

## Expression Pattern Implicates a Potential Role for Luman Recruitment Factor in the Process of Implantation in Uteri and Development of Preimplantation Embryos in Mice

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**Abstract.** Luman/CREB3 recruitment factor (LRF or CREBRF) was identified as a regulator of Luman (or CREB3) that is involved in the unfolded protein response during endoplasmic reticulum stress. Luman is implicated in a multitude of functions ranging from viral infection and immunity to cancer. The biological function of LRF, however, is unknown. In this paper, we report that uteri of pregnant mice and embryos displayed enhanced LRF expression at all stages, and the expressed LRF was found to be localized specifically at implantation sites. On the other hand, uteri of mice induced for delayed implantation or pseudopregnant mice showed low levels of LRF expression, suggesting that LRF mediates uterine receptivity during implantation. Further, expression of LRF was found to be modulated by steroid hormones such as progesterone and estradiol. This study thereby identifies a potential role for LRF in the process of implantation in uteri and development of preimplantation embryos in mice.

**Key words:** Estradiol, Implantation, Luman recruitment factor LRF/CREBRF, Progesterone, Uteri

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Implantation is a complex and crucial process in which where the uterus is made receptive for the attachment of the blastocyst (embryo). The prerequisites for implantation are development and differentiation of the embryo into the blastocyst stage. The differentiated blastocyst consists of 2 types of cells called the trophoblast and inner cell mass [1, 2]. Trophoblast cells are responsible for establishing contact with the uterine epithelium. Once materno-zygotic contact is established, the trophoblast cells invade into the luminal epithelium [3]. Numerous factors, such as hormones, growth factors, cytokines, vasoactive agents, etc., have been identified, which appear to play an essential role during implantation [4, 5]. The importance of implantation in reproduction is exemplified by the fact that loss of function of genes such as HOX [6] and COX2 [7], which are essential for implantation, leads to infertility.

Luman/CREB3 (also called LZIP) is a multifunctional protein that was initially identified as an interacting protein of herpes simplex virus (HSV)-related host cell factor 1 (HCF1) [8, 9]. It is an endoplasmic reticulum (ER)-associated transmembrane protein that is released from the ER by proteolytic cleavage during the unfolded protein response in response to ER stress [10], leading to the activation

of downstream stress response-related genes [11, 12]. In addition to HSV latency and reactivation [13], CREB3 is also implicated in human immunodeficiency virus (HIV) gene regulation [14], leukocyte migration and function [15–17], dendritic cell maturation [18], glucocorticoid receptor signaling [19] and cancer [20, 21].

Luman recruitment factor (LRF/CREBRF) was identified as a regulator of Luman that targets Luman to LRF nuclear bodies, and renders it inactive by the exclusion of factors such as HCF1 [22]. Binding of LRF to Luman also promotes proteasomal degradation of the latter [22]. Besides its cellular function, the biological role of LRF is largely unknown. Recently, we found a severe maternal behavioral defect in female LRF gene knockout mice accompanied by misregulation of glucocorticoid and prolactin signaling [23]. Our preliminary data also suggest a fertility deficit in these mutant mice (unpublished), suggesting a potential role for LRF in reproduction and related hormonal signaling. The present study was undertaken to investigate if there exists a role for LRF in embryonic implantation. To address this question, uteri isolated from pregnant mice at different stages, were analyzed for LRF expression both at the transcript and protein level. Here we report that the levels of LRF during implantation are regulated by progesterone and estradiol. The expression pattern suggests an important role of LRF in implantation and decidualization.

### Materials and Methods

#### Animals

Mature female Chinese Kunming White mice were obtained from

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the Experimental Animal Center of Xi'an JiaoTong University. The mice were housed 5 per cage in a temperature and humidity controlled environment with a 12-h light-dark cycle and fed standard diet. Mice had access to food and water *ad libitum*, and all procedures were approved by the Animal Care and Use Committee at Northwest A&F University. For every experiment, a minimum of 10 embryos for each stage were analyzed.

#### *Preparation of uteri from pregnant and pseudopregnant mice*

To examine implantation, female mice were mated with fertile or vasectomized males of the same strain to induce natural pregnancy or pseudopregnancy respectively. The day of observation of a vaginal plug was considered to be day 1 of pregnancy or pseudopregnancy. Pregnancy was further ascertained by flushing the embryos from the oviduct and uterus. Embryo implantation sites were visualized on day 5 of pregnancy by intravenous injection of 0.3 ml of 1% Trypan Blue in saline, 10 min prior to sacrificing the mice. The uteri of pregnant and pseudopregnant mice were collected at 0900 h during days 1 through 6. The left uteri were fixed in 4% paraformaldehyde for immunohistochemical analyses, while the right uteri were stored in liquid nitrogen for RNA extraction.

#### *Induction of delayed implantation*

Delayed implantation was induced in mice by the method described previously [24, 25]. In brief, anesthetized pregnant mice were ovariectomized under sterile conditions at 0900 h on day 4 of pregnancy. Delayed implantation was maintained from days 5 through 7 by subcutaneous injections of progesterone (1 mg/day/mouse, Sigma) daily. Delay of implantation was confirmed by flushing the embryos from one horn of the uterus. The uteri collected from progesterone-treated mice were considered to be delayed implantation uteri. In order to initiate implantation in these mice, estradiol-17 $\beta$  (25 ng/mouse, Sigma) was subcutaneously injected on day 7 of pregnancy. Uteri collected 24-h after estradiol treatment were considered to be implantation activated uteri.

