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Challenges and prospects of the embryo transfer industry from a European standpoint

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Review Article

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Abstract

In vivo derived (IVD) and *in vitro* produced (IVP) embryo transfers are respectively the 2nd and 3rd generations of the reproductive biotechnologies. Commercial application of these technologies began in the seventies for the former and in the eighties for the latter. After 40 years, they have clearly proved their sustainability to the livestock industry. The present article deals with bovine embryos only. The numbers of embryos collected or produced and transferred worldwide are reported in the first part. About one million bovine embryos are transferred each year. This clearly shows the significant impact they have on the cattle industry. The second part describes the three main criteria used to assess the goal of this technology, i.e. to benefit farmers. The three criteria are: (1) the genomic selection tools being developed for these reproductive biotechnologies through ongoing scientific research, (2) the technical expertise of veterinarians and technicians in achieving the highest conception rate as possible and (3) the sanitary care required for safe transfers of pathogen-free embryos. The challenge to the ET industry is to improve sustainability. The high level of scientific research, technical expertise and professionalism of all scientists, veterinarians and technicians involved in the germplasm industry shapes a bright future of these biotechnologies.

Keywords: artificial insemination, embryo transfer, cattle, Europe

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Introduction

Classically, four generations of animal reproductive biotechnologies (ARB) are recognized as illustrated on Figure 1 (Thibier, 1990a). They appeared gradually, starting in the middle of last century in the mid-forties after the last world war, with the implementation of artificial insemination. It was then not before thirty years later that the embryo transfer industry took off the ground with in vivo derived embryos (IVD) and then 15 years later with in vitro produced (IVP) embryos. The fourth generation has since then remained mostly for experimental purposes or for specific applications such as generating biopharmaceutical products.

As the technologies of the first to third generations were developed for the benefits of the farming industry, international exchanges of germplasm increased via shipments of doses of semen first and later of straws of embryos. For various technical and economic reasons, those technologies have been mostly and widely used in cattle, however there are also embryos transferred or oocytes collected, fertilized and transferred in other farm animal species such as in small ruminants, swine and horses. This article will only refer to bovine embryos.

The embryo transfer industry which will be discussed in the present paper has remained remarkably sustainable for more than 40 years of existence. The first part of this presentation will report the numbers of embryos both in vivo derived (IVD) and in vitro produced (IVP) transferred worldwide, showing the excellent current situation. There are reasons for such a success story. Moreover, the challenges discussed in the second part will refer to the comparative advantages of such technology as seen from a European standpoint.

The embryo transfer industry in numbers during the second decade of the 21st century

In vivo derived (IVD) cattle embryos: How big is the embryo industry worldwide? The following tables, figures and numbers from the remarkable report of Perry of the IETS (International Embryo Transfer Society) data retrieval committee (Perry, 2013) will provide the answers.

Table 1 shows that only a fifth of the world countries reported their embryo collections and transfers. The statistics collected by the IETS data retrieval committee provide us a good indication of how well and alive the ET industry is. However, Asia poses a problem as some large countries irregularly or partially report on their activity even though a large number are being transferred. Hence, only 10% of the countries of Asia reported their numbers. Japan is the leading country in terms of ET activity in this continent.

As shown in Table 2, more than 100,000 donors had their embryos collected in 2012. Asia collected close to 10,000 embryos from cattle. North America took 50% of this number, and Europe and Asia 19 and 14%, respectively. The number of embryos transferred per donor collected, which may to some extent reflect the level of technology, was in Asia around 6.5, which seems quite high and hence very satisfactory.

