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## Original research article

# Differential influence of ampullary and isthmic derived epithelial cells on zona pellucida hardening and *in vitro* fertilization in ovine



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## ABSTRACT

The central role of the oviduct, as the site of zona pellucida (ZP) maturation, fertilization and early embryogenesis, has been recognized. The objective of this study was to investigate whether ampullary and isthmic derived epithelial cells have different effects on *in vitro* ZP hardening, *in vitro* fertilization (IVF) and *in vitro* culture (IVC) of the resulting embryos. Cumulus oocyte complexes (COCs) were matured in a coculture system with ampullary/isthmic epithelial cells, TCM199 supplemented with insulin-like growth factor I (IGF-I) and epithelial derived growth factor (EGF) (GF treated group), conditioned media produced using ampullary (ACM), isthmic (ICM), COCs + ampullary, and COCs + isthmic epithelial cells, contactless culture system, oviductal fluid, GF + ACM/ICM, and drops of TCM199 (control), for 24 h. The matured oocytes were randomly divided into two groups: Group I was subjected to ZP digestion; Group II underwent IVF. The duration of the ZP digestion, in a coculture system with ampullary epithelial cells (AE) was significantly increased ( $p < 0.05$ ), compared with other groups. Penetrated oocytes and monospermic fertilization were significantly increased ( $p < 0.05$ ) in the AE group. The mean number of spermatozoa per penetrated oocyte was reduced dramatically for the AE group ( $p < 0.05$ ). A significant increase ( $p < 0.05$ ) in the embryo development was observed in all treated groups, compared to the control. Results revealed that epithelial cells harvested from the ampullary segment of the oviduct had *in vitro* specialized role in ZP hardening and have subsequent IVF and IVC outcomes.

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## 1. Introduction

For several years, it has been proposed that the oviduct served as a simple transporter for gametes. Oviducts consist of highly specialized regions. Three major segments of the oviduct, the infundibulum, ampulla and isthmus, are known. *In vivo*, each of the mentioned regions has its specialized and critical role in gametes physiology, fertilization and early embryo development. These critical roles are never bypassed *in vivo*. If one region of the oviduct does not work properly, a degree of infertility would be a consequence of the malfunction. In recent years, assisted reproductive techniques (ART) have been developed to overcome infertility problems. The use of ART in infertility treatment leads to the elimination of the critical functions of the reproductive organs. One of the victims of ART is the oviduct. *In vitro* maturation (IVM) and *in vitro* fertilization (IVF), which have been the basic procedures in ART, have led to the neglect of the oviduct as a crucial part of the reproductive system. In the last two decades, several studies have provided evidence of a dialog between gametes/early embryos and oviductal epithelial cells [1-3]. The oviduct is the natural site of zona pellucida (ZP) maturation, fertilization and early embryo development [4]. The period when the immature oocytes are cultured *in vitro* is mainly the time when they are released from the dominant follicle and should be in the ampullary region of the oviduct, *in vivo*. At this time, the ZP of the oocytes will undergo oviductal zona maturation, called "ZP hardening". The ZP hardening takes place in two steps. The two steps of the ZP maturation occur, respectively, at the dominant follicle and oviductal ampullary segment [4-8]. It is well known that the oviduct provides the best microenvironment for gametes and embryo development. Therefore, several groups used oviductal cells as the helper cells for coculture in assisted reproduction [9]. The majority of the recent studies have focused on coculture of early embryos. Their results showed major improvements in the rate of blastulation, hatching and the number of cells per blastocyst [9-11]. The possible *in vitro* specialized functions of ampullary and isthmic regions, with the main emphasis on oocyte maturation and ZP hardening, as an important factor in the prevention of polyspermy, have not been studied.

Studies in rats and golden hamsters showed that the oviductal fluid (OF) compositions changed during the estrous cycle [11,12]. On the other hand, electron probe analysis revealed that there are differences in element composition between ampullary and isthmic fluid regardless of the stage of the estrous cycle [13]. In addition, histological studies have shown regional variations in ultrastructural features of the secretory cells in the oviductal epithelium. Regional variations have been found in the number of putative secretory granules in the oviductal secretory cells. Histochemical and immunocytochemical studies have also demonstrated regional differences in the localization of various minerals and proteins in the oviductal epithelium [14].

Abnormal fertilization is a drawback of ART. One kind of abnormal fertilization is known as polyspermy. For many years, it has been proposed that ZP hardening is a post-fertilization event, contributing to polyspermy blocking. In light of recent studies, it has been clarified that ZP hardening is

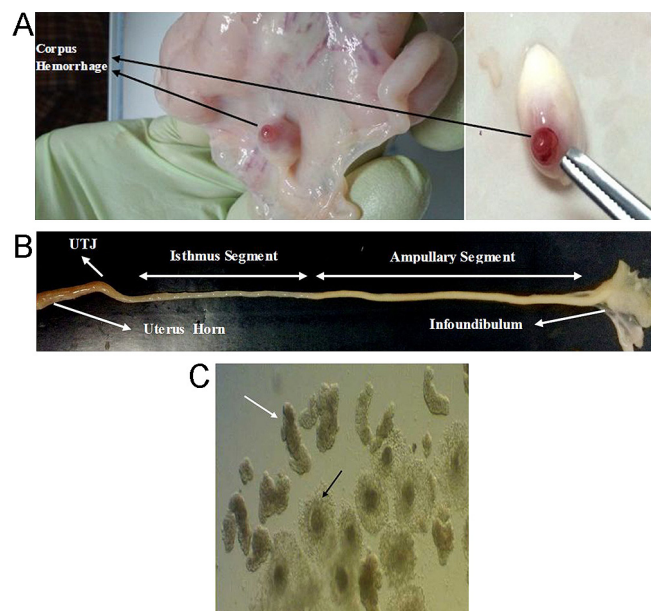
one of the important elements in the prevention of polyspermy. When the current *in vitro* culture systems are applied for *in vitro* embryo production (IVEP), the final step of ZP hardening is skipped and polyspermy becomes unavoidable.

The present study was designed to investigate if coculture of immature cumulus oocyte complexes (COCs) with epithelial cells harvested from different segments of an ovine oviduct can influence ZP hardening; and furthermore, whether this culture system at IVM has different effects on subsequent IVF and *in vitro* culture of embryos (IVC).

## 2. Materials and methods

### 2.1. Preparation of ovine oviduct epithelial cells

The ovine oviduct epithelial cells (OOECs) were obtained as described by Rottmayer et al. [15], with certain modifications. The reproductive tract was recovered after animal slaughter. Oviducts connected to ovaries with corpus hemorrhage were selected (Fig. 1A) for harvesting OOECs. These ovaries had ovulated in the last 24 h. Therefore, these OOECs had been



**Fig. 1 - (A) Ovary with corpus hemorrhage; oviducts connected to ovaries with corpus hemorrhage were selected for harvesting epithelial cells. (B) Different segments of ovine oviduct; identification of oviducts' segments was performed based on anatomical differences and histological evaluations. One-third of the oviduct length belonged to the isthmic region and the remaining two-thirds of the oviduct belonged to the ampullary segment. As can be seen, the isthmic region is the narrowest part of the oviduct. In addition, it is brighter than the ampullary segment. Changes in the diameter of the oviduct are the key points for the determination of each segment of the oviducts. The oviduct segments with their specific features are indicated by arrows. (C) The preferred size of the oviductal epithelial cells (white arrow) and typical cumulus oocyte complexes (black arrow) (40× magnifications).**

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