

Società Italiana di Embryo Transfer

Notiziario Luglio 2012

Cari colleghi,

come da tradizione estiva ecco alcune note riguardanti la società italiana di embryo transfer che ancora per pochi mesi ho l'onore di presiedere. Cominciamo con la parte più piacevole: il congresso annuale. Quest'anno ci ospiterà Roma, Torre in Pietra, nelle giornate di venerdì 5 e sabato 6 Ottobre . Il congresso anche quest'anno penso sia molto interessante, orientato a differenza degli ultimi appuntamenti verso argomenti più specialistici. Con grande piacere sarà nostro ospite Roger Sauvé, Canadese, (Clinique Veterinaire St. Luois-Embryobec) caro amico e mio grande maestro e Joao Henrique Viana (Embrapa Dairy Cattle Research Center) dal Brasile. Roger si occuperà di embryo transfer in tutte le sue particolarità. Condurrà interventi molto pratici essendo lui un veterinario di campo che a differenza delle abitudini italiane ha sempre raccolto i dati. Sicuramente nasceranno occasioni di confronto e spunti di riflessione. Joao è una figura emergente nella realtà brasiliana di produzione embrionale, suggeritaci dal nostro grande amico Pietro Baruselli, che si occupa principalmente delle problematiche in vitro. Grazie alle sue informazioni, cercheremo di capire come mai in Sud-America la produzione di embrioni in vitro sta di gran lunga aumentando a dispetto di quella in vivo. Un assaggio di tale valutazione è contenuto in un articolo appena pubblicato da Joao su una rivista brasiliana open-access (Animal Reproduction) che riportiamo di seguito in questo notiziario. Il programma si completa con la relazione del Dr. Biffani sulla genomica, una review sull'ovum pick up da parte dell'amico Raffaele Boni e un personale aggiornamento sull'utilizzo del seme sessato nell'ET. In occasione dell'assemblea annuale ci sarà il rinnovo del consiglio direttivo. Come più volte riferito non è mia intenzione ricandidarmi, penso che un ricambio ora sia vitale per il futuro della società. Quest'anno abbiamo ricevuto una proposta collaborativa da parte della Società Italiana di Buiatria per organizzare assieme il congresso del 2013 occupandoci noi della sezione che riguarda la riproduzione. La proposta mi è sembrata molto interessante. La Siet è una piccola società che però ha, in questi ultimi anni, lavorato bene e offerto aggiornamenti scientifici di alto livello nel campo della riproduzione. Ci fa quindi onore che società più strutturate si rivolgano a noi e, indipendentemente da schieramenti o posizioni politiche, penso che collaborazioni con altre società, qualunque esse siano, se motivate da nobili finalità scientifiche siano positive. Sarà comunque l'assemblea a decidere. Nel frattempo mi piacerebbe ricevere vostre considerazioni e consigli via mail a siet@sietitalia.it o gigi@embryovet.it.

Un arrivederci a Roma sperando come al solito di vedervi numerosi anche per passare un paio di giorni assieme.

Pierluigi Guarneri

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Features and perspectives of the Brazilian *in vitro* embryo industry

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Abstract

In the last decade *in vitro* fertilization emerged as an alternative to superovulation and in a few years become the technique of choice for bovine embryo production, especially in zebu breeds. The recent growth in the commercial use of *in vitro* technologies in Brazilian embryo industry is reviewed here, highlighting the features and trends in different periods, as well as future challenges and perspectives. The data presented here was provided by the Statistics Committee of the Brazilian Embryo Technology Society, and include reports from breeders associations, commercial IVF companies, and ET practitioners. Three different periods were characterized in the use of IVF technologies in Brazilian embryo industry: 1) the first years (1999-2003), when IVF growth was driven by the growing demand from the embryo market, although the technology was still labeled as elitist; 2) a period of exponential growth (2003-2006), when IVF overcome the conventional ET as the technique of choice for embryo production; and 3) a last period, when total numbers trend to stabilize but IVF started to increase in importance in dairy breeds. The whole picture shows IVF as an interesting example of innovation, since the development of these new embryo technologies provided new products, processes and possibilities to fit the demands and remarkably change the scenario of the Brazilian embryo industry.

