Original Article
Male reproductive health and infertility

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health 2024 Jul 42(3): 531-542
https://doi.org/10.5534/wjmh.230069

The World Journal of MEN's HEALTH

Impact of Hydroxyurea to Treat Haematological Disorders on Male Fertility: Two Case Reports and a Systematic Review

Simone Cilio[1,2,*], Giuseppe Fallara[1,3,*], Maria Teresa Lupo Stanghellini[4], Fabio Ciceri[3,4], Francesco Montorsi[1,3], Francesca Lunghi[4,†], Andrea Salonia[1,3,†]

[1]Division of Experimental Oncology, Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, [2]Department of Neurosciences, Reproductive Sciences and Odontostomatology, Urology Unit, University of Naples "Federico II", Napoli, [3]Vita-Salute San Raffaele University, [4]Hematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

Purpose: Hydroxyurea (HU) is a cytoreductive agent used as standard treatment option for sickle cell anaemia/disease (SCD), essential thrombocythemia (ET), and polycythaemia vera (PV). Despite its overall good safety profile, its use also in relatively young patients raises an interest on its potential impact on spermatogenesis. To perform a systematic review of all published articles investigating fertility in male patients affected by SCD, ET, and PV and treated with HU. Two paradigmatic case reports of patients affected by PV and ET, respectively, have been also reported.

Materials and Methods: PubMed, EMBASE, and Cochrane databases were queried for all the published studies indexed up to November 15th, 2022. A combination of the following keywords was used: "hydroxyurea," "fertility," "male," "sperm," "sickle cell anaemia," "sickle cell disease," "essential thrombocythemia," "polycythaemia vera."

Results: Of 48 articles identified, 8 studies, involving 161 patients, were eligible for inclusion. Overall, the number of spermatogonia per round cross section of seminiferous tubule were decreased in patients with SCD compared to healthy males. HU treatment was always associated with a worsening of semen parameters, even up to azoospermia. Notably, treatment discontinuation was associated with an improvement of semen parameters and a trend toward normalization in the case of PV and ET, with a less clear amelioration in men with SCD. In both our patients with either PV or ET, HU discontinuation was associated with a significant improvement of spermatogenesis with successful spontaneous pregnancies.

Conclusions: Published evidence do not consistently report normalization of spermatogenesis after HU discontinuation in SCD cases. Conversely, the literature almost consistently reported an improvement of semen parameters at the discontinuation of HU therapy in PV and ET cases. Our real-life two cases confirmed those findings. The willing of fatherhood and the need for effective fertility treatment warrant further research to improve work-up management in men with hematological disorders.

Keywords: Anemia, sickle cell; Fertility; Hydroxyurea; Polycythemia vera; Thrombocythemia, essential

Received: Jun 20, 2023 **Revised:** Jul 30, 2023 **Accepted:** Aug 3, 2023 **Published online** Jan 2, 2024
Correspondence to: Andrea Salonia https://orcid.org/0000-0002-0595-7165
Division of Experimental Oncology, Unit of Urology, Urological Research Institute, IRCCS Ospedale San Raffaele, Via Olgettina 60, 20132 Milan, Italy.
Tel: +39-2-26436763, **Fax:** +39-2-26432969, **E-mail:** salonia.andrea@hsr.it
*These authors contributed equally to this work as co-first authors.
†These authors contributed equally to this work as joint senior authors.

The World Journal of MEN's HEALTH

INTRODUCTION

Hydroxyurea (HU) is an anti-proliferative drug synthesized by Dresler and Stein in 1869 and firstly used as an anti-tumor agent in the 1960s in the setting of DNA synthesis through inhibition of the ribonucleotide reductase enzyme [1,2]. HU is currently used as first-line therapy of sickle cell anaemia/disease (SCD) [3-5], essential thrombocythemia (ET) [6-8], polycythaemia vera (PV) [7,9], leukemia [10,11], psoriasis [12], and HIV infection [13] and in combination with radio-chemotherapy for some malignancies [14,15]. The primary site of action of HU is the ribonucleotide reductase, also known as ribonucleoside diphosphate reductase, a crucial enzyme in DNA synthesis which acts by catalyzing the reduction of ribonucleotides to deoxyribonucleotides [16]. Its inhibition leads to the depletion of intracellular deoxynucleotide pools, to the impairment of DNA synthesis and repair, and to block cell mitosis in the S-phase [1]. Moreover, HU causes cell apoptosis by directly cleaving DNA molecules, breaking chromosomes in the metaphase's mitotic division, and, once converted to hydroxylamine, by cleaving acetyl-coenzyme A and disrupting oxidative phosphorylation [17]. HU related cytotoxicity relies both on its concentration and length of treatment, whilst the effects are usually reversible at drug discontinuation [18]. As a consequence of its pharmacodynamics, common adverse events include bone marrow suppression, bleeding, headache, dizziness, fatigue, various cutaneous disorders, nausea/vomiting, gastric pain, and diarrhea [19-21].

SCD is the most diffuse inherited hemoglobinopathy worldwide [22]. It is characterized by the production of hemoglobin S, which, when deoxygenated, crystallize and alter the structure of red blood cells giving the characteristic sickle shape [23]. The loss of the normal biconcave shape induces acute or chronic vasa-occlusion, haemolytic anaemia and vasculopathy with painful crises, sometimes accompanied even by acute chest syndrome, stroke, organ failure and eventually death [24]. SCD is a life-long process, with daily pain symptoms starting throughout childhood [25], and increasing over adolescence into adulthood [26].

ET is a Ph-negative chronic myeloproliferative tumor characterized by increased production of functionally poor platelets [7,27,28]. The clinical course of the disease is complicated by hemorrhagic and, more frequently, thrombotic events [29], with an overall dysfunction of the microcircular system [30]. Major risk associated with ET is the evolution to neoplasm and patients below 40 years of age seem to be at higher risk [31].