#### *Induction of decidualization*

Decidualization was induced by infusing 100  $\mu$ l sesame oil in one horn of the uterus on day 4 of pseudopregnancy. The contralateral, uninjected horn was used as a control. Mice were sacrificed on day 8 of pseudopregnancy, and the weights of the oil-infused and control uterine horns were used to assess the extent of decidualization [26].

#### *Steroid hormone treatments*

Female mice were ovariectomized two weeks prior to hormone treatments. The ovariectomized mice were given a hypodermic injection of either estradiol (100 ng/mouse), progesterone (1 mg/mouse) or a combination of both. Control mice received 0.1 ml sesame oil/mouse. Uteri were collected from mice, which were sacrificed 24 h post hormone treatment.

#### *Isolation of preimplantation embryos*

Female mice were treated with pregnant mare serum gonadotrophin (PMSG, Ningbo Sansheng Pharmaceutical, China) between 1500 h and 1700 h. After 48 h, the mice were administered human chorionic gonadotrophin (hCG, Ningbo Sansheng Pharmaceutical, China) to

induce superovulation. Treated mice were mated with fertile males of the same strain, and the result of fertilization (pregnancy) was determined by vaginal plug. Mice were then sacrificed by cervical dislocation at different time points to collect embryos of different stages: Zygotes and 2-cell and 4-cell embryos were collected from oviducts at 21–24 h, 43–44 h and 58–60 h respectively. However, the 8-cell stage embryo, morula and blastula were collected from the uterine horns at 64–66 h, 78–80 h and 96–100 h respectively.

#### *qRT-PCR (quantitative real-time polymerase chain reaction)*

Total RNA was extracted using TRIzol reagent (Takara, Dalian, China) and cDNA synthesis was carried out using PrimeScript<sup>TM</sup> RT reagent kit (Takara) according to the manufacturer's instructions. qRT-PCR was carried out using 2 $\times$  SYBR<sup>®</sup> Premix Ex Taq<sup>TM</sup> II and primers for mouse LRF (5'-TCTTCTCGGAAGAGAGGGAA-3' forward; 5'-CAGAAGGCCTCAGAAATCCTC-3', reverse). Each PCR reaction was performed in a 20.0- $\mu$ l reaction mixture containing 10.0  $\mu$ l of 2 $\times$  SYBR<sup>®</sup> Premix Ex Taq<sup>TM</sup> II, 2.0  $\mu$ l cDNA (equivalent of 20 ng total RNA) as template, 0.8  $\mu$ l of each primer at 10  $\mu$ M and 6.4  $\mu$ l of nuclease-free water. PCR cycling conditions comprised one cycle at 95 C for 30 sec, followed by 40 cycles at 95 C for 5 sec and at 60 C for 30 sec. Experiments were performed in triplicate for each data point, and the mean of all these values was used for the final analysis. Samples were run on Bio-Rad iQ5, and the data were analyzed using the Bio-Rad Optical System Software (Bio-Rad). Amplification of the mouse Rplpo gene was used for normalizing the data.

#### *Immunohistochemistry*

Immunohistochemical analysis of paraformaldehyde-fixed uterine sections was performed using a Histostain-Plus kit (Beijing 4A Biotech, China), according to the manufacturer's instructions. Briefly, the paraffin-embedded tissues were sliced into 6  $\mu$ m sections, which were treated with 0.3% H<sub>2</sub>O<sub>2</sub>-Methanol for 10 min to block endogenous peroxidase activity, and washed in 0.01 M PBS 3 times. Nonspecific binding was blocked in 10% normal goat serum in PBS for 1 h. Sections were incubated with anti-LRF antibody (2.0  $\mu$ g/ml) at 37 C (60 min) and washed in 0.01 M PBS 3 times, followed by incubation (30 min) with biotinylated goat anti-rabbit secondary antibody at 37 C. The slides were rinsed, incubated in streptavidin-horseradish peroxidase for 10 min at 37 C, rinsed again and then incubated (5–10 min) in 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen as the substrate. After a final rinse with ddH<sub>2</sub>O, the sections were counterstained with hematoxylin, ethanol dehydrated and mounted using neutral balsam. In the negative control sections, PBS was substituted for the primary antibody.

#### *Immunofluorescence*

Immunofluorescence analysis of mouse preimplantation embryos was carried out using a kit (Boster, Wuhan, China), according to the manufacturer's instructions. Briefly, embryos were washed in 0.01 M PBS (3  $\times$  3 min) and blocked in 5% normal goat serum (30 min). Embryos were then incubated with anti-LRF antibody (2.0  $\mu$ g/ml) at 37 C (60 min) and washed in 0.01 M PBS (3  $\times$  3 min), followed by incubation (30 min) with biotinylated goat anti-rabbit secondary antibody at 37 C. After washing in 0.01 M PBS (3  $\times$  3

min), SABC-FITC (1:100, Green) was added to the embryos, which were then incubated at 37 C for 30 min and washed in 0.01 M PBS (3 × 3 min). The nuclei were stained with DAPI (4',6-diamidino-2-phenylindole dihydrochloride) and washed in 0.01 M PBS (3 × 3 min) before mounting onto a slide. Quantitation of the data was performed using the ImageJ software.

