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Large-scale programs for recipients of *in vitro*-produced embryos

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Abstract

In vitro embryo production (IVEP) has become an accessible option for meat and milk producers, and Brazil is now the leader of IVEP worldwide. Recipient females represent one of the most significant costs in embryo transfer (ET) programs. Thus, hormonal protocols may increase the proportion of suitable recipients to receive embryos and improve the efficiency of IVEP programs. Besides improving the amount of available recipients, it is important to select high quality animals. Due to the great demand, the type of females that were considered ideal for ET has become scarce, especially for large-scale programs. Therefore, new approaches have successfully emerged, as the use of Nelore cows recently calved as embryo recipients. For being the most numerous category in Brazil, these animals can be acquired for fair prices. Embryo production from slaughterhouse ovaries also represents an innovative strategy for large-scale IVEP. With the use of sorted sperm and large amount of pregnancies, is has become an interesting alternative compared to AI. In this article, recent advances in embryo IVP are discussed, as well as some of the most used hormonal protocols for estrus synchronization of recipients in large-scale IVEP programs.

Keywords: *Bos taurus indicus,* estrus induction/ synchronization, FTET, IVP.

Introduction

In vitro embryo production (IVEP) has been a powerful tool for increase and dissemination of high quality genetic animals. IVEP has become more accessible in recent decades, and can currently be considered as a consolidated technique (Thibier, 2006).

Brazil occupies the first place in the world ranking of cattle IVEP (Thibier, 2006), and this leadership is certainly related to the predominance of Nelore cattle (*Bos taurus indicus*) in the national herd. Nelore has higher number of ovarian follicles and more oocytes recovered per ovum pick up (OPU) session when compared with european breeds (Pontes *et al*., 2009).

To ensure the success of IVEP programs, a crucial aspect concerns the availability of recipients. To achieve the best pregnancy rates, it would be ideal to have a large number of animals, to allow rigorous selection of the most suitable ones. However, the greater the number of recipients the higher the cost for maintaining these animals. High costs may be prohibitive and compromise the economic viability of the embryo industry. Thus, the efficiency of recipient preparation protocols becomes a factor of extreme importance for successfully obtaining products through the application of embryo transfer technology.

In dairy herds, more than 50% of cows in heat are not detected, by lack of mating behavior (Van Eerdenburg, 2002). This problem is more pronounced when high producing dairy cows are used as recipients, since high rates of estradiol metabolism make signs of estrus even less intense (Rodrigues *et al*., 2010). The failure to detect estrus also represents a great challenge in programs that use cattle with some proportion of *Bos indicus* blood due to the increased occurrence of short duration estrus and the high incidence of estrus at night (Bó *et al*., 2003).

In order to solve problems related to heat detection and to increase the proportion of suitable recipients to receive embryos, several hormonal protocols were developed, allowing embryo transfer (ET) without the need of estrus observation. These protocols enable the transfer of embryos in a fixed time (FTET), by using hormones that control follicular and luteal dynamics and promote synchronization of the moment of ovulation (Bó *et al*., 2002; Nasser *et al*., 2004).

This article describes the importance of large-scale recipient programming for IVEP, discussing some of the commonly used FTET protocols in programs involving large numbers of recipients.

Advances in large-scale IVEP

Until a few years ago, large-scale IVEP was limited by several drawbacks. One of them was the long distances between laboratories and recipient properties. Large herds of cattle are usually located in new areas of livestock production, such as the North of Brazil. Those areas are located thousands of miles away from oocyte donors and IVEP laboratories, which are concentrated mainly in South and Southeast regions. The limitations for cryopreservation of *indicus* IVP embryos greatly restricted the connection between production and transfer of IVP embryos. Thus, the disposal of embryos not transferred was a common practice.

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Another problem associated with the scarcity of recipients was the large number of calves of unwanted gender. When unsexed sperm is used, twice as many recipients are required to achieve the desired number of products of the expected gender.

Therefore, transportation of embryos over long distances and the use of sexed sperm are important procedures for a more rational use of recipients, improving the efficiency of large-scale embryo production (Pontes *et al*., 2010).

Transportation of embryos produced with sexed sperm over long distances

Pontes *et al*. (2010) conducted a large-scale IVEP program using exclusively sexed sperm from bulls of Holstein and Gir breeds. A total of 5,047 OPU sessions were conducted in Gir, Girolanda and Holstein females, and the embryos were transported over distances from 800 to 2000 km to be transferred to the recipients. Embryo production rates in relation to total oocytes were similar for all types of donors, *Bos indicus, Bos taurus* and *Bos indicus taurus* (17.4 to 18.9%), as well as pregnancy rates (36 to 40%, overall average of 39%).

