Gonadal function in male and female patients with classic galactosemia

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BACKGROUND: Hypergonadotropic hypoestrogenic infertility is the most burdensome complication for females suffering from classic galactosemia. In contrast, male gonadal function seems less affected. The underlying mechanism is not understood and several pathogenic mechanisms have been proposed. Timing of the lesion, prenatal or chronic post-natal, or a combination of both are not yet clear.

METHODS: This review focuses on gonadal function in males and females, ovarian imaging and histology in this disease. It is based on the literature known to the authors and a Pubmed search using the keywords galactosemia, GALT deficiency, (premature) ovarian failure/insufficiency/dysfunction, testicular function, gonadotrophins, FSH, LH (published between January 1971 and April 2009).

RESULTS: Male gonads are less affected, boys spontaneously reach puberty, although onset can be delayed. Semen quality has not been extensively studied. Several affected males are known to have fathered a child. Female gonads are invariably affected, although to a varied extent (hypergonadotropic hypoestrogenic ovarian dysfunction). Intriguingly, FSH is often already increased in infancy. Imaging usually shows hypoplastic and streak-like ovaries. Histological findings in some cases reveal the presence of morphologically normal but decreased numbers of primordial follicles, with the absence of intermediate and Graafian follicles.

CONCLUSION: Gonads in males seem less affected than in females who exhibit hypergonadotropic hypoestrogenic subfertility. FSH can be elevated in infancy, and ovarian histology sometimes shows the presence of normal primordial follicles with absence of intermediate and Graafian follicles. These findings are similar to other genetic diseases primarily affecting the ovary.

Key words: galactosemia / gonads / ovarian dysfunction / testicular function / FSH
to the latter category. Classic galactosemia is a hereditary disorder of galactose metabolism, caused by deficiency of one of the enzymes of the Leloir pathway, the main pathway responsible for galactose metabolism in the human body. The most important exogenous source of galactose is the disaccharide lactose present in dairy products. Lactose’s major role is that of an osmole that assures the production of a fluid milk (Vilotte, 2002), which would otherwise be viscous. Besides, the human body synthesizes considerable galactose quantities. The endogenous production of circulating free galactose in adults ranges from 0.53 to 1.05 mg/kg/h (Berry et al., 1999; Ning et al., 2000).

In the liver, galactokinase catalyses the first step forming Gal-1-P, which is together with UDP-Glu (uridine diphosphate glucose) converted into UDP-Gal (uridine diphosphate galactose) and glucose-1-phosphate by GALT (galactose-1-phosphate uridylyltransferase). Galactose can be converted into energy by entering the glycolytic pathway or be incorporated into glycopolymers and glycolipids. The UDP galactose-4’ epimerase (GALE) enzyme catalyses the interconversion of UDP-Gal and UDP-Glu. The condition known as classic galactosemia refers to the deficiency of the GALT enzyme. This enzyme deficiency leads to accumulation of galactose and its metabolites in body tissues and fluids and leads secondarily to glycosylation abnormalities. The GALT gene, located on the short arm of chromosome 9 (9p13), was fully cloned and sequenced in 1992 (Leslie et al., 1992) and since then more than 220 different mutations have been described (Calderon et al., 1992). The most common mutation in European populations and individuals of Northern descent is Q188R mutation (Tyfield et al., 1999). This sequence change is significant since its location is two amino acids away from the histidine–proline–histidine-binding sequence thought to be the active catalytic site (Field et al., 1989; Lai et al., 1999) and is associated with a severe phenotype. The incidence of the disease in Europe and North America is ~1:30 000–1:50 000. Other mutations the so-called variants, lead to a mild or even asymptomatic clinical phenotype. The S135L mutation for instance, predominant in the African and African-American populations, leads to a much less severe phenotype (Lai et al., 1996). The same holds true for the N314D mutation (the Duarte type) with a mild or even undetectable phenotype (Elsas et al., 2001).

Affected children usually present in the first weeks of life with food intolerance, failure to thrive and lethargy, symptoms associated with hepatocellular damage, renal tubular disease and cataract. Nursing infants must move large amounts of galactose through this pathway in order to utilize the carbon skeletons for energy. Treatment with a galactose restricted diet resolves the neonatal picture but despite early diagnosis and diet, long-term diet-independent complications occur (Waggoner et al., 1990; Schweitzer et al., 1993; Holton et al., 2001). Ovarian dysfunction with its subsequent infertility in women (Kaufman et al., 1981; Forges et al., 2006) and brain impairment with cognition and neurological sequelae in men and women (Ridel et al., 2005) are the most burdensome (Bosch et al., 2004). Bone mass (Kaufman et al., 1993; Rubio-Gozalbo et al., 2002; Panis et al., 2004), growth (Panis et al., 2007) and body composition abnormalities (Panis et al., 2005) have also been reported.

