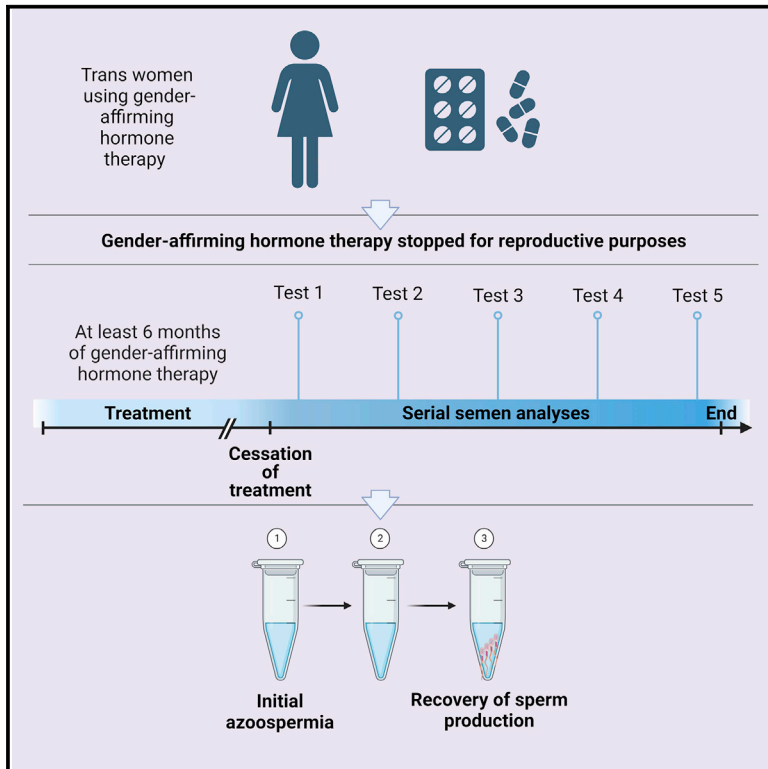


Successful restoration of spermatogenesis following gender-affirming hormone therapy in transgender women

Graphical abstract



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In brief

Trans women often commence gender-affirming hormonal therapy (GAHT) to promote feminization. But GAHT can also impair fertility. de Nie et al.'s report of successful restoration of spermatogenesis in trans women after discontinuation of GAHT suggests that GAHT's impact on fertility can be reversed, which has important clinical implications.

Highlights

- This study follows trans women after discontinuation of hormone therapy
- The authors examine the subsequent ability of these women to produce sperm
- Trans women exhibit spermatogenesis after GAHT cessation
- Hormone treatment for trans women does not lead to permanent infertility



Report

Successful restoration of spermatogenesis following gender-affirming hormone therapy in transgender women

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SUMMARY

Increasing numbers of transgender individuals are presenting for gender-affirming medical care. For trans women, gender-affirming hormone therapy (GAHT) promotes feminization but also inhibits spermatogenesis. There is a common untested assumption that this inhibition is permanent, resulting in infertility. In this longitudinal study, we report the recovery of viable spermatozoa in nine trans women who stopped GAHT for reproductive purposes. Our preliminary findings suggest that the negative impact of GAHT on spermatogenesis can be reversed, casting doubt on previous claims that GAHT in trans women inevitably leads to permanent infertility. Larger studies are needed to confirm our findings, which have implications not only for fertility counseling and the reproductive options of transgender individuals but also efforts to restrict access to GAHT based on fertility grounds.