Due to the large distance between the laboratory and the farms where the recipients were housed, transportation of embryos during the early stages of embryonic development was proposed. Embryos at different developmental stages (day 2 to day 5; day 0 = day of IVF) were subjected to transportation. To establish the moment to begin the transport, a calculation considering the distance and time needed for the trip was made. The end of the transportation had to coincide with day 7 of culture, and embryos should be at the morula or blastocyst stage. Thus, the latest stages of embryo development occurred during transport. The strategy was to place the embryos in groups of 35-40, in microtubes containing 400 µl of culture medium under 300 µl of mineral oil, in culture conditions similar to the laboratory (39°C and 5% CO_2 in air), but in a portable incubator (Ceafepe Veterinary Technology, Sorocaba, or WTA, Cravinhos, SP, Brazil). In situations of longer transportation time, the replacement of the medium and the gaseous atmosphere was carried out by the veterinarian who conducted the portable incubator. At the end of transportation, embryos were re-evaluated and loaded into straws for transferring.

In the program described above, the use of sexed sperm allowed a more rational use of the recipients, because the objective was to obtain female offsprings. Fertilization of oocytes with sexed sperm enables an accuracy of 85 to 95%, being the quality of the embryos similar to those produced with conventional sperm (Peippo *et al*., 2009, 2010). There were effects of bull and ejaculate, showing the need for a rigorous evaluation and previous selection of the best batches of sperm to obtain more advantageous results (Pontes *et al*., 2010).

Ideal type of recipient

Selection of high quality recipients is a challenging procedure, especially in countries like Brazil, where there is an excessive number of embryos in relation to the ability of direct transferring them to recipients. Good nutritional and health status, as well as adequate management, are important factors to ensure the success of IVEP programs.

The use of virgin heifers as recipients is usually indicated as the best option for achieving higher pregnancy rates. There used to be an informal concept that the best recipients would be crossbred heifers, usually half-blood Nelore and European breeds with some dairy ability, such as Simmental and Braunvieh. However, due to a limited supply and a high demand, this type of recipients became costly and scarce. Particularly for large ET projects, it became prohibitive to use this type of heifers as embryo recipients.

A successful alternative recently proposed (Pontes *et al*., 2009) refers to the use of Nelore cows recently calved as embryos recipients. This female category is the most numerous in the country, allowing an adequate supply with a fair price. Contradicting preconceived ideas, this animal category has allowed pregnancy rates around 40%, with more than 10,000 calves already born (In Vitro Brasil Ltda, Mogi Mirim, SP; unpublished data). The option of using cows with suckling calves should consider nutrition and health conditions, so that estrous cycles can restart soon after calving, in time to respond to synchronization protocols (Jones and Lamb, 2008).

If an adequate nutritional and health status has been established, the use of cows may effectively present certain advantages compared to heifers. In general, the use of cows may have a higher recipients transferred-to-treated rate on FTET, since they usually show a better response to the hormonal protocol and heifers often require a pre-synchronization. Another interesting aspect is that cows have been exposed more to pathogens. Thus, they may have greater disease resistance and better quality colostrum. Finally, the frequency of dystocia tends to be higher in heifers than in cows.

Importance of recipients on calf gene expression

Until recently, the importance of recipients has been based mainly on their ability to respond to synchronization protocols and to maintain pregnancy. However, with the recent advances in epigenetic area, it became evident that diet quality and maternal behavior can interfere on gene expression of the calf. Despite the fact that the genetic sequence is established since the first cell division, the expression of genes may be altered due to environmental factors. For example, during pregnancy, nutritional status of the recipient may interfere in the follicular ovarian reserve of the fetuses (Ireland *et al*., 2011). Then, a future donor may have its reproductive performance compromised if during its fetal formation the recipient has undergone nutritional deprivation.

Another important aspect of epigenetic events refers to maternal ability of the recipient. It was shown that females with high maternal ability influence DNA methylation of their products, interfering in the expression of important genes for environmental adaptation (Weaver *et al*., 2004).

Protocols for estrus synchronization

Prostaglandin F2α

The simplest treatment for estrus synchronization of recipients is based on a single administration of PGF2α, or on two injections 11 to 14 days apart. Although those procedures are relatively effective and inexpensive, treatment with PGF2α depends on the efficiency of heat detection, as well as the responsiveness of the corpora lutea of animals subjected to protocol.