It is still uncertain whether these long-term complications are initiated in early development, perhaps even prenatally, or whether they represent degenerative processes compounded by ongoing galactose exposure. Particularly interesting is the relative abundance of the different Leloir pathway enzymes in different cells and its relation with the observed long-term complications. Studies in rat tissue reveal that liver has the highest GALT mRNA and GALT activity. Kidney, ovary and heart have similar but lower mRNA and GALT activity, and skeletal muscle and testes have the least (Heidenreich et al., 1993). The long-term complications arise in organs with high physiological GALT expression. Interestingly, physiological GALT expression in the female rat pituitary gland changes during the estrous cycle, when the production of gonadotrophins reaches a peak, the GALT expression is also high (Daude et al., 1996).

Methods

This review focuses on the gonadal function in males and females in this disease. Ovarian imaging and histological data are reviewed and compared with several genetic diseases primarily affecting female reproduction. The information on gonadal function in male and female patients with classic galactosemia is based on the literature known to the authors complemented with a Pubmed search using the keywords galactosemia, GALT deficiency (premature) ovarian failure/insufficiency/dysfunction, testicular function, gonadotrophins, FSH, LH (publication dates from January 1971 to April 2009). Further articles were acquired from the citations in the articles obtained from the search. The literature was restricted to the papers in the English, Dutch, French, German and Spanish languages.

Results and Discussion

Gonadal function in classic galactosemia

Ovary

Premature ovarian failure (POF) in female galactosemia patients contrasts with the apparently normal gonadal function in males. POF occurs in almost all women homozygous for mutations in the GALT gene that nearly or completely abolish GALT activity and are associated with a severe phenotype (Waggoner et al., 1990). The first reported incidence of POF in galactosemia was 67%, but as the initial group aged it appeared that almost every female patient is affected sooner or later (Kaufman et al., 1986). The severity of this complication however varies even in patients harbouring the same genotype. Risk factors for the development of ovarian dysfunction are genotype associated with severe phenotype, mean erythrocyte Gal-1-P > 3.5 mg/dl during therapy and a recovery of 13C2O2 from whole body 13C-galactose oxidation reduced below 5% of administered 13C-galactose (Guerrero et al., 2000). The pattern of GALT mRNA reflects the GALT-specific enzymatic activity. In the anterior pituitary from adult rats, both GALT mRNA and GALT protein pattern of expression varies during the different stages of the estrous cycle being elevated in gonadotrophin-producing cells during proestrous and estrous phase, possibly related to the physiological requirements for galactose (Daude et al., 1996).

The first report of hypergonadotropic hypogonadism in female patients with galactosemia in the peer-reviewed literature dates from 1981 (Kaufman et al., 1981). Prior to this publication, three letters noting this complication were published in 1979 and 1981 (Hofnagel et al., 1979; Kaufman et al., 1979; Coulam, 1981). In one cohort of female patients with classic galactosemia, more than 90% manifested hypergonadotropic hypogonadism (Kaufman et al.,
1986). In many patients, the serum FSH level has been found elevated very early in life (4 months to 4 years) (Steinmann et al., 1981a, b; Beauvais and Guilhaume, 1984; Schwarz et al., 1984; Irons et al., 1986; Kaufman et al., 1986) and also between early childhood and onset of puberty (5–12 years) (Steinmann et al., 1981a, b; Irons et al., 1986; Rubio-Gozalbo et al., 2006). Etiology and timing of the lesion, prenatal, chronic post-natal or a combination of both are not yet clear. The investigation of pathogenic mechanisms in this disease is hampered by the failure of the GALT-knockout mice to replicate the clinical phenotype manifested in human patients (Leslie et al., 1996). Main proposed mechanisms, summarized by Liu et al. (2000),Forges and Monnier-Barbarino (2003) and Forges et al. (2006), are ovarian damage due to elevated metabolites (galactose-1-phosphate and galactitol); UDP-galactose deficiency causing apoptosis of oocytes and ovarian stromal cells, or aberrant glycosylation of glycoproteins and galactolipids involved in ovarian function. Other mechanisms such as abnormalities of the immune system (like unrecognized auto-ovarian antibodies) could account for ovarian dysfunction in classic galactosemia. This mechanism has been described in diabetes and other auto-immune-related diseases in which ovarian antibodies are released secondary to cellular damage and interfere with post-receptor function, but these antibodies have never been tested in galactosemics.

