Abstract

Klinefelter syndrome, 47,XXY (KS), is the most frequent sex chromosome aberration in males, affecting 1 in 660 newborn boys. The syndrome is characterized by testicular destruction with extensive fibrosis and hyalinization of the seminiferous tubules resulting in small testes, hypergonadotropic hypogonadism, and azoospermia in the majority of cases. Until recently, infertility was considered an untreatable condition in KS. However, with the development of new advanced assisted reproductive techniques such as testicular sperm extraction (TESE) combined with ICSI it seems that KS patients should no longer be labelled as infertile. Especially, microdissection (micro)-TESE has proved to be an advantageous procedure for the identification of testicular spermatozoa in KS. The aim of this review was to describe current knowledge on the testicular changes occurring in KS, the associated changes in reproductive hormones and spermatogenesis, and the existing possibilities of biological fatherhood in 47,XXY patients.

Introduction

Klinefelter syndrome, 47,XXY (KS), is characterized by a progressive testicular failure causing small firm testes, androgen deficiency, and azoospermia (1). Although it is the most prevalent chromosome aberration (occurring in ~1 in 660 newborn males (2)), and one of the most frequent genetic causes of infertility (occurring in 11% of azoospermic men and in 3% of infertile men (3, 4)), it is a profoundly underdiagnosed condition. Epidemiological studies have shown that only 25% of adult males with KS are ever diagnosed, and diagnosis is rarely made before the onset of puberty (2). The syndrome is generally diagnosed at three main stages in life: prenatally, around school age mainly because of tall stature, learning and behavioural difficulties, or in adulthood mainly because of infertility (2, 5).

The presence of a sex chromosome aneuploidy affects the patient at multiple organ levels. Although many individuals with sex chromosome variations can live functionally normal lives, others may experience physical, developmental, psychosocial, behavioural, and learning disabilities. The natural history of these clinical findings is not completely elucidated. Some may be a consequence of the hypogonadism typical for this syndrome, whereas others may be directly related to the chromosome abnormality. Although the vast majority of men with nonmosaic KS are azoospermic, motile sperms in the ejaculate and even spontaneous pregnancies resulting from KS fathers have been described, although such cases are rare (6, 7, 8, 9, 10). In general, Klinefelter mosaics (47,XXY/46,XY) are less severely affected and the chance of finding sperm in the ejaculate in these males is significantly higher than in nonmosaic cases. Thus, in the majority of cases use of donor semen (or more rarely by adoption) has been the only possible way of becoming a father. However, in recent years testicular sperm extraction (TESE) followed by ICSI have resulted in more than 100 cases of KS men worldwide who became biological fathers. In a recent nationwide register-based study from Denmark of 1049 KS patients and 100 824 matched controls, KS subjects had significantly fewer children and at a later age as compared with controls (11). Bojesen et al. found that only 25% of the expected number of KS men became fathers compared with controls.

Until recently, infertility was considered an untreatable condition in KS. However, with the development of new advanced assisted reproductive techniques such as TESE combined with ICSI it seems that KS patients should no longer be labelled as infertile. Especially, microdissection (micro)-TESE has proved to be an advantageous procedure for the identification of testicular spermatozoa in KS. In 1996 successful
recovery of spermatozoa by TESE in men with azoospermia and KS was reported for the first time (12), with the first pregnancies reported in 1997 (13). In the present review we describe current knowledge on the testicular changes occurring in KS, the associated changes in reproductive hormones and spermatogenesis, and the existing possibilities of biological fatherhood in 47,XXY patients.

Testicular histology in 47,XXY

Infertility in KS is a consequence of germ cell degeneration that commences already in utero, progresses slowly during infancy and early childhood, and accelerates during puberty and adolescence, eventually resulting in extensive fibrosis and hyalinization of the seminiferous tubules and hyperplasia of interstitium in the adult patient (for review see (14)). Testicular volume is significantly reduced in infants and prepubertal boys with KS as compared with similarly aged healthy boys (15, 16), indicating that the number of seminiferous tubules is significantly reduced before puberty.

As illustrated in Figs 1 and 2, germ cell degeneration is already noted in the pubertal Klinefelter boy. Accordingly, Wikstrom et al. (17) only found germ cells in the testes of 50% of peripubertal Klinefelter boys indicating that the fertility may already be impaired at this young age. In addition, Wikstrom et al. (18) showed that germ cell differentiation was arrested in spermatogonium or early spermatocyte stage in KS, and that the spermatogonia undergo apoptosis instead of entering meiosis at the onset of puberty. It has been shown that azoospermic Klinefelter men may have single residual foci with preserved spermatogenesis (4, 19, 20, 21, 22, 23, 24, 25) and may benefit from assisted reproductive techniques to father a child.

