelling the cost assuming superovulatory responses from 50 to 80% and three averages of transferable embryos (5–10 and 15) for all the fertility ranges (0–100%). All these calculations without taking in consideration embryo grading, (iii) a standardized cost of transferable embryos produced per breed was calculated in three conditions: (a) according to the superovulatory rates and the average of transferable embryos reported by Ake-López et al. (1995), (b) based on the observed results for these parameters in the present study and (c) an expected (modelled) estimation for any breed, with a 80% of superovulatory rate and two fixed means (8 and 12) of transferable embryos per cow.

Results

Descriptive statistics

From the total of 59 donor cows, 62.7% responded to treatment, with a significant difference ($p = 0.002$) in the

²http://www.inegi.org.mx/prod_serv/contenidos/espanol/bvinegi/productos/integracion/estd_perspect/qro/pers-qro.pdf

percentage of the response between breeds, being 90.5% (19/21) in Holstein and 47.4% (18/38) in Brahman. A total of 283 embryos were graded as transferable, while 141 as not transferable, without difference in the percentage of transferable embryo by breed ($p = 0.181$). The means, according to embryo grading, were also not different in all grades. The amount of embryos produced graded by quality, and its mean and standard deviation are described in Table 1.

Costs estimates

The estimated cost for the production and transferring of embryos regardless of their quality and based on the superovulatory response was 73% more expensive in Brahman than Holstein if the fertility estimated was only 10%; the cost is reduced to approximately 35% if the fertility augments to 80% (Fig. 1). This result, with the specific variations in the proportional differences, is similar for the two types of transferable embryos (grades I and II).

On the other hand, the most expensive embryos were those graded as II from Brahman cows, highly influenced by the low superovulatory response that leads to a low absolute production (Table 1). They were followed by those of Holstein graded as II; subsequently, with very similar results in embryos graded as I for Holstein and Brahman cows, especially when the percentage of embryo fertility is over 50%.

The highest difference in costs, regardless of its quality by breed, were seen in the lower levels of probable fertility of the embryos transferred, even reaching several hundred dollars; thus, for example, the Brahman embryos graded II cost \$2811.9 more than the Holstein at 10% fertility, of the same grade. This difference falls steadily through various probable fertility rates; however, even in the best of scenarios (100% fertility), the variance was \$281.2 (Fig. 1).

When modelling the expected costs for embryo produced and transferred, with rates of fertility to the possible range of superovulatory response (0–100%),

extreme values were obtained when the response rates are low and the number of transferable embryos is also small, with the consequent reverse condition when both elements are high. These differences can, for example, reach nearly \$2000.00 when the probable fertility is only 10%; however, the absolute and relative differences decrease as the probable fertility increase, reaching reduced absolute differences at the end of range (Fig. 2).

Figure 2 depicts six possible modelling scenarios, while only comparing 50% responders + 15 embryos or 80% responders with 10 embryos (values usually reported in the literature), similar results were observed when this comparison was carried out. However, if an scenario 50% responders but only averaging 10 embryos or 80% responders with five embryos, the costs rise considerably as even having a reasonable response from the donors (more than 50%) if the fertility averages the same, the costs augment between 90 and 150 US dollars.

When comparing the standardized cost per embryo produced in three different scenarios (i) using the data reported by Ake-López et al. (1995) as an average response reported in the literature, (ii) the data actually obtained in the field study and (iii) results obtained by modelling, costs are standardized using the figures in the data obtained in the field study. Holstein embryos obtained in Ake's study are more expensive to produce according to their data. On the contrary, in our field study, the Brahman embryos were the most expensive. Moreover, when the number of transferrable embryos changes from the hypothetical response of 8 or 12 embryos, there is a substantial reduction of 20–27% in the costs (Table 2).