#### Statistical analysis

All experiments were replicated at least three times for each group. Results are presented as means ± SEM. Data were analyzed with ANOVA, followed by Fisher's least significant difference test (Fisher LSD) using SPSS software (Version 13.0; SPSS, Chicago, IL, USA). Differences were considered significant for  $P < 0.05$ . For experiments involving embryos, a minimum of 10 embryos for each stage were analyzed, and the Student-Neuman-Keuls test was used as a post hoc test for multiple comparison.

## Results

#### *Implantation in mice is associated with expression of LRF*

Our preliminary observations indicate that female LRF gene knockout mice [23] have a severe fertility deficit (unpublished), suggesting a potential role for LRF in reproduction. To investigate the potential role of LRF, we examined the expression of LRF in the process of implantation. For this purpose, real-time PCR analyses were carried out in uteri isolated from pregnant and pseudopregnant mice. The results indicate that LRF transcripts were significantly higher in the uteri of pregnant mice at all stages from days 1 through 7 (Fig. 1a, black bars) when compared with those in uteri from pseudopregnant mice (Fig. 1a, white bars). Although the levels of LRF transcripts were found to be elevated at all stages of pregnant mice uteri, there was a specific increase at day 5 and day 7, which correspond to the time at which implantation occurs in mice. To confirm the above observation, immunohistochemical studies were carried out in the uteri of pregnant and pseudopregnant mice at days 1 through 7 (Fig. 2, panels a–h). The levels of LRF protein expression were elevated significantly in pregnant uteri at the sites of implantation compared with interimplantation sites without embryonic implantation on day 5 (Fig. 2, panel e vs. panel f), and LRF protein expression was found to be primarily localized in the luminal epithelium, glandular epithelium and stromal cells. These results therefore suggest a definitive role for LRF in the implantation process.

#### *Delayed implantation is associated with low levels of LRF*

To further test the hypothesis that LRF plays an important role in implantation, mice that were treated to induce delayed implantation were analyzed for the expression of LRF. RT-PCR analyses of LRF transcripts were carried out in uteri treated to induce delayed implantation, and the results were compared with that of implantation activated uterus. We found a significantly higher level of LRF transcripts in implantation activated uteri ( $P < 0.01$ ) as opposed to delayed implantation uteri (Fig. 1b). Similarly, immunohistochemical analyses revealed a significantly higher expression of LRF in the implantation activated uteri (Fig. 2, panel j) than in uteri treated to induce delayed implantation (Fig. 2, panel i). Consistent with our above observation in the RT-PCR analysis, LRF protein was

found to be specifically localized at the implantation sites and was extensively detected in the stromal cells of implantation activated uteri (Fig. 2, panel j) but weakly detected in the stromal cells of delayed implantation uteri (Fig. 2, panel i). These results therefore suggest that delayed implantation may be a consequence of decreased LRF expression.

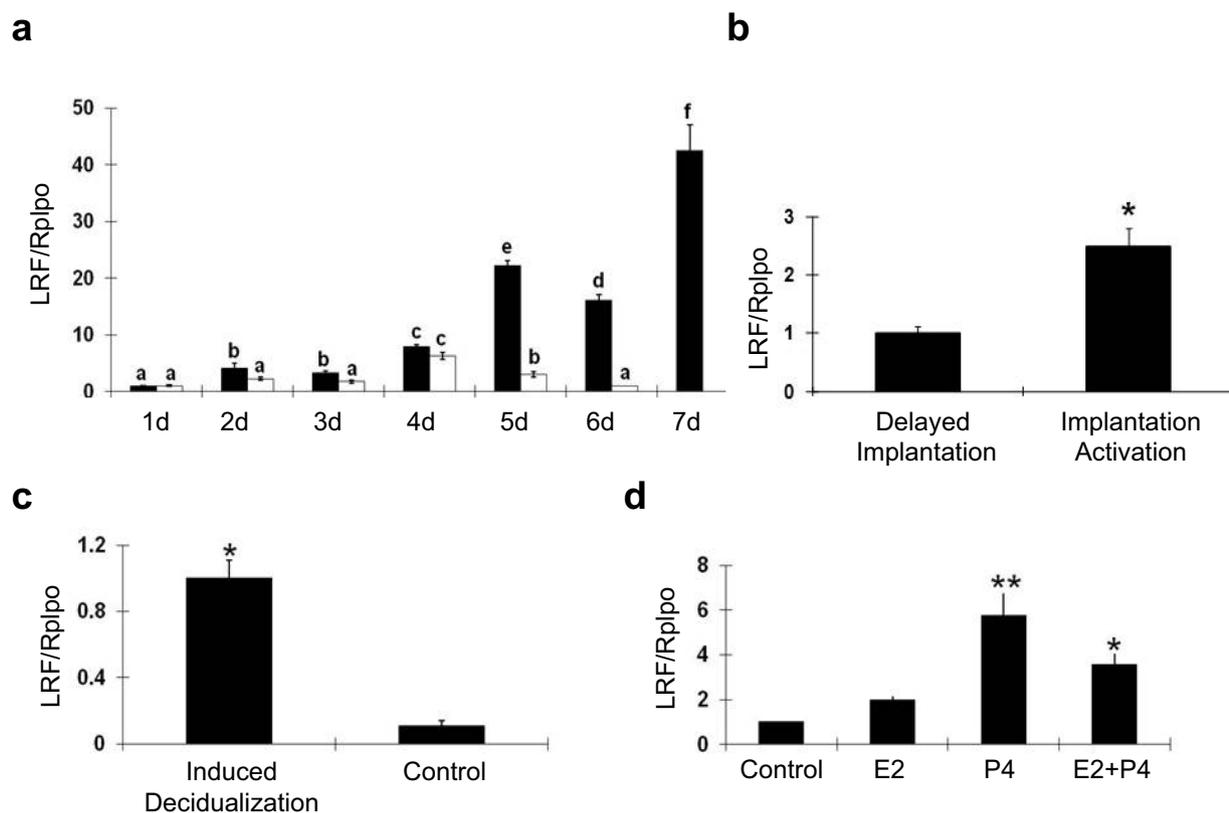
#### *Decidualization enhances the expression of LRF in the mouse uterus*

