

FIGURES

Figure 1: Expression of prolactin in eutopic endometrium from patients and healthy controls and in endometriotic lesions

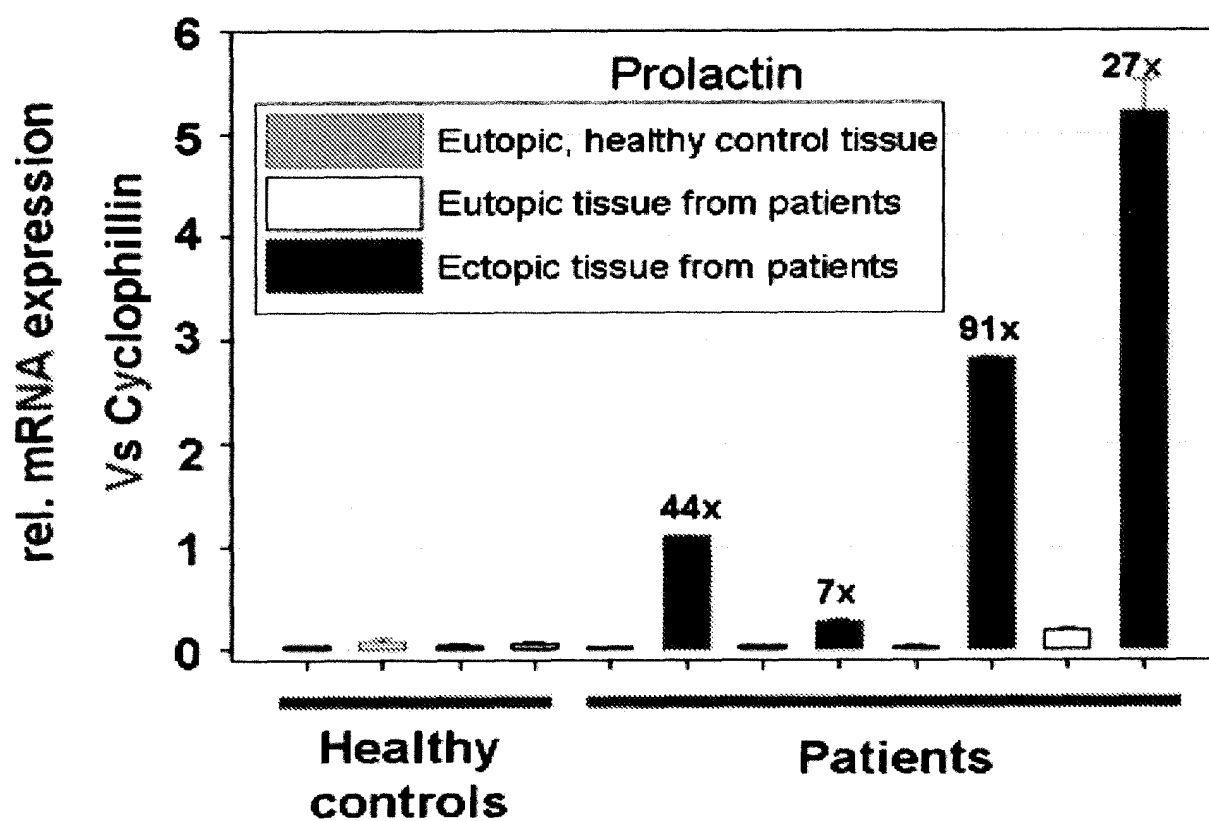


Figure 2: Expression of the prolactin receptor in eutopic endometrium from patients and healthy controls and in endometriotic lesions

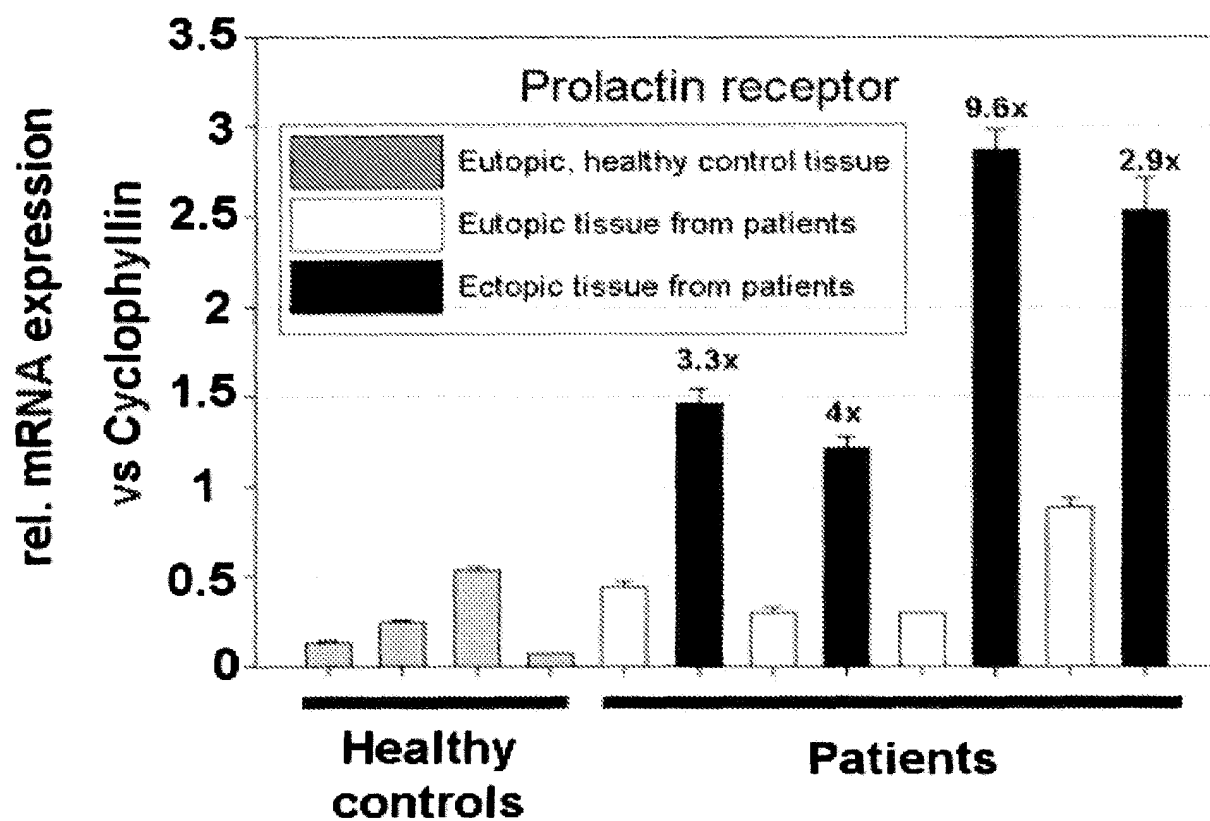
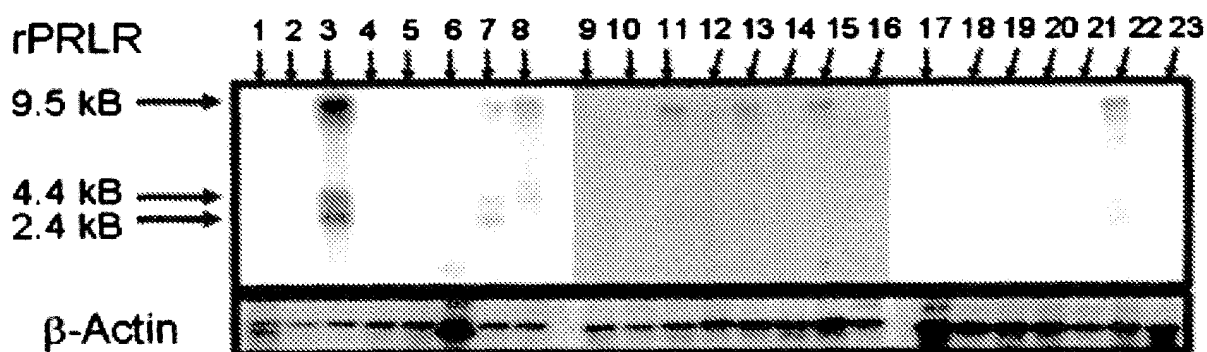


Figure 3: Analysis of prolactin receptor mRNA expression in rat tissues by Northern blot



- | | | |
|----------------------|----------------------------|--------------------------|
| 1. heart | 9. spleen | 17. stomach |
| 2. brain | 10. thymus | 18. thyroid |
| 3. placenta | 11. prostate | 19. spinal cord |
| 4. lung | 12. testis | 20. lymph node |
| 5. liver | 13. ovary | 21. trachea |
| 6. sk. muscle | 14. small intestine | 22. adrenal gland |
| 7. kidney | 15. colon | 23. bone marrow |
| 8. pancreas | 16. PBL | |

Figure 4: Analysis of prolactin receptor protein expression in rat prostate from castrated animals (gdx) and from intact animals treated with vehicle (intact), dihydrotestosterone (DHT) or estradiol (E2)



Figure 5: Inhibition of Baf cell proliferation (cells stably transfected with hPRLR) by different neutralising PRLR antibodies

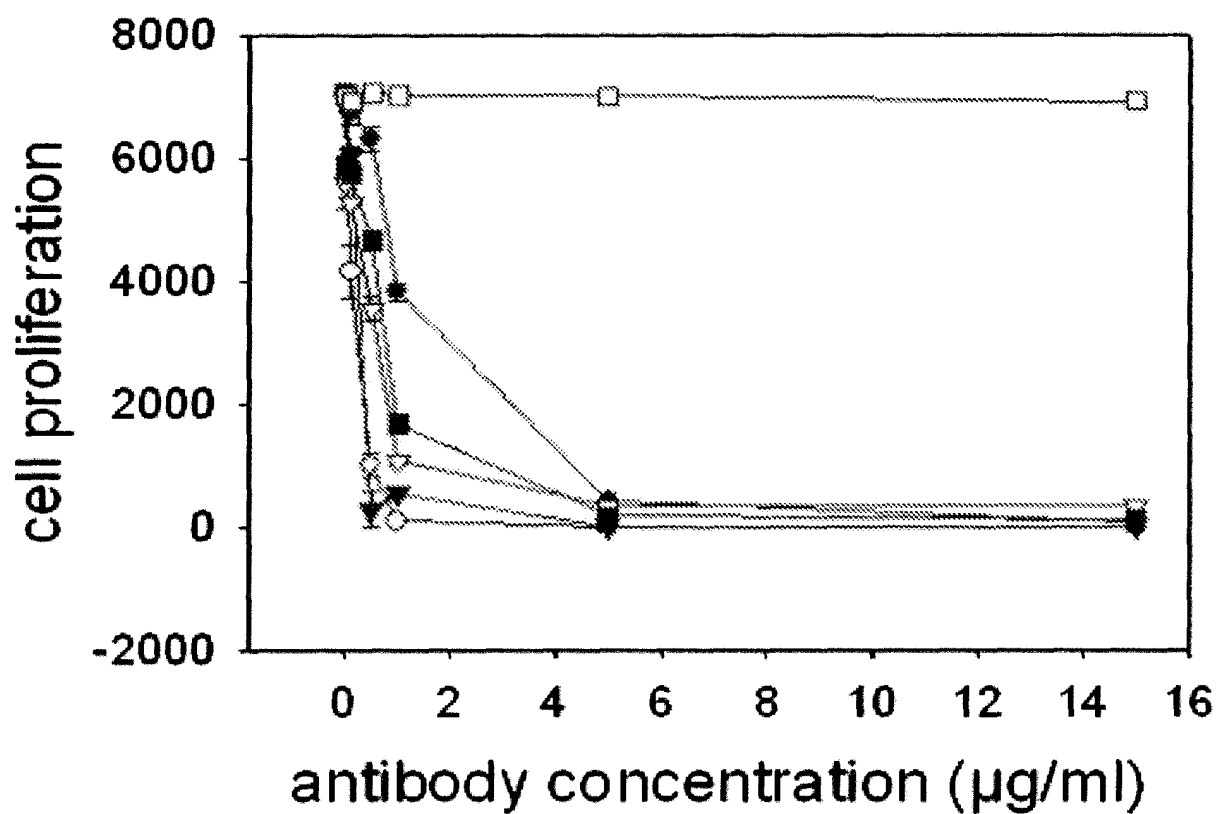


Figure 6: Inhibition of prolactin-induced rat lymphoma cell proliferation (NB2 cells) by neutralising prolactin receptor antibodies

