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In vitro embryo production in goats: Slaughterhouse and laparoscopic ovum pick up–derived oocytes have different kinetics and requirements regarding maturation media

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ABSTRACT

A total of 3427 goat oocytes were used in this study to identify possible differences during *in vitro* embryo production from slaughterhouse or laparoscopic ovum pick up (LOPU) oocytes. In experiment 1, one complex, one semi-defined, and one simplified IVM media were compared using slaughterhouse oocytes. In experiment 2, we checked the effect of oocyte origin (slaughterhouse or LOPU) on the kinetics of maturation (18 vs. 22 vs. 26 hours) when submitted to semi-defined or simplified media. In experiment 3, we determined the differences in embryo development between slaughterhouse and LOPU oocytes when submitted to both media and then to IVF or parthenogenetic activation (PA). Embryos from all groups were vitrified, and their viability evaluated *in vitro* after thawing. In experiment 1, no difference ($P > 0.05$) was detected among treatments for maturation rate (metaphase II [MII]; 88% on average), cleavage (72%), blastocyst from the initial number of cumulus oocyte complexes (46%) or from the cleaved ones (63%), hatching rate (69%), and the total number of blastomeres (187). In experiment 2, there was no difference of MII rate between slaughterhouse oocytes cultured for 18 or 22 hours, whereas the MII rate increased significantly ($P < 0.05$) between 18 and 22 hours for LOPU oocytes in the simplified medium. Moreover, slaughterhouse oocytes cultured in simplified medium matured significantly faster than LOPU oocytes at 18 and 22 hours ($P < 0.05$). In experiment 3, cleavage rate was significantly greater ($P < 0.001$) in all four groups of embryos produced by PA than IVF. Interestingly, PA reached similar rates for slaughterhouse oocytes cultured in both media, but improved ($P < 0.05$) the cleavage rate of LOPU oocytes. Slaughterhouse oocytes had acceptable cleavage rate after IVF (~67%), whereas LOPU oocytes displayed a lower one (~38%), in contrast to cleavage after PA. The percentage of blastocysts in relation to cleaved embryos was not affected by the origin of the oocytes ($P > 0.05$). Therefore, slaughterhouse oocytes developed a greater proportion of blastocysts than LOPU ones, expressed as the percentage of total cumulus oocyte complexes entering to IVM. Vitrified-thawed blastocysts presented similar survival and hatching rates between the oocyte origin, media, or method of activation. In conclusion, slaughterhouse and LOPU derived oocytes may have different IVM kinetics and require different IVM and IVF

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conditions. Although the IVM and IVF systems still need improvements to enhance embryo yield, the *in vitro* development step is able to generate good quality embryos from LOPU-derived oocytes.

1. Introduction

Goats are well adapted to many different environments, and they are very versatile as producers of food and raw matter such as milk, meat and skin. Efficient reproductive biotechnologies are essential to sustain worldwide production. Over the recent years, researchers have been trying to determine which conditions are needed during IVM, IVF, and *in vitro* development (IVD) processes to enhance embryo production to optimize the technique, allowing increasing its adoption in near future. Regarding maturation process in mammals, primary oocytes enter meiosis in perinatal life, progress to the diplotene stage of prophase I (germinal vesicle stage; GV), and remain arrested, eventually until shortly before the time of ovulation. Resumption of meiosis is mediated *in vivo* under the influence of hormonal stimuli and *in vitro* by releasing oocytes from the follicular meiotic inhibiting environment, and further culturing them in suitable conditions. However, it was well demonstrated that IVM oocytes are compromised in their developmental capacity compared with those matured *in vivo* [1,2]. The lower potential of IVM oocytes is probably related to the heterogeneity of the oocytes obtained, in terms of differentiation status, and to inappropriate conditions used in IVM [3]. This results in a relatively low rate of oocytes finally reaching the blastocyst stage, which is one of the main limitations of IVP from immature oocytes in mammals. Therefore, remains a challenge to enhance IVM rates to obtain good quality IVP embryos.

In most of the studies, the basic medium is supplemented with hormones and different concentrations of serum [3,4]. However, all complex supplements such as fetal calf serum (FCS), estrus goat and/or sheep serum or follicular fluid lead to a lack of reproducibility and risk of pathogen contamination. For these reasons, there is a trend to use defined or at least semi-defined maturation media, but this information for goat oocyte is still incipient. To make IVM simpler and more repeatable, we proposed a simplified maturation medium, tissue culture medium (TCM 199), supplemented with 10 ng/mL epidermal growth factor (EGF) and 100 μM cysteamine, and obtained good results in embryo development using slaughterhouse oocytes [5]. Currently, oocytes used for IVP are collected from slaughterhouse ovaries or by LOPU from live animals. Slaughterhouse ovaries provide a cheap source of large number of oocytes usually from unknown females, which are helpful for research and improvement of IVP conditions. However, the use of IVP for genetic improvement or diffusion requires collecting oocytes by repeated LOPU from given females with high economic or genetic merit. Therefore, both sources are equally important to be studied. Earlier trials performed in our laboratory suggest that oocyte requirements during maturation may differ according to their origin, i.e., slaughterhouse or LOPU derived and, thus, the same maturation media could have different efficiencies on both sources [6].

The time required for IVM varies among different species. Earlier studies reported that IVM of goat oocytes should last at least 27 hours [7] or even 32 hours [8], when comparing from 0 to 36 hours. The authors justified that this long time was related to the origin of oocyte (slaughterhouse ovaries that were not stimulated by gonadotropins). However, in the last decade, we have been using 22 to 24 hours of IVM for slaughterhouse goat oocytes with good results in terms of maturation rates and blastocyst production [3,5,9]. Interestingly, a recent study reported longer IVM for slaughterhouse oocytes (24–27 hours) than for LOPU (18–21 hours), probably because the latter were collected from stimulated goats, and already primed for maturation [10]. However, no studies were performed to evaluate the kinetics of maturation of goat LOPU oocytes or, even further, a direct comparison between the IVM kinetics of both sources of oocytes.

Besides the enhanced number of blastocysts at the end of the process, another challenge is to make sure that these embryos are of good quality. The best way to assess embryo quality or viability is to check their capacity of establishing pregnancy, and consequently give birth to normal offspring after transfer to synchronized recipient. However, embryo transfers being heavy and costly in domestic species, some other reliable indicators of embryo viability may be used: the evaluation of the level of expression of specific gene sets, the cell number and allocation (inner cell mass and trophectoderm), kinetic of development, and the resistance to cryopreservation [11]. Vitrification has proven to be as effective as slow cooling methods to cryopreserve mammalian embryos, it was tested in goats with good results [12,13], and can be used for goat blastocysts quality evaluation by postthawing *in vitro* survival score.

Most studies carried out to identify the factors influencing IVM, and subsequent embryo development of goat oocytes were performed using slaughterhouse ovaries. Therefore the aims of this study were to examine the (1) effect of IVM medium composition on maturation rate, fertilization, and embryo development for slaughterhouse oocytes; (2) IVM kinetics of slaughterhouse and LOPU-derived oocytes when submitted to different maturation media; and (3) the developmental competence of slaughterhouse and LOPU-derived oocytes when submitted to different maturation media, and submitted to IVF or parthenogenetic activation (PA).

2. Material and methods

Except otherwise indicated, chemicals were purchased from Sigma Chemical Co. (Saint Louis, MO, USA).

All the experiments were conducted at the Experimental Unit Unité expérimentale de Physiologie Animale de l'Orfrasière in Nouzilly (France, latitude 47°22'N, longitude