The corpus luteum (CL) is responsive to PGF2α only from day 5 to 16 of the estrous cycle. Also, by the time of the application each recipient is in a particular stage of the follicular wave, resulting in considerable asynchrony on the onset of estrus (Siqueira *et al*., 2009). For large scale programs, the limitations of estrus synchronization may impair the efficiency of the technique. Therefore, in those cases it would be more suitable to synchronize ovulation and to transfer the embryos in a fixed time (FTET).

FTET

In large programs of IVEP, where most of the embryos are transferred fresh, the availability of a significant number of recipients is needed. To reach this goal, protocols of FTET have been increasingly used.

In a recent study with repeat breeders dairy cows, Rodrigues *et al*. (2010) compared a single application of PGF2α to a FTET protocol using progesterone, eCG, estradiol benzoate and estradiol cypionate. Higher recipient transferred-to-treated rate (75.0 *vs*. 34.5%) and pregnancy at 60 days (29.3 *vs*. 16.2%) were observed in FTET group. In the same study, it was found that the FTET protocol was effective regardless of the presence of a CL at the beginning of the treatment. Thus, using the FTET protocol increased the amount of recipients suitable for ET and allowed the use of cows without a CL at the beginning of the protocol, with the same efficiency as those with a CL.

Estradiol benzoate x cypionate

In spite of having different pharmacokinetic properties, estradiol benzoate (EB) and estradiol cypionate (EC) are considered effective in inducing ovulation in *Bos taurus* and *Bos indicus* cattle. By having a shorter half-life, EB promotes an LH surge of greater amplitude and shorter duration than EC, resulting in greater synchrony of LH surges (Sales *et al*., 2012). Due to its low solubility in water, EB is released more slowly and promotes a longer lasting LH surge (Vynckier *et al*., 1990).

Protocols of FTET that use EB as ovulation inducer are widely used. One model consists of an injection of EB (2 mg) and insertion of a progestin-releasing implant on a random day of the estrous cycle, which is considered as day 0. On day 5, eCG is administered (400 IU), and on day 8 the implant is removed and an injection of PGF2α is given (0.5 mg). On day 9, another dose of EB is administered (1 mg). Day 10 is considered the day of estrus, and FTET is carried out on day 17.

In protocols that use EC to induce ovulation, it is administered on day 8, along with the removal of the progestin implant. Day 10 is considered the day of estrus and FTET is performed on day 17 (Rodrigues *et al*., 2010). Execution of the EB protocol requires handling animals four times, while replacing EB by EC allows the elimination of one handling, reducing labor costs time. Sales *et al*. (2012) evaluated the effect of both drugs for induction of ovulation on lactating *Bos indicus* cows submitted to FTAI. They concluded that, despite the greater synchronization of LH surge in animals that received EB, synchronization of ovulation was similar between the two groups, with no effect on fertility of the animals exposed to either protocol.

Use of eCG

Administration of eCG on day 5 of synchronization protocols increases pregnancy rates and proportion of recipients selected to ET (Bó *et al*., 2002; Nasser *et al*., 2004). For its ability to increase the size of the ovulatory follicle and to induce single and multiple ovulations, this drug provides a higher concentration of progesterone during the subsequent luteal phase (Baruselli *et al*., 2010).

Studies were performed in order to evaluate the effect of delaying eCG administration from day 5 to day 8 of the original protocol, thus avoiding the handling of recipients on the 5th day (Nasser *et al*., 2004, Reis *et al*., 2004). Treatment on day 5 results in higher number of CL and higher plasma progesterone concentration, with recipients treated on day 5 presenting higher pregnancy rates than those treated on day 8. One possible explanation for the better performance of recipients that received eCG on day 5 would be that the high concentration of progesterone from physiological CL together with progestin-releasing implant can affect LH pulsatility and follicular growth (Baruselli *et al*., 2010).

Thus, Ferreira *et al*. (2006) evaluated the effect of delaying the administration of eCG from day 5 to day 8, but replacing vaginal progestin implant for an auricular implant, containing less hormone. Results showed that, when ear implants were used, it was possible to reduce the number of animal handlings without compromising the efficiency of the synchronization protocol (Table 1). The protocol proposed by Ferreira *et al*. (2006) consists of insertion of a progesterone device and administration of EB on day 0; administration of PGF2α, eCG, CE and device removal of progesterone on day 8, and FTET on day 17. There is also the option of using intravaginal progesterone-releasing device, with the condition that a dose of PGF2α is administered on day 0 at the time of progesterone device insertion. For requiring only three handlings, the new protocol is easily implemented, even if a large number of recipients must be synchronized at the same time.