Table 1 Number and proportion of data collectors by region (Perry, 2013)

Region	No. of countries in region	No. countries submitting area	% countries submitting area
Africa	57	2	3.51%
Asia	53	5	9.43%
Central America	31	1	3.23%
Europe	45	26	57.78%
North America	3	3	100.00%
Oceania	23	2	8.70%
South America	13	2	15.38%
Globally	**225**	**41**	**18.22%**

Table 2 Collection and transfer of embryos by region (Perry, 2013)

Region	Embryo collection		Embryo transfer	
	Donors	% global	Total	% global
Africa	1,107	1.09%	6,347	1.25%
Asia	9,494	14.37%	64,770	12.80%
Europe	23,653	19.33%	106,463	21.05%
North America	52,701	50.87%	235,344	46.52%
Oceania	2,654	2.22%	15,050	2.98%
South America	15,274	12.12%	77,902	15.40%
Grand total	**104,883**	**100.00%**	**505,876**	**100.00%**

More than half a million of IVD embryos were transferred in 2012, around 65,000 embryos in Asia, i.e. about 13% of the world transfer.

Regarding the trend over the years since 1997, Figure 1 shows stabilization in the number of donors collected in Asia (grey line) as opposed to a slight increase up to 2006 in North America (blue line). However, both continents had similar trends of stabilization during the last six years.

The trend in the numbers of IVD cattle embryos transferred (Figure 2) was close to that described for the numbers of donors, indicating stabilization in both continents since 2006.

However, a global trend of an increase from less than 400,000 to over 500,000 from 1997 until 2012 can be seen. Examining the Asian data, some fluctuations can be seen with a peak of over 100,000 embryos transferred in 2006, related to some reports from the People Republic of China.

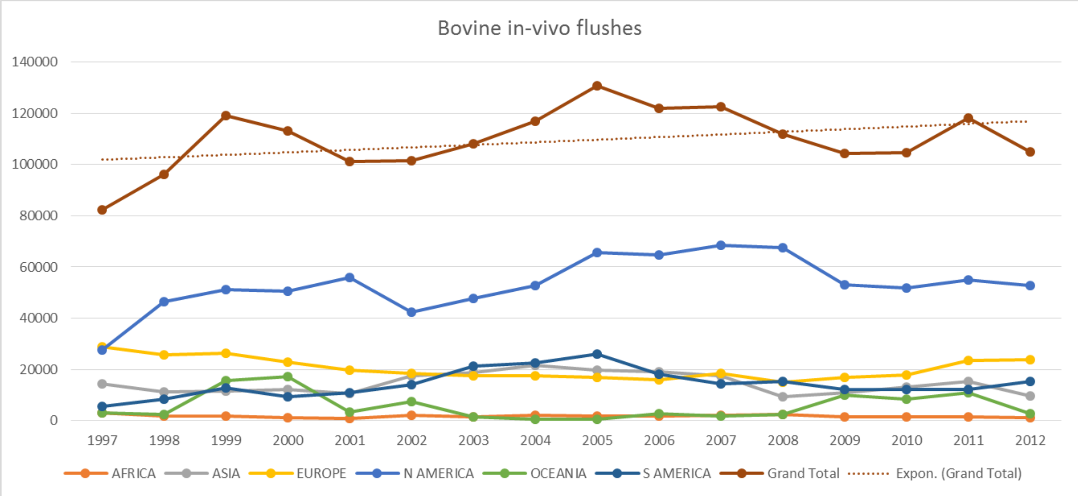

Figure 1 Graph of IVD embryo collection per region and year (Perry, 2013)

