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A history of farm animal embryo transfer and some associated techniques

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Abstract

Events over the last 125 years that have been particularly important to the development of embryo transfer in farm animals are reviewed, arguing that an appreciation of the history of a discipline helps shape its future. Special attention is paid to how the motivations of the scientists involved have changed over time, and how these changes have influenced the practical application of embryo transfer to animal breeding.

Keywords: Embryo transfer; Cattle; Sheep; Goats; Pigs; Horses; History

1. Introduction

> "... the history of science bores most scientists stiff. A great many highly creative scientists ... take it quite for granted, though they are usually too polite or too ashamed to say so, that an interest in the history of science is a sign of failing or unawakened powers" (Medawar, 1968).

Mea culpa, mea culpa. Even the briefest perusal of the contents of this volume will indicate just how broadly embryo transfer is now used as a tool in livestock breeding and burgeoning biotechnology industry. Even the most passing acquaintance with the mass media will indicate just how deeply work with embryos currently affects (and troubles) the general public, well beyond the earlier confines of the animal breeding industry. It was not always thus, and a consideration of how present circumstances came about has, in my opinion, lots to teach us about the likely future of embryo transfer. The history of our subject

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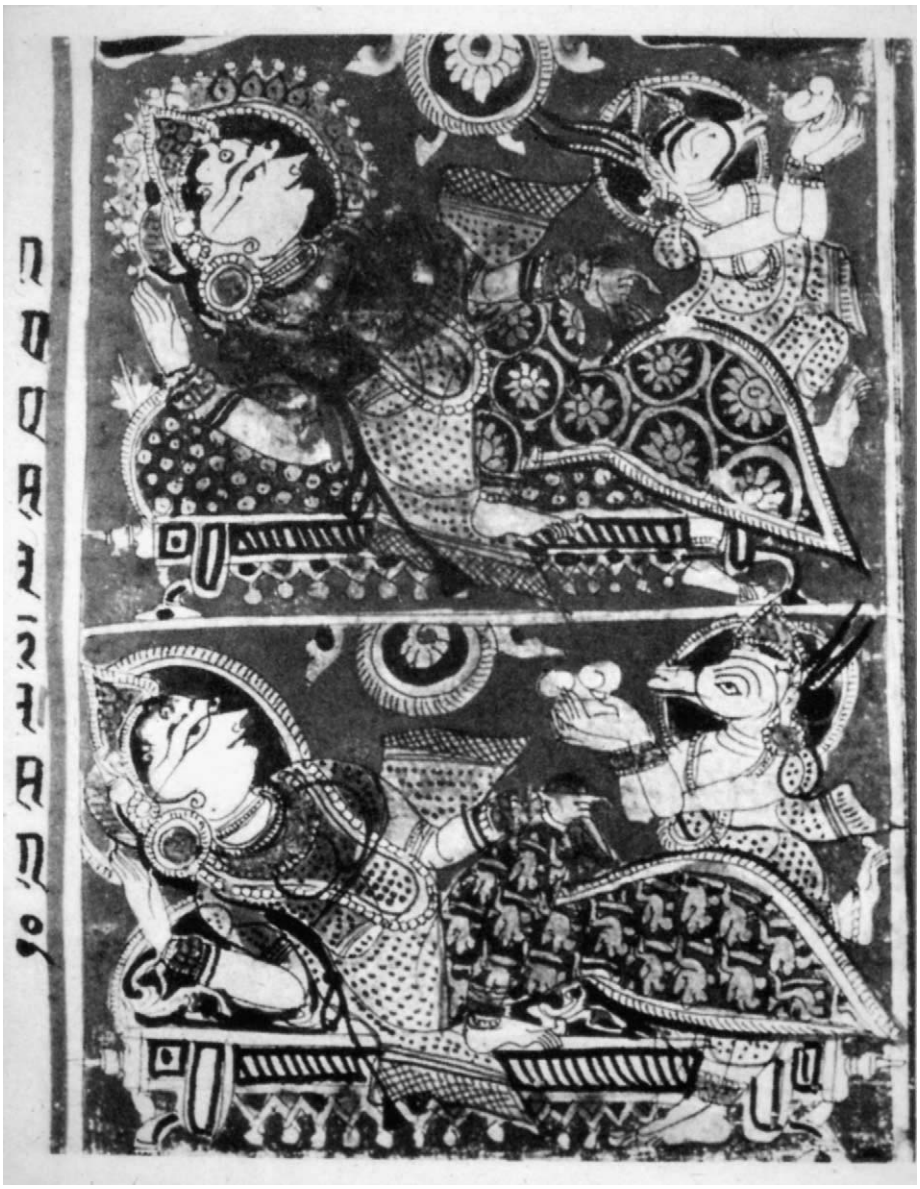

Fig. 1. A painting from the northern Indian state of Rajasthan, depicting the birth of the Mahavira, the founder of the Jain religion, in 599 B.C. Sleeping at the top is Devananda, the wife of a priest, in whom the Mahavira was conceived. Below, also asleep, is Queen Trisala, into whose womb the Mahavira was transplanted by the horse-headed creature, Harinegameshin, seen to the right of each panel. The transfer was necessary because saints and founders of religions could only be born of royalty (Guttman and Guttman, 1980).

also reveals a delicious scientific irony: the first successful embryo transfers showed that rabbits of one breed can be gestated in the reproductive tract of females of another breed without being affected by the uterine environment (Heape, 1891, 1897b); more than a century later, embryo transfer is central to investigations of just how profoundly embryos, fetuses and offspring *can* be affected by their earliest environment in vivo or in vitro.

So, reviewing the development of embryo transfer as a tool in the hands of biologists of widely varying interests presents opportunities to examine how our subject has changed, and been changed by, parallel developments in the scientific and social milieux since late Victorian times. Opportunities, too, for considering what our predecessors—the users of the tool and the shapers of the milieux—thought about how embryo transfer could, should, and should not be applied to the advancement of science.

Before embarking on such a strictly rational approach, however, let us remember that the concept of transferring a baby from one uterus to another has been a part of human religious thinking for a very long time (Guttman and Guttman, 1980; Figs. 1 and 2). Could it be that

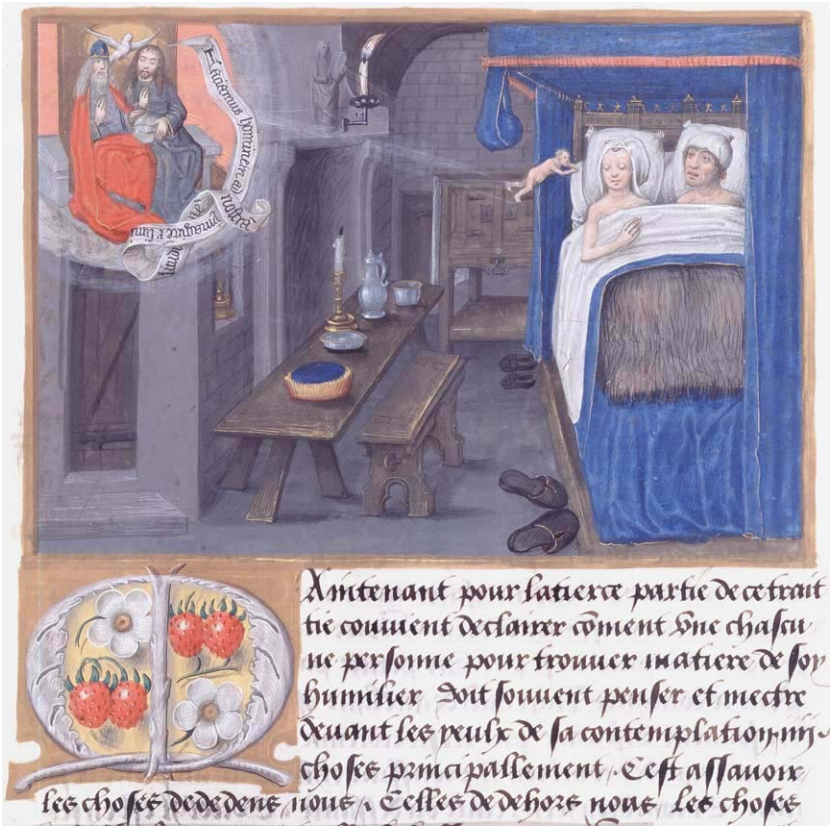

Fig. 2. A man and wife ‘receiving’ a baby from the Holy Trinity. Miniature from a 15th century manuscript bearing the inscription “Faciamus hominem ad ymaginem et simultudinem nostram (We make man in our own image)”. Bibliothèque de l’Arsenal, Paris (Brought to my attention by Dr. Wolfgang Jöchle).

any replacement of such wondrous religious mysteries with sterile clinical procedures is subconsciously resented and affects public opinion of what we do with embryos?

2. The late 19th century

Yes, it was indeed Walter Heape (1855–1929; Fig. 3) who first produced live young (rabbits) by embryo transfer in April 1890 and richly deserves the recognition that he

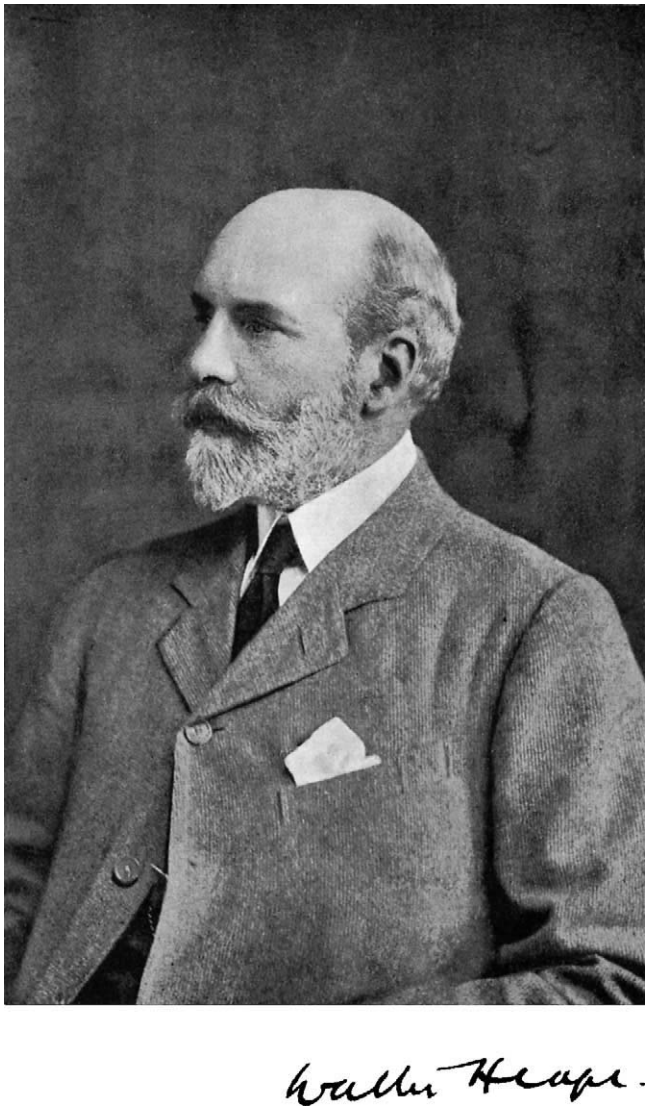

Fig. 3. Walter Heape (1855–1929) (from Marshall (1930); © The Royal Society).

Fig. 4. George John Romanes (1848–1894). The Elliott and Fry portrait used as frontispiece in Romanes (1908).

has earned for the feat (Betteridge, 1981; Biggers, 1991; Heap, 1992). We shall return to Heape and his rabbits shortly. Here, though, it is well to recognize George John Romanes (pronounced in the rhythm of Gonzales, 1848–1894; Fig. 4) whose first attempts at embryo transfer, albeit unsuccessful, may have preceded those of Heape (Biggers, 1991).

George John Romanes was born on 19 May 1848, in Upper Canada (Kingston, Ont.) where his Scottish father was Professor of Classical Literature at the newly founded Queen's

College (later Queen's University) and his mother a "vivacious, unconventional highland wife" (Ringereide (1979) cited by Forsdyke (2001)).[1] The family left Canada for England in 1850 and lived in London. There, the young George was educated mostly at home and by travel to Heidelberg and other German towns which left him with "a knowledge of German and a passion for music and poetry" (Ringereide, loc. cit.). After initial interest in an Oxford education and a calling to the Church, Romanes entered Gonville and Caius College, Cambridge, at the age of 19. It was at Cambridge that he became interested in Natural Science, inspired particularly by the lectures of Dr. Michael Foster. Romanes took honors in the Natural Science tripos in 1870 at which time he moved to London to work in the Physiological Laboratory at University College, then under the direction of Professor Burdon Sanderson. Romanes' devotion to science became absolute during his convalescence from typhoid fever in the early 1870s. An early letter of his to Nature (Romanes, 1873) caught the attention of Charles Darwin who wrote kindly to Romanes and so initiated a lifelong friendship and correspondence between the two. Many of their letters were published by George Romanes' wife, Ethel, after his death (Romanes, 1908). It was Romanes' interest in Darwinism and evolution that led him to use embryo transfer as an experimental tool. Unlike Heape, Romanes had no primary interest in agriculture and was best known for his work on the locomotion of medusae and echinoderms, and behavior and intelligence in animals and humans.