Specific localization of LRF protein in decidual cells implicates a possible role for LRF in the process of decidualization. To confirm this, RT-PCR and immunohistochemical analyses were carried out in induced decidual and non-decidual uteri. Although, LRF transcripts were detected both in the induced decidual and non-decidual uteri, the levels of LRF transcripts were found to be significantly higher in the decidual uteri ( $P < 0.01$ ; Fig. 1c). Similarly, LRF protein was highly expressed in the mass of decidual cells in the induced decidual uteri (Fig. 2, panel k) compared with the luminal and glandular epithelium of non-decidual uteri (Fig. 2, panel l). Further, the expression of LRF protein was found to be significantly higher in the primary decidual zone (PDZ) on day 6 (Fig. 2, panel g) and secondary decidual zone (SDZ) on day 7 (Fig. 2, panel h) of pregnancy. These results emphasize the importance of LRF in the process of decidualization.

#### *Estrogen and progesterone modulate the expression of LRF*

Proper levels of steroid hormones, such as estrogen and progesterone, are essential for the implantation process. As the results here implicate a crucial role for LRF in implantation, analyses of LRF expression were carried out in the presence of these hormones to check if steroid hormones can modulate LRF function. For this purpose, uteri of mice administered either estradiol ( $E_2$ ), progesterone ( $P_4$ ) or a combination of both  $E_2$  and  $P_4$ , and were subjected to RT-PCR analysis. Control mice were administered 0.1 ml of sesame oil per mouse.

Treatment with the steroid hormones independently or in combination resulted in an increase in the levels of LRF transcripts compared with controls. However, comparison among the treated groups indicated that the maximal induction in LRF expression was observed in mice treated with progesterone alone. Interestingly, estradiol was found to mitigate the LRF expression induced by progesterone (Fig. 1d). We speculated that overstimulation of molecules upstream of LRF may have triggered a negative feedback control mechanism. These results are consistent with the observations in pregnant mice, in which the maximum expression of LRF was concomitant with an elevation in progesterone levels and a decrease in estrogen levels. Immunohistochemical analyses revealed that the LRF protein levels were significantly elevated in the luminal epithelium and glandular epithelium of uteri obtained from mice treated with  $E_2$  (Fig. 2, panel n),  $P_4$  (Fig. 2, panel o) and  $E_2$  and  $P_4$  (Fig. 2, panel p), compared with the controls (Fig. 2, panel m). Consistent with the RT-PCR analyses, progesterone induced maximal induction of LRF in stromal cells, and estradiol mitigated the LRF expression induced by progesterone. These results therefore suggest that progesterone plays a very important role in implantation by regulating the levels of LRF protein.



**Fig. 1.** a: Uteri isolated from pregnant and pseudopregnant mice at different stages were analyzed for LRF transcripts using qRT-PCR as described in Materials and Methods. The figure represents the quantification of LRF transcripts, with the black and white bars corresponding to uteri isolated from pregnant and pseudopregnant mice, respectively. Different letters indicate significant differences in P value with  $P < 0.05$ . b: Comparative analysis of LRF transcripts by qRT-PCR, in uteri isolated from mice treated to induce delayed implantation and implantation activated mice. The figure represents the quantification of LRF transcripts. \*Indicates significant difference in P value with  $P < 0.01$ . c: Comparative analysis of LRF transcripts by qRT-PCR in uteri isolated from mice treated to induce decidualization and non-decidual mice. The figure represents the quantification of LRF transcripts. \*Indicates a significant difference in P value with  $P < 0.01$ . d: Analysis of LRF transcripts by qRT-PCR, in the uteri of mice treated with either estradiol ( $E_2$ ), progesterone ( $P_4$ ) or both  $E_2$  and  $P_4$ . Mice administered with sesame oil were used as controls. The figure represents the quantification of LRF transcripts. Asterisks (\*\* and \*) indicate significant differences in P value with  $P < 0.01$  and  $P < 0.05$ , respectively.

