The global mission of mammalian embryology: are we as good as supposed?

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Summary

Mammalian embryology has obtained considerable reputation during the past decades and is regarded now as one of the most developed branches of biomedical sciences with impressive impact on animal breeding and human reproduction. Although achievements are unquestionable, the advancement seems to slow down due to structural, administrative, financial setbacks as well as lack of innovative thinking. These tendencies may endanger the accomplishment of ambitious goals and delay realization of intrinsic possibilities of applied mammalian embryology. This review is an attempt to focus attention on these problems and call for changes in structure as well as mentality.
Reconsidering the frames seems to be indispensable for qualitative advancement in the laboratory work, to replacing obsolete techniques with fully automated procedures, and to increase radically the efficiency and accessibility of human and domestic animal embryology to fulfil its destiny.

KEY WORDS: embryology, IVF, assisted reproduction, human, domestic animal, perspective.

Introduction

The purpose of this work is to critically review the past 25 years of mammalian embryology, in order to answer the question of whether this discipline has fulfilled its role. Firstly, terms used in the title should be clarified. *Mammalian,* in this context, includes humans, domestic and wild animals, but excludes laboratory animals such as rats and mice. *Embryology* as used in assisted reproduction is restricted to the laboratory work involving oocytes and embryos. Sperm technologies are not discussed since artificial insemination in domestic animals developed to a large-scale industry before the period of interest. The clinical part of human infertility treatment is a related but different discipline with its own approaches, instrumentation and specialists; therefore it is discussed here only in a general sense. *Global* refers to a discussion that encompasses the status of embryology and overall efficiency of different methodological approaches around the world. Finally, *mission* means goals that are realistic, achievable and possibly promised, and meet demand and expectations. This analysis is self-critical, sometimes provocative, and conclusions do not necessarily agree with some widely held views. The intention is to challenge those views, and provoke thoughts and debates that may help eliminate obstacles to progress and accelerate development.

Expectations *vs* reality

Mammalian embryology is commonly regarded by laymen as a cutting-edge science with highly sophisticated instruments, perfectly standardised methods and top level scientists with great innovative skills. Achievements are described as amazing, and the whole discipline is ranked equal to or even higher than information technology, space research or theoretical physics. Needless to say, most embryologists are happy

to share this view. If we get criticism from the outside world, it would be blame for too rapid advancement, too ambitious goals and dangerous achievements. Lack of progress, low creativity, out-dated methods and primitive approaches are never discussed, and nobody mentions the low impact of our science on problems it was supposed to solve.

A close look may find the overall picture more contentious.

In domestic animals, the principal goal is to support breeding, and to facilitate genetic improvement by accelerating the propagation of individuals with superior traits. The use of embryology seems to be a uniquely appropriate approach for this purpose. However, the advancement falls far short of that which was predicted or expected. Cattle is the only species in which special features, i.e., large population – an industry at the scale of car production worldwide –, large individuals, multiple uses for different products – milk, beef, skin –, low efficiency of natural reproduction, and our previous experience in artificial insemination have offered fertile ground for large-scale application of embryology. Multiple Ovulation and Embryo Transfer (MOET), i.e. hyperstimulation, *in vivo* insemination, then flushing of D6-7 embryos from the uterus and transfer to less valuable females achieved considerable initial successes in the 1970's and 80's but in the past two decades we have seen a slow agony worldwide. In the early 90's IVF with the use of abattoir-derived ovaries was a great promise. Once technically resolved, it was almost immediately disregarded, apparently because of the lack of an efficient cryopreservation system for *in vitro* produced embryos. Finally, during the turn of the millennium, the standardisation and improvement of a decade-old method (trans-vaginal ovum pick-up, then *in vitro* maturation, *in vitro* fertilisation, blastocyst culture and transfer; OPU-IVF) rescued cattle embryology, at least in some parts of the world including South America. Currently, OPU-IVF is the only method with increasing application and real financial profit. Even so, together with MOET, it contributes to no more than 0.2% of the births of calves worldwide *(http://www.statista.com/statistics/263979/global-cattle-population-since-1990; http://www.ers.usda.gov/topics/animal-products/cattle-beef/statistics-information.aspx#.UzGPq1cqMgo; http://www.mla.com.au/Cattle-sheep-and-goat-industries/Industry-overview/Cattle; http://agebb.missouri.edu/mkt/bull12c.htm; http://issuu.com/itarget.2012/docs/o_embriao_51/7?e=5321994/1496796)*.

Still, this contribution is by far the most important impact of ART in animal breeding.

