Human Reproduction, Vol.27, No.4 pp. 1156–1161, 2012
Advanced Access publication on February 10, 2012 doi:10.1093/humrep/des001

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Pubertal development in ICSI children

F. Belva[1,*], M. Roelants[2], R. Painter[3], M. Bonduelle[1], P. Devroey[4], and J. De Schepper[5]

[1]Center for Medical Genetics, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, Brussels 1090, Belgium [2]Laboratory of Anthropogenetics, Vrije Universiteit Brussels, Brussels, Belgium [3]Department of Obstetrics and Gynecology, AMC, Amsterdam, The Netherlands [4]Center for Reproductive Medicine, UZ Brussel, Brussels, Belgium [5]Department of Pediatric Endocrinology, UZ Brussel, Brussels, Belgium

*Correspondence address. Tel: +32-2-4776071; Fax: +32-2-4776860; E-mail florence.belva@uzbrussel.be

Submitted on October 18, 2011; resubmitted on December 14, 2011; accepted on December 20, 2011

BACKGROUND: To date, information on the pubertal development of adolescents born after ICSI is scarce, since the very first cohort is only now reaching young adulthood. In this study, pubertal development at the age of 14 was characterized in a longitudinally followed cohort of ICSI-conceived teenagers and compared with that of a spontaneously conceived (SC) control group.

METHODS: Pubertal development was assessed by Tanner staging (breast, genital and pubic hair development) and age at menarche in 217 singleton ICSI-conceived children (116 boys, 101 girls) and 223 SC peers (115 boys, 108 girls). ICSI teenagers were part of a previously published cohort followed since birth; controls were a cross-sectional sample recruited from schools. Differences in pubertal development between ICSI and SC children were analyzed with logistic regression of current status data.

RESULTS: Mean age at menarche was similar in ICSI and SC girls (13.1 $\pm$ 1.2 versus 13.1 $\pm$ 1.4 years; $P = 0.8$). Breast developmental at the age of 14 years was less advanced in ICSI females compared with SC females, even after adjustment for demographic (age, BMI), genetic (maternal age at menarche), social (maternal educational level) and early life factors (birthweight, gestational age and maternal parity) [odds ratio (OR) 0.5; 95% confidence interval (CI) 0.3–0.8]. After adjustment, genital development in the 14-year-old boys was comparable in the ICSI and SC groups (OR 1.1; 95% CI 0.7–1.8), pubic hair development was comparable in the ICSI and SC groups, for both males (OR 0.9; 95% CI 0.7–1.6) and females (OR 0.7; 95% CI 0.4–1.3).

CONCLUSIONS: We found that pubertal development, characterized by menarche, genital development in males and pubic hair development in males and females, was comparable in the ICSI and SC groups. Breast developmental was less advanced in ICSI females compared with SC peers, even after adjustment for known potential confounders. In order to confirm that progression through subsequent stages of pubertal development occurs on a timely basis in ICSI teenagers, long-term follow-up studies up to adulthood are required.

Key words: ICSI / puberty / children / long-term / outcome

Introduction

Although the number of children born after assisted reproductive technology (ART) is increasing worldwide, few studies have been published on the pubertal development of children born after assisted reproduction (Ceelen *et al.*, 2008; Beydoun *et al.*, 2011). However, the evaluation of sexual maturation of offspring born after fertility treatment is important, since it reflects the reproductive axis function, which in turn may predict later life fertility. More particularly, pubertal outcome after ICSI has not been described, as the very first cohort is only now reaching young adulthood.

Since delayed puberty can be the first sign of gonadal failure, the study of pubertal development of male offspring from men who experience gonadal failure requiring ICSI to reproduce, is of particular interest, especially for those with congenital reproductive tract abnormalities (Funke *et al.*, 2010). On the other hand, children born after ICSI are more frequently born small-for-gestational age (SGA) (McDonald *et al.*, 2009), which itself has been shown to be related to early adrenarche and a higher incidence of functional ovarian hyperandrogenism in girls, and subfertility in boys (Francois *et al.*, 1997; Ibañez *et al.*, 1998).

We therefore investigated genital development in boys and breast development and age of menarche in girls as well as pubic hair growth in both genders in a group of 14-year-old children born after ICSI, in comparison with the sexual maturation of a control group born after spontaneous conception (SC). We hypothesized that ICSI children would be at risk of disturbed pubertal development.