Concepts of the nature of heredity during Romanes' regrettably short scientific career (he died prematurely from a brain tumor on 23 May 1894, at the age of 46) were in ferment, as has been succinctly described by Biggers (1991). Broadly speaking, biologists were struggling to interpret all that Darwin's "Origin of Species" had revealed and implied, but without the mechanistic understanding that was to come with the "rediscovery" of Mendel's work by de Vries, Correns, and Tschermak, and its publication in English in 1900 (see Bateson (1902); Forsdyke[2]). Views were polarized between those (the neo-Lamarckists) who held that acquired characteristics are passed on through the generations and those (notably Weismann and his followers) who insisted that they are not. Romanes accepted many of Weismann's ideas but felt that they went too far in proposing that the inheritance of acquired characteristics could be *absolutely* excluded (Romanes, 1899). With a view to prove his argument against Weismann, and bolstering Darwin's theory of pangenesis, Romanes described, in a letter dated 11 November 1889 to Professor Poulton[3] (Romanes, 1908) how:

> "Although I spent more time and trouble than I like to acknowledge (even to myself) in trying to prove Pangenesis between '73 and '80, I never obtained any positive results, and did not care to publish negative. Therefore, there are no papers of mine on the subject, although I may fairly believe that no other human being has tried so many experiments upon it".

Those experiments, which would have been done in London, included the replacement of the blood of single rabbits of a domestic breed with the blood of three wild rabbits,

[1] See also http://post.queensu.ca/~forsdyke/romanes.htm#Genealogy.

[2] http://post.queensu.ca/~forsdyke/bateson1.htm.

[3] Presumably the authorized translator of August Weismann's *Aufsoetze ber Verebung und verwandte biologischhe Fragen* (Essays Upon Heredity and Kindred Biological Problems; English translations by Edward B. Poulton, two volumes published by Clarendon Press, Oxford, 1892 and 1889).

the grafting of ovaries from one breed of rabbit to another, and—of most relevance to this paper:

> "... the transplantation of fertilized ova from one variety to another, for the purpose of ascertaining whether, if a parturition should take place under such circumstances, gestation by the uterine mother would affect the characters of the ovum derived from the ovarian mother—she, of course, having been fertilized by a male of her own variety" (Romanes, 1895).

It is interesting that Romanes took for granted "that both the mothers should be in season at about the same time"; the necessity for such synchrony would not be firmly established experimentally for another 40 odd years in rats (Nicholas, 1933) and for more than 60 years in rabbits and other species (Chang, 1950). One of the reasons that Romanes chose rabbits for his experiments was their almost constant estrus. The other reason was that the breeds he used (Himalayans and Belgian Hares) "are well-marked varieties, breed true, and in respect of colour are very different from one another". He subsequently lamented this choice, thinking, erroneously, that hybridization in rabbits fails to produce intermediate forms that might have revealed the effect of one breed on the other. For this reason he had planned, in the last year of his life, to repeat the between-breed ovarian grafting experiments in dogs, well known to produce clearly recognizable cross-breeds (Romanes, 1895). Much later, ovarian transplantation was used successfully in guinea pigs to answer the kind of question that Romanes was interested in, but provided an answer that Romanes would not have relished (Castle and Phillips, 1909).

It seems unlikely that we shall ever know exactly when, or how, Romanes performed his ovum transfers, the only account of them seeming to be the posthumous one referred to above (Romanes, 1895) and a passing reference in an appendix on telegony in his treatise on Weismannism (Romanes, 1899). Personally, I think that his experiments must have been contemporaneous with those of Heape because he says, very specifically (Romanes, 1895):

> "... *while* [italics added for emphasis] I was at work upon this experiment, it was also being tried, unknown to me, by Messrs. Heap [sic] and Buckley who, curiously enough, employed exactly the same material".

My assumption, though, is contradicted by the 1873–1880 dates mentioned in Romanes' letter to Poulton (above). More information on the date of Romanes' embryo transfers could also help define where they were done, for he moved from London to the Oxford Physiological Laboratory in November 1890 (Romanes, 1908). As to the 'how', Foster and Balfour's *The Elements of Embryology* seems to be a very likely source of Heape's embryo transfer methods (Betteridge, 1981; Biggers, 1991) and could also have served Romanes who, as has been said, drew great inspiration from Foster. This book was first published in 1874 and a second edition, edited by Sedgwick and Heape because of Balfour's tragic loss in a climbing accident in July 1882, appeared in 1883. "Practical Directions for Obtaining and Studying Mammalian Embryos" are given in an appendix to the second edition, and recommend the rabbit for class work. After reading how to dissect out the oviduct onto a glass slide, we learn that:

> "With the aid of a lens it is frequently possible to distinguish the ovum or ova, through the wall of the oviduct. In this case cut a transverse slit into the lumen of the duct with a fine pair of scissors a little to one side of an ovum; press with a needle upon the oviduct on the other side of the ovum, which will glide out through the slit, and can be with ease transported upon the point of a small scalpel, or what is better spear-headed needle. In case the ovum cannot be distinguished in the oviduct by superficial observation, the latter must be slit up with a fine pair of scissors, when it will easily be seen with the aid of an ordinary dissecting lens".

Without any experimental details, it is impossible to know if and how Romanes induced pregnancy or pseudopregnancy in his recipient does. Heape used mated recipients, but is it possible that Romanes' failures were due to the fact that the necessity for corpora lutea for pregnancy maintenance had yet to be demonstrated (by Fraenkel and Cohn (1901))?

Romanes would not have been human had he not been a little envious of Heape and Buckley's success in 1890. He graciously acknowledged that "they were the first to obtain a successful result". However, his footnote (Romanes, 1895) to the effect that Heape and Buckley were not concerned with any theory of heredity seems to me to split hairs. Heape (1891), after all, made rather clear his appreciation of how his experiment related to heredity, writing:

> "The experiment . . . was undertaken to determine in the first place what effect, if any, a uterine foster-mother would have upon her foster children, and whether or not the presence and development of foreign ova in the uterus of a mother would affect the offspring of that mother born at the same time. So far as this single case goes, the evidence is negative".

Was the investigation of that second question, concerning a putative effect of foreign on indigenous ova, which required the use of pregnant recipients, the serendipitous key to Heape's success?

Romanes' unsuccessful forays into embryo transfer are only incidental to his exceptional contributions to science in the fields of psychology and evolutionary biology. He is probably best remembered through the lecture series that he endowed at Oxford but Forsdyke (2001) considers that "Romanes' contribution to evolutionary biology is no less significant than that of Mendel" although it has taken more than a century for his major work (Romanes, 1897) to be appreciated.

Returning now to Walter Heape, it must be said that much has already been written about him and his transfers (Marshall and Hammond, 1946; Betteridge, 1981; Adams, 1982; Biggers, 1991; Heap, 1992). Here, let us just be reminded that he was a remarkable character whose influence on reproductive biology and agriculture extended far beyond embryo transfer. It was Heape's 1897 paper on artificial insemination (AI) (Heape, 1897a), for example, that rekindled practical interest in AI, particularly in horses (Heape, 1898).[4] However, his illustrious successors at Cambridge, F.H.A. Marshall and John Hammond, counted his most important work to be *The Sexual Season of Mammals* (1900) in which were coined the very terms we use today to describe the estrous cycle (Marshall and Hammond,

[4] Curious to note, given today's attitudes in the thoroughbred industry, that one of the earliest successes was the production of the first-class thoroughbred foal Sandflake out of Sandiway after insemination with semen from Trenton (Marshall and Hammond, 1946).

1946; Heape, 1900). The first text-book on reproductive physiology, Marshall's *Physiology of Reproduction* (Marshall, 1910) was dedicated to Walter Heape, and Heape's own book on *The Breeding Industry* (Heape, 1906) contains numerous prophetic passages calling for research on reproduction in the farm animals. One of special relevance to embryo work deserves to be quoted verbatim:

> "... it is the functional condition of the generative organs which require [sic] investigation; knowledge ... of the intimate physical relation of the embryo to the mother, and all the intricate physiological phenomena connected with the pre-natal and post-natal nutrition of the embryo that are here concerned; and it is work from which great benefit would undoubtedly be derived".

Biggers (1991) explains that Heape almost certainly performed his first transfer not in Cambridge, as the publication would suggest, but in a laboratory in his home in Prestwich, Manchester. Heape worked with the acknowledged assistance of Mr. Samuel Buckley, a well-known surgeon in the Manchester area at the time. The parallel with R.G. Edwards' partnership with Mr. Patrick Steptoe to perform their ground-breaking work on human IVF in another Lancashire town, Oldham, is striking. Also striking is the coincidence in the choice of rabbit breeds for the earliest embryo transfers: Angoras and Belgian Hares by Heape, Himalayans and Belgian Hares by Romanes. The name of the Belgian Hare breed has led to confusion in some quarters, with claims that the first transfers were between species![5]

Yet more striking as features of Victorian science in Britain are the relatively small size of the scientific world at that time, and the interconnections between its biologists and their patrons. We have seen (above) how both Romanes and Heape were connected to Foster at Cambridge. Romanes published and lectured with J. Cossar Ewart, professor of Natural History at the University of Edinburgh. Ewart hired Marshall to work with him on an experimental study of telegony. Ewart corresponded with Heape and with Alice Balfour, sister of Frank Balfour (co-author of *The Elements of Embryology* with Foster and friend of Romanes) and of Arthur Balfour, later Prime Minister, who sponsored a Chair at Cambridge for Bateson. Heape took up a Balfour fellowship shortly before communicating the success of his 1890 transfer. These networks—of which the preceding skein gives only a glimpse—are of much more than social interest; they gave rise to dynasties and institutions in reproductive physiology that influence British research in the area to this day (Adams, 1982; Hunter, 1995; Betteridge, 2001).

Just as the earliest transfers of embryos were not seen as being important to animal production, neither were contemporaneous studies of embryos themselves—studies undertaken for fundamental, not applied, reasons. In continental Europe, a "new" science—"Developmental mechanics" (Entwicklungsmechanik)—came into existence at the end of the 19th century, thanks chiefly to Wilhelm Roux (1850–1924) who is generally acknowledged as the "father" of experimental embryology. Belgium was a particularly strong center of these studies (also known as "causal mammalian embryology"), which were to become of

[5] In fact, rabbits of the Belgian Hare breed are handsome animals, once in favor with gold beaters who found their long, furry hind limbs useful for applying finely powdered gypsum to gold beaters' skins, so allowing the smooth expansion of gold over the skin!

increasing, unanticipated relevance to embryo transfer, as has been well told and illustrated in reviews by Mulnard (1986) and Alexandre (2001).

3. The first half of the 20th century

The first evidence of interest in embryo transfer beyond the British Isles seems to be the paper of Biedl et al. (1922) from the Institut fuer Allgemeine und Experimentelle Pathologie, Vienna, also in rabbits. Their work was done during 1911–1913 but publication was delayed by the war and Biedl's move from Vienna to Prague (Adams, 1982). They made their transfers into the uterus rather than the oviduct and, from 70 experiments, achieved only one pregnancy, which is hardly surprising in the light of what we now know about the necessity for synchrony between embryonic and reproductive tract development. Even their single pregnancy was viewed as "doubtful" (Pincus, 1936), probably because the young were born at night and had been eaten by next morning (Dowling, 1949). Arthur Biedl was an outstanding endocrinologist of the early 20th century, still known for describing the syndrome that bears his name (the Lawrence–Moon–Biedl syndrome). He should also be remembered "as the author of an astounding textbook of endocrinology, which came in two volumes of more than 1000 pages, and based on more than 8000 (!) references, reviewed the knowledge of basic and clinical endocrinology of that time in every detail".[6]

Rabbit embryos were also the first to be used in attempts to culture mammalian embryos in vitro, by Brachet in 1912, no doubt because of the popularity of the rabbit as an experimental animal at that time (Mulnard, 1986; Alexandre, 2001). Again, the objective was to make mammalian embryos accessible for experimental investigation, not transfer.

Writing in 1925, John Hammond arrived at an intriguing, though tentative, interpretation of Heape's results in terms of the effect of the influence of the embryo and fetus on the duration of pregnancy. Hammond noted that Heape's transferred (Angora) ova were older than the endogenous Belgian Hare ova (actually by ∼29 h), and that the two Angora and four Belgian Hare offspring were all born on the 32nd day after coitus. The Angoras were stronger than the others and Hammond concluded:

> "... it would appear that the youngest set of embryos determined the time of birth ... but the results are not conclusive".