Keywords: *in vitro* fertilization, innovation, reproductive biotechnologies, Zebu cattle.

Introduction

The acronym IVEP stands for *in vitro embryo production* and refers to a number of procedures performed in laboratory, including *in vitro* maturation (IVM), fertilization (IVF), and zygote culture (IVC), required to produce embryos from immature oocytes. These procedures emerged as an alternative to *in vivo* embryo production by superovulation, also known as conventional embryo transfer (ET). Until the end of the 1990s, IVEP in Brazil was performed almost only for research purposes and, consequently, had no commercial impact. Due to its complexity and high cost characteristic, IVEP was formerly expected to increase slowly, focused in fulfill specific market demands. In a period of only five years, however, the country became the world's largest producer of bovine embryos and a reference to the use of IVEP technologies in a commercial scale. We can identify three different moments in the recent history of commercial IVEP in Brazil, each one with some particular characteristics, which are like pieces of a puzzle that, putting together, lead to the understanding of the whole picture and explain why this new technology was not only commercially successful but also had the potential to change concepts and trends in beef and dairy production.

Early days of Brazilian IVEP industry: from the lab to the field (1999-2003)

The possibility of generating mammals by *in vitro* fertilization is known since the 1950s (Chang, 1959). It took, however, two decades for the first *in vitro* produced calf to be born (1981; Brackett *et al.*, 1982), and another decade for this technology to reach Brazilian zebu breeds (1993) (Rubin, 2005). Therefore, it was not a surprise that successful commercial use of IVEP in large scale only happened years later. Although there was an increasing demand from the private sector, a long process needed to be completed before the technology became ready for commercial use in the late 1990s. Research on IVEP has been performed in Brazil since the 1980s by different universities and research centers (Rubin, 2005), and gradually created a solid knowledge base in the area. Besides development of protocols for *in vitro* maturation, fertilization, and embryo culture, the whole process required a better understanding and control of the reproductive physiology in tropical zebu breeds, and the optimization of ovum pick-up (OPU) protocols, which currently is the technique of choice for collecting most of the oocytes used for IVEP in Brazil. Fortunately, during the same period of time the use of ultrasonography in cattle brought significant progresses in the characterization of different aspects of the follicular dynamics in zebu breeds (Figueiredo *et al.*, 1997, Viana *et al.*, 2000) and in the development of estrous synchronization protocols (Baruselli

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et al., 2006), further used to prepare donors and recipients. Embryo technologies were first used in Brazil in European dairy breeds, specially Holstein, but in the following years this activity turned out to be increasingly important in zebu beef breeds, such as Nelore and Brahman, which were the first large market for commercial IVEP. Since the very beginning, zebu breeds accounted for more than 90% of the *in vitro* produced embryos. Research advances on zebu reproduction, consequently, were a key point for the success of *in vitro* technologies.

Successful commercial IVEP activity began in Brazil in 1998-1999, and the gradual increase in *in vitro* embryo production resulted in a rise in the embryo industry thereafter (Fig. 1). The parallel growth of IVEP and conventional ET during this first period (1999-2003) clearly shows that there was a suppressed demand, i.e., conventional ET was not fulfilling the needs of the embryo market. At that time, however, most practitioners understood IVF as a complementary technique, or an alternative to be used in specific situations such as in infertile donors (Thibier, 2005). Actually, the complexity of the procedures, the high cost of equipment, and the need for laboratory infrastructure contributed for the misunderstanding regarding IVEP potential. The technique was therefore labelled as “elitist” or believed to be useful only for high-valuable show cows. This paradigm would be broken soon.