PV is a chronic clonal myeloproliferative tumor characterized by increased production of red cells with high levels of hemoglobin [32,33]. Patients experiment fatigue, pruritus, and splenomegaly together with an increased risk of thrombotic and cardiovascular events [7,34]. Despite being more common among the older, it can occur also in men younger than 20 years of age [35].

These three hematological diseases can be treated with HU, which should be offered as soon as possible after the diagnosis, starting from 9 months of age [36,37]. As these patients get older, one of the problems they may encounter is the impairment of spermatogenesis due to a decreased spermatogonial maturation, resulting in worsening of sperm parameters till azoospermia and eventually fertility issues [38,39].

Consequently, on the one hand considering the need for a relatively secure treatment for myeloproliferative diseases (ET and PV) and hemoglobinopathy (SCD), and, on the other, the willingness of fatherhood, current systematic review aims at summarizing the published evidence on the impact of HU toward male fertility. Likewise, to support published literature and provide further insight over the management work-up of men presenting for couple's infertility, we also present two clinical cases of patients under HU treatment because of ET and PV, respectively, who had been referred to our tertiary referral reproductive medicine center. The final aim of this work is to provide practical advice and guide andrologists through an effective clinical work-up and decision-making in this relatively neglected field.

MATERIALS AND METHODS

We conducted a systematic review of all published articles related to the effects of HU in terms of male fertility. PubMed, EMBASE, and Cochrane were queried for all the published studies indexed up to March 2022. A combination of the following keywords was used: "hydroxyurea"; "fertility"; "male"; "sperm"; "sickle cell anaemia"; "sickle cell disease"; "essential thrombocythemia"; "polycythaemia vera". Only original articles that included patients affected by SCD, ET, and PV, treated with HU as first-line therapy, and whose fertility was evaluated by means of sperm parameters or

testicular tissue analysis before (*i.e.*, naïve patients), during, or after HU treatment, were considered for inclusion in the current systematic review. Titles and abstracts of manuscripts were screened for initial study inclusion. Full text review was performed when the abstract was not sufficient to determine study inclusion. Abstracts, commentaries, editorials, articles that did not undergo peer-review, and studies on animal models were excluded. References lists of included studies were hand-searched for completeness. Full text review was performed when the abstract was not sufficient to determine study inclusion. In the case of multiple publications from the same cohort, the most updated one has been included. Finally, non-English studies, those without an evaluation of patients' fertility with semen analysis or testis specimen, and studies where different treatment modalities were used for SCD, ET or PV, were also excluded from the current systematic review.

This systematic review was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and registered to PROSPERO with ID number CRD42022375767. Two authors completed the study selection independently (S.C. and G.F.) according to PRISMA requirements. Potential disagreements were resolved by consensus among all co-authors. The risk of bias was assessed by use of the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I).

1. Variables and outcomes definition

Data were collected according to pre-defined form including authors' names, year of publication, number of included subjects and their mean age, type of hematological disease, number of treated and untreated men, duration of HU treatment, sperm parameters and/or testicular tissue analyses before (naïve patients), during, or after HU treatment, the presence of healthy/untreated controls, and sperm parameters or testicular tissue findings for healthy controls.

2. Data collection

Data collection followed the principles outlined in the Declaration of Helsinki; all patients had signed an informed consent agreeing to deliver their own anonymous information for future studies. The study was approved by Our local Ethical Committee (Prot. 2014—Pazienti Ambulatoriali).

Methods used to analyze sperm specimen complied to those in the Björndahl guidelines [40].

RESULTS

Overall, 48 articles have been identified; thereof, 40 articles were excluded (*i.e.*, 19 duplicates; 12 because only the abstract was available; 3 dealt with other diseases; 5 were pre-clinical/animal studies; and 1 was a review). Of all, 8 manuscripts accomplished our inclusion criteria and have been considered for this systematic review (Fig. 1). Risk of bias according to the ROBINS-I tool emerged to be severe in 3, moderate in 3 and low in 2 studies, respectively (Supplement Table 1).

Table 1 summarizes the characteristics of the included studies. Overall, 161 patients were included in the current systematic review. The oldest study was published in 2007 and the most updated in 2021. Of all, 6 studies investigated SCD, 1 study both SCD and PV, and 1 ET alone. Overall, median age of patients ranged

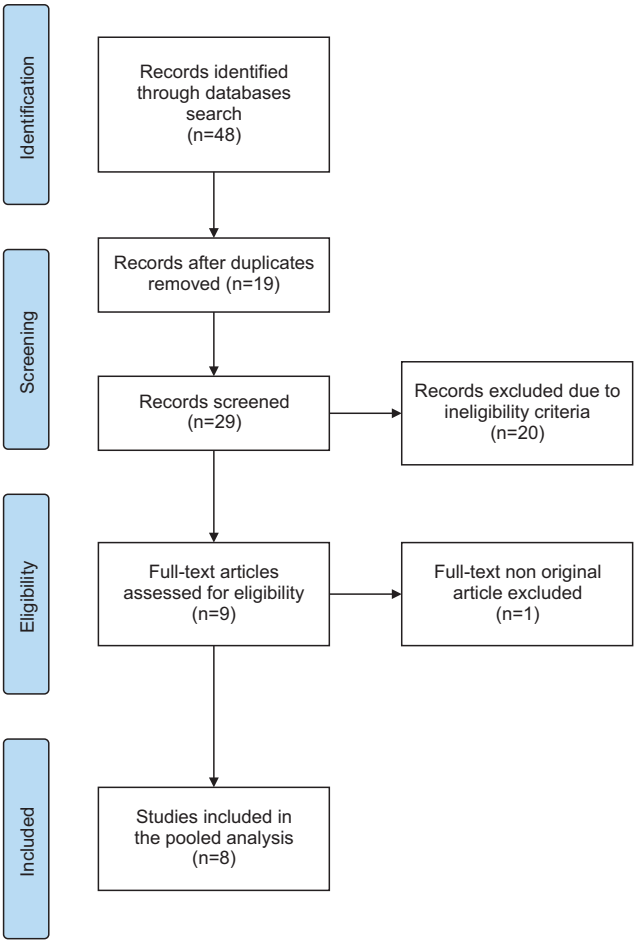

Fig. 1. Prisma flow chart—study selection with inclusion and exclusion criteria of reviewed studies.