**Toxic damage to the ovary**

Direct toxic damage to the ovary through either galactose or one of its metabolites is supported by several findings: galactose metabolism is of importance to the ovary, and the relative physiological abundance of galactose metabolic enzymes (GALK, GALT, GALE and UDP-glucose phosphorylase) may render ovarian tissue more prone to accumulation of galactose and its metabolites. Additionally, galactitol is metabolized poorly and causes accumulation in viable ovarian cells leading to swelling and cell dysfunction as well as decreased glutathione levels that predispose to hydrogen peroxide accumulation and promote apoptosis (Meyer et al., 1992). Apoptosis may also be increased by structurally abnormal gonadotrophins and growth hormones, and low estrogen-levels in galactosemia, since apoptosis is decreased by gonadotrophins, estrogen, growth hormone, cytokines and nitric oxide (Amsterdam et al., 1997). Furthermore, galactose may cause the accumulation of methylglyoxal by hampering the glutathione redox cycle and hence enhance apoptosis (Liu et al., 2000).

There are some data regarding the toxic effects of galactose on the ovary in rats studies. Chen et al. (1981) observed that when pregnant rats were fed a 50% galactose diet there was a striking reduction in oocyte number in the offspring. The most prominent effects were noted after exposure to galactose during the premeiotic stages of oogenesis. The authors concluded that prenatal exposure to galactose or its metabolites may contribute to the premature ovarian failure characteristic of human galactosemia. Swartz and Mattison (1988) reported that when adult female mice were fed a diet consisting of 50% galactose for either 2, 4 or 6 weeks, at all times there was a decrease in the normal ovariolytic response, as evidenced by a reduction in the number of corpora lutea when compared with controls. Additionally, the exposure of galactose-treated mice to a super-ovulatory regimen of pregnant mare’s serum gonadotrophin and human chorionic gonadotrophin (hCG) failed to induce an increased ovariolytic response. Morphologic alterations, such as the increase in the interstitial tissue and the appearance of lipofuscin, coupled with the failure to respond to exogenous gonadotrophins, suggesting ovarian damage. Bandopadhyay et al. (2003) conducted a study in which pregnant rats were fed pellets supplemented with or without 35% galactose from Day 3 of conception continuing through weaning of the litters. Female offspring were evaluated for serum levels of galactose and galactose-1-phosphate, growth rate, onset of puberty, reproductive cycle, ovarian complement of follicles, hypothalamo-pituitary-ovarian function and follicular response to gonadotrophins. Galactose toxicity delayed the onset of puberty and developed a state of hypergonadotropic hypoestrogenism. The characteristic low FSH levels at weaning followed by pubertal spurs of gonadotrophins and estradiol secretion of the controls was replaced by a sustained high level of FSH and a low level of estradiol under galactose toxicity. The ovary developed with apparently normal or deficient complement of follicles. Ovarian response to exogenous gonadotrophin stimulation was blunted, but the response improved significantly when the stimulation was preceded by pituitary desensitization.

**Aberrant FSH and FSH/FSH receptor interaction**

It has been suggested that the ovarian dysfunction in classic galactosemia might be due to abnormal FSH action due to aberrantly glycosylated FSH and/or its receptor. FSH under-stimulation of the ovary from an early age could result in an increased follicle atresia rate.

Classic galactosemics have a low UDP-galactose/UDP-glucose ratio (Ng et al., 1989). UDP-galactose is needed for synthesis of ovarian membrane glycoproteins and galactolipids, support of germ cells, follicle maturation and steroidogenesis. Furthermore, Gal-1-P can inhibit galactosyltransferases and disturb glycosylation (Lai et al., 2003).

Glycosylation abnormalities in galactosemia are well known but its relevance as pathogenic mechanism is not clear. The structures of the truncated glycans are consistent with a decreased capacity to galactosylate glycoproteins (Tedesco and Miller, 1979; Dobbie et al., 1990; Petry et al., 1991; Jaeken et al., 1992; Ornstein et al., 1992). The first suggestion of a glycosylation defect in classic galactosemia was made by Haberland et al. (1971). They found an abnormal pattern of glycoproteins when examining the brain of a galactosemic patient at autopsy. The truncated N-glycans persist in a small proportion despite dietary treatment (Charwood et al., 1998), and treated galactosemic patients may remain at risk for (sub) clinical endocrine dysfunction.

Interestingly, patients with primary congenital disorders of glycosylation type 1, due to mutations in genes encoding for products involved in the glycosylation machinery itself, also have ovarian failure based on hypergonadotropic hypogonadism, and remarkably also tend to have high concentration of FSH in infancy as seen in classic galactosemia, suggesting a similar pathophysiological etiology (Kristiansson et al., 1995). Gonadotrophins and their receptors undergo extensive post-translational modifications such as glycosylation, which are crucial for their proper functioning (Ulloa-Aguirre et al., 1995). The post-translational modification of gonadotrophins is essential for its regulatory action. Human FSH contains diantennary, triantennary and tetrantennary N-linked oligosaccharide structures capped by Gal-sialic acid. There is increasing evidence that the regulatory action of these hormones depends also on qualitative differences in the carbohydrate side-chains (Lambert et al., 1998). The amount of a carbohydrate side-chain’s terminal sialic acid (and/or sulfate) is the
primary determinant of the overall charge of glycoproteins. This charge is widely accepted as the major moderator of the in vivo clearance rate. Less acidic recombinant FSH has been found to have a shorter half-life (de Leeuw et al., 1996).

