Anim Reprod, v.9, n.3, p.341-344, Jul./Sept. 2012

Challenges in work with bovine gametes and embryos

H. Callesen[1]

[1]Department of Animal Science, Aarhus University, Tjele, Denmark

Abstract

Gametes (spermatozoa and oocytes) and embryos from domestic animals are routinely handled *in vi*tro in large and increasing numbers all over the world. Such manipulation causes various forms of damage to the gametes/embryos that can lead to different problems. A safe and reliable basis for continued practical use of these technologies in science and cattle industry requires further activities in research and development. Just as important is a continued close relation between science and industry so that the extent and results of this work can be collected, analyzed and reported for the benefit of all groups involved and interested such as scientists, consumers, industry and legislators.

Keywords*:* cattle, *in vitro* handling, oocytes, spermatozoa.

Working with gametes and embryos

Today it is a widespread routine to work with oocytes, spermatozoa and embryos in many mammalian species. For cattle, the worldwide extent of this activity has been estimated 10 yr ago for semen (Thibier and Wagner, 2002), while statistics for oocytes and embryos are collected and published every year by the International Embryo Transfer Society (IETS; Stroud, 2011). If all this is added together, every year more than two million oocytes and around one million embryos are handled worldwide, and these numbers continue to increase. One reason is that more and more techniques have been developed since artificial insemination (AI) was introduced 70-80 yr ago (Table 1), but also that the techniques are being used in more and more domestic animal species and last, but not least, have been implemented in practice. Many of the techniques are used in practice as part of systematic breeding plans, starting with AI in the 1950's, MOET in the 1980's, OPU/IVP in the 1990's, and today with the beginning of genomic selection of embryos. Even a technique such as SCNT is being used more often, mostly for single animals of certain high value such as "Starbuck II" (born in Canada in 2000; www.ciaq.com) and three copies of "Mtoto" (born in Italy in 2002; Galli *et al*., 2003); furthermore, different companies such as Cyagra and Bovance are offering cloning to farmers.

Table 1. Overview of the approximate decade for the first practically useful results with *in vitro* handling technologies on spermatozoa, oocytes and embryos from domestic animals.

In vitro handling technique	Gametes		Embryos
	Spermatozoa	Oocytes	
Artificial insemination (AI)	1930		
Biopsy of embryo			1990
Cryopreservation	1950	1990	1970
In vitro maturation (IVM)		1980	
In vitro fertilization (IVF)	1980	1980	
In vitro culture (IVC)			1990
Multiple ovulation and embryo transfer (MOET)			1970
Ovum pick-up (OPU)		1990	
Somatic cell nuclear transfer (SCNT)			1990
Sorting of semen	2000		
Splitting of embryo			1980

Consequences

Any *in vitro* handling of gametes and embryos is a threat to the cells, as they are under artificial conditions and will easily be damaged. Although such damages should be reduced as much as possible, working with these techniques results in a combination of biological, technical and practical reasons for damages. The biological reasons are reflections of our level of knowledge about the cells (e.g. media composition for IVM; superovulation protocol for MOET; voltage for electrofusion in SCNT); the technical reasons are related to the way the technique is performed (e.g. *in vitro* handling in a dish or in a microfluid system; *in vitro* culture after HandMade Cloning); the practical reasons are often dictated by the working conditions (e.g. distance between oocyte donors (slaughterhouse or stable) and the IVM-

[1]Corresponding author: henrik.callesen@agrsci.dk
Phone: +45-8715-7989; Fax: +45-8715-4249
Received: May 25, 2012
Accepted: July 6, 2012

laboratory; temperature variations in the environment where embryos are handled). In most cases it will be a combination of these three reasons, as performing one technique often means including one or several others - examples: "semen sorting" includes collection, eventually cryopreservation, and finally AI; "OPU" includes IVF, eventually biopsy and afterwards cryopreservation, and finally ET; and "SCNT" includes IVC and finally ET.