The World Journal of MEN's HEALTH

Table 1. Characteristics of the included studies classified according to year of publication (2021–2007)

Author	Year of publication	No. of patients	Haematological disease	Mean age (SD)	Treated patients, n (%)	Years of treatment, mean (range)	Parameter evaluated	Healthy/ untreated control
Gille et al [41]	2021	30	SCD	8.8 (5.4)	17 (56.7)	3 (0.7–5.6)	S/T ratio	Yes
Stukenborg et al [43]	2018	6	SCD	7.9 (3.6)	6 (100)	N/A	S/T ratio	Yes
Joseph et al [47]	2021	38	SCD	17 (N/A)	15 (39.5)	4 (0.5–10)	Seminal parameters	Yes
Portela et al [39]	2020	3	SCD	N/A	3 (100)	N/A	Spermatogonia's maturation	Yes
Berthaut et al [38]	2017	35	SCD	33.6 (9.2)	35 (100)	0.5	Seminal parameters	No
Berthaut et al [45]	2008	44	SCD	25.8 (N/A)	13 (29.5)	N/A (2–10)	Seminal parameters	No
Grigg [44]	2007	4	PV	29.3 (7.9)	1 (25.0)	10.5	Seminal parameters	No
			SCD		1 (25.0)	1.4		
			SCD		1 (25.0)	4.1		
			SCD		1 (25.0)	6.25		
Masood et al [48]	2007	1	ET	38 (N/A)	1 (100)	3	Seminal parameters	No

SD: standard deviation, SCD: sickle cell disease, N/A: not available, S/T ratio: number of spermatogonia (S) per round cross section of seminiferous tubule (T), PV: polycythaemia vera, ET: essential thrombocythemia.

from 7.9 to 38 years and the length of HU administration from 1.4 to 10.5 years. As a whole, male fertility was investigated with semen analysis in 5 studies (Table 2), and in 3 studies with testicular tissues cryopreserved before cytoreductive therapies; of these, 2 studies reported the number of spermatogonia per round cross section of seminiferous tubule (S/T ratio), and 1 study reported spermatogonia's maturation (Table 3).

1. Effects on fertility of hydroxyurea in prepubertal patients with sickle cell disease

Two studies investigated the effects of HU on fertility in prepubertal patients affected by SCD. The first study form Gille et al [41] in 2021 compared the characteristics of testicular tissue from 30 (13 HU-naïve and 17 HU-exposed) prepubertal males affected by SCD. Testicular tissue was available due to a previous cryopreservation obtained through testicular surgery before a hematopoietic stem cell transplantation. The overall median age was 8.8 years. The authors compared the S/T ratio of testicular tissues from HU-naïve and HU-exposed patients and found no statistical difference in the spermatogonial pool between the two groups (p=0.52). Moreover, by comparing the S/T ratio of both groups with the reference values of healthy individuals, it was confirmed that the spermatogonial number in patients with SCD is lower than in healthy prepuberal individuals [42]. Likewise, Stukenborg et al [43] analyzed testicular tissues of 6 young patients with SCD (mean age of 7.9 years) treated with HU for an average of 6 years. Testicular tissue was collected before patients have been provided with alternative disease-modifying therapies for SCD, which had been not specified in the manuscript; due to the potential sterilizing adverse effect, all patients underwent pretreatment testicular cryopreservation for potential subsequent fertility preservation programs. The authors found a lower mean S/T ratio in the testicular tissue of patients with SCD as compared with available control samples from the biobank of the Department of Pathology, Karolinska University Hospital (p=0.003).

2. Effects on fertility of hydroxyurea in adult patients with sickle cell disease

Four studies investigated the effects of HU on fertility in adult patients affected by SCD. The first study form Grigg [44] in 2007 analyzed semen parameters of 3 patients with SCD. One patient had a sperm count

Table 2. Semen parameters reported in analysed studies classified according to year of publication (2007–2021)

