

Inhibition of Stat3 activation in the endometrium prevents implantation: A nonsteroidal approach to contraception

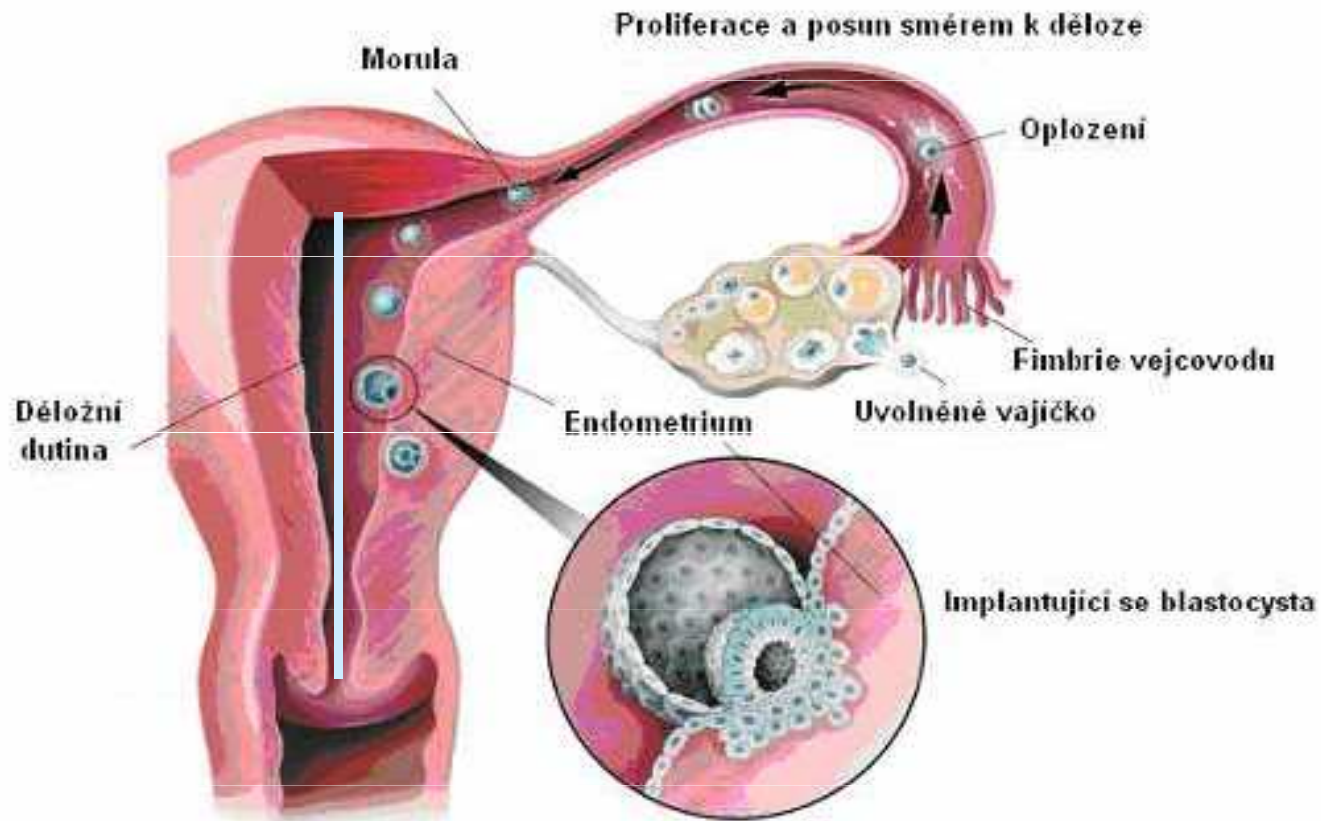
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Biology in UK




Implantace (nidace)