In the FSH receptor, N-glycosylation occurs co-translationally and plays a role in the maturation and processing of the receptor (Menon et al., 2005). Along with the identification of alternatively spliced FSH receptor variants, which might interact differently with FSH glycosylation variants, these findings represent an intricate scheme to regulate hormone signalling (Sairam and Babu, 2007).

Gonadotrophins play a key role as survival factors in both the ovary and the testis (Billig et al., 1996). Data on FSH malfunction or aberrant FSH/FSH interaction obtained from patients with mutations in gonadotrophins or their receptors have helped in the elucidation of the physiological effects of FSH and LH on ovary and testis reveal that the phenotypic effects of the ligand appear to be more profound than those of its receptor. FSH is essential for normal puberty and fertility in females, particularly ovarian folliculogenesis. In males, FSH is necessary for normal spermatogenesis and when FSH function is completely absent, infertility occurs. In partial FSH deficient males, spermatogenesis is affected, but fertility may still be maintained (Stewart-Bentley and Wallack, 1975; Maroulis et al., 1977; McConnon et al., 1979; Kumar et al., 1997; Simoni et al., 1999). In favour of this hypothesis, Prestoz et al. (1997) demonstrated basic (hypoglycosylated) FSH isoforms in the sera of four female galactosemia patients. However, FSH bioactivity studies in patients with classic galactosemia reveal normal results. Kaufman et al. (1981) applied an in vivo mouse method, based on the increase of ovarian weight in immature female rats when FSH is administered. They tested the function of the urinary gonadotrophins from 18 girls and women with classic galactosemia and detected no abnormality. Recently, Sanders et al. (2009) measured FSH bioactivity in a subset of 10 girls and women with galactosemia who were not on HRT. They used Chinese hamster ovary cells transfected to express the human FSH receptor. The cells produce cAMP in response to receptor activation by FSH. Eight of the 10 patients demonstrated FSH bioactivities that were within the control range, two had slightly elevated bioactivities. FSH malfunction due to hypoglycosylation has been made less probable by the fact that FSH bioactivity was found normal. Furthermore, investigation of six women with hypergonadotropic hypogonadism due to congenital disorders of glycosylation (CDG) (congenital disorders of glycosylation) type I, a primary defect in one of the enzymes of the glycosylation machinery, does not show any signs of affection of the terminal charged carbohydrate portion of the gonadotrophins (Kristiansson et al., 1995).

A functionally altered FSH receptor due to hypoglycosylation remains a possibility that has not yet been studied.

Epigenetic mechanisms
Another possible mechanism could have an epigenetic origin. The possibility of a prenatal or neonatal hit disturbing expression of genes involved in follicle development might explain the ovarian failure in classic galactosemia. Affectation of genes involved in follicular development is a possibility that needs to be explored. Recently, the human tumour suppressor gene alysia ras homolog I (ARH1), a novel imprinted tumour suppressor gene, member of the Ras superfamily, which is involved in ovarian folliculogenesis, has been proposed as a target of toxicity in classic galactosemia (Lai et al., 2008). This gene has been evolutionarily lost in rodents (Fitzgerald and Bateman, 2004), and re-expression of this gene in mice causes failure of ovarian follicular maturation, poor growth and impaired Purkinje cell development (Xu et al., 2000). ARH1 is expressed consistently in normal ovarian epithelial cells.

Liu et al. (2006) studied the possible role of growth differentiation factor-9 (GDF-9), which is thought to be an obligatory growth factor during the gonadotrophin-independent phase of folliculogenesis. After 10 immature Long-Evans rats were fed a diet consisting of 20% galactose for 19 days, whole body, ovary and uterine weights were measured. Serum estradiol and progesterone concentrations were measured by radioimmunoassay. Ovarian follicles were counted by morphometric analysis and GDF-9 expression was investigated by immunohistochemistry and immunoblot assay. Galactose treatment does not affect the onset of puberty as marked by the time of vaginal opening. The galactose diet significantly decreased the number of healthy growing follicles. The results of immunoblot assay showed that both bands corresponding to propeptide and mature forms of GDF-9 decreased with the galactose diet of about 90% and 70%, respectively. The results of immunohistochemical staining showed that the GDF-9 positive follicle number and the ratio of GDF-9 positive to GDF negative (primordial/non-growing) follicles significantly decreased with this high galactose diet. This study suggested that a high galactose diet inhibits follicular development, possibly through down-regulation of GDF-9 in the rat ovary.