Sample collection timing	Semen parameters	Haematological disease							
		Joseph et al [47] (2021)	Berthaut et al [38] (2017)	Berthaut et al [45] (2008)	Grigg [44] (2007)				Masood et al [48] (2007)
		SCD	SCD	SCD	PV	SCD	SCD	SCD	ET
Patients before treatment/ HU-naïve	Semen Volume (mL)	2.0 (0.25–5.90)	2.34	3.08 (1.67)	N/A	N/A	N/A	N/A	N/A
	Sperm concentration ($\times 10^6$/mL)	8.0 (0.00–120.00)	N/A	38.55 (43.12)	N/A	N/A	N/A	N/A	N/A
	Total sperm count ($\times 10^6$)	12.0 (0.00–523.90)	61.6	114.17 (124.12)	N/A	N/A	N/A	N/A	N/A
	Progressive motility (%)	35.0 (1.00–55.00)	N/A	28.66 (18.38)	N/A	N/A	N/A	N/A	N/A
	Normal morphology (%)	9.5 (0.00–38.00)	N/A	21.92 (14.6)	N/A	N/A	N/A	N/A	N/A
	Vitality (%)	58.0 (0.00–87.00)	N/A	59.75 (21.6)	N/A	N/A	N/A	N/A	N/A
Patients under treatment	Semen Volume (ml)	N/A	2.5	2.68 (1.28)	N/A	N/A	N/A	N/A	3
	Sperm concentration ($\times 10^6$/mL)	N/A	N/A	2.66 (3.75)	0	26	N/A	5	1
	Total sperm count ($\times 10^6$)	N/A	0.63	7.02 (10.18)	N/A	N/A	N/A	N/A	N/A
	Progressive motility (%)	N/A	N/A	34.50 (21.92)	N/A	35	N/A	41	0
	Normal morphology (%)	N/A	N/A	34.50 (21.9)	N/A	35	N/A	19	0
	Vitality (%)	N/A	N/A	52.00 (14.2)	N/A	N/A	N/A	N/A	N/A
Patients after treatment discontinuation	Semen Volume (mL)	2.7 (0.20–10.35)	N/A	2.99 (2.85)	N/A	N/A	N/A	N/A	5.5
	Sperm concentration ($\times 10^6$/mL)	25.3 (0.00–210.00)	N/A	18.46 (26.86)	30	15	15	N/A	200
	Total sperm count ($\times 10^6$)	77.5 (0.40–525.00)	N/A	61.12 (107.37)	N/A	N/A	N/A	N/A	N/A
	Progressive motility (%)	2.0 (0.00–75.00)	N/A	19.16 (16.3)	20	21	58	N/A	94
	Normal morphology (%)	6.0 (0.00–25.00)	N/A	19.16 (16.3)	6	6	50	N/A	83
	Vitality (%)	52.5 (12.00–77.00)	N/A	44.40 (20.1)	N/A	N/A	N/A	N/A	N/A
	Timing from HU discontinuation (years)	0–13	N/A	0.5–5	0.25	2.7	1	N/A	0.5
	p-value	All>0.05	<0.001	All>0.05	N/A				N/A

Values are presented as median (range), mean only, or mean (standard deviation).
SCD: sickle cell disease, N/A: not available, PV: polycythaemia vera, ET: essential thrombocythemia, HU: hydroxyurea.

The World Journal of MEN's HEALTH

Table 3. Tissue specimen data in analysed studies classified according to year of publication (2021–2007).

Author	Parameter evaluated	Testicular specimen origin	Without treatment	During treatment	p-value
Gille et al [41] (2021)	S/T ratio	Patients with SCD	3.1±4.0	1.5±0.7	<0.0001
Stukenborg et al [43] (2018)	S/T ratio	Patients with SCD	N/A	0.3±0.6	0.003
		Normal tissue from biobank	4.1±4.6	N/A	
Portela et al [39] (2020)	Spermatogonia maturation	Patients with SCD	N/A	Lower maturation	<0.016

Values are presented as mean±standard deviation.
S/T ratio: number of spermatogonia (S) per round cross section of seminiferous tubule (T), N/A: not available, SCD: sickle cell disease.

at the lower limit of the normal range and a sperm motility below the normal limits during the 17 months under HU therapy. HU was then ceased for 32 months, and the patient performed a semen analysis which showed persistently subnormal sperm count, motility, and morphology. A second patient had a modestly low sperm count but normal motility and morphology after one year form HU discontinuation (the author did not report sperm data at baseline or during the 4 years of HU therapy course). Lastly, another patient had substantially low concentration with normal motility but abnormal morphology during the 4 years course of HU therapy (the author did not report sperm data before or after the HU discontinuation).

In 2008 Berthaut et al [45] analyzed 108 ejaculates from 44 patients with SCD: 76 samples collected before HU treatment from 34 patients; 6 samples collected during treatment (lasting from 2 to 10 years) from 5 patients; and, 26 samples collected from 8 patients after the discontinuation of the treatment (ranging from 0.5 to 5 years from stop of treatment). Mean age was 25.8 years (range: 16–48 years). Before treatment, 91% of samples showed alteration of at least one sperm parameter, with severe impairment of sperm morphology in two-thirds of the cases. Moreover, a decrease of the semen volume was detected, likely suggesting an impairment of both the seminal vesicles and the prostate. During the treatment, the analysis of all samples showed oligoasthenoteratospermia. After HU discontinuation, no improvements were found in semen parameters, with comparable rates of oligoasthenoteratospermia, and a case of new onset of azoospermia was diagnosed 4 years after treatment discontinuation. However, despite alterations of semen parameters, fertility seemed to be conserved in young couples. Indeed, the 40% of men involved in the study achieved at least one pregnancy allowing the authors to consider the pregnancy outcomes in a normal range with 29 normal births, 3 spontaneous miscarriages (for the same patient) and 4 induced abortions. Thereafter, Berthaut et al [38] evaluated the changes in semen analysis in another cohort of 35 adults with SCD (mean age 33.6 years) treated with HU. The analysis was performed before HU was started and after 6 months of drug administration. At baseline, 40% of the patients had abnormal semen parameters, with only one case of cryptozoospermia and no cases of azoospermia. After 6 months of HU treatment, the number of cases of cryptozoospermia and azoospermia did significantly increase more than 10-fold (median sperm total count 61.6×10^6 before *vs.* 0.63×10^6 at 6 months during HU treatment), thus suggesting a detrimental effects of HU on spermatogenesis ($p<0.001$). In 2020, Portela et al [39] analyzed the density of spermatogonia in the testicular tissues of 3 patients with SCD under treatment with HU. Tissues samples were collected through cryopreservation for fertility preservation programs before treatment did start. As controls, the authors used free-from-disease parts of the testes taken from patients submitted to orchiectomy due to testicular cancer. They found no differences in the density of spermatogonia among the two sample groups, despite not being optimal as a control tissue, given the possibility of already compromised spermatogenesis because of testis cancer itself [46]. However, patients with SCD treated with HU presented a significantly decreased expression of the marker of maturation 5-mC, compared to the control arm ($p<0.016$), thus suggesting a condition of arrested spermatogenesis. Moreover, Joseph et al [47] compared the semen analyses of 38 patients (mean age 17 years) affected by SCD, 23 of which were HU-naïve and 15 had been treated with HU in childhood whilst no longer under HU therapy at the time of the analysis. Thus, semen samples have been collected from 26 and 46 men, respectively. The time of discontinuation ranged from 0 to 13 years, during which patients were all subjected to transfusion programs. Quantitative and qualitative semen abnormalities were detected in all

samples, and significant differences between patients previously exposed to HU and no longer in therapy and/or HU-naïve were found only in terms of greater sperm count (8.0×10^6 *vs.* 25.3×10^6) and higher concentration (12.0×10^6/mL *vs.* 77.5×10^6/mL).