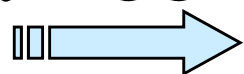
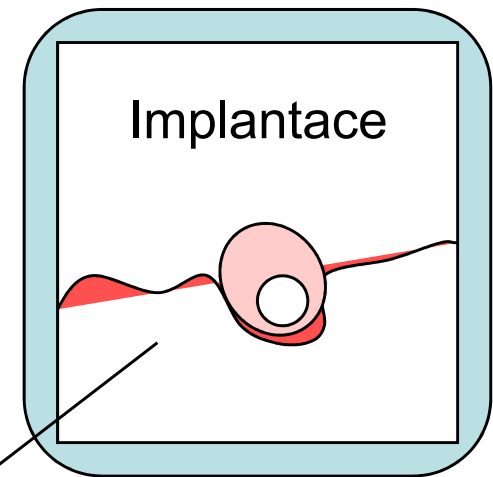
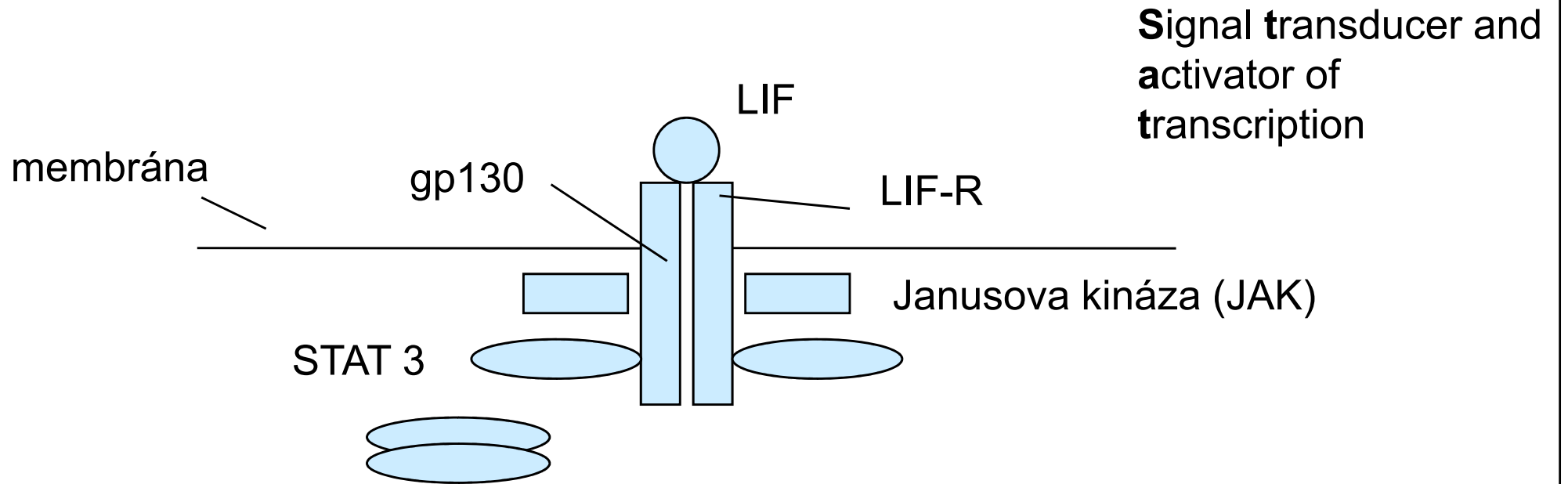
Implantace – faktory

- Blastocysta, 4 *dpc*  interakce trofoblastu a sliznice (luminal epithelium, LE)
- Přestavba endometria – decidualizace, sekreční fáze
 **receptivita**
- Pod vlivem **estrogenu** a **progesteronu** z ovarií

 Leukemia Inhibitory Factor (LIF)