Other genes associated with female infertility and causing a clinical picture similar to classic galactosemia could be affected. An example of this is the FOXL2 gene, which has been found mutated in blepharoepithelial erosion syndrome (epicanthus syndrome), and encodes a novel putative forkhead transcription factor that is expressed predominantly in the developing eyelid and the ovary. In the mouse, expression localizes to the follicular cells, implying that it is essential for follicular development. Infertility in this syndrome, as in classic galactosemia, is associated with ovarian failure, ranging from ovaries that look essentially normal by ultrasonography to streak gonads (Crisponi et al., 2001). The clinical spectrum, likewise, varies from primary amenorrhea to irregularity of menstrual periods and subsequent POI. Ovarian biopsies reveal a wide range of numbers of unstimulated primordial follicles (Fraser et al., 1988; Nicolino et al., 1995). Furthermore, in females with Turner syndrome and mosaicism in chromosome analysis, remaining follicles in their ovaries have been found, even in some girls with no spontaneous puberty and high FSH and LH serum concentrations and low anti-Müllerian hormone (AMH) levels (Borgstrom et al., 2009).

Testis
Although many studies have investigated female gonadal function in galactosemia, only few studies have looked at its male equivalent. At present, in male galactoseemics fertility is not considered to be impaired, but data are very limited. The onset of puberty has been reported to be delayed in two large population studies (Waggone et al., 1990; Schweitzer et al., 1993). Mean height corrected for target height has been found decreased in both genders (Panis et al., 2007); however, galactosemic children grow beyond the age of 18 years. These findings might also reflect delayed puberty in boys. Gonadotrophin levels measurements by Steinmann et al.
(1981b) revealed elevated levels in adult patients. Endocrine measurements comprising FSH, LH, E2, testosterone, SHBG (sex hormone-binding globulin), DHEAS (dehydroepiandrosterone-sulfate) and androstenedione in 12 males ranging in age between 6 and 19 years were all normal (Rubio-Gozalbo et al., 2006). Interestingly, three of the 12 males in this study had cryptorchidism, while its prevalence in the normal population at 1 year of age is ≤1% (Berkowitz et al., 1993). Testis descent is regulated by hormonal and mechanical factors including testosterone, dihydrotestosterone, AMH, the gubernaculum, intra-abdominal pressure and the genito-femoral nerve. AMH, secreted by Sertoli and granulosa cells, is high during fetal and neonatal life and decreases thereafter, with a minimum at puberty. Elevated AMH levels in 24 males, in whom the age range was not specified (Van der Ven et al., 2005, International Symposium Fulda, November 16–18, 2005; personal communication) have been described; however, we found normal AMH levels in 12 males aged 4–16 years (unpublished results). 1 (16 years) had a slight elevation above normal level. However, different ages and measurement methods might have been used, hampering comparison. Inhibit B was found to decrease in pubertal boys and normal in adult males (Van der Ven et al. 2005, International Symposium Fulda, November 16–18, 2005; personal communication). Semen quality, widely used as the best indirect parameter for male fertility, has been investigated in only two cases (Kaufman et al., 1986), showing normal results. One male galactosemia patient, reported by our group, is known to have fathered a child (Panis et al., 2006). We know of several other male galactosemics that have fathered a child (personal communications, European Galactosemia Association Scientific meeting, Paris, 2009).

Data regarding the toxic effects of galactose on the testis in rat studies are in line with a decreased vulnerability of the testis to galactose toxicity. Chen et al. (1984) performed a study in which rats were fed a 50% galactose diet during pregnancy and nursing, and the testes were later examined and hormone levels determined in male offspring. Exposure to galactose for various periods during pregnancy, throughout the entire gestation, or post-natally to nursing mother until pups were 5 weeks of age produced no significant differences from control testicular weight, seminiferous tubular diameter or microscopic appearance of the testis when the offspring became adult (Day 66 or 127). Serum LH, FSH and testosterone levels showed no significant differences from controls. Blood galactose-1-phosphate levels in animals receiving the 50% galactose diet were comparable to those observed in the human galactosemia.

The effects of classic galactosemia on the male reproductive system seem less obvious than the widely described effects on the ovaries in females. A suggested explanation is resistance of the testis to toxic damage. GALT mRNA levels are much lower in the testis (Liu et al., 2000).

Exogenous gonadotrophin administration and estradiol response

Several female patients with classic galactosemia and ovarian dysfunction have responded to exogenous gonadotrophin administration, either by ovulating, or by documented estrogen production. Kaufman et al., (1981) were able to elicit an estradiol response in a 15-year-old girl after human menopausal gonadotrophin (hMG) administration of 225 IU/day over 3 days. This patient had oligomenorrhea with menstruation every 70–90 days. A second patient stimulated with hMG also reported by Kaufman et al. (1981) showed a significant estrogen response, but during stimulation, however, the estrogen level decreased again despite increasing the hMG dosage up to 900 IU/day. This patient had amenorrhea since a pregnancy at age 26 and was almost 30 years old at the time of testing. Menezo et al. (2004), reported a woman with secondary amenorrhea since age 19 who became pregnant after stimulation: two cycles, with estrogen and progesterone pretreatment, stimulation with recombinant FSH (up to 150 IU/day) followed by ovulation triggered by hCG, both resulted in ovulation, with the second leading to conception of a healthy child.

