

Supplementary Table S7 - Pubertal development

Study ID	Country	Study design	Study quality	Treated sample	Average age CSH treatment started	Comparator	Intervention	Outcome (measure)	Follow-up specific to outcome	Study results
Hormone levels										
Beking 2020	Netherlands	Cohort	Moderate	21 brf	Mean age 16.1 y (SD 0.7) Mean treatment duration 9.8 m (SD 2.9 m)	41 (no GD)	Testosterone (with GnRHa)	Testosterone	Single (range 5.6-14.8m)	Increase in testosterone in brf at follow-up - lower than in male controls and higher than female controls.
Burke 2016	Netherlands	Cohort	Moderate	21 brf	Mean age 16.1 y (SD 0.8) Mean treatment duration 10 m (range 6-15)	41 (no GD)	Testosterone (with GnRHa)	Testosterone	Single (range 6-15m)	At follow-up, testosterone levels much higher than female controls and slightly lower than male controls.
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen	Oestradiol, LH, FSH	12m, 24m, 36m	LH and FSH remained the same over time, oestradiol increased over time.
Jarin 2017	US	Pre-post	Moderate	116 (72 brf, 44 brm)	Mean age brm: 18 y (range 14-25 y) Mean age brf: 16 y (range 13-22 y)	N/A	Hormones (brm plus/minus anti-androgens)	Testosterone, oestradiol	1-3m, 4-6m, 6m+	In brf, testosterone levels increased and oestradiol levels remained the same. In brm, testosterone levels decreased and oestradiol levels increased.
Laurenzano 2021	US	Pre-post	Moderate	119 brf	Mean age 16.5 (range 13.0-19.9)	N/A	Testosterone	Testosterone (total and free), oestradiol	Single (median 1.9y, range 6m to 5.5y)	Total and free testosterone levels increased. Oestradiol levels decreased.
Mullins 2021	US	Pre-post	Moderate	611 (428 brf, 183 brm)	Not reported	N/A	Hormones	Testosterone, oestradiol	Single (median 554/7d brm/f, range 283-1037)	In oestradiol users, level at follow-up was 47.2 pg/mL (IQR: 27.2-99.5), and testosterone was 189.5 ng/dL (IQR: 15.2-367). In testosterone users, level at follow-up was 413.0 ng/dL, and oestradiol was 33.2 pg/mL (IQR: 24.0-47.9).
Olson 2014	US	Pre-post	Moderate	36 brf	Mean age 18.7 y (SD 2.6 y)	N/A	Testosterone	Testosterone (total and free), oestradiol	6m	Total testosterone and free testosterone increased. Oestradiol levels decreased.
Olson-Kennedy 2018	US	Pre-post	Moderate	59 (34 brf, 25 brm)	Mean age 18 y (range 12-23 y)	N/A	Hormones (brm plus/minus progestins)	Testosterone (total and free), oestradiol	24m (range 21-31m)	Total and free testosterone levels decreased in brm and increased in brf. Oestradiol increased in brm and decreased in brf.
Peri 2020	Israel	Pre-post	Moderate	9 brf	Mean age 15.1 y (SD 0.9 y)	N/A	Testosterone	Testosterone, oestradiol, LH, FSH	Single (mean 4m, SD 2m)	Testosterone and oestradiol levels increased. LH and FSH levels remained constant.
Peri 2021	Israel	Pre-post	Moderate	15 brm	Mean age 16.1 y (SD 1.5 y)	N/A	Oestrogen (with GnRHa)	Testosterone, oestradiol, LH, FSH	Single (median 18.5m, range 3-63m)	Oestradiol levels increased, testosterone remained constant. LH remained constant, FSH levels decreased slightly.
Stoffers 2019	Netherlands	Pre-post	Moderate	62 brf	Median age 17.2 y (range 14.9-18.4 y)	N/A	Testosterone	Testosterone, oestradiol, LH, FSH	6m, 12m, 24m	Testosterone and oestradiol levels increased. LH and FSH levels remained constant.
Tack 2016	Belgium	Pre-post	Moderate	38 brf	Mean age 17 y 5 m	N/A	Testosterone (with Lynestrenol)	Testosterone (total and free), oestradiol, LH, FSH	6m, 12m	Total and free testosterone levels increased. Oestradiol remained same. LH and FSH remained constant.