### Preimplantation embryos express LRF

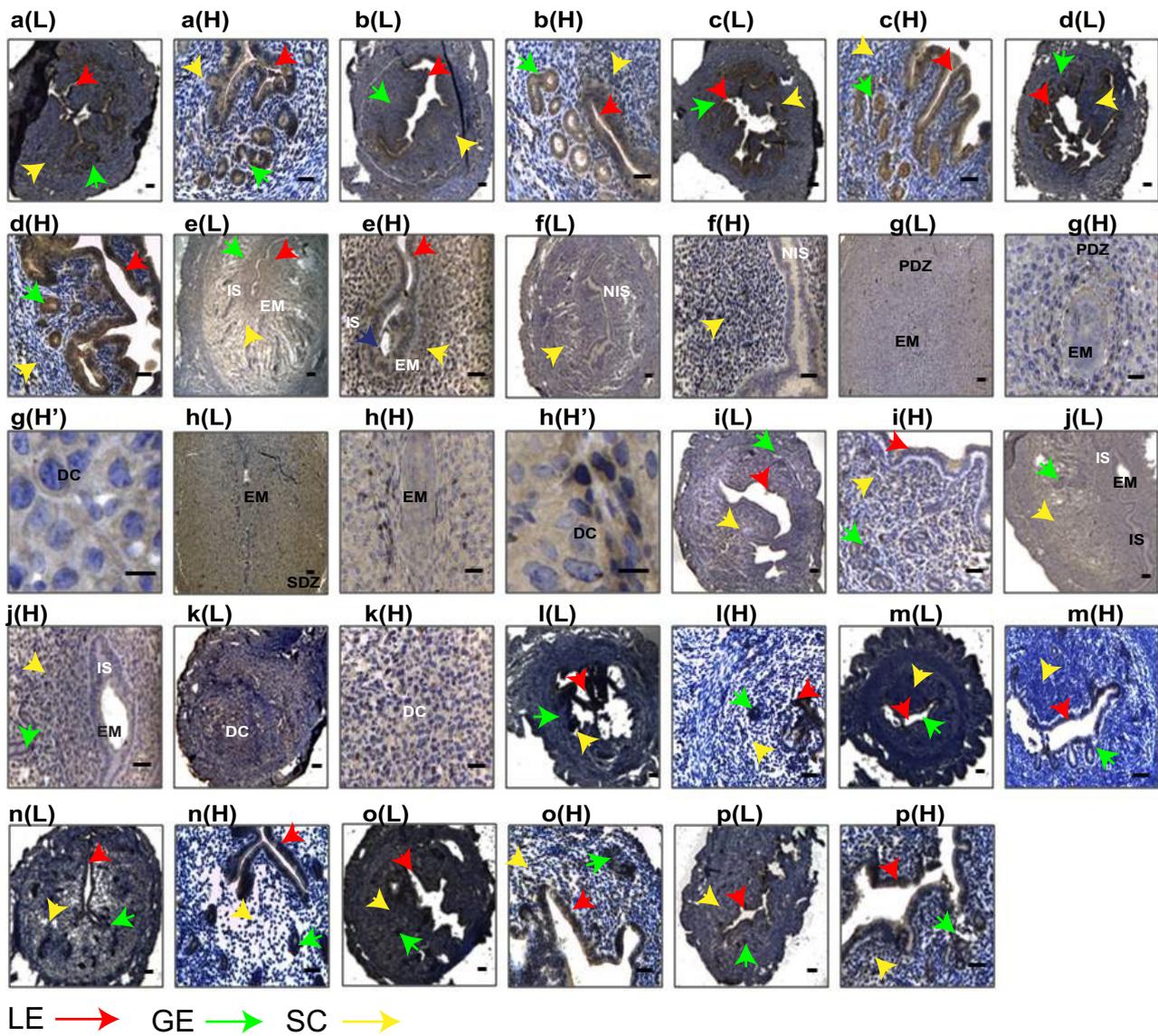
As implantation involves the attachment of embryonic trophoblast cells to the uterine epithelium, it is possible that LRF facilitates this attachment. To address this question, different stages of embryo during the preimplantation period were analyzed by immunohistochemistry for the expression of LRF. The results demonstrated that LRF is expressed in all the stages of mouse preimplantation embryos (Fig. 3A). Further, LRF protein was found in the cytoplasm, nucleus and polar body. Interestingly, the expression of LRF was found to be very high in the 4-cell stage embryo and in the early blastula stage when compared with the other embryonic stages (Fig. 3B,  $P < 0.05$ ). As the blastula stage is the stage at which the embryo undergoes implantation, increased expression of LRF may be a prerequisite for establishing contact between the blastula and uterine epithelium.

### Discussion

The present study evaluated the role of LRF during implantation, which is a reciprocal interaction between the blastocyst and uterus.

To address this question, expression of LRF was analyzed both at the transcript level and protein level in the uteri of pregnant mice. Expression of LRF was found to be highly elevated on day 5 of pregnancy, which correlates with the time of implantation. Further, LRF was not only found to be localized at the sites of implantation, but also was expressed in trophoblast cells, which are responsible for attachment to the uterine epithelium. These observations therefore implicate a potential role for LRF in implantation.