Conversely, Steinmann et al. (1981b) reported two patients, aged 14 and 15 years, with no estrogen response despite 5-day stimulation with 150 IU of hMG. The same treatment was administered to a young woman by Schwarz et al. (1986), again with no significant estradiol increase. Two sisters with secondary amenorrhea following hormone replacement therapy (HRT) after delayed pubertal development and spontaneous menarche, aged 31 and 33 years at the time of testing, failed to show follicle maturation after treatment with oral estrogens, clomiphene and exogenous gonadotrophins (Fraser et al., 1986). Another patient, reported by Sauer et al. (1991) did not ovulate despite hMG stimulation with up to 600 IU/day. This woman, who was treated with HRT for primary amenorrhea at age 16, later became pregnant by oocyte donation.

Furthermore, in females with CDG and hypergonadotropic hypogonadal dysfunction there is some anecdotic evidence of estradiol response after exogenous gonadotrophin administration: Ohzeki et al. (1993) reported a 14-year-old girl who responded and similar results were reported in two monozygotic twin-sisters aged 13 years by de Zegher and Jaeken (1995).

The fact that an estradiol response can be elicited after exogenous gonadotrophin administration in some galactosemic patients can be interpreted in several ways. It has been suggested that this might be due to inactivity of endogenous FSH; however, FSH bioactivity measurements in vitro reveals normal results (Kaufman et al., 1981; Sanders et al., 2009). Estradiol response after exogenous FSH administration in these patients could be due to ovarian stimulation by increasing the FSH concentrations above threshold levels with exogenous FSH, the principle of controlled ovarian hyperstimulation. However, ovarian function fluctuates even without exogenous stimulation, resulting in varying levels of estradiol and possibly even ovulations. Consequently, the impact of exogenous FSH on ovarian function is difficult to demonstrate in patients with galactosemia.

Imaging and histology of the ovaries

To gather evidence whether ovarian failure is a consequence of increased attrition of a normal number of follicles present within the ovary, or of normal attrition of a decreased number of follicles we have reviewed the imaging and histological data available in the literature (Table 1).