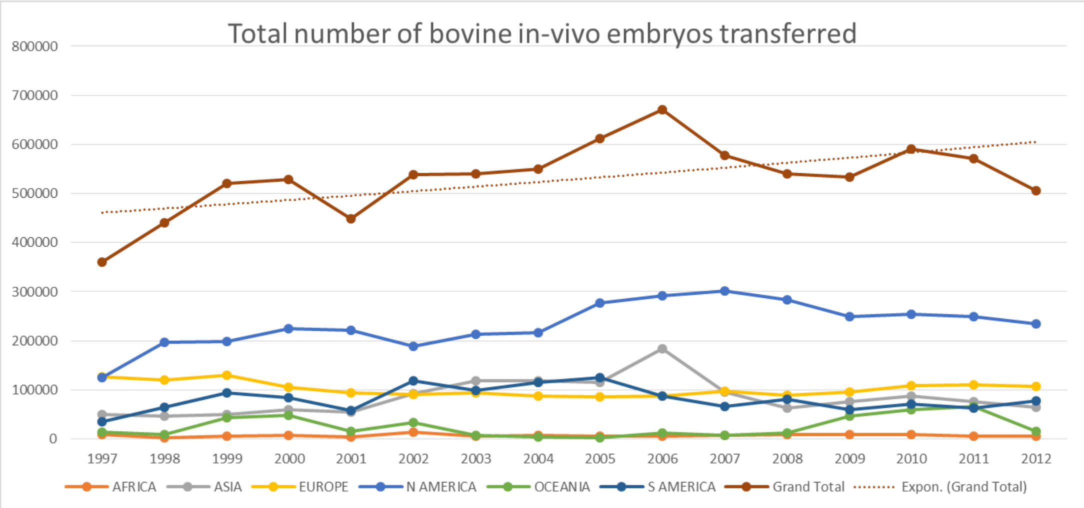

Figure 2 Total number of bovine IVD cattle embryos transferred (Perry, 2013)

In vitro produced (IVP) cattle embryos: As shown in Table 3, the worldwide total number of IVP embryos (close to 400,000) was close to that of IVD embryos and the vast majority of those came from the South American region, particularly from Brazil. Asia ranked second with more than 10,000 IVP embryos transferred, particularly from Japan. It is, however, of notice that South America and Japan do not proceed at all the same way. In Japan, most of the oocytes are collected from abattoirs and further cultivated *in vitro* in a laboratory some distance away from the abattoir. In contrast, Brazil produces most of its cattle embryos from oocytes collected from donor cows by ovum pickup.

Table 3 Bovine *in vitro* embryo production (Perry, 2013)

Region	Ovum pickup		Abattoir		Total Embryos Transferred
	Embryo produced	Embryo Transferred	Embryo produced	Embryo Transferred	
Asia	5,294	665	12,584	11,809	12,474
Europe	8,792	8,266	1,138	38	8,304
North America	74,242	40,546	200	1,916	42,462
South America	355,205	335,994	0	0	335,994
Oceania	0	125	0	0	125
Grand total	**443,533**	**385,596**	**13,922**	**13,763**	**399,359**

The trend over the years (Figure 3) as reported by Perry referring to OPU IVP cattle embryos is interesting in that it is likely that worldwide, in contrast to Europe, the numbers of IVP embryos were nearly equal to those of IVD embryos before perhaps overcoming the latter in the near future.