3. Effects on fertility of hydroxyurea in an adult patient with polycythaemia vera

We found only one study reporting the case of one patient diagnosed with PV, treated with HU and dealing with fertility issue. After more than 10 years on HU the patient was azoospermic; pre-treatment values were not unfortunately available. Therefore, HU treatment was stopped and, after 3 months of drug discontinuation, the patient recovered to normal sperm count, despite the presence of asthenoteratozoospermia. The patient was then able to perform sperm banking for further fertility purposes. Thereafter, HU was started again and azoospermia was found once again after 6 month of continuous treatment [44].

4. Effects on fertility of hydroxyurea in an adult patient with essential thrombocythemia

We found only one study reporting the case of one patient diagnosed with ET, treated with HU and dealing with fertility issue. More in details, Masood et al [48] reported the effects HU administered for 3 years in a 38-year-old patient diagnosed with ET. Semen analysis during ongoing treatment showed azoospermia; conversely, when HU was discontinued, sperm count recovered to a normal count in a period of 6 months.

5. Cases reports

1) A case of secondary couple's infertility with a diagnosis of polycythaemia vera under treatment with hydroxyurea

We present the case of a 40-year-old man who was referred to our center for secondary couple's infertility (the first child was 2.5 years old at patient presentation). The patient was diagnosed with myeloproliferative disease PV JAK2V617F+ according to WHO 2016 classification [49,50]. The diagnosis was performed in concomitance with diagnosis of Multiple Sclerosis during the hospitalization due to thrombosis of the left transverse sinus in 2020. The patient was classified as high vascular risk according to ELN and started treatment with HU in combination with antiplatelet therapy [51].

Trying to conceive, the patient presented in our fertility clinic with a semen analysis reporting cryptozoospermia (*i.e.*, 1 sperm per 25 High Resolution Fields). The only treatments ongoing at time of fertility evaluation were HU for PV and dimenthyl fumarate for Multiple Sclerosis.

His wife, followed at the same ART center, had no fertility issues after a comprehensive Gynecological evaluation [52].

As for each infertile male patient, a complete medical assessment with detailed medical and reproductive history taking and physical examination was performed,

Table 4. Semen parameters of the two case reports included in our study

Hematological disease	Semen parameters	Semen parameters under treatment with HU	Semen parameters after HU discontinuation
PV	Semen volume (mL)	5	2.5
	Sperm concentration ($\times10^6$/mL)	0	7.5
	Total sperm count ($\times10^6$)	0	18.75
	Progressive motility (%)	0	20
	Normal morphology (%)	0	1
	Vitality (%)	0	66
ET	Semen volume (mL)	2	2
	Sperm concentration ($\times10^6$/mL)	0	30
	Total sperm count ($\times10^6$)	1 SPZ/25 HPF	60
	Progressive motility (%)	1	61
	Normal morphology (%)	1	4
	Vitality (%)	5	87

HU: hydroxyurea, PV: polycythaemia vera, ET: essential thrombocythemia, SPZ/25 HPF: spermatozoa/25 high power field.

The World Journal of MEN's HEALTH

which revealed no additional medical issues. Blood test with complete hormonal profile was carried out without finding no additional issues. Ultrasound scan of the testis and of the lower abdomen revealed no morphologic abnormality in the reproductive system. As such, the most probable etiology of secondary infertility was attributed to the initiation of HU. Thereafter, HU treatment was discontinued in accordance with the treating Hematologist in January 2022, and a bloodletting regime was started. After 5 months from HU discontinuation, a spontaneous pregnancy did occur; moreover, at semen analysis performed 6 months after HU was stopped a significant improvement of all semen parameters was achieved up to normozoospermia (Table 4) [53].

2) A case of primary infertility with a diagnosis of essential thrombocythemia under treatment with hydroxyurea

We present the case of a 34-year-old man who had received six years before a diagnosis of ET according to 2008 WHO classification, with platelets count over $1{,}000\times10^9$/L, an increased megakaryocytes count at bone marrow biopsy, without driver mutation JAK2V617F–CALR–MPL negative) also confirmed at NGS study [54]. The patient was classified as high risk due to platelets count above 1.500×10^9/L and started HU according to ELN 2011 [51]. After 5 years of HU therapy, the patient was referred to our center because of primary couple's infertility and the finding of azoospermia at semen analysis, while first level investigations including physical examination, ultrasound assessment of the testes and the abdomen and circulating hormones were normal. Past medical history revealed the presence of Gilbert syndrome and a cholecystectomy, which were however not related to his current infertility problem. The partner, followed at the same ART center, had no fertility issues after a comprehensive Gynecological evaluation [52]. In the hypothesis that HU was the cause of his azoospermia, HU treatment was discontinued in accordance with the treating Hematologist and the patient did start salicylic acetyl acid treatment. After 3 months, a partial improvement in sperm concentration (6.0×10^6/mL) was depicted at semen analysis, with a diagnosis of oligoasthenoteratozoospermia. The further semen analyses at 5-, 6-, and 8-month investigation from HU discontinuation, confirmed the observed improvement in terms of sperm concentration (the last 7.5×10^6/mL) (Table 4). A full-term pregnancy was achieved after two years form the drug suspension, while patient was under salicylic acetyl acid treatment.