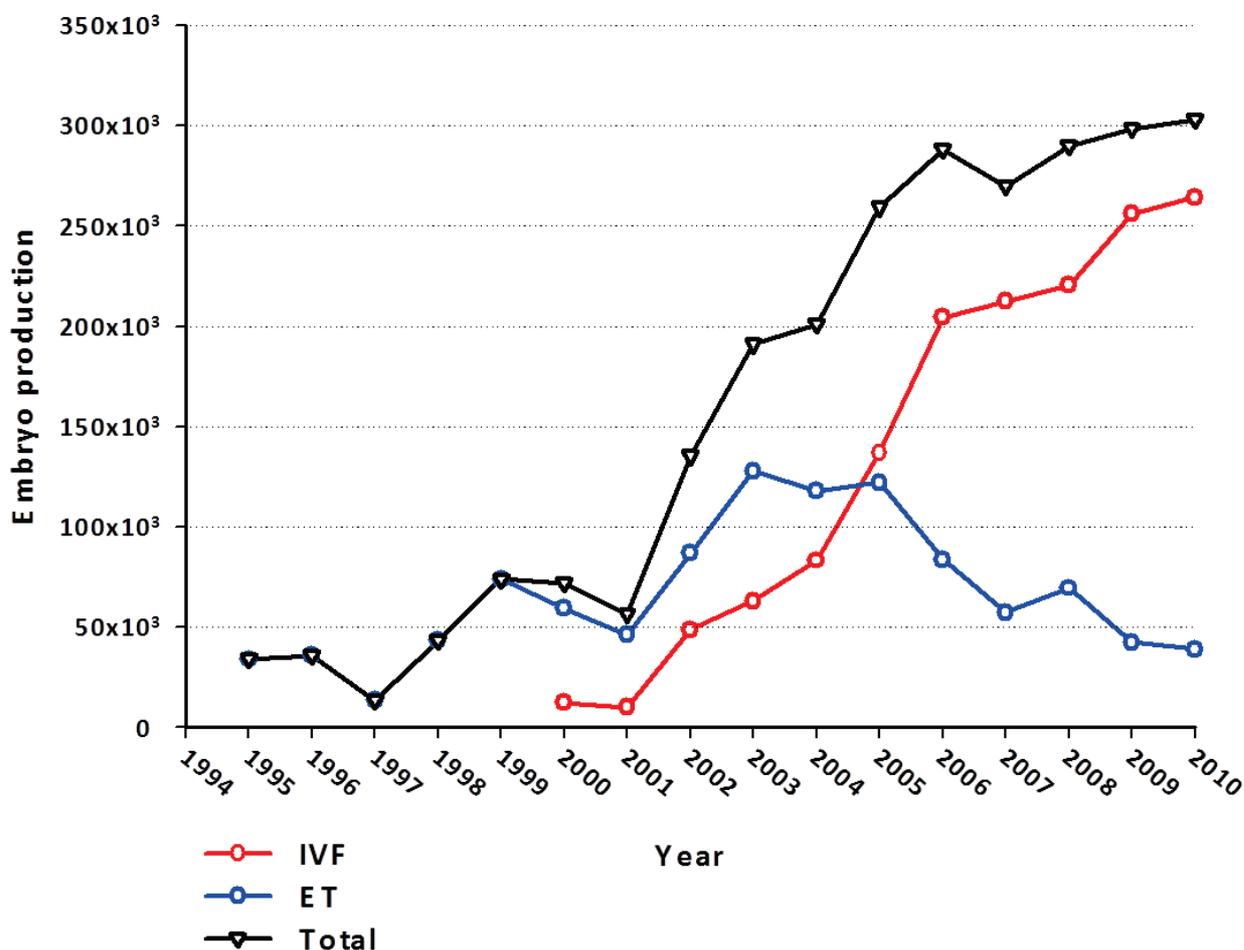


Figure 1. Production of bovine embryos in Brazil, according to the technique employed, in the period 1995 to 2010. (*in vivo*: embryos produced by superovulation (conventional ET); *in vitro*: embryos produced in laboratory; IVEP).