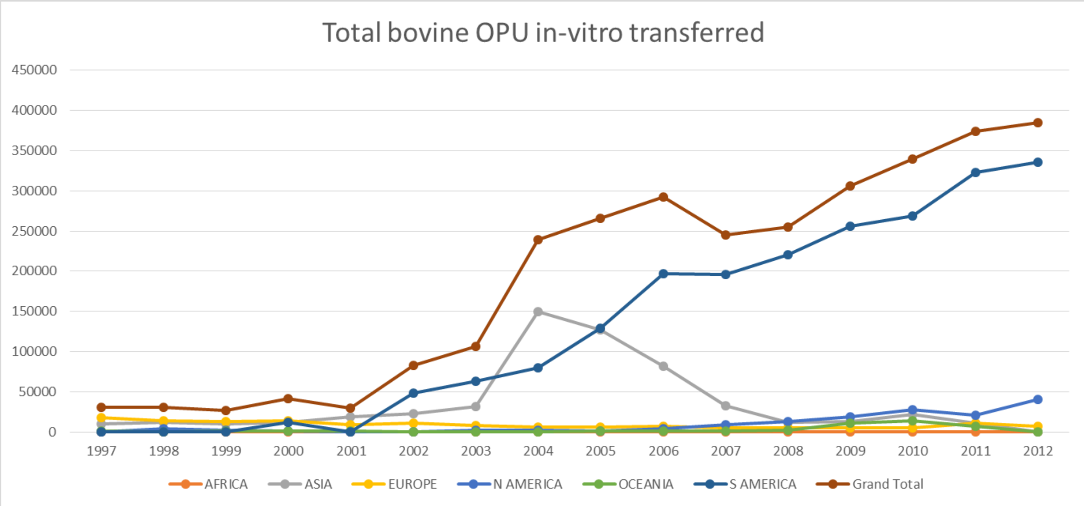

Figure 3 Numbers of bovine OPU IVP embryos transferred (Perry, 2013)

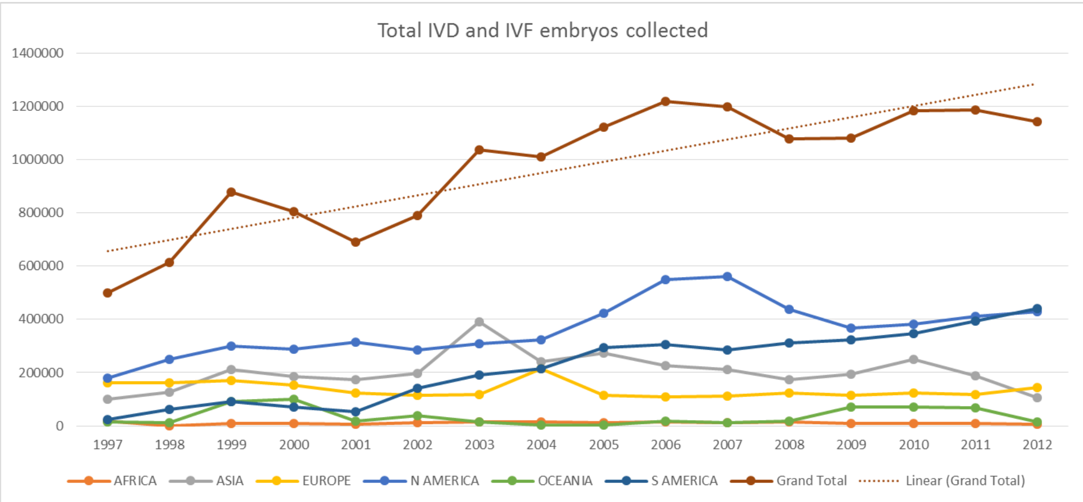

Figure 4 Total cattle embryos transferred worldwide (Perry, 2013)

Table 4 Numbers of cattle embryos produced *in vitro* and transferred in 2013 and 2014 in Europe and North and South America (Perry, 2015)

Region	Embryos produced		Embryos Transferred	
	2013	2014	2013	2014
Europe	13,722	15,693	11,000	13,937
North America	112,300	206,139	66,602	92,930
South America	376,459	356,960	304,988	251,273

As shown in Table 4, the production of IVP embryos dramatically increased in North America in 2014 compared to the previous year; it slightly increased in Europe and dropped a little in South America due to severe drought conditions in major beef areas and falling beef prices.

Another interesting feature of the ET industry for cattle IVP embryos is the drop in the percentage of IVP embryos transferred as fresh, which means that freezing IVP embryos which has often been reported as unsuccessful has a tendency to increase (Table 5).

Table 5 Fresh bovine IVP Embryos transferred as % of all IVP embryos transferred (Perry, 2015)

Region	%IVP Embryo transferred fresh	
	2013	2014
Africa	66.5%	87.6%
Asia	57.2%	-
Europe	76.6%	78.8%
North America	81.0%	76.7%
Oceania	44.4%	39.2%
South America	95.1%	84.0%
Grand total	**89.8%**	**81.3%**

Comparative impact of AI and ET in cattle herds: Considering those figures in a prospective approach, it is also interesting to realize that the impact of ET on the worldwide cattle population is still very low (Table 6), allowing foreseeing some progress provided that the cost of this technology remains reasonable.