DISCUSSION

This systematic review of the literature provides findings from 8 studies on the impact of HU treatment in men with SCD, PV, or ET toward male fertility outcomes. Of these, only two case reports dealt with infertility issues in patients with either PV or ET. Overall, evidence on infertility in patients treated with HU are scant and definitive conclusions cannot be drawn. To sum published literature up, in prepubertal patients with SCD, testes quality seemed similar between treated and untreated patients, despite being lower compared to healthy individuals. In adult patients with SCD several sperm abnormalities have been reported already before therapy with HU, with a further worsening of semen parameters during ongoing treatment with HU, and without consistent evidence of improvement after drug discontinuation. Moreover, the analysis of testicular sample revealed no differences in spermatogonial density between HU-treated and healthy tissues—although potentially biased since derived from orchiectomy performed in patients with testicular cancer—despite a decreased spermatogonial maturation. Conversely, azoospermia was more clearly reported to occur during HU therapy in men with ET or PV, and normozoospermia was eventually restored after treatment discontinuation. The length of HU discontinuation before restoration or at least improvement in semen parameters ranged between 1 month and 13 years. Similarly, here we presented the case of one patient with PV and of one patient with ET, both treated with HU and seeking medical help at our andrology center for couple's infertility. Of clinical relevance, both patients had a significant improvement of all semen parameters after HU discontinuation, at 3 and 6 months, respectively. Moreover, both patients eventually spontaneously fathered.

These data deserve further considerations.

First, there is evidence that SCD itself might cause an impairment of spermatogenesis. In fact, our review found that HU-naïve patients diagnosed with SCD have worse quantitative and qualitative semen abnormalities compared to healthy individuals [5,36,41,43,47].

Gonadal dysfunction due to SCD could results from vasa-occlusion phenomenon of either hypothalamic-pituitary blood vessels, with the result of secondary hypogonadism, or of the testicular vessels, thus resulting in organ failure and primary hypogonadism [55]. Indeed, the hypoxic-ischemic damage of testicular tissues in men with SCD creates alterations similar to the ones observed in testicular tissues in men with varicocele, postulating that hypoxia is a contributing factor to sperm abnormalities in these patients [56,57].

Second, literature is poor on this issue despite SCD, PV and ET are quite frequent diseases and HU represents often their first-line treatment. Of note, HU is indicated as primary therapy in patients affected by PV and ET classified as high risk, and also in low-risk patient with at least one among adverse features including leucocytosis, poor hematocrit control from phlebotomy-only, symptomatic progressive splenomegaly, and in patients with progressive and persistent leucocytosis, extreme thrombocytosis and inadequate hematocrit control from phlebotomy-only (>6/year), as it has been highlighted by the ELN 2021 recommendation [58]. This will potentially increase the number of young patients candidate to cytoreductive treatment unveiling the need for a fertility counseling and management. Infertility should be considered among the issues relevant for the selection of a second line treatment in high-risk patients according to ELN 2021; thereof, further data are needed to corroborate this hypothesis.

Third, published data regarding HU discontinuation as a potential modality to improve semen parameters, supporting the post-hoc hypothesis that treatment with HU might cause an impairment in spermatogenesis, are conflicting. Indeed, animal studies have shown that HU in mice increases testicular germ cell apoptosis and reduces spermatogenesis [37]. Results from our systematic review suggested that HU could worsen the testicular function in patients with hematological diseases. Following this reasoning, HU discontinuation has the potential of improving spermatogenesis, especially for patients with ET and PV. Of note, data are much more conflicting for SCD, since the only three studies which had analyzed the impact of HU in this setting, both during HU treatment and after HU discontinuation, showed that improvement was not present at drug discontinuation, regardless of the timing from HU suspension.

Overall, males with SCD might suffer from semen abnormalities that are also independent from the HU itself and therefore not reversible after HU discontinuation. Conversely, for PV and ET, the two published case reports and the two clinical cases presented here are concordant in supporting HU discontinuation to achieve better semen parameters and eventually even pregnancy and live births. This strategy should be discussed with the treating hematologist.

The present manuscript and the findings of our systematic review are certainly not devoid of limitations. First, the small number of studies published on this issue, the small sample size of these studies, their high heterogeneity in both patients' characteristics and HU treatment duration and time frame since discontinuation, prevent to formulate definitive recommendations on the management of infertility in case of treatment with HU in patients with SCD, PV and ET. In this context, the very few studies published on this topic have involved a very small number of patients and were all retrospective in nature, thus implying a high risk of relevant biases. In addition, there is a high heterogeneity in the study populations, follow-ups and outcome definitions. Moreover, most of the selected studies had a "moderate risk" of bias according to the ROBINS-I tool. Overall, these issues largely prevented us from drawing definitive recommendations regarding the use of HU in patients affected by hematological diseases. Finally, the biological underpinnings of these findings are not known, and achieving an adequate explanation is key to justify any clinical decision making in this delicate setting.

Overall, with the specific aim of both preserving and restoring fertility potentials in male patients who wish to fathering but who require HU for their relevant hematological disorders, further research is needed to i) better understand testicular and accessory glands alterations in SCD patients, ii) depict the actual effects of HU discontinuation, and iii) find new strategies, together with the hematological team.

CONCLUSIONS

In conclusion, considering the several limitations of this current systematic review and recognizing the rarity of these conditions, no definitive conclusion could be drawn especially in men with SCD, and further studies are needed. However, a comprehensive discussion in a multidisciplinary team both involving the an-

drologist and the hematologist is of utmost importance when dealing with male infertility. It is likely that HU has a detrimental effect on spermatogenesis, due to its pharmacodynamic profile and irrespective of the baseline disease. In addition, in men with PV and ET, HU discontinuation might significantly improve semen parameters and increase pregnancy probability, whereas evidence in case of SCD is more conflicting, probably because SCD per se might cause irreversible damage to the testis and hence to the spermatogenesis.

Conflict of Interest

The authors have nothing to disclose.

Funding

None.

Acknowledgements

None.

