

(Review)

Extragonadal LH/hCG action - not yet time to rewrite textbooks

Tomi Pakarainen^a, Petteri Ahtiainen^a, Fu-Ping Zhang^b, Susana Rulli^c, Matti Poutanen^a and

Ilpo Huhtaniemi^{a,d}

^a*Department of Physiology, University of Turku, Kiinamylynkatu 10, 20520 Turku, Finland,*

^b*Department of Physiology, University of Helsinki, Biomedicum Helsinki, Haartmaninkatu 8, 00014 Helsinki, Finland, ^cInstitute of Biology and Experimental Medicine, CONICET, Vuelta de Obligado 2490 Buenos Aires, Argentina, and ^dInstitute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN,*

UK

Correspondence:

Prof. Ilpo Huhtaniemi
Institute of Reproductive and Developmental Biology
Imperial College London
Hammersmith Campus, Du Cane Road
London W12 0NN, UK
FAX: +44-2+-75942104
E-mail: ilpo.huhtaniemi@imperial.ac.uk

Abstract

Gonadotropins are indispensable in both sexes in the regulation of gonadal sex steroid production and gametogenesis. In addition to their well established classical actions, numerous recent publications have indicated the presence and function of luteinizing hormone/chorionic gonadotropin receptors (LH/hCG-R) in a variety of extragonadal tissues. However, the physiological significance of such effects has remained unclear. We have generated two genetically modified mouse models, one with excessive production of hCG and the other with targeted disruption of *LH/hCG-R* gene, and used them to address the functions of LH and hCG. Numerous gonadal and extragonadal phenotypes were found in the models with the two extremes of LH/hCG action. However, when the extragonadal effects were scrutinized in greater detail, they all appeared to arise through modification of gonadal function, either through enhanced or inhibited response to LH/hCG stimulation. Hence, further evidence is needed before the extragonadal LH/hCG-R expression can be considered functionally significant.

Keywords: luteinizing hormone (LH), human chorionic gonadotropin (hCG), LH/hCG receptor (R), testis, ovary, transgenic mouse, knockout mouse, gonadal function, extragonadal LH/hCG action

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1. Introduction

The role of pituitary luteinizing hormone (LH) in the regulation of normal reproductive functions of males and females is well established. According to a generally accepted dogma, LH is secreted from the anterior pituitary gland under the stimulatory control of hypothalamic gonadotropin-releasing hormone (GnRH), and it acts by binding to its specific receptors (LH/hCG-R), which belong to the family of seven-times membrane spanning G-protein coupled receptors (McFarland *et al.*, 1989; Ascoli *et al.*, 2002). In gonads, the LH/hCG-Rs are situated in testicular Leydig cells and in ovarian theca, granulosa and luteal cells (Segaloff and Ascoli, 1993; Dufau, 1998). LH has two roles in the regulation of gonadal function, i.e. to stimulate sex steroid synthesis and to regulate gametogenesis. Another hormone, human (h) chorionic gonadotropin (CG), is structurally related to LH, and both hormones bind to the common LH/hCG-R. The production of CG is confined to human, primate and equine placentas, whereas only pituitary LH is produced in rodents, including the mouse. hCG is essential for the maintenance of human pregnancy by stimulating progesterone production of corpus luteum gravidarum, and it also stimulates testosterone production of fetal Leydig cells (Huhtaniemi *et al.*, 1977; Huhtaniemi and Pelliniemi, 1992; Huhtaniemi, 1994). In contrast to humans, the mouse genome does not contain a gene encoding chorionic gonadotroin. In contrast to humans, the sexual differentiation of male mice *in utero* can occur totally independent of LH/LHR action (El-Gehani *et al.*, 1998; Pakarinen *et al.*, 2002).

Numerous recent studies have provided evidence for the expression of LH/hCG-Rs in several extragonadal tissues, thus suggesting additional non-classical actions for LH and hCG (Rao, 2001; Zhang et al., 2001b; Fields and Shemesh, 2004; Filicori et al., 2005). The novel actions suggested include improvement of blastocysts implantation (Han et al., 1999), regulation of oviduct and cervix function (Lei et al., 1993a; Lin et al., 2003), improvement of endometrial angiogenesis and growth (Filicori et al., 2005), and a role in brain development and sexual behaviour (Lei et al., 1993b; Apaja et al., 2004). However, despite the numerous findings on existence of extragonadal LH/hCG-Rs their physiological significance has remained unclear, and *in vivo* evidence for their functionality and necessity is lacking. We have generated two genetically modified mouse models to address LH/hCG actions *in vivo* from two opposite directions. One model is the hCG overexpressing transgenic (TG) mouse, producing either moderately or highly elevated levels of circulating bioactive LH/hCG (Rulli et al., 2002; Rulli et al., 2003). The other model is the *LHR* knockout mouse (LuRKO), totally devoid of functional LH/hCG-Rs and responsiveness to LH/hCG stimulation (Zhang et al., 2001a). We concentrate in this review on the findings in these two mouse models that are relevant for the unravelling of a physiological role for the extragonadally expressed LH/hCG-Rs.

2. Classical gonadal actions of gonadotropins

In females both gonadotropins are essential for fertility. LH/hCG-Rs are located in the ovary in theca cells, luteinizing granulosa cells and after ovulation in luteal cells, in the testis they are expressed in Leydig cells (Segaloff and Ascoli, 1993). Follicle-stimulating hormone (FSH) -R, in turn, is exclusively expressed in ovarian granulosa cells and testicular Sertoli cells (Simoni et al., 1997). Follicular maturation and reproduction in females need the synergy and interaction of both follicular cell types; theca and granulosa cells (Gougeon, 1996). Under the influence of LH action, theca cells produce androgens, which thereafter are aromatized to estrogens in granulosa

cells. Both gonadotropins stimulate the expression of cytochrome P450-aromatase (P450-arom), which is needed for the aromatization of androgens to estrogens in granulosa cells (Hickey et al., 1988). Furthermore, FSH stimulus increases the expression of LHR and other genes in the granulosa cells of preovulatory follicles (Richards, 1994). At the end of follicular development an LH peak is needed for triggering the cascade of events finally leading to ovulation. Thereafter, LH maintains the progesterone production of corpus luteum. In males, LH is indispensable for the Leydig cell production of androgens and indirectly for the maintenance of spermatogenesis through stimulation of Sertoli cell function. In light of the recently produced mouse knockouts and human mutations discovered in the gonadotropin and gonadotropin receptor genes, the need of FSH for male fertility is relative (Themmen and Huhtaniemi, 2000; Huhtaniemi and Themmen, 2005).

