Thyroid hormones in ovarian follicular fluid: Association with oocyte retrieval in women undergoing assisted fertilization procedures

Mónica Rosales1,2, Myriam Nuñez3, Andrea Abdala4, Viviana Mesch1,2*, Gabriela Mendeluk2,5*

1 Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica, Cátedra de Bioquímica Clínica I, Laboratorio de Endocrinología. Buenos Aires, Argentina.
2 Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires, Argentina.
3 Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Matemática. Buenos Aires, Argentina.
4 Universidad de Buenos Aires. Hospital de Clínicas "José de San Martín". División Ginecología. Buenos Aires, Argentina.
5 Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Departamento de Bioquímica Clínica II, Laboratorio de Fertilidad Masculina. Buenos Aires, Argentina.

* These authors equally contributed to this work

ABSTRACT

Objective: Our aim was to analyze the role of thyroid hormones in follicular fluid (FF) in relation to the number of oocytes retrieved in women recruited for an assisted fertilization procedure.

Methods: Retrospective cohort study of 51 women 37.5±3.3 years, range 29-42, evaluated after a controlled ovarian stimulation protocol in a University Hospital. FF was sampled by transvaginal ultrasound-guided aspiration after ovarian hyperstimulation and we measured T3 (T3f), T4 (T4f), TSH (TSHf) and free T4 (T4ff). The oocyte maturation rate was calculated as: Number of metaphase II oocytes/Number of oocytes retrieved x 100. Statistical analysis was performed using the SPSS-19 software.

Results: Hormone levels in FF were: TSHf 1.3µIU/ml (0.4 - 2.7), T3f: 1.52±0.46 nmol/L, T4f 88.8±30.9nmol/L and T4ff: 15.44±2.57pmol/L. The number of oocytes recovered was dependent onT4f following the equation: Log (oocyte) = 0.379+0.042*T4f (r:0.352, p=0.012). After a logistic regression model analysis, T3f showed a tendency to be associated with the OMR: OR (95% CI)= 0.977 (0.954 to 1.001), p=0.057.

Conclusions: The correlation found between thyroid hormones and the number of oocytes retrieved suggests an interaction between thyroid and gonadal axes in relation to follicular development.

Keywords: thyroid hormones, follicular fluid, oocyte, assisted fertilization procedures, follicular development, oocyte maturation

INTRODUCTION

Assisted reproductive technology (ART) has grown by leaps and bounds in recent decades; the optimization of these procedures comes from basic reproduction physiology knowledge (Ishihara et al., 2015). In this sense the gonadal axis is explored to adjust the optimal stimulation schemes in assisted reproduction programs. However, the role of the thyroid axis in this process has been less studied, despite large evidence that emphasizes its importance in natural fertility (Vissenberg et al., 2015; Alemu et al., 2016).

In brief, thyroid hormones play an important role in conception and pregnancy, and are essential for normal adult health, fetus and childhood development (Alexander et al., 2017; Yassaee et al., 2014). Alterations in thyroid physiology can lead to menstrual irregularities, ovulation disturbances and therefore, reduced possibilities of a successful pregnancy (Vissenberg et al., 2015). However, their physiological mechanism in fertilization is not elucidated yet.

Many studies have shown an association between maternal hypothyroidism with obstetric complications and/or psychomotor impairment in the offspring (Committee of the American Society for Reproductive Medicine, 2015; Maraka et al., 2017). Although there is only limited evidence on the possible positive effects of T4 treatment in such cases, there is widespread agreement among clinicians about the need for treatment of clinical hypothyroidism during pregnancy (Velasco & Taylor, 2018). Oocyte maturation and embryo development are controlled by hormones as well as intra-ovarian factors such as cytokines and growth factors. In assisted reproduction the number of mature oocytes retrieved is a key point (Milachich & Shterev, 2016), although oocyte quality is more important than its quantity (Verberg et al., 2009).

It is well known that spermatozoa exposure to FF favors the acrosomal reaction, its motility and ability to penetrate the ovum (De Jonge, 2017). In this sense, we have previously reported that in vitro addition of T4 stimulates sperm hyperactive movement, increasing the recovery rate after enrichment techniques like “swim up” (Mendeluk & Rosales, 2016). We were interested in evaluating the role of thyroid hormones in the female reproductive tract.

