Reduced recruitment and survival of primordial and growing follicles in GH receptor-deficient mice

Karin A Slot^{1,2}, Jan Kastelijn¹, Anne Bachelot³, Paul A Kelly³, Nadine Binart³ and Katja J Teerds¹

¹Department of Animal Sciences, Human and Animal Physiology Group, Wageningen University, Haarweg 10, 6709 PJ Wageningen, The Netherlands, ²Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands and ³Hormone Targets, INSERM U584, Faculty of Medicine, René Descartes-Paris 5, France

Correspondence should be addressed to K J Teerds; Email: katja.teerds@wur.nl

Abstract

GH influences female fertility. The goal of the present study was to obtain more insight into the effect of loss of GH signalling, as observed in humans suffering from Laron syndrome, on ovarian function. Therefore, serial paraffin sections of ovaries of untreated and IGF-I-treated female GH receptor knock-out (GHR/GHBP-KO) mice were examined to determine the follicular reserve and the percentage of follicular atresia in each ovary. Our observations demonstrate that the amount of primordial follicles was significantly elevated in GHR/GHBP-KO mice, while the numbers of primary, preantral and antral follicles were lower compared with wild-type values. The reduced number of healthy growing follicles in GHR/GHBP-KO mice was accompanied by a significant increase in the percentage of atretic follicles. IGF-I treatment of GHR/GHBP-KO mice for 14 days resulted in a reduced number of primordial follicles, an increased number of healthy antral follicles, and a decreased percentage of atretic follicles. The results of the present study suggest that GH may play a role, either directly or indirectly, via for instance IGF-I, in the recruitment of primordial follicles into the growing pool. Furthermore, GH seems to protect antral follicles, directly or indirectly from undergoing atresia.

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Introduction

It has been assumed for many decades that a fixed number of dormant primordial follicles was endowed during early life in the cortex of the ovary, often in close vicinity to the ovarian surface. This dogma has recently been questioned by Johnson and colleagues (2004), who suggested that up to several weeks after birth new primordial follicles were formed as a result of proliferation of oogonial stem cells, thus contributing to the resting pool of primordial follicles. Independently of whether new follicles continue to develop after birth, primordial follicles are uninterruptedly recruited to the growing pool of follicles until the end of reproductive life. Depending on the species, only 1–10% of these growing follicles will reach the preovulatory stage and ovulate. The remaining follicles degenerate as a result of follicular atresia, a process with apoptosis as the underlying mechanism (Hsueh *et al.* 1994). The fate of follicles is controlled by peptide and steroid hormones as well as intraovarian growth factors. Gonadotrophins are the most important survival factors of follicular development around and beyond the antral stage. Preantral follicular

development has generally been considered to be largely gonadotrophin-independent; the mechanisms that control the initiation of primordial follicle growth and the subsequent exhaustion of the dormant primordial follicle pool at the end of fertile life, are still largely unknown (McGee & Hsueh 2000). The possibility has been raised that metabolic hormones, such as growth hormone (GH) may play a role in these processes (Hull & Harvey 2001).

Until now, it is unclear whether GH acts directly on the ovary or acts via an indirect endocrine route through stimulation of the release of insulin-like growth factor I (IGF-I) by the liver (Hull & Harvey 2000). Both GH and IGF-I affect numerous processes associated with ovarian function, like gonadotrophin release and ovarian steroidogenesis, as well as follicular growth, development and atresia (Chun *et al.* 1994, Zhou *et al.* 1997a, Danilovich *et al.* 1999, 2000, Cushman *et al.* 2001, Bartke *et al.* 2002, Ptak *et al.* 2004). GH receptors (GHRs) and IGF-I receptors as well as IGF-I are widely expressed in the ovary together with receptor-derived soluble binding proteins, GH-binding protein (GHBP) and IGF-binding proteins (IGFBPs), that regulate the bioavailability and