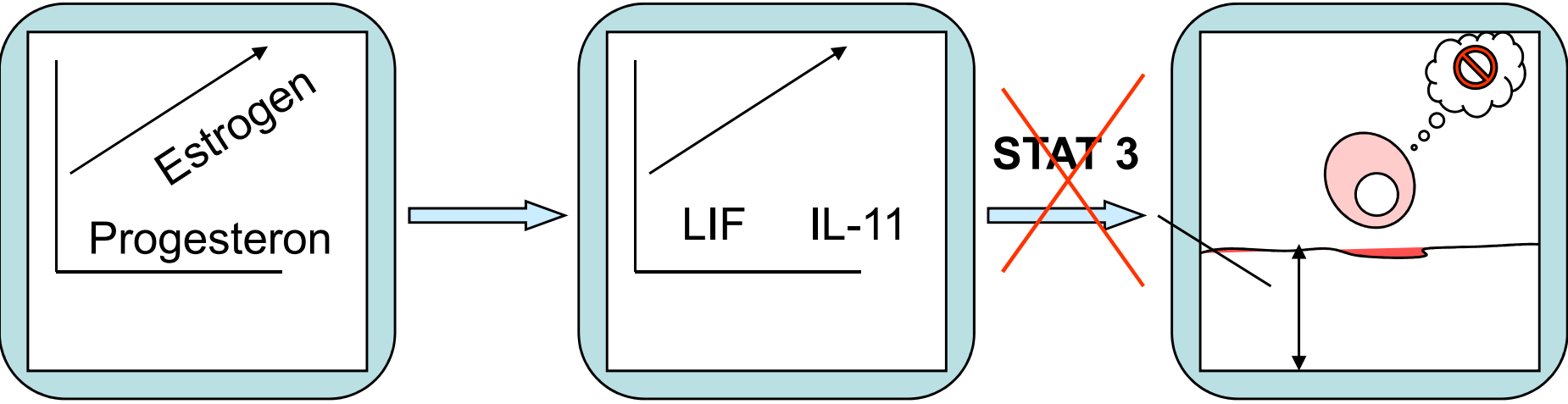
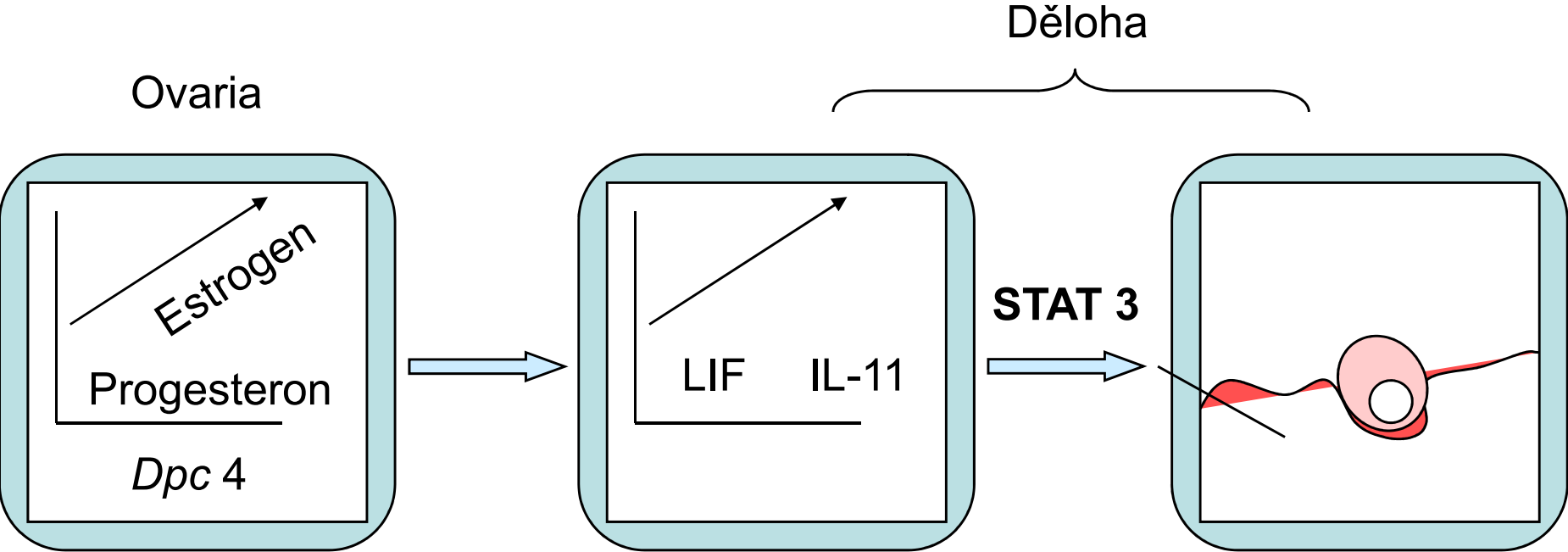
 Interleukin-11 (IL-11)

Signální dráha gp130 – JAK/STAT



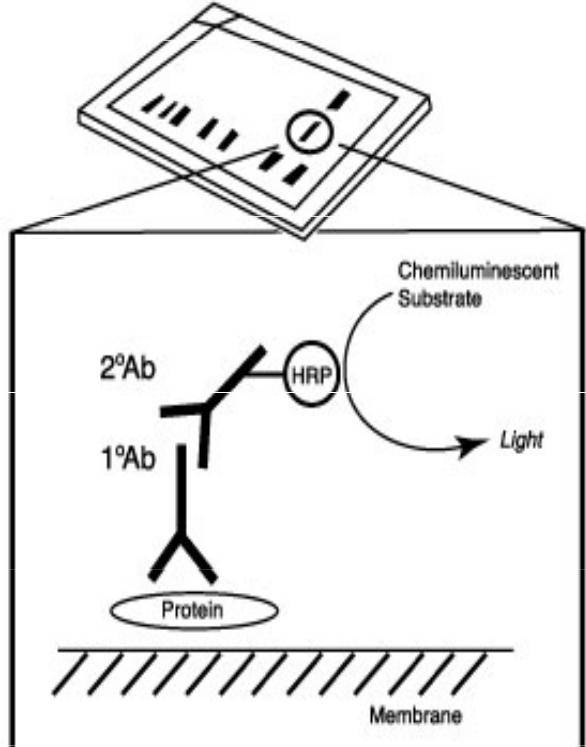
Spuštění transkripce vybraných genů (*Irg1*, *cochlin*, *amphiregulin*...)

Summary



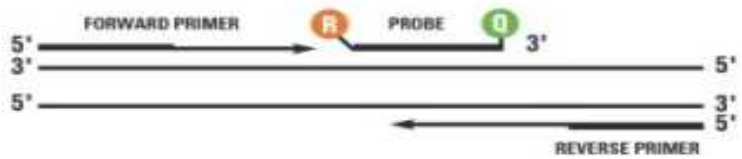
Metody

Western blotting

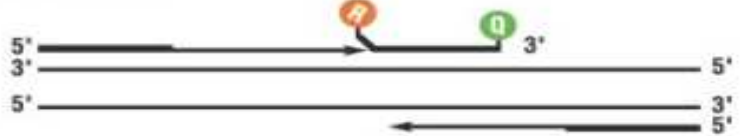


Real time RT PCR

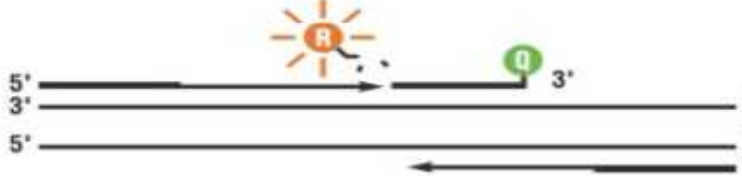
1. **Polymerizace:** Fluorescenční substrát (reporter - R) a jeho zhášec (Q) jsou navázány na 5' a 3' konce TaqMan sondy.