Discussion

The average response of embryos recovered is in agreement with current figures gathered recently by the International Embryo Transfer Society (IETS) (Perry 2013). However, as in many studies, there is a bias when comparing two breeds and moreover when using the average as a response variable. In effect, the

Table 1. Descriptive statistics of embryos collected from Holstein and Brahman cows, graded by quality and transferable/not transferable condition

| Embryo grading | Holstein | | | Brahman | | | p Value* |
|-----------------------------|----------|------|------|---------|------|------|----------|
| | n | Mean | SD | n | Mean | SD | |
| I | 102 | 6.00 | 3.37 | 109 | 6.06 | 3.61 | 0.963 |
| II | 36 | 1.89 | 3.48 | 36 | 2.00 | 2.66 | 0.919 |
| III | 30 | 1.76 | 1.52 | 53 | 2.94 | 3.86 | 0.242 |
| IV | 29 | 1.53 | 2.01 | 29 | 1.61 | 2.12 | 0.901 |
| Transferable (I,II) | 138 | 7.26 | 6.44 | 145 | 8.06 | 4.40 | 0.667 |
| Not transferable (III, IV) | 59 | 3.11 | 2.05 | 82 | 4.56 | 3.42 | 0.131 |
| Superovulatory response (%) | | | | | | | 90.5 |
| Transferability rate (%) | | | | | | | 47.4 |
| | | | | | | | 70.1 |
| | | | | | | | 63.4 |
| | | | | | | | 0.181 |

SR, percentage of cows with superovulatory response.

*p value for differences of means according to the embryo quality between breeds.

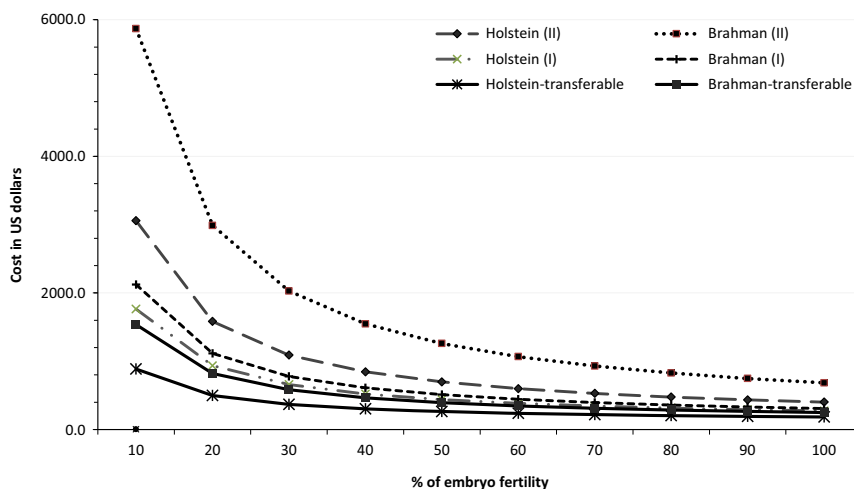


Fig. 1. Estimated costs of embryos according to grading in different possible fertility rates, obtained from the superovulatory response (90.5% for Holstein and 47.4% for Brahman)

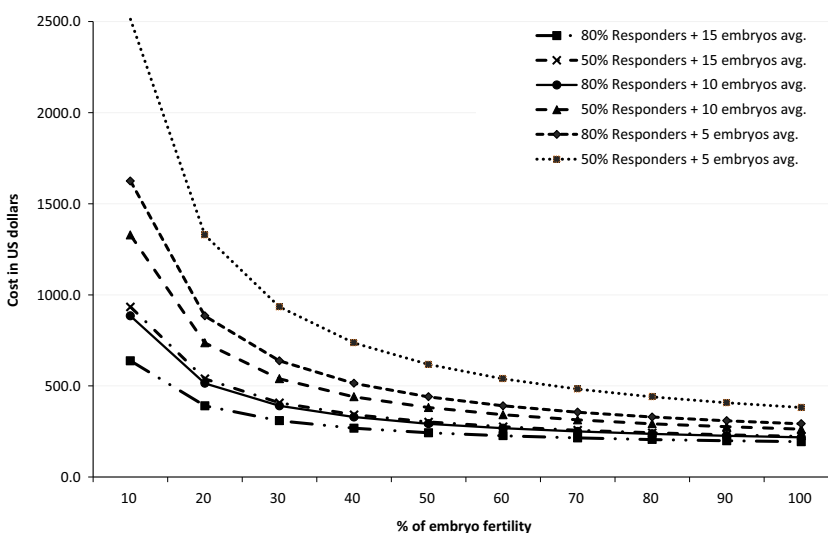


Fig. 2. Expected costs of transferable embryos at different percentages of fertility, fixing the rate of cows with superovulatory response in two levels (80% and 50%) and three different means of embryos obtained per cow (15, 10 and 5)

