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Review Article

# Advances in *in vitro* production of sheep embryos

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ABSTRACT

Sheep is an important livestock in the world providing meat, milk and wool for human beings. With increasing human population, the worldwide needs of production of sheep have elevated. To meet the needs, the assistant reproductive technology including ovine *in vitro* embryo production (ovine IVP) is urgently required to enhance the effective production of sheep in the world. To learn the status of ovine IVP, we collected some publications related to ovine IVP through PubMed and analyzed the progress in ovine IVP made in the last five years (2012–2017). We made comparisons of these data and found that the recent advances in ovine IVP has been made slowly comparable to that of ovine IVP two decades ago. Therefore, we suggested two strategies or approaches to tackle the main problems in ovine IVP and expect that the efficiency of ovine IVP could be improved significantly when the approaches would be implemented.

## 1. Introduction

Since the first success of sheep *in vitro* fertilization (IVF) was reported in Cambridge, the UK in1986 [1], sheep reproductive technology entered a new era, the great efforts had been made in the field by the scientists worldwide. Until the second half of 1980s, the IVF became entirely *in vitro* systems, called "*in vitro* embryo production" (IVP) including the three procedures, namely *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* embryo culture (IVC). Up to the early1990s, the basic systems of ovine IVP including the three procedures had been well established and have been utilized until now. IVP is a valuable tool to aid the understanding of early mammalian development with applications ranging from therapeutic treatment of human reproductive failure to the preservation of gametes from animals of high genetic merit [2] and speeding up genetic improvement in livestock. However, the process in sheep is still inefficient: approximately 70–90% of immature oocytes undergo maturation, from prophase I to metaphase II; 50–80% undergo fertilization and cleave to at least the two-cell stage at 24 to 48 h post-insemination; only 20% to 50% of immature oocytes ever reach the blastocyst stage, on day 7 to 8 post fertilization shown in Table 1, these results are similar to that reported by Walker et al. [3] in 1996. Additionally, *in vivo* produced embryos are, in general, of greater quality than *in vitro*-produced embryos, because of greater implantation rates, high birth rate and high survival rate. The differences imply a great potential in improving ovine IVP. According to the statistical data reported by the United Nation Food and Agricultural Organization; production of sheep in the world has increased from 1060 million in 2000 to 1196 million heads in 2014 (cited from FAOSTAT-DATA 2017 online) (Fig. 1). This tendency indicates that the IVP systems as a new reproductive technology may play an important role for production of sheep in the future to accelerate sheep breeding and to improve the efficiency of production. However, we are currently facing many technical challenges in improving the efficiency of ovine IVP system such as low efficiency and poor quality of embryos, the system remains important, especially in sheep genetic breeding's compared to natural reproduction and could be used to ensure the sustainable development of sheep production. Therefore, we urgently need to find solutions to overcome the problems so that the system could significantly be improved. Additionally, there are recently many excellent reviews on IVP in sheep [4–9], which not only described the advances in the field, but also pointed out the direction of the technology in the future. Likewise, based on recent publication associated with ovine IVP, in this review, we summarized the recent advances and challenges in sheep IVP including IVM, IVF and IVC procedures and suggested two possible approaches to tackle the problems. At the end, we predict the prospects of applications of sheep IVP systems, particularly in biomedical research.

### 1.1. *In vitro* maturation of ovine oocytes

Immature oocytes to become fertilizable must undergo cytoplasmic and nuclear maturation. Subsequently, oocytes extrude the first polar

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**Table 1**
Summary of ovine embryo *in vitro* production in recent years (2012–2017).