Becoming the technique of choice (2003-2006)

In vivo embryo production, which has progressively grown before the onset of IVEP activity and even during its beginning (1999-2003), stabilized thereafter and started to decline. Conversely, in the period of 2004-2006 IVEP industry increased remarkably, taking the Brazilian embryo industry to a new level (>200,000 embryos transferred/year). This contrast was the result of the progressive adoption of IVEP as the technique of choice for embryo production, mainly in zebu breeds. In fact, IVEP overcame the main limitation of superovulation: poor and inconsistent ovarian response to exogenous FSH stimulation, commonly observed in most of the zebu breeds



(Baruselli *et al.*, 2006). There were, however, at least two other reasons for the success of IVEP in Brazil. The process of IVEP is still characterized by low efficiency. Different studies reported COCs recovery rates of approximately 70%, by using transvaginal follicular aspiration in non-stimulated animals (Seneda *et al.*, 2001; Viana *et al.*, 2004); 10 to 40% of embryo production (including maturation, fertilization, and culture to the blastocyst stage; van Wagendonk-de Leeuw, 2006; Lonergan and Fair, 2008; Rizos *et al.*, 2008); pregnancy rates ranging from 30 to 40% (Peterson and Lee, 2003; Pontes *et al.*, 2009; Siqueira *et al.*, 2009); and a quite high incidence of abortion and stillbirths (Peterson and Lee, 2003). Consequently, the overall efficiency (considering number of calves born relative to aspirated follicles) is hardly higher than 10% (van Wagendonk-de Leeuw, 2006). In spite of a great effort into the development of each of the steps involved in IVEP, substantial gains are unlikely to occur, once they are primarily limited by the quality of the recovered COCs (Blondin *et al.*, 2002; Merton *et al.*, 2003). Therefore, the efficiency and consequent economic viability of IVEP are closely related to the number of follicles available for aspiration (OPU) on the donors' ovaries, and to the quality and developmental potential of the recovered oocytes.

Females of zebu breeds are known for presenting many differences in ovarian physiology, including a greater number of follicles recruited in each follicular wave and a greater number of follicular waves per cycle (Figueiredo *et al.*, 1997; Viana *et al.*, 2000), a lower persistency and diameter of the dominant follicle at deviation (Sartorelli *et al.*, 2005; Viana *et al.*, 2010a), and differences in the acquisition of ovulatory capacity by the dominant follicle (Gimenes *et al.*, 2008). These differences also account for a greater number of growing follicles throughout the estrous cycle and, as a consequence, more COCs are recovered by OPU (Table 1) compared to European breeds (Lopes *et al.*, 2006; de Roover *et al.*, 2008). Based on partial results obtained from different commercial IVEP companies in Brazil (Table 2), we can estimate an average of 2.7 pregnancies per donor/aspiration session. In contrast, the compilation of IVEP activity in Europe from 2000 to 2003 (Thibier, 2001, 2002, 2004) showed a mean production of 1.6 embryo per aspiration session (28,209 embryos/18,140 OPU), which, even considering a hypothetical pregnancy rate of 50%, would result in less than one pregnancy per aspiration, i.e., less than a third of the result observed in zebu breeds in Brazil. The European average number of embryos/aspiration is five to seven COCs recovered per donor, which is in agreement with the oocyte recovery rates reported in different studies involving *Bos taurus* (Gibbons *et al.*, 1994; Goodhand *et al.*, 1999; Lopes *et al.*, 2006; de Roover *et al.*, 2008; Merton *et al.*, 2009). Besides the greater number of recovered COCs, the results of commercial companies evidenced higher embryo production rates in zebu breeds (Table 2). These fairly superior results can be a consequence of an intrinsic better oocyte quality, or a reflection of the positive cooperation effect resulting from a greater number of COCs in culture (Ferry *et al.*, 1994; Donnay *et al.*, 1997). The difference between *Bos indicus* and *Bos taurus* performance in IVEP systems resulted in a discrepancy in the percentage of embryos produced *in vivo* and *in vitro*, relative to breed: zebu account for 97.3% of IVP embryos, but only 48.5% of the embryos produced by superovulation in Brazil.

Table 1. Results consolidated from four different IVEP companies in Bra.