INTRODUCTION

Increasing numbers of transgender, gender diverse, and non-binary (henceforth, trans) people are seeking hormonal intervention as part of gender-affirming medical care. For trans women, gender-affirming hormone therapy (GAHT) typically consists of estrogen combined with an anti-androgen. Such treatment promotes feminization but is also known to impair spermatogenesis.¹ Specifically, multiple studies have observed that most trans women receiving GAHT are not producing mature sperm at the time of gender-affirming surgery.^{2–8} Some of these studies reported relatively normal spermatogenesis in a variable minority of trans women (e.g., 11%–40%), but Vereecke et al. found that none of the 97 individuals they studied had histological evidence of complete spermatogenesis at gonadectomy.^{2,7,8}

Current international clinical guidelines therefore recommend that trans women “should be informed about sperm preservation options and encouraged to consider banking their sperm prior to hormone therapy.”⁹ Such advice seems sensible, especially in light of prominent claims from leaders in the transgender health field that GAHT “eventually results in irreversible infertility.”¹ However, proof that GAHT causes “irreversible infertility” re-

quires longitudinal follow up and, importantly, an assessment of whether or not loss of spermatogenesis can be reversed after GAHT is ceased. With that in mind, we identified a small cohort of trans women, each of whom had elected to stop GAHT for reproductive purposes. We assessed their subsequent ability to produce sperm and observed the recovery of spermatogenesis following azoospermia in several individuals.

RESULTS

In total, nine trans women met our study criteria (Table 1). Four of the nine had stopped GAHT to conceive with current partners; the remaining five stopped because they were hoping to bank sperm for potential future reproductive purposes. Their mean age was 26.1 years (range: 18–32 years), and the median duration of GAHT was 36 months (range: 6–216 months). All used estrogen in combination with an anti-androgen (spironolactone or cyproterone acetate). Seven individuals had been on oral estradiol (median dose: 4 mg daily; range: 2–4 mg daily), while the other two had been on topical estradiol.

Following cessation of GAHT, viable spermatozoa were eventually documented in all nine cases (Table 1). Initial semen



Table 1. Clinical characteristics of nine trans women who stopped GAHT and their subsequent semen analyses

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Initial presentation to gender clinic	Location	The Netherlands	The Netherlands	The Netherlands	The Netherlands	The Netherlands	The Netherlands	Australia	The Netherlands	The Netherlands
	age at first presentation to gender clinic	26	18	25	28	26	32	28	26	27
	age at diagnosis of gender dysphoria	28	21	25	28	27	33	28	27	29
	relevant medical history	left testicular torsion	androgenetic alopecia	N/A	commenced hormone treatment on own initiative after purchasing online	started hormone treatment in Lebanon; depression	N/A	N/A	N/A	used oral contraceptives for 2 months in 2012
	concurrent medication	N/A	finasteride 1mg/day (for alopecia)	N/A	N/A	paroxetine 20 mg/day	N/A	N/A	N/A	N/A
	baseline serum hormone levels									
	estradiol (pmol/L)	123	72	82	705	ND	95	122	127	124
	testosterone (nmol/L)	27	19	15	<0.5	ND	21	14.8	29	33
LH (IU/L)	2.8	3.6	2.4	<0.1	ND	5.2	4.2	5.1	9	
FSH (IU/L)	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Fertility history and previous counseling	previous children	nil	nil	nil	nil	nil	nil	nil	nil	nil
	reproductive desires prior to commencing GAHT	wish for fertility preservation, wants children in the future.	used illicitly obtained hormones, no prior fertility counseling	not discussed	used illicitly obtained hormones, no prior fertility counseling	used illicitly obtained hormones, no prior fertility counseling	no prior fertility counseling	fertility counseling was provided; not wanting children, planning to adopt; fertility preservation declined	fertility counseling was provided; declined fertility preservation	fertility counseling was provided; declined fertility preservation
	prior fertility preservation	semen cryopreservation: 43 vials suitable for ICSI	none	none	none	none	none	none	none	none