Hammond (1925) went on to advocate research on this subject by transplantation of fertilized ova of a definite stage into animals with corpora lutea of different ages, and vice versa.

The late 1920s and 1930s saw the beginnings of activities on both sides of the Atlantic (and, indeed, transatlantic exchanges) that ushered in the notion that embryo transfer could be of practical value to agriculture. A pivotal exchange was between Cambridge, Massachusetts, and Cambridge, England, and I can do no better than to repeat a previous account of the importance of this (Betteridge, 1981). Marshall and Hammond (1946), writing of research at Marshall's Institute of Nutrition at Cambridge University, follow a section on the advantages of AI with the following statement:

[6] http://www.akh-wien.ac.at/expatho/history.html.

> "... the first steps towards a similar extension of the reproductive powers of good genetic stock on the female side ... [were] ... begun in 1929 by G. Pincus, a visiting National Research Council fellow from Harvard University, who, following work initiated by Heape, cultivated the fertilized eggs of the rabbit outside the body and later successfully transplanted them into other does. By the use of anterior pituitary hormones ... he was able to obtain up to fifty or more fertilized eggs at one time for this purpose. These experiments have yet to be extended to farm animals, in which its [sic] possibilities are great".

Pincus (1930) describes a successful outcome of one of the three control transfers and cites Asdell and Hammond as obtaining 3 litters from a series of 21 transfers. According to Adams (1982), the actual transfers were performed by Hammond and Walton at this stage. While there may be some question as to the precise chronology of these developments (Betteridge, 1981), there is no doubt about Marshall and Hammond's enthusiasm for applying science to agriculture in ways such as these. In 1940, for example, John Hammond wrote (Hammond, 1940):

> "The economic success of the animal industry depends on the successful production and proper development of the next generation of our farm animals. ... experience gained on domestic animals will in the future enable man the more easily to control his development also. During the last 100 years great advances have been made in the physical sciences, as witness the steam-engine, motor-car, telephone and wireless. The experiments now proceeding in biological laboratories are beginning to show promise that during the next 100 years an equal advance will be made in the control of the animal".

Pincus (1936) and his colleagues used light anesthesia (ether after atropine premedication, or urethane), laparotomy to expose both oviducts and ovaries, and a special transfer pipette. The pipette had an opening in the tube above the capillary to minimize the volume of fluid taken up with the ova and to control expulsion of the ova and fluid, which could only be accomplished when the hole was covered. Their manipulative skills were already remarkable; in 1936, Pincus stated that he had transferred single blastomeres of 2-cell rabbit embryos into the oviduct and obtained normally differentiating, but small sized, blastodermic vesicles from the pseudopregnant uteri of recipient does. Pincus and Enzmann (1934) felt that they had achieved fertilization in vitro in rabbits, followed by the birth of live young. However, as explained in a 1951 review by Audrey Smith, this claim was not acceptable because the fertilization may have been effected in vivo, following transfer of ova with sperm attached into the recipient oviduct (Smith, 1951). Pincus and Enzmann were also transferring mouse ova under a dissecting microscope by this time (Pincus, 1936) while, in the rat, successful transfer of oviductal embryos into the uterus had already been reported at Yale University (Nicholas, 1933).

The embryo's tolerance of a total lack of synchrony for its early development in the oviduct was also demonstrated by Pincus and Kirsch (1936) who obtained early blastocysts from 1- and 2-cell embryos transferred into the oviduct of rabbit does in estrus and therefore lacking corpora lutea. This knowledge was to become very important to the development of embryo transfer; as we shall see, the rabbit oviduct became a vital incubator of embryos of various species being manipulated or transported over long distances.

While Pincus was beginning his work in Cambridge, England, Hartman and his colleagues, collaborating at the Carnegie Laboratory of Embryology in Baltimore and the United States Department of Agriculture, took another vital step upon the road to making embryo transfer applicable to agricultural species. On 15 March 1930, they were the first to recover and observe a bovine 2-cell embryo (Hartman et al., 1931; Miller et al., 1931). A commentator at the time (Buchanan Smith, 1932) recognized that recovery as being a physiological tour de force, comparable with scaling the Matterhorn, undiminished by the "fact" that it had ". . . no immediate bearing upon the practice of breeding better Ayrshire cows" (Betteridge, 1980). A motion picture, *Ovulation, Fertilization and Early Development of the Mammalian Egg* (1935)[7] made by W.H. Lewis of USDA (a co-author of the bovine embryo recovery papers) shows how excellent were techniques of time-lapse cinematography in the late 1920s (Lewis and Gregory, 1929) and 1930s, though it is rabbit, not bovine, embryos that are featured.

The first recorded use of embryo transfer in farm species was at the Agricultural and Mechanical College of Texas, by Warwick, Berry and Horlacher who, in 1932 and 1933, used the technique in both sheep and goats to investigate the causes of the in utero loss of hybrids between these species (Warwick et al., 1934; Warwick and Berry, 1949). Their excitement at encountering sheep and goat embryos for the first time in December 1932, bears repeating:

> "Five days after we had seen our first living ovine egg, we operated on a goat which had been bred two days previously. We examined both ovaries for corpora hemorrhagica and found one typical corpus. We then removed the corresponding oviduct and proceeded to attempt to find the egg. We were successful; and soon were gazing in awe at a beautiful four-celled goat in saline under the binocular. We wondered why we had been so skeptical of our abilities, and regretted that we did not have a recipient animal of either species ready to become the uterine foster parent of this handsome animal. This animal seemed so beautiful to us that we could not bear to let it die of neglect, so we hastily decided to put it back in its original home for five months. When, 147 days later, we again looked at this same animal after normal parturition, a good many changes had take place" (Warwick and Berry, 1949).

Dr. Berry was the first recipient of the Pioneer award of the International Embryo Transfer Society (IETS, about which more later; Table 1) and died in Texas in December 1992. He was also a pioneer in studies of reproductive immunology and has a lectureship named after him at Texas A and M University for that aspect of his work.

In France, Charles Thibault used rabbits when he began his studies of fertilization and early development in rabbits before the Second World War (Thibault and Gérard, 1940). His subsequent body of work was of great consequence to the later development of in vitro fertilization and was recognized by the IETS in 1989 (Table 1). From the very beginning, Thibault included domestic animals in his studies, showing his foresight in seeing his fundamental research work in a practical context at a time when any such link must have seemed tenuous to those of less courage.

[7] Available as a videotape from the University of Guelph library.

Table 1
Recipients of the pioneer award of the international embryo transfer society, 1982–2003

Year of award	Awardee	Principal place of work	Citation in Theriogenology (volume, issue, pages)
1982	R.O. Berry	Department of Genetics, Texas A and M University, College Station, TX, USA	–
1983	M.C. Chang	Worcester Foundation, Shrewsbury, MA, USA	19 (3), 294–303
1984	L.E. Casida	University of Wisconsin, Madison, WI, USA	21 (1), 1–2
1985	L.E.A. Rowson	ARC Unit of Reproductive Physiology and Biochemistry, Cambridge, England	23 (1), 1–2
1986	T. Sugie	National Institute of Animal Industries, Chiba, Japan	25 (1), 1–2
1987	E.J.C. Polge	ARC Unit of Reproductive Physiology and Biochemistry, Cambridge, England	27 (1), 1–3
1988	A.L. McLaren and D. Michie	ARC Unit of Animal Genetics, Institute of Animal Genetics, University of Edinburgh, Scotland	29 (1), 1
1989	C.G. Thibault	INRA, Station centrale de physiologie animale Jouy-en-Josas, France	31 (1), 1–2
1990	J.D. Biggers	Harvard University, Cambridge, MA, USA	33 (1), 1–3
1991	A.K. Tarkowski	Warsaw University, Warsaw, Poland	35 (1), 1–3
1992	R.L. Brinster	University of Pennsylvania, Philadelphia, PA, USA	37 (1), 1–3
1993	R.G. Edwards	Physiological Laboratory, University of Cambridge, England	39 (1), 1–4
1994	N.W. Moore	Department of Animal Husbandry, University of Sydney, Australia	41 (1), 1–2
1995	C.R. Austin	Physiological Laboratory, University of Cambridge, England	43 (1), 1–2
1996	W.K. Whitten	University of Sydney and Australian National University, Australia	45 (1), 1–2
1997	S. Wintenberger-Torres	INRA, Station centrale de physiologie animale, Jouy-en-Josas, France	47 (1),1–2
1998	I. Gordon	Department of Animal Husbandry, University College Dublin, Eire	49 (1), 1–2
1999	R.M. Moor	ARC Unit of Reproductive Physiology and Biochemistry, Cambridge, England	51 (1), 1–2
2000	R. Yanagimachi	Department of Anatomy and Reproductive Biology, University of Hawaii, Honolulu, HI, USA	53 (1), 1–2
2001	P.J. Dziuk	Department of Animal Sciences, University of Illinois, Champaign-Urbana, IL, USA	55 (1), 1–2
2002	R.H. Foote	Department of Animal Science, Cornell University, Ithaca, NY, USA	57 (1), 1–3
2003	K.J. Betteridge	Agriculture Canada; CRRA, Université de Montréal; Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Canada	59 (1), 1–2

Seemingly unconnected with the world of embryo transfer, there appeared, in 1938, Hans Spemann's *Embryonic Development and Induction*, a monumental summary of a life's research in Germany into fundamental aspects of amphibian embryology, extending back to the earliest years of the 20th century (Spemann, 1938). His techniques were elegant (Fig. 5). Like Romanes, Spemann was intrigued by the ideas of Weismann, in particular the hypothesis that the "potency of the genome" declines as differentiation progresses.

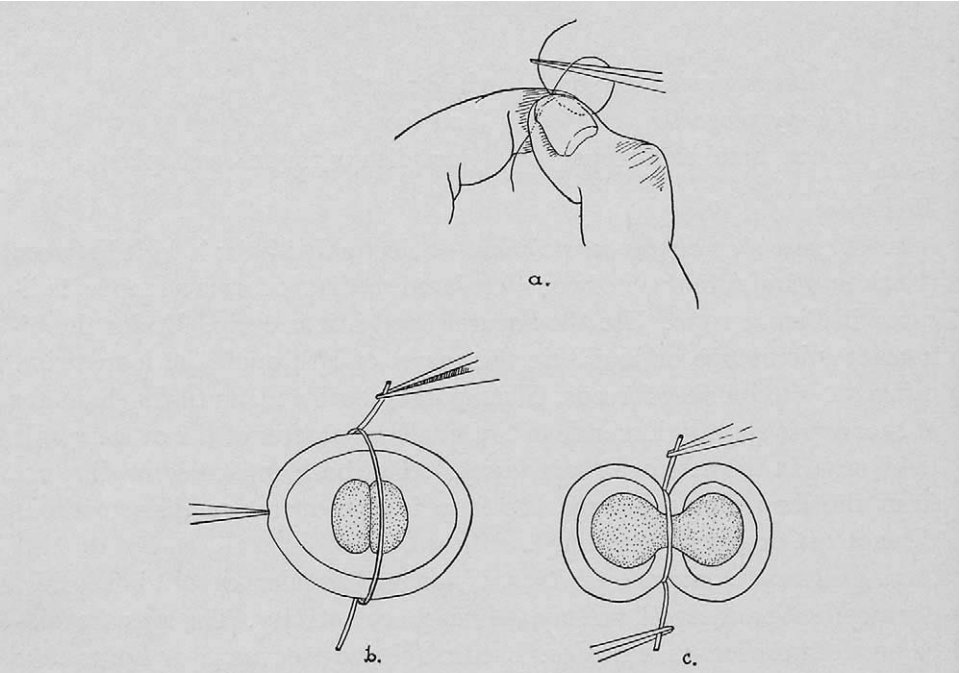

FIG. 19.—Constriction of a urodele egg (2-cell stage) with a hair. *a*=preparation of the hair loop; *b*=placing of the egg in the loop; *c*=constriction.

Fig. 5. Egg bisection with a human hair, as practised by Spemann and illustrated in an embryology textbook by his student Viktor Hamburger (Hamburger, 1942).

Presciently, on page 211 of his book he stated:

> "Decisive information about this question may perhaps be afforded by an experiment which appears, at first sight, to be somewhat fantastical. It has been shown . . . in the egg of the sea urchin . . . and the newt . . . that a piece of the egg protoplasm which contains no nucleus may be induced to develop, may be 'fertilized' as it were by a descendant of the fertilized egg nucleus. . . . Probably the same effect could be attained if one could isolate the nuclei of the morula and introduce one of them into an egg or egg fragment without an egg nucleus. The first half of this experiment, to provide an isolated nucleus, might be attained by grinding the cells between two slides, whereas for the second, the introduction of an isolated nucleus into the protoplasm of an egg devoid of a nucleus, I see no way for the moment. If it were to be found, the experiment would have to be extended so that older nuclei of various cells could be used. This experiment might possibly show that even nuclei of differentiated cells can initiate normal development in the egg protoplasm. Therefore, though it seems an anticipation of exact knowledge to say that 'every single cell possesses the whole apparatus of potencies' (Petersen, 1922, p. 116), yet this opinion may be right".