3. Extragonadal effects of gonadotropins

Most of the extragonadal, and also many of the gonadal effects of gonadotropins are indirect consequences of the stimulation of gonadal steroid biosynthesis. However, LH/hCG-R expression has been found in various non-gonadal tissues in humans and many animal species (Table 1). This has given rise to numerous suggestions for their novel physiological functions. Reports on extragonadal FSH-Rs are fewer, but there are reports on their presence in the bovine cervix, suggesting for them a role in the relaxation and opening of this structure (Mizrachi and Shemesh, 1999). Moreover, extrapituitary GnRH receptors have been found, and a role has been suggested for them as autocrine/paracrine regulators in the embryo-endometrial interaction during implantation (Raga et al., 1998). However, despite numerous studies showing the existence of extragonadal gonadotropin receptors with different molecular and biochemical methods, the physiological importance of these receptors *in vivo* has remained uncertain. The extragonadal LH/hCG-R expression differs in many respects from that of the gonadal receptors

(Stewart, 2001). For instance, reports of the molecular weights of the receptor protein and mRNA transcripts of the non-gonadal LHRs are at variance with the gonadal receptor. Although most of the data on extragonadal LH/hCG-R expression and function appear at face value convincing, several caveats remain. Often only fragments of the LHR mRNA have been identified using RT-PCR, without evidence for functional full-length message. The size of immunoreactive LHR protein detected does not always agree with the authentic gonadal receptor, and the use heterologous antibodies reduce the specificity of immunodetection. Most of the functional data have been obtained from *in vitro* incubations and cell cultures, and compelling *in vivo* data are scarce. Moreover, human inactivating mutations of *LHR* have only gonadal phenotype, and all extragonadal effects can be explained by altered gonadal function (Themmen and Huhtaniemi, 2000).

In addition to extragonadal gonadotropin receptors, there are also reports on production of gonadotropin subunits outside the pituitary gland. *LH β* , *FSH β* and *common- α subunit* expression at mRNA and protein level has been found in gonads, suggesting a paracrine pituitary independent component of gonadotropin action (Markkula et al., 1995; Zhang et al., 1995b; Zhang et al., 1995a; Markkula et al., 1996; Wong and Zohar, 2004). However, as with the receptors, the evidence for a physiologically significant role of the extrapituitary sources of gonadotropins is missing.

4. hCG overexpressing transgenic mice

Transgenic (TG) mice overexpressing *hCG β* and *common α -subunits* under the human ubiquitin C promoter were produced by conventional pronuclear injection techniques (Rulli et al., 2002; Rulli et al., 2003). In these mice, the ubiquitin C promoter maintains low level TG expression in most tissue types, and its activity has been shown to start on embryonic day 10.5 (Schorpp et al., 1996). In the *hCG β* TG mice (hCG β +), the *hCG β* transgene is expressed also in

the pituitary cells expressing the endogenous *common α -subunit*. This enables dimerization of the TG and endogenous subunits to form biologically active hCG (human β /mouse α -subunit), inducing 3-4 fold elevation in hCG/LH bioactivity in males, but 30-fold increase in females compared to wild-type (WT) littermates (Rulli et al., 2002; Rulli et al., 2003). Phenotypic characterization of the hCG β + males revealed no obvious alterations, with the exception of slightly decreased testis weights most probably due to decreased FSH-levels seen in these males (Rulli et al., 2003). Simultaneously with our study, another group developed mice expressing the *hCG β -subunit* under the metallothionein (MT-1) promoter (Matzuk *et al.*, 2003). The male mice were infertile without elevated bioactive LH/hCG and without obvious testicular phenotype, and the authors speculate that high circulating hCG β -subunit could bind to the LH/hCG-R and antagonize the effect of normal circulating LH, thus interfering with spermatogenesis in a quantitative manner (Matzuk *et al.*, 2003). In the other TG mouse model, which expresses a fusion protein of the bovine LH β subunit and the hCG β C-terminal peptide (bLH β -CTP) under common α -subunit promoter, males did not show elevated LH/hCG-bioactivity and presented with no apparent phenotype (Risma *et al.*, 1995).

In contrast to hCG β + males, the hCG β + females presented with precocious puberty, disrupted estrous cycle, infertility and massive ovarian luteinization already as young adults (Rulli *et al.*, 2002). As expected, we found elevated estradiol concentrations in prepubertal females, but surprisingly not in adult age, apparently due to the massive hCG-induced luteinization of all ovarian structures. Accordingly, serum progesterone concentration was 40-100-fold and testosterone 3-8 fold elevated in adult hCG β + females (Rulli *et al.*, 2002). Clear extragonadal phenotypes were also observed: the females developed obesity, pituitary macroprolactinomas, mammary gland adenocarcinomas, and their bone density was elevated (Rulli *et al.*, 2002; Yarram *et al.*, 2003) (see below). However, all the extragonadal phenotypes of the hCG β + females were abolished by gonadectomy, indicating that ovarian hyperstimulation

and abnormal gonadal hormone production, rather than direct extragonadal hCG effects, was responsible for the extragonadal phenotypes observed in the hCG β + females (Figs. 1 and 2). Although there are some differences between ovarian steroidogenesis and ovarian tumor formation, many similarities exist between bLH-CTP overexpressing female mice and our mice, including the formation of pituitary and mammary gland tumors, obesity and infertility (Risma *et al.*, 1995; Rulli *et al.*, 2002; Kero *et al.*, 2003; Mann *et al.*, 2003; Mohammad *et al.*, 2003). The ovarian tumor formation in bLH β -CTP mice was found to be dependent on mouse strain (Keri *et al.*, 2000), and we have not yet bred our mice to those strains susceptible to formation of ovarian tumors (Keri *et al.*, 2000). Because the bioactive LH/hCG-levels and thus the ovarian steroidogenesis are higher in our mice than in the bLH β -CTP mice, we expect to see ovarian tumor formation also in our mice when bred into an appropriate strain of mice. In the MT-1 promoter driven hCG β -females, infertility and in some cases ovarian cysts were seen, and the authors speculated that this is a result of inhibition of the normal LH-binding and/or disturbing some ovarian paracrine factors by free hCG β -subunit rather than being a results of ovarian hyperstimulation, because no dimeric hCG was detected (Matzuk *et al.*, 2003).