Materials and Methods

Set-up and study population

This study is part of a larger prospective follow-up study on cardiometabolic risk in the very first cohort of ICSI children conceived at the

Centre for Reproductive Medicine of the UZ Brussel. At the age of 14 years, pubertal development was assessed, and compared with that of a control group of children born after SC.

ICSI and SC children were eligible for inclusion if they were Caucasian, singleton and born after at least 32 weeks of gestation. Children born SGA were not excluded.

Of the 501 eligible children, 217 (116 boys, 101 girls) born after a transfer of fresh embryos obtained by ICSI, and reaching the age of 14 years between January 2008 and March 2011, were examined; 116 could not be reached and 168 refused to participate. The participation rate (tested/reached) was 56%. ICSI was predominantly performed because of male factor infertility (86%); and less frequently because of female factor infertility (6%), combined infertility (5%) or unexplained infertility (3%). The proportion of highly educated mothers was lower in the non-participants (53 versus 66%; $P = 0.01$), but birthweight, gestational age, preterm birth rate (<37 weeks of gestation) and maternal age were comparable between participants and non-participants (those not reached + those who refused). Upon refusal, parents were asked minimal information on the health of their child, which was obtained for 105 children (63%). ICSI children who refused did not differ from participating peers regarding gender (51 versus 53% males, $P = 0.8$), level of education (general education 70 versus 74%, $P = 0.5$), weight (females: 50.9 ± 7.8 versus 53.1 ± 8.7 kg, $P = 0.1$; males: 52.1 ± 9.7 versus 53.0 ± 12.1 kg, $P = 0.6$) and height (females: 163.3 ± 7.9 versus 164.0 ± 6.6 cm, $P = 0.5$; males: 164.5 ± 7.5 versus 165.8 ± 9.7 cm, $P = 0.3$).

The comparison group consisted of 223 children (115 boys, 108 girls), born after SC and without use of hormonal stimulation. SC children were recruited by letter from schools in the same geographical region of Belgium (Flanders) and with a comparable type of education (general and vocational) as those attended by ICSI children. Of the 553 invited children, 327 agreed to participate, of whom 278 children met the inclusion criteria; 83 refused (72 refused because of lack of time or interest) and 143 did not respond to our invitation. It is not known if parents of the latter group were reached, since invitation letters were distributed anonymously to the pupils at school. Finally, 223 children were clinically examined. Response rates are described in detail elsewhere (Belva *et al.*, 2012). Children who refused did not differ from their participating peers with respect to the proportion of males (43 versus 51%), birthweight (3403 ± 500 versus 3436 ± 481 g, $P = 0.6$), gestational age (39.1 ± 1.5 versus 39.4 ± 1.5 weeks, $P = 0.2$), attending general education (73 versus 81%, $P = 0.2$), current body weight (females: 52.8 ± 7.6 versus 51.6 ± 7.2 kg, $P = 0.4$; males: 54.6 ± 10.9 versus 56.1 ± 11.5 kg, $P = 0.5$), current height (females: 165.1 ± 5.8 versus 163.3 ± 6.8 cm, $P = 0.1$; males: 168.9 ± 8.6 versus 170.3 ± 7.8 cm, $P = 0.4$) and maternal age (29.3 ± 3.9 versus 28.5 ± 4.4 years, $P = 0.2$). As in the ICSI group, non-participating mothers were however less likely to be highly educated (50 versus 68%, $P = 0.01$).

The set-up of this study is similar to a previously published follow-up study at the age of 8 years (Belva *et al.*, 2007). In brief, data from a physical examination were supplemented with data on the child's personal and family history, obtained from a parental questionnaire. This included anthropometric, medical and socio-economic characteristics. Regarding the parents, age, maternal age at menarche, BMI, educational level and the medical history were recorded. Educational level was dichotomized as high (bachelor or higher degree) or low (the remaining). The medical history contained items such as hypertension, hypercholesterolemia, thyroid, diabetes and pregnancy-induced disorders (hypertension, pre-eclampsia, gestational diabetes and thyroid). Regarding the children, information on birth characteristics, school type (general or vocational education) and sports participation (hours/week) was obtained.

The study was approved by the ethics committee of the UZ Brussel. Written informed consent was obtained from the parents of all participants.