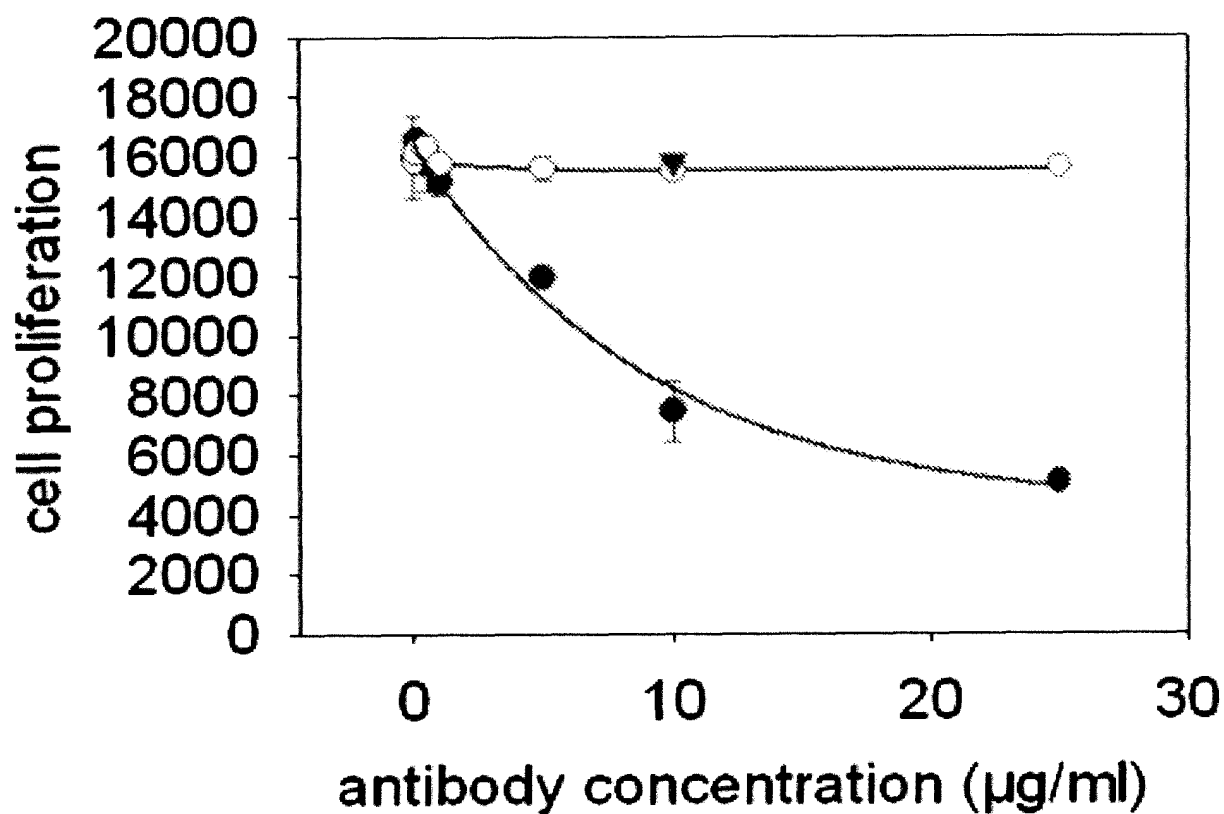
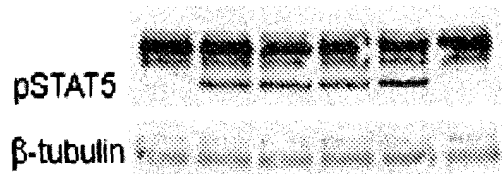


Figure 7: Inhibition of prolactin-induced STAT5 phosphorylation in T47D cells by neutralizing prolactin receptor antibodies and unspecific control antibody

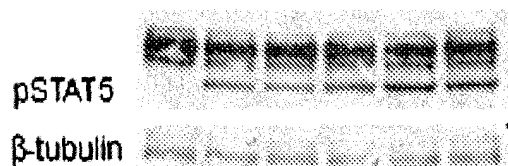
XHA06.642

Prolactin	0	+	+	+	+	+
Antibody	0	0	0.01	0.1	1	3 $\mu\text{g/ml}$



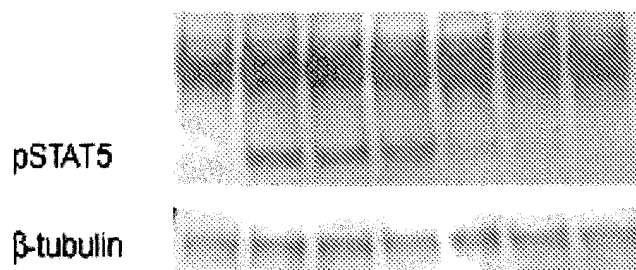
FITC

Prolactin	0	+	+	+	+	+
Antibody	0	0	0.01	0.1	1	3 $\mu\text{g/ml}$



IgG1 005_C04

Prolactin	0	+	+	+	+	+	+
Antibody	0	0	0.01	0.1	1	3	10 $\mu\text{g/ml}$



IgG1 006_H08

Prolactin	0	+	+	+	+	+	+
Antibody	0	0	0.01	0.1	1	3	10 $\mu\text{g/ml}$

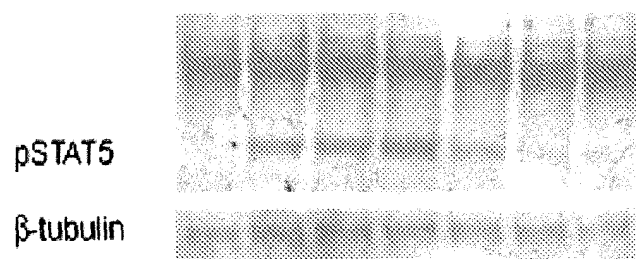


Figure 8: Luciferase assay in HEK293 cells stably transfected with hPRLR and transiently transfected with luciferase under the control of LHRE's. Inhibition of luciferase activity by specific and unspecific antibodies.

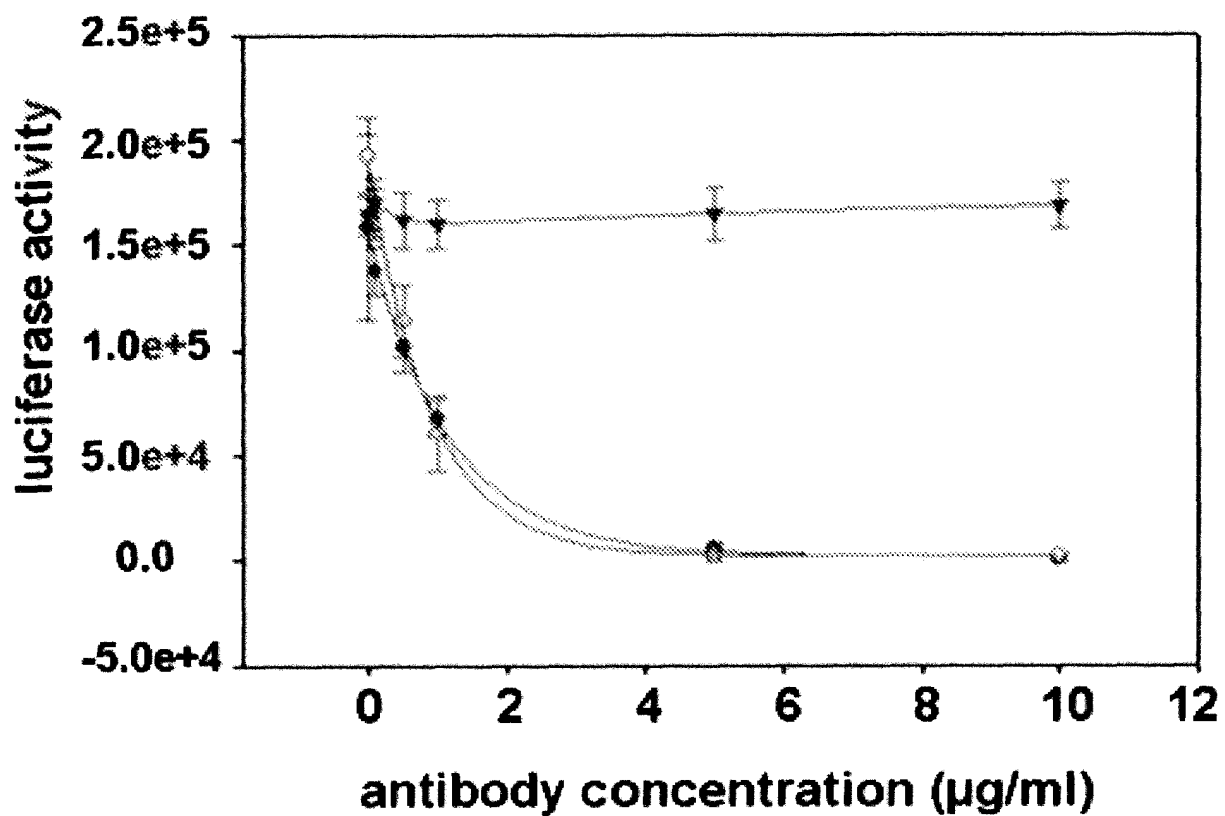


Figure 9: Luciferase assay in Hek293 cells stably transfected with murine PRLR and transiently transfected with luciferase under the control of LHREs – analysis of inhibition of luciferase activity by specific and unspecific antibodies