In order to further facilitate recipient management in large-scale programs, different durations of the progesterone device have been tested. Synchronization protocol is initiated at the same day in the entire group of females (day 0), with the possibility of removing the device in 7, 8 or 9 days. It is possible to start the program in all animals and remove the implant from one third of the lot, starting from day 7 (In Vitro Brasil Ltda, Mogi Mirim - SP, unpublished data). The ovulation inducer (EC), the luteolytic agent and eCG are applied at the same day of the progesterone device removal. Thereby, the group that has the implant removed on day 7 receives embryos on day 16, and so on, making it viable to adjust the synchronization between embryonic developmental stage and recipient uterus. In a program conducted in 2011, 357 Nelore cows with suckling calves were subjected to FTET with P4 removal on days 7, 8 or 9 of the protocol. There was no difference in transferred-to-treated rate or in pregnancy rates among animals that remained with the implant for 7, 8 or 9 days (Table 1).

Table 1. Transferred-to-treated rate and pregnancy rates obtained in synchronization protocols using cows as recipients for IVEP programs.

Protocol	Breed of recipients	Prior evaluation of CL	Utilization rate %	Pregnancy rate %	Source
P4 and BE + eCG, PGF2α and CE	Holstein	without CL	61.2	38.2	Rodrigues *et al*. (2010)
P4 and BE + eCG, PGF2α and CE	Holstein	with CL	75.0	42.9	Rodrigues *et al*. (2010)
P4 and BE + eCG, (D5) + PGF2α and BE	*taurus x indicus*	with CL	76.1	31.0	Siqueira *et al*. (2009)
P4 and BE + eCG, PGF2α and CE	*taurus x indicus*	with CL	96.0	59.0	Ferreira *et al*. (2006)
P4 and BE + eCG, PGF2α and CE (D7)	Nelore	No evaluation	88.0	43.0	In Vitro Brasil
P4 and BE + eCG, PGF2α and CE (D8)	Nelore	No evaluation	88.0	42.0	In Vitro Brasil
P4 and BE + eCG, PGF2α and CE (D9)	Nelore	No evaluation	88.0	44.0	In Vitro Brasil
P4 and BE + PGF2α (D7) + eCG, and CE (D9)	Nelore	No evaluation	61.8	42.1	In Vitro Brasil
P4 and BE + $PGF_{2\alpha}$ (D7) + eCG, and CE (D8)	Nelore	No evaluation	67.9	37.2	In Vitro Brasil
	taurus x indicus	No evaluation	73.8	45.4	In Vitro Brasil

Replacement of AI by ET and of FTAI by FTET

In general, embryos are more resistant than gametes, particularly when heat stress is considered. As a result, for dairy cattle, the use of embryos to obtain pregnancies may be more advantageous, especially in months and/or regions with greater average temperatures.

Some dairy farms have completely replaced IA by ET, achieving better pregnancy rates and accelerating genetic gain between generations. In those situations, recipients and donors are dairy cows from the same herd, the ones with best genetic potential are used as embryo donors and the remainder as embryos recipients.

In beef herds, a new perspective is the embryo production using oocytes obtained from slaughterhouse and Y-sorted sperm. With large amounts of pregnancies, a competitive price can be achieved for the embryo, turning it into a commercially attractive alternative compared to semen.

Conclusions

Despite the wide spread use of ET in the last years, the use of this technology has been restricted by failure on heat detection, in both beef and dairy herds. In both cases, the acquisition and maintenance of the recipient in the herd represent a major cost in IVEP programs. Expenses with food, estrus synchronization and ET rise in each unsuccessful transfer. Therefore, protocols that increase the number of pregnant recipients by synchronization treatment represent the best solution for economically viable programs.

Likewise, several studies were conducted to reduce the number of times that recipients are handled during synchronization protocols. The results obtained to date are considered to be very satisfactory, since the currently available protocols are considered practical and easily applied.

The FTET is an interesting alternative that could result in the achievement of good pregnancy rates all year round, even in repeat-breeder cows and milk-producing cows in the warmer months of the year. Finally, advances in the use of sexed sperm and embryo transport over long distances have enabled the effectiveness of large-scale IVEP programs, benefiting beef and milk livestock.

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