The consequences of the different damages can be fatal with death of the cells, but with the techniques today most damages are moderate or minor. The severity of the damages for the given gamete/embryo depends on the robustness (quality) and type of technique (some are more demanding than others with stronger physical or chemical stress or with longer duration of the *in vitro* handling). It is therefore important to realize the extent of the damage and evaluate its importance. In that context, damage to a fraction of cells from an ejaculate with millions of spermatozoa can be more acceptable than on a small group of oocytes or a single embryo.

The moderate and the minor damages are often more difficult to detect and evaluate. Minor damages may be within the range of "optimal conditions" that are considered to be accepted as normal by us (and by the cells!). The moderate damages may be more difficult to detect as they may reveal themselves later; perhaps as a low developmental rate, soon after transfer or around implantation as abortion, or perhaps first at birth or even later. Therefore it is often considered that a conclusive evaluation of a technique includes post-transfer embryonic development and calf characteristics (Merton *et al*., 2012).

Challenges

In the work with gametes and embryos, there are two types of challenges. One is regarding the damage to the cells themselves caused by the techniques, and the other is the way we perform the techniques. This is illustrated in the following three examples.

Efficiency

The extensive and increasing use of OPU-IVP in cattle industry is a very clear example of a set of techniques that have found a solid place in practice. Basic biological and technical problems have been sufficiently solved, but also the practical problems have found a convincing solution as described in a large study from Brazil (Pontes *et al*., 2010). Long distances between site of oocyte collection, the IVP-laboratory and the site of transfer to recipients is no longer a practical problem, illustrating that the real limitations of a given technology are often first realized when it is used under large-scale practical working conditions where robust solutions are needed.

Even though this technology is used routinely and in large scale, which is a significant milestone in its development, new additions and optimizations continue to be investigated. Two examples of this are:

Oocyte donors

There is a large variation between number of oocytes collected from donors used for OPU, and with the established role of this technique in more and more breeding programs, factors responsible for this are naturally being investigated. Breed has a significant influence (Guyader-Joly *et al*., 2010; Pontes *et al*., 2010), but there is also a strong influence of the oocyte donor on the blastocyst production (Tamassia *et al*., 2003). At least part of this seems to be related to genetic parameters, where some traits have been found to be of potential value for future genetic selection (Merton *et al*., 2009).

Sexed semen

This technology is today used both for AI, superovulation and after OPU-IVP (e.g. Blondin *et al*., 2009; DeJarnette *et al*., 2010; Pontes *et al*., 2010), but large individual differences between bulls are described. Part of the issue is related to the sorting process itself as well as the low dosage used for AI (Frijters *et al*., 2009). However there is also the influence of the IVP caused by the *in vitro* conditions and eventual additional cryopreservation (Blondin *et al*., 2009). However, even though sexed sperm is affected by the sorting procedure, the calves are not different from calves born after non-sorted semen (Seidel, 2009).

Both of these examples have been revealed in larger commercial settings, where the large scale of the activities allows for such data collection and analysis. Other examples can be mentioned that are more focused on technical developments, such as development of new methods for cryopreservation of *in vitro* produced embryos.

Animal welfare

In the earlier years with use of IVP and SCNT, it was shown (Kruip and den Daas, 1997) that an increased proportion of calves born after IVP or SCNT were abnormal in a number of different ways compared to AI calves. This phenomenon became commonly known as Large Offspring Syndrome (LOS; Renard *et al*., 1999), because increased birth weight was one of the very visible problems. Due to the many other problems with the calves, the term AOS was later suggested (Abnormal Offspring Syndrome; Farin *et al*., 2006).

Since then, only few larger comparisons between AI, MOET and IVP calves have been made, being the occurrence of LOS/AOS confirmed in 2000 (van Wagtendonk-de Leeuw *et al*., 2000). It was also

demonstrated that the problem could be minimized with reduced serum concentrations in the IVP-medium. This was gradually implemented, and today it is the impression that LOS/AOS is no longer an issue in the IVP field (e.g. Merton *et al*., 2012).