It should be noted that cryopreservation of *in vitro* produced bovine embryos – that could radically increase the application fields of OPU-IVF – has never become a routine technique although isolated groups reported excellent survival-pregnancy and calf-on-the-ground (the bovine analogue of take-home babies) results with vitrification since the end of the 90's.

In other domestic species (in spite of the most respected efforts of our colleagues working in these fields) the quantitative impact of IVF and related techniques on breeding is close to zero.

The infamous somatic cell nuclear transfer – even in the few tolerant countries – has remained and may remain forever just a curiosity, a luxury and a shopwindow item, with negligible impact on animal breeding. The same is true for the preservation of endangered (not even speaking about extinct) domestic breeds and wild species by embryo technologies, including but not restricted to cloning. Controversially, in most countries, for example the entire European Union, achieving success with somatic cell nuclear transfer has done more harm than good, creating a rather hysteric, hostile atmosphere and damaging the reputation of embryo technologies in general.

The only utility of embryology in domestic species seemed to be the production of transgenic animals for predominantly biomedical purposes, including live bioreactors, human disease models, and xenograft organ donors. Embryo technologies, primarily somatic cell nuclear transfer, have offered elegant solutions for the creation of transgenic domestic animals (1-6). However, when the technical problems were resolved, and increasing numbers of transgenic animals carrying and expressing various human disease genes were produced, they were almost entirely disregarded by the target industry, pharmaceutical companies and experimental medical institutions, leading to the closure of expensive embryology facilities and forcing qualified scientists to move to other areas.

Even more frustrating is the fact that not a single ground-breaking technical innovation has occurred in the animal field since the birth of

Dolly in 1996.

In humans, the situation is less disappointing. The number of IVF babies is well above 5 million worldwide. In some countries up to one in twenty children born are from IVF (7), and the number of IVF treatments is increasing 5 to 10% annually. On the other hand, the overall biological efficiency of IVF is still low (8) even in Europe, differences in success rates among different countries may be as high as twentyfold, and there are signs of a slowdown even in countries with the most advanced ART techniques *(http://www.eshre.eu/guidelines-and-legal/art-fact-sheet.aspx)*. Considering that one in six couples is infertile worldwide, the total contribution of ART to births could reach 15% but the estimated actual percentage is only 0.27% (350,000 ART babies /130,000,000 births worldwide) *(http://esa.un.org/wpp/Documentation/WPP%202010%20publications.htm)*.

Obviously ART will never be able to resolve all infertility and related situations, and there are serious external factors that hamper application of these sophisticated technologies to all who need them (9-11). Still, the 55-fold difference between the theoretical need and actual recipients of care is too high, and even with an extremely optimistic expected annual increase of 10% in the number of treatments, more than 30 years would be needed to provide children to a lucky third of infertile couples around the globe. Also considering that the annual increase in developed countries seems to be slowing down, and many poor countries will not be able to keep up the 10% rate, the actual prospects are probably much worse.

Accordingly, the sincere answer to the original question is that, in spite of impressive achievements, mammalian embryology has failed to fulfil its mission, and with the present tendency the situation will not change dramatically in the foreseeable future, not even for the next generation.

The background

The optimist would expect the future to bring sparkling new ideas and exciting new technologies that would improve efficiency and increase access to ART. However, this view is not well founded. The era of ground breaking innovations in mammalian embryology ended in the early 1990s. During the past 20 years, we were successful in exploiting past resources by fine-tuning applications, adjusting parameters and refining tools, but without any innovative approaches being introduced. IVF, SUZI, ICSI, embryo biopsy, assisted hatching, vitrification, even time-lapse were established and available. Single phase media were abandoned but reintroduced recently based on the principles established more than two decades ago. Even low oxygen and individual small incubators were staples of the best bovine embryo laboratories in the early nineties, to be rediscovered 10 years later by laboratories engaging in human embryo work. There are some fashionable new trends now and then with enthusiastic reports, then some controversial outcomes, finally a silent death and return to the old routine.

A world-class molecular genetics laboratory in the late 80s consisted of a few centrifuges, water baths, horizontal and vertical gel electrophoresis boxes, a PCR machine, and UV illuminators. Even the Southern blots were performed with paper towels derived from the ladies/men's rooms. Just imagine a researcher of this lab, after 20 years in coma, returning to a modern genomics facility, loitering helplessly among the fully automated, hermetically closed machines with mysteriously blinking computer screens.