In another model, we intercrossed the common- α and hCG β overexpressing TG mice to achieve double-TG mice expressing both subunits (hCG $\alpha\beta$ +). These mice presented with more than 1000-fold increase in serum LH/hCG bioactivity, because coexpression and dimerization of the two transgenic hCG subunits took place in most tissues, not only in the pituitary gland (Rulli *et al.*, 2003). The hCG $\alpha\beta$ + males presented prepubertally with Leydig cell adenomas originating from fetal Leydig cells, but these neoplasms disappeared around the time of puberty, along with the regression of fetal Leydig cells according to their normal life span (Ahtiainen *et al.*, 2005). In adult age, the hCG $\alpha\beta$ + males presented with elevated testosterone production accompanied by aggressive behaviour, hyperplasia of prostate and lower urinary tract obstruction with hydronephrosis and dilated urinary bladder without clear anatomical obstruction. The hCG $\alpha\beta$ +

males displayed qualitatively normal spermatogenesis, but were infertile apparently because of their aggressive behaviour upon breeding or the physiological (i.e. functional) obstruction of their lower urinary tract hindering normal ejaculation (Rulli et al., 2003). The extragonadal phenotypes observed in the hCG $\alpha\beta$ + males can be explained by the elevated testosterone production. However, further studies are needed because LH/hCG-R expression has been demonstrated in male urogenital organs (Table 1). Similar phenotype of adult male mice was observed in the other TG mouse model expressing both hCG-subunit under metallothionein promoter, although the development of fetal Leydig cell adenomas was not reported (Matzuk et al., 2003).

The hCG $\alpha\beta$ + females were a close phenocopy of the hCG β + females except for one particular difference. Instead of the highly luteinized ovaries seen in hCG β + females, the hCG $\alpha\beta$ + females presented with germ cell tumors already before age of two months, and the tumors grew rapidly causing death of the mice before age of 6 months, before mammary gland adenocarcinomas and large macroprolactinomas were developed (Rulli et al., unpublished results). Gonadectomy prevented all phenotypic changes also in the hCG $\alpha\beta$ + females. In contrast to our mice, female mice expressing both hCG-subunits under metallothionein promoter showed cystic and hemorrhagic ovaries with the presence of hyperplastic theca-cell layers resembling ovarian thecomas. No reports on the formation of germ cell tumors exist on these mice (Matzuk et al., 2003).

In summary, the TG models described above show that even in the presence of a very high level of hCG there were no extragonadal phenotypes in mice caused by extragonadal LH/hCG-R receptor activation. This was proven by the observation that all extragonadal phenotypes disappeared when the gonadal contribution to the responses was eliminated by gonadectomy.

5. LH/hCG-R knockout (LuRKO) mice

The *LHR* knockout (LuRKO) mouse was developed by homologous recombination replacing exon 11 of the mouse *LHR* gene with the *neo* gene (Zhang et al., 2001a). The 11th exon encodes the cytoplasmic and transmembrane parts of the LHR and its targeted disruption totally inactivates the function of the receptor. LHR inactivation causes multiple reproductive and non-reproductive phenotypes in the mice. The mice are born healthy with normal male and female appearance. However, in both sexes the LHR inactivation leads to multiple alterations in the reproductive tract starting at puberty. The lack of gonadal negative feedback on the hypothalamic-pituitary level induces elevated LH and FSH levels, whereas due to the gonadal unresponsiveness to LH circulating sex steroid concentrations are dramatically decreased, resulting in infertility in both sexes. The intratesticular testosterone level is reduced by 98% and the intraovarian estradiol level by >90%. The final preovulatory maturation of follicles is blocked, and consequently the females do not ovulate. The minimal sex steroid levels cause besides the absence of gametogenesis severe underdevelopment of the accessory sex organs. In males, spermatogenesis is arrested at the round spermatid stage, and the number and size of Leydig cells are remarkably reduced (Zhang et al., 2001a). Similar observations have been reported by Lei *et al.* with their LHR-KO mouse model (Lei et al., 2001). In females, the Graafian follicles do not develop beyond the antral stage and the ovaries reveal increased follicular apoptosis (Pakarainen et al., 2005b). In conclusion, poorly developed secondary sex characteristics and infertility are observed in both sexes. Further detail about the phenotype of this model are in our previous reports (Zhang et al., 2001a; Zhang et al., 2003; Zhang et al., 2004; Pakarainen et al., 2005c; Pakarainen et al., 2005b; Pakarainen et al., 2005a).

LHβ KO phenotype has been recently reported (Ma et al., 2004). As expected, the phenotype closely resembles that of *LHR* KO mice, with infertility of both sexes, hypoplastic sex organ development and reduced androgen and estrogen levels. Spermatogenesis was arrested, similar to

LHR KO mice, at the round spermatid stage and follicular development was arrested at the antral follicular stage. However, the *LH β* KO mice, at variance with *LHR* KO mice, were reported to have unaltered FSH levels. As expected, exogenous hCG treatment showed a response in *LH β* KO mice and could even lead to ovulation in females.

6. Bone phenotypes of LuRKO and hCG TG mice

There are data on LHR expression in the bone based on Western blotting, immunolocalization and RT-PCR of mouse osteoblasts (Yarram et al., 2003). However, these studies were unable to show specific binding of iodinated hCG or hCG-stimulation of cAMP or phosphorylated ERK1/2, which findings left the functional significance of the positive findings open. This prompted us to look for a bone phenotype in the LuRKO and hCG $\alpha\beta$ + mice as evidence for or against direct actions of LH/hCG on the bone. LuRKO males but not females had decreased bone mineral density (BMD), whereas the hCG $\alpha\beta$ + females had increased, but males unaltered BMD in comparison to WT controls (Fig. 2). When the hCG $\alpha\beta$ + or WT females were ovariectomized, there was in both cases a significant decrease in BMD to a similar reduced level (Fig. 2). Taken together these data indicated that all effects found on bone density in the WT and the two genetically modified mouse models could be explained by modifications of gonadal activity. Most conspicuously, the increase in BMD seen in hCG $\alpha\beta$ + females was totally abolished and reversed to the same low level as in ovariectomized WT females, despite the persistent elevation of hCG in TG females (Yarram et al., 2003). It is apparent that ovarian estrogen production was the main factor determining BMD in these models. The findings, in fact, were quite similar with many of the other data on extragonadal LH/hCG action; they demonstrate low level of mRNA or protein expression, but the functional data often remain inconclusive.