To further elucidate the function of LRF in implantation, mice were treated to induce delayed implantation and analyzed for the levels of LRF. The results indicate that these mice displayed low levels of LRF when compared with implantation activated uteri, which showed a marked increase in LRF expression in the regions of the luminal epithelium, glandular epithelium and stromal cells. These results therefore complement the above observation that LRF has a significant function during implantation. Further, the presence of LRF in the luminal and stromal cells suggests that it may act as an adhesion molecule and favor the apposition of trophoblast cells of the blastula to the luminal epithelia of the uterus, thereby



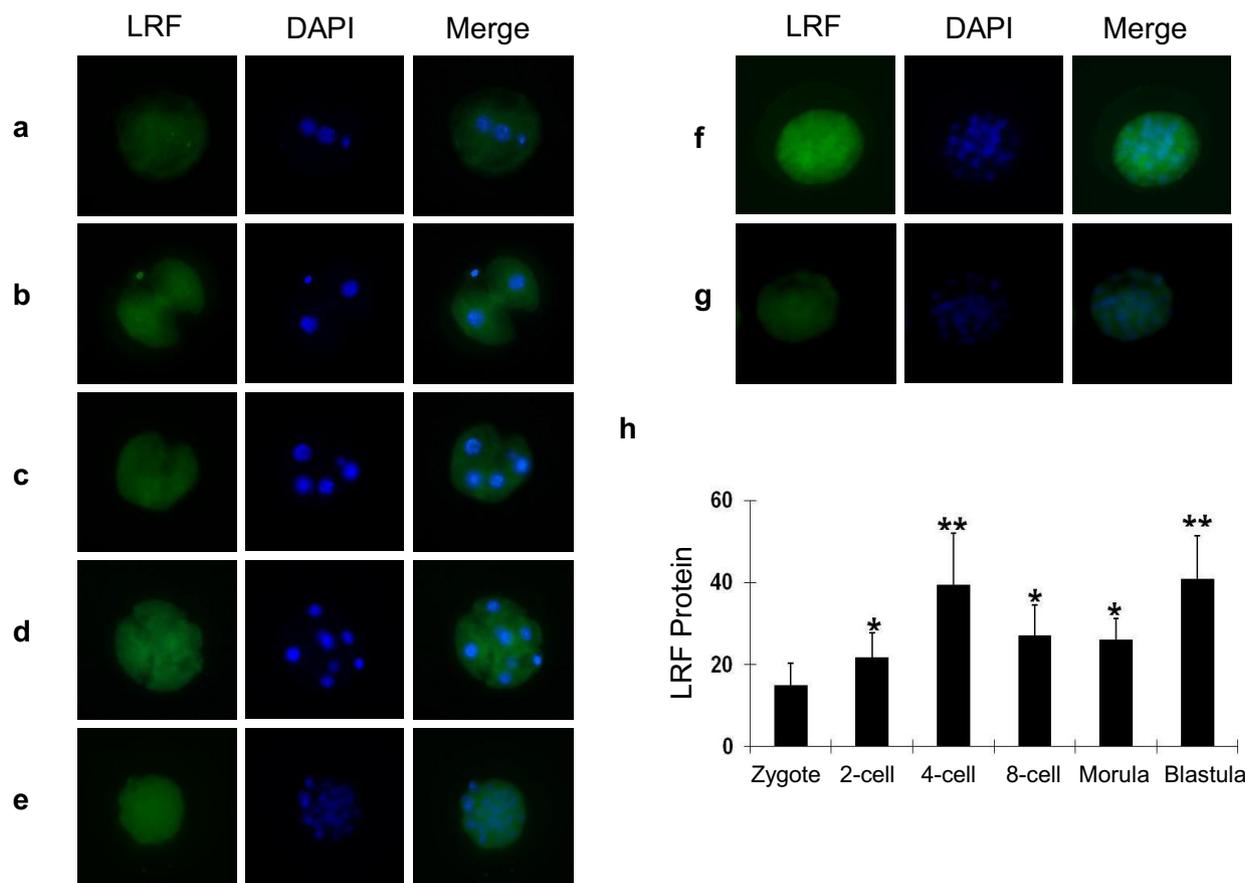
L= Low magnification, scale bar= 100  $\mu$ m.  
 H= High magnification, scale bar= 40  $\mu$ m.  
 H'= High magnification, scale bar=20  $\mu$ m.

**Fig. 2.** The figure represents the immunohistochemistry of LRF protein in uteri of pregnant mice during embryonic implantation at days 1 through 5 (panels a–e, respectively), at day 5 without embryonic implantation (panel f), at days 6 and 7 showing embryonic implantation (panels g and h, respectively), during delayed implantation (panel i), during activated implantation (panel j) and during induced decidualization (panel k); in the control uterus (panel l), in the untreated control uterus (panel m); after estradiol treatment (panel n); after progesterone treatment (panel o); and after treatment with both estradiol and progesterone (panel p). Each panel shows the images taken at both low and high magnifications. H and H' in the panels indicate images taken at higher magnifications, while L indicates the images taken at lower magnifications. The scale bars for H, H' and L represent 40  $\mu$ m, 100  $\mu$ m and 20  $\mu$ m, respectively. DC, decidual cell; EM, embryo; GE, glandular epithelium; IS, implantation site; LE, luminal epithelium; NIS, non-implantation site; PDZ, primary decidual zone; SDZ, secondary decidual zone.

enhancing uterine receptivity.