action of GH and IGF-I respectively (Adashi *et al.* 1997, Monget & Bondy 2000, Hull & Harvey 2001). Animal studies have shown that IGF-I is absolutely required for reproduction; IGF-I-deficient female mice are sterile as they fail to ovulate (Baker *et al.* 1996). In contrast, GHR-null female mice (GHR/GHBP knock-out (KO) mice) are fertile, although their litter size is significantly reduced (Bachelot *et al.* 2002, Zaczek *et al.* 2002). In addition, women suffering from GH resistance or insensitivity (the so-called Laron syndrome) commonly require assisted reproductive treatment to induce ovulation, suggesting deficits in reproductive function (Hull & Harvey 2001).

GHR/GHBP-KO mice have characteristics mimicking the Laron phenotype in humans (Zhou *et al.* 1997b, Kopchick & Laron 1999), such as severe postnatal growth retardation, and have elevated GH, reduced IGF-I, IGFBP-3 and oestradiol levels in serum (Zhou *et al.* 1997b, Danilovich *et al.* 1999, Bachelot *et al.* 2002). As indicated above, most female GHR/GHBP-KO mice are fertile and have a regular oestrous cycle, although their start of puberty is delayed. The number of preovulatory follicles and corpora lutea, as well as the ovulation and embryo implantation rate are significantly reduced, resulting in smaller litter sizes compared with wild-type mice (Bachelot *et al.* 2002, Zaczek *et al.* 2002). Early (primordial, primary and early secondary) follicular development has, however, not been studied in much detail in these GH/GHBP-KO mice as yet. Moreover, it is unclear whether the effect of the absence of GH signalling is directly or indirectly a result of reduced IGF-I signalling. Therefore, we have investigated in the present study whether the early stages of follicular development, i.e. primordial follicle recruitment and primary follicular development, are affected by the absence of functional GHRs. Furthermore, it was studied whether supplementation with IGF-I could improve follicular development in GHR/GHBP-KO mice.

Materials and Methods

Animals

GHR/GHBP-KO mice were produced as described previously (Zhou *et al.* 1997b) and were subsequently established on a pure Sv129Ola background. Adult GHR/GHBP-KO females were mated with either GHR/GHBP-KO or wild-type males. Mice were housed in a room with a photoperiod of 12 h light:12 h darkness (lights on from 0700–1900 h) and at a temperature of 20°C. Mice were given free access to a nutritionally balanced diet and tap water. One group of GHR/GHBP-KO mice was treated with recombinant human IGF-I (Genentech, Inc., South San Francisco, CA, USA) by using micro-pumps (Alzet, Palo Alto, CA, USA) releasing 6 mg/kg per day for 14 days. These micro-pumps were inserted dorsally at the age of 4 weeks (Bachelot *et al.* 2002) and IGF-I treatment was started at the age of 7 weeks. Groups of mice, i.e. wild-type mice ($n = 5$), GHR/GHBP-KO mice

($n = 8$) and GHR/GHBP-KO mice treated with IGF-I for 14 days ($n = 4$), were killed at the age of 9 weeks and the ovaries were excised. All experimental designs and procedures were in accordance with the guidelines of the Animal Ethics Committee of the French Ministère de l'Agriculture.