2. **Elongace řetězce:** Dokud je sonda intaktní záření emitované substrátem R je pohlcováno blízkým "zhášečem".



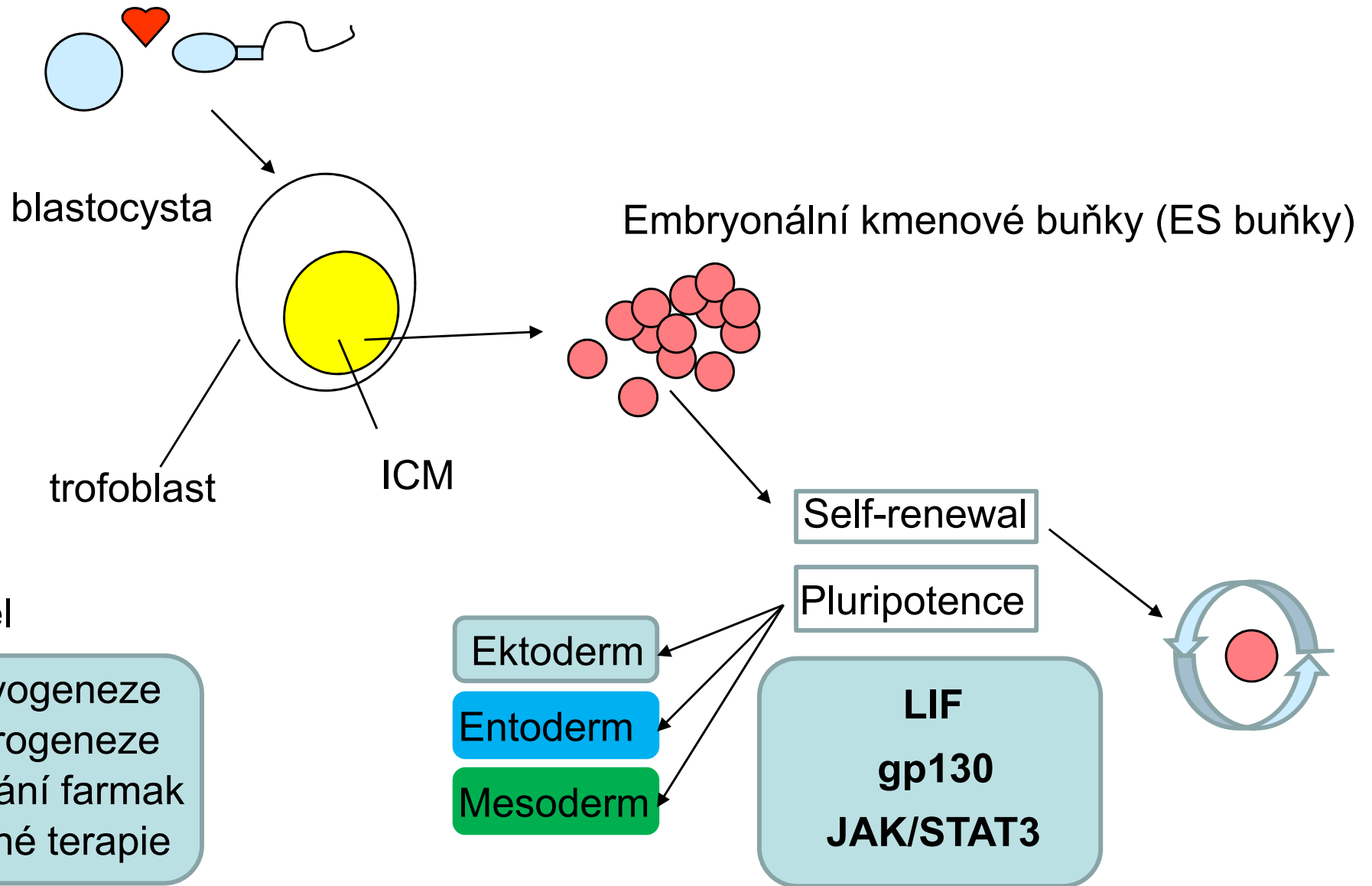
3. **Odštěpení:** když Tag polymeráza dorazí k začátku sondy, postupně ji odchlípuje až odštěpí fluorofor. Ten se tímto oddálí od zhášeče a emitované světlo přestane být pohlcováno - detekovaná fluorescence stoupá.



4. **Polymerizace ukončena:** reporterová barva oddělená od zhášeče emituje charakteristickou fluorescenci.