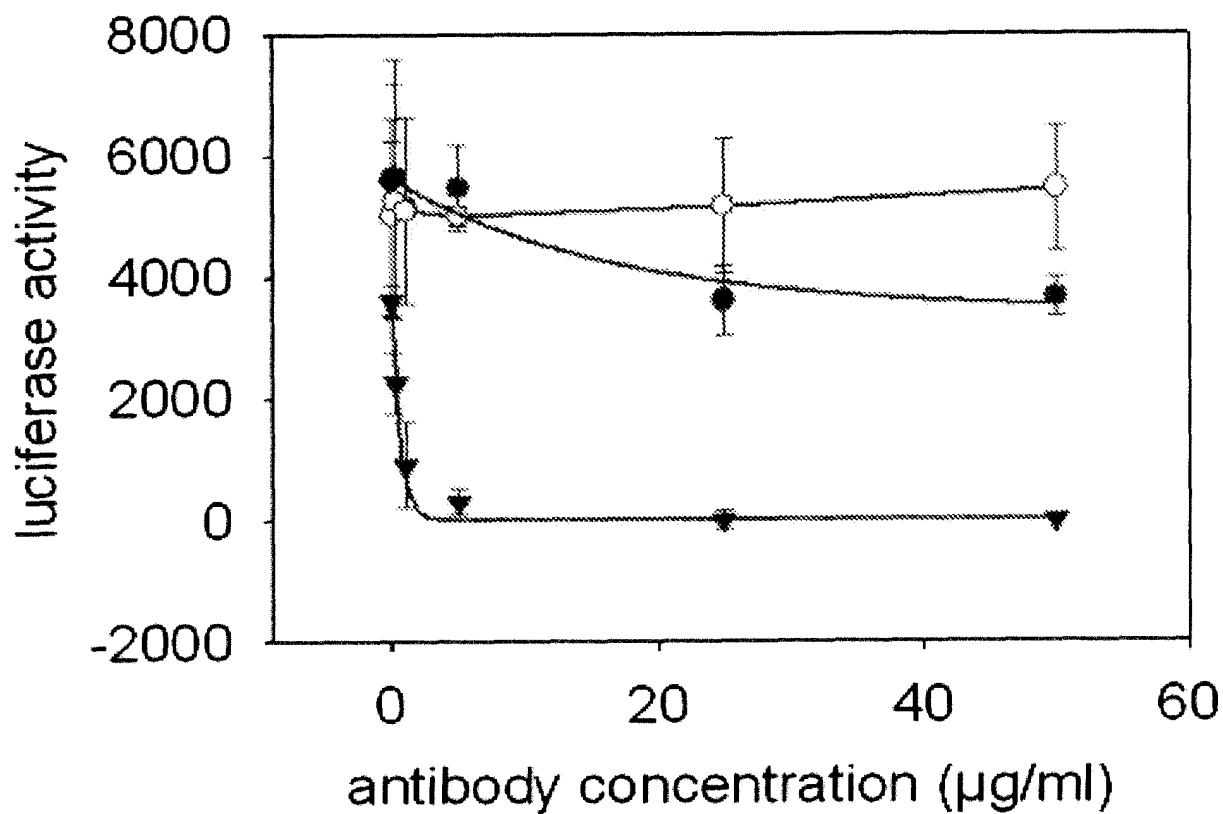


Figure 10: Inhibition of Baf cell proliferation (cells stably transfected with murine PRLR) by different neutralizing PRLR antibodies

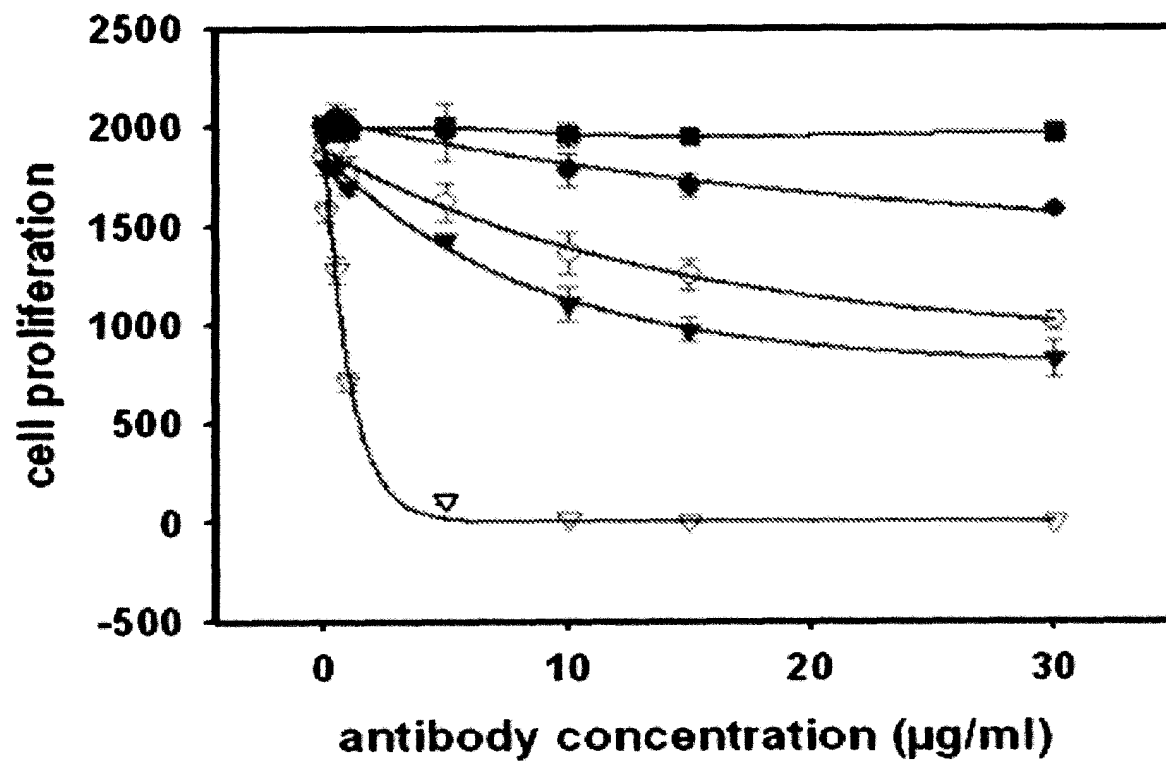


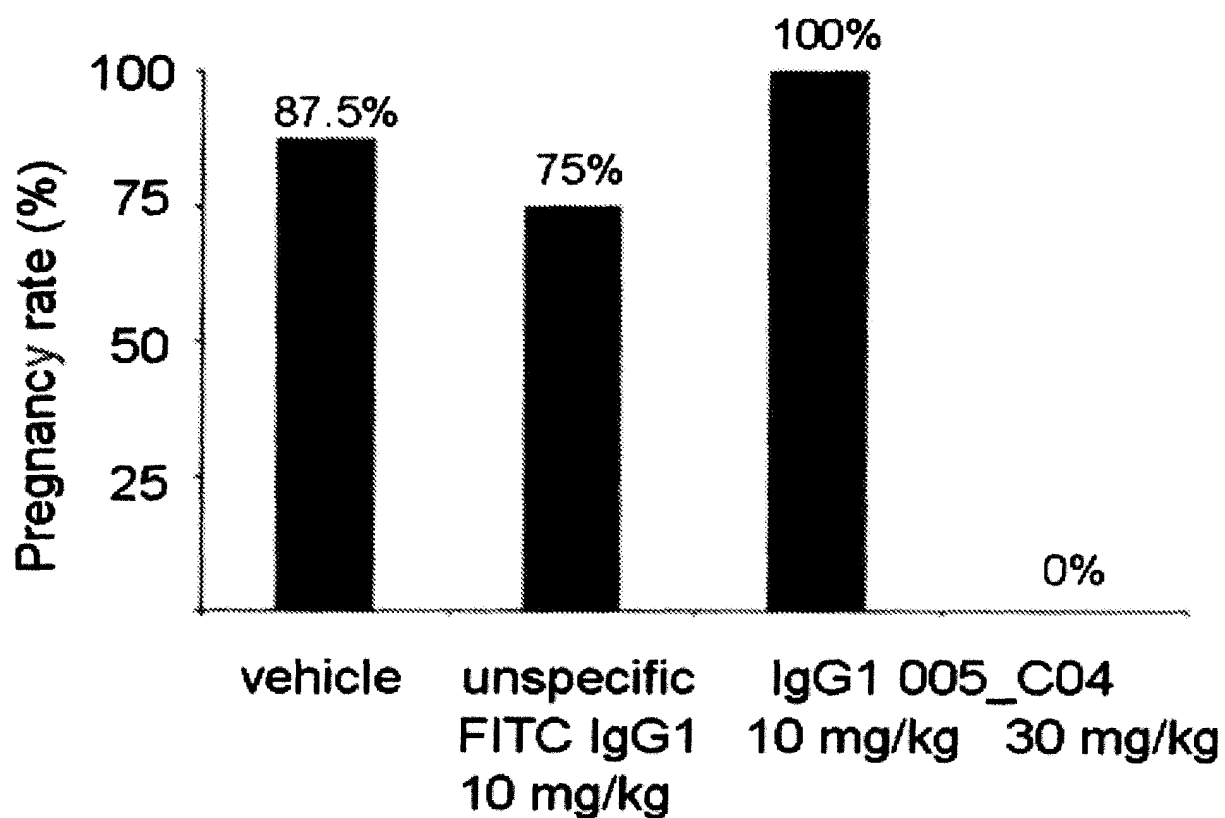
Figure 11: Contraceptive effect of neutralizing PRLR antibodies in mice**A Pregnancy rates**

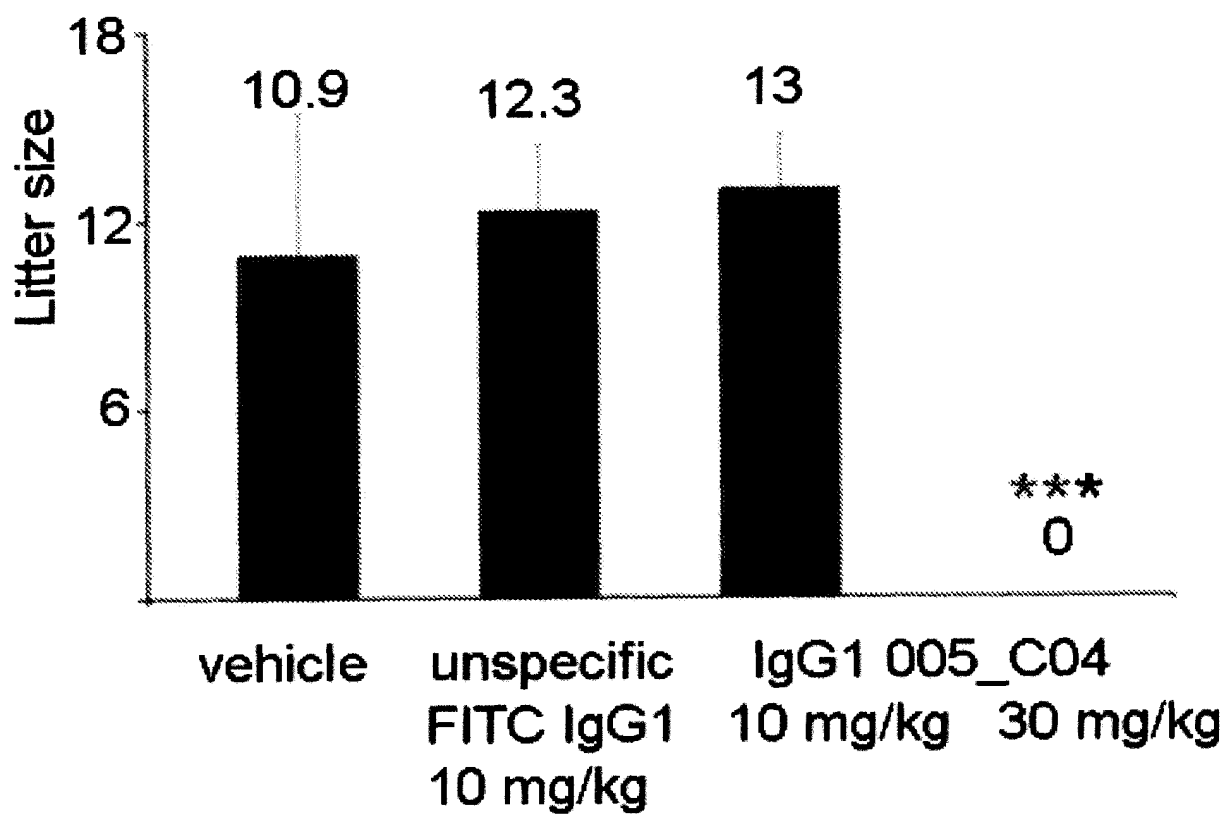
Figure 11: Contraceptive effect of neutralizing PRLR antibodies in mice**B Litter size**

Figure 12: Kabat Numbering of framework amino acid positions according to Johnson and Wu (Nucleic Acids Res. 2000, 28, 214-218)