average in our study was 7.65 comparing favourably to Perry’s worldwide data 6.67 embryos per flush. First of all, studies depend largely in what is measured as transferrable embryo and consideration such as distance and season (Bastidas and Randel 1986; Bényei et al. 2006) management on a farm (Stroud and Hasler 2006) and embryo grading (Aguilar et al. 2002) play an important role in establishing the average response. Perry’s survey (2013) also illustrates the difficulty in gathering sufficient and accurate information about the accountability of the technique as only countries with an established embryos transfer programme, that is large countries provide the information. For example, the majority of African and Asian countries have not responded to the IETS surveys. Coincidentally, these are the regions where small community farmers predominate and where international organizations have vested considerable resources to make this technique available and affordable. For example, in a study in rural México sponsored by FAO (Montiel et al. 2006), it became apparent the variability in the response between farmers. One later study in the same region, calculated that

the cost for producing a replacement heifer soared to almost 3000 US dollars (Alarcón et al. 2010). Considering the added value in milk production when the cost of this replacement of an undefined breed is 900 dollars, it is uneconomical at the moment to choose changing the replacement programme in a small farm using embryo transfer. The difference in price does not compensate for the revenues obtained in disparity on milk production between the two systems.

In spite of the embryos produced and catalogued as transferable, 33.3% of the total number of cells recovered were not suitable for embryo transfer. This result is in accord with Hasler (2003) and many others (for review, see Bó and Mapletoft 2014) and remains as an important added cost in embryo production. The differences observed between breeds in relation to embryos production and quality are widely acknowledged (for review, see Barros and Nogueira 2001), and again many factors such as housing, nutrition and management play an important role in this disparity (Bényei et al. 2006; Betteridge 2003). In effect, Hasler (2014) in a review underlines the economical importance

Table 2. Costs (in US dollars) of transferred embryos for Brahman and Holstein cows in three different scenarios (reference, observed and modelled) at different rates of superovulatory response, average of transferable embryos and embryo fertility

| Calculation base | Brahman | | | | Holstein | | | | Modelled ^c | | | |
|------------------------|--------------|----------------|--------|------------|--------------|----------------|--------|------------|-----------------------|----------------|--------|------------|
| | % SOv. Resp. | Transf. Embryo | Cost | Emb. Fert. | % SOv. Resp. | Transf. Embryo | Cost | Emb. Fert. | % SOv. Resp. | Transf. Embryo | Cost | Emb. Fert. |
| Reference ^a | 75.0 | 7.8 | 244.46 | 63.4 | 86.4 | 6.6 | 269.59 | 70.0 | 80.0 | 12.0 | 182.51 | 70 |
| | | | 301.86 | 44.8 | | | 337.43 | 50.0 | | | 213.03 | 50 |
| | | | 303.62 | 44.8 | | | 339.51 | 40.0 | | | 239.74 | 40 |
| Observed ^b | 47.4 | 8.1 | 318.16 | 63.4 | 90.5 | 7.3 | 229.30 | 70.0 | 80.0 | 8.0 | 220.66 | 70 |
| | | | 406.16 | 44.8 | | | 280.41 | 50.0 | | | 266.44 | 50 |
| | | | 408.87 | 44.8 | | | 281.98 | 40.0 | | | 306.50 | 40 |
| | | | | | | | | | | | | |

% SOv. Resp., percentage of cows with superovulatory response; Transf. Embryo, Transferable embryo; Emb. Fert., Embryo fertility.

^aReference = from Ake-López et al. (1995).