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Table 1. Continued

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Estrogen therapy	age started	28	18	23	24	24	33	28	27	30
	agent	estradiol patches	estradiol valerate	estradiol valerate	estradiol valerate	estradiol valerate	estradiol valerate	estradiol valerate	estradiol valerate	estradiol valerate
	starting dose	100 mcg/24 h twice a week	4 mg/day	2 mg/twice a day	2 mg/twice a day	2 mg/twice a day	2 mg/twice a day	4 mg/day	2 mg/twice a day	2 mg/twice a day
	age stopped	31	18	41	32	27	36	29	33	33
	dose at cessation	100 mcg/24 h twice a week	2 mg/day	estrogen 0.06% 1.25 mg twice a day	estradiol valerate 2 mg /twice a day	estradiol valerate 2 mg/day	estradiol valerate 2 mg/twice a day	4 mg/day	estradiol valerate 2 mg/twice a day	estradiol valerate 2 mg/twice a day
	duration of estrogen therapy (months)	22	6	216	99	36	36	14	56 (period of cessation in 2014)	36
Anti-androgen therapy	age started	28	18	23	24	24	33	28	27	29
	agent	cyproterone acetate	spironolactone	cyproterone acetate	cyproterone acetate	spironolactone	cyproterone acetate	cyproterone acetate	cyproterone acetate	cyproterone acetate
	starting dose	25 mg/day	100 mg/day	100 mg/day	50 mg/day	100 mg/day	50 mg/day	25 mg/day	50 mg/twice a day	50 mg/day
	age stopped	31	18	41	32	27	36	29	33	33
	dose at cessation	10 mg/day	100 mg/day	50 mg/day	50 mg/day	100 mg/day	12.5 mg/day	25 mg/alternate days	20 mg/day	50 mg/day
	duration on anti-androgen (months)	17	6	216	93	36	36	14	56 (period of cessation in 2014)	40
Gender affirming surgery	None	None	Breast augmentation	Breast augmentation	None	None	None	None	None	None
Physical response to hormone treatment	physical changes	breast development, decreased muscle strength	unknown	unknown	unknown	unknown	breast development, decreased facial hair growth	Tanner stage 3-4 breast development	breast development	decreased muscle strength, decreased body hair growth, softer skin, breast development
	subsequent serum hormone levels									
	timing	20 months after commencement	ND	204 months after commencement	93 months after commencement	ND	15 months after commencement	13 months after commencement	24 months after re-initiation of hormones	36 months after commencement of estradiol
	estradiol (pmol/L)	641	ND	120	286	ND	246	556	320	154
	testosterone (nmol/L)	0.4	ND	0.9	0.2	ND	4.4	0.9	0.7	0.4
	LH (IU/L)	0.1	ND	0.3	<0.1	ND	1.1	0.2	1.2	<0.1
	FSH (IU/L)	n/d	ND	ND	ND	ND	ND	ND	ND	ND

(Continued on next page)

Table 1. Continued

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Cessation of anti-androgens/estrogen	rationale	wishes natural conception with new partner	wants to cryopreserve semen to keep all options open	wants to conceive with partner	wants to cryopreserve semen to keep all options open, partner has active child wish	prefers genetically related offspring over adoption	wants to conceive with partner	wants to conceive with partner	wants to cryopreserve semen to keep all options open	strong parental desire
	physical changes after cessation	increased hair growth and higher volume of ejaculate	unknown	none, no hot flushes, no increased hair growth	acne	increased hair loss/baldness	increased facial hair growth	breast atrophy, testicular volume increase	higher volume of ejaculation, increased sexual function, increased muscle strength	increased hair growth (body and face)
	subsequent serum hormone levels									
	timing	3 months after cessation	ND	ND	ND	3 months after cessation	22 months after cessation	6 months after cessation	7 months after cessation	13 months after cessation
	estradiol (pmol/L)	82	ND	ND	ND	ND	ND	164	ND	ND
	testosterone (nmol/L)	17.0	ND	ND	ND	18	11	18.8	21	16
	LH (IU/L)	3.1	ND	ND	ND	ND	ND	5.2	9.2	11
	FSH (IU/L)	9.0	ND	ND	ND	3.3	6.2	17.2	19	23
Semen specimen #1 after stopping hormones	time after stopping GAHT (months)	3	3	27**	4	3	7	1	4	7
	volume (mL)	1.2	4.5*	2.1*	3.5*	1.3	3.4*	0.2	unknown	1
	sperm concentration (million/mL)	3.3	48*	77.7*	0.2	1.2	31.3*	0	0	0
	sperm motility (%)	20	69*	51*	19	17	40*	nil	0	0
Semen specimen #2 after stopping hormones	time after stopping GAHT (months)	7	3	N/A	N/A	3	22	8	7	7
	volume (mL)	1.3	5*	N/A	N/A	1.7*	1.6*	1	unknown	2.5*
	sperm concentration (million/mL)	3.8	55*	N/A	N/A	0.8	22.3*	<2	0	0
	sperm motility (%)	41*	55*	N/A	N/A	49*	63*	6	0	0