7. Orthotopic ovarian transplantation in LuRKO mice

Due to the difficulties in discriminating between direct and indirect actions of LH/hCG and to clarify the real functional significance of the extragonadal LH/hCG-R *in vivo*, we carried out orthotopic ovarian transplantations in LuRKO mice (Pakarainen et al., 2005a). We replaced bilaterally the ovaries of LuRKO mice with pieces of WT ovaries by a method described previously (Lavebratt et al., 1998). Thus, the transplanted mice were devoid of functional LHRs in all other tissues except for the transplanted WT ovarian tissue. A dramatic suppression of endogenous LH level of LuRKO mice indicated onset of sex steroid production by the transplanted tissue, which in turn induced accessory sex organ growth, and macroscopically the transplanted LuRKO (LuRKO-TR) mice were indistinguishable from WT mice at the age of 3 months. Furthermore, the transplanted ovaries functioned in a normal cyclic manner and follicles of all developmental stage could be seen upon histological study (Fig. 3). Test breedings of the LuRKO-TR mice indicated that they had gained normal fertility and were able to deliver healthy offspring (Table 2). Fertility rate of the LuRKO-TR females was slightly lower than that of similarly transplanted WT control females. However, the difference was not statistically significant, and it was likely due to the fact that transplantations were technically more demanding in LuRKO mice because of their smaller ovaries and uteri at the time of transplantation. The complete recovery of fertility in LuRKO females after ovarian transplantation, including normal estrous cycle, mating behaviour, pregnancy rate, delivery and normal nursing and feeding of offspring, clearly indicated that extragonadal LH/hCG-R are redundant for mouse reproductive functions.

Rao et al. studied the implantation capability of their *LHR* KO model by stimulating the mice with estradiol, PMSG and hCG, and made *in vitro* fertilizations (IVF) (Rao and Lei, 2002). They failed to induce implantation and pregnancy, and considered this as evidence for a functional role of the uterine LHRs. However, there are multiple technical limitations in this type of

experiments. Especially if the results are negative, in the absence of positive control, it remains a possibility that the exogenous hormone treatment has not been able to simulate completely that caused by endogenous hormones. The same authors were also unable to achieve pregnancies following orthotopic transplantations of WT ovarian tissue in their *LHR* KO mice (Chudgar et al., 2005), which negative finding also remains inconclusive.

7. Concluding remarks

In contrast to the recent suggestions on direct actions of LH/hCG-R in multiple non-gonadal tissues, our studies with the two mouse models were unable to provide data in support of such actions. In contrast, in light of our findings, the extragonadal LH/hCG-R appeared redundant. The hCG overexpressing TG mouse models produce pharmacological levels of hCG, which was shown to induce tumorigenic effects in female mice. However, the tumor formation was directly related to the gonadal sex steroid production, and the pituitary and mammary gland tumorigenesis could be prevented by ovariectomy. Thus, even at pharmacological levels of hCG we were not able to recognize apparent extragonadal phenotypes induced in the absence of gonadal response. Furthermore, all the phenotypes so far detected in LuRKO mice could be explained on the basis of reduced sex steroid production. The ovarian transplantations confirmed this and demonstrated that the infertility of LuRKO females was caused solely by the lack of LH signalling in the ovaries, and the extragonadal LH/hCG-Rs did not play a role in fertility of the female mice. Hence, on the basis of these observations it is too early to call for a paradigm shift in the classification of gonadotropin action from being specifically targeted to gonads to a more general hormone also regulating functions of non-gonadal organs.

References

- Ahtiainen, P., Rulli, S.B., Shariatmadari, R., Pelliniemi, L.J., Toppari, J., Poutanen, M. and Huhtaniemi, I.T. (2005) Fetal but not adult Leydig cells are susceptible to adenoma formation in response to persistently high hCG level: a study on hCG overexpressing transgenic mice. *Oncogene* 24, 7301-9.
- Apaja, P.M., Harju, K.T., Aatsinki, J.T., Petaja-Repo, U.E. and Rajaniemi, H.J. (2004) Identification and structural characterization of the neuronal luteinizing hormone receptor associated with sensory systems. *J Biol Chem* 279, 1899-906.
- Ascoli, M., Fanelli, F. and Segaloff, D.L. (2002) The lutropin/choriogonadotropin receptor, a 2002 perspective. *Endocr Rev* 23, 141-74.
- Chudgar, D., Lei, Z. and Rao Ch, V. (2005) Orthotopic transplantation of LH receptor knockout and wild-type ovaries. *Life Sci* 77, 2656-62.
- Dufau, M.L. (1998) The luteinizing hormone receptor. *Annu Rev Physiol* 60, 461-96.
- El-Gehani, F., Zhang, F.P., Pakarinen, P., Rannikko, A. and Huhtaniemi, I. (1998) Gonadotropin-independent regulation of steroidogenesis in the fetal rat testis. *Biol Reprod* 58, 116-23.
- Fields, M.J. and Shemesh, M. (2004) Extragonadal luteinizing hormone receptors in the reproductive tract of domestic animals. *Biol Reprod* 71, 1412-8.
- Filicori, M., Fazleabas, A.T., Huhtaniemi, I., Licht, P., Rao Ch, V., Tesarik, J. and Zygmunt, M. (2005) Novel concepts of human chorionic gonadotropin: reproductive system interactions and potential in the management of infertility. *Fertil Steril* 84, 275-84.
- Gougeon, A. (1996) Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev* 17, 121-55.
- Han, S.W., Lei, Z.M. and Rao, C.V. (1999) Treatment of human endometrial stromal cells with chorionic gonadotropin promotes their morphological and functional differentiation into decidua. *Mol Cell Endocrinol* 147, 7-16.
- Hickey, G.J., Chen, S.A., Besman, M.J., Shively, J.E., Hall, P.F., Gaddy-Kurten, D. and Richards, J.S. (1988) Hormonal regulation, tissue distribution, and content of aromatase cytochrome P450 messenger ribonucleic acid and enzyme in rat ovarian follicles and corpora lutea: relationship to estradiol biosynthesis. *Endocrinology* 122, 1426-36.
- Huhtaniemi, I. (1994) Fetal testis--a very special endocrine organ. *Eur J Endocrinol* 130, 25-31.
- Huhtaniemi, I. and Pelliniemi, L.J. (1992) Fetal Leydig cells: cellular origin, morphology, life span, and special functional features. *Proc Soc Exp Biol Med* 201, 125-40.
- Huhtaniemi, I.T. and Themmen, A.P. (2005) Mutations in human gonadotropin and gonadotropin-receptor genes. *Endocrine* 26, 207-17.
- Huhtaniemi, I.T., Korenbrot, C.C. and Jaffe, R.B. (1977) HCG binding and stimulation of testosterone biosynthesis in the human fetal testis. *J Clin Endocrinol Metab* 44, 963-7.
- Keri, R.A., Lozada, K.L., Abdul-Karim, F.W., Nadeau, J.H. and Nilson, J.H. (2000) Luteinizing hormone induction of ovarian tumors: oligogenic differences between mouse strains dictates tumor disposition. *Proc Natl Acad Sci U S A* 97, 383-7.
- Kero, J.T., Savontaus, E., Mikola, M., Pesonen, U., Koulu, M., Keri, R.A., Nilson, J.H., Poutanen, M. and Huhtaniemi, I.T. (2003) Obesity in transgenic female mice with constitutively elevated luteinizing hormone secretion. *Am J Physiol Endocrinol Metab* 285, E812-8.
- Lavebratt, C.I., J., Petersson, S., Iwarsson, K., Schalling, M. and Ährlund-Richter, L. (1998) Ovary transplantation method resulting in high reproductive performance in mice. *Scand. J. Lab. Anim. Sci* 25, 53-58.

