Ferroportin mRNA is down-regulated in granulosa and cervical cells from infertile women

José Maria Moreno-Navarrete, Ph.D.,[a,b] Eva López-Navarro, Ph.D.,[c] Luz Candenas, Ph.D.,[e] Francisco Pinto, Ph.D.,[e] Francisco J. Ortega, Ph.D.,[a,b] Mònica Sabater-Masdeu, M.Sc.,[a,b] Manuel Fernández-Sánchez, M.D., Ph.D.,[f] Victor Blasco, M.Sc.,[f] Antonio Romero-Ruiz, Ph.D.,[g,h] Marina Fontán, M.Sc.,[d] Wifredo Ricart, M.D., Ph.D.,[a,b] Manuel Tena-Sempere, M.D., Ph.D.,[b,g,h] and José M. Fernández-Real, M.D., Ph.D.[a,b]

[a] Department of Diabetes, Endocrinology and Nutrition, Institut d'Investigació Biomèdica de Girona, Girona; [b] Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y la Nutrición, Instituto de Salud Carlos III, Madrid; [c] Obstetrics and Gynecology Service, Institut d'Investigació Biomèdica de Girona, Girona; [d] Department of Biochemistry, Institut d'Investigació Biomèdica de Girona, Girona; [e] Instituto de Investigaciones Químicas, Consejo Superior de Investigaciones Científicas, Sevilla; [f] Instituto Valenciano de Infertilidad, Seville; [g] Department of Cell Biology, Physiology and Immunology, University of Cordoba, Cordoba; and [h] Instituto Maimonides de Investigacion Biomedica de Cordoba, Cordoba, Spain

Objective: To explore the relationship between iron and infertility by investigating iron-related gene expression in granulosa and uterine cervical cells.
Design: Case-control study.
Setting: Two tertiary hospitals.
Patient(s): Two independent cohorts of fertile (n = 18 and n = 17) and infertile (n = 31 and n = 35) women.
Intervention(s): In vitro fertilization.
Main Outcome Measure(s): Gene expression levels of ferritin light chain (*FTL*), ferritin heavy chain (*FTH*), transferrin receptor (*TFRC*), and ferroportin (*SLC40A1*) mRNA were analyzed in granulosa and cervical cells.
Result(s): In the first cohort, fertile and infertile women were similar in body mass index. Ferroportin mRNA levels were decreased in granulosa cells from infertile women in parallel with increased serum hepcidin levels. A positive association between ferroportin and *TFRC* mRNA, a gene associated with intracellular iron deficiency, was observed only in granulosa cells from fertile women. The major findings were replicated in a second independent cohort.
Conclusion(s): Ferroportin mRNAs and circulating hepcidin identify a subset of infertile women and may constitute a target for therapy. (Fertil Steril® 2017;107:236–42. ©2016 by American Society for Reproductive Medicine.)
Key Words: Ferroportin, ferritin, ovarian granulosa cells, uterine cervical cells, infertility, sterility, in vitro fertilization

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/12569-22718

Reactive oxygen species (ROS) are produced in humans under physiologic conditions. Free radicals play a key role in the origin of life and biologic evolution. ROS are involved in physiologic processes linked to female reproduction, such as folliculogenesis, oocyte maturation, ovulation, corpus luteum formation, endometrial cycle, luteolysis, implantation, embryogenesis, and pregnancy (1). Imbalance in homeostatic control of ROS exposure causes oxidative stress, which can affect fertilization and induce apoptosis.

The importance of iron-induced ROS production and lipid peroxidation in the pathophysiology of male infertility has been documented (2). Uncomplexed iron together with superoxide, which reduces Fe^{3+} and hydrogen peroxide, provides a lethal mixture

Received July 4, 2016; revised September 27, 2016; accepted October 6, 2016; published online November 16, 2016.
J.M.M.-N. and E.L.-N. should be considered similar in author order.
J.M.M.-N. has nothing to disclose. E.L.-N. has nothing to disclose. L.C. has nothing to disclose. F.P. has nothing to disclose. F.J.O. has nothing to disclose. M.S.-M. has nothing to disclose. M.F.-S. has nothing to disclose. V.B. has nothing to disclose. A.R.-R. has nothing to disclose. M.F. has nothing to disclose. W.R. has nothing to disclose. M.T.-S. has nothing to disclose. J.M.F.-R. has nothing to disclose.
Supported in part by research grant FIS PI15/01934 from the Instituto de Salud Carlos III Spain, Fondo Europeo de Desarrollo Regional, Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y la Nutrición and projects BFI2014-58571-P and PIE14/00005 (to M.T.-S.); and Institut d'Investigació Biomèdica de Girona, which had no any role in the study's design, conduct, or reporting.
Reprint requests: José M. Fernández-Real, M.D., Ph.D., Institut d'Investigació Biomèdica de Girona (IdIBGi), Section of Diabetes, Endocrinology and Nutrition (UDEN), Hospital "Dr. Josep Trueta" of Girona, Avinguda de França s/n, Girona 17007, Spain (E-mail: jmfreal@idibgi.org).

