Reduced fertility with impairment of early-stage embryos observed in mice lacking *Lgr4* in epithelial tissues

Lgr4 is one of the genes identified as novel G protein–coupled receptor genes designated *Lgr4–Lgr8*, with high homology with FSH receptor, LH receptor, and TSH receptor genes, but studies of *Lgr4*-mutant mice have suggested that Lgr4 has essential functions in development. This is the first report describing the relationship between the functions of Lgr4 and female reproductive systems. (Fertil Steril® 2010;94:2878–81. ©2010 by American Society for Reproductive Medicine.)

Key Words: *Lgr4, K5-Cre*–transgenic mice, reproductive tract

Ovulation and fertilization are key processes in female reproduction, and they are highly regulated by transforming growth factor β, activins, bone morphogenetic proteins, FSH, LH, steroid hormones, and their receptors. These growth factors and hormones, acting through their receptors, induce a number of preovulatory processes, including follicular development, oocyte maturation, cumulus expansion, and rupture of the antral follicles (1–4). The ovulated oocytes move to the oviduct, which supplies the critical environment for interaction between oocytes and sperm leading to successful fertilization.

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Lgr4 (leucine-rich repeat–containing G protein–coupled receptor 4) is one of the genes identified as novel G protein–coupled receptor genes designated *Lgr4–Lgr8*, from an expressed sequence tag database with high homology with glycoprotein hormone receptors including FSH receptor (FSHR), LH receptor (LHR), and TSH receptor (TSHR) genes (5, 6). Because *Lgr4* showed high homology with FSHR, LHR and THSR, it was first thought to be involved in reproductive systems. However, several studies of *Lgr4*-mutant mice suggested that Lgr4 had essential and critical functions in embryonic development (7–10). *Lgr4*-knockout mice had small kidneys, in which the total number and density of the glomeruli were decreased (8). The conditional knockout mice generated by crossing with the *K5-Cre*–transgenic line suggested that *Lgr4* was a new gene class regulating keratinocyte migration or the formation of hair follicle placodes (9, 10). Additionally, Mendive et al. (11) and Hoshii et al. (12) reported that *Lgr4* gene-trap lines exhibited circumvention of lethality and defective male reproductive tract during the postnatal period, in contrast to the embryonic/neonatal lethality seen in our *Lgr4*-knockout mice on a 129Ola × C57BL/6 hybrid background (8). Recently, it was reported that Lgr4 was involved in the development of the male epididymis and efferent ducts through regulation of estrogen receptor α and androgen receptor expressions via the cyclic adenosine monophosphate (AMP)/protein kinase A signaling pathway (13). Furthermore, disruption of the Lgr4 function could lead to an impairment in eye development through down-regulation of Pitx2, a key transcription factor in anterior segment development (14). Also, *Lgr4* is expressed in liver and bone at embryonic stages, and its down-regulation impairs definitive erythropoiesis at midgestation through the suppression of c-Myc, cyclin D1, and ATF4 pathways, showing a dramatic delay in osteoblast differentiation and mineralization (15, 16). Although possible functions of Lgr4 in male reproductive systems have been suggested by others, so far there has been no suggestion that Lgr4 functions in the female reproductive tract.

We tested the Cre-activity of a *K5-Cre*–transgenic line (17) by crossing the transgenic mice with R26R conditional reporter mice (18), and the induction of β-galactosidase was observed in oviduct and uterus ($K5\text{-}Cre^{\pm};R26R^{\pm}$; data not shown). On the