Table 6 Impact of AI and ET on worldwide cattle population (Thibier, 2005)

Regions	Total number of bovine females of breeding age (*)	Total first service AI	Impact ratio of AI (**) ×100	Total number of IVD and IVP embryos (***)	Impact ratio of ET (****) ×100
Africa	51,577,000	870,892	1.68	6,347	0.01
North America	42,296,000	11,203,880	24.80	275,890	0.65
South America	124 460 000	1 366 678	1.09	413,896	0.33
Asia	236,850,000	58,181,005	24.56	65,435	0.02
Near East	23,433,000	1,068,991	4.55	0	0
Europe	61,750,000	37,738,142	61.11	114,099	0.18
Total	**543 276 000**	**110,429,588**	**20.32**	**875,667**	**0.16**

(*) 40% of total cattle and buffaloes
(**) calculated from the total first service of AI divided by the total number of females of breeding age
(***) data from Perry (2013)
(****) calculated from the total number of IVD and IVP embryos divided by the total number of females of breeding age

Economic impact on international trade of embryos: It is hard to have a good estimate of the international movement of embryos for many reasons, including the lack of reports from some countries and illegal imports. A preliminary approach to the international movement of germplasm was reported by Thibier (2009) as well as Thibier and Wrathall (2012). Fortunately, a major feature of the 2015 IETS data retrieval committee report was the improved ET data for exports of bovine IVD embryos around the world.

As shown in Table 7, 17 of 38 countries provided ET export activity data in 2014, as opposed to seven countries the previous year, indicating vast improvement. Of the 32,285 bovine IVD embryos exported, 21,467 (66%) were embryos collected from dairy cattle, the rest were from beef cattle.

Table 7 Number of IVD embryos exported from countries known to export (Perry, 2015)

Animal Spp.	Country	Exported
Bovine IVD	Argentina	2827
	Australia	32
	Belgium	90
	Canada	10920
	Denmark	61
	Finland	8
	France	562
	Hungary	15
	Luxembourg	50
	Netherlands	1040
	South Africa	926
	Spain	66
	Switzerland	151
	United States	15537
	TOTAL	**32285**
Bovine IVP OPU	Canada	821
	Panama	60
	Russian federation	100
	TOTAL	**981**

Animal Spp.	Country	Exported
Sheep IVD	Australia	414
	Canada	200
	New Zealand	79
	TOTAL	**693**
Goat IVD	New Zealand	3
	United States	199
	TOTAL	**202**
Swine IVD	France	112
	TOTAL	**112**

The main technical reasons for a sustainable technology in the world: Illustration from Europe: Obviously, if the ET technology remains active more than 40 years after its beginning, it shows that the market is there and that the economic benefit the farmers get from applying it with its diverse possibilities is real. It also clearly means that the results the practitioners perform every day are up to the expected high standards. The 3 main technical reasons for such success in implementing this embryo transfer technology on the ground will be reported briefly, according to my personal opinion.

A technology well adapted for improvement in genetic value of cattle population: It was early recognized that embryo transfer was the tool of choice for good genetic management to allow generating particular bull sires with top breeding value and being the top of the pyramid of progeny and performance testing. This was particularly true for the sophisticated programs in place in Europe for dairy cattle. It has contributed to significantly improve the efficiency of such programs as judged by the mean annual improvement rate, notably in reducing the interval between generations simultaneously to intensify the selection of candidates and improve the precision of such selection.

Transfer of high value embryos became, in 1980-2005, the core of all the genetic programs and this explains also why in Europe, many ET teams were closely related to the artificial insemination centers or companies in charge of conducting genetic improvement schemes.

