IN VITRO EMBRYO PRODUCTION IN SHEEP: A USEFUL TOOL FOR FARMING AND BENCH

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The latest advances in *in vitro* embryo technology in sheep have improved its use in diverse systems and applications. Major developments and some limitations found in our Laboratory are summarized at the INITRA meeting. With the aim to improve the use of live animals as donors for embryo production, hormonal follicular manipulation to enhance oocyte quality for laparoscopic assisted follicle aspiration is discussed. The *in vitro* production (IVP) system used in our Lab, including oocyte maturation, fertilization and embryo culture has been standardized to achieve acceptable and highly repeatable blastocyst rates usually close to 30-40%. However, the main limiting factor of expansion of *in vitro* embryo production is related to the low cryotolerance of IVP embryos, and thus, the need for continuous availability of recipient females. For this reason, new protocols for fixed time embryo transfer (FTET) are discussed avoiding estrous detection and making easier recipient management, facilitating the implementation of this technology on-farm. Relative to the low cryotolerance of IVP embryos, it seems to be partially solved by the novel vitrification methods with minimum volume such as the Cryotop like systems. In a recent experiment, we obtain acceptable pregnancy rate after FTET of IVP blastocysts submitted to vitrification/warming by this minimum volume method, which was around 40% vs. <10% obtained with conventional slow freezing with ethylene glycol. Furthermore, the use of Cryotop method also improved pregnancy outcome of *in vivo* derived embryos when compared with conventionally frozen embryos (around 68% *vs*. 46%, respectively; $P<0.05$). Interestingly, on 453 transferred embryos, IVP vitrified blastocysts using Cryotop method had a similar survival rate (fetuses/transferred embryos) compared with the currently default method using *in vivo* derived blastocysts frozen with ethylene glycol (P=NS). This finding suggests that Cryotop could be applied to *in vitro* embryo programs, and also may be interesting for conventional MOET programs. In addition, the optimal number of IVP embryos to be transferred per female is depending on the maternal ability of the breed used as recipients, but in general, in our experience this should be adjusted to obtain no more than one lamb per ewe. Although we found that more embryos transferred per female improved the percentage of pregnant/transferred ewes, multiple embryo transfer did not improve the number of fetuses/transferred embryos. In addition, birth of twins resulted in lower birth weight with reduced lamb survival rate when compared to singleton. As consequence, the number of live lambs from total transferred embryos was greater in singleton births. Along with these enhancements, we use the IVP embryos in our Lab for genetic improvement programs, research projects, and also for the generation of transgenic and CRISPR genome edited sheep. Thus, the latest refinements of *in vitro* embryo technology have enabled its implementation in a wide range of systems, generating great progress for basic and applied science.