Decidualization is a critical process in early pregnancy that involves creation of a specialized environment for embryo implantation, invasion and placenta formation by altering the factors such as metalloproteinases, cytokines, surface integrins and major

histocompatibility complex molecules [27]. Analyses of LRF in the decidual uterus revealed a significant increase in the levels of LRF transcripts compared with the controls. However, LRF protein was observed in the mass of decidual cells of the induced decidual uteri, and in primary and secondary decidual regions of pregnant uteri at



**Fig. 3.** a–g: Immunofluorescence analyses of LRF expression in embryos at different stages of preimplantation, i.e., a– zygote, b– 2-cell embryo, c– 4-cell embryo, d– 8-cell embryo, e– morula; f– early blastocyst and g–hatching blastocyst. Scale bar: 100  $\mu$ m. h: Quantification of the immunofluorescence data obtained in panels a–g. \*\* $P$ <0.05; \* $P$ <0.01.

day 6 and 7 respectively. These results therefore suggest that LRF may also aid in invasion of the luminal epithelium and differentiation of stromal cells into decidual cells through paracrine signaling and thereby help in establishing a maternofetal vascular connection [1, 2, 24, 28]. Previous studies have demonstrated the ability of decidual cells to secrete several factors including prolactin, relaxin, rennin and insulin-like growth factor binding protein-1 (IGFBP-1) [29, 30] and specific extracellular matrix (ECM) proteins such as laminin and fibronectin [31]. Therefore, it is also possible that the role of LRF in implantation could be a result of its interaction with factors involved in implantation and decidualization.

Steroid hormones such as progesterone ( $P_4$ ) and estradiol ( $E_2$ ) are essential for establishment and maintenance of pregnancy. Although, progesterone alone is required throughout pregnancy, estradiol is essential only during early pregnancy, especially during implantation [32]. In rodents,  $E_2$  is required along with  $P_4$  to prepare the uterus for implantation [33] and decidualization [32, 34]. The present work on the uteri of mice injected with estradiol, progesterone or both revealed that LRF expression was modulated by these hormones. Similar effects were observed in pregnant and pseudopregnant mice, in which the LRF levels were elevated from days 1 through 4 in

the case of the uteri of pregnant mice, and from days 1 through 6 in the case of the uteri of pseudopregnant mice. Expression of LRF coincides with an elevation in progesterone levels gradually from day 1 through day 4 alongside a decrease in estradiol from day 1 through day 3. After day 3, estradiol levels remain fairly constant [1, 26]. These results strongly suggest that progesterone and estradiol, dictate the success of implantation, possibly by modulating the expression of LRF.

In conclusion, the results of this study suggest that LRF may be a part of the progesterone and estradiol hormone signaling during pregnancy and plays a critical role in implantation and decidualization.