Morphological normal ovaries with abundant oocytes and normal folliculogenesis have been reported in two neonates (Levy et al., 1984; Levy, 1996). In a patient aged 16 years, laparoscopy showed hypoplastic ovaries and serial sections of ovarian tissue showed two morphologically normal primary follicles on light microscopy—far fewer than expected for age. Electron microscopy failed to reveal oocytes but showed normal ovarian stroma (Robinson et al., 1984).
<table>
<thead>
<tr>
<th>Number</th>
<th>Age at examination</th>
<th>Puberty</th>
<th>Menses</th>
<th>Obstetrics</th>
<th>Abdominal/ transvaginal ultrasound</th>
<th>Laparoscopy/ laparotomy</th>
<th>Ovarian histology</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>5 days</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Abundant oocytes, normal folliculogenesis*</td>
<td>Levy et al., 1984</td>
</tr>
<tr>
<td>3</td>
<td>7 years</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>Normal</td>
<td>n.d.</td>
<td>Kaufman et al., 1981</td>
</tr>
<tr>
<td>4</td>
<td>13.7 years</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>Hypoplastic</td>
<td>Streak like</td>
<td>n.d.</td>
<td>Steinman, 1981a, b</td>
</tr>
<tr>
<td>5</td>
<td>15 years</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>One single small ovary</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Kaufman et al., 1981</td>
</tr>
<tr>
<td>6</td>
<td>15 years</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>Hypoplastic</td>
<td>Possibly absent</td>
<td>n.d.</td>
<td>Steinman, 1981a, b</td>
</tr>
<tr>
<td>7</td>
<td>15.8 years</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>One single small ovary</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Kaufman et al., 1981</td>
</tr>
<tr>
<td>8</td>
<td>16 years</td>
<td>Delayed</td>
<td>Primary amenorrhea</td>
<td>n.r.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Hypoplastic</td>
<td>Few follicles</td>
</tr>
<tr>
<td>9</td>
<td>16 years</td>
<td>None spontaneously (16 years)</td>
<td>Primary amenorrhea, then HRT, after withdrawal of HRT</td>
<td>n.r.</td>
<td>Small prepubertal ovaries</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>10</td>
<td>16.2 years</td>
<td>Delayed</td>
<td>Primary amenorrhea, HRT at 16 years</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.r.</td>
<td>Rare primordial follicles, absence of intermediate and Graafian follicles</td>
</tr>
<tr>
<td>11</td>
<td>16.8 years</td>
<td>Normal</td>
<td>Primary amenorrhea, then HRT</td>
<td>n.r.</td>
<td>Growing on yearly follow-up</td>
<td>n.r.</td>
<td>Hypoplastic, streak ovaries</td>
<td>No ovarian parenchyma</td>
</tr>
<tr>
<td>No.</td>
<td>Age</td>
<td>Diagnosis</td>
<td>Menarche</td>
<td>Menarche detail</td>
<td>Egg count</td>
<td>Maturation</td>
<td>Size</td>
<td>Size description</td>
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</tr>
<tr>
<td>12</td>
<td>17</td>
<td>None spontaneously</td>
<td></td>
<td>n.r.</td>
<td>Small,</td>
<td>Streak like</td>
<td>n.r.</td>
<td>Fibrous parenchyma, few follicles</td>
</tr>
<tr>
<td>13</td>
<td>17</td>
<td>Delayed</td>
<td></td>
<td>n.r.</td>
<td>n.r.</td>
<td>Small</td>
<td>n.d.</td>
<td>No follicles</td>
</tr>
<tr>
<td>14</td>
<td>17.3</td>
<td>n.r.</td>
<td></td>
<td>n.r.</td>
<td>n.r.</td>
<td>Not visualized</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>15</td>
<td>18.5</td>
<td>Delayed</td>
<td>Spontaneous menarche at 18 years</td>
<td>n.r.</td>
<td>Small</td>
<td>Not visualized</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>16</td>
<td>18.7</td>
<td>n.r.</td>
<td></td>
<td>n.r.</td>
<td>n.r.</td>
<td>Not visualized</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>18</td>
<td>20.5</td>
<td>Delayed, not competed spontaneously</td>
<td>Primary amenorrhea</td>
<td>n.r.</td>
<td>Small</td>
<td>Small</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>19</td>
<td>21</td>
<td>n.r.</td>
<td>Primary amenorrhea, HRT at 21 years</td>
<td>n.r.</td>
<td>n.d.</td>
<td>Small</td>
<td>Streak ovaries</td>
<td>n.d.</td>
</tr>
<tr>
<td>20</td>
<td>26</td>
<td>Delayed</td>
<td>Spontaneous menarche at 18 years, oligomenorrhea, then secondary amenorrhea</td>
<td>n.r.</td>
<td>n.r.</td>
<td>n.r.</td>
<td>Streak ovaries</td>
<td>n.d.</td>
</tr>
<tr>
<td>21</td>
<td>26</td>
<td>Delayed</td>
<td>Spontaneous menarche at 16 years, oligomenorrhea</td>
<td>3 spontaneous pregnancies</td>
<td>Normal</td>
<td>Inactive looking</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>23</td>
<td>30</td>
<td>Delayed</td>
<td>Oligomenorrhea</td>
<td>n.r.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Very small, almost streaks</td>
<td>Two primordial follicles; signs of follicular activity</td>
</tr>
</tbody>
</table>

n.d., not done; n.r., not reported.
*aObduction.*
In a patient aged 17 years, ovarian biopsy yielded a fibrous parenchyma with many collagen fibres and small vessels, not resembling ovarian stroma; rarely, a follicle without signs of maturation was seen in a slide, but in the majority of sections no follicles were seen (Beauvais and Guilhaume, 1984). In another female, also aged 17 years, laparotomy revealed a small uterus and normal fallopian tubes, with bilateral streak ovaries. Histological examination of one complete ovary showed ovarian stroma and a small group of hilar cells but no follicles were present (Morrow et al., 1985). Laparoscopy in a woman aged 21 years showed a uterus of normal appearance, but both ovaries were severely hypoplastic and streak like, measuring 2 × 0.5 cm each. The surface of the ovaries was white to yellowish and gave the impression of having small underlying follicles, at least in some areas. Histological examination of a biopsy from one ovary showed some smooth muscle and fibrous tissue, but no ovarian parenchyma (Schwarz et al., 1986). Histological examination in another woman at the same age showed ovarian tissue capped by a single layer of mesothelial cells. The tunica albuginea was thin and merged with the underlying stroma, which was made up of undulating fibrocytes and collagen fibres forming a herring bone pattern. The predominantly eosinophilic stroma supported a few widely scattered hyalinized atretic follicles and rare primordial follicles (one per eight high power fields of cortex), and no intermediate evolving Graafian follicles were identified (Kaufman et al., 1989). Biopsy examination in a third woman aged 21 years demonstrated a fibrotic stroma and an absence of intermediate and Graafian follicles (Sauer et al., 1991). Bilateral large laparoscopic biopsies taken from normal sized but inactive looking ovaries in a female at age 26 showed numbers of primordial follicles and oocytes within the normal range, but no developing follicles were found even on extensive review of the material. Bilateral ovarian biopsies in a second woman were carried out at the age of 30 years. A major segment was removed from each very small ovary and multiple sections revealed only two recognizable primordial follicles containing oocytes (Fraser et al., 1986). Ovarian biopsy in one of our patients at age 16 years revealed normal ovarian tissue with morphologically normal primordial follicles, but less than normal for this age (Fig. 1). Interestingly, in a patient described by Kaufman et al. (1981) apparently normal ovaries (on abdominal exploration for gallstones) were seen at 7 years of age, whereas at 17 years streak gonads on laparoscopy were found.