Fertility and Sterility® Vol. 107, No. 1, January 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.10.008

containing ROS that can directly damage DNA, lipids, and proteins (3). Very recently, high seminal iron was found in patients with transfusion-dependent thalassemia associated with azoospermia or oligospermia (4). Elevated systemic non–transferrin-bound iron and labile plasma iron, together with low seminal glutathione, in these patients, which correlate with sperm motility, strongly suggest that iron-induced oxidative stress may have a major role in subfertility (4).

Despite this evidence, the potential role of iron excess in female infertility has been less well studied. In women with thalassemia major, infertility seems to be attributed to iron deposition and iron-induced oxidative stress in various endocrine organs, such as the hypothalamus, pituitary, and female reproductive system (5). Given the pathophysiologic role of iron in women with β-thalassemia, endometriosis, and polycystic ovary syndrome (PCOS) (6–8), we designed the present observational study to test the hypothesis that the expression of iron-related genes might be deregulated in women with infertility.

MATERIALS AND METHODS

Cohort 1: Discovery

Follicular fluid and granulosa cells were collected from the ovulatory follicles of 49 euthyroid women, aged 22–38 years, undergoing oocyte retrieval at Hospital Universitari Dr. Josep Trueta and at Gynecologic Clinic Girexx (Girona, Spain). Thirty-one were infertile (unexplained primary infertility and no previous endocrine diseases or previous infertility treatments), and 18 were fertile women (as demonstrated by a history of at least one previous pregnancy or success of subsequent egg donation program) who voluntarily donated eggs.

All women were subjected to a standard short antagonist protocol with the use of subcutaneous gonadotropins and received 6,500 UI choriogonadotropin alpha or 0.2 mg triptorelin as an ovulation inductor when the leading follicle/s exceeded 16 mm in diameter. Granulosa cells and follicular fluids were harvested by means of ultrasound-guided transvaginal follicular aspiration 34–36 hours later. While the woman was under anesthesia, exocervical cytology was performed. After isolation of oocytes, follicular fluid containing granulosa cells and cervical cells suspended in physiologic saline solution were centrifuged at 1,000*g* for 3 minutes at room temperature. Cell samples were collected as a pellet. All samples were frozen at −80°C.

Every subject gave written informed consent after the purpose of the study was explained to her. The Institutional Review Board approved the protocol, so we certify that all applicable institutional regulations concerning the ethical use of information and samples from human volunteers were followed during this research.

Cohort 2: Replication

Follicular fluid and granulosa cells were collected from the ovulatory follicles of 52 euthyroid women, aged 19–43 years, undergoing oocyte retrieval at IVI Center for Reproductive Care (Seville, Spain). Thirty-five were infertile (unexplained primary infertility and no previous endocrine diseases, except for four women who had polycystic ovary syndrome and nine who had endometriosis), and 17 were healthy oocyte donors whose fertility was evidenced by the success of subsequent egg donation programs.

Women were subjected to a standard short antagonist protocol (n = 46) or to a long agonist protocol (n = 6 infertile patients) with the use of subcutaneous gonadotropins, and received 6,500 UI choriogonadotropin alfa or 0.2 mg triptorelin as an ovulation inductor when the leading follicle/s exceeded 16 mm in diameter. Granulosa cells and follicular fluids were obtained by means of ultrasound-guided transvaginal follicular aspiration 34–36 hours later.

After isolation of oocytes, the follicular fluids were pooled and granulosa cells collected with the use of the Dynabeads methodology as described previously (9). Briefly, granulosa cells were separated from erythrocytes by means of density-gradient centrifugation with the use of Histopaque 1077 (Sigma). The middle layer was collected and resuspended in red blood cell lysing buffer (Hybri-max; Sigma). Granulosa cells were then recovered and incubated with magnetic beads coated with monoclonal antihuman CD45 antibody (Dynabeads pan mouse IgG; Invitrogen). The plastic tube containing the mixture was placed next to a fixed magnet and the unlabeled granulosa cells collected. All samples were frozen at −80°C.