- Lei, Z.M., Toth, P., Rao, C.V. and Pridham, D. (1993a) Novel coexpression of human chorionic gonadotropin (hCG)/human luteinizing hormone receptors and their ligand hCG in human fallopian tubes. *J Clin Endocrinol Metab* 77, 863-72.
- Lei, Z.M., Rao, C.V., Kornyei, J.L., Licht, P. and Hiatt, E.S. (1993b) Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. *Endocrinology* 132, 2262-70.
- Lei, Z.M., Mishra, S., Zou, W., Xu, B., Foltz, M., Li, X. and Rao, C.V. (2001) Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. *Mol Endocrinol* 15, 184-200.
- Lin, P.C., Li, X., Lei, Z.M. and Rao Ch, V. (2003) Human cervix contains functional luteinizing hormone/human chorionic gonadotropin receptors. *J Clin Endocrinol Metab* 88, 3409-14.
- Ma, X., Dong, Y., Matzuk, M.M. and Kumar, T.R. (2004) Targeted disruption of luteinizing hormone beta-subunit leads to hypogonadism, defects in gonadal steroidogenesis, and infertility. *Proc Natl Acad Sci U S A* 101, 17294-9.
- Mann, R.J., Keri, R.A. and Nilson, J.H. (2003) Consequences of elevated luteinizing hormone on diverse physiological systems: use of the LHbetaCTP transgenic mouse as a model of ovarian hyperstimulation-induced pathophysiology. *Recent Prog Horm Res* 58, 343-75.
- Markkula, M., Hamalainen, T., Loune, E. and Huhtaniemi, I. (1995) The follicle-stimulating hormone (FSH) beta- and common alpha-subunits are expressed in mouse testis, as determined in wild-type mice and those transgenic for the FSH beta-subunit/herpes simplex virus thymidine kinase fusion gene. *Endocrinology* 136, 4769-75.
- Markkula, M., Kananen, K., Klemi, P. and Huhtaniemi, I. (1996) Pituitary and ovarian expression of the endogenous follicle-stimulating hormone (FSH) subunit genes and an FSH beta-subunit promoter-driven herpes simplex virus thymidine kinase gene in transgenic mice; specific partial ablation of FSH-producing cells by antiherpes treatment. *J Endocrinol* 150, 265-73.
- Matzuk, M.M., DeMayo, F.J., Hadsell, L.A. and Kumar, T.R. (2003) Overexpression of human chorionic gonadotropin causes multiple reproductive defects in transgenic mice. *Biol Reprod* 69, 338-46.
- McFarland, K.C., Sprengel, R., Phillips, H.S., Kohler, M., Rosemblyt, N., Nikolics, K., Segaloff, D.L. and Seeburg, P.H. (1989) Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. *Science* 245, 494-9.
- Mizrachi, D. and Shemesh, M. (1999) Follicle-stimulating hormone receptor and its messenger ribonucleic acid are present in the bovine cervix and can regulate cervical prostanoid synthesis. *Biol Reprod* 61, 776-84.
- Mohammad, H.P., Abbud, R.A., Parlow, A.F., Lewin, J.S. and Nilson, J.H. (2003) Targeted overexpression of luteinizing hormone causes ovary-dependent functional adenomas restricted to cells of the Pit-1 lineage. *Endocrinology* 144, 4626-36.
- Pakarainen, T., Zhang, F.P., Poutanen, M. and Huhtaniemi, I. (2005a) Fertility in luteinizing hormone receptor-knockout mice after wild-type ovary transplantation demonstrates redundancy of extragonadal luteinizing hormone action. *J Clin Invest* 115, 1862-8.
- Pakarainen, T., Zhang, F.P., Nurmi, L., Poutanen, M. and Huhtaniemi, I. (2005b) Knockout of luteinizing hormone receptor abolishes the effects of follicle-stimulating hormone on preovulatory maturation and ovulation of mouse graafian follicles. *Mol Endocrinol* 19, 2591-602.
- Pakarainen, T., Zhang, F.P., Makela, S., Poutanen, M. and Huhtaniemi, I. (2005c) Testosterone replacement therapy induces spermatogenesis and partially restores fertility in luteinizing hormone receptor knockout mice. *Endocrinology* 146, 596-606.