And now, imagine the same situation with an embryologist. What would he or she find: incubators, laminar flow hoods, stereomicroscopes, and micromanipulators. All the same as 20 years ago, with minor added conveniences like ready-to-use micropipettes and culture media (albeit with unknown or partially known composition), and inconveniences like banned mouth pipettes. So, in five minutes, the time-traveller scientist could sit at the bench and start his work – just as he had done twenty years before.

In this regard, IVF is in a very special situation when compared to molecular genetics, stem cell research, practically all fields of biomedical science, not to mention computers and mobile phones. Time has stopped, not only in the liquid nitrogen containers but also in the whole laboratory.

"High technology? Forget it. IVF is a low technology". The (unfortunately unpublished) words of Rodney Wade from 2004 are still valid, more than ever.

The cause I. Structural, financial and legislative issues

Factors listed in the title hamper innovative research in both human and animal embryology, although the specific situation and impact are different.

Most *bovine industrial applications* are joint ventures of breeding companies and academic laboratories, or fully private ventures directed by former academics. The purpose of these companies is to establish/exploit new embryo technologies for large scale commercial application. Unfortunately research is financed by the industry and directed by scientists. Investors do not understand the unpredictable outcomes, hate missed deadlines and unfulfilled promises; scientists dislike the rigid hierarchy and commercial pressure. Most laboratories are bad hybrids lacking the standards and efficiency of a viable commercial venture and the freedom of academic environments. The budget is limited, and the purchase of "scientifically indispensable" makes it even more limited, although items really needed for efficient embryo work may be surprisingly inexpensive. The lack of money restricts most research activities to the laboratory phase. Although it provides a rapid feedback to a researcher (the typical length of an IVF or cloning experiment is 9 days), the information is insufficient for an investor who wants live offspring or fully mature elite animals, not blastocyst rates. The fate of these ventures depends on the patience and tolerance of investors. Obviously this tolerance is limited, and so is the lifespan of the laboratory.

Only few companies are directly exposed to the hard effects of the market. These may acquire appropriate industrial standards in a given technology to get commercially viable and may survive, but the chance for productive research in these ventures is zero.

Applied human embryology – including research and development – is done almost exclusively in ART centres. Many of these centres are independent private ventures; others are run by universities or hospitals. Although some of them were or still are directed by IVF pioneers with respectable (past) innovative skills the only purpose of the vast majority of these centres is to become or remain commercially successful – here and now. The competition is strong, and the profit has to be re-invested. Research is regarded as a luxury. Moreover, legal frameworks are strict, in some situations suffocating, hampering even the routine work, and making innovative research extremely complicated and difficult.

The (almost) complete lack of support from various funding agencies is a typical albeit rarely discussed feature of human embryology research (12-14). Reasons may be diverse and are out of the scope of this review. It should be emphasised, however, that the consequences are serious for the present and may be even more harmful for the future.

Accordingly, academic participation is usually restricted to collaborations for improvement of a given method or characterisation of a given phenomenon. If there is any independent scientific activity in these centres, it is typically done as a by-product of the routine work: to compare two similar approaches in embryo culture, cryopreservation, etc.

Contribution of the industry to research and development activities is also controversial (15). Indirect support is provided in form of financing conference participation, etc. Direct participation is usually restricted to selling items (tools, solutions). It can be helpful for routine work, but may even hamper research (media with unknown constituents, etc.) (16). Instrument development follows small-scale laboratory innovations slowly, and does not provide radically new solutions - in sharp contrast to genomics, information technology, etc. Sporadic development of some complicated instruments and simple equipment reflects a lack of profound knowledge of the needs of an embryo lab and the possibilities of advanced technology, with disappointingly inadequate outcomes.

The cause II. The human factor

One might suppose that the prestige and public attention may create a favourable environment for embryology research. Unfortunately, the contrary is true. The reputation is controversial, and may result in a negative selection. Capable but shy researchers get frightened by the unavoidable attention that follows their work, and those who like publicity may prefer to achieve it in a less ambivalent area.

On the other hand, the work in an embryo laboratory requires a strict and demanding schedule, high level of precision, manual skills and most

of all devotion. Although these conditions are regarded by laymen as obvious for all laboratory research, many candidates are discouraged by them and select an easier, more convenient topics.

In domestic animal embryology research, the main problem is the abovementioned strong influence of the academic structure and mentality. In an academic institution the laboratory work is usually done by PhD students, who are obviously inexperienced in the given technology, at least for most of the period of their scholarship. Postdocs are supposed to teach them, but their role is usually restricted to supervision, with less and less direct involvement in the manual work, fading memories and decreasing practical skills. Moreover, most senior scientists have only superficial knowledge about the practical work that is going on in the laboratory, they only enter there for presenting their empire to visitors. Unfortunately, these leaders make all decisions regarding the goals, needs and costs.