Histological evaluation of follicle numbers

The ovaries were fixed in Bouin's fluid for 24 h and embedded in paraffin. From each animal one ovary was serial sectioned at a thickness of 7 µm. Every fifth section of each ovary was mounted on glass slides, stained with periodic acid and Schiff's reagent (PAS) and Mayer's haematoxylin, and examined by light microscopy. The total number of sections counted per ovary varied from 29 to 44 depending on the treatment of the animal. In all of these sections the numbers of healthy primordial, primary, preantral and antral follicles were counted according to the method of Flaws *et al.* (1997). Briefly, primordial and primary follicles were identified as healthy when they contained an intact oocyte surrounded by a single layer of (pre)granulosa cells that showed no signs of apoptosis. Preantral and antral follicles were identified as healthy when they contained an intact oocyte, an organized granulosa layer with proliferating cells and less than 5% apoptotic cells. The surrounding theca layer should have a healthy appearance and not show any signs of hypertrophy. Follicles were scored as primordial when they contained an intact oocyte with a healthy nucleus and nucleolus surrounded by a single layer of squamous pregranulosa cells. Follicles that included some cuboidal granulosa cells but in which the majority of the surrounding cells still had a squamous appearance, were also classified as primordial. Primary follicles were identified by the presence of a intact, enlarged oocyte with a healthy nucleus and nucleolus, surrounded by a single layer of cuboidal granulosa cells. Follicles in which the oocytes were surrounded by a single layer of cuboidal and squamous cells, in which the cuboidal cells predominated, were scored as primary (Britt *et al.* 2004). Preantral follicles consisted of more than one layer of granulosa cells, an oocyte with a nucleus and nucleolus and a developing theca layer. Antral follicles were identified by the presence of a healthy oocyte with nucleus and nucleolus and an antral space, of which the diameter was at least the size of the oocyte, several layers of granulosa cells and a theca layer. To obtain an estimate of the total number of follicles per ovary, the number of primordial, primary, preantral and antral follicles counted in the mounted sections was multiplied by five to account for the fact that every fifth section was used in the analysis (Flaws *et al.* 1997, Tilly 2003).

Histological evaluation of atresia

Using morphological criteria, follicles were classified as healthy or atretic as described previously (Logothetopoulos

et al. 1995, Teerds & Dorrington 1995). In atretic preantral follicles, the oocyte had degenerated and was surrounded by either a disorganized granulosa layer with apoptotic cells (more than 5% of the cells showed signs of apoptosis) and/or a hypertrophied theca layer. In atretic antral follicles the oocyte was usually intact, whereas the layer of granulosa cells contained more than 5% apoptotic cells and the theca layer showed signs of hypertrophy. As atresia proceeded, the granulosa cells were lost completely and the oocyte degenerated. Due to the risk of counting the same (pre)antral atretic follicle more than once in two or more successive sections, an estimate of the percentage of atretic follicles was made according to the method of Dijkstra *et al.* (1996). Briefly, in three sections of the ovary (at a quarter, half and three-quarters of the ovary), all preantral and antral healthy and atretic follicles were counted, independently of the presence of an oocyte. Since the counted numbers reflected only part of the total follicle population in an ovary, the mean percentage of non-atretic and atretic follicles was calculated in each group of mice and analysed statistically. Primordial and primary follicles were often arranged in small or large clusters. The number of follicles in these clusters varied considerably; therefore, primordial and primary follicles were excluded from these calculations and counted separately.

Atresia in primordial and primary follicles was identified by the presence of a reduction in the size of the oocyte, condensation of the nuclear chromatin (Johnson *et al.* 2004) and sometimes extensive PAS staining of the cytoplasm of the oocyte. Due to the small size of these follicles, there was no risk of counting the same follicle twice, and thus the percentage of atretic primordial and primary follicle was determined by counting the healthy and atretic primordial and primary follicles in which a nucleus was present in all mounted sections.

Statistical analysis

Statistics were performed by a one-way ANOVA, unless otherwise mentioned. Differences between group variances were determined by Tukey's multiple comparison test; differences between two groups were determined by Student's *t*-test. Values were considered to be statistically significant when $P < 0.05$.

Results

Effect of GHR deficiency on follicular development

To obtain more insight into the physiological role of GH on ovarian function, the effect of the absence of GH signalling on follicular development was studied in GHR/GHBP-KO mice by determining the number of healthy and atretic follicles in the ovaries. Moreover, we investigated whether IGF-I supplementation could antagonize the effects on follicular development and atresia, caused by defective GH signalling.