"Extended" those experiments were to be, and "right" the opinion was to prove; the life of "Dolly" (5 July 1996–14 February 2003) has immeasurably changed the complexion of embryo transfer in farm animals.

The Second World War, of course, almost totally interrupted progress in the development of embryo transfer techniques themselves in Europe. However, there were great efforts made to improve the production of equine chorionic gonadotrophin (ecG) for the purposes of increasing calf production in Britain (Parkes and Hammond, 1940; Folley and Malpress, 1944). This hormone (as "pregnant mares' serum", PMSG), first discovered at the University of California, Davis (Cole and Hart, 1930) would later, along with pituitary extracts, become enormously important for superovulating farm species other than the horse (Betteridge, 1981).

Food shortages at that time also spurred the British government to establish Britain's first AI center for cattle breeding at Cambridge in 1942, under the direction of a young, recently qualified veterinary surgeon, L.E.A. (Tim) Rowson (Fig. 6; Polge, 2000), ably assisted by Pilot Officer Jim Henderson, seconded out of the Royal Canadian Air Force. Rowson, as we shall see, was to become generally regarded as a founding father of embryo transfer in farm animals; the international recognition of his stature is exemplified by his being made the founding honorary president of the IETS, and an early recipient of its Pioneer award (Table 1).

Things were a little different in North America during World War II and two important and independent developments occurred in the 1940s. The first of these again involved Gregory Pincus, long since returned from his fellowship in England. He was introduced to Mr. Tom Slick, of San Antonio, TX, by Ray Umbaugh of Indiana for the purposes of initiating embryo transfer in cattle in the early 1940s (Umbaugh had formerly been an assistant of Pincus). There, they formed the Foundation for Applied Research (FAR), clearly foreseeing practical advantages of embryo transfer, once improved. In 1945, Pincus brought M.C. Chang over from Cambridge, England, for 1 year to work specifically on superovulation. Chang had earned his Ph.D. with Hammond and Walton, studying the low temperature preservation of ram semen for AI. It needed Hammond, apparently, to convince Pincus that there remained much to do with laboratory animal models before embarking on work with cattle, so liberating Chang to do most of his egg transfer work in rabbits (Chang, 1983). That decision immediately paid dividends; Chang (1947) published his early successes with transferring rabbit embryos that had been cooled to 10 °C—an event which he later (1971) described as ". . . one of my happiest moments, and I still remember the excitement" (Chang, 1971).

It was FAR that convened the First National Egg Transfer Breeding Conference in 1949 (Foundation for Applied Research, 1951). By that time farm journals were drawing public attention to the potential advantages of Umbaugh's work (Hervey, 1949). However, as I have stated elsewhere (Betteridge, 2000), with no calves to show for 8 years' work despite the use of 750 cows and, no doubt, a great deal of money, I suspect that the San Antonio conference was called in the realization that progress could only be made by exchanging information rather than keeping it secret. This suspicion is supported by the interesting preface to the conference proceedings, published in 1951. There, the FAR's director, Harold Vagtborg, stated the purpose of the conference as having been "to stimulate increased activity in egg transfer by *disclosing* the research of the

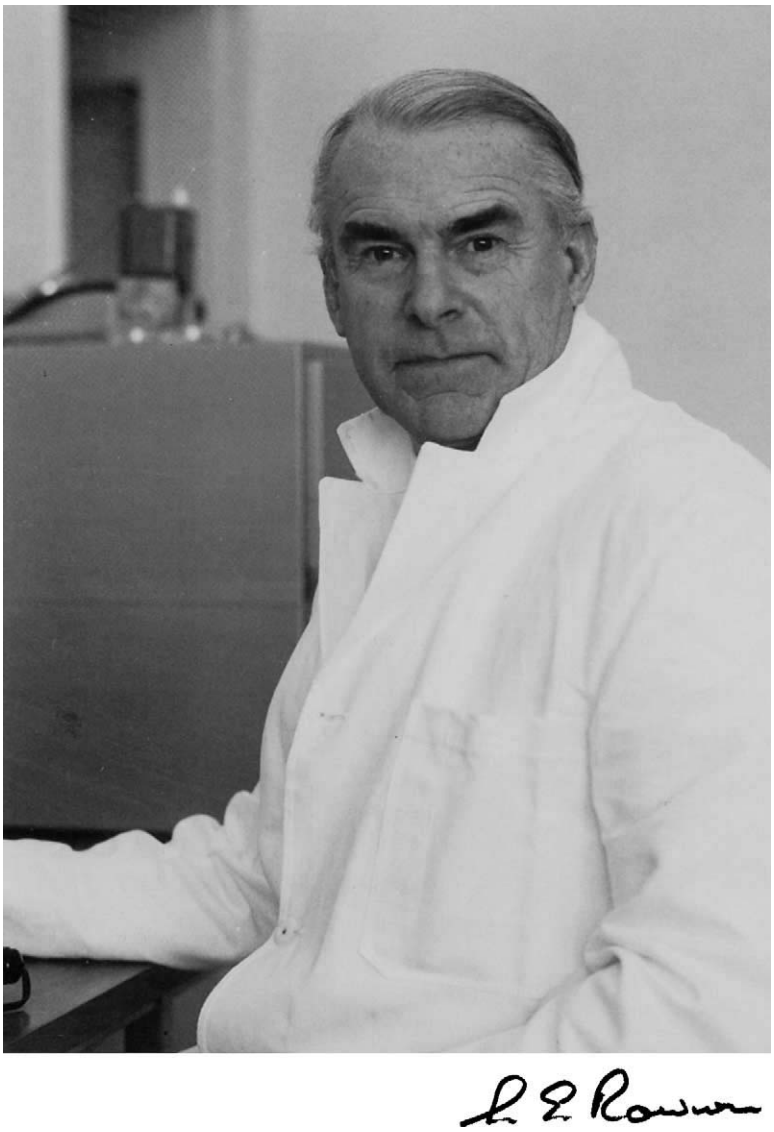

Fig. 6. Lionel Edward Aston Rowson (1914–1989) (from Polge (2000); by permission of Godfrey Argent, London).

investigators working on this and correlated phases of reproduction" (my emphasis added).

The parallel development in the United States that affected the future of embryo transfer in cattle was in Wisconsin. It began in about 1946 with the foundation of the American Foundation for the Study of Genetics (AFSOG), a private research organization created by John Rockefeller Prentice near Madison (Betteridge, 2000). Prentice was a businessman with no scientific training who owned five bull studs throughout the USA, including the

Wisconsin Scientific Breeding Institute near Madison. He saw ET as a commercial opportunity to accomplish genetically on the female side what was being done through AI on the male side in cattle.

By 1949, others, too, were thinking along the same lines; "... the increase in rate of genetic improvement from selection that would be possible if these techniques (superovulation and embryo transfer) are perfected" was certainly under discussion in the United States (Comstock, 1949). In Britain, Hammond (1950) foresaw the use of embryo transfer as a means of producing beef from the dairy herd and as a tool for producing high quality AI sires. Hammond's foresight received governmental support when, in 1949, the Agricultural Research Council (ARC) established the Unit of Animal Reproduction at 307 Huntingdon Road in Cambridge to expand reproductive work in farm animals. The study of farm animal reproduction already had a long pedigree by that time, having been initiated in a sub-department of the Institute of Animal Nutrition under Marshall's direction in 1919 and transferred to Huntingdon Road from its original Field Station in 1923 (Marshall and Hammond, 1946). An early, and singular, publication from the ARC unit was a description (Rowson and Dowling, 1949) of a catheter for transcervical recovery of ova and embryos from cattle. Similar efforts were underway in Minnesota (Dracey and Petersen, 1950; Dziuk et al., 1958). The design of Rowson and Dowling's instrument has scarcely changed since, yet it did not come into widespread use until more than 25 years later (Brand and Drost, 1977a). Elsewhere in England in 1949, the cryoprotective effect of glycerol on mammalian spermatozoa was discovered, with immeasurable effects on animal production in general, and embryo transfer in particular (Polge et al., 1949; Parkes, 1957). Amongst many other honors, Chris Polge received the IETS Pioneer award for this and other achievements in 1987 (Table 1).

Thus, the 1940s drew to a close with distinct interests in applying embryo transfer techniques to cattle, advances in our knowledge of gamete physiology, publication of two reports of bovine transfers—one on each side of the Atlantic (Umbaugh, 1949; Dowling, 1949)—but still no calves. That situation was to change as the 20th century entered its second half.

4. The second half of the 20th century

4.1. The 1950s

Embryo transfer in sheep was already in progress in the Soviet Union by 1950 and Poland by 1957 (Lopyrin et al., 1950, 1951; Kardymowicz and Stepinski, 1957). Lopyrin et al. reported the birth of lambs after the transfer of not only fertilized ova to unmated ewes but also of follicular oocytes to mated ewes. The work of Lopyrin et al. would seem to be the first to show that the maternal environment in utero can, indeed, affect the phenotype of offspring resulting from transferred embryos.

Pig embryo transfer began behind the Iron Curtain. Until recently, the history behind the development of the technique in those countries remained largely undocumented in English (Kvasnitski, 1951). However, a conference held in Kiev in May 2000 to commemorate the 50th anniversary of the first successful embryo transfer in the pig, has led to an English translation of Kvasnitski's landmark 1950 paper (Kvasnitski, 2001). This paper is especially interesting in showing how embryo transfer was seen at that time, at least in the Ukraine, as

Fig. 7. Elwyn Willett at work in the laboratory of the American Foundation for the Study of Genetics at about the time that he and his colleagues produced the first live calf by embryo transfer, born on 19 December 1950 (Willett et al., 1951; Betteridge, 2000) (from a photo by courtesy of Dr. Marvin Pace and Infigen).

a means of demonstrating the power of ". . . the spirit of Michurin biology" (as espoused by T.D. Lysenko) in the face of "biased and unscrupulous" interpretation of findings favoring "formal genetics". Thus, the relatively stronger performance of the Angora offspring (from transplanted eggs) than of their indigenous littermates in Heape's mixed litter of rabbits is cited as supporting the concept that the principles of "vegetative hybridization" would be "completely applicable to animals in general and farm animals in particular. . . . another blow to Mendel–Morgan supporters . . . ".

At AFSOG in Wisconsin in 1950, 33-year-old Elwyn Willett (Fig. 7; a former graduate student of Glenn Salisbury at Cornell) had been recruited from the University of Hawaii to become Director of Research. He was assigned by Prentice to work on superovulation and embryo transfer in cattle in collaboration with the Bureau of Dairy Industry of the United States Department of Agriculture and the Department of Genetics at the University of Wisconsin. Their efforts resulted in the birth of the first calf to result from embryo transfer on 19 December 1950, as has been described in some detail (Betteridge, 2000). Yet, despite the overall business goals of Prentice's AFSOG, the motivation for the work that led to the first calf was predominantly scientific. Willett et al. (1951) made very clear their opinion that:

> ". . . this technique, with improvements, may be valuable in the study of certain fertility problems in cows where a question of normality of the ovum vs. normality of the genital tract is involved".

This was because they recognized the practical limitations of a technique that produced limited number of eggs and required surgery. These, they reasoned, precluded the wide-scale application of embryo transfer in the fashion that AI was being practised. Thus:

> "Unless ways are found to procure eggs in large numbers, or to compensate for the limited number of eggs by finding ways of successfully obtaining and transplanting individual blastomeres, the improvement of the population by mass application of superovulation and egg transplantation is out of the question" (Willett, 1953).

It was over the next three decades that the necessary improvements gradually became realized. In the United States, there were extensive efforts in Minnesota during the 1950s to develop methods of superovulating cattle, collecting and transferring their embryos through the cervix, handling embryos pending transfer, and synchronizing the estrous cycles of donors and recipients (Dziuk et al., 1958). However, no pregnancies were obtained from 16 non-surgical transfers. Most advances came, it must be said, as a result of research either at the ARC unit in Cambridge or by scientists who had passed through that unit (Polge, 2000). Tim Rowson was already working on the superovulation of cattle there in 1950 (Rowson, 1951). Polge joined the unit at Huntingdon Road in the early 1950s, initially to field test the use of frozen bull semen (Parkes, 1957, 1985; citation in Table 1), shortly before a changing of the guard there when John Hammond retired in 1954. Hammond's place was taken by Thaddeus Mann and, for the next 22 years (until Dr. Mann's retirement in 1976), the newly named Unit of Reproductive Physiology and Biochemistry concentrated much of its research effort on embryo transfer under the direction of Rowson. The influence on our field of Rowson, Polge and their colleagues and students was, and remains, immense, as has been summarized by Adams (1982) and Gordon (1994). Adams, who undertook monumental reviews of the literature during the 1970s, describes (1982) how, from 1950:

> "The initial trickle of papers from just a few laboratories grew at first slowly and then apace so that by 1977, nearly 1000 articles on egg transfer had been published, with an ever increasing number on cattle".