Clinical data

Pubertal development was assessed using Tanner scores (Tanner and Whitehouse, 1976) for breast development (M) and pubic hair development (P) in girls, and genital development (G) and pubic hair development (P) in boys. Date of menarche was obtained from the questionnaire. Anthropometric data on body weight and height were collected with standard equipment to the nearest 0.1 kg and 0.1 cm. BMI was calculated as weight divided by height squared (kg/m^2). Height, weight and BMI were converted to standard deviation scores (SDS) using recent Flemish reference curves (Roelants *et al.*, 2009), and thus correcting for age and gender. Birthweight was expressed as SDS in order to correct for gestational age and gender.

Statistical analysis

Continuous data are presented as means and SDS, and were compared among groups by a Student's *t*-test. Categorical data are presented as the number of children and the percentage in a certain category and were compared among groups by a χ^2 test.

Mean age at menarche was estimated with a parametric survival curve assuming a Gaussian distribution and taking into account that some girls had not yet reached menarche.

Having reached menarche was analyzed with binary logistic regression, with adjustment for current anthropometric characteristics (age and BMI of the child), genetic factors (maternal age at menarche), social factors (maternal educational level) and early life factors (birthweight, gestational age and parity), with known associations from the literature (Wronka and Pawlinska-Chmara, 2005; Karapanou and Papadimitriou, 2010; Rubin *et al.*, 2011). Results are presented as the odds ratio (OR) for having reached menarche in ICSI compared with SC girls, both unadjusted and fully adjusted for all known confounders. Elimination of non-significant confounders using backward selection did not significantly alter the effect size observed in the ICSI group.

Ordinal logistic regression (proportional odds model) was used to estimate differences in the present pubertal stages for breast, genital and pubic hair development, according to the mode of conception. Results are presented as the OR for having reached a subsequent stage of breast, genital and pubic hair development in the ICSI group compared with the SC group. The models were adjusted for the same factors as for menarche (i.e. current anthropometric characteristics, social factors and early life factors), except for the genetic factor, maternal age at menarche. Elimination of non-significant confounders using backward selection did not significantly alter the effect size observed in the ICSI group for any of three parameters of pubertal development. By using proportional odds models, we assume that the OR for being in a particular stage of pubertal development or above, is independent of the pubertal stage that is taken as the threshold. For example, the OR for being in Stage 2 or above versus Stage 1 is the same as the OR for being in Stage 3 or above versus Stages 1 or 2. This assumption was tested and confirmed for all models with the Score test.

Results

Baseline clinical characteristics

As expected, birthweight, birthweight SDS and gestational age were significantly lower in children conceived by ICSI compared with their SC counterparts (3280 ± 496 versus 3436 ± 481 g; -0.3 ± 1.1

Table I Clinical characteristics of 14-year-old ICSI and SC children stratified by gender.

	Boys ICSI (n = 116)	SC (n = 115)	P-value	Girls ICSI (n = 101)	SC (n= 108)	P-value
Age (years)	14.0 ± 0.4	14.3 ± 0.3	<0.01	14.0 ± 0.5	14.3 ± 0.3	<0.01
Weight SDS	−0.2 ± 1.1	0.0 ± 0.9	0.3	0.0 ± 0.9	−0.2 ± 0.9	0.06
Weight (kg)	53.0 ± 12.1	56.1 ± 11.5	0.04	53.1 ± 8.7	51.6 ± 7.2	0.2
Height SDS	−0.1 ± 1.2	0.3 ± 0.9	0.02	0.2 ± 1.0	0.0 ± 1.0	0.1
Height (cm)	165.8 ± 9.7	170.3 ± 7.8	<0.01	164.0 ± 6.6	163.3 ± 6.8	0.5
BMI SDS	−0.2 ± 1.1	−0.2 ± 1.0	0.9	−0.1 ± 0.9	−0.3 ± 0.8	0.2
BMI (kg/m^2)	19.1 ± 3.1	19.2 ± 3.0	0.8	19.7 ± 2.8	19.3 ± 2.2	0.3
Tanner stage of genital (boys) and breast (girls) development (n, %)						
2	8 (7)	5 (4)	0.3	3 (3)	2 (2)	0.04
3	40 (34)	29 (25)		33 (32)	18 (17)	
4	55 (48)	65 (57)		53 (53)	69 (64)	
5	13 (11)	16 (14)		12 (12)	19 (17)	
Reached menarche (n, %)	—	—		78 (77)	85 (79)	0.9

Table II ORs for menarche and pubertal development in ICSI compared with SC girls, after adjustment for current, early life and social factors.