- Pakarinen, P., Kimura, S., El-Gehani, F., Pelliniemi, L.J. and Huhtaniemi, I. (2002) Pituitary hormones are not required for sexual differentiation of male mice: phenotype of the T/ebp/Nkx2.1 null mutant mice. *Endocrinology* 143, 4477-82.
- Raga, F., Casan, E.M., Kruessel, J.S., Wen, Y., Huang, H.Y., Nezhat, C. and Polan, M.L. (1998) Quantitative gonadotropin-releasing hormone gene expression and immunohistochemical localization in human endometrium throughout the menstrual cycle. *Biol Reprod* 59, 661-9.
- Rao, C.V. (2001) Multiple novel roles of luteinizing hormone. *Fertil Steril* 76, 1097-100.
- Rao, C.V. and Lei, Z.M. (2002) Consequences of targeted inactivation of LH receptors. *Mol Cell Endocrinol* 187, 57-67.
- Richards, J.S. (1994) Hormonal control of gene expression in the ovary. *Endocr Rev* 15, 725-51.
- Risma, K.A., Clay, C.M., Nett, T.M., Wagner, T., Yun, J. and Nilson, J.H. (1995) Targeted overexpression of luteinizing hormone in transgenic mice leads to infertility, polycystic ovaries, and ovarian tumors. *Proc Natl Acad Sci U S A* 92, 1322-6.
- Rulli, S.B., Kuorelahti, A., Karaer, O., Pelliniemi, L.J., Poutanen, M. and Huhtaniemi, I. (2002) Reproductive disturbances, pituitary lactotrope adenomas, and mammary gland tumors in transgenic female mice producing high levels of human chorionic gonadotropin. *Endocrinology* 143, 4084-95.
- Rulli, S.B., Ahtiainen, P., Makela, S., Toppari, J., Poutanen, M. and Huhtaniemi, I. (2003) Elevated steroidogenesis, defective reproductive organs, and infertility in transgenic male mice overexpressing human chorionic gonadotropin. *Endocrinology* 144, 4980-90.
- Schorpp, M., Jager, R., Schellander, K., Schenkel, J., Wagner, E.F., Weiher, H. and Angel, P. (1996) The human ubiquitin C promoter directs high ubiquitous expression of transgenes in mice. *Nucleic Acids Res* 24, 1787-8.
- Segaloff, D.L. and Ascoli, M. (1993) The lutropin/choriogonadotropin receptor ... 4 years later. *Endocr Rev* 14, 324-47.
- Simoni, M., Gromoll, J. and Nieschlag, E. (1997) The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocr Rev* 18, 739-73.
- Stewart, E.A. (2001) Gonadotropins and the uterus: is there a gonad-independent pathway? *J Soc Gynecol Investig* 8, 319-26.
- Themmen, A.P.N. and Huhtaniemi, I.T. (2000) Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocr Rev* 21, 551-83.
- Wong, T.T. and Zohar, Y. (2004) Novel expression of gonadotropin subunit genes in oocytes of the gilthead seabream (*Sparus aurata*). *Endocrinology* 145, 5210-20.
- Yarram, S.J., Perry, M.J., Christopher, T.J., Westby, K., Brown, N.L., Lamminen, T., Rulli, S.B., Zhang, F.P., Huhtaniemi, I., Sandy, J.R. and Mansell, J.P. (2003) Luteinizing hormone receptor knockout (LuRKO) mice and transgenic human chorionic gonadotropin (hCG)-overexpressing mice (hCG alphabeta+) have bone phenotypes. *Endocrinology* 144, 3555-64.
- Zhang, F.P., Rannikko, A. and Huhtaniemi, I. (1995a) Isolation and characterization of testis-specific cDNAs for luteinizing hormone beta-subunit in the rat. *Biochem Biophys Res Commun* 210, 858-65.
- Zhang, F.P., Markkula, M., Toppari, J. and Huhtaniemi, I. (1995b) Novel expression of luteinizing hormone subunit genes in the rat testis. *Endocrinology* 136, 2904-12.
- Zhang, F.P., Poutanen, M., Wilbertz, J. and Huhtaniemi, I. (2001a) Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. *Mol Endocrinol* 15, 172-83.

- Zhang, F.P., Pakarainen, T., Poutanen, M., Toppari, J. and Huhtaniemi, I. (2003) The low gonadotropin-independent constitutive production of testicular testosterone is sufficient to maintain spermatogenesis. *Proc Natl Acad Sci U S A* 100, 13692-7.
- Zhang, F.P., Pakarainen, T., Zhu, F., Poutanen, M. and Huhtaniemi, I. (2004) Molecular characterization of postnatal development of testicular steroidogenesis in luteinizing hormone receptor knockout mice. *Endocrinology* 145, 1453-63.
- Zhang, M., Shi, H., Segaloff, D.L. and Van Voorhis, B.J. (2001b) Expression and localization of luteinizing hormone receptor in the female mouse reproductive tract. *Biol Reprod* 64, 179-87.

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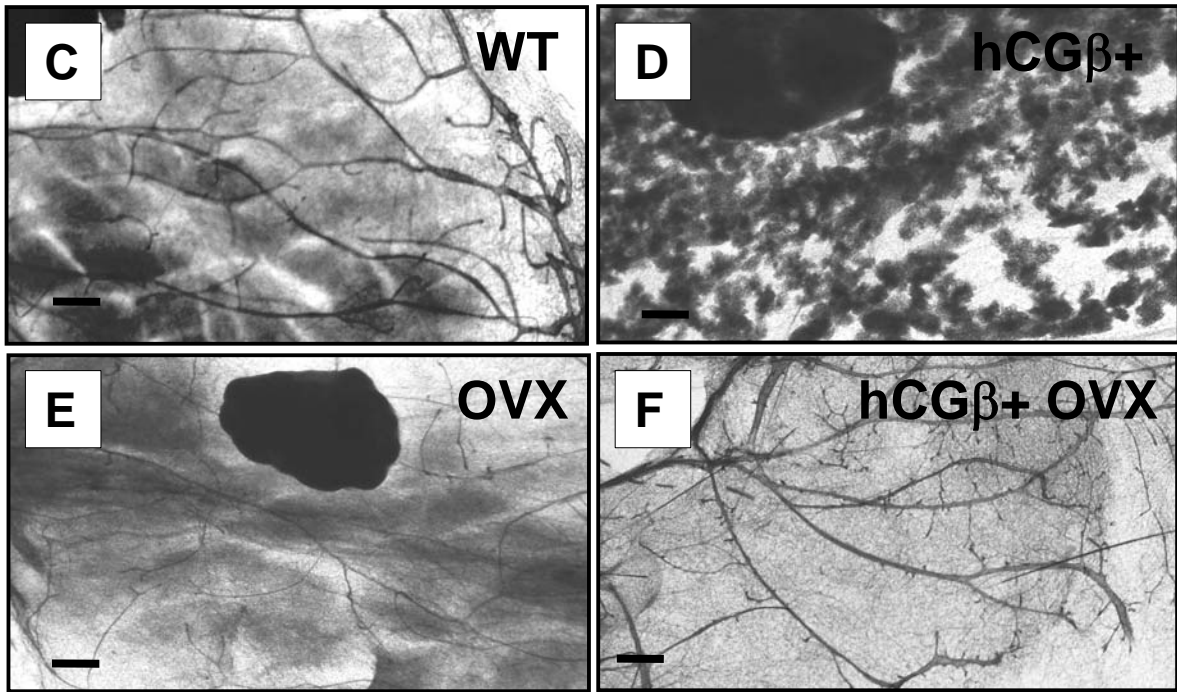
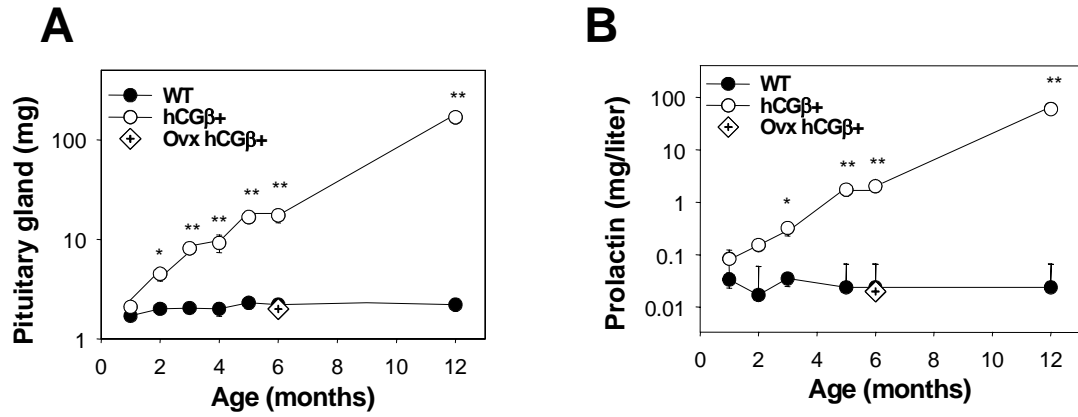
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Fig. 1. Effect of gonadectomy on some extragonadal phenotypes of the hCG β + females. The *upper part* of the figure shown pituitary gland weights (*panel A*) and serum prolactin concentrations (*panel B*) of WT control (filled circles) and hCG β + (open circles) females. Diamond-shaped symbol shows the values of gonadectomized hCG β + females, which are at level of WT controls. The *lower part* of the figure shows whole-mount images of mammary gland lobuloalveolar development in 6-month-old WT (*panel C*), hCG β + (*panel D*), ovariectomized (ovx) WT (*panel E*) and ovx hCG β + (*panel F*) females. Lobuloalveolar development is not seen in ovx WT and hCG β + females. Scale bars = 400 μ m, modified from Rulli et al. (2002).