The pool of resting follicles from 9-week-old control ovaries of wild-type mice contained approximately 2600 primordial follicles per ovary (Fig. 1A). In contrast, ovaries of GHR/GHBP-KO females contained approximately 42% more primordial follicles per ovary ($P < 0.01$). Treatment of GHR/GHBP-KO mice with IGF-I for 14 days resulted in a significant reduction in the number of primordial follicles per ovary ($P < 0.01$) to levels similar to those observed in wild-type mice (Fig. 1A). The number of growing follicles per ovary in GHR/GHBP-KO mice also differed significantly from wild-type mice (Fig. 1A). The percentages of primary, preantral and antral follicles were reduced by 35, 52 and 84% respectively in GHR/GHBP-KO mice compared with wild-type mice ($P < 0.05$). IGF-I treatment of GHR/GHBP-KO mice for 14 days resulted in an increase in the number of antral follicles to levels similar to those observed in wild-type mice. The number of

Figure 1 Effect of IGF-I on follicle number per ovary in GHR/GHBP-KO mice. (A) The total numbers of primordial, primary, preantral and antral follicles per ovary in wild-type (open bars; $n = 5$), GHR/GHBP-KO (filled bars; $n = 8$) and GHR/GHBP-KO mice treated with IGF-I (stippled bars; $n = 4$) were determined using the criteria as described in the Materials and Methods. (B) The sum of the total number of follicles per ovary. Values represent means $\pm$ S.E.M. ^aSignificantly different from wild-type mice. ^bSignificantly different from GHR-KO mice ($P < 0.05$, Tukey's multiple comparison test).

primary and preantral follicles per ovary, however, remained low upon IGF-I treatment compared with wild-type mice (1099 ± 385 and 253 ± 29 vs 1634 ± 460 and 657 ± 121 respectively). Remarkably, the total follicle count per ovary was 20% lower in IGF-I-treated GHR/GHBP-KO mice compared with untreated GHR/GHBP-KO and wild-type mice (Fig. 1B), although this difference did not reach the level of significance.

Effect of GHR deficiency on follicular atresia

To investigate whether deficient GH signalling affects follicular atresia, we determined the percentage of atretic follicles in the ovaries, ranging from early to late atretic follicles. Representative examples are depicted in Fig. 2. The reduced number of healthy growing follicles in GHR/GHBP-KO mice (Fig. 1A) was accompanied by a nearly significant increase in the percentage of atretic primordial follicles (Fig. 3A, $P = 0.058$, Student's *t*-test), while the percentage of (pre)antral atretic follicles was significantly higher compared with wild-type mice (Fig. 3B, $P < 0.01$). In IGF-I-treated GHR/GHBP-KO mice, the increased number of healthy antral growing follicles (Fig. 1A) was accompanied by a significant decrease in the percentage of atretic follicles from the preantral stage onwards compared with untreated GHR/GHBP-KO mice (Fig. 3B, $P < 0.05$), suggesting increased follicular survival.

A considerable number of follicles in GHR/GHBP-KO mice contained two or more oocytes (Fig. 4A–C), a phenomenon which was almost never observed in wild-type mice. These multi-oocyte follicles, which

have been demonstrated in other KO models as well (e.g. anti-Müllerian hormone (AMH)-KO mice (MA Visser, Erasmus Medical Centre, Rotterdam, The Netherlands, personal communication)) were identified at the primary, preantral and early antral stage of development but never in large antral follicles.

Discussion

The results of this study indicate that the ovaries of adult GHR/GHBP-KO mice contained higher numbers of primordial follicles, lower numbers of healthy growing primary, preantral and antral follicles and had an increased percentage of atretic follicles compared with wild-type animals. Our results extend data from earlier reports on GHR/GHBP-KO mice that investigated the effects of absence of GHR signalling on secondary (preantral) and antral follicles. These studies reported a marked reduction in the number of healthy growing antral follicles (Bachelot *et al.* 2002, Zaczek *et al.* 2002). We showed for the first time that in the KO mice primordial and primary follicles are also affected by this mutation. Moreover, the present study further demonstrates for the first time that continuous treatment of GHR/GHBP-KO mice with IGF-I for 14 days reduced the number of primordial follicles significantly to wild-type control levels, while the number of growing antral follicles increased. These data suggest that GH may at least in part affect follicular recruitment and growth through the action of IGF-I.