Testicular endocrinology

In infancy, around the age of 3 months, the hypothalamic–pituitary–gonadal (HPG) axis is transiently activated in the so-called mini-puberty (26, 27, 28, 29). The initial activation of the HPG axis is believed to be important for genital development, including renewal and differentiation of the germ cells (30). Dysfunction of any of the components of the mini-puberty may underlie the lowered eventual sperm counts in boys with hypogonadotropic hypogonadism by decreasing the number of spermatogonia produced for the future (31). The mini-puberty represents a window suitable for studying the function of the pituitary–gonadal axis at this young age by measuring the spontaneous, basal hormone levels (32). Although controversies exist as to whether the HPG axis is impaired in KS infants (16, 33, 34, 35), the latest and largest study on the mini-puberty in KS demonstrated normal testosterone concentrations (35). Importantly, however, the testosterone concentrations were below the median of the controls, which may indicate a subtle Leydig cell dysfunction, although this was not supported by an elevation of LH concentrations (35). This could most likely reflect the fact that androgen receptors are not highly expressed at 3 months and that the normal feedback system is not functioning.

In childhood, KS boys are characterized by normal concentrations of testosterone, FSH, LH, anti-Müllerian hormone (AMH), inhibin B, and insulin-like factor 3 (INSL3) until the onset of puberty (17, 18, 36, 37, 38, 39, 40, 41, 42, 43). KS boys usually enter puberty at the expected age with an initial normal increase in testicular volume (Fig. 3) and appropriate rise in serum concentrations of testosterone, INSL3, and inhibin B. However, from around midpuberty the testicular deterioration occurs, as evidenced by a regression of testicular volume (5, 41) (Fig. 3) and levelling off in the serum concentrations of testosterone and INSL3, both
of which remain in the low–normal range through puberty (17, 36, 37, 39). Concomitantly, a dramatic decline is observed in inhibin B concentrations, which are most often undetectable at the end of puberty in Klinefelter patients (17, 38, 39). The physiological pubertal decline in serum AMH is also observed in KS, although this occurs later than observed in healthy boys (18, 43, 44). At midpuberty, a relative hypogonadism is usually evident by increasing LH and FSH concentrations to hypergonadotropic levels. FSH increases earlier and more markedly than LH (5, 36, 37, 41). Adults with KS are characterized by hypergonadotropic hypogonadism with highly elevated serum concentrations of FSH and LH. The serum concentration of testosterone is most often in the lower half of the reference range of healthy males, and rarely below the reference range. Inhibin B is below the detection limit in the vast majority of KS adults reflecting the absent spermatogenesis (39), whereas the circulating concentrations of AMH and INSL3 are significantly reduced compared with healthy males (40, 45, 46). Mean testes volume in KS adults is 3.0 ml (range 1.0–7.0) as compared with 22 ml in healthy adult males (Fig. 3) (5).

**Figure 2** Testicular biopsies from patients with KS at various ages. (A) Fourteen-year-old boy with complete hyalinization of seminiferous tubules. Note the tubules appear as ‘ghost tubules’ without germ cells and Sertoli cells as marked with green lines. (B) Fifteen-year-old boy with tubule containing fully differentiated Sertoli cells only, next to an area with many ‘ghost tubules’ marked with green dotted lines. (C) Eighteen-year-old male with heterogeneous, adult-type pattern with tubules with spermatogenesis (left), and a few Sertoli cell-only tubules (right) mixed with completely hyalinized tubules and Leydig cell hyperplasia. (D) Nineteen-year-old male with heterogeneous adult-type pattern with differentiated Sertoli cells, type A (left) and incompletely differentiated Sertoli cells (type B) (right), fibrosis and Leydig cell hyperplasia. (E) Twenty-five-year-old male presenting with heterogeneous adult pattern showing type A (top) and type B (middle) Sertoli cells. (F) Twenty-nine-year-old male with adult pattern showing large Leydig cell clumps, a few Sertoli cell-only tubules, and ghost tubules marked with L. (G) Thirty-year-old male with adult pattern. Note the tubules with spermatogenesis embedded in large Leydig cell clumps denoted by L. (H) Thirty-one-year-old male. Martius/scarlet/blue (MSB) staining. Adult pattern with impairedd spermatogenesis embedded in Leydig cells and hyalinized tubules (arrows). (I) Forty-seven-year-old male. Adult pattern with scattered Sertoli cell-only tubules (marked with S) embedded in large Leydig cell clumps. All bars are 100 µm except F, G, and I where it denotes 250 µm. All staining are H&E except A and H, which are periodic acid-Schiff and MSB respectively (reprinted from Akslagde L, Skakkebaek NE, Almstrup K & Juul A. Clinical and biological parameters in 166 boys, adolescents and adults with nonmosaic Klinefelter syndrome: a Copenhagen experience. *Acta Paediatrica* 2011 **100** 793–806, with permission).