For SCNT, the problems still exist with the calves having a range of abnormalities and reduced viability. However, since 1997 (Kruip and den Daas, 1997) much has been learned, and already 10 yr ago it was concluded from a literature review both that "the abnormalities observed in cloned offspring are also seen with natural reproduction" and in a higher rate, but "the great majority of clones appear to develop normally" (Cibelli *et al*., 2002).

For both IVP and SCNT, it is as important as ever to follow the development of the techniques, both because new changes are introduced in the procedures, but also because of the intense focus on the outcome. With the already large commercial use of IVP and increasingly use of SCNT, the industry will be an important partner in providing information about further developments and improvement of techniques.

Product quality

The outcome of the practical use of the many techniques mentioned in this article must be a range of end products of high quality. There are several examples that this is a strong point of concern for many groups, e.g. farmers, consumers, industry and legislators. This is in particular the situation related to SCNT, where major concerns have been addressed to the actual food products such as meat and milk from cloned cattle and their offspring. Many experiments have been made to test for differences between such products from cloned vs. non-cloned cattle, especially in Japan (Watanabe, 2011). Many investigations have also been made with focus on other types of products such as sperm characteristics (Couldrey *et al*., 2011), embryo development (Yamanaka *et al*., 2011) and production and health characteristics (Wang *et al*., 2011). Furthermore, extensive reviews have been performed by FDA (Rudenko *et al*., 2007) and European Food Safety Authority (EFSA, 2012). From all these documents, a common conclusion is that no differences have been found in food or other products between cattle clones and their offspring compared to non-cloned cattle. One remaining issue is life length and senescence that has not been possible to study due to the long generation intervals of domestic animals (Cibelli *et al*., 2002).

Final remarks

The *in vitro* handling of gametes and embryos of domestic animals is routine, and the extent of this work is growing rapidly with even better results in practice, and with even more techniques being added. This is paralleled by new developments and refinements of the various techniques. Such development is good for the techniques and their uses, and also for the companies and persons working with them. However, there is a tendency not to inform about these recent modifications and improvements as much as it was done in the first years. This is probably related to the practical situation of companies where the techniques are intensively used, though with focus on things other than to collect, analyze and publish data from the daily activities. However, the last 20 yr's history has clearly shown the importance of contributions to the literature from both science and industry. As illustrated above, there is a need to continue having such focus especially on the consequences of the practical use of the techniques in domestic animals, both for the animals as well as for their use in food production. Practically, the already existing role of several scientific societies in the collection and communication of such information can only be urged to continue and even expanded in parallel with the use of existing and new developments of *in vitro* technologies in domestic animals.

References

Blondin P, Beaulieu M, Fournier V, Morin N, Crawford L, Madan P, King WA. 2009. Analysis of bovine sexed sperm for IVF from sorting to the embryo. *Theriogenology*, 71:30-38.

Cibelli JB, Campbell KH, Seidel GE, West MD, Lanza RP. 2002. The health profile of cloned animals. *Nat Biotechnol*, 20:13-14.

Couldrey C, Wells DN, Lee RSF. 2011. DNA methylation patterns are appropriately established in the sperm of bulls generated by somatic cell nuclear transfer. *Cell Reprogram*, 13:171-177.

DeJarnette JM, Nebel RL, Marshall CE. 2010. Use of flow cytometrically sex-sorted semen in single and superovulated cows and heifers. *In*: Proceedings from 26th Annual Meeting of Association Européenne de Transfert Embryonnaire, 2010, Kuopio, Finland. Kuopio: AETE. pp. 79-95.

European Food Safety Authority (EFSA). 2012. Update on the state of play of animal cloning. *EFSA J*, 10:2794-2835.

Farin P, Piedrahita JA, Farin CE. 2006. Errors in development of fetuses and placentas from in vitro-produced bovine embryos. *Theriogenology*, 65:178-191.

Frijters ACJ, Mullaart E, Roelofs RMG, van Hoorne RP, Moreno JF, Moreno O, Merton JS. 2009. What affects fertility of sexed bull semen more, low sperm dosage or the sorting process? *Theriogenology*, 71:64-67.