The reduction in the primordial follicle pool in GHR/GHBP-KO after postnatal IGF-I treatment suggests that GH may indirectly, via IGF-I, play a role in the

Figure 2 Representative photomicrographs of atretic follicles in ovaries of GHR/GHBP-KO (A, B) and wild-type mice (C–E). (A) An atretic primordial follicle (†) in which the nucleus of the oocyte shows signs of condensation and the cytoplasm is strongly PAS-positive (see also Materials and Methods), and a healthy primordial follicle (*). (B) An atretic primary follicle (†) in which the oocyte shows signs of nuclear condensation and shrinkage, and a healthy primary follicle (*). (C) Early atretic preantral follicle displaying a degenerating oocyte (†). The granulosa layer (GC) shows some signs of apoptosis (arrow) and the theca layer (TC) is in the early stages of hypertrophy. (D) Severe atretic (pre)antral follicle (†) in which the granulosa cells have disappeared, the oocyte has degenerated while the zona pellucida (arrowhead) is still present. The theca layer is severely hypertrophied. (E) Late atretic (pre)antral follicle (†), in which most theca cells (TC) have now undergone apoptosis, but the zona pellucida (open arrowhead) of the degenerated oocyte is still present. Bar represents 20 μm .

Figure 3 Effect of IGF-I on the percentage of atretic follicles per ovary in wild-type (open bars; $n = 5$), GHR/GHBP-KO (filled bars; $n = 8$), and GHR/GHBP-KO mice treated with IGF-I (stippled bars; $n = 4$). (A) Percentage of apoptotic primordial and primary follicles. (B) Percentage of (pre)antral atretic follicles per ovary. The percentages of atresia were estimated as described in Materials and Methods. Values represent means $\pm$ S.E.M. ^aSignificantly different from wild-type mice. ^bSignificantly different from GHR-KO mice ($P < 0.05$, Tukey's multiple comparison test).

recruitment of primordial follicles into the growing pool. A role for IGF-I in follicular growth has previously been suggested by Baker *et al.* (1996), who observed that in IGF-I-null mice the number of growing follicles was reduced. Whether these IGF-I-null mice also have an increased primordial follicle pool was not investigated by Baker and colleagues. In contrast to the present *in vivo* observations, *in vitro* studies using a rat ovarian culture system have shown that IGF-I was unable to affect recruitment of primordial follicles (Kezele *et al.* 2002). One possible explanation for this discrepancy may be that there are differences between mice and rats concerning the functioning of the intraovarian IGF-I system (Adashi *et al.* 1997). On the other hand, it is also very well possible that the actions of GH and IGF-I *in vivo* may be through (in)direct stimulation of other regulatory factors, such as insulin, which has been shown to play a role in primordial follicle recruitment *in vitro* (Kezele *et al.* 2002). Beside severely reduced IGF-I levels, GHR/GHBP-KO mice have also greatly reduced insulin levels (Hauck *et al.* 2001), implying that these low insulin levels could be a cause of the reduced primordial to primary follicle transition. IGF-I can bind, although with low affinity, to the insulin receptor (Laron 2001). The micro-pumps in the IGF-I-treated GHR/GHBP-KO mice released large amounts of IGF-I on a daily basis. The observation that smaller numbers of primordial follicles were observed upon IGF-I treatment in GHR/GHBP-KO mice, may,

therefore, be due to a non-specific effect of these high levels of IGF-I acting on the insulin receptor, thus stimulating follicular recruitment. In relation to this hypothesis, it would be of interest to investigate whether there exists an interaction between GH/IGF-I and other factors beside insulin that affect primordial follicle growth and recruitment, like stem cell factor, growth differentiation factor 9, kit ligand, basic fibroblast growth factor, nerve growth factor (Huang *et al.* 1993, Vitt *et al.* 2000, Nilsson & Skinner 2004) and AMH (Durlinger *et al.* 2002).