**Cryopreservation of ejaculated spermatozoa**

Cryopreservation of spermatozoa is currently offered to boys undergoing gonadotoxic treatments, which may render them sterile. The success rate of obtaining sperm from masturbation in adolescent boys depends on the degree of pubertal maturation, but also psychological factors influence whether or not it is possible to obtain a semen sample by masturbation. In our experience it may be possible to obtain sperm for cryopreservation from boys with testicular volumes of 6 ml and more, but we have not been successful in identifying other predictive factors (e.g. hormone levels) for a positive outcome (47). In subjects where shyness and/or other psychological factors and immaturity disable a positive outcome, penile vibration, or electroejaculation under general anaesthesia may result in sperm for cryopreservation before chemotherapy (47). In the few KS patients where motile sperms are seen in the ejaculate, cryopreservation should be offered (6). Anecdotally, it has been proposed that the chance of finding motile sperm in the ejaculate would be higher in semen samples from early pubertal KS boys before the destruction of the seminiferous tubules has been completed. However, we were not successful in 12 out 12 KS adolescents aged 15–20 years (6). In contrast, Lanfranco et al. found spermatozoa in the ejaculate of 11 of 131 (8.4%) men with KS (including one 47,XXY/46,XY mosaic) aged 18.6–34.8 years. Ejaculated semen from men with KS has been used for ICSI.
and to date at least eight live born children have been reported (48, 49, 50, 51, 52).

During recent years, cryopreservation of spermatogonial stem cells (SSCs) has been offered on an experimental basis to immature boys prior to chemo- or radiotherapy with the purpose of being (hypothetically) able to reintroduce the SSCs in the patient’s own testis by SSC transplantation. So far, in vitro spermatogenesis of human SSCs has not been possible, but this technique might become an option in the future since the in vitro differentiation of mouse SSCs up to mature sperm cells has recently been reported (53, 54). Unfortunately, only 10% of KS patients are diagnosed before puberty, explaining the limited experience on testicular tissue banking in KS adolescents (2). Since KS testes are characterized by extensive fibrosis and hyalinization of the seminiferous tubules, the ultimate use of the SSCs is likely to differ from that of boys with a normal karyotype. At present, cryopreservation with or without preceding TESE must be considered an experimental approach in adolescents with KS. Thus, it may be interesting as part of a research protocol, but it clearly remains to be seen if this will become a standard offer to such boys.

A new sperm retrieval method

Until recently, the only way to become a father for males with KS was by the use of donor insemination or adoption. During the 1990s a new technique, TESE, was developed and subsequently refined by the so-called micro-TESE (55). Conventional TESE is based on multiple blind testis biopsies, whereas micro-TESE is based on microsurgery to identify individual seminiferous tubules with active spermatogenesis. The micro-TESE technique has proved superior to TESE with respect to minimizing the damage to the testicular tissue, and maximizing the success rate of sperm retrieval (55).

An overview of the published studies on success rates and predictors of sperm retrieval in men with KS according to method (TESE vs micro-TESE) is presented in Table 1. A total of 741 patients were included with an average sperm retrieval rate of 50% distributed on 42% by the use of TESE and 57% by the use of micro-TESE (56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77). A total of 14 mosaics were included in these studies (56, 72). Exclusion of these did not change the success rates.