Approval for the use of granulosa cells was obtained from the Institutional Ethics Committees of Consejo Superior de Investigaciones Cientificas and Hospital Virgen Macarena (Seville, Spain), and every patient gave informed written consent.

Analytic Determinations

After 8 hours of fasting, blood was obtained for measurement of serum lipids and glucose in the discovery cohort (cohort 1). Serum glucose concentrations were measured in duplicate by means of the glucose oxidase method with the use of a Beckman Glucose Analyzer II. We used a Roche Hitachi Cobas c711 instrument to do the determinations. Total serum cholesterol was measured by means of an enzymatic colorimetric method through the cholesterol esterase/cholesterol oxidase/peroxidase reaction (Cobas Chol2). High-density lipoprotein cholesterol was quantified by means of a homogeneous enzymatic colorimetric assay through the cholesterol esterase/cholesterol oxidase/peroxidase reaction (Cobas HDLC3). Total serum triglycerides were measured by means of an enzymatic colorimetric method with the use of glycerol phosphate oxidase and peroxidase (Cobas Trigl). Low-density lipoprotein (LDL) cholesterol was calculated with the use of the Friedewald formula. Serum iron also was determined on a Roche Hitachi Cobas c711 by means of a colorimetric assay.

Serum ferritin was determined in patients from the discovery cohort with the use of a microparticle enzyme immunoassay (Axsym; Abbot Laboratories), with an intra- and interassay coefficient of variation <6%. Circulating hepcidin levels in serum were measured by a solid-phase ELISA (DRG Hepcidin 25 [Bioactive], EIA-5258; DRG International). Detection limit was 0.35 ng/mL. Intra- and

interassay coefficients of variation were 5%–15%. Analytic determinations and blood tests were not performed in cohort 2.

RNA Extraction

Total RNA was extracted and purified with the use of the RNeasy Mini Kit (Qiagen). Thawed pellets were homogenized in 0.6 mL Qiazol Lysis Reagent (Qiagen), a monophasic solution of phenol and guanidine thiocyanate which facilitates sample disaggregation and inhibits RNases. After addition of chloroform (0.4 volumes), the homogenate was separated into aqueous and organic phases by centrifugation (15 minutes at 12,000*g* and 4°C). Then the upper aqueous RNA-rich phase was isolated and ethanol absolute (1.5 volumes) was added to provide appropriate binding conditions for RNA. The solution was then applied to the RNeasy Mini spin column (Qiagen), where RNA binds to the membrane while phenols and other compounds are washed away. High-quality RNA was finally eluted in 30 μL RNase-free water. Final RNA concentrations were assessed with the use of a Nanodrop ND-1000 Spectrophotometer (Thermo Fischer Scientific). The integrity was checked by means of the Nano lab-on-a-chip assay for total eukaryotic RNA with the use of Bioanalyzer 2100 (Agilent Technologies). In the validation cohort, total RNA was extracted with the use of Trireagent (Sigma) or the RNA/Protein purification kit (Norgen Biotek), and residual genomic DNA was removed with the use of RNase-free DNase I and RNasin (Promega).

Retrotranscription and Real-Time Polymerase Chain Reaction

The expression of genes codifying for iron-related genes (ferritin heavy chain [*FTH*], ferritin light chain [*FTL*], ferroportin [*SLC40A1*], and transferrin receptor [*TFRC*]) was assessed in granulosa and cervical cells from 18 fertile and 31 infertile women (cohort 1) and in granulosa cells from 17 fertile and 35 infertile women (cohort 2). Three micrograms total RNA was reverse transcribed to cDNA with the use of the High Capacity cDNA Archive Kit (Applied Biosystems) according to the manufacturers' protocol. Commercially available and prevalidated Taqman primer/probe sets were used for gene expression determinations (Applied Biosystems). Expression was assessed by means of real-time polymerase chain reaction with the use of the Lightcycler 480 Real-Time PCR System (Roche Diagnostics) or the Bio-Rad iCycler iQ real-time detection apparatus (Bio-Rad Laboratories) along with Taqman technology suitable for relative gene expression quantification. The reaction was performed in a final volume of 12.5 μL. The cycle program consisted of an initial denaturing of 10 minutes at 95°C then 40 cycles of 15 seconds denaturizing phase at 95°C and 1 minute annealing and extension phase at 60°C. A threshold cycle (Ct value) was obtained for each amplification curve and a ΔCt value was first calculated by subtracting the Ct value for human cyclophilin A (*PPIA*) RNA from the Ct value for each sample. Fold changes compared with the endogenous control were then determined by calculating $2^{-\Delta Ct}$, so gene expression results are expressed as expression ratio relative to PPIA gene expression. Replicates and positive and negative