	Menarche			Breast development			Pubic hair development		
	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
Unadjusted	0.9	0.5–1.8	0.8	0.5	0.3–0.8	0.01	0.8	0.5–1.3	0.4
Adjusted for current characteristics									
Age	1.2	0.6–2.7	0.5	0.7	0.4–1.2	0.1	0.9	0.6–1.7	0.7
Child's BMI[a]	1.1	0.5–2.4	0.7	0.5	0.3–0.9	0.02	0.8	0.5–1.5	0.4
Adjusted for early life characteristics									
Birthweight[a]	1.3	0.6–2.7	0.5	0.7	0.3–1.2	0.1	0.9	0.5–1.7	0.8
Gestational age[a]	1.4	0.6–2.9	0.4	0.7	0.3–1.2	0.1	0.9	0.5–1.7	0.8
Parity[a]	1.1	0.5–2.5	0.7	0.6	0.3–1.1	0.1	0.9	0.5–1.5	0.5
Adjusted for social characteristics									
Maternal educational level[a]	1.2	0.6–2.6	0.5	0.7	0.3–1.2	0.09	0.9	0.5–1.6	0.7
Maternal age at menarche[a]	1.3	0.6–3.1	0.5	0.7	0.3–1.2	0.1	0.9	0.5–1.6	0.6
Adjusted for current, early life and social characteristics	1.4	0.6–3.7	0.4	0.5	0.3–0.8	0.02	0.7	0.4–1.3	0.1

Each row represents a separate regression analysis; only the ORs for ICSI treatment are shown in the table.
[a]Additional adjustment for age.

versus 0.0 ± 1.3; 38.7 ± 1.3 versus 39.4 ± 1.5 weeks, respectively; all $P < 0.01$). The overall number of SGA children in this study is low, but nevertheless higher in the ICSI group (8.3 versus 4.1%; $P = 0.07$). Genital malformations that required surgery were reported in 5/116 (4.3%) of the ICSI boys [hypospadias (one), cryptorchid testis (two), varicocoele (two)] and in 4/115 (3.5%) of the SC boys [hypospadias (one), cryptorchid testis (two), chordae penis (one)] ($P = 1.0$).

Mothers of children born after ICSI were older (32.0 ± 4.6 versus 28.5 ± 4.4 years; $P < 0.01$) but the maternal education level was similar (66 versus 68%; $P = 0.7$). The mothers of the ICSI and SC groups had a comparable BMI (23.6 ± 3.7 versus 23.3 ± 3.3 kg/m^2; $P = 0.3$) and age at menarche (13.3 ± 1.4 versus 13.2 ± 1.3 years; $P = 0.7$) and did not experience more gestational diabetes or pregnancy-induced hypertension (5 versus 4%; $P = 0.4$). ICSI boys (14.0 ± 0.4 years) and girls (14.0 ± 0.3 years) were slightly younger compared with SC boys (14.3 ± 0.3 years) and girls (14.3 ± 0.3 years) at the time of examination ($P < 0.01$). Table I shows an overview of clinical data in our sample of 14-year-old boys and girls.3

Pubertal outcome data

Mean age at menarche was 13.1 ± 1.2 years in the ICSI group and 13.1 ± 1.4 years in the control group ($P = 0.8$). Having reached menarche was associated with older age ($P = 0.01$), a higher BMI ($P < 0.001$), a higher maternal education level ($P = 0.01$), and an earlier age at menarche in the mother ($P < 0.001$). After adjusting for these factors as well as for early life factors, as reported in Table II, the probability of having reached menarche was comparable

Table III ORs for pubertal development in ICSI compared with SC boys, after adjustment for current, early life and social factors.