PhD students focus on the highest possible number of publications with appropriate impact factor written in the shortest period, with the least effort. They consider their presence temporary, are not interested in the whole system but just their particular project. They leave the same mess in the delicate technology as on the lab bench. The once up-to-date basic system – established by an exceptionally enthusiastic colleague – erodes continuously, the overall efficiency drops, and neither bosses, nor students are able to restore it.

In fact, there is no immediate need to restore it. Unjustified self-confidence impaired by incompetence is sustained by the whole structure of scientific hierarchy, financing and publication system in many countries, and seems to be resistant to all attempted reforms and rationalisations. The pressure to achieve real, top level results is minimal, and in most cases not the decisive factor that determines the support given and the future of a research group.

In human embryology, the situation is different. Most embryologists are devoted, precise, have good manual skills and accept the demanding workload. Unfortunately features required for innovative research including creativity, theoretical and practical problem-solving ability and a restless desire to improve are less typical.

Even more regrettably some characters are almost completely missing: scientists who are independent, headstrong and autonomous, with no gods no masters. Those who focus on the solution, not the problem; in whom the stoutness and sovereignty meets with superior judgement to keep their lurid personality on the right track. Scientists like the great pioneers of domestic animal and human IVF – 30 to 40 years ago.

Today, a typical human IVF lab cannot host such scientists. In contrast, the training period aims to extinguish all attempts to think independently and search for alternative solutions. The "learn first what is going on, then you may improve it" principle sounds reasonable, but the second half of the sentence is forgotten later. The big machine governed by financial and legal imperatives crushes individuals and creates obedient employees. The situation serves the interests of the present but closes the route towards the future.

Obviously there are exceptions with open-minded leaders and matching staff members who may risk the safe future for a bright idea, but their numbers are disappointingly low. A handful of large clinics have established and maintain a more or less independent research group, although the impact of these groups on the advancement of human embryology has been modest so far.

The consequence

Present legal frameworks, regulations, guidelines and lab manuals don't allow such freedom for research as 40 or 30 years ago. Experimentation with animal and especially human embryos is seriously restricted. Established approaches hold the "experimental" label for a long time, and in many countries require special permission for clinical application (11). To lose the label of experimental, these procedures have to prove their benefits and harmlessness both in short and long term, preferably by large-scale multicentre prospective randomised and subsequent follow-up studies (17-20).

In fact, it is very hard to meet these conditions. Today's situation would successfully prevent the birth of Louise Brown and the five million other children who have followed her. Recent procedures that have gained large scale acceptance in the human IVF laboratories haven't passed but escaped these restrictions. PGD and PGS have achieved considerable successes

mainly because of the rapid development of the diagnostic methods, not the embryology part. Time-lapse was rapidly acquired worldwide because "taking photos in the incubator" did not require special permissions. The most impressive change of the past decade, large-scale application of vitrification has occurred as a watershed as the result of a few clinics with special courage and/or in a special situation; it has spread silently across many countries for years and was acknowledged only recently as a routine procedure in some of them (21) while half of the world uses the same methods in everyday practice.

The absence of human embryology research in academic institutions, lack of governmental support for independent units and lack of support by the industry leaves research and development on the budget of IVF clinics, but their typical size and financial restrictions does not allow them to fulfil this role. Many publications reflect the "we had some interesting results recently, let's see if we can publish them" approach, others deal with a potentially exciting problem but the design is poor, or the basic system is handicapped and the value of the claimed improvement is hard to assess. The lack of sound studies is painfully visible in the Methods description of a typical Systematic Review dealing with the most common and very simple questions such as vitrification *vs* traditional freezing, low vs. normal oxygen concentration, etc. An automated search results in thousands of hits, the subsequent manual sorting reduces the outcome to several dozens of relevant publications of which only a handful meet (more or less) the strict selection criteria required to answer the given question correctly (14, 22, 23). Eventually, most systematic reviews lack the expected conclusion, saying that "more research is needed", although said research in the proper from will probably never be accomplished and our science will proceed on unpaved roads.