VL chain:	
Kabat No. LFR1	L1 L2 L3 L4 L5 L6 L7 L8 L9 L11 L12 L13 L14 L15 L16 L17 L18 L19 L20 L21 L22 L23 Q/D S/I V L T Q P P S A S G T P G Q R V T I S C
Kabat No. LCDR1	L24 - L34 (X)n
Kabat No. LFR2	L35 L36 L37 L38 L39 L40 L41 L42 L43 L44 L45 L46 L47 L48 L49 W Y Q Q L P G T A P K L L I Y
Kabat No. LCDR2	L50 - L56 (X)n
Kabat No. LFR3	L57 L58 L59 L60 L61 L62 L63 L64 L65 L66 L67 L68 L69 L70 L71 L72 L73 L74 L75 L76 L77 L78 G V P D R F S G S K S G T S A S L A I S G L
Kabat No. LFR3	L79 L80 L81 L82 L83 L84 L85 L86 L87 R S E D E A D Y Y
Kabat No. LCDR3	L88 - L97 (X)n
Kabat No. LFR4	L98 L99 L100 L101 L102 L103 L104 L105 L106 L107 L108 L109 F G G T K L T V L G Q

VH chain:

Kabat NO. HFR1	H1 H2 H3 H4 H5 H6 H7 H8 H9 H10 H11 H12 H13 H14 H15 H16 H17 H18 H19 H20 H21 H22 E/Q V Q/E L L E S G G G L V Q P G G S L R L S C
Kabat NO. HFR1	H23 H24 H25 H26 H27 H28 A A S G F T
Kabat NO. HCDR1	H29 - H36 (X)n
Kabat NO. HFR2	H37 H38 H39 H40 H41 H42 H43 H44 H45 H46 H47 V R Q A P G K G L E W
Kabat NO. HCDR2	H48 - H66 (X)n
Kabat NO. HFR3	H67 H68 H69 H70 H71 H72 H73 H74 H75 H76 H77 H78 H79 H80 H81 H82 H82A H82B H82C H83 H84 H85 F T I S R D N S K N T L Y L Q M N S L R A E
Kabat NO. HFR3	H86 H87 H88 H89 H90 H91 H92 D T A V Y Y C
Kabat NO. HCDR3	H93 - H102 (X)n
Kabat NO. HFR4	H103 H104 H105 H106 H107 H108 H109 H110 H111 H112 H113 W G Q Q G T L V T V S S

Figure 13:

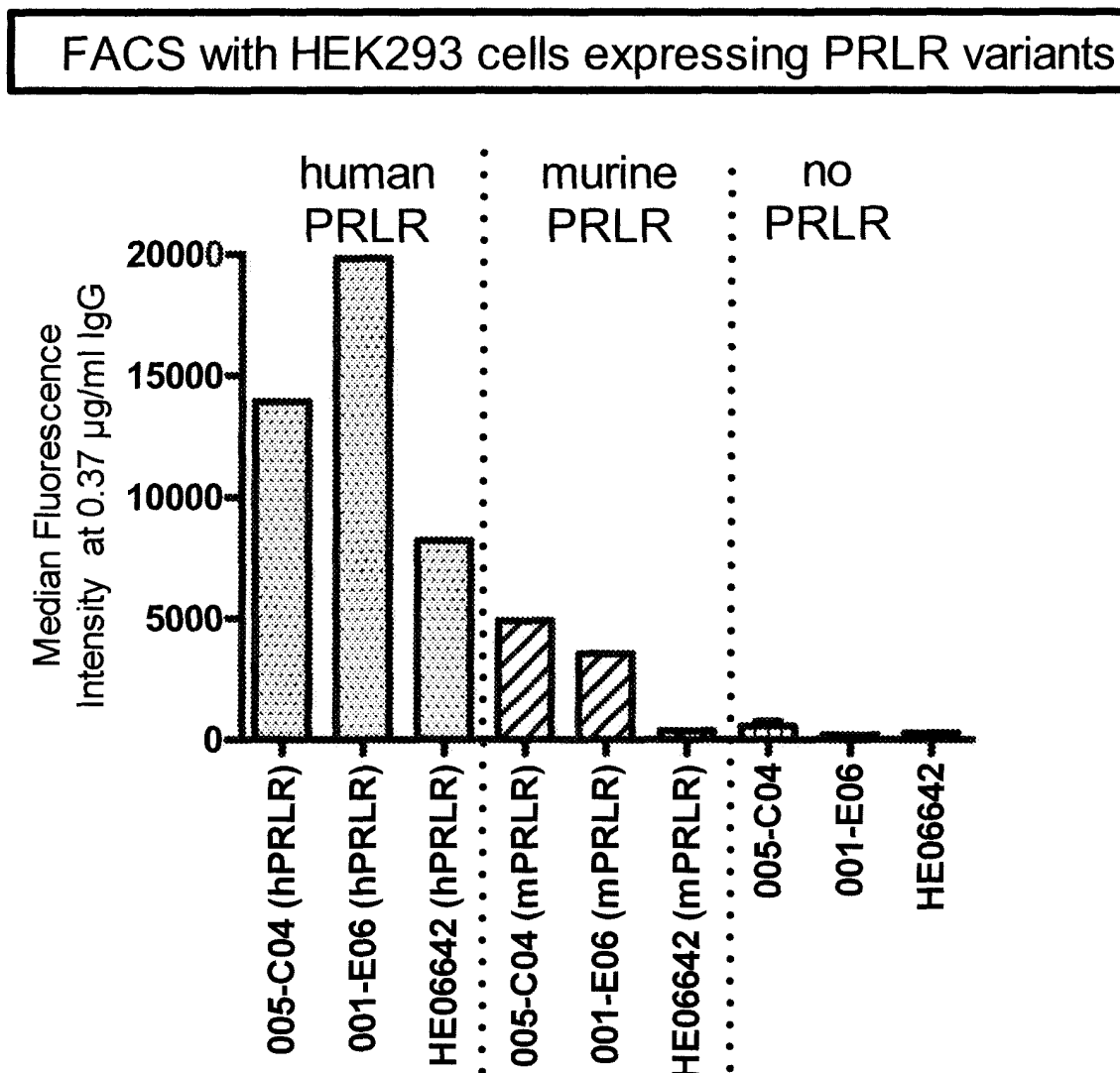


Figure 14: Effect of neutralizing PRLR antibodies on lactation in mice

A Percentage of daily litter weight gain if compared to litter weight on day 1 post partum

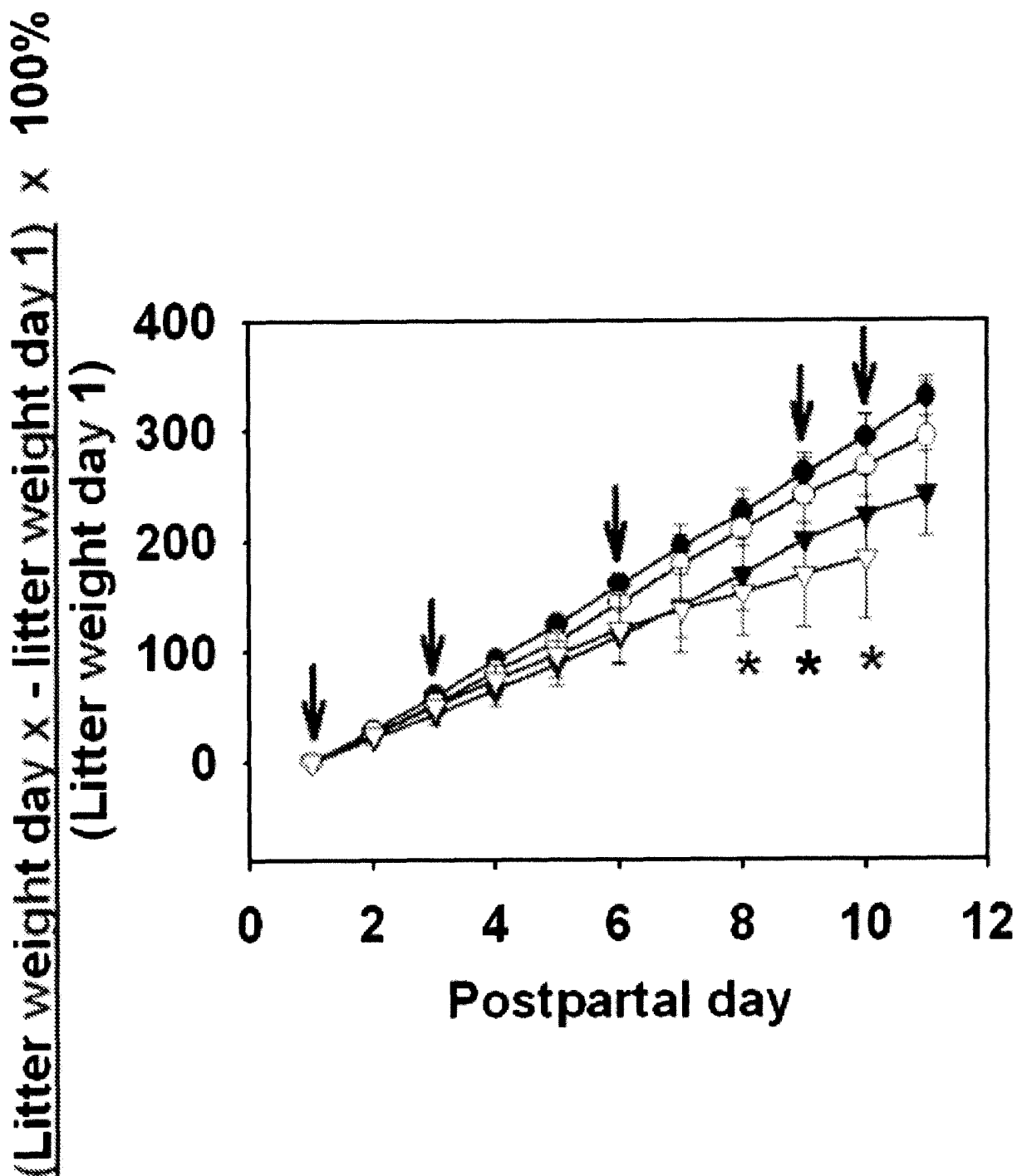


Figure 14: Effect of neutralizing PRLR antibodies on lactation in mice

B Litter weight gain from day to day expressed as percentage of litter weight on postpartal day 1