Kmenové buňky



Effect of intrauterine injection of STAT 3 inhibitor on implantation rates

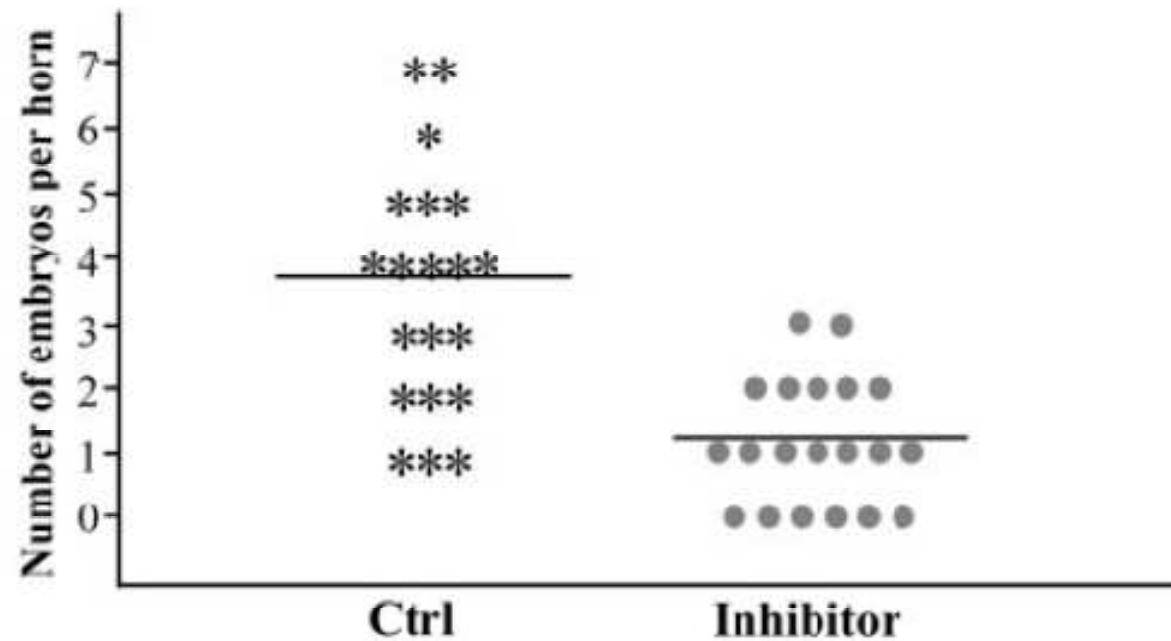


Fig. 1. Effect of intrauterine injection of Stat3 inhibitor on implantation rates. Pregnant mice were injected on the evening of day 3 in both horns with either control peptide (controls, $n = 10$) or Stat3 peptide inhibitor ($n = 10$), and the number of implanted embryos per uterine horn was counted on day 8. The mean number of implanted embryos in the control group was 3.7 compared with 1.1 in the inhibitor-treated group, indicated by the line ($P < 0.001$) corresponding to a reduction in implantation rate of 70%.

Stat3 phosphorylation status and response to LIF in LE isolated on day 3 (noon) or day 4 (noon) of pregnancy