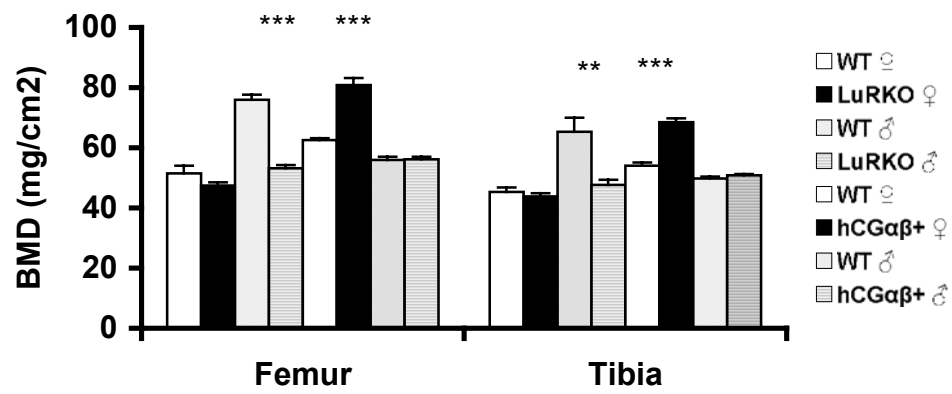
Fig 2. Bone mineral densities (BMD) of LuRKO and hCG $\alpha\beta$ + mice assessed by DXA. *Panel A* show BMDs of untreated 9-10 week-old LuRKO females, 5-months-old LuRKO males and 6-month-old hCG $\alpha\beta$ females and males. LuRKO males and hCG $\alpha\beta$ + females present significant difference between age-matched WT controls in BMD of both femur and tibia (***, $P < 0.0001$, **, $P < 0.003$). *Figure B* shows the effect of ovariectomy (ovx) at the age of 3 weeks on hCG $\alpha\beta$ + females, sacrificed at the age of 8 weeks. Ovariectomy reduces BMD of the hCG $\alpha\beta$ + females to the same level as in ovariectomized WT controls. Letter *a* indicates significant difference ($P < 0.001$) between the hCG $\alpha\beta$ + females and all other group. Asterisks denote significant difference between the WT females and ovariectomized WT and hCG $\alpha\beta$ + females (**, $P < 0.01$, ***, $P < 0.001$). Letters *ns* in the hCG $\alpha\beta$ + ovx bar denotes that significant differences between the WT ovx and hCG $\alpha\beta$ + ovx groups were not found. Modified from Yarram et al. (2003).

Figure 3. Representative light microscopic images of the ovaries of LuRKO (**A**), WT ovary-transplanted LuRKO (**B**), WT ovary-transplanted WT (**C**) and control WT (**D**) mice. CL, corpus luteum. Scale bars 500 μ m. Modified from Pakarainen et al. (2005a).

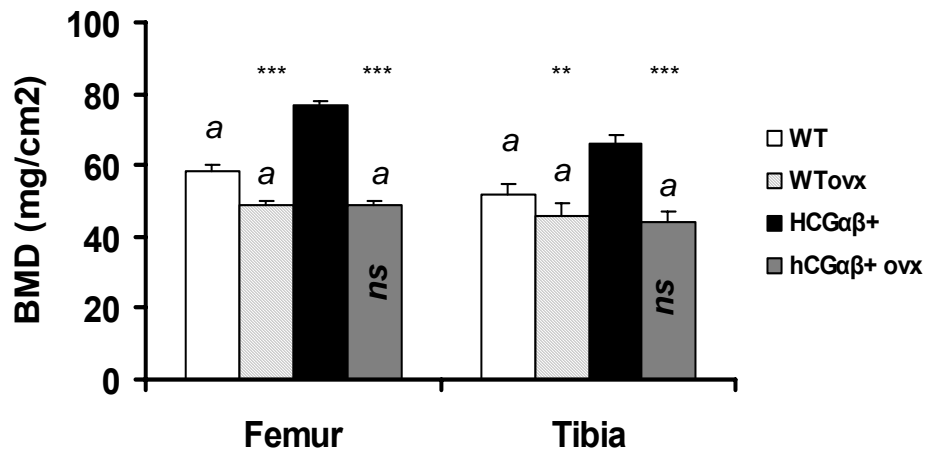
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A