Several isoforms of thyroid hormone receptors mRNA were described in 1997 to be expressed in human oocytes, suggesting a probable hormonal direct effect either on the oocyte per se as well as on the granulose cells (Zhang et al., 1997). In an indirect way, the effect on cumulus cells may affect the oocytes as well. Recently, the enzyme involved in thyroid hormones biosynthesis, thyroid peroxidase, was revealed for the first time in granulose cells, supporting the hypothesis that the ovarian follicle is an independent thyroid hormone producing unit (Monteleone et al., 2017). Although iodine concentration in thyroid gland is higher than in other organs, ovarian uptake and buildup was also described. The physiological importance of this process is not yet completely known.

The process of oocyte maturation, a key point in fertilization, is highly related to its environment, the FF (Chang et al., 2016). By studying its composition, important information may be obtained to clarify the whole process. Our aim was to analyze the role of thyroid hormones in FF in relation to the number of oocytes retrieved in women recruited for an assisted fertilization procedure.
MATERIALS AND METHODS

In a retrospective cohort study, we included 51 women of 37.5±3.3 years of age, ranging between 29-42; 11 women≤35 years, 24 between 35 and 39 years and 16≥40 years. All of them were evaluated after a controlled ovarian stimulation protocol at the Gynecology Division, Hospital de Clínicas "José de San Martín". Universidad de Buenos Aires, Argentina. The inclusion criteria were: woman with infertility for more than 12 months before being included in the study, having regular menstrual cycles of 24-35 days, presumably ovulating, ultrasound visualization of both ovaries without evidence of abnormalities in their first treatment cycle, with FSH serum levels in the early follicular phase lower than 12 IU/l, antral follicle count (diameter 2-10 mm) greater than 2 for both ovaries, with no endometriosis nor diseases of genetic origin, and body mass index between 18-25kg/m². To be included in the study, the women should have serum TSH levels and antithyroidperoxidase antibodies within the reference range. Women with endocrine diseases, autoimmunity or medication affecting thyroid function were excluded. The study was approved by the Institutional Review Board of the Hospital. Informed consent was obtained from all individual participants included in the study.

Ovarian reserve was evaluated in all patients through anti-Müllerian hormone (AMH) levels and antral follicle count by ultrasound. Only one cycle per woman was selected for this study. The women were scheduled for controlled ovarian stimulation with three different protocols: recombinant human FSH (rhFSH) only (150-300 UI); rhFSH plus recombinant LH (75 UI) and rhFSH plus human menopausal gonadotrophin (HMG, 75-150UI). In all cases, the hormones were administered subcutaneously, daily from day 2 of the cycle. Treatments lasted between 7 and 10 days. In order to avoid endogenous LH peaks, the women received a GnRH antagonist (0.25 mg) from day 7 (± 1) of the menstrual cycle until ovulation induction. Human chorionic gonadotrophin (hCG 10000 UI) was administered subcutaneously to induce ovulation. Oocyte retrieval was performed 36 hours after hCG administration. ICSI was performed in all cases.

FF was sampled and a transvaginal ultrasound-guided aspiration of the hyperstimulated ovary was performed; each follicle was individually aspirated and collected. For each follicle, the presence or absence of an egg was recorded immediately under a stereoscope and the residual follicular fluid was placed into a 15 ml sterile Falcon conical tube. The FF was cleared by centrifugation at room temperature for 10 minutes at 300× g, aliquoted and placed at -80°C for later analysis. The remnant FF collected was thawed and T3 (T3f), T4 (T4f), TSH (TSHf) and free T4 (T4ff) were measured using chemiluminescence immunoassay on Advia Centaur XP autoanalyzer. All oocytes retrieved were evaluated to analyze the complex cumulus corona expansion degree and the oocytes maturational stage was determined after denudation of oocytes by enzymatic and mechanical methods. Only those with a visible polar body were classified as mature or in metaphase II (MII). The oocyte maturation rate was calculated as: Number of metaphase II oocytes/Number of oocytes retrieved x 100. We employed a logistic regression model to determine whether a relationship exists between OMR and independent variables: T3f, T4f, TSHf, and T4ff. The response variable was coded considering an OMR cutoff value ≥ 60.