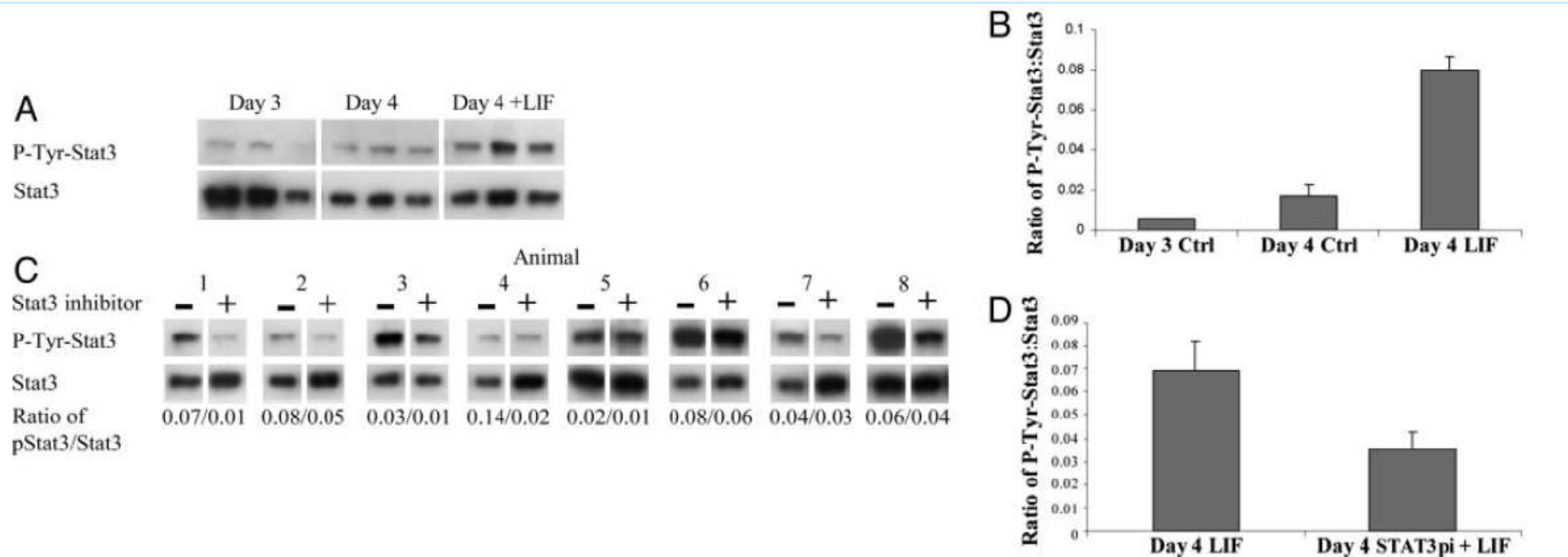


Fig. 2. Stat3 phosphorylation status and response to LIF in LE isolated on day 3 (noon) or day 4 (noon) of pregnancy. Day-4 LE isolated from one horn was treated with LIF after recovery in culture, and the other horn represented an untreated control. (A) Levels of Stat3 phosphorylation and total Stat3 in day 3, day 4 (control), and day 4 plus LIF LE were analyzed by Western blotting. Note that exposure to film was considerably longer for phospho-Stat3 than total Stat3. (B) After densitometry with the phosphoimager, the ratio of phospho-Stat3 to Stat3 was calculated. LIF treatment of day-4 LE dramatically increased levels of phosphorylated Stat3 ($P < 0.005$, $n = 3$). (C) Stat3 inhibitor reduced phosphorylation of Stat3 in the LE in response to LIF *in vitro*. LE was isolated on day 4 (noon), allowed to recover in culture for 2 h, and then incubated with or without Stat3 inhibitor for 1 h. It was then stimulated with LIF, and the levels of phosphorylated Stat3 and total Stat3 in control or inhibitor-treated LE were measured. The ratio of Stat3 phosphorylation to total Stat3 is given below each lane of paired samples ($n = 8$). (D) Mean ratio of phosphorylated Stat3 to total Stat3 after LIF stimulation of horns treated with or without Stat3 inhibitor. The inhibitor reduced phosphorylation of Stat3 after LIF stimulation by an average of 49% ($P < 0.007$).

Effect of Stat3 inhibitor on the expression of LIF-regulated genes *in vitro*

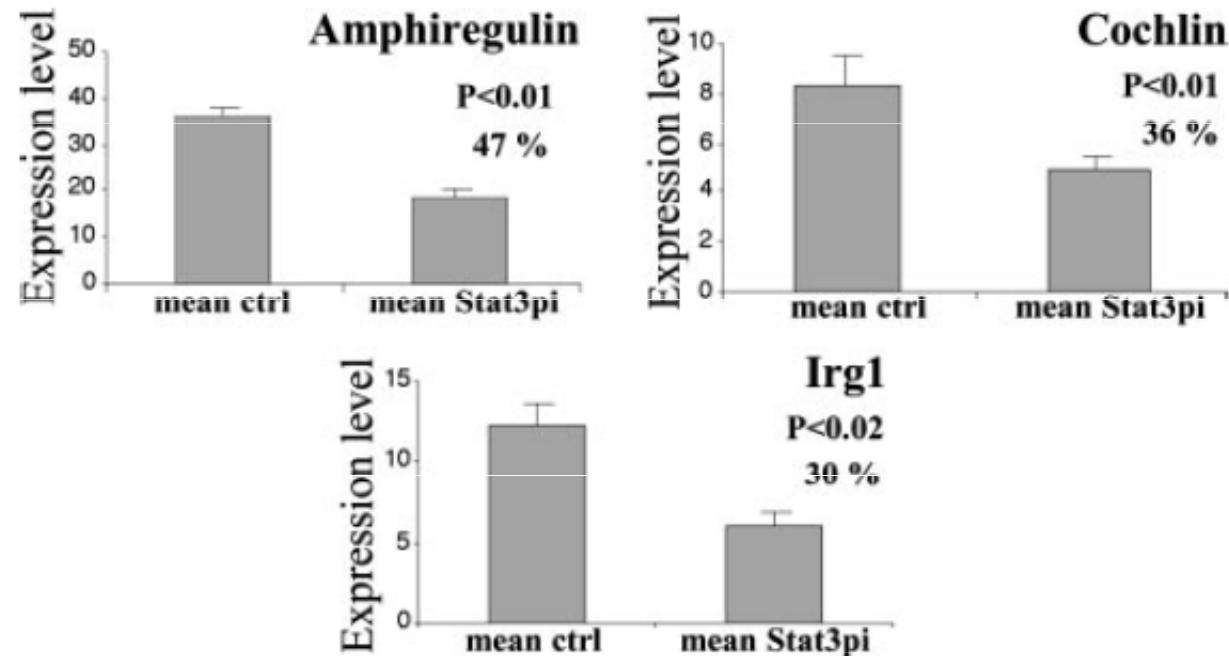


Fig. 3. Effect of Stat3 inhibitor on the expression of LIF-regulated genes *in vitro*. LE isolated from day-4 (noon) uterine horns were divided into two equal pieces and treated with or without 1 mM Stat3 peptide inhibitor. One hour later, samples were stimulated with LIF (50 ng/ml) and cultured for a further 4 h, and total RNA was extracted. The expression levels of LIF-regulated genes were determined by real-time PCR. The mean reduction compared with control between paired treatments ($n = 8$) is indicated as a percentage.