Rowson's activities in cattle during the early 1950s were concentrated on efforts to transfer embryos through the cervix—efforts that were frustrated by problems of infection and egg expulsion (e.g. Lamming and Rowson, 1952; Harper et al., 1961). At the same time, he and his colleagues and students were perfecting surgical methods of embryo recovery and transfer in sheep (e.g. Hunter et al., 1955; Averill and Rowson, 1958; Moore et al., 1960) which would later be applied in cattle.

It should not be thought that the birth of the first embryo transfer calf in 1950 ushered in an era of research conducted directly in the farm species. Chang, for example, used laboratory species throughout his career in using embryo transfer for experimental studies and achieving unequivocal vitro fertilization for the first time, work that earned him the second IETS Pioneer award (Table 1). So, too, did Austin who was similarly honored (Table 1) for, amongst other things, the discovery of sperm capacitation in rats at the same time as Chang's discovery of the phenomenon in rabbits (Austin, 1951; Chang, 1951). The 1950s also saw pioneer studies (which are described more fully in the citations for their respective IETS Pioneer awards, Table 1) in the culture of laboratory animal embryos in vitro (McLaren and Michie; Biggers; Whitten) and the micromanipulation of embryos

(Tarkowski). Biggers (2001) has provided an entertaining personal account of how Whitten's 1956 success with culturing mouse embryos in Australia led to the first successful transfer of cultured embryos (Four bottled babies!) at the Royal Veterinary College in London 2 years later. It is also interesting to note that embryology in the early post-war era benefited from scientists such as Dalcq and Seidel changing their research focus from fundamental studies of lower vertebrates and invertebrates to analogous investigations in mammals (Mulnard, 1986). The first mammals to be born following the transfer of blastomeres isolated at the 2-cell stage, for example, were rabbits produced by Friedrich Seidel who was interested in the regulative capacity of the mammalian egg. He began a series of publications on this topic as early as 1952 (Mulnard, 1986).

In a study that would have seemed totally unrelated to embryo transfer in the early 1950s, Briggs and King succeeded in transplanting a nucleus from a frog blastula cell into an enucleated frog egg and getting normal development into tadpoles (Briggs and King, 1952). Their paper concluded that "although the method of nuclear transplantation should be valuable principally for the study of nuclear differentiation, it may also have other uses". One year later, another understated conclusion—"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material"—(Watson and Crick, 1953) ushered in the molecular era, even less immediately connectable with embryo transfer!

Chang's success with cooling rabbit embryos for storage was followed up with freezing work in the early 1950s. Not surprisingly this was by a member of the Medical Research Council team at Mill Hill, England, that was involved in the investigation of the cryoprotective action of glycerol; Audrey Smith gave the first paper on the subject in 1952 (Smith, 1952).[8] Soon afterwards, mouse and rat oocytes were shown to survive freezing and thawing in ovarian tissue and, by 1960, mice had been born from frozen-thawed transplanted ovaries (see Leibo (in press)). How Romanes would have enjoyed that development!

Chang, in the meantime, pioneered yet another development of critical importance to the future of embryo transfer. In 1952, with Marden, he sent rabbit embryos by air back to Cambridge, England, from Worcester, MA, packed with ice balloons in a vacuum flask. Transfer of those embryos resulted in a 10% development rate, considered low but none the less demonstrating the potential of moving livestock internationally as embryos (Marden and Chang, 1952). The next stage in this approach also began in the 1950s, with shipment of sheep embryos from Huntingdon Road to Pietermaritzburg, South Africa, in the oviducts of rabbits (Adams, 1982). Early attempts failed but lambs were eventually born in March 1961 (Hunter et al., 1962). It was this project that brought R.M. Moor from South Africa to Cambridge and initiated studies that were to earn him recognition as an IETS Pioneer awardee (Table 1).

4.2. The 1960s

Bob Moor was one among many graduate students, post-doctoral fellows and visiting scientists from around the world who made 307 Huntingdon Road such a vibrant and productive

[8] Another important member of the Mill Hill laboratory working in this field at the time was James Lovelock, later to become much better known for his Gaia hypothesis (Parkes, 1985).

unit during the 1960s and 1970s. During the 1960s, work was progressing on parallel fronts in sheep, pigs and cattle. With personnel easily exceeding the 'critical mass' essential for progress, and facilities (especially for surgery) to match, advances came rapidly. The efficiency of surgical embryo transfer as a research tool in sheep, for example, led to pivotal physiological information about the 'critical period' for the 'recognition of pregnancy' (Moor and Rowson, 1966a,b).

It was, in part, the availability of surgical facilities and expertise in Cambridge that led to curious changes and counterchanges in methods of collecting embryos from cattle. Surgical methods of recovering embryos were first developed in dogs for application in humans (Allen et al., 1928) and were used in early work with sheep and goats (Warwick et al., 1934) and in some of the Texan work in cattle (Umbaugh, 1949). However, in cattle, other early work on embryo recovery, on both sides of the Atlantic as well as in Japan, often involved transcervical methods (see Brand and Drost (1977a,b) for references)—the first report of a calf born from non-surgical embryo transfer was in 1964 (Mutter et al., 1964). In Japan, Sugie (1965) also achieved success with a transvaginal approach, by-passing the cervix, in conjunction with CO_2 insufflation to make sure that the needle used to deposit the embryo was indeed in the uterine lumen. Sugie's work in Japan led Rowson and Moor (1966) to use CO_2 insufflation of the uterus after transcervical transfer, in the belief that this prevented expulsion of the embryo. Despite some success, however, the transcervical approach temporarily fell from favor in the face of the vastly superior results achieved through the use of surgery at Cambridge towards the end of the 1960s (Rowson et al., 1969). It was not until the middle 1970s that transcervical methods were 'rediscovered' for both recovery and transfer, and eventually replaced surgical methods completely (Brand and Drost, 1977a,b).

Sugie (another recipient of the IETS Pioneer award, Table 1) and his colleagues, also did important early work on embryo transfer in goats in Japan (Niwa et al., 1960) but appreciation of this in the West has been impeded by language difficulties.

Embryo transfer work in the United States during the 1960s was sporadic, with only occasional reports in cattle (Avery et al., 1962; Vincent et al., 1969), sheep (Alliston and Ulberg, 1961) and pigs (Vincent et al., 1964). Sheep studies were also reported from Germany (Schmidt, 1961) and the Royal Veterinary College in London (Hancock and Hovell, 1961; Dickinson et al., 1962). Pig work outwith the Soviet Union had begun early in the decade in London (Hancock and Hovell, 1962) and the surgical technique involved (which has remained very much the same ever since; cf Youngs, 2001; Hazeleger and Kemp, 2001) was demonstrated by Rowson (through the 'new' medium of closed circuit television!) at the Fifth International Congress on Animal Reproduction and AI in Trento in 1964. Research into embryo transfer in pigs expanded considerably at Cambridge during the 1960s (Polge, 1966; Hunter et al., 1967) and benefited from separate productive working visits by B.N. Day and P.J. Dziuk from Missouri and Illinois, respectively, during their establishment of influential centers of pig research in their home state universities. Among the results of these collaborations were the first successful transcervical embryo transfer in pigs (Polge and Day, 1968) and the early use of embryo transfer as a research tool for answering physiological questions (Dziuk et al., 1964). Dziuk would later become a recipient of the IETS Pioneer award (Table 1) for his development and use of embryo transfer. By the end of the decade, embryo transfer in pigs had become a well established and valuable as a tool in research (e.g. Bazer et al., 1969a,b) but not yet in commerce.

During N.W. Moore's study leave in Huntingdon Road at the end of the 1960s, the micromanipulation of rabbit and pig embryos was explored, with some indications that single blastomeres might develop into whole animals (Moore et al., 1968, 1969). Neil Moore's pioneer work in our field was recognized by the IETS in 1994 (Table 1). Gardner and Edwards (1968) showed that it was possible to sex rabbit embryos by removing a few cells for analysis of their sex chromatin. However, embryo survival was low and, since sex chromatin is not easily detectable in farm animals, the relevance of this seemed limited.

Overall then, the decade drew almost to a close without any new developments that might put the spotlight on embryo transfer, in cattle, as a practical procedure. The relative quiet was to be shattered with the explosion of embryo transfer activity that characterized the following decade.

4.3. The 1970s

Detonation of the explosion was by the coincidence of two quite separate events. The first of these was the publication of the excellent results achieved in cattle by Rowson et al. (1969). They compared two media (homologous serum and TCM 199) and two methods of transfer (surgical and non-surgical). To quote Polge (2000):

> "Homologous serum had been used in all previous experiments on embryo transfer in cattle and was used routinely in sheep, with good success. TCM 199 was chosen as an alternative medium simply because it was readily available in the laboratory at that time. The results were clear-cut and dramatic. No pregnancies at all were obtained following surgical or non-surgical transfer of embryos in serum. By contrast, with TCM 199 a few successful transfers were obtained non-surgically, but with surgical methods for collection and transfer of embryos the pregnancy rate was 91%".

The second event, with which the above publication coincided, was a decision by the Canadian government to import so-called 'exotic' (European) beef breeds. However, importation was to include very strict and expensive testing and quarantine procedures to prevent the concomitant importation of disease, notably foot-and-mouth disease. There was a demand for these 'new' breeds because of their superior performance when compared with the British breeds then populating the ranch country in the West particularly. The demand extended to the United States, where producers could import livestock from Canada but not directly from Europe. The supply of 'exotics' was very limited because they had to be held in quarantine stations specially built on Grosse Ile, Québec, and the French islands of St Pierre and Miquelon in the Gulf of St Lawrence. Consequently, the price of imported animals was extremely inflated and the incentive to obtain offspring from them correspondingly high—a tailor-made call for the application of superovulation and embryo transfer. The call was answered by veterinarians and their backers who set out to learn the techniques and apply them in commercial companies. Most aspiring transferers traveled to Cambridge to learn from Rowson and his colleagues but a few, convinced of the ultimate necessity for transcervical methods, crossed the Pacific to learn from Sugie in Japan.

Characteristic of Tim Rowson was a total lack of guile, and a willingness to provide free access to all that he and his colleagues had learned and developed in the years leading up to their 1969 success. Like Hammond before him, he was anxious to see practical application

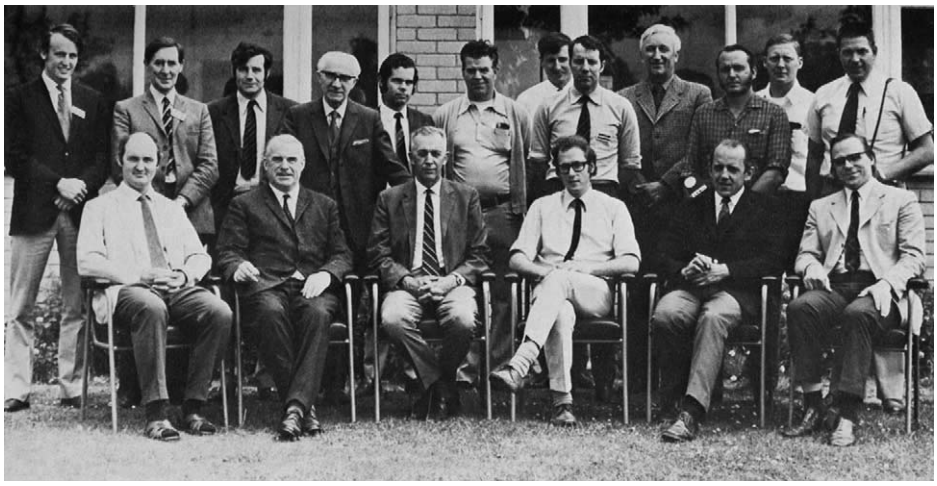

Fig. 8. Participants in the short course on embryo transfer held at the ARC Animal Research Station, 307 Huntingdon Road, Cambridge, in 1972. Seated (left to right): R.M. Moor (Staff), United Kingdom; L.E.A. Rowson (Staff), United Kingdom; P.T. Cupps, United States; H.R. Tervit (Staff), New Zealand; A. Brand (Staff), The Netherlands; M. Phillipppo, Scotland. Standing (left to right): M. Davison, New Zealand; L. Bell, United Kingdom; R.W.J. Plenderleith, Wales; T.R.R. Mann (Staff), United Kingdom; J.F. Cunningham, Ireland; K.R. Abbey, Canada; R. Newcomb, United Kingdom; S. Angus, Isle of Mann; P.J. Konkin, Canada; R.A. Carmichael, United States; M.M. Jacobson, Canada; E.D. Giebelhaus, Canada (from Carmichael (1980)).

of this new technology. As a consequence, in 1972 he organized a course of instruction in Cambridge that brought together a group of 13 veterinarians from around the world (Fig. 8) who took Rowson's knowledge and skills back to their home countries. Besides setting up numerous independent commercial embryo transfer companies, these attendees, 2 years later, formed the International Embryo Transfer Society (IETS; Carmichael, 1980; Schultz, 1980). After surviving some lean years in the 1970s, the IETS has become the main forum for scientific and regulatory exchange and discussion in the field of embryo transfer and associated techniques. Indeed, the Proceedings of the Annual Conference of the Society, which have been published as the first issue of 'Theriogenology' every year since 1978, serve as a yard-stick with which to measure changes in emphasis and intensity of activity in embryo transfer as time progresses. In the years since, sister national and regional embryo transfer associations (e.g. the American Embryo Transfer Association, the Canadian Embryo Transfer Association, and the Association Européenne de Transfert Embryonnaire) have complemented the activities of the IETS by focusing especially on subjects of commercial and/or regional concern, making invaluable bridges between research into, and practice of, embryo transfer.