	Genital development			Pubic hair development		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Unadjusted	0.6	0.4–1.0	0.08	0.6	0.4–1.0	0.07
Adjusted for current characteristics						
Age	1.0	0.6–1.7	0.9	1.0	0.6–1.7	0.9
Child's BMI[a]	1.0	0.6–1.7	0.9	0.9	0.6–1.7	0.9
Adjusted for early life characteristics						
Birthweight[a]	1.1	0.6–1.9	0.8	1.0	0.6–1.7	0.9
Gestational age[a]	1.0	0.6–1.9	0.9	0.9	0.6–1.7	0.9
Parity[a]	1.0	0.6–1.7	0.8	1.0	0.7–1.5	0.9
Adjusted for social characteristics						
Maternal educational level[a]	1.1	0.6–1.9	0.8	0.9	0.6–1.7	0.9
Adjusted for current, early life and social characteristics	1.1	0.7–1.8	0.6	0.9	0.7–1.6	0.7

Each row represents a separate regression analysis; only the ORs for ICSI treatment are shown in the table.
[a]Additional adjustment for age.

in ICSI compared with SC females [OR 1.4; 95% confidence interval (CI) 0.6–3.7; $P = 0.4$].

Having reached subsequent stages of breast development and pubic hair development in girls was associated with older age ($P = 0.01$), a higher BMI ($P < 0.001$) and an earlier age at menarche in the mother ($P < 0.001$), but not with maternal educational level ($P > 0.05$). After adjustment for these current and social characteristics as well as for early life factors, ICSI girls were found to be less advanced than SC girls regarding breast development (OR 0.5; 95% CI 0.3–0.8; $P = 0.01$), but comparable in terms of pubic hair development (Table II).

In boys, only age ($P = 0.001$) and BMI ($P = 0.01$) were significant predictors for being in a more advanced pubertal stage of genital or pubic hair development. After adjustment for possible confounders, the pubertal development in ICSI boys was comparable with that in SC boys in terms of genital (OR 1.1; 0.7–1.8; $P = 0.6$) and pubic hair development (OR 0.9; 0.7–1.6; $P = 0.7$) (Table III). In a subanalysis of boys from parents requiring ICSI because of male factor or combined male and female factor infertility, comparable results were obtained for genital and pubic hair development in comparison with the SC group (data not shown).

Discussion

This study presents the first data on pubertal development in 14-year-old adolescents conceived after ICSI. The possible effect of this ART on later pubertal development was assessed by comparing the proportion of ICSI children at subsequent stages of pubertal development with that of SC controls using ordinal logistic regression. These models allowed adjustment of the observed differences for several personal, genetic and early life characteristics. In girls, mean age at menarche, having reached menarche and pubic hair development in the ICSI group were comparable with the SC group, but breast development was less advanced. In boys, both genital and pubic hair development were comparable in the ICSI and SC groups.

Mean age at menarche in ICSI (12.6 ± 1.0 years) and SC (12.5 ± 1.0 years) girls who had already reached menarche, falls within the range of 12.5–13.5 years reported in European studies (Engelhardt *et al.*, 1995; Lindgren, 1996; Whincup *et al.*, 2001; Danubio *et al.*, 2004; Juul *et al.*, 2006; Roelants *et al.*, 2009). These estimates are however biased toward younger ages, since 23% of the ICSI girls and 21% of the SC girls had not yet reached menarche at the time of examination. Mean age at menarche obtained from survival analysis, which accounts for this bias, are very close to the population estimates of 13.1 years (Roelants *et al.*, 2009). The strong relationship between age at menarche in mothers and their daughters reported here is consistent with the literature (Graber *et al.*, 1995). Some, but not all studies, reported an association between being born SGA and an earlier age of menarche and precocious pubarche (Ibañez *et al.*, 1998; Hokken-Koelega, 2002; Ibañez and de Zegher, 2006). We did not find an association between birthweight and age at menarche (data not shown), and the number of SGA children (ICSI: $n = 18$; SC: $n = 9$) is unfortunately too small to perform a separate analysis. Nevertheless, no premature onset of puberty was observed in a previous report on 8-year-old ICSI-conceived children (Belva *et al.*, 2007).

Our findings that menarche and pubic hair development in girls, and genital and pubic hair development in boys were not affected by the ICSI technique, are in line with previous reports on pubertal development in children born after IVF. Ceelen *et al.* (2008) found no differences in the mean age of distinct pubertal stages in 233 age- and gender-matched IVF and control children. Pubertal development assessed by retrospective self-scoring questionnaires in a small group of young adults conceived by IVF, did not show any pubertal abnormalities (Beydoun *et al.*, 2011).