TABLE 1

Main characteristics and relative expression (relative units [RU]) in granulosa and cervical cells from fertile and infertile women in the discovery cohort, expressed as a ratio relative to endogenous control (cyclophilin A [*PPIA*]).

Variable	Fertile	Infertile	*P* value (Student)
Main characteristic			
n	18	31	
Age (y)	26.82 ± 4.12	33.64 ± 3.20	<.0001[a]
BMI (kg/m^2)	22.93 ± 3.24	22.41 ± 3.01	.542
Smokers (%)	63	27	.01[a]
Fasting glucose (mg/dL)	83 ± 6.3	85.8 ± 7.8	.14
Total cholesterol (mg/dL)	162.7 ± 20.5	181.4 ± 29.1	.006[a]
LDL-cholesterol (mg/dL)	93.4 ± 21	107.4 ± 26.3	.03[a]
HDL-cholesterol (mg/dL)	54.7 ± 12.2	58.7 ± 12.7	.23
Fasting triglycerides (mg/dL)	73.2 ± 26.2	76.3 ± 35.8	.72
Serum ferritin (ng/mL)	33.7 ± 20.5	46.1 ± 32.4	.1
Serum hepcidin (ng/mL)	7.5 ± 3.4	11.3 ± 6.2	.004[a]
Granulosa cells			
FTH mRNA (RU)	0.0135 (0.0073–0.0345)	0.0111 (0.0065–0.0171)	.567
FTL mRNA (RU)	3.5 (1.5–9.2)	1.5 (1.01–3.2)	.446
TFRC mRNA (RU)	0.0352 (0.0234–0.045)	0.0486 (0.0288–0.0683)	.560
SLC40A1 mRNA (RU)	0.0194 (0.0108–0.0393)	0.0061 (0.0032–0.0095)	.031[a]
Cervical cells			
FTH mRNA (RU)	0.768 (0.325–1.23)	1.09 (0.39–1.79)	.222
FTL mRNA (RU)	39.2 (19.6–63.2)	34.5 (20.5–54.7)	.581
TFRC mRNA (RU)	0.111 (0.076–0.222)	0.0693 (0.0364–0.1582)	.064
SLC40A1 mRNA (RU)	0.0819 (0.0613–0.137)	0.0436 (0.0178–0.0656)	.001[a]

Note: Data are expressed as mean ± SD or median (interquartile range). FTH = ferritin heavy chain; FTL = ferritin light chain; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SLC40A1 = ferroportin; TFRC = transferrin receptor.
[a] Significant difference.

Moreno-Navarrete. Iron and infertility. Fertil Steril 2016.

control samples were included. The following commercially available and prevalidated Taqman primer/probes sets were used: *FTH*, Hs01694011_s1; *FTL*, Hs00830226_gH; *TFRC*, Hs00951083_m1; *SLC40A1*, Hs00205888_m1; and *PPIA*, Hs99999904_m1.