End points	Mean value (n)	Range
COC recovered per OPU	19.9 (528,743/26,598)	15.2-24.4
Embryo rate*	35.4% (123,624/348,957)	32.9-41.2%
Pregnancy rate*	38.5% (30,729/79,798)	36.0-41.0%

* Only embryos and pregnancies which could be linked to the number of COCs in culture and to embryos produced, respectively, were computed. (Autor, 2010; Instituição da autor; personal communication).

Table 2. Comparison of embryo production rates in a commercial IVEP company in Brazil.

Subspecies	COCs (n)	Embryos (n)	Embryo rate (%)
<i>Bos taurus</i> breeds	8,200	2,098	25.6 ^a
Synthetic breeds	30,496	8,408	27.6 ^b
<i>Bos indicus</i> breeds	763,344	245,123	32.1 ^c
Total	802,040	255,629	31.9

^{a,b,c}Values followed by different letters, in the same row, differ (χ^2 , $P < 0.001$). Adapted from Watanabe *et al.* (2008).

Another reason for the success of IVEP in Brazil is the scale effect. The IVEP activity has high fixed-costs, but allows for the optimization in the use of high-cost semen straws, and also an improvement in the logistic of recipients' synchronization and management, mostly due to a better predictability of oocyte yield per donor (Boni *et al.*, 1997). The gain in predictability is more evident if compared to the *in vivo* embryo production, a technique that



often shows coefficients of variation greater than 100% (Hahn, 1992). Consequently, when used in large scale, the cost per pregnancy from IVEP can be lower than from conventional ET. Brazil has the largest commercial cattle herd in the world, approximately 205 million animals (Instituto Brasileiro de Geografia e Estatística, 2010), and one third of those are estimated to be cows and pubertal heifers. This large and genetically heterogeneous herd created a huge demand for yearling bulls (in beef herds) and heifers (in dairy herds) each year, that could not be met by artificial insemination (AI) or conventional ET. In this scenario, the usefulness of *in vitro* technologies to increase the number of offspring per donor in a short period of time (Thibier, 2005; van Wagendonk-de Leeuw, 2006) seemed very attractive, disregarding eventual increases in the economic costs. The scale effect also affected the commercial activity itself. The requirement for a whole set of new knowledge and procedures resulted in the appearance of the first embryo companies focussed only in *in vitro* technologies. After IVEP started to be the technique of choice for embryo production in the zebu and overcame conventional ET, traditional ET companies were pushed to adopt IVEP. In the end, *in vitro* technologies became the standard procedure for the whole embryo industry.

IVF reaches the dairy sector (2006-present)

After a period of exponential growth, sustained mainly by beef breeds, Brazilian embryo production showed a trend to stabilize at about 300,000 transfers/year. The subsequent increase in IVEP was, at least partially, counterbalanced by the reduction in conventional ET. Although beef breeds were still responsible for 78.4% of the embryos transferred in 2010, a noticeable advance in IVEP after 2005 occurred mainly in dairy zebu breeds. The use of IVEP in the major zebu beef breed, Nelore, showed a negative trend in the period 2005-2010 (-24%), whereas in the most important zebu dairy breed, Gyr, IVEP increased 764% in the same period. The increasing importance of embryo technologies in dairy breeds can be also noticed if one considers the proportion of embryos produced in relation to the total number of births recorded (RGNs). Nelore and Gyr breeds, for example, accounted for 68.5% and 16.5% of the embryos produced, but for 83.0% and 4.9%, respectively, of all the RGNs among zebu breeds (Associação Brasileira dos Criadores de Zebu, 2010). Also, figure 2 shows that this expansion in embryo activity in dairy breeds was related to an increase in the use of IVEP, but not ET. This shift towards dairy breeds can be attributed in a great extent to the onset of using sexed semen, since the greater proportion of males born after IVF with conventional semen (Camargo *et al.*, 2010) was one of the main reasons impairing economic viability of IVEP in dairy breeds.