Statistical Analysis

We ran the statistical analysis using the SPSS-19 software, considering values of p<0.05 statistically significant. The results are expressed as mean±SD or median (range) according to data distribution. The differences among treatment groups were assessed by Kruskal-Wallis ANOVA. The number of oocytes retrieved in relation to the different variables (T3f, T4f, TSHf, and T4ff) was evaluated by multiple regression analysis. We used logistic regression to determine if any of the hormones tested was associated with OMR.

RESULTS

Table 1 shows women’s mean ages, treatment used for each group of patients, the number of oocytes retrieved, the number of metaphase II oocytes and infertility etiology.

The number of oocytes retrieved and the number of oocytes in MII were not significantly different among the three treatments groups, neither among the three age groups: ≤35, 35-39 and ≥40 years (Kruskal-Wallis ANOVA).

Serum TSH levels were 1.8µIU/ml (0.4-4.0). Hormone levels in FF were: TSHf: 1.3µIU/ml (0.4 - 2.7), T3f: 1.52±0.46nmol/L, T4f: 88.8±30.9nmol/L and T4ff: 15.44±2.57pmol/L.

The number of oocytes recovered was 5 (0-18), and the number of oocytes in metaphase II was 3 (0-12), in both cases the data are expressed as median (range).

There was only one cancellation in which case no oocytes were recovered. In order to determine if there is any relation among the number of oocytes retrieved and the following independent variables: T3f, T4f, TSHf, T4ff, we performed a multiple regression analysis. We found that the number of oocytes recovered was only dependent on

| Table 1. Characteristics of the studied population according to treatment and results obtained |
|-----------------|-----------------|-----------------|
| Treatment       | rhFSH n=11      | LH-rhFSH n=30   | HMG-rhFSH n=10 |
| Mean age (years)| 34.7±3.1 (29-39)| 38.1±3.2 (29-42)| 38.7±2.0 (34-41)|
| Male factor     | 5               | 3               | 2               |
| Tubal factor    | 1               | 10              | 1               |
| Decreased ovarian reserve | 1 | 0 | 0 |
| Mixed           | 4               | 15              | 6               |
| Idiopathic      | 0               | 2               | 1               |
| No of Oocytes retrieved | 6.9±3.8 (2-13) | 5.1±3.7 (0-18) | 5.4±2.9 (3-12) |
| No of Oocytes MII | 4.7±2.2 (2-8) | 3.2±2.4 (0-12) | 2.9±2.2 (0-7) |

rhFSH: recombinant human FSH; LH-rhFSH: rhFSH along with recombinant LH; HMG-rhFSH: rhFSH plus human menopausal gonadotrophin; MII: metaphase II oocytes.
T4f. As the assumption of normality was not met, a log-
arithmetic transformation of the dependent variable (log
(oocyte)) was carried out. The resultant equation was as
follows:

\[
\log (\text{oocyte}) = 0.379 + 0.042 \times \text{T4f} \quad (r: 0.352, p=0.012)
\]

The median of OMR was 66, ranging from 57 to 74. In order to evaluate if thyroid hormones were related with
OMR, we applied a Logistic Regression Model. T3f showed
a tendency to be related with the OMR: OR (95% CI) =
0.977 (0.954 to 1.001), \( p=0.057 \). No relationship between
OMR and T4f, TSHf and T4ff was found.

**DISCUSSION**

The thyroid axis is currently evaluated in women en-
tering a fertilization program, since an euthyroid state
is mandatory to reach successful outcomes. In this way,
hypothyroid women are supplemented with levothyroxine
in order to reach an euthyroid status. These arguments
explain the well-known idea that the hypothalamus-hy-
pophysis-gonadal axis plays a major role in fertilization,
while the thyroid axis has a facilitating one (Colicchia
et al., 2014). Less is known about the molecular mechanisms
involved in thyroid hormone actions in this process.