Preoperation hormonal therapy

Early androgen replacement therapy in the peripubertal period is generally recommended to allow for normal pubertal development and age-appropriate attainment of muscle and bone mass, although no randomized controlled trial evaluating the effect of this approach exists at present. In patients already receiving androgen replacement therapy, it has been suggested to discontinue this treatment for at least 6 months prior to micro-TESE (72). Alternative therapeutic options with aromatase inhibitor (testolactam or anastrozol), human chorionic gonadotropin (hCG), or clomiphene are often applied, but no controlled trials exist. Uncontrolled studies have reported moderate positive effects on sperm retrieval rates in patients with nonobstructive azoospermia (NOA) (78, 79). However, controversy exists and a very recent study on 1054 men with NOA found that neither baseline testosterone nor the response to preoperation hormonal therapy had any effect on overall sperm retrieval, clinical pregnancy, or live birth rates (80).

Predictive factors of successful TESE in 47,XXY

The levels of FSH, inhibin B, and the inhibin B/FSH ratio are known predictive factors for fertility in males with normal karyotype (81, 82, 83), but this does not seem to be the case in KS. In fact, even patients with inhibin B below the detection limit underwent successful TESE in one study (64). Likewise, we reported two KS subjects with motile spermatozoa in their ejaculate (one of the patients fathered a child spontaneously); both had undetectable inhibin B and highly elevated FSH levels, and could not be distinguished from KS patients with persistent azoosperma (6).

Several authors demonstrated that age at TESE might affect the outcome of TESE in Klinefelter patients (Table 1) (66, 67, 69, 70, 73). This is in accordance with the concept of a progressive degradation of the
spermatogonia, and based on single case reports, declining spermatogenesis with ageing in Klinefelter men has been reported (9, 10). In addition, one study found a positive predictive value of testicular volume, testosterone levels and response to hCG test for successful TESE (62), but this association was not confirmed in others (66, 70, 84). Furthermore, no association between outcome of TESE and testicular ultrasonography, intratesticular blood-flow resistance, or degree of virilization has been found (66, 70, 84).

In the study by Ramasamy et al. the serum testosterone concentration and the testosterone–oestradiol ratio after preoperative medical therapy were higher in men in whom sperm were found than in men in whom no sperm were found ($P = 0.002$ and $P = 0.05$ respectively). Men with low baseline testosterone, who responded to the medical therapy with a resultant testosterone of $> 8.7$ nmol/l (250 ng/dl) had a higher chance of sperm retrieval than men who did not (75).

The age of the patient is the only consistent positive prognostic factor for successful TESE in KS. However, the existing results are contradicting and no single parameter has been identified so far.

## Complications to micro-TESE

It has been shown that micro-TESE causes fewer acute and chronic complications than conventional TESE (55, 85, 86, 87). Ramasamy et al. (86) studied 435 patients with NOA without KS undergoing either micro-TESE or conventional TESE and reported fewer acute and chronic changes as evaluated by ultrasound in the microdissection group than in the conventional group. In that study an 80% decrease in serum testosterone at 3–6 months after TESE and an increase to 85% after 12 months and 95% after 18 months was found (86).

### Table 1 Success rates of sperm retrieval with TESE and micro-TESE in patients with Klinefelter syndrome.

<table>
<thead>
<tr>
<th>References</th>
<th>No. of patients</th>
<th>No. of mosaics</th>
<th>No. of attempts</th>
<th>Success rate (%)</th>
<th>Parameter(s) affecting success rate (significance)</th>
<th>Parameter(s) not affecting success rate</th>
<th>Live born children</th>
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<td>8/15 (53)</td>
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<td>9</td>
<td>4/7 (57)</td>
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<td>1</td>
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<td>8/20 (40)</td>
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<td>5/12 (42)</td>
<td>NE</td>
<td>NE</td>
<td>6</td>
</tr>
<tr>
<td>(62)</td>
<td>20 None</td>
<td>NA</td>
<td>NA</td>
<td>9/20 (45)</td>
<td>Higher basal testosterone concentration ($P &lt; 0.005$)</td>
<td>Response to hCG ($P &lt; 0.002$)</td>
<td>Testicular volume ($P &lt; 0.05$)</td>
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<td>(63)</td>
<td>24 None</td>
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<td>NA</td>
<td>12/24 (50)</td>
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<tr>
<td>(64)</td>
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<td>5/18 (28)</td>
<td>Inhibin B</td>
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<tr>
<td>(56)</td>
<td>36 11</td>
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<td>NA</td>
<td>10/36 (28)</td>
<td>Mosaicism (significant)</td>
<td>Not FSH, testosterone, testicular volume, and age</td>
<td>Testosterone, gynecomastia, testicular volume, and age</td>
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<tr>
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<td>Low FSH ($P = 0.04$)</td>
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<td>Testosterone, LH, FSH, and testicular volume</td>
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<td>NA</td>
<td>9/24 (38)</td>
<td>Age and reproductive hormones</td>
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<td>29/42 (69)</td>
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<tr>
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<td>Age ($P = 0.002$)</td>
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<tr>
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<td>NA</td>
<td>50/106 (47)</td>
<td>Age</td>
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<tr>
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<td>NA</td>
<td>NA</td>
<td>27/50 (54)</td>
<td>NE</td>
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<td></td>
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<tr>
<td>Total</td>
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<td></td>
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NA, not available; NE, not evaluated.
Similar results have been reported in studies on patients with nonmosaic KS. Okada et al. (88) reported a decrease in serum testosterone concentration which did not improve after 12 months, whereas a recovery in serum testosterone to 50% of baseline after 12 months was reported in another study (89). Similar results have been published by Ishikawa et al. (90). They concluded that the decline in serum testosterone may be related to the small testicular volume and Leydig cell loss near the scars after the procedures. KS patients should therefore be followed endocrinologically after a TESE/micro-TESE procedure and substituted with androgens when indicated.