Tack 2017	Belgium	Pre-post	Moderate	27 brm	Mean age 17 y 7 m	N/A	Oestrogen (with Cyproterone acetate)	Testosterone (total and free), oestradiol, LH, FSH	6m, 12m	Oestradiol levels increased. Total and free testosterone levels decreased. LH and FSH decreased.
van der Loos 2021	Netherlands	Pre-post	Moderate	322 (106 brm, 216 brf)	Early-puberty group median age 15.7 y (IQR 15.3-16.0 y) Mid-puberty 16.0 y (IQR 15.8-16.6 y) Late-puberty 16.4 y (IQR 16.0-17.4)	N/A	Hormones (with GnRHa)	Testosterone, oestradiol	Single (> 2y, median 3-4y across groups)	Testosterone levels increased in brf and oestradiol levels increased in brm. Oestradiol levels increased in brf and testosterone levels decreased slightly in brm.
Nokoff 2020	US	Cross-sectional	Moderate	35 (21 brf, 14 brm)	Mean age at data collection for brm 16.3 y (SD 1.4 y, mean treatment duration 12.3 m (9.9m)) Mean age at data collection for brf 17.0 y (SD 1.4 y, mean treatment duration T 11.2 m (5.9m))	108 (no GD)	Hormones (plus/minus GnRHa)	Testosterone, oestradiol, LH, FSH	N/A	Brf higher testosterone than female controls, slightly lower than males. Oestradiol lower than females, higher than males. Brm higher testosterone than female controls, lower than males. Oestradiol levels similar to females, higher than males.
Induced pubertal progression										
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRHa)	Tanner breast stage	12m, 24m, 36m	Tanner breast stage increased over time.
Stoffers 2019	Netherlands	Pre-post	Moderate	62 brf	Median age 17.2 y (range 14.9-18.4 y)	N/A	Testosterone	Hair growth (facial, abdominal, chest and extremities) and deepening of voice	3m, 6m, 12m	Presence of hair (facial, abdominal, chest and extremities) increased over time. Voice deepened in all followed up.
Tack 2017	Belgium	Pre-post	Moderate	27 brm	Mean age 17 y 7 m	N/A	Oestrogen (with Cyproterone acetate)	Tanner breast stage	6m, 12m	Majority reached Tanner breast stage 3, with a small percentage reaching stage 4, but objectively small breast volume in most cases.
Vlot 2017	Netherlands	Pre-post	Moderate	70 (28 brm, 42 brf)	Median age for brm: 16.0 y (range 14.0-18.9 y) Median age for brf: 16.3 y (15.9-19.5)	N/A	Hormones (with GnRHa)	Tanner breast stage (brf only), genital stage (brm only) and pubic hair stages (brf and brm).	24m	Tanner breast stage remained same in brf. Tanner genital stage remained same in brm. Tanner pubic hair stage remained same for both.
Height/growth										
van de Grift 2020	Netherlands	Cohort	Moderate	43 (17 brf, 26 brm)	Mean age Tanner 2/3 group: 15 y (SD 0.5 y) Mean age Tanner 4/5 group: 17 y (SD 1.0 y)	157	Hormones	Height	At initiation of surgery	Brf who had hormones for longer (started puberty suppression earlier) were taller at follow-up than those who started later. For brm no difference.
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRHa)	Height and height SD score (reference to natal sex and affirmed gender)	12m, 24m, 36m	Height increased over time. Height SD score with reference to natal sex and female sex remained the same over time.

Klink 2015	Netherlands	Pre-post	Moderate	34 (15 brm , 19 brf)	Mean age for brm: 16.6 y (SD 1.4 y) Mean age for brf: 16.4 y (SD 2.3 y)	N/A	Hormones	Height and height SD score (reference to natal sex)	Single (22 years of age)	Absolute height increased for both groups. Height SD score increased for brm and remained the same for brf.
Stoffers 2019	Netherlands	Pre-post	Moderate	62 brf	Median age 17.2 y (range 14.9-18.4 y)	N/A	Testosterone	Height and height SD score (reference to natal sex and affirmed gender)	6m, 12m, 24m	Height increased over time. Height SD score with reference to natal sex and female sex remained the same.