Thyroid hormones seemingly influence the maturation
of human oocytes (Vissenberg et al., 2015), their receptors
have been isolated in mural granulose and cumulus cells
and the mature oocyte of the human ovarian follicle (Xie
et al., 2010). Enzymes involved in the chain that regulate
the generation of thyroid hormones have also been found
in granulose cells (Monteleone et al., 2017). Many reports
show the presence of thyroid hormones and their receptors
in FF, stating that they would be involved in human endo-
metrial physiology through a probable positive role during
folliculogenesis and ovulation (Colicchia et al., 2014).

Knowledge about the influence of thyroid hormones on
reproduction is being applied to animal production. In this
sense, Costa et al. (2013) hypothesizes that T3 may have
a beneficial effect on the kinetics of embryo development
in bovines. In our study we demonstrated that T3f should
be a predictor of OMR≥60. This OMR cut-off value is which
in our experience has clinical value, although other authors
use slightly higher values (Abbara et al., 2018).

Recent studies have revealed that thyroid hormones al-
ter estrous cyclicity and antioestrogen status in the ovary
of the rat acting through the nitric oxide synthase signaling
pathway (Zheng et al., 2015; Wei et al., 2018). It was also
reported that ovarian follicles of the laying hen express
mRNAs of thyroid hormone-nuclear receptors, as well as
integrin (αVβ3) plasma membrane receptors, indicating a
genomic and non-genomic action of thyroid hormones in
the chicken ovary (Sechman, 2013).

Data reported in the literature support the idea that
thyroid hormones would play a direct role in ovulation, ear-
ly follicular development, differentiation and stimulation of
steroidogenic capacity of granulose cells (Vissenberg et al.,
2015). Thyroid hormones are considered biological amplifiers
of the gonadotropins stimulatory action. In combination with
FSH, thyroid hormones increase the proliferation and inhibit
the apoptosis of these cells by the PI3K/Akt pathway (Vis-
zenberg et al., 2015; Monteleone et al., 2017). Thyroid hor-
mones may play a key role in the regulation of reproductive
processes (Cedkóvá et al., 2012). Our study agrees with the
above statement through the analysis of human FF in order
to contribute to the knowledge about human ovarian function
and disorders related to the reproductive process.

Different results have been reported while compar-
ing thyroid hormone levels in serum and FF in humans
and animals. Wakim et al. (1993) found that T3 and T4
levels in FF were similar to serum values, with a positive
correlation between serum and FF T4 values in humans. In

turn, Sleskodziński (2005) refers lower values for T4 and
within the normal range or higher for T3 in serum vs. FF
in animals. FF values in our patients are similar to those
in serum concentrations. Although we could not measure
thyroid hormones in the serum, our results are in agree-
ment with those reported by Wakim et al. (1993) and
Cedkóvá et al. (2012).

According to the standards of our Hospital Ethics Com-
mittee, we can only obtain FF from infertile women who
enter an ART program, so we did not manage to get a
group of fertile women to compare with the infertile ones.
Nevertheless, we consider that this is not mandatory, tak-
ing into account that the aim of the study was to report our
findings concerning thyroid hormones in FF in association
with the number of oocytes retrieved in assisted fertili-
zation procedures.

One limitation of this study is the lack of serum T4
levels. However, due to clinical data and considering that
TSH levels and anti thyroperoxidase antibodies were within
the reference range, we assume euthyroid condition in all
women studied. We must also take into account the wide
range of patient’s ages that could be the cause of the large
variation observed in the mean number of recovered and
mature oocytes.

To our knowledge, our study is the first to report a
correlation between T4 in follicular fluid and the number
of oocytes retrieved in an assisted reproductive program,
based on a mathematical equation determined in our pop-
ulation, which reflects a biological event. This evidence
suggests an interaction between thyroid and gonadal axes,
in relation to follicular development and oocyte matura-
tion. Given that the critical events of oocyte and follicular
maturation take place in a follicular fluid environment, a
thorough identification of the specific components that are
involved in this process is mandatory. Prospective studies
with larger number of patients should be carried out to
check our results.