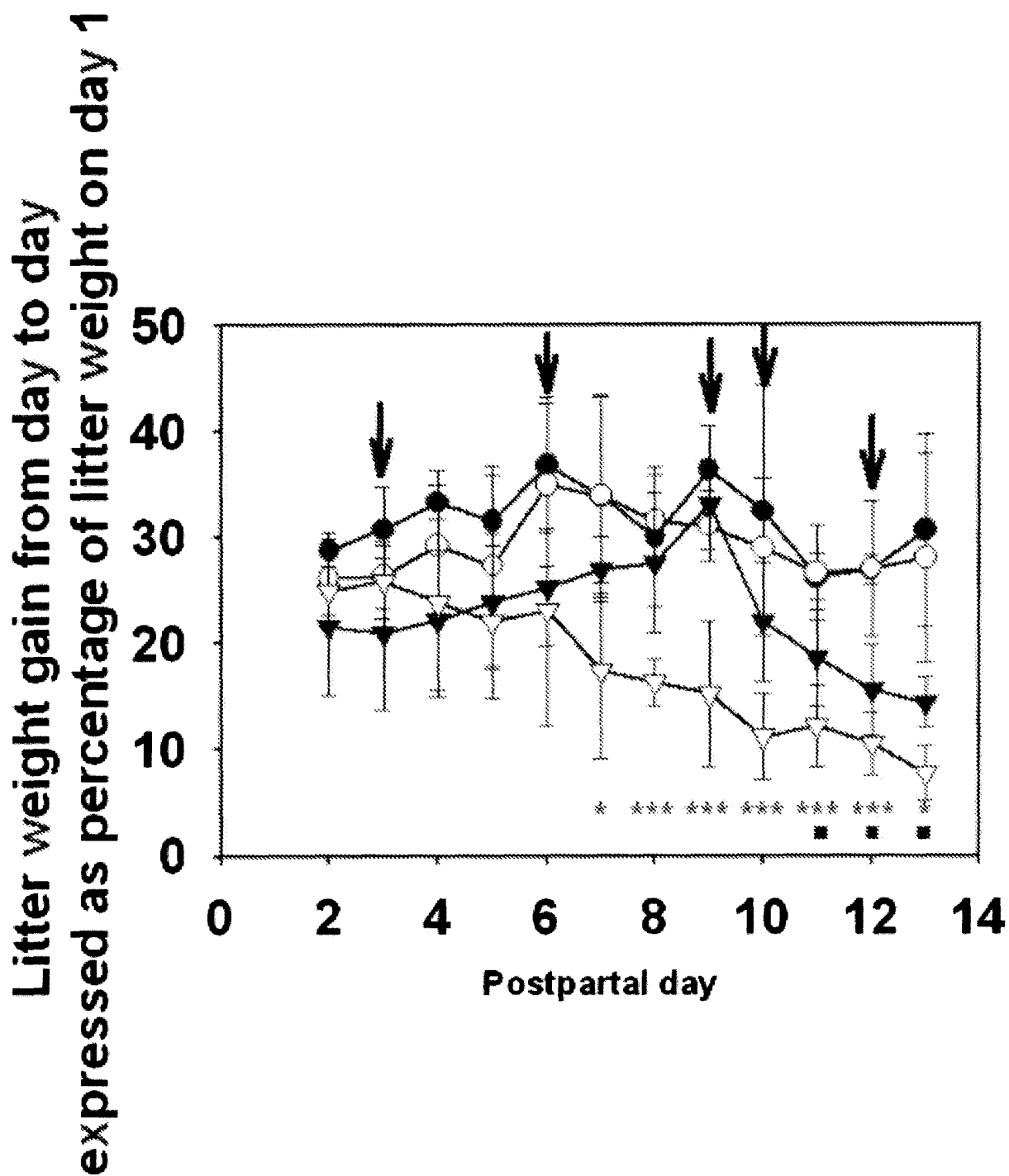


Figure 14: Effect of neutralizing PRLR antibodies on lactation in mice

C Involution of mammary glands in lactating mice treated with neutralizing PRLR antibodies

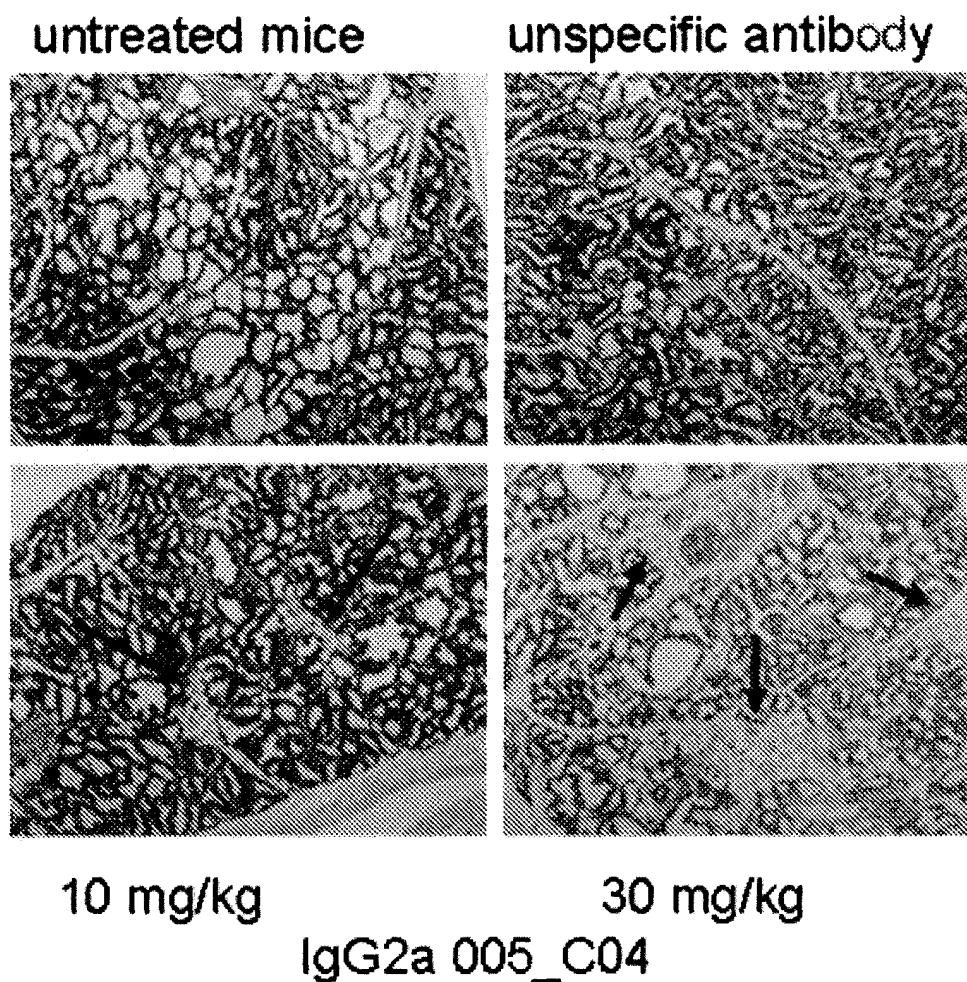


Figure 14: Effect of neutralizing PRLR antibodies on lactation in mice

D Expression of the milk proteins whey acidic protein (WAP), IGF1 and beta-casein (Csn2) is severely reduced in mammary glands from lactating mice treated with neutralizing PRLR antibodies

Part 1 : Beta-casein (snCsn2) expression:

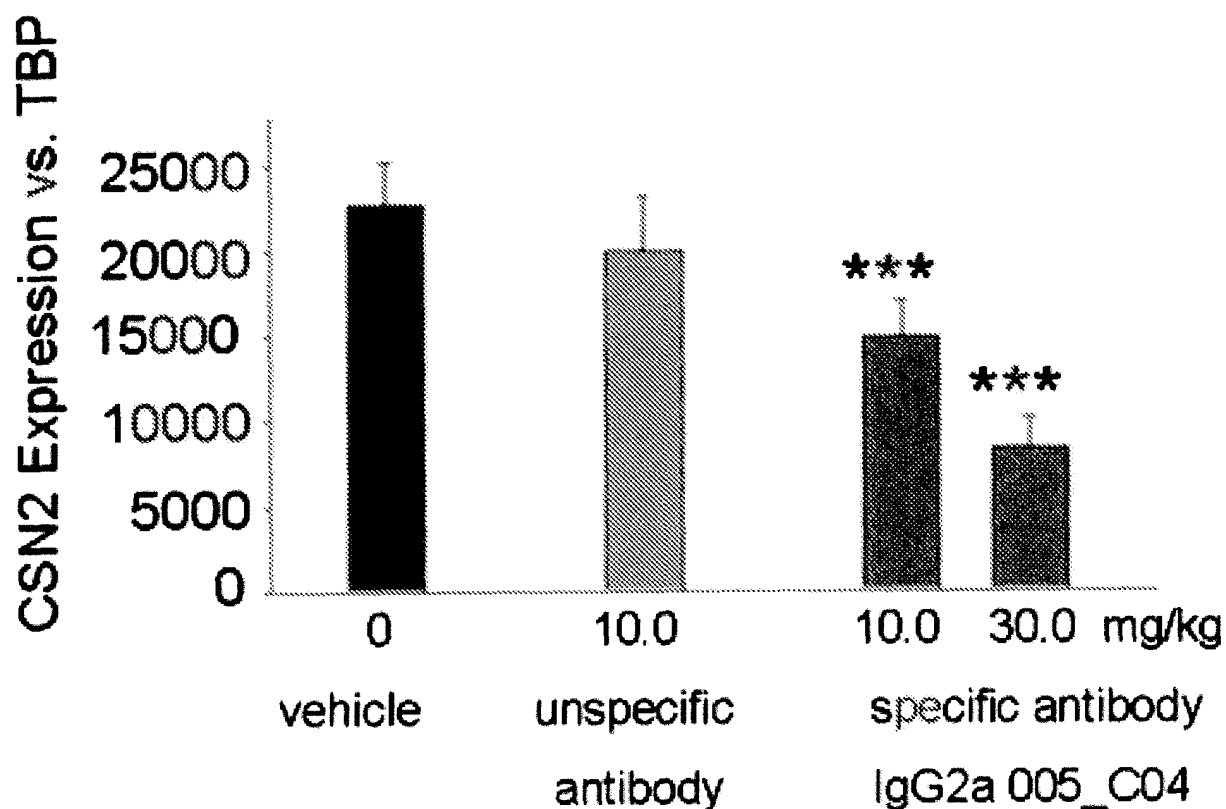


Figure 14: Effect of neutralizing PRLR antibodies on lactation in mice

D Expression of the milk proteins whey acidic protein (WAP), IGF1 and beta-casein (Csn2) is severely reduced in mammary glands from lactating mice treated with neutralizing PRLR antibodies

Part 2: WAP expression:

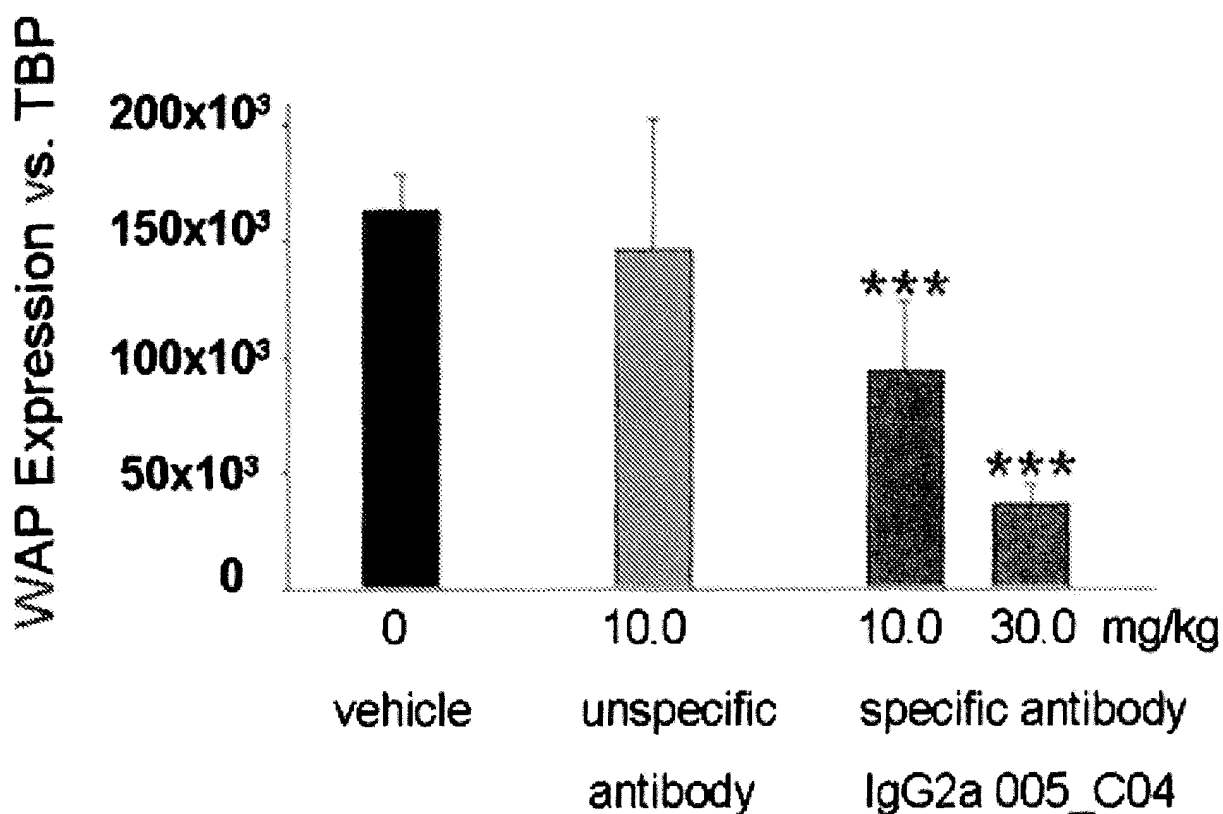


Figure 14: Effect of neutralizing PRLR antibodies on lactation in mice

- D** Expression of the milk proteins whey acidic protein (WAP), IGF1 and beta-casein (Csn2) is severely reduced in mammary glands from lactating mice treated with neutralizing PRLR antibodies