Author Contribution

Conception and design of the study: FL, AS, SC, GF, MTLS. Acquisition of data: FL, AS, SC, GF, MTLS. Data interpretation: FL, AS, MTLS, FC, FM, SC, GF. Writing – original draft: SC, GF, MTLS. Writing – review & editing: FL, AS, MTLS, FC, FM. All authors have approved the final version of the manuscript.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.230069.

REFERENCES

1. Singh A, Xu YJ. The cell killing mechanisms of hydroxyurea. Genes (Basel) 2016;7:99.
2. Saban N, Bujak M. Hydroxyurea and hydroxamic acid derivatives as antitumor drugs. Cancer Chemother Pharmacol 2009;64:213-21.
3. Monus T, Howell CM. Current and emerging treatments for sickle cell disease. JAAPA 2019;32:1-5.
4. Nevitt SJ, Jones AP, Howard J. Hydroxyurea (hydroxycarbamide) for sickle cell disease. Cochrane Database Syst Rev 2017;4:CD002202.
5. McGann PT, Ware RE. Hydroxyurea therapy for sickle cell anemia. Expert Opin Drug Saf 2015;14:1749-58.
6. Tefferi A, Vannucchi AM, Barbui T. Essential thrombocythemia treatment algorithm 2018. Blood Cancer J 2018;8:2.
7. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. Am J Hematol 2020;95:1599-613.
8. Guglielmelli P, Vannucchi AM. Current management strategies for polycythemia vera and essential thrombocythemia. Blood Rev 2020;42:100714.
9. Besses C, Alvarez-Larrán A. How to treat essential thrombocythemia and polycythemia vera. Clin Lymphoma Myeloma Leuk 2016;16 Suppl:S114-23.
10. Trepte ML, Auten JJ, Clark SM, van Deventer HW. Dose-related mucositis with hydroxyurea for cytoreduction in acute myeloid leukemia. J Oncol Pharm Pract 2019;25:801-5.
11. Moyo TK, Savona MR. Therapy for chronic myelomonocytic leukemia in a new era. Curr Hematol Malig Rep 2017;12:468-77.
12. Menter A, Gelfand JM, Connor C, Armstrong AW, Cordoro KM, Davis DMR, et al. Joint American Academy of Dermatology-National Psoriasis Foundation guidelines of care for the management of psoriasis with systemic nonbiologic therapies. J Am Acad Dermatol 2020;82:1445-86.
13. Stein J, Storcksdieck Genannt Bonsmann M, Streeck H. Barriers to HIV cure. HLA 2016;88:155-63.
14. Salama JK, Haraf DJ, Stenson KM, Blair EA, Witt ME, Williams R, et al. A randomized phase II study of 5-fluorouracil, hydroxyurea, and twice-daily radiotherapy compared with bevacizumab plus 5-fluorouracil, hydroxyurea, and twice-daily radiotherapy for intermediate-stage and T4N0-1 head and neck cancers. Ann Oncol 2011;22:2304-9.
15. Desjardins A, Quinn JA, Vredenburgh JJ, Sathornsumetee S, Friedman AH, Herndon JE, et al. Phase II study of imatinib mesylate and hydroxyurea for recurrent grade III malignant gliomas. J Neurooncol 2007;83:53-60.
16. Musiałek MW, Rybaczek D. Hydroxyurea-the good, the bad and the ugly. Genes (Basel) 2021;12:1096.
17. Madaan K, Kaushik D, Verma T. Hydroxyurea: a key player in cancer chemotherapy. Expert Rev Anticancer Ther 2012;12:19-29.
18. Agrawal RK, Patel RK, Shah V, Nainiwal L, Trivedi B. Hydroxyurea in sickle cell disease: drug review. Indian J Hematol Blood Transfus 2014;30:91-6.
19. Spivak JL, Hasselbalch H. Hydroxycarbamide: a user's guide for chronic myeloproliferative disorders. Expert Rev Anticancer Ther 2011;11:403-14.
20. Harrison CN, Campbell PJ, Buck G, Wheatley K, East CL,