**ACKNOWLEDGMENTS**

We thank Dr. Patricia Maidana, Dr. Darío Jacobsen and
Dr. Mariel Cano, for assistance with hormone assessment
in the Instituto de Fisiopatología y Bioquímica Clínica (IN-
FIBIOC), Faculty of Pharmacy and Biochemistry, University
of Buenos Aires, and Ernesto Gomez Pasanante MD, Javier
Singla MD, Sergio Provenzano MD and Lucio Ratto MD for
clinical assistance of the patients recruited in the Unit of
Assisted Reproduction Gynecology Division, Tocoginecology
Department, Hospital de Clínicas José de San Martín.

This study was supported by Clinical Grants from the
University of Buenos Aires (UBACYT 009BA and UBACYT
003BA). It is a collaborative work between the Laboratory
of Male Fertility, the Laboratory of Endocrinology from the
University Clinical Hospital “José de San Martín”, Clinical
Biochemistry Department, INFIBIOC, Faculty of Pharma-
cy and Biochemistry, University of Buenos Aires, Argentina
and the Unit of Assisted Reproduction Gynecology Division,
Tocoginecology Department, Hospital de Clínicas José de
San Martín”, Buenos Aires, Argentina.

**CONFLICT OF INTERESTS**

The authors declare that they have no conflict of interest.

**Corresponding author:**

Mónica Rosales

Laboratory of Endocrinology

Department of Clinical Biochemistry

Faculty of Pharmacy and Biochemistry

University of Buenos Aires.

Buenos Aires, Argentina.

E-mail: monales0@hotmail.com
REFERENCES

Abbara A, Clarke SA, Dhillo WS. Novel Concepts for Inducing Final Oocyte Maturation in In Vitro Fertilization Treatment. Endocr Rev. 2018;39:593-628. PMID: 29982525 DOI: 10.1210/er.2017-00236

Alemu A, Terefe B, Abebo B, Biadgo B. Thyroid hormone dysfunction during pregnancy: A review. Int J Reprod Biomed (Yazd). 2016;14:677-86. PMID: 27981252 DOI: 10.29252/ijrm.14.11.677

Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ, Peeters RP, Sullivan S. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid. 2017;27:315-89. PMID: 28056690 DOI: 10.1089/thy.2016.0457

Cedíková M, Babuška V, Rajdl D, Zech NH, Kališ V, Králíčková M. Comparison of prolactin, free T3 and free T4 levels in the follicular fluid of infertile women and healthy fertile oocyte donors. Ceska Gynekol. 2012;77:471-6. PMID: 23116354

Chang HM, Qiao J, Leung PC. Oocyte-somatic cell interactions in the human ovary-novel role of bone morphogenetic proteins and growth differentiation factors. Hum Reprod Update. 2016;22:131-18. PMID: 27797914 DOI: 10.1093/humupd/dmw039

Colicchia M, Campagnolo L, Baldini E, Ulisse S, Valensise H, Moretti C. Molecular basis of thyrotropin and thyroid hormone action during implantation and early development. Hum Reprod Update. 2014;20:884-904. PMID: 24943836 DOI: 10.1093/humupd/dmu028

Costa NN, Cordeiro MS, Silva TV, Sastre D, Santana PP, Sá AL, Sampaio RV, Santos SS, Adona PR, Miranda MS, Ohashi OM. Effect of triiodothyronine on developmental competence of bovine oocytes. Theriogenology. 2013;80:295-301. PMID: 23683691 DOI: 10.1016/j.theriogenology.2013.04.011

De Jonge C. Biological basis for human capacitation revisited. Hum Reprod Update. 2017;23:289-99. PMID: 28115407 DOI: 10.1093/humupd/dmw048

Ishihara O, Adamson GD, Dyer S, de Mouzon J, Nygren KG, Sullivan EA, Zegers-Hochschild F, Mansour R. International committee for monitoring assisted reproductive technologies: world report on assisted reproductive technologies, 2007. Fertil Steril. 2015;103:402-13.e11. PMID: 25516078 DOI: 10.1016/j.fertnstert.2014.11.004