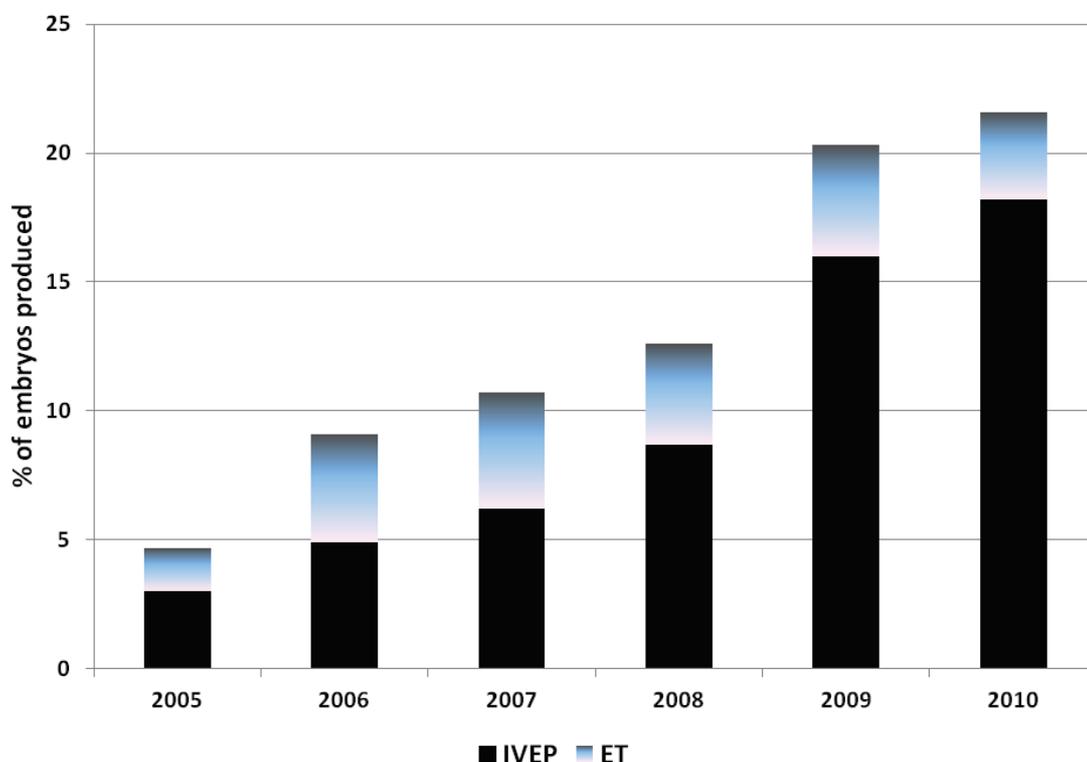


Figure 2. Participation of dairy breeds in the total of embryos produced in Brazil in the period 2005 to 2010, and the contribution of each technique to the total. (*in vivo*: embryos produced by superovulation; *in vitro*: embryos produced in laboratory).



The availability of sexed semen also opened a new set of possibilities in the dairy industry, exemplified mostly by the production of crossbred Zebu-Holstein animals. A dairy breed called Girolando (Gyr x Holstein) is the only crossbred with significant participation both in ET and in IVEP activity, responding for 2.5% of the total. It is interesting to highlight that embryo technologies can be used in dairy breeds not only for the production of donor sires and dams, but also to provide replacement heifers for commercial farms (major income comes from selling milk, not animals). There are some ongoing experiences in the use of IVEP in large scale to produce F1 Gyr x Holstein female calves (Pontes *et al.*, 2009), with promising results. The resulting first generation crosses (F1) can also be used as an oocyte donor to produce other crosses, such as 3/4 and 1/4 (further used to produce 5/8). The performance of F1 (1/2) and 1/4 donors in the IVEP is close or even better than the performance of Gyr donors, considering both the number of oocytes collected and embryos produced (Pontes *et al.*, 2009). Another interesting feature of this new market is the use of IVEP to produce F1 and other crosses from some very high-genetic merit Gyr and Holstein donors, a strategy that would probably not be used with AI or even ET. This new approach resulted in the appearance of a class of “elite” crossbred, high producing (>10,000 Kg/lactation) dairy cows, similar to what is observed in purebred animals.

