Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Domestic Animal Endocrinology 29 (2005) 97–103
DOMESTIC ANIMAL ENDOCRINOLOGY
www.journals.elsevierhealth.com/periodicals/dae

# Ovarian iodide uptake and triiodothyronine generation in follicular fluid The enigma of the thyroid ovary interaction

A.B. Ślebodziński *

*Professor emeritus, Polish Academy of Sciences, ul. Promienista 166A/34, 60-157 Poznań, Poland*

**Abstract**

Since 1928, the iodine concentration in the ovary has been known to be higher than in every other organs except the thyroid. The ovarian iodide uptake varies with sexual activities, is enhanced by estrogens and a hypothyroid state and blocked by goitrogens. The recent discovery of a sodium iodide symporter (NIS) in ovaries has offered a possible mechanism for ovarian iodide uptake and other functional similarities to its thyroid counterpart. Nevertheless, the physiological significance of ovarian iodine uptake and accumulation remains unknown. The presence of thyroid hormones (TH) in follicular fluid (FF) has been established recently. Our preliminary studies on TH in FF (1996–1998) in rabbits, pigs, horses showed that the concentration of $T_4$ is generally lower than that in serum and that for $T_3$ is within the normal range or higher. A positive correlation exists between the $T_4$ levels in FF and serum but not between the corresponding $T_3$ levels. These studies revealed, for the first time, the presence of the ovarian 5′-monodeiodinase system in FF capable of generating $T_3$ (ovary-born $T_3$) by outer ring deiodination of $T_4$. In mares, seasonal polyestrus, ovarian 5′-monodeiodinase (MD) activity and FF $T_3$ levels have been found to be higher during the ovulatory period than in the anovulatory one. The exact physiological significance of this system generating $T_3$ and coexisting with isoforms of TH receptors in granulosa cells has not been elucidated. A direct role of $T_3$ for the early follicular development, differentiation and for the steroidogenic capability of granulosa cells, although strongly suggested by data obtained from in vitro studies, has to be elucidated.
© 2005 Elsevier Inc. All rights reserved.

*Keywords:* Iodide; Follicular fluid; Ovarian deiodinase; Ovary-born $T_3$; Thyroxine

* Tel.: +48 61 868 5328; fax: +48 61 868 5328.
*E-mail address:* endocrin@rose.man.poznan.pl.

0739-7240/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.domaniend.2005.02.029

## 1. Introduction

From the wide field of thyroid–ovary interaction this retrospective and current research concentrates on two aspects of the interaction in normal mature, non-pregnant females—the involvement of the ovaries in iodide uptake and the presence and origin of thyroid hormones in follicular fluid.

## 2. The ovaries and body iodide economy

### *2.1. Ovarian iodine uptake*

The concentration of iodine in mammalian ovaries depends largely on sexual activity: lower in the pre-puberal and in post-menopausal periods, higher during the stage of follicular growth [1,2] and reduced during pregnancy. As shown by Elmer in his review [2] the amount of iodine found in ovarian tissue varies greatly across species, reflecting both species variability and differences in sexual activity at the time of ovarian dissection. Expressed as the concentration of iodine (μg iodine /100 mg wet tissue) the following values (mean or range) were found: in man 741, in pig 600–648, in rabbit 60–2150, in guinea pig 340 and in dog 104–161.

The accumulation of radioiodide in ovarian follicles occurs first in the walls of large Graafian follicles. It reaches a maximum 4 h later and shows a higher concentration in the central parts than in others. After 4 days, the accumulation disappears from the follicular walls and is then higher in the follicular fluid (FF) than in surrounding tissues and the blood [1]. The accumulation rate constant ($K_m$) is higher for small oocytes and can be within the range for $K_m$ in the thyroid, as found in Japanese quail [3]. Although ovarian tissue does not synthesize hormonal iodo-proteins, the ovary's ability to take up and accumulate iodine is inhibited by thiocyanate, perchlorate and excessive doses of iodide (the Wolff–Chaikoff effect), as in thyroidal tissue.