Maraka S, Mwangi R, McCoy RG, Yao X, Sangaralingham LR, Singh Ospina NM, O’Keeffe DT, De Ycaza AE, Rodriguez-Gutierrez R, Coddington CC 3rd, Stan MN, Brito JP, Montori VM. Thyroid hormone treatment among pregnant women with subclinical hypothyroidism: US national assessment. BMJ. 2017;25:356:i6865. PMID: 28122781 DOI: 10.1136/bmj.i6865

Mendeluk GR, Rosales M. Thyroxin Is Useful to Improve Sperm Motility. Int J Fertil Steril. 2016;20:142-9. PMID: 27584608 DOI: 10.5935/1518-0557.20160032

Monteleone P, Favia P, Artini PG. Thyroid peroxidase identified in human granulose cells: another piece to the thyroid-ovary puzzle? Gynecol Endocrinol. 2017;33:574-6. PMID: 28277109 DOI: 10.1080/09513590.2017.1296424

Practice Committee of the American Society for Reproductive Medicine. Subclinical hypothyroidism in the infertile female population: a guideline. Fertil Steril. 2015;104:545-53. PMID: 26239023 DOI: 10.1016/j.fertnstert.2015.05.028

Sechman A. The role of thyroid hormones in regulation of chicken ovarian steroidogenesis. Gen Comp Endocrinol. 2013;190:68-75. PMID: 23631902 DOI: 10.1016/j.ygcen.2013.04.012

Slebodziński AB. Ovarian iodide uptake and triiodothyronine generation in follicular fluid. The enigma of the thyroid ovary interaction. Domest Anim Endocrinol. 2005;29:97-103. PMID: 15927769 DOI: 10.1016/j.domaniend.2005.02.029

Velasco I, Taylor P. Identifying and treating subclinical thyroid dysfunction in pregnancy: emerging controversies. Eur J Endocrinol. 2018;178:1-12. PMID: 29070512 DOI: 10.1530/EJE-17-0598

Verberg MF, Eijkemans MJ, Macklon NS, Heijnen EM, Baart EB, Hohmann FP, Fauser BC, Broekmans FJ. The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: a meta-analysis. Hum Reprod Update. 2009;15:5-12. PMID: 19091754 DOI: 10.1093/humupd/dm0053

Vissersen R, Manders VD, Mastenbroek S, Fliers E, Afink GB, Ris-Stalpers C, Goddijn M, Bisschop PH. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Hum Reprod Update. 2015;21:378-87. PMID: 25634660 DOI: 10.1093/humupd/dmv004
Wakim AN, Polizzotto SL, Buffo MJ, Marrero MA, Burholt DR. Thyroid hormones in human follicular fluid and thyroid hormone receptors in human granulosa cells. Fertil Steril. 1993;59:1187-90. PMID: 8495763 DOI: 10.1016/s0015-0282(16)55974-3

Wei Q, Fedail JS, Kong L, Zheng K, Meng C, Fadlalla MB, Shi F. Thyroid hormones alter estrous cyclicity and antioxidative status in the ovaries of rats. Anim Sci J. 2018;89:513-26. PMID: 29214681 DOI: 10.1111/asj.12950

Xie D, Chen CC, Ptaszek LM, Xiao S, Cao X, Fang F, Ng HH, Lewin HA, Cowan C, Zhong S. Rewirable gene regulatory networks in the preimplantation embryonic development of three mammalian species. Genome Res. 2010;20:804-15. PMID: 20219939 DOI: 10.1101/gr.100594.109

Yassaee F, Farahani M, Abadi AR. Prevalence of subclinical hypothyroidism in pregnant women in Tehran-Iran. Int J Fertil Steril. 2014;8:163-6. PMID: 25083181

Zhang SS, Carrillo AJ, Darling DS. Expression of multiple thyroid hormone receptor mRNAs in human oocytes, cumulus cells, and granulosa cells. Mol Hum Reprod. 1997;3:555-62. PMID: 9268132 DOI: 10.1093/molehr/3.7.555

Zheng K, Suleiman FJ, Li J, Wei Q, Xu M, Shi F. Nitric oxide and thyroid hormone receptor alpha 1 contribute to ovarian follicular development in immature hyper- and hypo-thyroid rats. Reprod Biol. 2015;15:27-33. PMID: 25726374 DOI: 10.1016/j.repbio.2014.11.002