Statistical Analyses

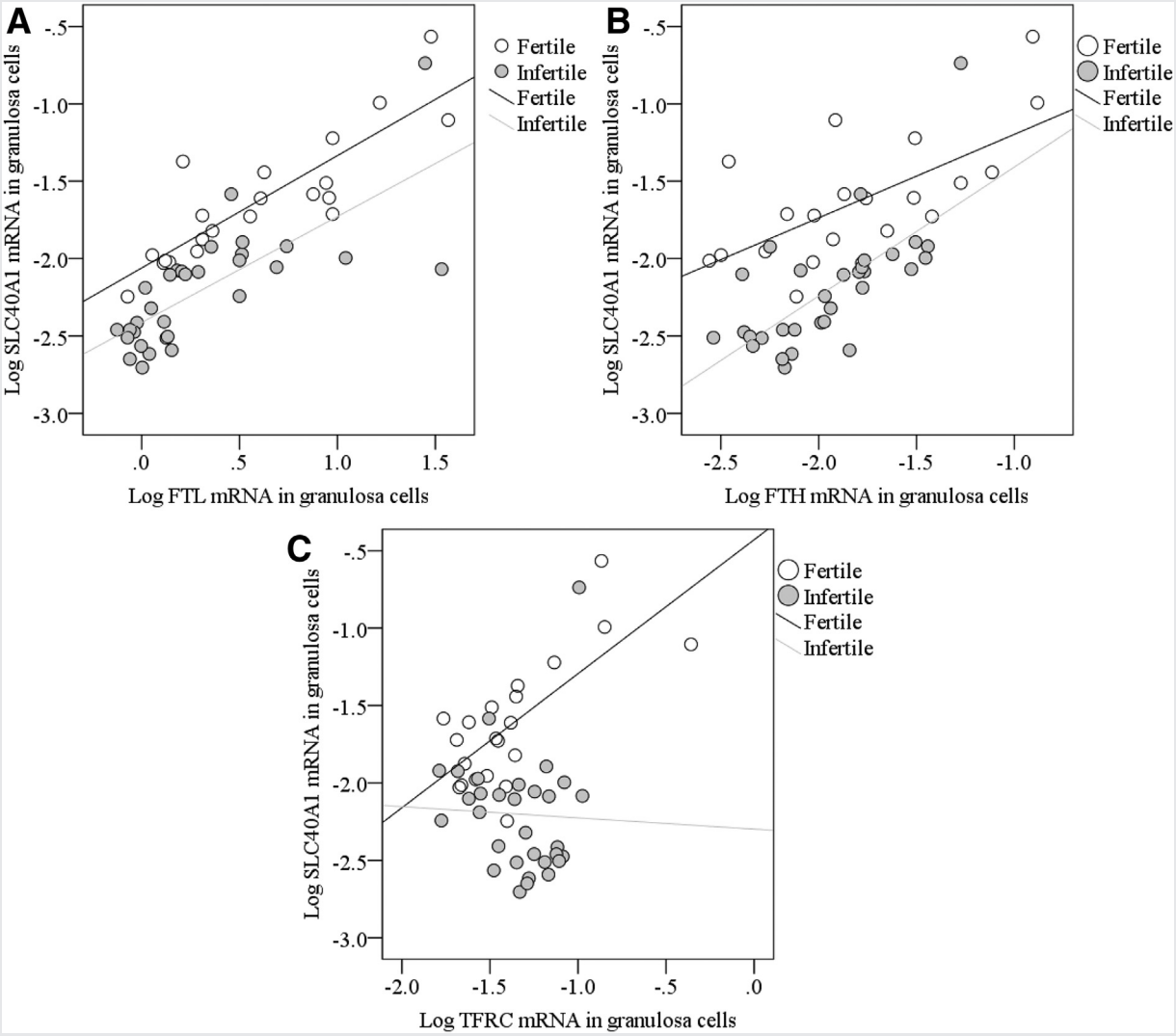
Descriptive results of continuous variables are expressed as mean ± SD or median (interquartile range). Before statistical analysis, normal distribution and homogeneity of the variances were evaluated with the use of the Levene test. Variables were given a base log10 transformation when necessary. Those parameters were analyzed and tested for significance on that logarithmic scale. The antilog-transformed values of the means (geometric means) are reported in the tables. The relation between variables was tested with the use of the Pearson test. Multiple linear regression analyses (enter method) controlling for confounding variables were also performed. The statistical analyses were performed with the use of the SPSS program (version 13.0).

RESULTS

Cohort 1

Subjects' characteristics are described in Table 1. Both groups were similar in body mass index and serum ferritin levels. Infertile women were significantly older and less frequently smokers (Table 1). Age-adjusted serum cholesterol and LDL concentrations were not significantly different between groups. Expression of all of the examined genes was not significantly influenced by the type of ovulation inductor administered.

FIGURE 1



Linear regression analysis of the association between ferroportin (*SLC40A1* mRNA) and (**A**) ferritin light chain (*FTL* mRNA), (**B**) ferritin heavy chain (*FTH* mRNA), and (**C**) transferrin receptor (*TFRC* mRNA) in granulosa cells from infertile and fertile women.

