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Ovum pick up, intracytoplasmic sperm injection and somatic cell nuclear transfer in cattle, buffalo and horses: from the research laboratory to clinical practice

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ABSTRACT

Assisted reproductive techniques developed for cattle in the last 25 years, like ovum pick up (OPU), intracytoplasmic sperm injection (ICSI), and somatic cell nuclear transfer, have been transferred and adapted to buffalo and horses. The successful clinical applications of these techniques require both the clinical skills specific to each animal species and an experienced laboratory team to support the *in vitro* phase of the work. In cattle, OPU can be considered a consolidated technology that is rapidly outpacing conventional superovulation for embryo transfer. In buffalo, OPU represents the only possibility for embryo production to advance the implementation of embryo-based biotechnologies in that industry, although it is still mainly in the developmental phase. In the horse, OPU is now an established procedure for breeding from infertile and sporting mares throughout the year. It requires ICSI that in the horse, contrary to what happens in cattle and buffalo, is very efficient and the only option because conventional IVF does not work. Somatic cell nuclear transfer is destined to fill a very small niche for generating animals of extremely high commercial value. The efficiency is low, but because normal animals can be generated it is likely that advancing our knowledge in that field might improve the technology and reduce its cost.

1. Introduction

Breeding techniques have always been at the center of any livestock enterprise, motivated by curiosity and consolidated by breeder's needs and interests. This has been the case, for example, when artificial insemination was developed as an hygienic measure to prevent disease transmission or at present in the genomics era, where embryos can be genotyped and/or propagated by somatic cell nuclear transfer (SCNT) [1,2] to reproduce the desired genotype from selected parents. Livestock species have provided for decades the knowledge for assisted reproduction techniques that have been translated to humans. Because of the easy availability of both gametes and pre-implantation embryos, the closer similarities with the human counterpart and the different ethical requirements, scientists working with livestock have contributed to the advancement and consolidation of the human field [3]. In turn, many of the more advanced techniques developed for humans have provided a model for animal scientists after the birth of the first human being conceived *in vitro* [4] and following the subsequent developments, including transvaginal ovum pick up (OPU) [5–7].

The practical application of assisted reproduction technologies in livestock requires the integration of the laboratory techniques with the clinical management of donors, recipients and newborn animals, because what matters to

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the client requesting such services is a live offspring. The drive behind the developments of advanced assisted reproduction techniques discussed in this article for cattle and buffalo is the need to generate large numbers of embryos, preferably of predetermined sex and known genotype, for genetic improvement of the herds. On the contrary, in horses the main reason for applying these technologies has been for addressing both female as well as male infertility [8], very much as it is done in human assisted reproduction, including pre-implantation genetic diagnosis.

In this paper, we revisit the major developments that played a role in turning experimental findings into specialized clinical practices in the breeding of high genetic merit livestock. We discuss the impact of the techniques as well as the bottlenecks and the possible future developments. It is more of a personal view and experience than a comprehensive review of all the literature.

2. Ovum pick up

2.1. Cattle

The development of OPU in cattle followed the establishment of reproducible techniques for *in vitro* maturation [9], fertilization [10,11] and culture of sheep [12] and cattle embryos [13], bringing to the birth of normal offspring [14], techniques. All the experimental work done to develop *in vitro* embryo production (IVP) procedures was essentially based on oocytes that were recovered from slaughterhouse ovaries in large numbers. However, for practical application, it was clearly desirable to recover oocytes from living donors of known genetic value.

According to the International Embryo Transfer Society statistics (Fig. 1), the number of embryos produced *in vitro* and transferred into recipients has increased more than 10 times [15] in the last dozen years and are now approaching the numbers of embryos produced *in vivo* by superovulation. This indicates that OPU and IVP is considered a reliable and cost-effective technique and has acquired a role in cattle breeding.

The first attempts to use ultrasound-guided follicular aspiration for embryo production *in vitro* were reported by Pieterse and other authors in the late 1980s [16–18] by using a human endovaginal probe adapted for the use in

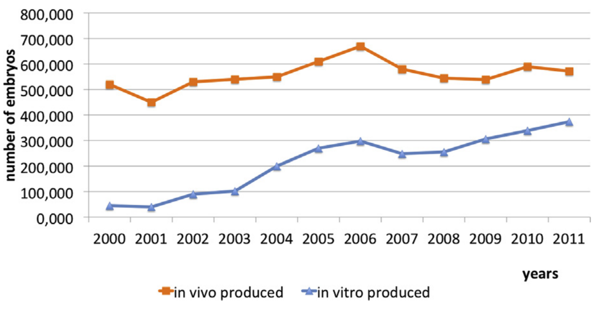

Fig. 1. Summary of the International Embryo Transfer Society statistical data collected by the data retrieval committee. *In vitro* production of embryos is steadily growing and the majority of them are now produced by ovum pick up (OPU).

cattle. They reported a recovery rate of 55%, repeatability of the procedure, and absence of side effects on the donor cows. Although the procedures for embryo production in those days still required major refinements that came later [19], the basics of OPU described by Pieterse et al. are still the same used today by many practitioners. Recovery rates have been improved in the excess of 70% owing to the use of better ultrasound equipment with 6- or 7-MHz convex array probes that provide a better resolution on smaller follicles and the use of gonadotrophin priming that increases the size of smaller follicles that otherwise would not be picked up by inexperienced operators. Native endovaginal probes are available essentially for human use and need to be adapted to hold longer 60 cm long single lumen needles (Fig. 2). Several manufacturers of ultrasound equipment have provided custom-made plastic holders that can house generic convex array transducers together with the needle guide. A partial simplification of the equipment was described by Bols, et al. [20] that used simple hypodermic disposable needles. However, this simpler setting has the disadvantage of a bigger size that, although it is fine for cows, can be traumatic for very young heifers. The use of native endovaginal probes allows the complete replacement, for each donor animal, not only of the probe latex cover, but also of the needle guide together with the needle. This solution uses one sterile long needle and one needle guide for each OPU session/donor, requiring more equipment and more labor for cleaning, sterilizing, and packaging; as a consequence, it is more expensive. However, from an hygienic standpoint and quality control, it is a system that provides no cross-contamination between donors and therefore provides the highest biosafety standards, as it is done in humans. On the contrary, the setting with a generic probe enclosed in a plastic adapter together with the needle guide make it convenient to replace the disposable and less expensive needle used for aspiration. However, the needle guide is normally not replaced from donor to donor, being enclosed in the plastic adapter. Therefore, there is always some blood that by capillarity will infiltrate in the needle guide and in the surroundings of the probe cover when the vaginal wall is punctured. The needle in both settings is connected through a tubing and a test tube to a vacuum pump that usually provides a vacuum set at flow rate between 15 and 25 mL/min [21] to ensure maximum recovery with the least damage to the cumulus oocytes complexes. Flow rates are more indicative than vacuum pressure because the gauge of the needle and the length of the tubing can make a big difference. Simple flushing media for embryo transfer, supplemented with heparin to avoid follicular fluid or blood clotting, are currently used for oocyte recovery.

The OPU technique was initially applied on problem cows that did not respond to superovulation [22,23], but later on it was applied on a wider scale also on pregnant cows and heifers, including prepubertal heifers [21]. It is difficult and often not relevant to make comparisons between different dataset because there are so many variables involved, most of which are not even manageable. Beef breeds perform better than dairy cows, dry cows do better than lactating ones, and cows perform better than heifers (Table 1). Climate conditions with high temperature and