Part 3: IGF-1 expression

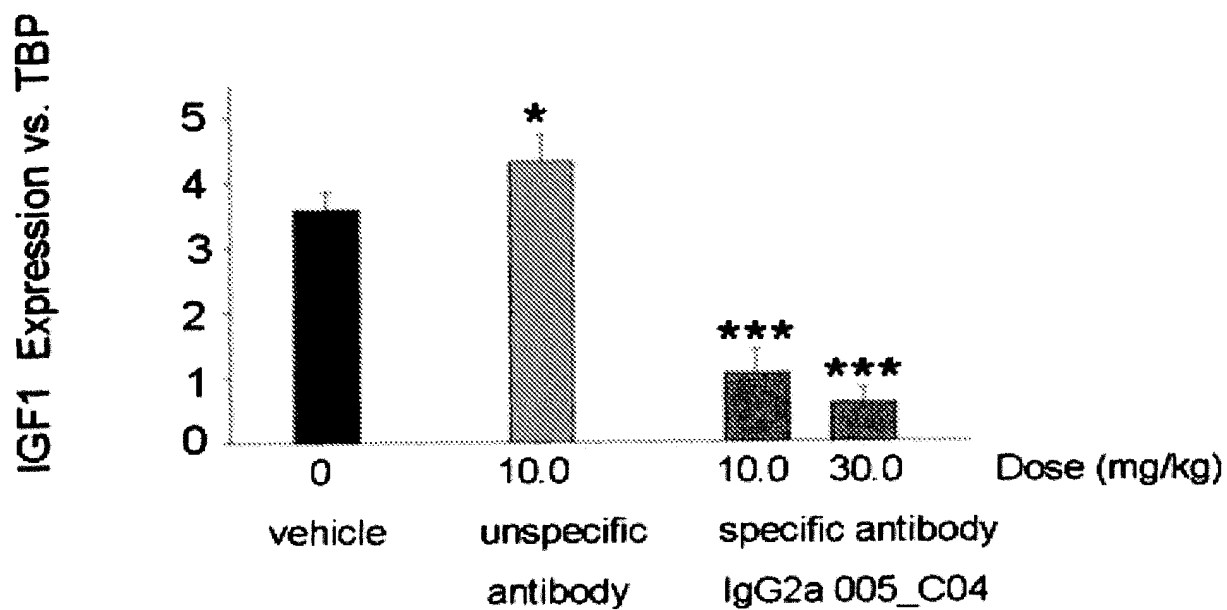


Figure 15: Effect of neutralizing PRLR antibodies on mammary gland epithelial cell proliferation and hyperplasia in a hyperprolactinemic model of benign breast disease

A Inhibition of side-branching in the mammary gland by neutralizing PRLR antibodies

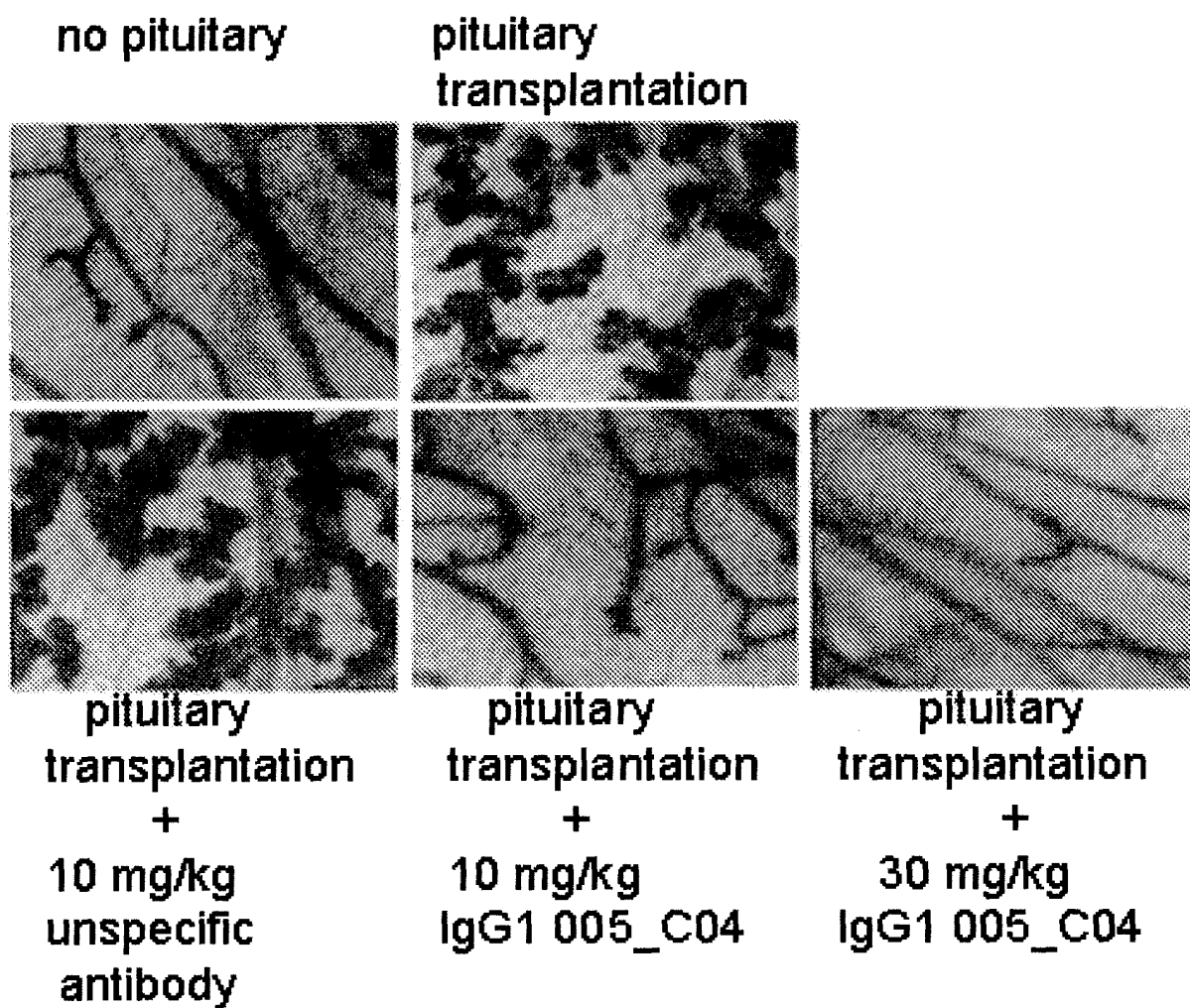


Figure 15: Effect of neutralizing PRLR antibodies on mammary gland epithelial cell proliferation and hyperplasia in a hyperprolactinemic model of benign breast disease

B Inhibition of mammary epithelial cell proliferation by using neutralising PRLR antibodies

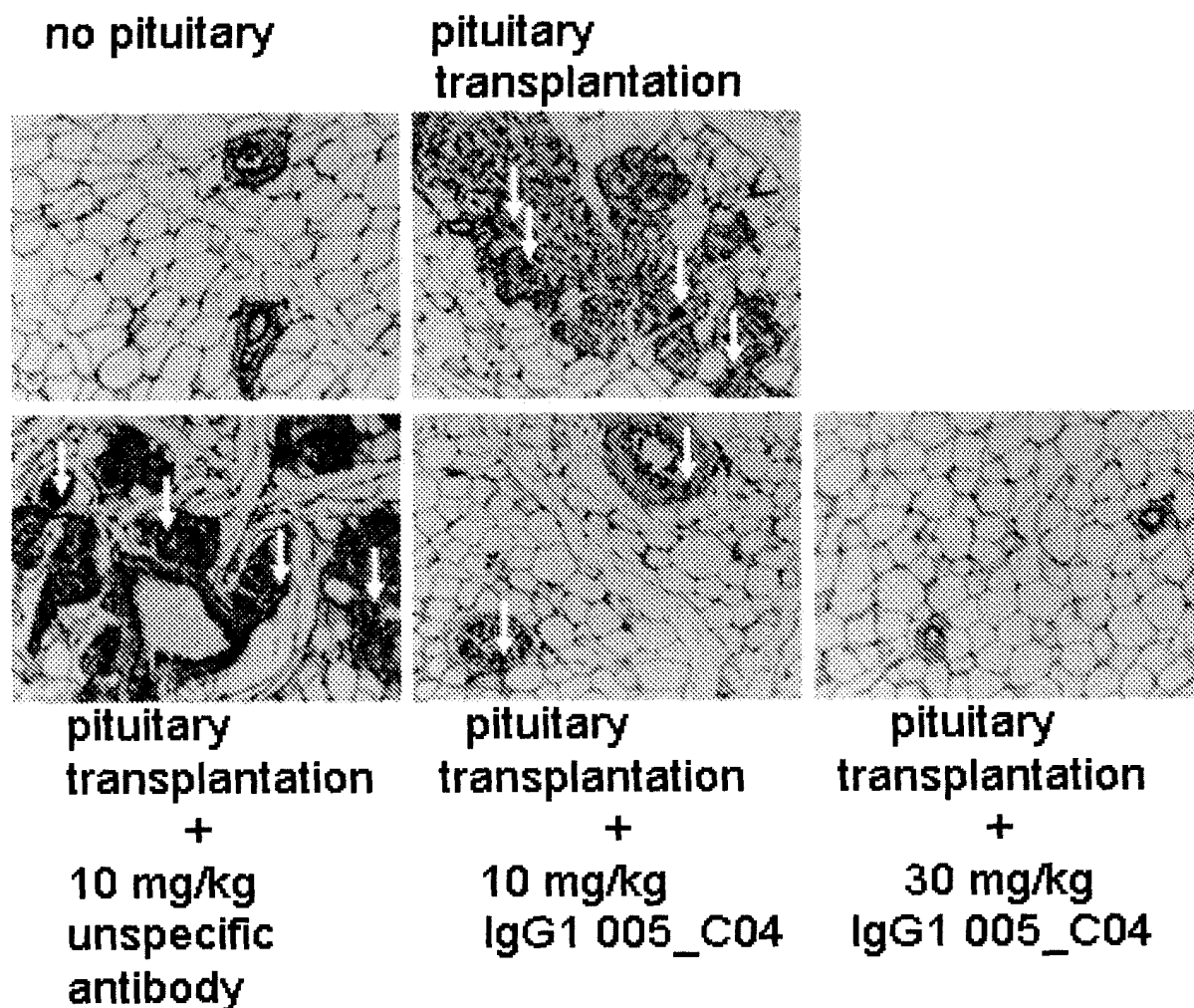


Figure 15: Effect of neutralising PRLR antibodies on mammary gland epithelial cell proliferation and hyperplasia in a hyperprolactinemic model of benign breast disease

C Inhibition of STAT5 phosphorylation in mammary epithelial cells by application of neutralising PRLR antibodies