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## References

1. Dey SK, Lim H, Das SK, Reese J, Paria B, Daikoku T, Wang H. Molecular cues to implantation. *Endocr Rev* 2004; **25**: 341–373. [Medline] [CrossRef]
2. Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. *Nature Reviews Genetics* 2006; **7**: 185–199. [Medline] [CrossRef]
3. Kimber SJ, Spanswick C. Blastocyst implantation: the adhesion cascade. *Semin Cell Dev Biol* 2000; **11**: 77–92. [Medline] [CrossRef]
4. Lee KY, DeMayo FJ. Animal models of implantation. *Reproduction* 2004; **128**: 679–695. [Medline] [CrossRef]
5. Yoshinaga K. Review of factors essential for blastocyst implantation for their modulating effects on the maternal immune system. *Semin Cell Dev Biol* 2008; **19**: 161–169. [Medline] [CrossRef]
6. Daftary GS, Taylor HS. Implantation in the human: the role of HOX genes. *Semin Reprod Med* 2000; **18**: 311–320. [Medline] [CrossRef]
7. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 1997; **91**: 197–208. [Medline] [CrossRef]
8. Freiman RN, Herr W. Viral mimicry: common mode of association with HCF by VP16 and the cellular protein LZIP. *Genes Dev* 1997; **11**: 3122–3127. [Medline] [CrossRef]
9. Lu R, Yang P, O'Hare P, Misra V, Luman. A new member of the CREB/ATF family, binds to herpes simplex virus VP16-associated host cellular factor. *Mol Cell Biol* 1997; **17**: 5117–5126. [Medline]
10. Raggio C, Rapin N, Stirling J, Gobeil P, Smith-Windsor E, O'Hare P, Misra V. Luman, the cellular counterpart of herpes simplex virus VP16, is processed by regulated intramembrane proteolysis. *Mol Cell Biol* 2002; **22**: 5639–5649. [Medline] [CrossRef]
11. DenBoer LM, Hardy-Smith PW, Hogan MR, Cockram GP, Audas TE, Lu R. Luman is capable of binding and activating transcription from the unfolded protein response element. *Biochem Biophys Res Commun* 2005; **331**: 113–119. [Medline] [CrossRef]
12. Liang G, Audas TE, Li Y, Cockram GP, Dean JD, Martyn AC, Kokame K, Lu R. Luman/CREB3 induces transcription of the endoplasmic reticulum (ER) stress response protein Herp through an ER stress response element. *Mol Cell Biol* 2006; **26**: 7999–8010. [Medline] [CrossRef]
13. Lu R, Misra V. Potential role for Luman, the cellular homologue of herpes simplex virus VP16 ( $\alpha$  gene trans-inducing factor), in herpesvirus latency. *J Virol* 2000; **74**: 934–943. [Medline] [CrossRef]
14. Blot G, Lopez-Vergès S, Treand C, Kubat NJ, Delcroix-Genête D, Emiliani S, Benarous R, Berlioz-Torrent C. Luman, a new partner of HIV-1 TMgp41, interferes with Tat-mediated transcription of the HIV-1 LTR. *J Mol Biol* 2006; **364**: 1034–1047. [Medline] [CrossRef]
15. Ko J, Jang SW, Kim YS, Kim IS, Sung HJ, Kim HH, Park JY, Lee YH, Kim J, Na DS. Human LZIP binds to CCR1 and differentially affects the chemotactic activities of CCR1-dependent chemokines. *FASEB J* 2004; **18**: 890–892. [Medline]
16. Jang SW, Kim YS, Kim YR, Sung HJ, Ko J. Regulation of human LZIP expression by NF- $\kappa$ B and its involvement in monocyte cell migration induced by Lkn-1. *J Biol Chem* 2007; **282**: 11092–11100. [Medline] [CrossRef]
17. Sung HJ, Kim YS, Kang H, Ko J. Human LZIP induces monocyte CC chemokine receptor 2 expression leading to enhancement of monocyte chemoattractant protein 1/CCL2-induced cell migration. *Exp Mol Med* 2008; **40**: 332–338. [Medline] [CrossRef]
18. Eleveld-Trancikova D, Sanecka A, van Hout-Kuijter MA, Looman MWG, Hendriks IAM, Jansen BJH, Adema GJ. DC-STAMP interacts with ER-resident transcription factor LUMAN which becomes activated during DC maturation. *Mol Immunol* 2010; **47**: 1963–1973. [Medline] [CrossRef]
19. Kang H, Kim YS, Ko J. A novel isoform of human LZIP negatively regulates the trans-activation of the glucocorticoid receptor. *Mol Endocrinol* 2009; **23**: 1746–1757. [Medline] [CrossRef]
20. Jin DY, Wang HL, Zhou Y, Chun ACS, Kibler KV, Hou YD, Kung H, Jeang KT. Hepatitis C virus core protein-induced loss of LZIP function correlates with cellular transformation. *EMBO J* 2000; **19**: 729–740. [Medline] [CrossRef]
21. Kim HC, Choi KC, Choi HK, Kang HB, Kim MJ, Lee YH, Lee OH, Lee J, Kim YJ, Jun W, Jeong JW, Yoon HG. HDAC3 selectively represses CREB3-mediated transcription and migration of metastatic breast cancer cells. *Cellular and molecular life sciences. CMLS* 2010; **67**: 3499–3510. [Medline] [CrossRef]
22. Audas TE, Li Y, Liang G, Lu R. Luman/CREB3 recruitment factor (LRF) regulates human activation of the unfolded protein response. *Mol Cell Biol* 2008; **28**: 3952–3966. [Medline] [CrossRef]
23. Martyn AC, Choleris E, Gillis DJ, Armstrong JN, Amor TR, McCluggage ARR, Turner PV, Liang G, Cai K, Lu R. Luman/CREB3 recruitment factor (LRF) regulates glucocorticoid receptor activity and is essential for prolactin-mediated maternal instinct. *Mol Cell Biol* 2012; **32**: 5140–5150. [CrossRef] [Medline]
24. Hayashi K, Erikson DW, Tilford SA, Bany BM, Maclean JA II, Rucker EB III, Johnson GA, Spencer TE. Wnt genes in the mouse uterus: potential regulation of implantation. *Biol Reprod* 2009; **80**: 989–1000. [Medline] [CrossRef]
25. Chen W, Han BC, Wang RC, Xiong GF, Peng JP. Role of secretory protease inhibitor SPINK3 in mouse uterus during early pregnancy. *Cell Tissue Res* 2010; **341**: 441–451. [Medline] [CrossRef]
26. Ni H, Yu XJ, Liu HJ, Lei W, Rengaraj D, Li XJ, Yang ZM. Progesterone regulation of glutathione S-transferase Mu2 expression in mouse uterine luminal epithelium during preimplantation period. *Fertil Steril* 2009; **91**: 2123–2130. [Medline] [CrossRef]
27. Lunghi L, Ferretti ME, Medici S, Biondi C, Vesce F. Control of human trophoblast function. *Reprod Biol Endocrinol* 2007; **5**: 6. [CrossRef] [Medline]
28. Lim H, Song H, Paria B, Reese J, Das SK, Dey S. Molecules in blastocyst implantation: uterine and embryonic perspectives. *Vitam Horm* 2002; **64**: 43–76. [Medline] [CrossRef]
29. Brosens JJ, Hayashi N, White JO. Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology* 1999; **140**: 4809–4820. [Medline] [CrossRef]
30. Salamonsen LA, Dimitriadis E, Jones R, Nie G. Complex regulation of decidualization: a role for cytokines and proteases—a review. *Placenta* 2003; **24**: S76–S85. [Medline] [CrossRef]
31. King A. Uterine leukocytes and decidualization. *Hum Reprod Update* 2000; **6**: 28–36. [Medline] [CrossRef]
32. Kim TH, Lee DK, Franco HL, Lydon JP, Jeong JW. ERBB receptor feedback inhibitor 1 regulation of estrogen receptor activity is critical for uterine implantation in mice. *Biol Reprod* 2010; **82**: 706–713. [Medline] [CrossRef]
33. Marcus GJ, Kraicer P, Shelesnyak M. Studies on the mechanism of decidualization. *J Reprod Fertil* 1963; **5**: 409–415. [Medline] [CrossRef]
34. Schatz F, Krikun G, Caze R, Rahman M, Lockwood CJ. Progesterin-regulated expression of tissue factor in decidual cells: implications in endometrial hemostasis, menstruation and angiogenesis. *Steroids* 2003; **68**: 849–860. [Medline] [CrossRef]