It is interesting to me that, 20 years ago, I described the 1970s as "the commercial era" and wrote of "... advances that have been made over the past decade as embryo transfer has been put to work commercially on scales that dwarf all previous developmental work" (Betteridge, 1981). So what were those advances and how did they come about?

To answer the second question first, there is no doubt that the development of embryo transfer in cattle owes a great deal to the private veterinary practitioners and small

commercial companies who had the courage to take techniques from the laboratory to the field in those early years. Probably the earliest company was Livestock Breeders International (LBI), established in Oklahoma by James Dula and Duane Kraemer. Kraemer was then at the Southwest Foundation for Research and Education in San Antonio—the very place that the Foundation for Applied Research (FAR) had been formed with commercial intent almost 30 years earlier. LBI produced their first calf commercially on 28 June 1971 (Kraemer and Dula, 1972). The best established (longest surviving) and largest of the early companies was also in operation by 1971; Alberta Livestock Transplants (ALT) in Calgary, founded by Drs. David Dyrholm and Ted Mitenko with links to the University of Calgary and the enthusiasm of Dr. R.B. Church, accepted 11 donors into their program in that year and 10 times that number in 1972 (Church and Shea, 1976). ALT can certainly be credited as being the first enduring commercial operation of which the sole intent was to provide embryo transfer services to cattle breeders for profit—a service still offered by ALT's direct descendant, the Alta Embryo Group. These pioneers encountered many practical difficulties and it is salutary to recall that the IETS was founded by people with the good sense to realize that open discussion of those difficulties would be necessary if progress was to be made. The parallel with the convening of the FAR conference some 25 years earlier is striking. Solution of the problems called for academic effort, too. Thanks to the growing interest in the practical use of embryo transfer, governments and universities became more and more interested in fostering research into the procedures in cattle. Colorado State University, for example, set up its flourishing embryo transfer unit in the early 1970s when George Seidel moved there from Ithaca, New York.[9] Other units started in the University of California, Davis, Washington State University, and the University of Saskatchewan, Saskatoon. Also in Canada, the foresight of the Veterinary Director General at the end of the 1960s, Dr. Ken Wells, facilitated the formation of an embryo research group at the Animal Diseases Research Institute (ADRI), Canada Department of Agriculture. The mandate of the ADRI group was to establish procedures and then use them in investigating both the risks of transmitting disease with embryos and the potential of the technique for eliminating those risks. The first calf to result from embryo transfer in Canada was born at the ADRI in Hull, Quebec, in May 1972. Another was born very shortly thereafter in Cardston, Alberta, where LBI had arranged to use embryo transfer as a means of importing the 'exotics' into the United States from Canada. Duane Kraemer left LBI in 1974 and took a position at Texas A and M University in 1975; he and his students would go on to produce the first embryo transfer offspring in baboons, dogs and cats before the 1970s were out (see Adams (1982)).[10]

In Europe, units were established mainly at AI units (especially in West Germany, as it was then) and research institutes (e.g. INRA in France, to which Thibault had moved in 1950; the Agricultural Institute in Ireland). Embryo transfer also became a focus of research in several universities (e.g. in Utrecht in The Netherlands, Gent in Belgium, Hanover and Munich in Germany, University College, Dublin and the Royal Veterinary College in Copenhagen).

[9] George, and his wife Dr. Sarah Seidel, deserve enormous credit for nursing the fledgling IETS through its lean years in the 1970s. For this, and his many other contributions, George received the Distinguished Service award of the IETS in 2001.

[10] In addition to his scientific and teaching contributions, Duane Kraemer instituted the Pioneer Award of the IETS which provides a useful window onto the history of embryo transfer through the citations listed in Table 1.

As to the advances, they were numerous and, fortunately, well documented at the time as a result of discussions at three major meetings. Two of the meetings, in 1975 in Cambridge and in 1977 in Galway, were organized by the Commission of European Communities and resulted in two collections of papers on the practice and genetic usefulness of embryo transfer in cattle (Rowson, 1976; Sreenan, 1978). The other meeting was a panel discussion at the Eighth International Congress on Animal Reproduction and Artificial Insemination in Krakow, Poland, in 1976. This gave rise to a multi-authored monograph on embryo transfer in all the farm species, paying attention as much to potential applications in commerce and research as to procedural details (Betteridge, 1977). Important reviews of superovulation and embryo transfer were also published early in the decade from Cornell and University College, Dublin (Foote and Onuma, 1970; Gordon, 1975, respectively).

One of the most far-reaching advances occurred early in the decade. After a false start (Whittingham, 1971), mouse embryos were successfully frozen, thawed and transferred to give live young (Whittingham et al., 1972).[11] Ian Wilmut and Tim Rowson produced the first calf from a frozen-thawed blastocyst soon afterwards (Wilmut and Rowson, 1973). However, as explained by Polge (2000) and Leibo (in press) this advance was not followed immediately by practical application because younger embryos—cleavage stage or early morulae favored for transfer at the time—did not survive the procedure. Practical application came later in the decade, following further work by Alan Trounson and Steen Willadsen as research fellows at Cambridge, and the shift to transcervical recovery and transfer of late morulae and early blastocysts, which are much more tolerant of freezing and thawing.

The 'rediscovery' of transcervical (non-surgical) methods in cattle has already been referred to. Here, though, it is worth noting that a major factor contributing to this development was the practical necessity of avoiding surgery if embryo transfer was to become practicable in the field. Necessity really was the mother of invention, with early meetings of the IETS being dominated by discussion of the merits and demerits of this and that catheter.

Another major advance was the advent of using prostaglandin $F_{2\alpha}$ and its analogues to synchronize estrus in farm animals (Phillippo and Rowson, 1975). How hard it is to think back to the times when a reliance on naturally cycling animals required the maintenance of very large and expensive recipient herds.

Practical considerations also underlay early demonstrations of how livestock could be moved internationally as embryos. As methods of storing mammalian embryos in vitro improved, so, too, did methods of transporting them without resort to the rabbit oviduct as an incubator. Pig and sheep embryos were the first species to be successfully transported over long distances in this way (Baker and Dziuk, 1970; Wrathall et al., 1970; Baker et al., 1971; Kardymowicz and Kremer, 1971). In 1974, the potential for transporting frozen embryos was demonstrated in mice (Whittingham and Whitten, 1974),[12] corroborated by the export of frozen cattle embryos from England to New Zealand in 1976 (Polge, 2000). The use of the oviduct for transport purposes was not yet over, however; the rabbit was again used to

[11] This, for me, constitutes a classic example of the value of internationalism and interdisciplinary research. Would one really have been able to *plan* for a young Welsh veterinary surgeon, and two Americans working on freezing bacteriophages in an atomic weapons research center, to come together to show that mammalian embryos can be successfully frozen?

[12] Wes Whitten was the IETS Pioneer awardee in 1996 (Table 1) for his development of culture media.

transport horse embryos from Cambridge to Poland in 1976 (Allen et al., 1976). Horse work began in Cambridge with the participation of 'Twink' Allen in the early 1970s, at about the same time as the program in Japan which produced the first foal by embryo transfer (Oguri and Tsutsumi, 1974).

Intermediate recipients were used in an interesting way to import 'exotic' breeds of cattle into New Zealand. Embryos of the 'exotics' were transferred into Jersey and Friesian recipients in England and were therefore eligible to pass the importing country's health regulations (Morcan, 1972). As a corollary to these developments, the 1970s also saw a growing awareness of the potential hazards of inadvertently transmitting disease along with embryos, as well as the potential usefulness of embryo transfer for disease control (Mitchell and Betteridge, 1977). The latter potential was already being exploited by 1975, using embryo transfer to introduce new stock into pathogen-free pig herds (Curnock et al., 1975).

Gardner and Edward's approach to sexing embryos at the time of transfer was modified (by using chromosome rather than sex chromatin analysis) and applied successfully to sheep (Dain and Rowson, cited by Polge and Rowson (1973)) and cattle, the first sexed calf being born on Christmas Day, 1975 (Hare et al., 1976).

Very much finer micromanipulation of mammalian embryos was reported at about the same time by Bromhall (1975) in Oxford who had limited success in introducing nuclei of embryonic rabbit cells into unfertilized eggs by both micro-injection and virus-induced fusion. The significance of this may have seemed arcane to most practitioners of farm animal embryo transfer at the time. Not so to Ian Gordon (IETS Pioneer awardee in 1998; Table 1); in 1977 he wrote (Gordon, 1977):

> "Although the difficulties in working with the minute mammalian egg are formidable, it is perhaps not impossible to think forward into a future in which calves can be obtained from eggs provided with transplanted nuclei that would also determine sex according to their gender of origin".

A landmark publication on the production of identical twins at the end of the decade brought that vision a step closer. Willadsen (1979) started to manipulate cleavage-stage sheep and goat embryos at Huntingdon Road with the same facility that Tarkowski and Mintz had shown when, separately, they produced chimeric mice almost 20 years earlier. Some chimeric sheep had been produced in Cambridge 5 years earlier (Tucker et al., 1974) but Willadsen's ingenuity included the invention of a means of culturing manipulated embryos in agar 'chips' (very small cylinders) inserted into the oviducts of ewes, thereby greatly increasing the efficiency of the approach.

Moderate progress was made during the 1970s in research aimed at maturing farm animal follicular oocytes and fertilizing them in vitro, and there was optimism that embryos would one day be obtained from abattoir material although results in cattle were still particularly disappointing (reviewed in Betteridge (1977)). An early attempt was that of Sreenan (1970) in Ireland, but it was in Japan that Iritani and Niwa (1977) first fertilized bovine oocytes in vitro. In Canada, Bedirian et al. (1975) demonstrated that bovine follicular oocytes could be fertilized in the oviducts of other cattle or of pigs. By the end of the decade, it had been demonstrated that oocytes matured in vitro could be fertilized in the cow oviduct and produce calves in some instances (Newcomb et al., 1978). However, whatever progress had

been made in animals was eclipsed by the birth of Louise Brown, the first 'test tube' baby (Steptoe and Edwards, 1978). Bob Edwards' contributions to embryo transfer and associated techniques have been enormous, as was summarized in the citation that accompanied the presentation of the IETS Pioneer award in 1993 (Table 1).

Needless to say, Louise Brown's birth focused public attention on all aspects of embryo work as never before, with consequences that will always persist. An immediate effect was a demand for mammalian embryologists capable of applying their skills in a human clinical setting, i.e. those with experience of animal work. One of the first and most influential such scientists was Alan Trounson who had returned to Australia in 1977 from research fellowships in Cambridge and Calgary. In the Department of Obstetrics and Gynecology of Monash University, Trounson's work soon led to one of the first textbooks on human IVF (Trounson and Wood, 1984) and his appointment as Director of the Centre for Early Human Development in 1985. Remarkably, he has maintained a concurrent research program in farm animals, stressing the comparative value of such an approach. Until 1978, the histories of embryo studies in farm animals and humans were obviously one and the same (Biggers, 1984); from then onwards, however, they diverged and the clinical applications in human medicine are beyond the scope of this paper.

The elapse of 20 years between the first successful embryo transfer in cattle in 1951 and the wide-scale applications that got underway in the 1970s may seem puzzling. In a personal communication (see Betteridge (2000)), Dr. Harold Hafs has explained that part of the reason is probably that Willett's efforts were diverted by his employer from embryo to semen work in 1953. Willett was therefore unable to resume work on embryo transfer until he moved to Michigan State University in 1956. Regrettably, Willett died of a brain tumor after less than 2 years in his new position.

Scientifically, then, the 1970s were buoyant years for embryo transfer; commercially they were equally buoyant to start with, but less so later on. The bubble of proliferating the exotic beef cattle in North America was bound to burst, and so it did. However, this recession in demand had its beneficial effects in selecting out the most efficient units that would carry the industry into the 1980s. One of those units was Rio Vista which, at the turn of the decade, was performing some 3000 transfers a year very close to where Umbaugh's laudable but precocious efforts on behalf of FAR had foundered some 30 years previously.