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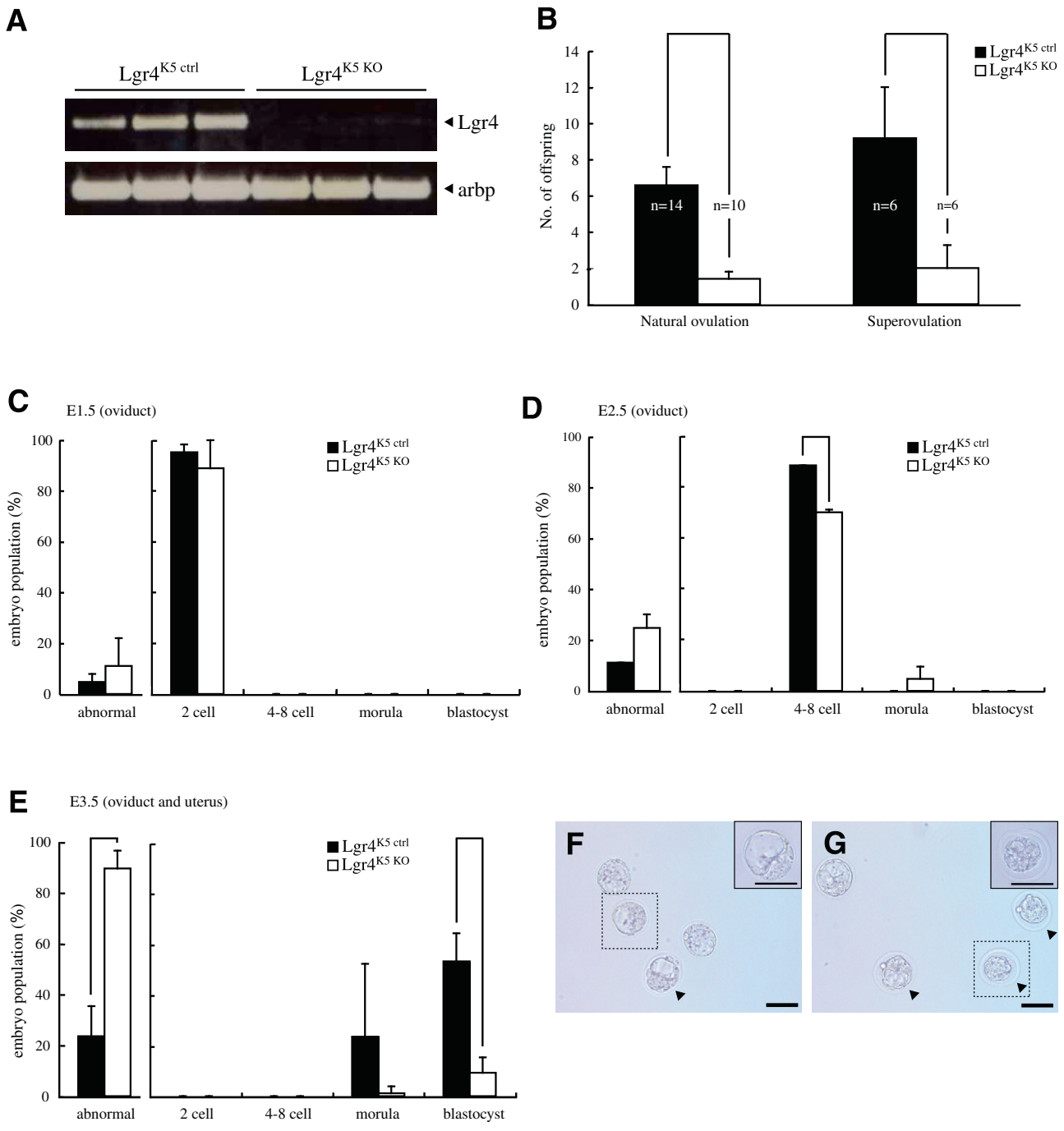
FIGURE 1



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other hand, the induction was not observed in ovaries of the same mice or in any tissues of negative control mice (data not shown). We next performed polymerase chain reaction (PCR) analysis on genomic DNA to confirm the tissue-specific deletion of floxed *Lgr4* by the Cre-activity. The genomic DNAs prepared from *K5-Cre*$^{-/-}$;*Lgr4*$^{+/fx}$ and *K5-Cre*$^{\pm}$;*Lgr4*$^{+/fx}$ mice oviducts were subjected to PCR analysis. *K5-Cre* was activated in the oviduct, accounting for the presence of the knockout allele in the oviduct (data not shown). We performed similar PCR detection using genomic DNA prepared from the ovary and did not detect the knockout allele (data not shown). It was reported that the expression of *Lgr4* appeared in ovarian granulosa cells, epithelial cells of oviduct and smooth muscle cells of the uterus (19). This information and our observation implied the coexpression of *K5-Cre* and