In vivo administration of Stat3 inhibitor reduces phosphorylation of Stat3 in the LE in response to LIF

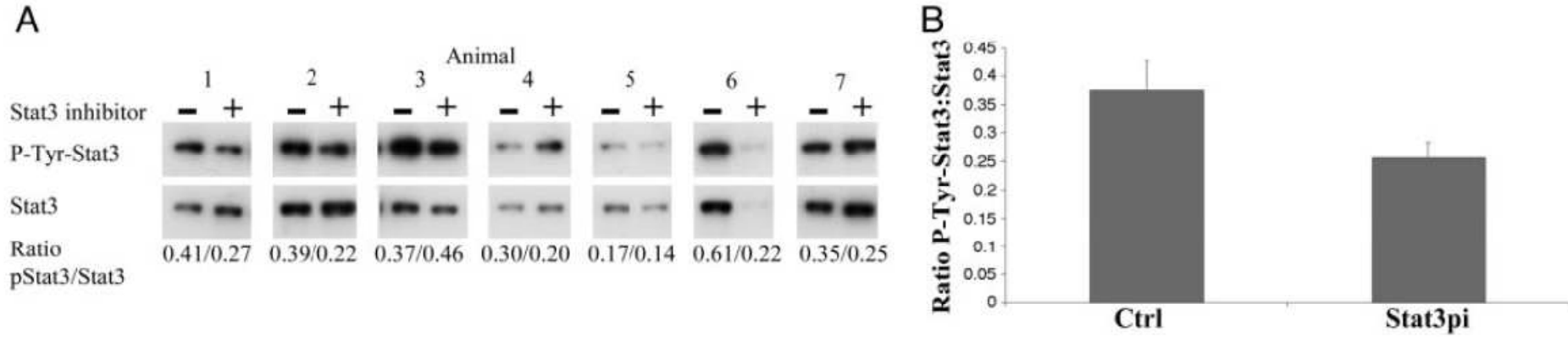


Fig. 4. *In vivo* administration of Stat3 inhibitor reduces phosphorylation of Stat3 in the LE in response to LIF. Mice were given intrauterine injections of PBS (control) in one horn and Stat3 inhibitor (Stat3pi) in the other on day 3 of pregnancy. LE was isolated on day 4 (noon), allowed to recover in culture for 3 h, and then stimulated for 15 min with LIF to assess the response of Stat3. (A) Relative levels of phospho-Stat3 and total Stat3 in the samples were determined by Western blotting, followed by densitometry with the phosphoimager, and the ratio is shown below each sample. (B) Stat3 inhibitor reduced Stat3 phosphorylation after LIF stimulation by an average of 32% 18 h after *in vivo* administration of the inhibitor ($P < 0.04$).