(Continued on next page)

Table 1. Continued

		Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Semen specimen #3 after stopping hormones	time after stopping GAHT (months)	8	3	N/A	N/A	3	22	9	10	13
	volume (mL)	4.8*	6*	N/A	N/A	0.9	2.5*	2*	3.2*	3.2*
	sperm concentration (million/mL)	20.2*	53*	N/A	N/A	0.4	7.1	<2	9	0
	sperm motility (%)	48*	75*	N/A	N/A	56*	13	50*	44*	0
Semen specimen #4 after stopping hormones	time after stopping GAHT (months)	N/A	N/A	N/A	N/A	N/A	22	N/A	11	N/A
	volume (mL)	N/A	N/A	N/A	N/A	N/A	2.2*	N/A	2.7*	N/A
	sperm concentration (million/mL)	N/A	N/A	N/A	N/A	N/A	21*	N/A	11.8	N/A
	sperm motility (%)	N/A	N/A	N/A	N/A	N/A	43*	N/A	36*	N/A

Additional follow-up Patient 1: Partner became pregnant 4 months after cessation of hormone treatment through natural conception
 Patient 3: Partner became pregnant 40 months after cessation of hormone treatment through natural conception
 Patient 6: Partner became pregnant 20 months after cessation of hormone treatment through natural conception
 Patient 9: After failing to identify any mature sperm on repeated semen specimens, further physical examination and investigations were performed. Examination revealed a right testicle volume of 2 ml, left testicle volume of 3 ml and normal consistency on both sides without any abnormalities of the epididymis or vas deferens. Scrotal ultrasound revealed: right testicle volume of 2.0 ml, left testicle volume 3.7 ml, normal testicular structure and vascularisation, normal epididymis. Karyotype was 46XY with no Y chromosome abnormalities seen. Testicular sperm extraction was subsequently performed. Complete spermatogenesis was sporadically encountered in both testicles, and mature spermatozoa were harvested 17 months after stopping hormones.

The single asterisk (*) indicates that semen parameters were above the normal WHO reference range for semen volume (≥ 1.5 mL), sperm concentration (≥ 15 million/mL), or progressive motility ($\geq 32\%$).

The double asterisk (**) indicates that Patient 3's first semen analysis was significantly delayed due to a move across continents, during which time she was unable to be with her partner. They were eventually reunited after which the semen analysis was performed.

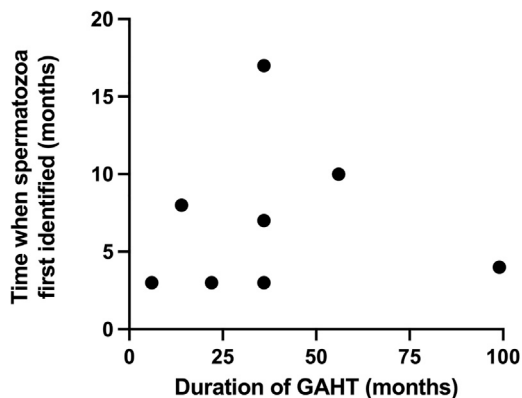


Figure 1. Relationship between GAHT duration and the time when spermatozoa were first identified after stopping GAHT

Note: patient 3 was excluded from this analysis given that their first semen analysis was significantly delayed (see Table 1).

collection and analysis occurred 3–27 months after GAHT was ceased. Six had sperm present on their initial semen analyses, but the other three had documented azoospermia on their initial analyses at 1, 4, and 7 months. Spermatozoa were later recovered from the semen of two of these individuals at 8 and 10 months. For the remaining individual, azoospermia was still present at 13 months, but eventually, testicular sperm extraction at 17 months recovered mature spermatozoa from both testes. Four individuals yielded at least one normal semen analysis; the other four had either low volume, low sperm concentration, and/or low sperm motility (see Table 1 for full description of semen volume, sperm concentration, and motility at each semen analysis). There was no obvious relationship between the duration of GAHT and the timing of when spermatozoa were first identified (Figure 1), although our small sample size and the heterogeneity in the timing of semen collection within the cohort limit any firm conclusions in this regard.