B



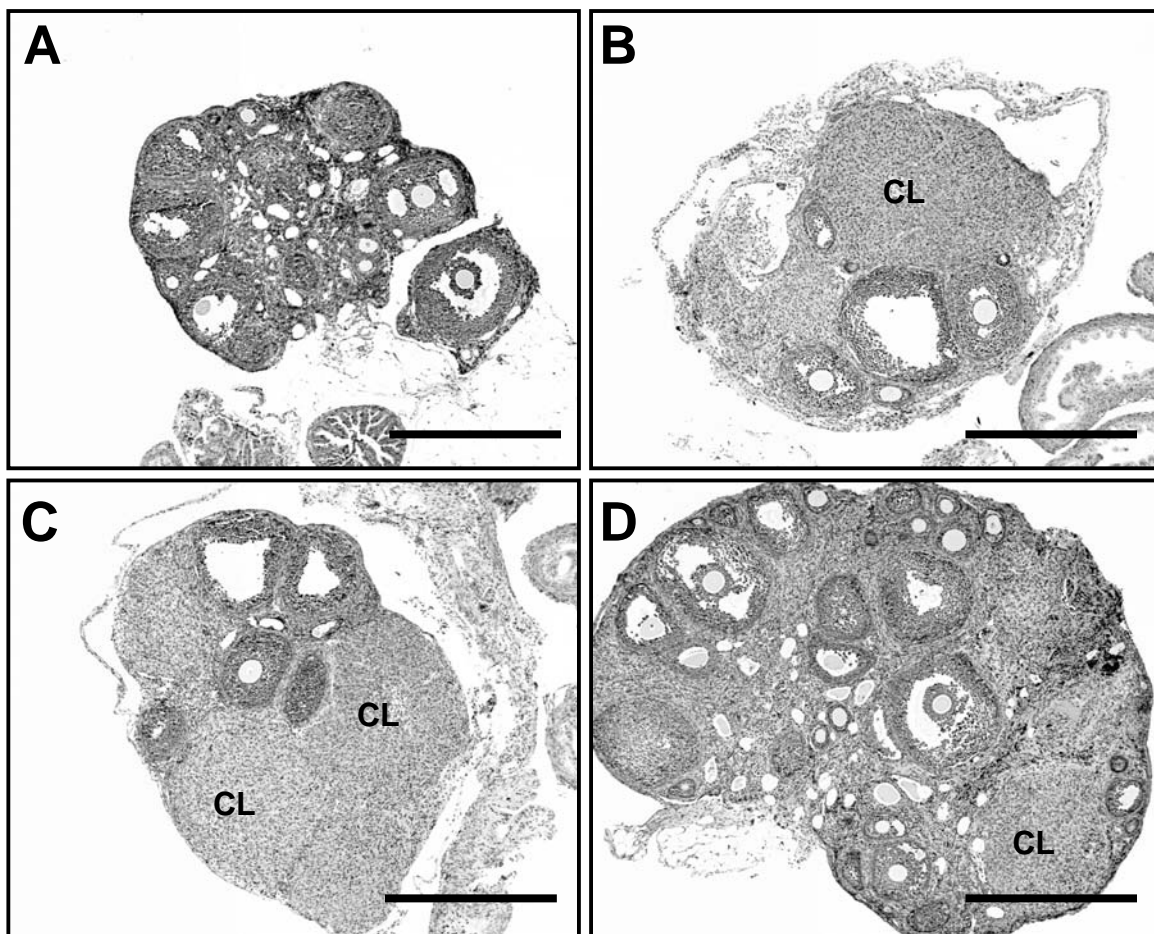


Table 1. LH/hCG receptors found in humans and rodents in extragonadal tissues.

Tissue	Species	References
Adrenal gland	human	Pabon <i>et al.</i> 1996b
	rat	Apaja <i>et al.</i> 2005
Blood vessel*	human	Reshef <i>et al.</i> 1990
		Lei <i>et al.</i> 1992
Brain	rat	Lei <i>et al.</i> 1993b
		Apaja <i>et al.</i> 2004
Cervix	human	Lin <i>et al.</i> 2003
Fetal tissues**	human	Abdallah <i>et al.</i> 2004
Kidney	rat	Apaja <i>et al.</i> 2005
Lymphocyte	human	Lin <i>et al.</i> 1995
Mammary gland	rat	Tao <i>et al.</i> 1997
Oviduct	human	Lei <i>et al.</i> 1993a
		Han <i>et al.</i> 1996
	mouse	Zhang <i>et al.</i> 2001b
Placenta	human	Reshef <i>et al.</i> 1990
Prostate	rat	Tao <i>et al.</i> 1995
		Reiter <i>et al.</i> 1995
Seminal vesicle	rat	Tao <i>et al.</i> 1998
Skin	human	Pabon <i>et al.</i> 1996a
		Venencie <i>et al.</i> 1999
Sperm	human	Eblen <i>et al.</i> 2001
Spinal cord	rat	Rao <i>et al.</i> 2003
Umbilical cord	human	Rao <i>et al.</i> 1993
Uterus	human	Han <i>et al.</i> 1997
		Reshef <i>et al.</i> 1990
	mouse	Zhang <i>et al.</i> 2001b

*endometrial and myometrial blood vessels of the uterus; **fetal kidneys, liver, pancreas, lung, small and large intestines, and adrenals.

Table 2. Mating incidence on the 1st breeding, and litter sizes following the 1st, 2nd and 3rd pregnancy of LuRKO-TR, WT-TR, WT and heterozygous (HZ) mice (Pakarainen et al., 2005a).

	1st pregnancy					2nd pregnancy			3rd pregnancy		
	Vaginal plug	1 st Litter Size	N	% /all	% /normal	2 nd Litter Size	N	% /all	3 rd Litter Size	N	% /all
LuRKO-TR	19/20 (95 %)	4.2 ± 2.4	9	45 % ^A	56 % ^A	4.0 ± 2.1 ^A	9	100 %	2.9 ± 1.1 ^A	9	100 %
WT-TR	21/22 (95 %)	4.4 ± 2.3	16	73 % ^A	80 % ^A	5.2 ± 3.0 ^{AB}	12	75 %	4.8 ± 2.7 ^{AB}	8	67 %
WT	10/10 (100 %)	6.0 ± 2.4	10	100 % ^B	100 % ^B	7.9 ± 2.2 ^B	9	100 %	7.2 ± 2.8 ^B	9	100 %
HZ	-	5.5 ± 2.6	52	-	-	5.6 ± 2.6 ^{AB}	37	-	6.7 ± 2.4 ^B	22	-

N = number of litters born; % /all = % of pregnancy of females mated; % /normal = % of pregnancy rate of females with no obvious explanation for infertility. The groups provided with different superscripts are significantly different ($p < 0.05$).-, not studied. All figures are mean ± SD.