Galli C, Duchi R, Crotti G, Turini P, Ponderato N, Colleoni S, Lagutina I, Lazzari G. 2003. Bovine embryo technologies. *Theriogenology*, 59:599-616.

Guyader-Joly C, Moulin B, Mariller F, Curin V, Ponchon S, Gonzalez C, Humblot P, Ponsart C. 2010. Biological factors affecting oocyte collection and embryos produced in a commercial ovum pick-up

system in Holstein and Montbeliard breeds. *Reprod Fertil Dev*, 22:378. (abstract).

Kruip ThAM, den Daas JHG. 1997. In vitro produced and cloned embryos: effects on pregnancy, parturition and offspring. *Theriogenology*, 47:43-52.

Merton JS, Ask B, Onkundi DC, Mullaart E, Colenbrander B, Nielen M. 2009. Genetic parameters for oocyte number and embryo production within a bovine ovum pick-up-in vitro production embryo-production program. *Theriogenology*, 72:885-893.

Merton JS, de Roos APW, Koenen EPC, Roelen BAJ, Vos PLAM, Mullaart E, Knijn HM. 2012. Bovine OPU-derived oocytes can be matured in vitro for 16-28 h with similar developmental capacity. *Reprod Domest Anim*. doi: 10.1111/j.1439-0531.2012.02010.x.

Pontes JHF, Silva KCF, Basso AC, Rigo AG, Ferreira CR, Santos GMG, Sanches BV, Porcionato JPF, Vieira PHS, Faifer FS, Sterza FAM, Schenk JL, Seneda MM. 2010. Large-scale in vitro embryo production and pregnancy rates from *Bos taurus*, *Bos indicus*, and *indicus-taurus* dairy cows using sexed sperm. *Theriogenology*, 74:1349-1355.

Renard J-P, Chastant S, Chesné P, Richard C, Marchal J, Cordonnier N, Chavatte P, Vignon P. 1999. Lymphiod hypoplasia and comatic cloning. *Lancet*, 353:1489-1491.

Rudenko L, Matheson JC, Sundlof SR. 2007. Animal cloning and the FDA - the risk assessment paradigm under public scrutiny. *Nat Biotechnol*, 25:39-43.

Seidel Jr GE. 2009. Sperm sexing technology - the transition to commercial application. *Theriogenology*, 71:1-3.

Stroud B. 2011. IETS Statistics and Data Retrieval Committee Report. The year 2010 worldwide statistics of embryo transfer in domestic farm animals. *IETS Newsletter*, 29(4):14-23. Available on: http://www.iets.org.

Tamassia M, Heyman Y, Lavergne Y, Richard C, Gelin V, Renard JP, Chastant-Maillard S. 2003. Evidence of oocyte donor cow effect over oocyte production and embryo development in vitro. *Reproduction*, 126:629-637.

Thibier M, Wagner HG. 2002. World statistics for artificial insemination in cattle. *Livest Prod Sci*, 74:203-212.

van Wagtendonk-de Leeuw AM, Mullaart E, de Roos APW, Merton JS, den Daas JHG, Kemp B, de Ruigh L. 2000. Effects of different reproduction techniques: AI, MOET or IVP, on health and welfare of bovine offspring. *Theriogenology*, 53:575-597.

Wang H, Zhang JX, Zhao MB, Zhang XL, Sun QY, Chen DY. 2011. Production and health assessment of second-generation cloned Holstein cows derived by somatic cell nuclear transfer. *Anim Reprod Sci*, 126:11-18.

Watanabe S. 2011. Somatic cell cloned cattle and their progeny produced in Japan: a report for animal health and characteristics of animal products. *Mem Natl Inst Livest Grassl Sci*, 12:1-44.

Yamanaka K, Kaneda M, Inaba Y, Saito K, Kubota K, Sakatani M, Sugimura S, Imai K, Watanabe S, Takahashi M. 2011. DNA methylation analysis on satellite I region in blastocysts obtained from somatic cell cloned cattle. *Anim Sci J*, 82:523-530.