Our finding that ICSI girls have a less advanced breast development is novel and rather unexpected. We recently reported a higher body fat mass and a higher BMI in ICSI girls compared with SC girls (Belva *et al.*, 2012) and therefore expected that their excess of adiposity

could have contributed to a more advanced pubertal development since excess adiposity is known to advance puberty in girls (Burt Solorzano and McCartney, 2010). In this study, we could confirm that a high BMI is a strong predictor for menarche and for being in a more advanced pubertal stage. On the other hand, the less advanced breast development might be caused by a disturbed hypothalamic reactivation of gonadotrophin-releasing hormone (GnRH) release. Normal pubertal development is, after all, the result of reappearance, after a quiescence during childhood, of increasing pulsatile secretion of GnRH which stimulates the release of LH and FSH and in turn elicits gonadal activity, by secreting sex steroids which are eventually responsible for the development of secondary sex characteristics (Brook, 1999; Ebling, 2005). Since ICSI and SC children were examined at various time periods of the day and due to the known diurnal pulsatility and/or cyclic pattern of LH, FSH and estradiol, hormonal assessment was not included in this observational study. Whether the *in vitro* technique can permanently affect the functioning or activity of the hypothalamic–pituitary–gonadal axis remains to be elucidated. We must, however, not forget that puberty is a dynamic period of physical maturation which includes early and late developers. This study was a cross-sectional evaluation and hence can only comment on the pubertal status at the studied age span, but not at the tempo of progression of puberty and achievement of final pubertal maturation.

Some limitations of our study should be noted. Participation bias cannot be excluded since no reply or reason for refusal was obtained from a quarter of the eligible controls. However, non-participation analysis did not reveal any differences between participants and non-participants regarding clinical characteristics, and a higher educational level was observed in mothers of both participating ICSI and SC children.

Reassuringly, no delayed puberty in ICSI offspring was observed. In addition, the subgroup of boys born to fathers with fertility problems were found not to be at particular risk of a more delayed pubertal development. Despite the lack of longitudinal data on sexual maturity, our investigation provides some robust cross-sectional data on pubertal development in ICSI teenagers, particularly, since our group of ICSI offspring are a homogeneous group born predominantly because of male factor infertility. The normal sexual maturation, described here, together with the normal salivary testosterone and serum inhibin B measurements, described previously (Belva *et al.*, 2010, 2011) in a cohort of adolescent boys born predominantly because of male factor infertility, adds to the assumption that the reproductive capacity in ICSI male offspring is normal.

In conclusion, breast development was less advanced in ICSI girls compared with SC girls, but no differences in the pubertal development were observed in boys. Mean age at menarche was comparable between ICSI and SC girls. In order to confirm that progression through subsequent stages of pubertal development occurs on a timely basis in ICSI teenagers (girls should reach menarche within 5 years after M2, and boys should reach G5 four and a half years after G2), longitudinal follow-up studies of teenagers and young adults born after ART are required.

Acknowledgements

The authors are grateful to all the parents and children who took part in this study. The authors are extremely thankful to our study nurse Leen Ausloos for the recruitment of the ICSI families and to Walter Meul for database support.

Authors' roles

The current study was designed by F.B., M.B., R.P. and J.D.S. F.B. collected the data. M.R. analyzed the data. All co-authors interpreted the data. F.B. wrote the paper and it was finalized by all co-authors. All co-authors approved the definitive version of the manuscript.

Funding

This study was supported by research grants from Fonds voor Wetenschappelijk Onderzoek Vlaanderen, Onderzoeksraad Vrije Universiteit Brussel and Wetenschappelijk Fonds Willy Gepts. Unconditional grants from MSD Belgium, Merck International, IBSA Institut Biochimique and Ferring International Center are kindly acknowledged.

Conflict of interest

M.B. stated that the UZ Brussel receives funding for maintenance of the database on FU of ART children, from MSD, MSD Belgium and IBSA.

References

Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born ≥32weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod* 2007;**22**:506–515.

Belva F, Bonduelle M, Painter RC, Schiettecatte J, Devroey P, De Schepper J. Serum inhibin B concentrations in pubertal boys conceived by ICSI: first results. *Hum Reprod* 2010;**25**:2811–2814.

Belva F, Bonduelle M, Schiettecatte J, Tournaye H, Painter RC, Devroey P, De Schepper J. Salivary testosterone concentrations in pubertal ICSI boys compared with spontaneously conceived boys. *Hum Reprod* 2011;**26**:438–441.