Despite of the recent increase in the use of *in vitro* technologies in dairy breeds, there are some important challenges ahead. The benefits of the scale effect, for example, can be easily achieved in beef, but not in dairy herds. Most dairy farms don't have enough area to support an independent recipient herd, and the use of lactating cows as recipients would require either the use of frozen IVP embryos or the increase in voluntary waiting period, which would probably impair calving interval and/or calving distribution throughout the year. Although some progress has been recently accomplished in cryopreservation of *in vitro*-produced embryos, frozen-thawed embryos accounted for only 5 to 6% of the total IVP embryos transferred in 2009 (Viana *et al.*, 2010b). The low efficiency of the cryopreservation procedures for *in vitro*-produced embryos in Brazil seems to be directly related to inherent characteristics of the *Bos indicus* embryo, since similar pregnancy rates have been reported for frozen-thawed *in vitro* and *in vivo* produced embryos in *Bos taurus* (Galli *et al.*, 2001; Thibier, 2005). In regard to the restriction in number of recipients available, an alternative would be the segmentation of the dairy industry, with the production of replacement heifers being concentrated in a few specialized reproductive centers. This organization, albeit very common in poultry and swine, is still new for the Brazilian dairy industry. The use of *in vitro* technologies to produce crossbred animals may consolidate this trend in the future.

Final remarks

The IVEP technology is an interesting example of innovation in livestock production in Brazil. Firstly, the development of reproductive protocols was based on the confluence of research efforts in correlated areas. Secondly, IVEP should not be considered merely an improvement in the way to produce bovine embryos, if compared to conventional ET, since it resulted in new products, procedures, applications, and, why not, a new market. Thirdly, the technology was quickly adopted by the private sector, and replaced the preceding one (*in vivo* embryo production) as the standard procedure in zebu. Finally, IVEP caused a significant change in the scenario of the Brazilian embryo industry, first in beef and later in dairy industries, with predictable consequences for genetic progress and herd productivity.

Acknowledgments

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Comunicazioni ai soci

Congresso SIET 2012

5-6 ottobre 2012 Torre in Pietra – ROMA presso La Corte di Arenaro (Casale del Fagocchiaro)

Giovedì 4 Ottobre

Ore 17.00 Gigi Guarneri invita gli interessati ad un Practitioners' forum: un incontro informale con Roger Sauvè.

Venerdì 5 Ottobre

Ore 8.30 Apertura segreteria

Ore 9,30 Saluto del Presidente SIET e degli organizzatori locali

Ore 9,45 La produzione embrionale nella bovina in Canada. Evoluzione dei protocolli operativi. **Roger Sauvè**

Ore 11.30 Coffee break

Ore 12.00 Utilizzo della tecnica di embryo-splitting in condizioni di campo. **Roger Sauvè**

Ore 13.00 **Pausa Pranzo**

Ore 15.00 Utilizzo del seme sessato nella superovulazione della bovina. **Pierluigi Guarneri**

Ore 15.30 La tecnica Ovum Pick-Up a 25 anni dalla sua messa a punto. **Raffaele Boni**

Ore 16.15 Coffee break

Ore 16.45 La genomica nella selezione dei bovini da latte. **Stefano Biffani**

Ore 17.30 Problematiche legate all'applicazione della superovulazione nel Settentrione d'Italia. **Davide Bolognini.**

Ore 17.45 Problematiche legate all'applicazione della superovulazione nell'Italia Centrale. **Riccardo Fortunati.**

Ore 18.00 Problematiche legate all'applicazione della superovulazione nel Meridione d'Italia. **Michelangelo La Spisa.**

Ore 18.15 Assemblea dei soci

Ore 20.30 Cena sociale

Sabato 6 Ottobre

Ore 9.00 Caratteristiche e prospettive della produzione embrionale in vitro in Brasile. **Joao Henrique Viana**

Ore 10.30 Coffee break

Ore 11.00 Dinamica follicolare e produzione embrionale nella bovina *indicus vs taurus*. **Joao Henrique Viana**

Ore 13.00 Chiusura lavori

Quota iscrizione SIET annua 30 euro

Iscrizione congresso prima del 10 settembre 100 euro

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