Three of the four trans women who stopped GAHT to naturally conceive with their partners successfully did so after 4, 20, and 40 months (for the latter individual, it should be noted that she was separated from her partner for a substantial duration after GAHT cessation, which is likely to have contributed to the extended time it took to conceive). For the remaining trans woman, there had not been any report of a natural conception at the most recent follow up 28 months after stopping GAHT.

DISCUSSION

The prevailing dogma in transgender health is that GAHT in trans women “eventually results in irreversible infertility.”¹ This view has fueled controversy over the provision of GAHT, especially to adolescents, and is reflected in arguments to support legal efforts to restrict young people’s access to gender-affirming care.¹⁰

While there are numerous reports that GAHT impairs spermatogenesis, there is also indirect evidence to suggest that the impact may be transient. For example, Alford et al. recently described a trans woman who—prior to gender-affirming genital surgery and

following a course of follicle-stimulating hormone (FSH) and clomiphene—successfully cryopreserved sperm from semen specimens collected 6–10 weeks after GAHT cessation.¹¹ Similarly, Adeleye et al. reported the presence of mature sperm among three trans women for whom GAHT had been withdrawn 3–6 months earlier.¹² However, for all four cases, the extent to which spermatogenesis had been previously impaired by GAHT was uncertain. As noted earlier, up to 40% of trans women have evidence of normal spermatogenesis at the time of gonadectomy despite GAHT.⁸ Thus, it is conceivable that spermatogenesis in each of these four cases had not been impaired. Consequently, these previous reports do not resolve the question of whether spermatogenesis can recover after GAHT cessation.

Our observation that six trans women had sperm identified from their initial semen analyses after GAHT cessation provides further indirect evidence that GAHT-induced impairment of spermatogenesis can be reversible. *A priori*, the probability that at least one of these six patients had ceased producing mature sperm due to GAHT is very high ($p(\text{azoospermia in at least one of the 6 patients}) = 1 - p(\text{finding mature sperm in all six patients}) = 1 - p(\text{finding mature sperm in one patient})^6 = 1 - 0.4^6 = 99.6\%$), indicating that spermatogenesis is likely to have recovered in some of these individuals. More noteworthy, though, is our observation that three trans women with documented azoospermia subsequently produced mature sperm afterward. This provides evidence that spermatogenesis can recover following GAHT cessation.

In summary, our data strongly suggest that the impact of GAHT on spermatogenesis can be reversed and cast doubt on claims that GAHT inevitably leads to permanent infertility. Our findings also have important implications for fertility counseling in transgender health. For example, many trans women (or non-binary individuals) receiving feminizing GAHT and who have their gonads intact believe that they are permanently infertile, as do their clinicians. Our observations will help such women make better informed reproductive choices moving forward. Similarly, for trans women (or non-binary individuals) wanting to commence GAHT in the future, our findings may influence their decision-making regarding fertility preservation (e.g., some may be less inclined to freeze their sperm knowing that they may be able to produce sperm should they stop GAHT later on). Nonetheless, we would still recommend sperm cryopreservation prior to GAHT for anyone who might want to be a genetic parent in the future. After all, recovery of spermatogenesis took many months in some cases, during which time testosterone levels increased and are likely to have had negative physical and psychological consequences. Moreover, previous studies found that semen quality is decreased in individuals with a history of GAHT compared with individuals without GAHT.¹³ This is in line with our observation that invasive testicular sperm extraction was required in one case, and >55% (5/9) of individuals had impaired semen quality after stopping GAHT.