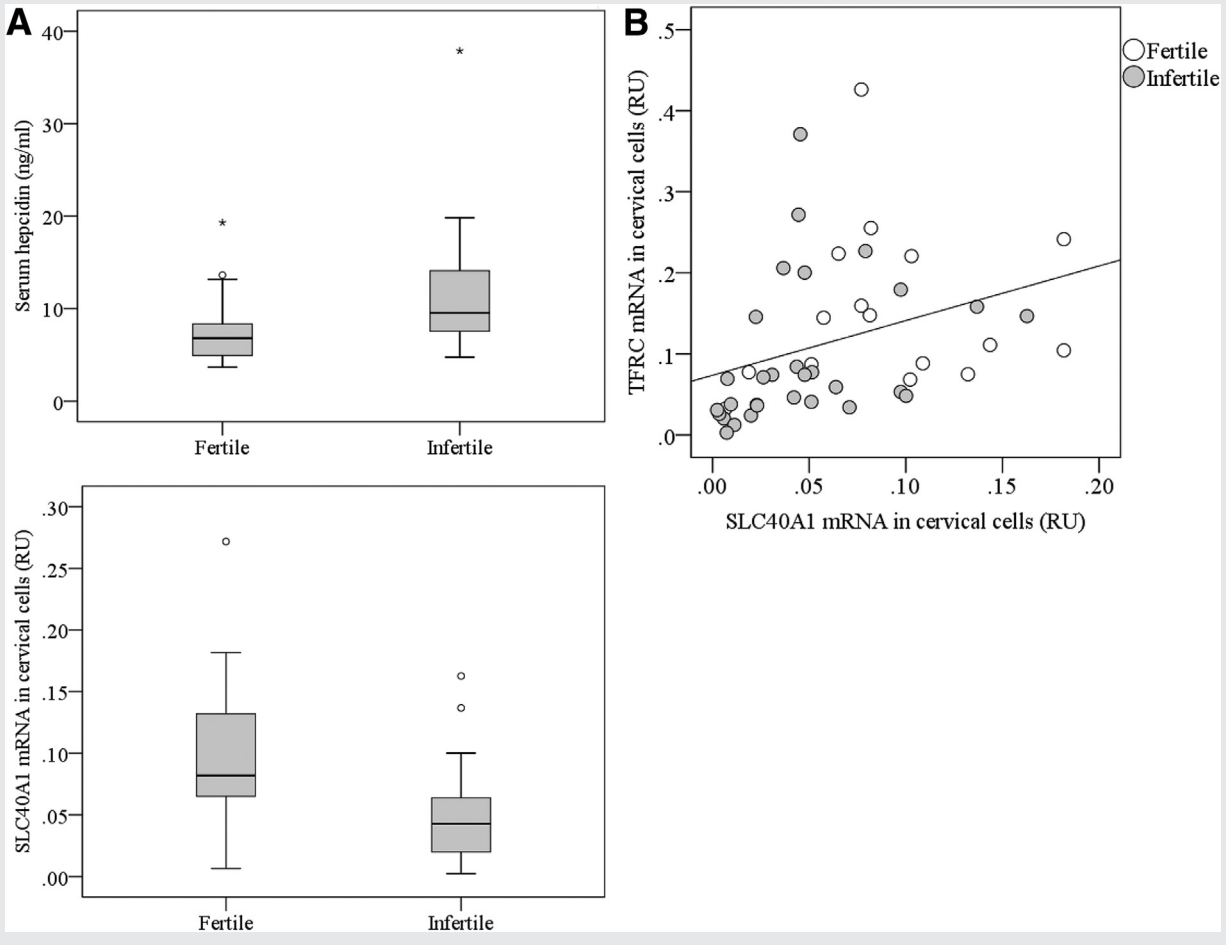
Moreno-Navarrete. Iron and infertility. Fertil Steril 2016.

The gene expression of different iron-related genes (*FTH*, *FTL*, and *TFRC*) was similar in granulosa cells from both groups (Table 1). Ferroportin (*SLC40A1*) mRNA levels were down-regulated in granulosa cells from infertile women (Table 1). In a multiple linear regression analyses, being infertile was independently associated with lower ferroportin mRNA levels ($\beta = -0.53$; $P=.002$) after adjusting for age and smoking status, contributing to 34.3% of its variance. Reinforcing these findings, circulating hepcidin levels were significantly increased among infertile women (Table 1). Circulating hepcidin correlated positively with serum ferritin in both fertile ($r = 0.58$; $P=.003$) and infertile women ($r = 0.77$; $P<.0001$). However, the relationship between hepcidin and *FTH* mRNA expression in granulosa cells was significant only among infertile women ($r = 0.41$; $P=.021$) and not in control women ($r = -0.11$; $P=.66$).