Tack 2017	Belgium	Pre-post	Moderate	27 brm	Mean age 17 y 7 m	N/A	Oestrogen (with Cyproterone acetate)	Height SD score (reference to natal sex)	6m, 12m	Height SD score increased over time.
van der Loos 2021	Netherlands	Pre-post	Moderate	322 (106 brm, 216 brf)	Early-puberty group median age 15.7 y (IQR 15.3-16.0 y) Mid-puberty 16.0 y (IQR 15.8-16.6 y) Late-puberty 16.4 y (IQR 16.0-17.4)	N/A	Hormones (with GnRHa)	Height	Single (> 2y, median 3-4y across groups)	For both males and females, average height at follow-up was higher for those who started hormones at an earlier age compared to those who started later.
Vlot 2017	Netherlands	Pre-post	Moderate	70 (28 brm, 42 brf)	Median age for brm: 16.0 y (range 14.0-18.9 y) Median age for brf: 16.3 y (15.9-19.5)	N/A	Hormones (with GnRHa)	Height	24m	Height increased in both males and females.
Waist and hip measurements										
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRHa)	Waist and hip circumference and SD scores, waist-hip ratio and SD score (references for males and females)	12m, 24m, 36m	Waist circumference remained the same and waist-hip ratio decreased. SD score with reference to males and females decreased. Hip circumference increased, no increase in SD score.
Body composition										
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRHa)	Fat percentage and lean body mass percentage	12m, 24m, 36m	Fat percentage and lean body mass percentage remained the same over time.
Nokoff 2020	US	Cross-sectional	Moderate	35 (21 brf, 14 brm)	Mean age at data collection for brm 16.3 y (SD 1.4 y, mean treatment duration 12.3 m (9.9m) Mean age at data collection for brf 17.0 y (SD 1.4 y, mean treatment duration T 11.2 m (5.9m)	108 (no GD)	Hormones (plus/minus GnRHa)	Total body fat percentage, fat mass percentage, lean tissue percentage and lean mass percentage	N/A	Brf had lower body fat and higher lean tissue than female controls but the converse to males. Brm higher body fat and lower lean tissue than male controls, but the converse than females.
Menstrual suppression										
Grimstad 2021a	US	Pre-post	Moderate	232 brf	Mean age 16.3 y	N/A	Testosterone	Breakthrough bleeding - defined as bleeding after more than 1 year of treatment	Single (>12 months)	Approximately 20% experienced breakthrough bleeding during the duration of the study.
Laurenzano 2021	US	Pre-post	Moderate	119 brf	Mean age 16.5 (range 13.0-19.9)	N/A	Testosterone	Cessation of menses and time to cessation	Single (median 1.9y, range 6m to 5.5y)	Cessation in nearly all participants on 200mg dose, just over half on 140mg dose. Average time between start of hormones and cessation was 4.7 months (SD 3.0).
Olson 2014	US	Pre-post	Moderate	36 brf	Mean age 18.7 y (SD 2.6 y)	N/A	Testosterone	Cessation of menses and time to cessation	Single (6m)	Cessation of menses obtained in 85% of participants by 6 months. Average number of months to cessation was 2.9 months (SD 1.5 months).
Bone age and geometry										
Hannema 2017	Netherlands	Pre-post	Moderate	28 brm	Median age 16.0 y (range 13.9-18.9)	N/A	Oestrogen (with GnRHa)	Bone age	12m, 24m, 36m	Bone age increased over time.
van der Loos 2021	Netherlands	Pre-post	Moderate	322 (106 brm and 216 brf)	Early-puberty group median age 15.7 y (IQR 15.3-16.0 y) Mid-puberty 16.0 y (IQR 15.8-16.6 y) Late-puberty 16.4 y (IQR 16.0-17.4)	N/A	Hormones (with GnRHa)	Subperiosteal width and endocortical diameter of the hip bone	Single (> 2y, median 3-4y across groups)	In brm, subperiosteal width and endocortical diameter increased in those who started GnRHa in mid or late puberty, but not early. In brf there was no change.
Vlot 2017	Netherlands	Pre-post	Moderate	70 (28 brm, 42 brf)	Median age for brm: 16.0 y (range 14.0-18.9 y) Median age for brf: 16.3 y (15.9-19.5)	N/A	Hormones (with GnRHa)	Bone age	24m	Bone age increased.
Abbreviations: brf – birth-registered females; brm – birth-registered males; CSH – cross-sex hormones; d – days; FSH – follicle-stimulating hormone; GD - gender dysphoria; GnRHa - Gonadotropin-Releasing Hormone analogues; IQR – inter-quartile range; LH – luteinising hormone; m – months; SD – standard deviation; y – years.										