^bObserved = from this study.

^cIt represents any breed in an expected superovulatory response of 80% with 12 and 8 transferable embryos on average.

of other factors related to ET; for example, in 1978, the cost per dose of the original Armour FSH-P was \$6.50 and the ET practitioner charge for his services \$500. Today, the cost of the available FSH preparations exceeds \$100 per dose and the ET practitioner fees are half or less than what they were 35 years ago. What impact will this have on the economics of embryo transfer?

The purpose of this communication is centered in establishing points of equilibrium where the technique could be feasible under the conditions of small community farmers. This goal can be achieved simply by calculating the cost of producing an embryo vs purchasing it commercially, or with the complexity related to the long-term investment of improving a particular breed during a lifetime production compared to the existing livestock in the area. Concerning the former, bearing in mind a commercial cost of \$250 to \$350 USD per gestation, these figures compared favourably to the ones of the present study, \$318 to \$408 USD for Brahman and \$230 to \$340 USD for the Holsteins. Bolivar and Maldonado working in Colombia (2008) reported costs close to \$360 USD. Nonetheless, this investment per embryo transferred terminating in a pregnancy does not take in consideration the added advantage of the local adaptation of the donor cow to the existing conditions in the area. This effect could only be measured in the production of their offspring which is a calculation still lacking in the embryo transfer industry in general.

Many studies particularly in Holstein cows exposed to heat stress (Ambrose et al. 1999; Al-Katanani et al. 2002; Stewart et al. 2011) indicate a higher fertility obtained using ET embryos instead of conventional AI, perhaps these publications are a good example that by not measuring the cost of comparing these two biotechnologies, one could assume that 'ET is better than AI' to solve an old problem of low summer fertility in dairy cattle. In effect in a study on small-scale farming in the south of Brazil, Brockington and Martínez (1996) found that even by improving the number of viable embryos from four with a 50% fertility rate to six and a

corresponding 70% of pregnancies, the producer faces a waiting period of some 15–20 years for a significant genetic returns from the use of ET.

This lack of response in 37.3% of the donor animals added to the cost of embryos not considered of optimum quality which can be as high as 40% (Ake-López et al. 1995) rise the cost of the technique considerably. Moreover, if the donor animal does not produce any embryos, there is an additional loss of days where the animal is open which ranges in dairy cows from \$3.2 USD to \$5.4 USD (De Vries 2004).

Again there is a wealth of information in the convenience of producing cross-bred embryos (Cunningham 1989), and the resultant heterosis (Madalena 1993) has afforded sufficient information to make this technique attractive to low-scale farming for improving their incomes. However, it does not seem feasible to produce embryos in these enterprises as the failures elevated the costs significantly; for example, it will be necessary to obtain at least an 80% response in the donor cows with a mean of 4–6 transferrable embryos, three embryo flushings per year and a fertility of 50% to make this technique economically feasible (Brockington and Martínez 1996). This event is more evident as there are fewer animals in the farm, thus one could place in jeopardy the whole enterprise. At the moment, it seems to be that options to organize an ET programme in small community farmers can be reduced to two possible scenarios (a) purchase embryos from commercial companies (b) produce embryos from large beef or dairy cattle enterprises to obtain the F1 cross-bred animal that could improve milk production under the conditions of small farmers. Both techniques have the disadvantage of a possible poor adaptation of the offspring to the harsh environmental conditions in the tropics. To conclude, embryo production using donor animals from a small enterprise does not seem economically feasible under the present results. However, a good management and selection of the donors might greatly improve the current situation.

The modelling analysis indicate that it will be necessary to augment by almost twofold the response of

donor animals and the quality of embryos graded as good or excellent to make this technique an attractive proposition to small community farmers in the tropics.

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Conflict of interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or

entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Author contributions

Zazil Sanchez and I were in charge of executing and analysing the data, Marco Alarcón and Miguel A. Lammoglia were involved in the field work, and Juan José Romero participated in the modelling.

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Author's address (for Correspondence): CS Galina, Departamento de Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico.
E-mail: cgalina@unam.mx