4.4. The 1980s

Progress was rapid on all fronts throughout the 1980s, fueled to a great extent by concomitant advances in embryo micromanipulation, in vitro fertilization, and molecular biology which were to totally transform the practice of embryo transfer within the decade. One measure of the pace of events was the increase in public awareness of the work that we do. Cover stories in scientific journals and popular magazines became increasingly common (e.g. Seidel, 1981, Fig. 9; Fehilly et al., 1984; Hammer et al., 1985)[13] and, with the emergence of human IVF clinics, every discussion in the mass media had to draw parallels

[13] The prominence accorded these papers in top-ranked scientific journals (Nature and Science) argues against the later assertion (Kolata, 1998) that embryo studies in farm animals were considered "backwaters" of developmental biology by mainstream scientists.

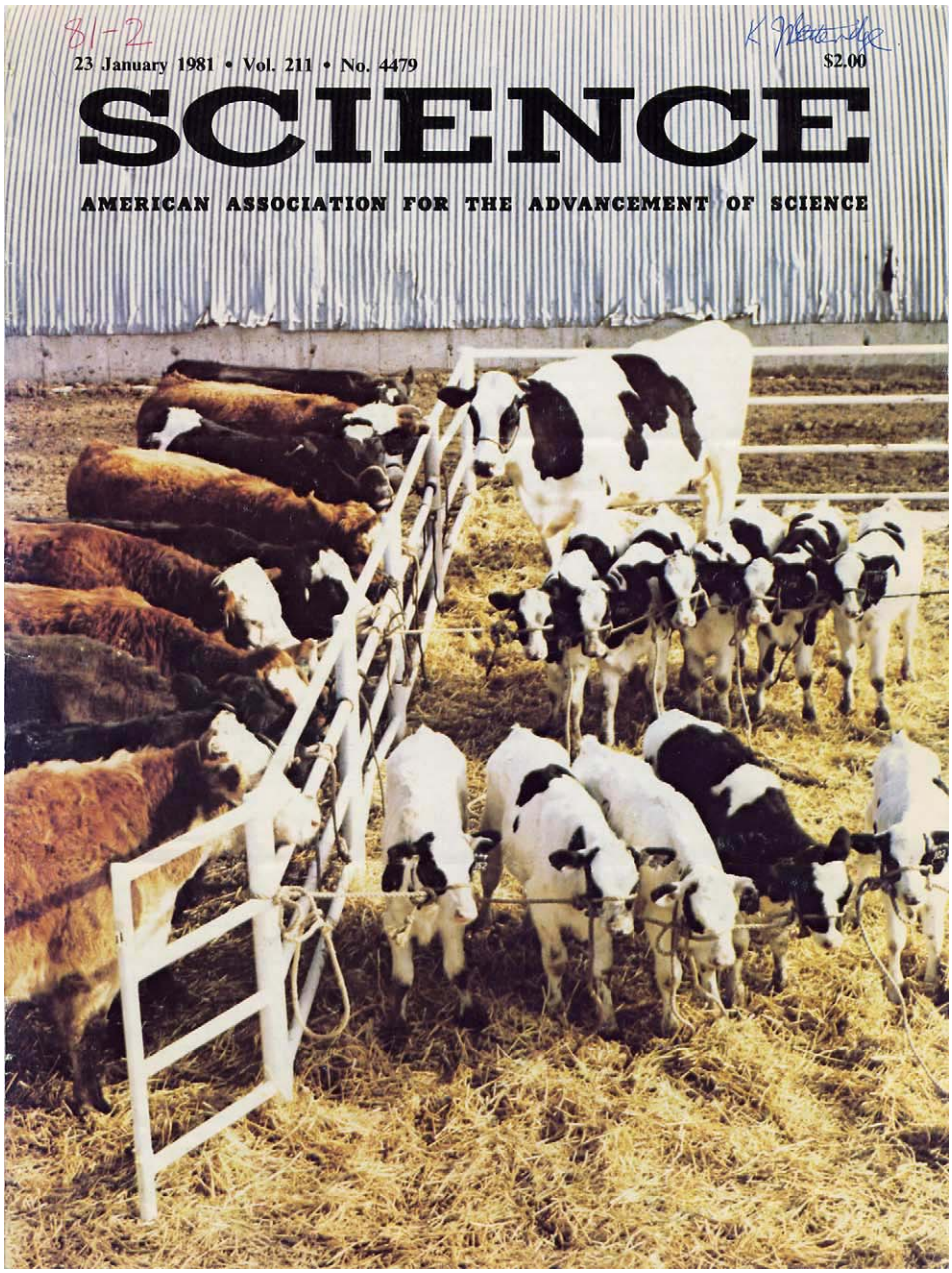

Fig. 9. Cover of ‘Science’, 23 January 1981. The legend reads: “The dairy cow (upper right) is the genetic mother of the ten calves. She was superovulated, and the embryos were recovered from her uterus 1 week after conception. After 3–10 h of culture in vitro, the embryos were transferred to the uteri of the ten recipient cows (left) for gestation to term. The cattle are owned by Colorado State University. See page 351. [J. Messineo, Fort Collins, Colo.]”. The reference “page 351” is to Seidel (1981), from which this figure is reproduced, with permission. Copyright, 1981, American Association for the Advancement of Science.

between animal and potential human applications. In addition to many embryo-specific conferences during the decade (e.g. Greve et al., 1989; the IETS annual conferences), every animal reproduction meeting held sessions on the role of embryo transfer in their discipline.

Seidel's masterly review of the whole field of bovine embryo transfer in 1981 brought the subject to the fore and reflected the enormous contributions being made (as they still are) by Colorado State University, advancing not only the science itself, but also its application in the field. Field work also proved a useful source of revenue for farm animal embryo research at some universities early in the decade. For example, the Reproduction Research Trust established by Rueben Mapletoft at the University of Saskatchewan, Saskatoon, used income from its cattle work to help its programs of research, training, and international collaboration.

Commercial embryo transfer in cattle grew rapidly throughout the decade (Fig. 10), as did the literature on the subject. Much of that literature will be more appropriately considered as it relates to specific topics discussed in other papers in this special edition. Here, however, a

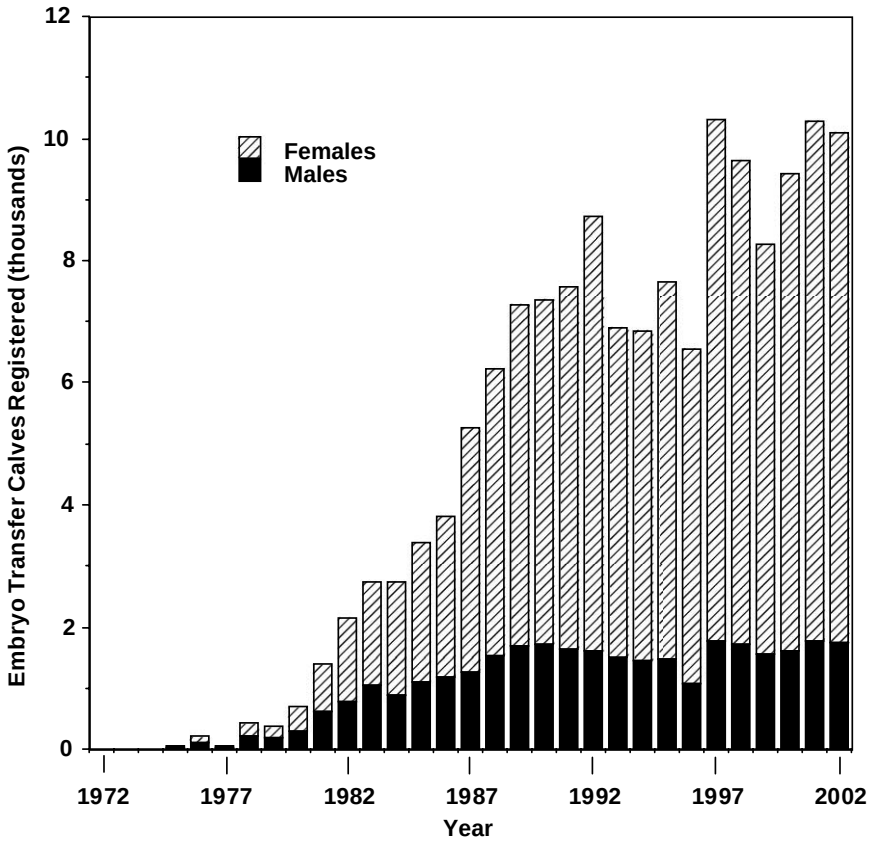

Fig. 10. Embryo transfer activity in Holstein dairy cattle in Canada up until 2002 (data kindly provided by the Canadian Holstein Association).

broad survey of research trends over the last two decades of the 20th century seems relevant to the historical context. It is extraordinary, for example, to think that when Adams wrote his comprehensive review of the history of embryo transfer in 1982, embryo micromanipulation was still a novelty and in vitro fertilization in farm animals merely "a popular subject with reviewers".

The 'splitting' of bovine embryos by micromanipulation is one of the best examples of how rapidly a technique can be transposed from the laboratory to the field when the demand is there, and simplified in the process. One minute, in the early 1980s, the method was a technical tour de force in the hands of Steen Willadsen in his laboratory; the next, it was accurately illustrated as a commercial service advertised in a beef producers' magazine (Fig. 11). The simplification came when it was shown that the splitting could be just as well accomplished on post-compaction morulae and blastocysts recovered from the uterus as on the cleavage-stage embryos originally used. This discovery, by Jean-Paul Ozil and his colleagues in France, Tim Williams and his colleagues in Colorado, and Bob Godke's group in Louisiana, removed the necessity of incubating the divided embryos in agar chips in an ewe's oviduct. In no time, commercial kits for splitting were available. Yet, surprisingly, the technique has never been very widely practised for economic reasons. Two reviews of embryo micromanipulation published in the 1980s were by Fehilly and Willadsen (1986) and Picard and Betteridge (1989).

Embryo splitting opened the door to embryo biopsy for sexing at much earlier stages than had been used in the original work in the 1970s; and improved embryo cryopreservation allowed more time for the analysis of the biopsy sample. Sexing of the biopsy sample by chromosome analysis did produce some sexed calves from frozen embryos during the 1980s (Picard et al., 1985) but, because it depended on having good metaphase spreads, this approach could never be 100% effective. It was the advent of the polymerase chain reaction and the use of Y-specific probes in the second half of the 1980s that made embryo sexing much more efficient.

Micromanipulative skills were also applied to the production of chimeric livestock (including interspecies chimeras), and transgenic livestock during the 1980s. It remains to be seen whether incorporating transfected stem cell lines into chimeric embryos will be as valuable a route to making transgenic animals in livestock species as it is in mice, or whether it will be superseded by cloning. The first transgenic animals (mice) resulted from pronuclear injection, first by Tom Wagner and his colleagues in Ohio, then by Richard Palmiter, Ralph Brinster and their colleagues in Philadelphia in the early years of the decade. Again, transposition to farm animals was almost immediate; in September 1985, a conference entitled *Genetic Engineering of Animals*: *An Agricultural Perspective* attracted over 300 delegates from 11 countries to a 4-day conference in Davis, CA.

Willadsen soon turned his skills to the sub-cellular level, producing the first 'cloned' lambs by nuclear transfer in 1986 (Willadsen, 1986). At the time, amphibian work led everyone to believe that only embryonic nuclei would be usable in this procedure and that "The more extravagant claims concerning 'cloning' of valuable adult animals are therefore quite unrealistic at present; it is only unproven embryos that can be duplicated by any existing, or immediately foreseeable, technique" (Betteridge, 1986)! Such thoughts did not deter some very large commercial companies investing heavily in the scientific effort required to produce groups of identical cattle; the W.R. Grace company backed Neal First's