Bareford D, et al.; United Kingdom Medical Research Council Primary Thrombocythemia 1 Study. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. N Engl J Med 2005;353:33-45.
21. Karimi M, Cohan N, Mousavizadeh K, Falahi MJ, Haghpanah S. Adverse effects of hydroxyurea in beta-thalassemia intermedia patients: 10 years' experience. Pediatr Hematol Oncol 2010;27:205-11. Erratum in: Pediatr Hematol Oncol 2011;28:538.
22. Piccin A, Murphy C, Eakins E, Rondinelli MB, Daves M, Vecchiato C, et al. Insight into the complex pathophysiology of sickle cell anaemia and possible treatment. Eur J Haematol 2019;102:319-30.
23. Pinto VM, Balocco M, Quintino S, Forni GL. Sickle cell disease: a review for the internist. Intern Emerg Med 2019;14:1051-64.
24. Lidonnici MR, Ferrari G. Gene therapy and gene editing strategies for hemoglobinopathies. Blood Cells Mol Dis 2018;70:87-101.
25. Colombatti R, Casale M, Russo G. Disease burden and quality of life of in children with sickle cell disease in Italy: time to be considered a priority. Ital J Pediatr 2021;47:163.
26. Callaway D, Chawla A, Sprinz P. Physical therapy in pediatric and young adult patients with sickle cell disease: assessing potential benefits and barriers. J Pediatr Hematol Oncol 2020;42:463-6.
27. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016;127:2375-90.
28. Faraz M, Mansoori H, Ali M, Ali SA. Different shapes of megakaryocytes in essential thrombocythemia. J Ayub Med Coll Abbottabad 2022;34:389-91.
29. Rumi E, Cazzola M. How I treat essential thrombocythemia. Blood 2016;128:2403-14.
30. Lussana F, Femia EA, Pugliano M, Podda G, Razzari C, Maugeri N, et al. Evaluation of platelet function in essential thrombocythemia under different analytical conditions. Platelets 2020;31:179-86.
31. Gangat N, Szuber N, Jawaid T, Hanson CA, Pardanani A, Tefferi A. Young platelet millionaires with essential thrombocythemia. Am J Hematol 2021;96:E93-5.
32. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405. Blood 2016;128:462-3.
33. Iurlo A, Cattaneo D, Bucelli C, Baldini L. New perspectives on polycythemia vera: from diagnosis to therapy. Int J Mol Sci 2020;21:5805.
34. Mesa R, Verstovsek S, Kiladjian JJ, Griesshammer M, Masszi T, Durrant S, et al. Changes in quality of life and disease-related symptoms in patients with polycythemia vera receiving ruxolitinib or standard therapy. Eur J Haematol 2016;97:192-200.
35. Putter JS, Seghatchian J. Polycythaemia vera: molecular genetics, diagnostics and therapeutics. Vox Sang 2021;116:617-27. Erratum in: Vox Sang 2022;117:872.
36. DeBaun MR, Jordan LC, King AA, Schatz J, Vichinsky E, Fox CK, et al. American Society of Hematology 2020 guidelines for sickle cell disease: prevention, diagnosis, and treatment of cerebrovascular disease in children and adults. Blood Adv 2020;4:1554-88.
37. Qureshi A, Kaya B, Pancham S, Keenan R, Anderson J, Akanni M, et al.; British Society for Haematology. Guidelines for the use of hydroxycarbamide in children and adults with sickle cell disease: a British Society for Haematology guideline. Br J Haematol 2018;181:460-75.
38. Berthaut I, Bachir D, Kotti S, Chalas C, Stankovic K, Eustache F, et al. Adverse effect of hydroxyurea on spermatogenesis in patients with sickle cell anemia after 6 months of treatment. Blood 2017;130:2354-6.
39. Portela JMD, Heckmann L, Wistuba J, Sansone A, Pelt AMMV, Kliesch S, et al. Development and disease-dependent dynamics of spermatogonial subpopulations in human testicular tissues. J Clin Med 2020;9:224.
40. Björndahl L, Barratt CL, Mortimer D, Jouannet P. 'How to count sperm properly': checklist for acceptability of studies based on human semen analysis. Hum Reprod 2016;31:227-32.
41. Gille AS, Pondarré C, Dalle JH, Bernaudin F, Chalas C, Fahd M, et al. Hydroxyurea does not affect the spermatogonial pool in prepubertal patients with sickle cell disease. Blood 2021;137:856-9.
42. Masliukaite I, Hagen JM, Jahnukainen K, Stukenborg JB, Repping S, van der Veen F, et al. Establishing reference values for age-related spermatogonial quantity in prepubertal human testes: a systematic review and meta-analysis. Fertil Steril 2016;106:1652-7.e2.
43. Stukenborg JB, Alves-Lopes JP, Kurek M, Albalushi H, Reda A, Keros V, et al. Spermatogonial quantity in human prepubertal testicular tissue collected for fertility preservation prior to potentially sterilizing therapy. Hum Reprod 2018;33:1677-83.
44. Grigg A. Effect of hydroxyurea on sperm count, motility and morphology in adult men with sickle cell or myeloproliferative disease. Intern Med J 2007;37:190-2.
45. Berthaut I, Guignedoux G, Kirsch-Noir F, de Larouziere V, Ravel C, Bachir D, et al. Influence of sickle cell disease and

treatment with hydroxyurea on sperm parameters and fertility of human males. Haematologica 2008;93:988-93.
46. Minhas S, Bettocchi C, Boeri L, Capogrosso P, Carvalho J, Cilesiz NC, et al.; EAU Working Group on Male Sexual and Reproductive Health. European Association of Urology guidelines on male sexual and reproductive health: 2021 update on male infertility. Eur Urol 2021;80:603-20.
47. Joseph L, Jean C, Manceau S, Chalas C, Arnaud C, Kamdem A, et al. Effect of hydroxyurea exposure before puberty on sperm parameters in males with sickle cell disease. Blood 2021;137:826-9.
48. Masood J, Hafeez A, Hughes A, Barua JM. Hydroxyurea therapy: a rare cause of reversible azoospermia. Int Urol Nephrol 2007;39:905-7.
49. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. World Health Organization; 2017.
50. Barbui T, Thiele J, Gisslinger H, Kvasnicka HM, Vannucchi AM, Guglielmelli P, et al. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. Blood Cancer J 2018;8:15.
51. Barbui T, Tefferi A, Vannucchi AM, Passamonti F, Silver RT, Hoffman R, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. Leukemia 2018;32:1057-69.
52. Pozzi E, Boeri L, Candela L, Capogrosso P, Cazzaniga W, Fallara G, et al. Infertile couples still undergo assisted reproductive treatments without initial andrological evaluation in the real-life setting: a failure to adhere to guidelines? Andrology 2021;9:1843-52.
53. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16:231-45.
54. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood 2009;114:937-51.
55. Osegbe DN, Akinyanju OO. Testicular dysfunction in men with sickle cell disease. Postgrad Med J 1987;63:95-8.
56. DeBaun MR. Hydroxyurea therapy contributes to infertility in adult men with sickle cell disease: a review. Expert Rev Hematol 2014;7:767-73.
57. Nickel RS, Maher JY, Hsieh MH, Davis MF, Hsieh MM, Pecker LH. Fertility after curative therapy for sickle cell disease: a comprehensive review to guide care. J Clin Med 2022;11:2318.
58. Marchetti M, Vannucchi AM, Griesshammer M, Harrison C, Koschmieder S, Gisslinger H, et al. Appropriate management of polycythaemia vera with cytoreductive drug therapy: European LeukemiaNet 2021 recommendations. Lancet Haematol 2022;9:e301-11.