Belva F, Painter RC, Bonduelle M, Roelants M, Devroey P, De Schepper J. Are ICSI adolescents at risk for increased adiposity? *Hum Reprod* 2012; **27**:257–264.

Beydoun H, Sicignano N, Beydoun M, Bocca S, Stadtmauer L, Oehninger S. Pubertal development in the first cohort of young adults conceived by *in vitro* fertilization in the United States. *Fertil Steril* 2011; **95**:528–533.

Brook CGD. Mechanism of puberty. *Horm Res* 1999;**51**(Suppl 3):52–54.

Burt Solorzano CM, McCartney CR. Obesity and the pubertal transition in girls and boys. *Reproduction* 2010;**140**:399–410.

Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen F, Delemarre-van de Waal H. Pubertal development in children and adolescents born after IVF and spontaneous conception. *Hum Reprod* 2008;**23**:2791–2798.

Danubio ME, De Simone M, Vecchi F, Amicone E, Altobelli E, Gruppioni G. Age at menarche and age of onset of pubertal characteristics in 6–14-year-old girls from the province of l'Aquila (Abruzzo, Italy). *Am J Hum Biol* 2004;**16**:470–478.

Ebling FJ. The neuroendocrine timing of puberty. *Reproduction* 2005; **129**:675–683.

Engelhardt L, Willers B, Pelz L. Sexual maturation in East German girls. *Acta Paediatr* 1995;**84**:1362–1365.

Francois I, de Zegher F, Spiessens C, D'Hooghe T, Vanderschueren D. Low birth weight and subsequent male infertility. *Pediatr Res* 1997;**42**:800–901.

Funke S, Flach E, Kiss I, Sandor J, Vida G, Bodis J, Erti T. Male reproductive tract abnormalities: more common after assisted reproduction? *Early Hum Dev* 2010;**86**:547–550.

Graber JA, Brooks-Gunn J, Warren MP. The antecedents of menarcheal age: heredity, family environment and stressful life events. *Child Dev* 1995;**66**:346–359.

Hokken-Koelega AC. Timing of puberty and fetal growth. *Best Pract Res Clin Endocrinol Metab* 2002;**16**:65–71.

Ibañez L, de Zegher F. Puberty and prenatal growth. *Mol Cell Endocrinol* 2006;**254–255**:22–25.

Ibañez L, Potau N, Francois I, de Zegher F. Precocious pubarche, hyperinsulinism and ovarian hyperandrogenism in girls: relation to reduced fetal growth. *J Clin Endocrinol Metab* 1998;**83**:3558–3562.

Juul A, Teilmann G, Scheike T, Hertel NT, Holm K, Laursen EM, Main KM, Skakkebaek NE. Pubertal development in Danish children: comparison of recent European and US data. *Int J Androl* 2006;**29**:247–255.

Karapanou O, Papadimitriou A. Determinants of menarche. *Reprod Biol Endocrinol* 2010;**8**:115.

Lindgren G. Pubertal stages 1980 of Stockholm schoolchildren. *Acta Paediatr* 1996;**85**:1365–1367.

McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A; Knowledged Synthesis Group. Preterm birth and low birth weight among *in vitro* fertilization singletons: a systematic review and meta-analyses. *Eur J Obstet Gynecol Reprod Biol* 2009;**146**: 138–148.

Roelants M, Hauspie R, Hoppenbrouwers K. References for growth and pubertal development from birth to 21 years in Flanders (Belgium). *Ann Hum Biol* 2009;**36**:680–694.

Rubin C, Maisonet M, Kieszak S, Monteilh C, Holmes A, Flanders D, Heron J, Golding J, McGeehin M, Marcus M. Timing of maturation and predictors of menarche in girls enrolled in a contemporary British cohort. *Paediatr Perinat Epidemiol* 2011;**23**:492–504.

Tanner JM, Whithehouse RH. Clinical longitudinal standards for height, weight,, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976;**51**:170–179.

Whincup PH, Gilg JA, Odoki K, Taylor SJ, Cook DG. Age of menarche in contemporary British teenagers: survey of girls born between 1982 and 1986. *Br Med J* 2001;**322**:1095–1096.

Wronka I, Pawlinska-Chmara R. Menarcheal age and socioeconomic factors in Poland. *Ann Hum Biol* 2005;**32**:630–638.