When the genomic revolution occurred in the mid-2000's, it was impressive to see how quickly the ET industry embarked in such a revolution, modifying strongly the ET and dam schemes and providing the most useful service to the new schemes. Genomic tools are now available for most livestock species and are used routinely for genomic selection (GS) in cattle (Ponsart et al., 2014a). With this genomic selection in effect, it is necessary to generate a larger number of candidates to evaluate in a given period of time. Whatever the strategy in place, it will be necessary to identify a large number of elite females which will then either be bred with sexed semen or from which a batch of IVP embryos would be collected in order to be later transferred on recipients. A good combination of semen and embryo biotechnologies will make it possible to act on all parameters involved to speed up the genetic gain. One of the most important developments resulting from the introduction of genomic testing for dairy cattle is the application of reasonably priced low-density single nucleotide polymorphism technology in the selection of females. Moreover, multiple markers have been detected in biopsies of preimplantation stage embryos, thus paving the way to develop new strategies based on preimplantation diagnosis and genetic screening of embryos (Ponsart et al., 2014b). The biopsies of embryo can now be used with success in order to diagnose the genomic value of such entities. At the same time, a balance needs to be reached between removing a small number of blastomeres to preserve viability and sampling a sufficient quantity of DNA for further analysis. Ponsart et al. (2014b) reported some interesting results (Table 8).

Table 8 Number of IVD embryos exported from countries known to export (Perry, 2015)

Reference	Variation Factor	Class	N	Conception rate (%)
Lacaze et al.	Embryo stage	Morula	167	47.3
(2008)		Early Blastocyst	39	48.7
		Blastocyst	16	62.5
	Biopsy size	<3	26	55.6
	(no. cells)	3-7	65	47.8
		>7	37	49.4
Cenariu et al.	Embryo stage	Morula	186	44.1
(2012)		Blastocyst	114	43.0

A technology applied by well-trained and competent practitioners and continuous innovation: One of the striking features in this area has been, since the beginning, the high professionalism of all involved in embryo transfer. Very quickly, due to the critical importance of even the smallest of all details, people in charge and all staff, both in veterinary clinics and artificial insemination cooperatives, realize that training of excellence is absolutely necessary in order to obtain the best results possible. Good training and professionalism are key points to ensure that the market will be sustainable and farmers applying the technology are keen on applying it in their own herds. In addition to training and professionalism, the third component is innovation, a major feature of the ET industry. Innovation is constantly under scrutiny at both ends, that of daily practice and institutional research. This allowed the technology to positively evolve and practitioners to keep abreast of discoveries of all kinds to the farmers' benefits (Thibier, 2005).

Examples of such European contribution (as recently reported at the Association Européenne de Transfert Embryonnaire (AETE) meeting, Thibier, 2014) are provided here below. This does not minimize at all contributions from other parts of the world which have also been most critical for the development of the technology.

Research and innovation in Europe have been most instrumental in this area with the leadership of extraordinary pioneers as recognized as such, worldwide by the Pioneer Award of the International Embryo Transfer Society (IETS) (Table 9). In particular such pioneers in embryo transfer and reproductive physiology as a whole, such as J. Hahn (Germany) and C.H. Thibault (France), were very supportive since the beginning, thanks to them.

Table 9 Names of European IETS pioneer awardees

Name	Years	Names	Years
J.P. Renard	2015	R.G. Edwards	1993
J. Hahn	2013	A.K. Tarkowski	1991
I. Wilmut	2011	C.H. Thibault	1989
S. Willadsen	2005	A.L. McLaren	1988
I. Gordon	1998	E.J.C. Polge	1987
S. Winterberger Torres	1997	L.E.A. Rowson	1985

Some of these IETS awardees were also recognized by the AETE and the first AETE awards were precisely given to J. Hahn and C.H. Thibault in 1993 and 1994, respectively.