The fact that normal folliculogenesis has been observed in two neonates, a well as normal appearance of the ovaries in a young girl at age 7, coupled with the observed severe reduction of follicles in older females, tentatively suggests gonadal failure after embryologic follicular development with an increased attrition of a normal number of follicles present within the ovary. A striking feature is the absence/scarcity of follicle maturation, pointing to a maturation arrest. Unfortunately, no histological data are available in girls between infancy and adolescence. These histological and imaging findings have also been described in FSH receptor inactivating mutations (Aittomäki, 1994; Aittomäki et al., 1996; Beau et al., 1998; Touraine et al., 1999). FSH β-subunit mutations (Matthews et al., 1993; Layman et al., 1997; Matthews and Chatterjee, 1997) and Kallmann syndrome (Gauthier, 1960; Muller, 1964; Sparkes et al., 1968; Tagatz et al., 1970; Muller and Dellenbach, 1971; Bell et al., 1973; Goldenberg et al., 1976; Ettiging et al., 1976; Spitz et al., 1977; Rjosk and Goebel, 1978; Lieblich et al., 1982; Janicke et al., 1983; Chryssikopoulos, 1986; Sungurtekin et al., 1995; Kousta et al., 1996; Persson et al., 1999; Battaglia et al., 2000; Sipe and Van Voorhis, 2007) and Turner syndrome (Hreinsson et al., 2002; Borgstrom et al., 2009).

As far as we know, there are no imaging or histological data of the male gonads in patients with classic galactosemia.

**Course of ovarian failure and pregnancy chances in classic galactosemia**

Intriguingly, a fluctuating POF course has been noticed in galactosemic women. Rather than ovarian failure this has been called resistant ovary syndrome or primary ovarian insufficiency (Fraser et al., 1986; Twigg et al., 1996; Berry, 2008; Welt, 2008). Recently, a galactosemic woman with a fluctuating course and three pregnancies was reported (Gubbels et al., 2009). The mechanism behind this fluctuating course remains unclear, as in most other causes of POF.

Women with classic galactosemia are considered to be infertile at a very young age, but despite the high incidence of POF and subsequent infertility, spontaneous pregnancies do occur (Roe et al., 1971; Tedesco et al., 1972; Samuels et al., 1976; Sardharwalla et al., 1980; de Jongh et al., 1999; Briones et al., 2001; Kimonis, 2001; Noelmans et al., 2006; Ohlsson et al., 2007; Gubbels et al., 2008, 2009), and may not be as rare as is generally assumed (Gubbels et al., 2008).

When counseling young women concerning the occurrence of POF and its consequences for fertility, it is important to determine predictors for pregnancy and to discuss the impact of pregnancy on the galactosemic mother and (generally) heterozygous child and to propose surveillance during pregnancy and the post-partum period. These aspects have not been evaluated systematically.

The interpretation of well-established pregnancy chance predictors might be difficult in this group of women, as illustrated by serum AMH levels in female galactosemia patients. This is one of the identified growth factors involved in the initiation and inhibition of primordial folli...
Gonads and galactosemia

Conclusions

Gonads in males with classic galactosemia seem less affected than in females who exhibit hypergonadotropic hypoestrogenic subfertility. The timing of the damage to the ovary (prenatal, peri-natal or post-natal) as well as the pathophysiological mechanisms are unclear. Intriguingly, in many patients, the serum FSH level has been found elevated very early in life (4 months to 4 years) and also between early childhood and onset of puberty (5–12 years). Morphological normal ovaries with abundant oocytes and normal folliculogenesis have been reported in two neonates. Ovarian histology in young adult ovaries with abundant oocytes and normal folliculogenesis have been published (Sanders et al., 2009). Interestingly our group has reported a woman with fluctuating POF, low AMH and spontaneous pregnancy (Gubbels et al., 2009). Low AMH levels in women with classic galactosemia might not only be due to follicle depletion, but might also reflect the fact that only a minority of primordial follicles are making the transition to the growing stage. In this group of patients, AMH levels and chances of spontaneously achieving pregnancy need to be interpreted with caution.

Funding

This work was financially supported by grants from the Maastricht University Medical Center and Dutch Patients Galactosemia Research Foundation.

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Submitted on April 29, 2009; resubmitted on July 24, 2009; accepted on August 21, 2009.