Surprisingly, the reduced number of primordial follicles in IGF-I-treated GHR/GHBP-KO mice was not accompanied by increased primary and preantral healthy follicle numbers, nor by increased degeneration of primordial and primary follicles. Due to the fact that we have only investigated the effects of IGF-I at 14 days after the initiation of treatment, we can not exclude that loss of primordial and/or primary follicles from the stockpile occurred at an earlier stage of IGF-I treatment. The manifestations of apoptosis in primordial and primary follicles are thought to be of short duration; within 3–4 days after the initiation of atresia these atretic follicles have been eliminated completely from the ovary. Hence, atresia of primordial and primary follicles is difficult to detect histologically (Hirshfield 1994, Johnson *et al.* 2004). When this process would have been initiated fairly early after the onset of treatment, it may have passed unnoticed. It is also possible that the continuous high levels of IGF-I in our experimental setup may have inhibited further stimulatory effects by IGF-I, as a result of, for instance, receptor down-regulation or feedback mechanisms. High IGF-I concentrations have been shown to trigger apoptosis in mouse blastocysts via down-regulation of the IGF-I receptor (Chi *et al.* 2000). Although further experiments with IGF-I-treated wild-type mice are required to elucidate whether IGF-I treatment has indeed detrimental effects on the primordial/primary follicle pool, there is some further support in the literature for the assumption that high IGF-I levels may have a negative effect on the primordial follicle pool. Women suffering from premature ovarian failure (POF), a syndrome in which gonadal function ceases prematurely before the age of 40 years due to unexplained depletion of the primordial follicle pool, have significantly higher IGF-I levels compared with their age-matched controls with normal menstrual cycles or post-menopausal women (Hartmann *et al.* 1997a,b).

Figure 4 Abnormal follicles in GHR/GHBP-KO mice. (A) Early atretic follicle with two oocytes (*). (B) Healthy preantral follicle with two oocytes (*). (C) Healthy early antral follicle with two oocytes. Bar represents 40 μ m.

Whether the elevated IGF-I levels are the cause of POF or a consequence of POF is not clear and requires further investigation.

Although our results suggest that GH is important for the recruitment of primordial follicles into the growing pool, another explanation for the increased size of the primordial follicle pool in the GHR/GHBP-KO mice may be that as a consequence of the elimination of functional GHRs and GHBP the proliferation and apoptosis of the oogonia during fetal life is influenced. This would then lead to a rise in the size of the primordial follicle pool at the time of birth when compared with the wild-type animals. Increased numbers of primordial follicles after birth have been observed in studies in which genes involved in apoptosis were knocked out, resulting in an arrest in the naturally occurring process of apoptosis (Perez *et al.* 1999, Reynaud & Driancourt 2000). There are, however, no indications that this is also the case when hormones like GH or growth factors are knocked out. We would, therefore, hypothesize that the increased primordial follicle pool as observed in the present study in GHR/GHBP-KO mice is the result of reduced primordial follicle recruitment and not due to increased oogonial proliferation or oocyte survival during fetal life.