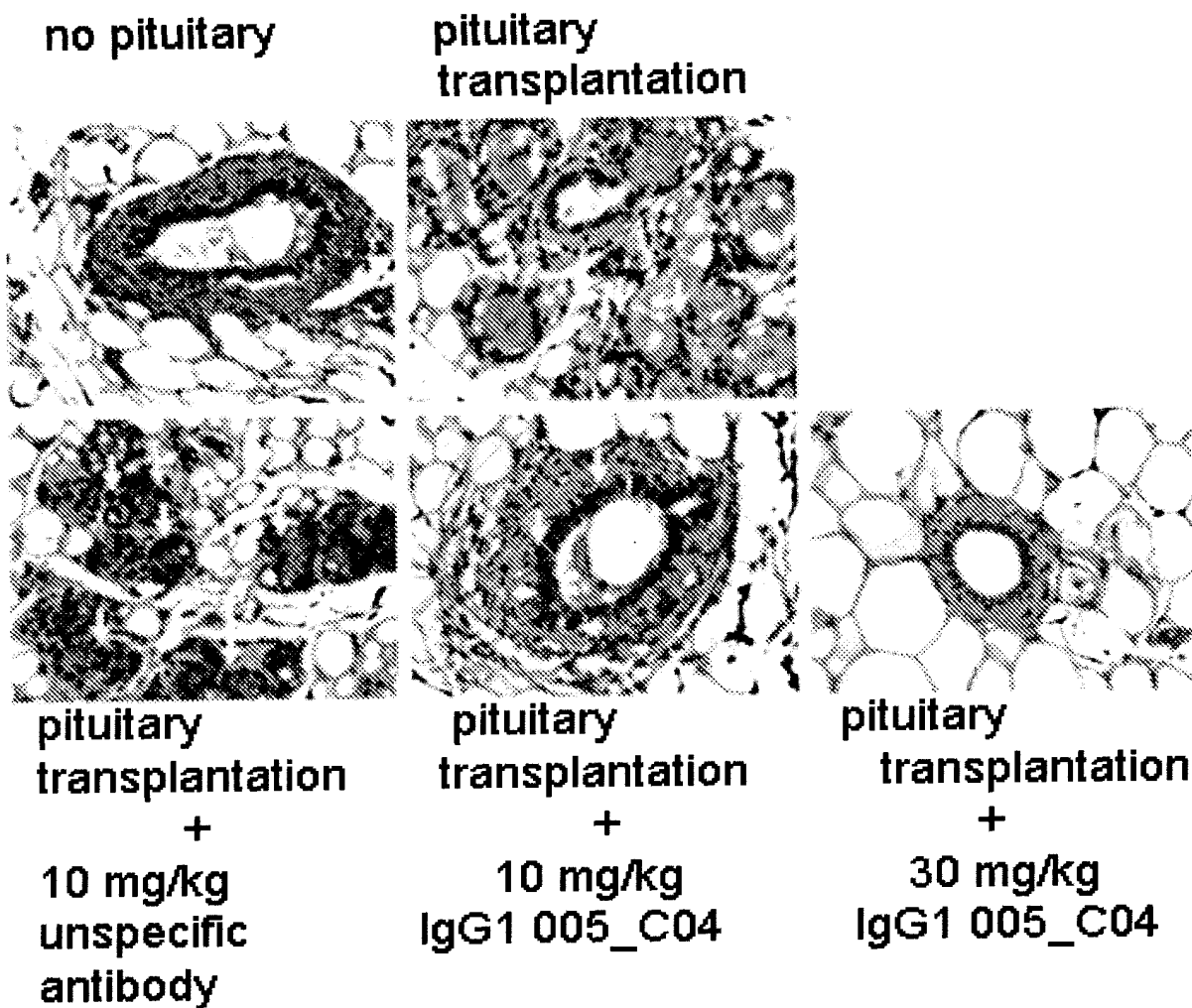


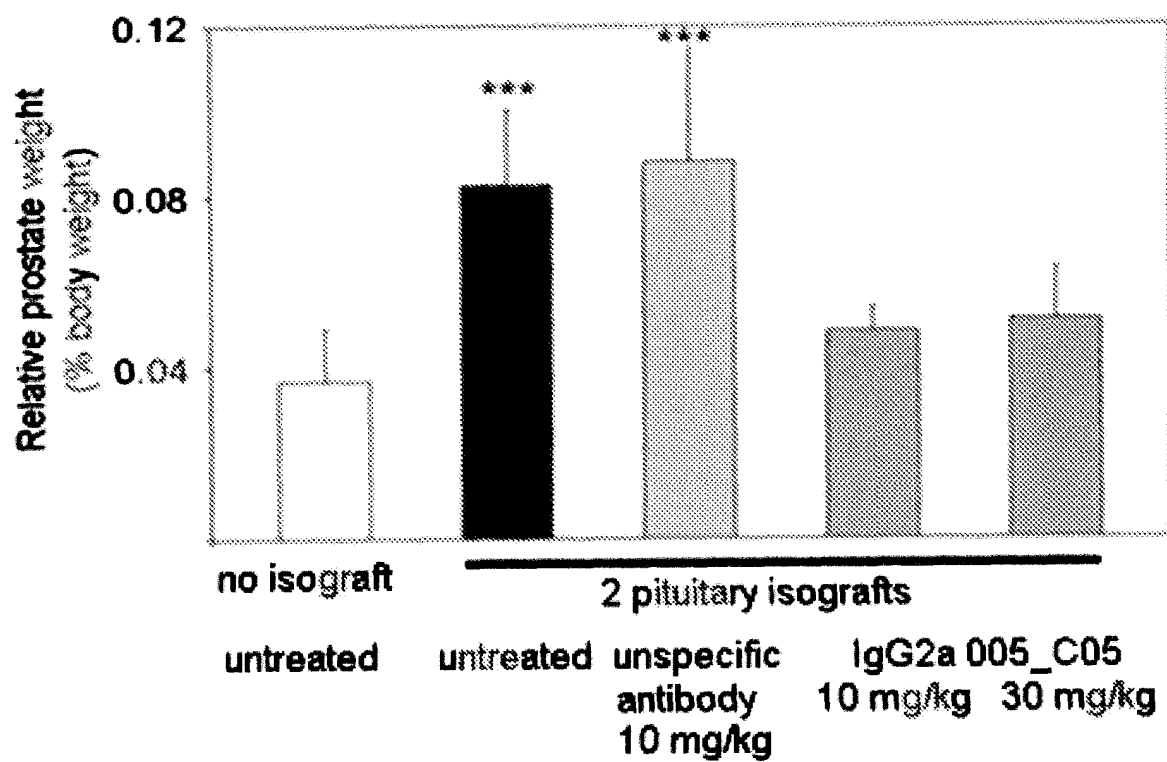
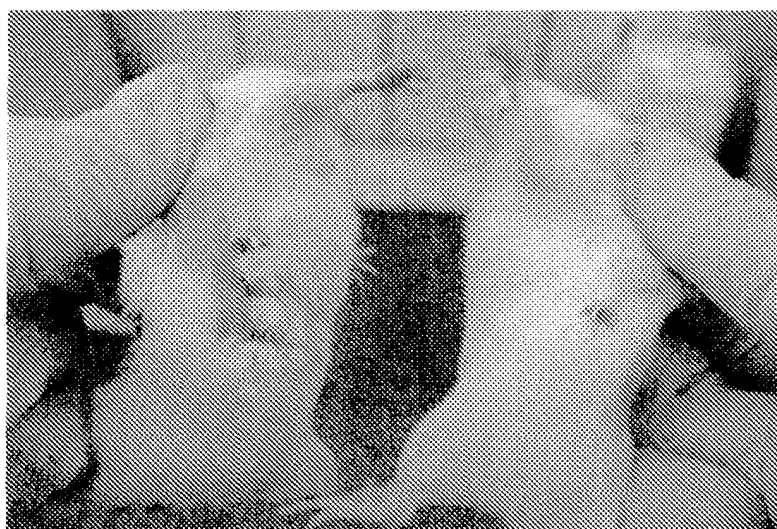
Figure 16: Effect of neutralising PRLR on inhibition of prostate hyperplasia

Figure 17: Effect of neutralizing PRLR antibodies on hair growth in the presence of hyperprolactinemia



No treatment

**IgG2a 005_C04
30 mg/kg**



**unspecific
antibody
10 mg/kg**

**IgG2a 005_C04
10 mg/kg**

Figure 18: Effect of neutralizing PRLR antibodies on hair regrowth in male and female mice under normo- and hyperprolactinemic conditions.

A Analysis of female mice

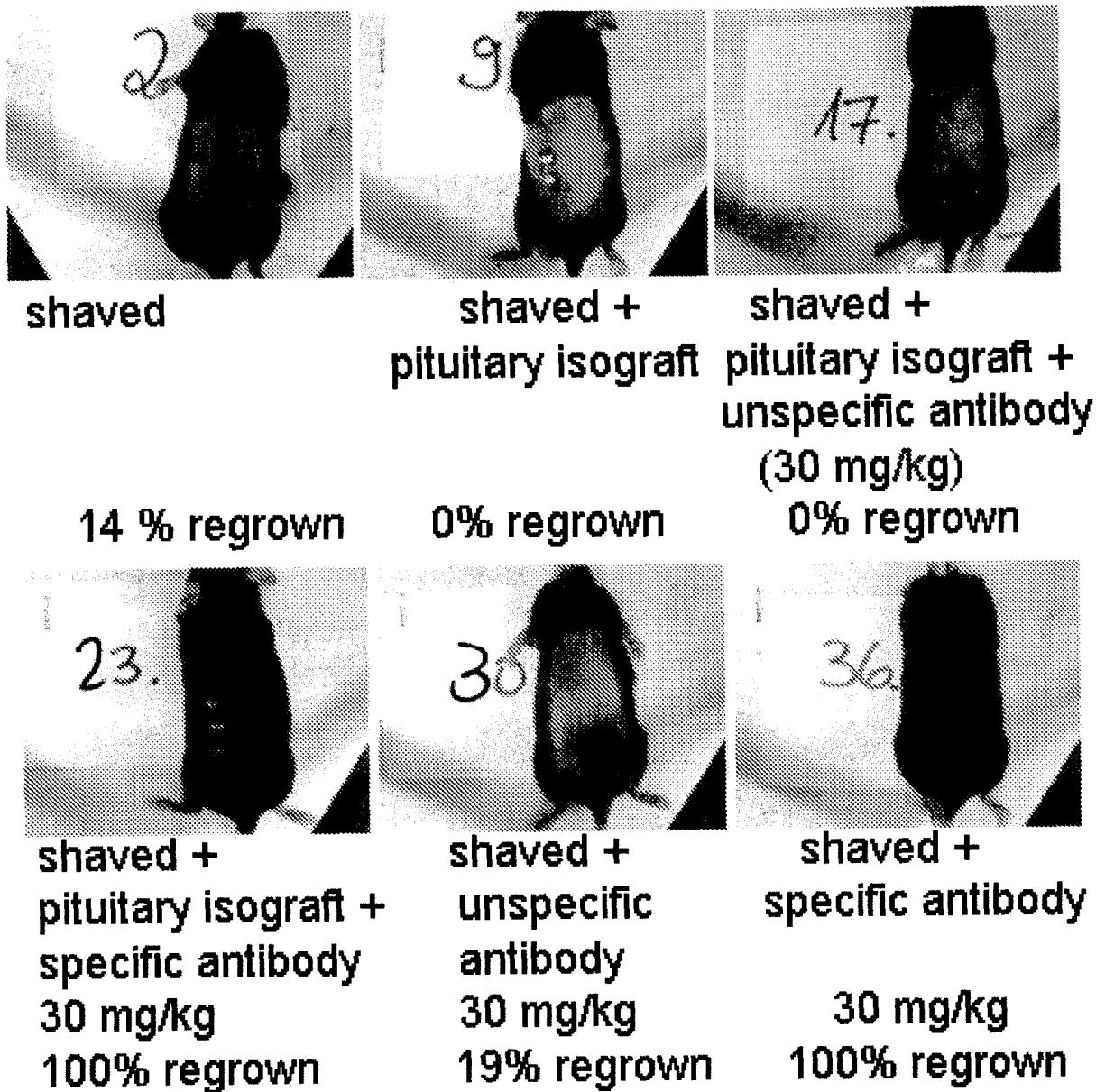


Figure 18: Effect of neutralizing PRLR antibodies stimulation hair regrowth in male and female mice under normo- and hyperprolactinemic conditions.