Looking back at the table of contents of the AETE meetings, one has a very good idea of the evolutions of ideas, and the techniques discussed, and further implemented once back on the field following the meetings. In the first years, three topics dominated the meetings and most of the discussion in sessions or out at coffee breaks. The first related to superovulation which was the main part of the meeting of the first year of AETE with published proceedings and subsequent meetings. In 1987, there were 6 invited presentations on superovulation by scientists who further had a major influence on this technology worldwide, namely:

- Superovulation chez les bovins…………………..by J. Saumande
- Induction of superovulation in cattle…….………...by J.F. Beckers
- Improved embryo yield ……………………..…..by S.J. Dieleman
- Comparison of 2 to 3 days FSH treatment………by H. Callesen
- Effect of LH on FSH induced superovulation……by D. Chupin

The two other major topics treated at AETE at that time were fresh vs frozen *in vivo* derived embryos and modes of embryo transfer.

However, very quickly, the Association extended its points of interest and as soon as 1988, in Lyon, I. Wilmut presented a famous lecture on "Biotechnology and the bovine embryo: at present and in the future". This was way before the birth of Dolly. By the same token, it was in 1990 that two other famous lectures were given by G. Brem on "State of the art, limitations and prospective of gene transfer in domestic mammals" and J.P. Renard on "the state of the art, limitations and prospective of cloning in domestic mammals". Those two lectures had a profound influence on the world's thought about biotechnologies in farm animals.

It is about at that time, in the late 80's, that *in vitro* production of embryos also became a major subject of oral presentations, posters, round tables and debates. The presentation by P.L.A.M. Vos and colleagues in 1990 of the methodology in use for applying what was since then called OPU (Ovum Pick Up) was also one of the highlights of the innovation realized in Europe.

Looking retrospectively, it is clear that one of the major reasons for this sustainability of ET in cattle in Europe is the constant search for innovation at both levels, practically on the animals' side and more fundamentally on the bench.

Embryo transfers: the safest mean of exchanging genes: This statement was presented by Thibier in 1990 (Thibier, 1990b) and was one of the major points claimed to the world and to the society as a whole, explaining why this critical comparative advantage from now on "allows any farmer in the world to obtain any gene that "Dame Nature" has provided the world with, at no risk health wise".

This statement results from a long chain of involvements and of strong sound research from the veterinary community in close association with the embryo transfer industry including that in Europe. Research in the early 80's showed that pathogens such as Foot and Mouth Disease virus, for example, behaved in a specific manner and in such a way that there was a possibility to eliminate pathogens associated with the embryo zona pellucida. Without getting into too many details which have been widely reported (Thibier, 1990b), it was then elaborated together with scientists and practitioners one specific set of procedures to follow in order to secure this high level of safety. This relies on the concept of the "official embryo collection teams" further extended to the "official embryo production teams" officially designated and approved by the national veterinary authorities. The EU, for example, publishes a list of such official ET teams of which the total for the EU is 229, with those from Norway and Switzerland making a total of 235. The countries with the most ET teams are Germany, France and United Kingdom at 39, 36 and 31, respectively.

This concept was somewhat opposed to what then (early 80's) was the dogma of the veterinary community to limit and prevent the extension of animal diseases. For example, for AI centers, the policy relies on the fact that only specific disease free males are allowed to enter an AI center, itself free from such specific diseases. Here for ET, it was found uneconomic and impracticable in many circumstances and particularly in the European context to manage embryo donors in closed facilities such as AI centers.

A major step to allow the ET industry to work efficiently and safely was the organization of a round table in 1985 at the World Organization for Animal Health (OIE) which agreed on publishing and further implementing this concept of the "official embryo transfer team", which had the responsibility to follow all the rules and guidelines put forward in order to prevent all association of pathogens with embryos. This was further "translated" in international guidelines on one hand and further legal directives, laws, decrees or rules on the other hand. The former was the elaboration of the relevant chapters of the OIE Terrestrial Animal Health Code, which since then has been the template of any legislation implemented domestically. Regarding the European Union, it is with the guidelines elaborated following the 1985 OIE round table was prepared, proposed and discussed the famous EU Directive 89/556/EEC published on 25 September 1989.