In vitro experiments have suggested that the effect of GH on preantral follicular growth in immature mice is independent of IGF-I (Kumar *et al.* 1997, Liu *et al.* 1998, Kobayashi *et al.* 2000). Our *in vivo* results are in agreement with these data, since IGF-treatment did not affect preantral follicular growth in GHR/GHBP-KO mice. The mechanism by which GH regulates growth in ovarian follicles is not exactly known yet. *In vitro* studies have shown that the stimulatory effect of GH on preantral follicular growth could be antagonized by follistatin (Liu *et al.* 1998). In addition, follistatin binds and inactivates activin, a potent stimulator of preantral follicle growth *in vitro* (Liu *et al.* 1998). *In vivo*, GH administration increased the number of small preantral follicles in cattle (Gong *et al.* 1991, 1993) and horses (Cochran *et al.* 1999). Moreover, GH-binding activity was highest in granulosa cells of preantral follicles compared with large antral follicles in porcine and fish ovaries (Gomez *et al.* 1999, Quesnel 1999), suggesting that GH is important for preantral follicular growth, possibly through increasing ovarian activin production (Liu *et al.* 1998).

Our results on follicular development beyond the preantral stage in GHR/GHBP-KO mice are in line with earlier data obtained in GHR/GHBP-KO mice, which showed a markedly reduced number of healthy growing follicles (Bachelot *et al.* 2002, Zaczek *et al.* 2002) and an increased percentage of atresia in follicles from 200 μ m (antral stage) onwards (Bachelot *et al.* 2002). However, the number of atretic follicles may be underestimated in these studies since only early atretic preantral and antral follicles were included. In the present study late atretic follicles were also counted, resulting in more pronounced effects of the GHR/GHBP mutation on the percentage of atretic follicles.

This may also explain why in another study no increase in follicular atresia was observed, using TUNEL labelling of granulosa cells as a method to detect atretic follicles (Zaczek *et al.* 2002). TUNEL labelling is only detected in early apoptotic cells. In advanced preantral and antral atretic follicles where the granulosa cells have become severely apoptotic or completely disappeared and only the hypertrophied theca cells and sometimes the zona pellucida are left, the number of TUNEL-positive cells will be negligible (Kim *et al.* 1998, Slot *et al.* in press).

In GH-overexpressing transgenic mice the incidence of apoptosis in preovulatory follicles has been reported to be significantly reduced (Liu *et al.* 1998, Danilovich *et al.* 2000). This stimulatory effect of GH may reflect indirect actions mediated through the (local) production of IGF-I (Chun *et al.* 1994), since treatment with IGF-I also suppresses apoptotic DNA fragmentation in preovulatory follicles. Indeed, we observed increased follicular development accompanied by reduced atresia beyond the preantral stage in ovaries of GHR/GHBP-KO mice upon IGF-I treatment. Direct effects of IGF-I in the GHR/GHBP-KO mice on antral follicle development can also not be excluded. In the present investigation high levels of IGF-I were established by the osmotic micro-pumps. Several studies have shown that administration of IGF-I stimulates granulosa cell proliferation and suppresses apoptosis when these cells are isolated from antral follicles (Chun *et al.* 1994, 1996, Hu *et al.* 2004). It is possible that GH, indirectly through IGF-I, enhances follicle-stimulating hormone (FSH) responsiveness by augmenting FSH receptor expression in granulosa cells (Zhou *et al.* 1997a), thereby allowing antral follicles to escape atresia. GH may also enhance follicular survival and cell proliferation by potentiating the action of luteinizing hormone (LH), since GH deficiency is associated with decreased LH receptor gene expression and LH responsiveness in rats (Chase *et al.* 1998, Liu *et al.* 1998). Exogenous GH *in vivo* corrects both effects (Advis *et al.* 1981), and increases the number of large antral/preovulatory follicles in GH-deficient dwarf rats (Danilovich *et al.* 2000).

In conclusion, the ovaries of adult GHR/GHBP-KO female mice contained more primordial follicles compared with wild-type females, suggesting that GH affects the size of the primordial follicle pool. The reduction in the primordial follicle pool in GHR/GHBP-KO after post-natal IGF-I treatment suggests that GH may indirectly, possibly via IGF-I and/or other growth factors, play a role in the recruitment of primordial follicles from the resting pool. Treatment of KO mice with IGF-I increased the number of healthy antral follicles, suggesting that GH either directly or indirectly via IGF-I affects follicular survival.

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