Apart from listing many objective factors, we also have to mention our own contribution, by acknowledging and tolerating the controversial situation. As said, although embryologists are called euphemistically as scientists in many IVF units, their real role is closer to a technician. Even more frustrating is that we seem to accept it. What was previously regarded as restrictive is stomached today or even regarded as ordinary, perhaps because there are few other options available. The blocking of creative thinking has serious consequences on innovation, and also impedes on routine work. Tools with obvious design flaws and media with unknown constituents continue to be just purchased and used. There is a very modest intention to understand what is needed and why; the most important principle is to follow the codified procedure. As a consequence, the ability for troubleshooting is restricted and there is an increasing tendency to turn to external help even in situations when a little practical thinking or search in the available scientific literature would provide the obvious answer.

The solution

Being a pathologist by academic education, I regard my primary task to establish a diagnosis. The therapy – if therapy was still needed – was always decided by others. However, to give some opinion, suggestions – and if possible, some hope – was part of my work.

Firstly it has to be acknowledged that efficiency improvements during the past decades have been highly impressive. The lack of groundbreaking innovations may also be interpreted as normal, natural consequences of cyclic revolutions and evolutions. One may refer to the example of cars being essentially the same for the past 80 years: four tyres, steering wheel, brake, clutch, etc. Unfortunately, the laboratory equivalent is that today's embryology is on the level of counting red blood cells in haemocytometers. Fine-tuning an optimisation may still have some potential and related disciplines including molecular biology and genomics may help us increase our efficiency further. However, we cannot just look forward to external help. We have to make fundamental changes in our laboratory work as well. Firstly, we have to change the way we think about the future.

Instead of measuring scrupulously individual variations between embryologists regarding the efficiency of ICSI each week, we have to make fully automated ICSI machines with adjustable but precisely accomplished parameters and without the impact of the manual skills and mental-emotional status of the operator. Is it an extremely demanding technological task? Maybe. Would it be very expensive? Maybe. However, just fifteen years ago tons of plastic pipette

tips and 3,000,000,000 dollars were spent to decode a single human genome. With the development of new generation machines every year, sequencing is now fully automated and costs have been reduced by 3,000,000 times, to 1,000 dollars. Further reduction to 300 or even 100 dollars is predicted and may happen in 1-3 years. Was it worth to invest? Did we achieve any reduction in the cost, at least the laboratory cost of an average IVF cycle since 2001, while increasing the accuracy and overall efficiency?

A major problem with our approach is that we cannot escape from the trap of our handheld pipettes, petri dishes, air-flow boxes, stereomicroscopes, micromanipulators and gassed incubators. In fact, the next generation IVF might be done in laptop-sized compact machines, disposable microchannel networks controlled by multiple cameras and sensors, and the role of the embryologists could be to operate the input (aspirated follicle fluid, ejaculated semen), approve or correct automatic decisions at several checkpoints and transport the embryos to the operating theatre. Just as is done in aviation, car production, genomics and – a very close example – in our laboratory assay machines.

One may be concerned with the small market, the high costs, and the technical difficulties. However, 3,000 clinics worldwide with 10% increase every year may together provide the resources, considering the need for multiple machines in most of them. The rapid spreading of time-lapse machines has demonstrated the purchasing capacity of clinics. Microchannels (if microchannels are the solution) are obviously more demanding and complicated, but the inability to achieve rapid advancement for the past 15 years is disappointing. Problems like "we cannot get rid of bubbles" etc. hamper applications and stop promising projects, while the system has been proven suitable for (almost) everything we need for IVF or even somatic cell nuclear transfer (24-28).

We just need somebody – well, a capable team, a considerable sum of money and a determined industry – to put the pieces together properly. Maybe we need a little more: a few competing teams and wide scientific, technical and emotional support from the community of embryologists.

Another concern is to allow machines to determine the fate of potential human beings. This concern may seem justified, but we allow machines to run subway trains, airplanes, diagnostic and therapeutic interventions, and very soon we will put our lives in their hands routinely every time we get into our cars. Are we sure that our trembling hands and often divided attention will always handle embryos better than sophisticated robots designed for that specific purpose? Of course control will always be required. This is also the answer for the next concern: the future role of embryologists. This role should evolve as well. It will open another level of professional opportunity – (open responsibility??) much like that given to pilots controlling the automatic landing of an Airbus 380. The more we contribute to the development, the more fascination we may find in the outcome.

Conclusion

It is beyond the goals of this review to suggest specific changes in the structural, financial and legal frameworks of research and development in embryo laboratories. However, to overcome the barriers, and to accelerate advancement changes are inevitable. We must re-create the enthusiastic and innovative atmosphere of embryo research of the past to meet the demands of the future.

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