**Outcome of TESE/ICSI in 47,XXY**

With the increasing chances for KS males of fathering children by the use of assisted reproductive techniques, the chromosomal condition of the germ cells in the testis from KS patients is both of scientific and practical medical interest. Investigations of the ejaculated or testicular spermatozoa in KS with the fluorescent in-situ hybridization technique have shown varying frequencies of normal spermatozoa ranging from 50.0 to 93.7% (60, 91, 92). Accordingly, it has been proposed that adults with KS have a substantially higher proportion of hyperhaploid spermatozoa (46,XY and 46,XX) than healthy males (93, 94), giving these males a theoretically increased risk of fathering a child with 47,XXY or 47,XXX (for review see (95)). Furthermore, an increased frequency of autosomal aneuploidy 13, 18, and 21 in spermatozoa from KS has been proposed (52, 93, 96). Importantly, Blanco et al. (97) suggested that the abnormal cells at the primary and the secondary spermatocyte or the spermatid level were arrested, giving rise to a continuous elimination of abnormal cells in the germ cell line along spermatogenesis.

Despite a substantial evidence that only diploid, XY, germ cells can turn into meiosis in the XXY mice (98), and that XXY germ cells are absent in the testes of adult XXY mice (99), this subject remains controversial in humans (4, 97, 100, 101, 102). However, in two recent studies of nonmosaic KS patients all meiotic spermatocytes were normally euploid and thus able to mature into haploid spermatozoa (25, 103). Thus, at least 149 healthy live born babies without anomalies were chromosomally normal and thus able to mature into haploid spermatozoa (25, 103). Hence, the vast majority of males with 47,XXY are chromosomally normal and thus able to mature into haploid spermatozoa (25, 103).

Even if the conception of 47,XXY pregnancies has been reported (61, 109) it appears relatively safe, but preimplantation genetic diagnosis (PGD) is generally offered to couples with KS who undergo successful TESE and ICSI. This technique allows for selecting chromosomally abnormal embryos in order to avoid transferring abnormal embryos. Staessen et al. (52) compared the result of PGD in 113 embryos from 20 couples with KS with 578 embryos from control couples with X-linked disease undergoing PGD for gender determination and found a significantly higher percentage of sex chromosome (13.2 vs 3.1%) and autosomal (15.6 vs 5.2%) abnormalities in embryos from KS couples as compared with the X-linked couples. With respect to the sex chromosome abnormalities monosomy X, monosomy Y, 47,XXX, 47,XYY, and mosaicisms were identified. Interestingly, no embryo with 47,XXY from KS couples was identified. Analyzing the autosomes separately, the difference was only significant for chromosomes 18 and 21 with both monosomies 18 and 21 and trisomies 18 and 21 present. Overall, 54.0% of the embryos from KS couples were normal with an almost equal sex ratio.