B Analysis of male mice

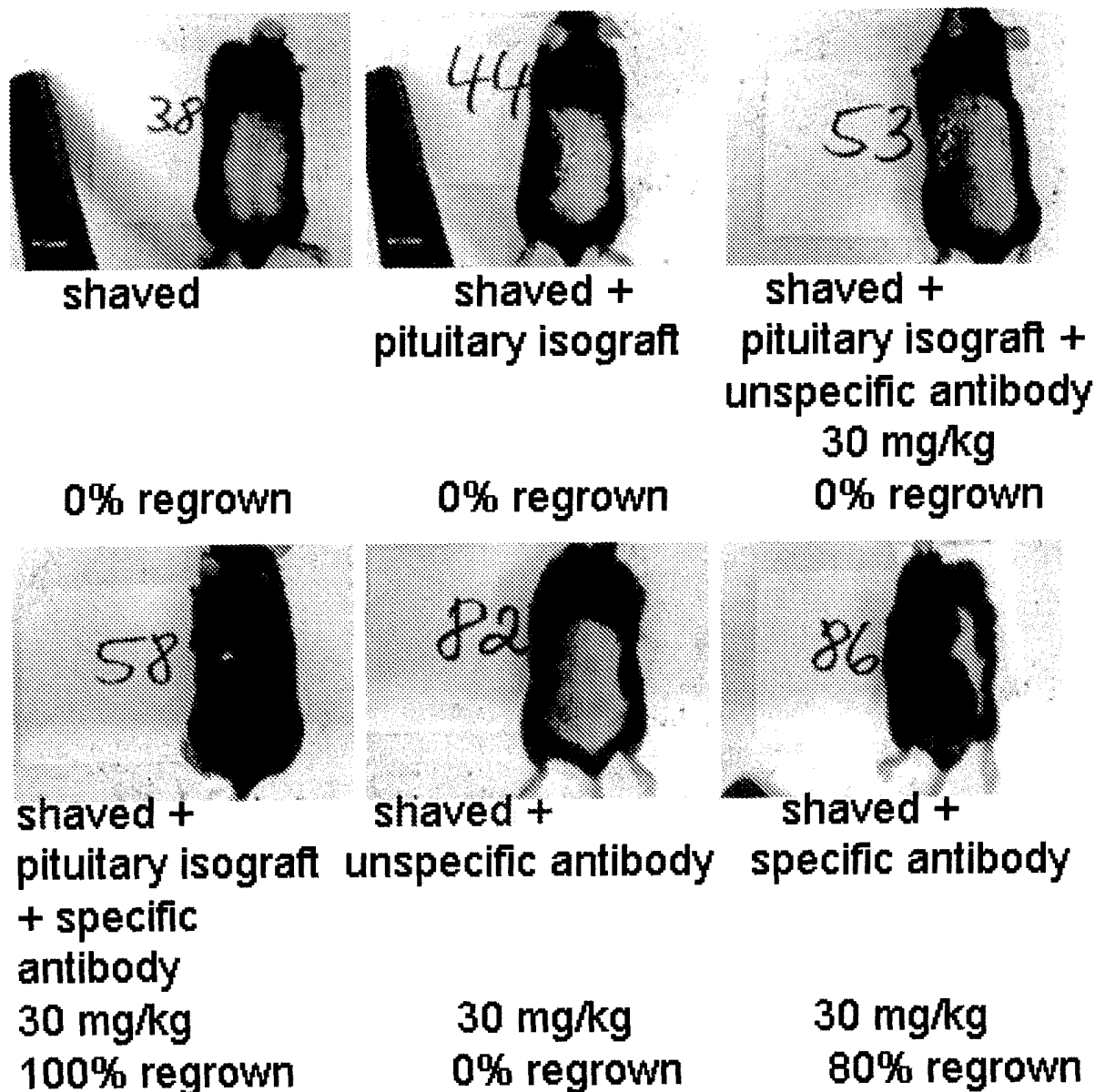
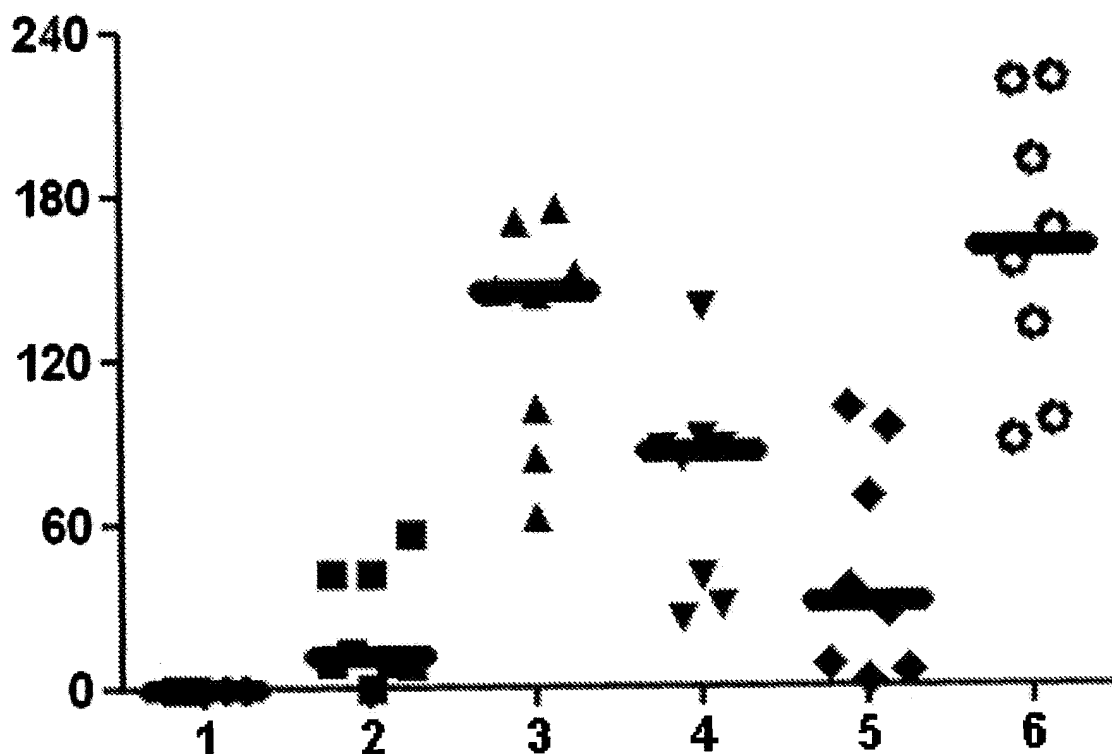


Figure 19: Effect of neutralizing PRLR antibodies on epithelial cell proliferation in the mammary gland under combined estrogen plus progestin hormone therapy



group 1: ovariectomized mice treated with vehicle

group 2: ovariectomized mice treated with 100 ng estradiol

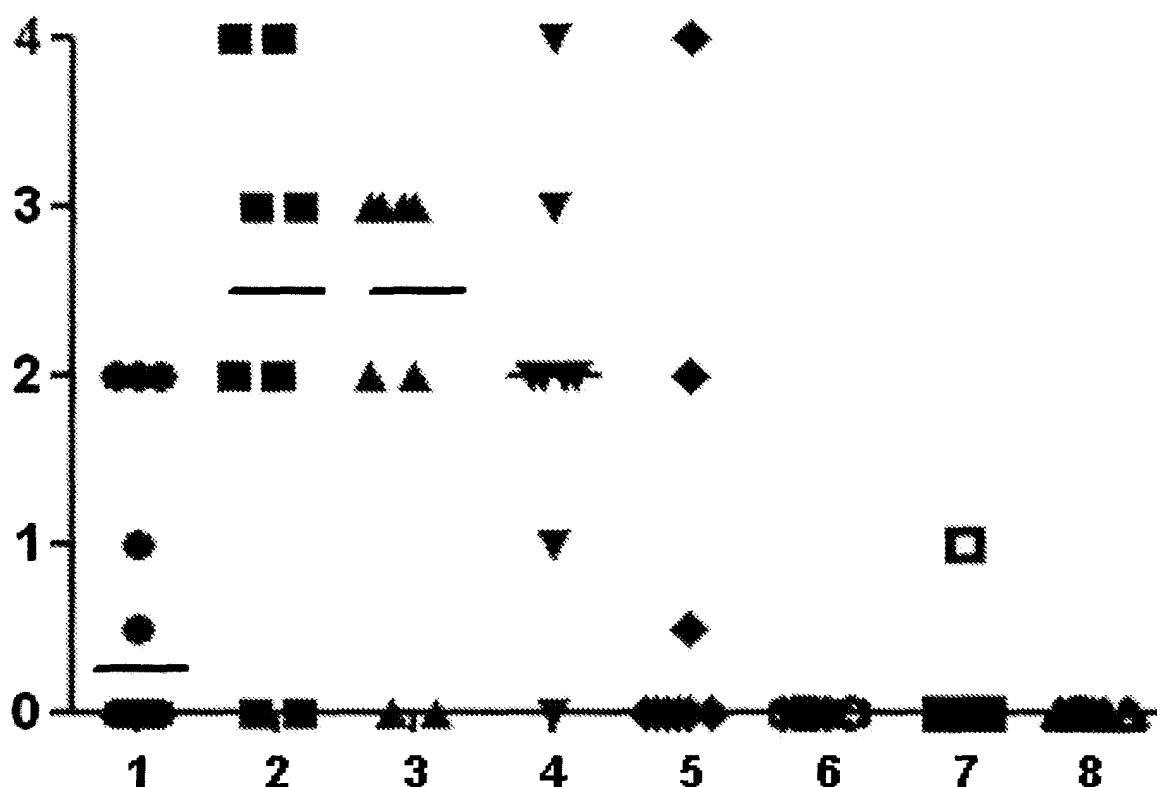
group 3: ovariectomized mice treated with 100 ng estradiol (E) + 100 mg/kg progesterone (P)

group 4: ovariectomized mice treated with E + P and specific antibody
005 C04 at a dose of 10 mg/kg once weekly

group 5: ovariectomized mice treated with E+P and specific antibody
005 C04 at a dose of 30 mg/kg once weekly

group 6: ovariectomized mice treated with E + P and unspecific control antibody at a dose of 30 mg/kg once weekly

Figure 20: Effect of neutralizing PRLR antibodies on endometriosis interna (adenomyosis uteri)



group 1: no pituitary isograft

group 2: pituitary isograft

group 3: pituitary isograft + unspecific antibody 30 mg/kg once weekly

group 4: pituitary isograft + unspecific antibody 30 mg/kg twice weekly

group 5 : pituitary isograft + specific antibody 005_C04 10 mg/kg once weekly

group 6: pituitary isograft + specific antibody 005_C04 10 mg/kg twice weekly

group 7: pituitary isograft + specific antibody 005_C04 30 mg/kg once weekly

group 8 : pituitary isograft + specific antibody 005_C04 30 mg/kg twice weekly

Figure 21. Binding studies with matured 006-H08 variants (Fabs from E. coli supernatants). HTRF assay results measured at different time points using the extracellular domain of the human PRLR.