And the end point, as published elsewhere (Thibier, 2011), ET has proven on the ground and for more than 30 years that it is indeed the safest mean of exchanging genes with no confirmed report of any contamination of recipients or offspring from transfers of IVD or IVP embryos.

Conclusion

The challenges to the ET industry are clearly of three types: facilitate the genomic selection in applying the most modern reproductive biotechnologies, perform the best results possible so as to contribute to level off the price of this technology and make sure the ET teams strictly apply the veterinary rules and regulations at no risk health wise. Observations made those last forty years or so make us feel quite confident that the future of the ET industry is bright. This optimism is due to the high level of technical expertise and extreme professionalism of all veterinarians and technicians involved in this germplasm industry technology. Major key points will be to ensure continued integrity and track record for the safe movement of germplasm, ethical conduct of the officially approved embryo production teams and, in terms of risk management, their adherence to specified criteria that should be met at all times.

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บทคัดย่อ

ความท้าทายและรูปแบบการย้ายฝากตัวอ่อนในเชิงอุตสาหกรรมจากจุดยืนของสหภาพยุโรป

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การย้ายฝากตัวอ่อนโดยการเก็บตัวอ่อนจากตัวสัตว์ (*In vivo* derived, IVD) เริ่มต้นในยุค 70 และจากการผลิตตัวอ่อน (*in vitro* produced, IVP) เริ่มต้นในยุค 80 ซึ่งเป็นยุคของการนำเทคโนโลยีชีวภาพ ทางการสืบพันธุ์ในระยะที่สองและสามตามลำดับ ไปใช้ในเชิงพาณิชย์ หลังจากผ่านไป 40 ปี มีบทพิสูจน์ความเป็นไปได้ในการนำเทคโนโลยีเหล่านี้ไปใช้ในอุตสาหกรรมปศุสัตว์ บทความฉบับนี้เกี่ยวข้องกับตัวอ่อนในโคเท่านั้น ในส่วนแรกรายงาน จำนวนของตัวอ่อนที่เก็บได้หรือผลิตได้ และขนส่งไปทั่วโลกประมาณ 1 ล้านตัวอ่อนของโคถูกขนส่งในแต่ละปี แสดงให้เห็นชัดเจนถึงผลกระทบอย่างมีนัยสำคัญต่ออุตสาหกรรมโค ในส่วนที่สองอธิบาย เกณฑ์ 3 ข้อ ที่นำมาใช้เพื่อให้บรรลุวัตถุประสงค์หลักของเทคโนโลยีนี้ คือเพื่อทำกำไรให้แก่เกษตรกร ได้แก่ (1) เป็นเครื่องมือในการคัดเลือกพันธุกรรม เพื่อพัฒนาเทคโนโลยีชีวภาพทางการสืบพันธุ์ผ่านทางการวิจัยทาง วิทยาศาสตร์ (2) เพิ่มความชำนาญให้กับสัตวแพทย์และนักเทคนิค ในการทำให้อัตราการผสมติด สูงที่สุดเท่าที่จะเป็นไปได้ และ (3) การดูแลด้านสุขาภิบาล เพื่อให้เกิดความปลอดภัยในการขนส่งตัวอ่อน ที่ปราศจากเชื้อโรค ความท้าทายในการคงอยู่ทางอุตสาหกรรมการย้ายฝากตัวอ่อน คือ การพัฒนาให้คงอยู่อย่างถาวร การทำวิจัยในระดับสูง ความเชี่ยวชาญทางด้านเทคนิค และความเป็นมืออาชีพ ของนักวิทยาศาสตร์ทุกคน สัตวแพทย์ และนักวิทยาศาสตร์ที่เกี่ยวข้องกับเซลล์ทางระบบสืบพันธุ์ จะช่วยขัดเกลาให้อนาคตของเทคโนโลยีชีวภาพเหล่านี้สดใสขึ้น

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