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ARTICLE

Spatial and temporal distribution of Oct-4 and acetylated H4K5 in rabbit embryos

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Abstract Rabbit is a unique species to study human embryology; however, there are limited reports on the key transcription factors and epigenetic events of rabbit embryos. This study examined the Oct-4 and acetylated H4K5 (H4K5ac) patterns in rabbit embryos using immunochemistry staining. The average intensity of the Oct-4 signal in the nuclei of the whole embryo spiked upon fertilization, then decreased until the 8-cell stage and increased afterwards until the compact morula (CM) stage. It decreased thereafter from the CM stage to the early blastocyst (EB) stage, with a minimum at the expanded blastocyst (EXPB) stage and came back to a level similar to that of the CM-stage embryos in the hatching blastocysts (HB). The Oct-4 signal was observed in both the inner cell mass (ICM) and the trophectoderm (TE) cells of blastocysts. The average H4K5ac signal intensity of the whole embryo increased upon fertilization, started to decrease at the 4-cell stage, reached a minimum at the 8-cell stage, increased again at the EXPB stage and peaked at the HB stage. While TE cells maintained similar levels of H4K5ac throughout the blastocyst stages, ICM cells of HB showed higher levels of H4K5ac than those of EB and EXPB.

KEYWORDS: H4K5 acetylation, inner cell mass, Oct-4, preimplantation, rabbit, trophectoderm

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Introduction

A series of genetic and epigenetic events define early embryo development. Disturbances in these highly co-ordinated processes are believed to contribute to developmental failures and defects in mammals (Corry et al., 2009; Jaenisch and Bird, 2003; Palini et al., 2011). For example, cell lineage formation at the blastocyst stage is regulated by the POU-domain transcription factor Oct-4 (also known as Pou5f1) (Ovitt and Scholer, 1998; Pesce and Scholer, 2001). When *Oct-4* was mutated, mouse embryos failed to establish an inner cell mass (ICM) and eventually died prematurely (Boiani and Scholer, 2005; Nichols et al., 1998). Therefore understanding key genetic and epigenetic events during early embryo development will help to identify the factors contributing to embryo losses and consequently improve embryo survival rates in human and other mammalian species (Maher et al., 2003; Mamo et al., 2008). It is reported that about 15—50% of mammalian embryos die during the preimplantation period (Mamo et al., 2008; Warner et al., 1998).

The majority of studies on early embryo development use mouse models; however, mouse embryos are not always representative of the earliest stages of mammalian development (Berg et al., 2011; Winston and Johnson, 1992). For example, the restrictive expression of *Oct-4* in the ICM, but not in the trophectoderm (TE), appears to be unique in the mouse (Dietrich and Hiiragi, 2007; Ovitt and Scholer, 1998; Palmieri et al., 1994; Pesce et al., 1998; Yeom et al., 1996). In human, cattle, pig and rabbit embryos, *Oct-4* expression was present in both ICM and TE cells even until the expanded blastocyst stage (Berg et al., 2011; Hansis et al., 2000; Kirchhof et al., 2000). It was suggested that the regulatory circuitry determining ICM/TE identity has been rewired in the mouse, to allow rapid TE differentiation and early blastocyst implantation (Berg et al., 2011). Alternative animal models are needed for better understanding of human embryology and stem cell biology.

The rabbit is a classic agricultural species and a useful model animal for biomedical research (Fan and Watanabe, 2003). Rabbits are genetically and physiologically closer to humans than mice. In comparison with larger animals, such as pigs and monkeys, rabbits can be housed indoor, have a short gestation and produce multiple-offspring litter. These advantages make rabbit a unique species for the study of human physiology. As a preferred laboratory species for many human disease studies such as atherosclerosis, it is also a pioneer species in the development of several embryo biotechnologies, such as IVF, transgenesis, animal cloning, embryo cryopreservation and embryonic stem cells (ESC) (Fan and Watanabe, 2003; Lin et al., 2011).

There are limited studies on key transcription factors and epigenetic programming events in preimplantation-stage rabbit embryos. Using quantitative real-time PCR, the gene for Oct-4, one of the few transcription factors studied in rabbit embryos, was found abundantly expressed in oocytes and zygotes, then gradually reduced until the activation of the embryonic genome and thereafter continuously increased until the blastocyst stage (Kobolak et al., 2009; Mamo et al., 2008). *Oct-4* mRNA was found in both the ICM and the TE, a pattern similar to that of the human embryos. Studies on epigenetic events during early rabbit embryo development are mostly based on nuclear transfer experiments. Immunostaining results showed that the acetylation patterns of histones H3K14, H4K12 and H4K5 were different between fertilized and cloned embryos. When cloned embryos were treated with trichostatin A, a histone deacetylation inhibitor, they displayed an acetylation pattern of H3K14, H4K12 and H4K5 more similar to that of normally fertilized embryos than those not treated with trichostatin A, suggesting that increased levels of global acetylation in cloned embryos may improve genetic reprogramming and consequently embryo development competence in rabbits (Shi et al., 2008).

As far as is known, the distribution pattern of Oct-4 has not been thoroughly examined in preimplantation-stage rabbit embryos at the protein level. Previous studies have only examined at the mRNA expression level (Kobolak et al., 2009; Mamo et al., 2008). The acetylated H4K5 (H4K5ac) pattern in different cell lineages (i.e. ICM and TE cells) in rabbit blastocysts is not available yet. H4K5ac is a good indicator for the global activation of genes (Grunstein, 1997; Rundlett et al., 1998; Shi et al., 2008), as the acetylation of histone H4 occurs initially at lysine 16 (H4K16), then at K8 or K12, and ultimately at K5 (Corry et al., 2009).

The present study used the immunochemistry approach to examine the spatial and temporal profiles of Oct-4 and H4K5ac in rabbit embryos at different developmental stages from zygotes to hatching blastocysts. This study also compared the patterns of these two important biomarkers in ICM and TE cells in blastocyst-stage embryos.

Materials and methods

All chemicals were purchased from Sigma Chemical Co (St. Louis, MO, USA), unless otherwise indicated.

Animal maintenance and hormone administration

All animal maintenance, care and use procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the National Taiwan University and the University Committee on the Use and Care of Animals of the University of Michigan. Sexually mature (6—18 months old) New Zealand White female rabbits were maintained under a 12/12 light/dark cycle and superovulated with hormones using a routine regime (Du et al., 2009), consisting of two 0.3-mg, two 0.4-mg and two 0.6-mg injections of FSH (folltropin-V; Bioniche Animal Health Canada, Belleville, Ontario, Canada) at intervals of 12 h, followed by 200 IU of human chorionic gonadotrophin (HCG; Chorulon; Intervet, Millsboro, DE, USA). Superovulated rabbits were either mated with fertile males and served as embryo donors or not mated and served as oocyte donors.

Embryo collection and culture

Embryo collection and culture were performed as described previously (Lin et al., 2011). Briefly, Dulbecco's phosphate-buffered saline (DPBS; 15240-013; Gibco, Grand Island, NY, USA) containing 0.1% polyvinyl alcohol (P-8136) was used for flushing embryos from oviducts (PBS-PVP). Medium 199 with Earle's Salts, l-glutamine, 2.2 g/l sodium