Limitations of the study

There are several important limitations that should be noted. Firstly, there was a lack of data on semen quality before and during GAHT. In this way, it is possible that some of our participants may have had abnormal semen parameters even before

treatment, in which case their abnormal semen analysis results after GAHT should be interpreted with caution. Secondly, assessment of hormone levels, such as testosterone, luteinizing hormone (LH), and FSH, following GAHT cessation in our cohort would have been useful as a guide to help indicate the resumption of normal reproductive endocrine function—including at the time of semen collection—but monitoring of these levels was haphazard among our patients. Finally, our sample size was obviously limited and ultimately only included three patients in whom reversible azoospermia could be confirmed. Looking ahead, future studies that include a larger number of such patients will therefore be important to validate and expand upon our findings.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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AUTHOR CONTRIBUTIONS

Conceptualization, K.C.P., N.M.v.M., and I.d.N.; methodology, K.C.P., N.M.v.M., and I.d.N.; validation, K.C.P. and I.d.N.; formal analysis, K.C.P. and I.d.N.; investigation, E.V., I.d.N., A.P., and C.C.; writing – original draft, K.C.P.; writing – review & editing, I.d.N., N.M.v.M., C.C., A.P., M.d.H., A.M., J.H., E.V., and K.C.P.; visualization, C.C., A.P., M.d.H., A.M., J.H., and E.V.; supervision, M.d.H., A.M., and J.H.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Transgender women's semen samples	Sexual health clinic in Coffs Harbor, Australia, and Amsterdam UMC, Netherlands	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to Ken Pang (ken.pang@mcri.edu.au).

Materials availability

This study did not generate new unique reagents or materials.

Data and code availability

Data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND HUMAN PARTICIPANT DETAILS

Human participant details

Trans women were identified based on: i) their attendance at either a sexual health clinic in Coffs Harbor, Australia, or the Center of Expertise on Gender Dysphoria at the Amsterdam UMC, Netherlands; ii) their desire to temporarily stop GAHT for reproductive purposes; and iii) a minimum of at least six months of GAHT use. Informed consent to participate in this study was subsequently obtained from nine eligible participants. As this study adhered to a case series design, no sample size calculation was performed. This study was conducted in accordance with the Medical Ethics Review Committee of VU University Medical Center (IRB00002991).

METHOD DETAILS

Semen samples were collected in accordance with standard sperm banking procedures. This included instructions to patients to ensure a suitable abstinence period (e.g., avoiding intercourse or masturbation for several days before collection), to avoid use of any lubricants, and to deliver samples for analysis within an hour of collection (in any cases where the specimen was produced off-site). Semen characteristics (semen volume, sperm concentration and sperm motility) were manually assessed by qualified clinical laboratory technicians. Volume was determined using a wide-bore volumetric pipette, and sperm concentration and motility were assessed using a Makler counting chamber (Sefi-Medical Instruments LTD, Haifa, Israel) and a phase contrast microscope (200–400x). Consistent with expert andrology advice, patients generally waited several months before re-attempting semen collection if semen analysis revealed azoospermia, since the timeline for sperm production is typically at least two months. Relevant clinical data were extracted from the medical records and included: confirmed gender incongruence, age at commencement of GAHT; type of estrogen and anti-androgen therapy, duration and dose; reasons for stopping GAHT; subsequent semen analysis results; and reproductive outcomes.

QUANTIFICATION AND STATISTICAL ANALYSIS

Baseline characteristics are presented as mean with SD when normally distributed, and as median with interquartile range (IQR) when non-normally distributed. In [Table 1](#) the characteristics for all participants of this study are displayed and semen parameters that were above the reference values for human semen as determined by the World Health Organization (semen volume (≥ 1.5 mL), sperm concentration (≥ 15 million/mL) or progressive motility ($\geq 32\%$)) were indicated with an asterisk. Patient 3's first semen analysis was significantly delayed due to a move across continents, during which she was unable to be with her partner. They were eventually reunited after which the semen analysis was performed. Therefore, patient 3 was excluded from [Figure 1](#) where the relationship was plotted between GAHT duration and time when spermatozoa were first identified after stopping GAHT.