Effect of Stat3 inhibitor on the expression of LIF-regulated genes *in vivo*

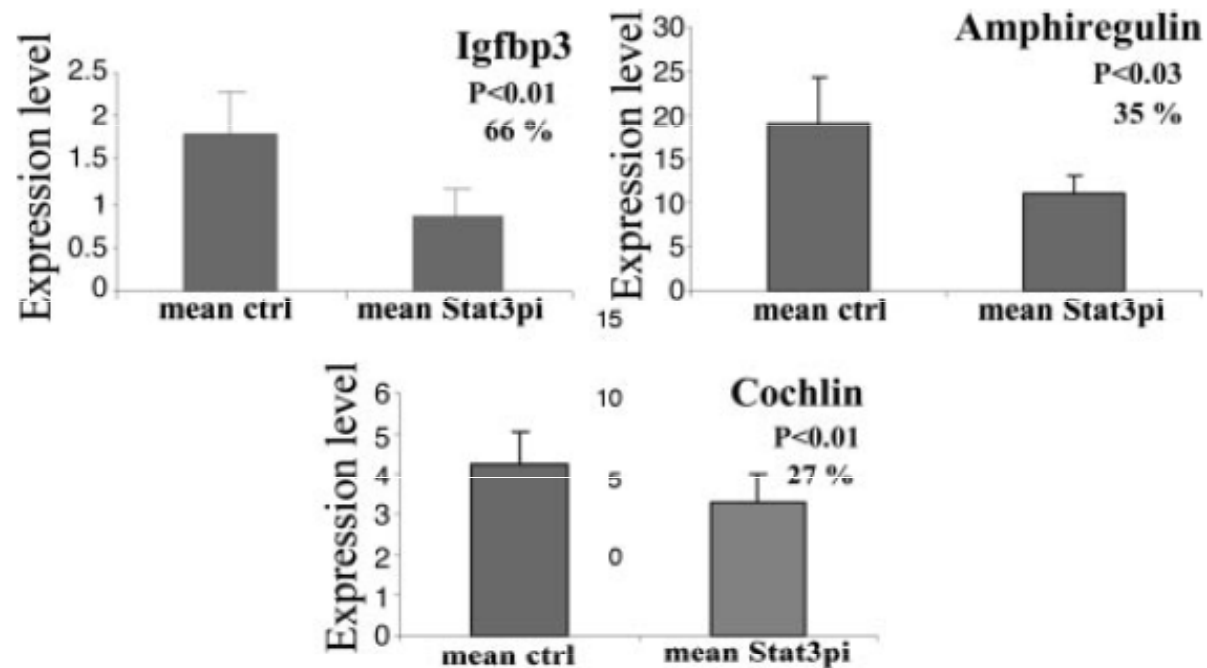


Fig. 5. Effect of Stat3 inhibitor on the expression of LIF-regulated genes *in vivo*. Mice were given intrauterine injections of PBS (control) in one horn and Stat3 peptide inhibitor in the other on day 3 of pregnancy. LE was isolated on day 4 (1600 hours), and total RNA was extracted. The expression levels of LIF-regulated genes were determined by real-time PCR. The mean reduction compared with control between paired treatments ($n = 10$) is indicated as a percentage.

In vivo administration of Stat3 inhibitor reduces phosphorylation of Stat3 in the LE in response to LIF

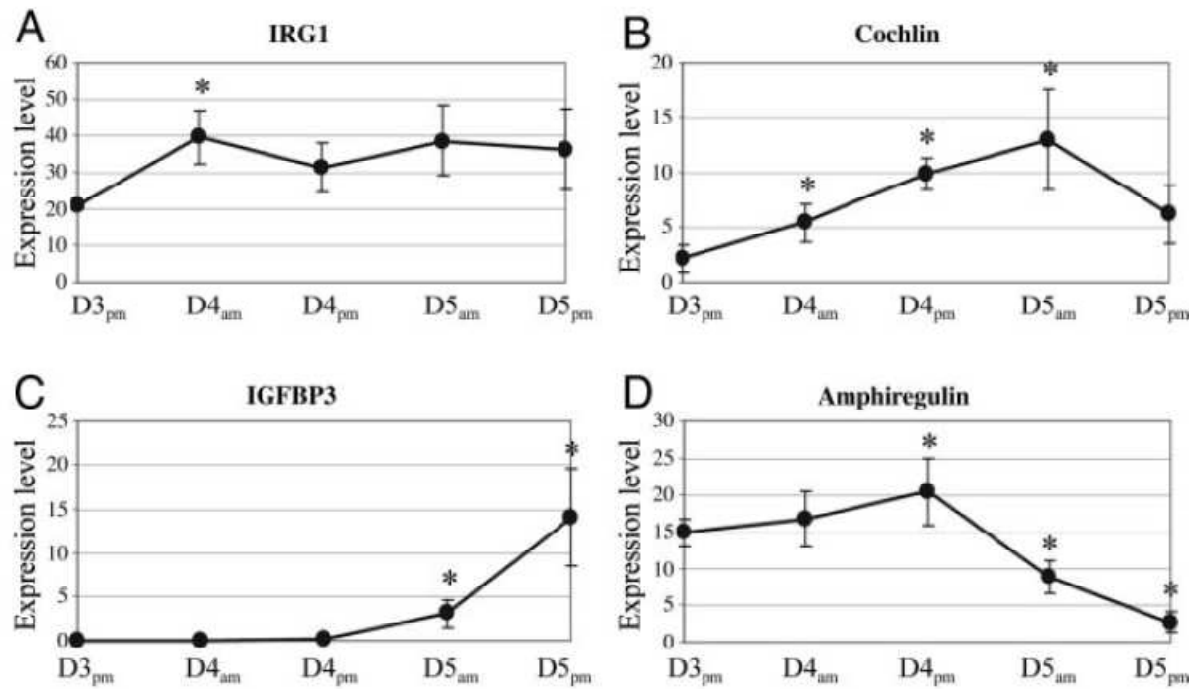


Fig. 6. Expression levels of LIF-regulated genes in the LE during early pregnancy. LE was isolated from day 3 (9 p.m., $n = 5$), day 4 (9 a.m., $n = 5$), day 4 (9 p.m., $n = 4$), day 5 (9 a.m., $n = 4$), and day 5 (9 p.m., $n = 3$). RNA was extracted, and expression levels were determined by real-time PCR. Mean expression values for each time point are indicated. Asterisks indicate time points where expression is different from the level at 9 p.m. on day 3 ($P < 0.05$).