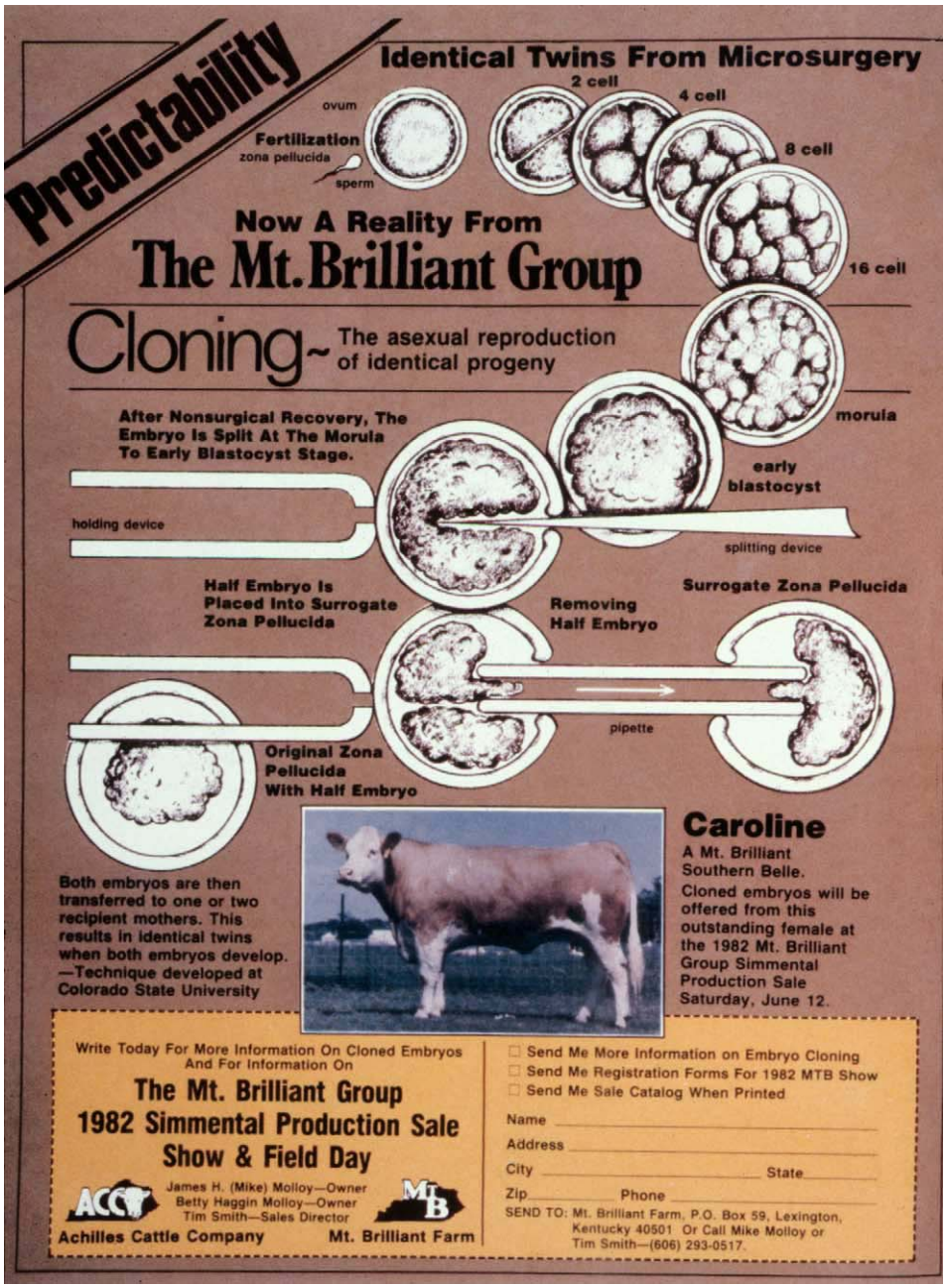

Fig. 11. An advertisement for embryo splitting from The Simmental Journal in 1982.

research group in Wisconsin, while Granada Genetics attracted Steen Willadsen and others to new facilities in Texas. Within the decade, the Wisconsin team had produced live young following nuclear transfer from blastomeres in cattle (Prather et al., 1987), rabbits (Stice and Robl, 1988) and pigs (Prather et al., 1989).

The pace of development in bovine IVF was equally rapid and equally affected by commercial interests. The logistics behind the production of Virgil, the first IVF calf, by Ben Brackett and his colleagues at the University of Pennsylvania's School of Veterinary Medicine in June 1981 were daunting to say the least (Brackett et al., 1982). This was because of the need to mature the ovum in vivo in preparation for fertilization in vitro, and then have a suitably synchronous recipient of the zygote. Progress remained slow over the next few years which also saw the birth of the first IVF pigs and lambs in Cambridge in 1983 and 1984, respectively, where Winston Cheng showed what an improvement could be had by raising the incubation temperature by 2 °C (Cheng et al., 1986)! Laparoscopic retrieval of follicular oocytes from cows made a brief appearance (Sirard and Lambert, 1985), only to be superseded by transvaginal 'ovum pick up' (OPU) by aspiration under ultrasonographic guidance. The latter technique was pioneered in The Netherlands (Pieterse et al., 1988) and has been reviewed by Galli et al. (2001). The oviducts of sheep or rabbits came back into their own temporarily as incubators of bovine zygotes when they improved results somewhat at Laval University in Quebec City and in Madison, WI. However, as has been described by Gordon (1994), very rapid progress in our understanding of how to prepare sperm for fertilization, and of culture requirements for ovum maturation and fertilization, and for early embryo development, all in vitro, supervened in the latter half of the 1980s. The systematic research that brought this about has been reviewed particularly comprehensively by Leibfried-Rutledge et al. (1997). It is remarkable that the transition to entirely in vitro systems resulted from work in both university settings (notably in First's laboratory in Wisconsin, in relation to their cloning work) and new commercial laboratories (for example, Ovamass associated with University College, Dublin, and Animal Biotechnology Cambridge on the Huntingdon Road premises in Cambridge) that aimed to produce high quality, low cost embryos for wide-scale transfer.

Embryo transfer in horses expanded during the 1980s, though not as rapidly as in cattle, primarily because of the mare's intractability to superovulation. The first foal to result from the transfer of a frozen-thawed embryo was born in Japan very early in the decade (Yamamoto et al., 1982) and a series of conferences on equine embryo transfer was initiated in 1985 and has been held every 4 years since, with proceedings published as supplements to the Equine Veterinary Journal and, more recently, by the Havemeyer Foundation.

The potential usefulness of embryo transfer for the conservation of endangered species came to the fore in the 1980s, newsworthy events in this connection including the birth of a Gaur from a Holstein cow, and of a Grant's zebra from a quarter horse mare (see Betteridge (1986)).

Very significant expansion of research into the potential for the spread of infectious disease, and for its prevention, by embryo transfer occurred as a result of collaborative studies between Agriculture Canada and the United States Department of Agriculture (e.g. Hare et al., 1988). This body of work, together with discussions in the Import/Export Committee of the IETS and an international meeting on the subject in Montreal, set standards recognized internationally by l'Office international des epizooties (OIE) for preparing embryos

for international movement. The procedures required were described in the IETS Manual, first published in 1987 and now in its third edition (Stringfellow and Seidel, 1998).

Montreal was also the site of the annual meeting of the IETS in 1985. Over and above the scientific discussions, this was significant for two reasons: it was the first time that the IETS met outside the United States, and it was the only time that Tim Rowson came to North America (to receive the Pioneer award). Rowson died in July 1989, just 10 years after his retirement, and with him passed an era.

The atmosphere surrounding farm animal embryo work during Rowson's time had been one of openness and free exchange of information. In my opinion, this changed gradually during the 1980s to one of preoccupation with commercial secrecy and patent protection. Amongst academic research workers, a mixture of shock and disbelief greeted the news of 'defections' of colleague after colleague to commercial companies and the lack of full disclosure in scientific presentations. In retrospect, that was only the thin edge of the wedge; as public funding of basic research dwindled, and pressure to recruit research partners from the private sector mounted, the community prepared itself for the 1990s.

4.5. The 1990s and into the new millennium

Though a sense of perspective becomes progressively more difficult to maintain as one approaches the present day, no account of the history of our field would be complete without mention of some momentous events of obviously lasting significance in a period that included both the birth of Dolly, and the President of the United States of America addressing his people, on prime-time television, on the subject of embryonic stem cells.[14]

Too much has been written about the impact of Ian Wilmut, Keith Campbell, and their colleagues' achievements in producing Dolly to bear summary here. However, to what extent that impact will be on animal production, as opposed to the production of pharmaceuticals and the like, it is still too early to say. Similarly, it is not yet clear whether the principal outcome will be efficient methods of cloning adult animals, or a much fuller understanding of the physiology of pregnancy as the persistent problems associated with nuclear transfer are gradually unraveled. What is clear is the enormous shift of emphasis in embryo research away from agricultural towards pharmaceutical and other ends; at the IETS meeting in January 2003, for example, of the 331 abstracts presented, 65 were directly about nuclear transfer and just 4 were concerned with the physiology of early pregnancy. Pendulums swing, and it seems to me unlikely that the efficiency of embryo transfer (and consequently its applicability in all sorts of fields) will improve without some redress of this imbalance.

Nevertheless, cloning apart, most of the trends in bovine embryo work that emerged in the 1980s continued into the 1990s. Production of embryos in vitro became routine, complemented by increasingly efficient methods of retrieving follicular oocytes per vaginam (OPU) (Fig. 12) molecular and imaging techniques to study their structure and function were made ever more sensitive and informative; there was more and more use of domestic animal embryos for comparative studies; and the interest in using embryo transfer for conserving endangered species increased. Domestic animal embryonic stem cells have resisted isola-

[14] It is doubtful that John Hammond would have foreseen this development when, in about 1940, he was required by the Committee of the Church Council to defend AI against allegations of immorality (Hammond, 1962)!

Fig. 12. Production of bovine embryos in vitro for commercial transfer at Boviteq, St. Hyacinthe, Québec, Canada. The technicians are processing embryos produced from the in vitro fertilization of oocytes collected from mature, non-lactating dairy cows kept on the premises and used for repeated ultrasound-guided transvaginal ovum pick-up, as described by Bousquet et al. (1999).

tion and exploitation in the way they have been used in mice. On the other hand, the prospect of using intratesticular injection of male germ cells as a route to transgenesis may be yet one more contribution for which we will thank IETS Pioneer (Table 1) Ralph Brinster. The most promising avenue to produce livestock of predetermined sex has veered markedly away from embryo sexing towards the separation of X-bearing and Y-bearing spermatozoa; it will be interesting to see whether this trend is permanent. Intracytoplasmic sperm injection (ICSI), which became so prevalent in human IVF during the 1990s, is hardly used in any animals other than horses at the moment, though Goto's achievement of producing calves with dead sperm in this way surely deserves to be singled out (Goto et al., 1990). Several foals have now been produced by ICSI since the first report by Squires et al. (1996) (references in Li et al. (2001)). This makes up somewhat for the general lack of success with IVF in this species, with only one or two foals born to date, and these from oocytes matured in vivo (Palmer et al., 1991). Healthy mule foals and a horse foal have also resulted very recently from the use of somatic cell nuclear transfer (Woods et al. (2003) and Galli et al. (2003), respectively).

And, to end on a positive note, it can be said that the efforts that have gone into farm animal embryo transfer over the last 50 years have had tangible results that would have pleased the Heapes, Hartmans, Hammonds, Berrys, Rowsons and Willetts of our world. The increase in the use of the technique has continued—modulated, of course, by fluctuations in the profitability of agriculture and by scourges such as bovine spongioform encephalopathy (BSE) and foot-and-mouth disease.[15] For many years, Michel Thibier has annually assembled best estimates of embryo transfer activities throughout the world. He reports (Thibier, 2002) that there were almost half-a-million transfers in cattle in 2001, about half of those with frozen-thawed embryos and just over 6% of them with embryos produced in vitro. This pattern of growth is exemplified (for Canadian dairy cattle) in Fig. 10. Cryopreserved embryos also made up about half of the 3800 sheep and goat embryos transferred. In pigs and horses, however, cryopreservation remains difficult and so the 83,000 and 8000 embryos transferred in these respective species were freshly collected. In considering the genetic effects of the practice of embryo transfer, while the promise of "accomplishing genetically on the female side what was being done through AI on the male side in cattle" has hardly been realized, it needs to be underlined that most bulls standing in AI studs these days have been produced by embryo transfer. Our forebears would also have been gratified by the extent to which private companies and cooperatives (e.g. Alberta Livestock Transplants, John Hasler's Em Tran, Holland Genetics, Granada Genetics, Rio Vista, UNCEIA, The Livestock Improvement Association of Japan, Boviteq and several others) have collected, analyzed, and shared data from their large-scale operations ever since the foundation of the IETS. Whether fundamental biologists or dedicated agriculturists, those same forebears would have been astonished (and, perhaps, less pleased?) to learn that a major market for students trained in our laboratories is in the human IVF industry rather than agriculture.

But here we stray into the present, much too close for perspective, and into conjecture. It must be for someone else one day to take up the history of embryo transfer in the 21st century; there is little doubt that there will be much to chronicle and that, in another 60 years, the "States of the Art" set out in this special edition will seem as quaint to our successors as Hammond's 1940 references to "the motor-car, telephone and wireless" seem to us.

5. Conclusion

I am conscious of how prone I am, as a scientist and not a professional historian, to the perils of inaccuracy—through omission or commission—in describing the development of even such a circumscribed subject as embryo transfer. This paper, obviously, is only *A* history of the topic, written from my own perspective and colored by my own 40 years of enjoyment of the field (what one reviewer has already called "a healthy dose of anglocentricism"!). Such bias, I'm afraid, is almost inevitable, despite my efforts to maintain balance. Thus, to those whose contributions have not been mentioned, perhaps for reasons of language and

[15] How ironic that superovulation of cattle with eCG, introduced in the UK as part of a war effort, may make a comeback as a result of the BSE-associated risks posed by the use of gonadotropins purified from pituitary extracts.

cultural parochialism, I can only end as I began—*mea culpa, mea culpa*—and look forward to the corrections of these shortcomings.

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