| Date (year) | Country | Season | Sheep age (Year) | Sperm types | Resources of COCs | IVM medium used | Hormones used | IVM duration (H) | Conditions of IVM | IVF medium | Chemicals for sperm capacitation | Sperm concentration (x$10^6$) | Duration of IVF (h) | Feralization rate (%) | Embryo culture medium | Blast rate (%) | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2016 | Italy | N/A | 4–6 | Frozen | Slaughter-house | M 199 + 10-% OSS | 0.1 IU m-$L^{-1}$ FSH /LH | 24 | 38.5 °C 5% $CO_2$ | SOF + 2% ESS | 1 mg/ mLheparin,1-μg/ mLhypotaur-ne | 1 | 22 h | 74.5% | SOFaa +- 0.4%BSA | 59.2% | 1 |
| 2016 | Brazil | N/A | 4–6 | Frozen | Slaughter-house | M199 10%FCS Roscoviti-ne | 0.1 IU/mL FSH/ LH | 24 | 38.5 °C 5% $CO_2$ | SOF + 2% ESS | 2% ESS | 1 | 22 | 71.6% | SOFaa +- 04% BSA | 48.9% | 2 |
| 2014 | Uruguay | N/A | N/A | Frozen | Slaughter-house | M199 + -10% ESS | 10 μg/mL FSH/ LH | 24 | 39 °C 5% $CO_2$ | SOF + 2% ESS | 10 μg/mL heparin, 10 mg/mL hypotaurine | 1 | 22 | 79% | SOFaa +- 0.4% BSA | 41.3% | 3 |
| 2015 | Iran | N/A | N/A | Frozen | Slaughter-house | M199 10% FBS α-linoleic acid | 1 μg/mL E2, 0.5 μg/ mL FSH/ LH | 24 | 38.5 °C 5% $CO_2$ | SOF + 2% ESS | TALP, 0.6% BSA, 1 mg/ mL heparin, 50 ng/mL epineph rine, + 50 ng/mL hypotaurine | 1 | 18 | 63% | SOF +- 10% SCF | 20% | 4 |
| 2013 | China | N/A | N/A | Frozen | Slaughter-house | M199 4 mg/mL BSA | 10 μg/mL FSH/LH, 1 μg/mLE2, 50 ng/mL ghrelin | 24 | 38.5 °C 5% $CO_2$ | SOF + 2% SS | 2% SS | 1 | 20 | 76.5% | SOFaa + 8-mg/mL BSA + 50 -ng/mL ghrelin | 36.7% | 5 |
| 2013 | Spain | N/A | 4 years | Frozen | Slaughter-house | TCM199- + 10% FCS | 10 μg/mL FSH/ LH | 24 | 38.5 °C 5% $CO_2$ | SOF + 2% ESS | 2% ESS + hepari-n + hypotaurine | 1 | 24 | 52% | SOF + BSA | N/A | 6 |
| 2013 | UK | N/A | N/A | Frozen | Slaughter-house | TCM199- + 10% FBS | 5 10 μg/mL FSH/LH, 10 μg/ mLE2 | 24 | 39 °C 5% $CO_2$ | SOF + 2%SS | 2% SS | 2 | 18 | 74% | SOFaa + B-SA | 45.1% | 7 |
| 2012 | Iran | N/A | N/A | Frozen | Slaughter house | M199 + -10% FBS | 5.0 μg/mL LH, 0.5 μg/ mLFSH 1 μg/mL E2 | 24 | 39 °C, 5% $CO_2$ | SOF + 2% ESS | 4 IU/mL heparin + P-HE+2% ESS | 2 | 18 | 71.7% | SOFaa + 8-mg/ mLBSA +- 1mMGlut-amine | 48.1% | 8 |
| 2012 | UK | N/A | N/A | Frozen | Slaughter-house | M199 + -10% FBS | 5 μg/mL FSH/ LH,1 μg/mL E2 | 24 | 39 °C, 5% $CO_2$ | SOF + 2% SS | 2% SS | 2 | 18 | 76.6% | SOF + BSA | 59.2% | 9 |
| 2016 | Italy | Breeding | 30–40 days | Fresh | Slaughter-house | M199 + -10% ESS CeO2 NPs | 0.1 U/mL FSH/LH 44 mg/mL $CeO_2$ NP | 24 | 38.5 °C, 5% $CO_2$ | SOF + 2% ESS | 2% ESS | 1 | 22 h | 77.8% | SOF +- 0.4% BSA | 35.8% | 10 |
| 2012 | Iran | N/A | N/A | Fresh | Slaughter-house | M199 + -10%FBS | 0.1 IU/mL FSH | 22 | 39 °C 5% $CO_2$ | SOF + 20% SS | 20% ESS | 1 | 22 | 50% | SOF + BS-A. | 40% | 11 |
| 2016 | Iran | N/A | N/A | Frozen | Slaughter-house | M199 + -10% FBS | 0.05 U/mL FSH | 24 | 39 °C 5% $CO_2$ | SOFaaBSA +- 20% SS | 20% ESS + 1% heparn | 1 | 18 | 85% | SOF + BSA | 35.4% | 12 |
| 2012 | Spain | N/A | 3–6 m | Frozen | | | | 24 | 38.5 °C 5% $CO_2$ | SOF + 20% ESS | 20% ESS | 1 | 20 | N/A | N/A | 24.1% | 13 |

(*continued on next page*)