In conclusion, low–normal testosterone and elevated LH serum concentrations are typical findings in KS and merit androgen supplementation in a majority of patients. The vast majority of males with 47,XXY are usually azoospermic, but the chances of fathering a child by the use of assisted reproductive techniques are increasingly encouraging. Based on the existing literature, we here report an average sperm retrieval rate of 50%, ranging from an average of 42% by the use of TESE to an average of 57% by the use of micro-TESE based on studies on a total of 741 males with KS. Although approximately half of cases are successful in retrieving sperm, the reported number of live born children of couples with KS is still limited. Spermatozoa may occasionally be found in the ejaculate, and we therefore recommend always performing analysis of ejaculated semen before considering TESE/micro-TESE.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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**References**

168 Ichioka K, Utsunomiya N, Kohei N, Ueda N, Inoue K & Terai A.  
169 Wikstrom AM, Raivio T, Hadziselimovic F, Wikstrom S, Tuuri T &  
170 Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF &  
171 Palermo GD, Schlegel PN, Sills ES, Veeck LL, Zaninovic N,  
172 Aksglaede L, Wikstrom AM, Rajpert-De ME, Dunkel L,  
173 Laron Z, Dickerman Z, Zamir R & Galatzer A. Paternity in  
174 subjects affected by classic Klinefelter’s syndrome.  
176 (doi:10.1210/jcem-42-4-679)  
177 4 Denschlag D, Tempfer C, Kunze M, Wolff G & Keck C. Assisted  
178 reproductive techniques in patients with Klinefelter syndrome:  
179 a critical review. Fertility and Sterility 2004 82 775–779.  
180 Sciarra RO, Luna Hisano CV, Rahn MI, Brugo Olmedo S,  
181 Rey Vazacchi G, Coco R & Solari AJ. Focal spermatogenesis  
182 originates in euploid germ cells in classical Klinefelter patients.  
184 humrep/dep180)  
185 Winter JS, Hughes IA, Reyes FI & Faiman C. Pituitary–gonadal  
186 relations in infancy: 2. Patterns of serum gonadal steroid  
187 concentrations in man from birth to two years of age. Journal  
189 (doi:10.1210/jcem-42-4-679)  
189 27 Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T,  
190 Simell O & Skakkebaek NE. Longitudinal reproductive hormone  
191 profiles in infants: peak of inhibin B levels in infant boys exceeds  
192 levels in adult men. Journal of Clinical Endocrinology and  
193 Metabolism 1998 83 675–681. (doi:10.1210/jc.83.2.675)  
194 28 Forest MG, Sizonenko PC, Cathiard AM & Bertrand J. Hypophyso-  
195 gonadal function in humans during the first year of life. 1. Evidence  
195 for testicular activity in early infancy. Journal of Clinical  
196 Investigation 1974 53 819–828. (doi:10.1172/JCI107621)  
197 29 Corbier P, Edwards DA & Robb J. The neonatal testosterone surge:  
197 a comparative study. Archives Internationales de Physiologie, de  
199 13813459209035274)  
200 30 Zivkovic D & Hadziselimovic F. Development of Sertoli cells during  
202 (doi:10.1007/s001200050301)  
203 Hadziselimovic F, Zivkovic D, Bica DT & Emmons LR. The  
203 importance of mini-puberty for fertility in cryptorchidism.  
205 0000181306.97839.b0)  
206 31 Main KM, Schmidt IM, Toppari J & Skakkebaek NE. Early  
206 perinatal treatment of hypogonadotropic hypogonadism with  
206 recombinant human FSH and LH. European Journal of Endo-  
207 crinology 2002 146 75–79. (doi:10.1530/eje.0.1460075)  
208 32 Akslaede L, Petersen JH, Main KM, Skakkebaek NE & Juul A.  
209 High normal testosterone levels in infants with non-mosaic  
210 33 Lahlou N, Fennoy I, Carel JC & Roger M. Inhibin B and anti-  
211 Müllerian hormone, but not testosterone levels, are normal  
211 in infants with non-mosaic Klinefelter syndrome.  
211 34 Topper E, Dickerman Z, Prager-Lewin R, Kaufman H, Maimon Z  
211 & Laron Z. Puberty in 24 patients with Klinefelter syndrome.  
212 35 Cabrol S, Ross JL, Fennoy I, Bouvieratt C, Roger M & Lahlou N.  
212 Assessment of Leydig and Sertoli cell functions in infants with  
212 nonmosaic Klinefelter syndrome: insulin-like peptide 3 levels are  
212 normal and positively correlated with LH levels. Journal  
213 (doi:10.1210/jc.2003-031624)  
213 36 Topper E, Dickerman Z, Prager-Lewin R, Kaufman H, Maimon Z  
213 & Lahlou N. Puberty in 24 patients with Klinefelter syndrome.  
214 BF00442070)