Ferroportin mRNA was positively associated with both *FTL* and *FTH* mRNA levels in fertile and infertile women (Fig. 1A and B), suggesting that intracellular iron evoked a compensatory ferroportin response to extrude iron from the cell. However, this response seemed to be efficient only in fertile women, given the positive association with *TFRC* mRNA, a gene associated with intracellular iron deficiency (Fig. 1C). The association remained significant after controlling for age and smoking status in a multiple linear regression analyses ($P=.028$). In contrast, this relationship was absent in infertile women (Fig. 1C).

We also investigated whether the expression of ferroportin was down-regulated in other more accessible cells. Importantly, we found that age- and smoking status–adjusted ferroportin mRNA was also down-regulated in cervical cells from infertile women (Fig. 2A). The functionality of this mRNA in cervical cells was suggested by the positive association with *TFRC* mRNA levels (Fig. 2B): the higher the ferroportin, the higher the iron export leading to intracellular ferropenia and increased *TFRC* mRNA levels. Again, the relationship persisted in significance after controlling for age and smoking status.

FIGURE 2



(**A**) Boxplots showing the medians and interquartile ranges of circulating hepcidin and ferroportin (*SLC40A1*) mRNA levels in cervical cells from infertile and fertile women. (**B**) Linear regression analysis of the association between *SLC40A1* mRNA and *TFRC* mRNA in cervical cells from infertile and fertile women. *Outlier.

Moreno-Navarrete. Iron and infertility. Fertil Steril 2016.

TABLE 2

Main characteristics and relative expression in granulosa cells from fertile and infertile women in the replication cohort, expressed as a ratio relative to endogenous control (cyclophilin A [*PPIA*]).

Variable	Fertile	Infertile	*P* value (Student)
Main characteristic			
n	17	35	
Age (y)	24.82 ± 3.28	37.14 ± 3.63	< .0001[a]
BMI (kg/m^2)	22.85 ± 4.02	22.34 ± 5.06	.542
Granulosa cells			
FTH mRNA (RU)	0.044 (0.027–0.069)	0.014 (0.009–0.0179)	< .0001[a]
FTL mRNA (RU)	2.15 (1.79–3.14)	2.11 (1.51–2.81)	.620
TFRC mRNA (RU)	0.121 (0.043–0.193)	0.017 (0.011–0.035)	< .0001[a]
SLC40A1 mRNA (RU)	0.029 (0.021–0.052)	0.007 (0.004–0.012)	< .0001[a]

Note: Data are expressed as mean ± SD or as median (interquartile range). Abbreviations as in Table 1. FTH = ferritin heavy chain; FTL = ferritin light chain; SLC40A1 = ferroportin; TFRC = transferrin receptor.
[a] Significant difference.

Moreno-Navarrete. Iron and infertility. Fertil Steril 2016.

Cohort 2

To strengthen these associations, the same analysis was performed in granulosa cells from an independent cohort (cohort 2). Of note, granulosa cells from cohort 2 were purified with the use of Dynabeads, an accurate methodology to increase the purity. The analysis of these gene expressions in cohort 2 confirmed reduced ferroportin mRNA levels in granulosa cells from infertile women (Table 2). Ferroportin mRNA levels were positively correlated with markers of intracellular iron excess (*FTL* [$r = 0.28$; $P=.04$] and *FTH* [$r = 0.49$; $P<.0001$]). In multiple linear regression analysis, being infertile (28.2%; $\beta = -0.52$; $P=.02$) contributed to ferroportin mRNA level variance after adjusting for age.

DISCUSSION

We found that ferroportin mRNA was down-regulated in granulosa and cervical cells from infertile women. This decrease persisted in significance after adjusting for age and smoking status in a multiple linear regression analysis. Although the mechanistic implication of this finding remains to be solved, several observations indicate that low ferroportin mRNA levels are a marker for disturbed iron metabolism linked to infertility status:

1) Ferroportin mRNA ran in parallel with both *FTH* and *FTL* mRNA in granulosa cells from fertile and infertile women. This finding suggests that up-regulation of ferroportin mRNA is the normal physiologic response to raised intracellular iron, resulting in increased *FTH* and *FTL* mRNA transcription. However, this up-regulation in infertile women did not reach the levels observed in fertile women at each level of *FTH* and *FTL* mRNAs (Fig. 1A and B).
2) Relatively low ferroportin mRNA levels were not associated with *TFRC* mRNA in granulosa cells from infertile women (Fig. 1C). In contrast, the relationship between ferroportin mRNA and *TFRC* mRNA was strong in fertile women (Fig. 1C). Because *TFRC* mRNA is usually a surrogate marker of intracellular iron (3), being up-regulated with intracellular iron deficiency, the positive relationship between ferroportin and *TFRC* mRNAs suggests that increased ferroportin leads to iron export, intracellular ferropenia, and therefore raised *TFRC* mRNA in granulosa cells from fertile women. This was not the case in granulosa cells from infertile women.
3) Finally, some of the findings were also confirmed in cervical cells, which are more accessible than granulosa cells. Decreased ferroportin mRNA was identified in cervical cells from infertile women (Fig. 2A). As in granulosa cells, ferroportin mRNA was positively associated with *TFRC* mRNA levels, suggesting that ferroportin was functional (Fig. 2B). The present data suggest that the measurement of ferroportin gene expression in these cells could aid in the diagnosis of infertility.

In this study, granulosa cells were obtained by means of different methods in two different clinical settings (public hospital and private IVF clinic). In one, we purified granulosa cells with the use of Dynabeads to eliminate white blood cells, whereas in the other we only centrifuged cells and studied the pellet. The methods for RNA extraction and quantification also were different. Even taking into account that the populations of cells studied were different, as well as the different methodologies, the findings were quite similar. The fact that similar results were obtained in both cohorts reinforces the validity of our observations and strongly argues for a general role of deregulated iron homeostasis in infertility, independently from its underlying cause.

The hormone hepcidin binds to ferroportin and induces its degradation, thus regulating iron homeostasis. In fetal and neonatal periods, because of the red cell compartment's rapid growth and expansion, hepcidin gene expression is drastically repressed (10). Current findings point toward the importance of hepcidin repression even before embryo formation, suggesting a possible hepcidin role in oocyte maturation and embryogenesis. In the present study, circulating hepcidin levels were significantly increased among infertile women, further suggesting the existence of a relationship between altered iron homeostasis and infertility.

In summary, evaluation of circulating hepcidin and ferroportin mRNA in granulosa and cervical cells could aid in the identification of infertile women and may constitute a target for therapy.

Acknowledgments: The authors acknowledge the technical assistance of E. Loshuertos and O. Rovira, both from Endocrinology, Institut d'Investigació Biomèdica de Girona.

REFERENCES

1. Agarwal A, Gupta S, Sekhon L, Shah R. Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications. Antioxid Redox Signal 2008;10:1375–403.
2. Aitken RJ, Harkiss D, Buckingham D. Relationship between iron-catalysed lipid peroxidation potential and human sperm function. J Reprod Fertil 1993;98:257–65.
3. Fernández-Real JM, Manco M. Effects of iron overload on chronic metabolic diseases. Lancet Diabetes Endocrinol 2014;2:513–26.
4. Singer ST, Killilea D, Suh JH, Wang ZJ, Yuan Q, Ivani K, et al. Fertility in transfusion-dependent thalassemia men: effects of iron burden on the reproductive axis. Am J Hematol 2015;90:E190–2.
5. Roussou P, Tsagarakis NJ, Kountouras D, Livadas S, Diamanti-Kandarakis E. Beta-thalassemia major and female fertility: the role of iron and iron-induced oxidative stress. Anemia 2013;2013:617204.
6. Yamaguchi K, Mandai M, Toyokuni S, Hamanishi J, Higuchi T, Takakura K, et al. Contents of endometriotic cysts, especially the high concentration of free iron, are a possible cause of carcinogenesis in the cysts through the iron-induced persistent oxidative stress. Clin Cancer Res 2008;14:32–40.
7. Sanchez AM, Papaleo E, Corti L, Santambrogio P, Levi S, Viganò P, et al. Iron availability is increased in individual human ovarian follicles in close proximity to an endometrioma compared with distal ones. Hum Reprod 2014;29:577–83.
8. Escobar-Morreale HF. Iron metabolism and the polycystic ovary syndrome. Trends Endocrinol Metab 2012;23:509–15.
9. García-Ortega J, Pinto FM, Fernández-Sánchez M, Prados N, Cejudo-Román A, Almeida TA, et al. Expression of neurokinin B/NK3 receptor and kisspeptin/KISS1 receptor in human granulosa cells. Hum Reprod 2014;29:2736–46.
10. Nicolas G, Bennoun M, Porteu A, Mativet S, Beaumont C, Grandchamp B, et al. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proc Natl Acad Sci U S A 2002;99:4596–601.