### *2.2. The effects of hypothyroidism*

There is an increase in ovarian iodide uptake during hypothyroidism induced by thiouracil. The hypothyroid state is characterized by an absolute or relative depletion of thyroid hormones (TH), altered sensitivity and ovarian response to gonadotropins leading to a rise in the content of mucopolysaccharides, followed by a tendency in some species towards the development of polycystic ovaries [4,5]. In fact, ovarian cyst formation is greatly intensified in women with primary hypothyroidism and in experimentally hypothyroid animals exposed to hyperstimulation with gonadotropins [6,7].

### *2.3. Exogenous estrogens*

Estrogens administered to euthyroid intact female guinea pigs alter their iodine economy. Small quantities of stilboestrol increase the bodily retention of iodine in a dose-dependent manner [8]. During 24 h post-injection the highest accumulation of radioiodine, amongst the

extrathyroidal tissues, is found in ovaries. By contrast, the thyroidal uptake of radioiodine is enhanced by low, and inhibited by high doses [9]. In conditions of reduced iodine availability, a precise mechanism securing adequate supplementation of iodine to the ovary takes place [9].

Exogenous estrogens inducing thyroidal and ovarian iodide uptake may simultaneously lead to a compensatory increase in the peripheral utilization of TH to counteract the effects of reducing tissue metabolism [10,11]. Although thyrotropin (TSH) involvement in the ovarian iodide uptake has not been documented, the presence of TSH in human FF, in concentrations similar to, or above, that in serum, has recently been reported [12].

A possible iodine concentrating mechanism for the nutrition of developing follicles and the production of estrogenic hormones was postulated by earlier workers [1]. The latter postulate is in a general agreement with the recent finding of a possible TSH action on FSH receptors in the ovary, best explained by the structural homology of TSH and gonadotropins (FSH, hCG) and their respective receptors [13].

### *2.4. Ovarian sodium iodide symporter (NIS)*

The recent discovery of a $Na^+/I^-$ symporter (NIS) in non-thyroidal tissues has suggested a mechanism for ovarian iodine uptake and accumulation and for other functional similarities to its thyroid counterpart. This transmembrane protein is known to catalyze the transport of iodide into thyroid, which is an initial and limiting step in the formation of thyroid hormones controlled by TSH. In addition to sodium iodide NIS also transports technetium, pertechneate and several anions which may competitively inhibit iodide uptake [14]. TSH up-regulates, while estradiol increases proliferation and down-regulates, sodium iodide symporter gene expression [15].

In ovaries, the expression of sodium iodide symporter mRNA was detected by RT-PCR and Southern hybridization in humans and mice [16–19]. The ovary, prostate and adrenal glands display lower level of expression when compared with other tissues [20]. At present, most of the data on NIS characteristics and manifestation have been obtained from a Chinese hamster ovary (CHO) cell lines studied in vitro. The cDNA of NIS has been cloned [21] and rat rNIS-gene was transfected into a CHO cell (CHO-4J) line, stably expressing a number of rat NIS [22]. Nucleotide sequences of hNIS cDNA derived from the parotid gland, gastric mucosa and mammary gland were found identical to the hNIS cDNA sequence of the thyroid [16]. Similar studies on ovarian NIS cDNA sequencing are lacking.

The significance of the ability of ovarian tissue for exceptionally high iodine trapping and accumulation in follicular cells and follicular fluid in vivo remains unknown and needs further elucidation. There is, however, little doubt that the presence of an optimal level of elemental iodine is a characteristic phenomenon bound to the developmental stages of growing follicles. Small and the growing follicles take up more iodine than large ones and the accumulation of iodine seems to be crucial for the process supporting ovarian granulose cell secretory activities, as suggested by comparable works on the functional activity of INS in the physiology of some secretory tissues (discussed below). No interrelationships between the accumulation of iodine in the ovary and the activity of the pituitary–thyroid axis have been proved or postulated.

## 3. The presence and origin of thyroid hormones in follicular fluid

Preliminary measurements of radioimmunoassayable (RIA) triiodothyronine ($T_3$) and thyroxine ($T_4$) in the gonads of farm animals have revealed that the levels of $T_4$ and $T_3$ in seminal plasma are low or undetectable [23,24]. The $T_4$ level in FF is generally lower while $T_3$ is either within the broad range of the concentrations for serum or is higher [25]. The inter-gonad variation in $T_4$ levels is apparently due to differences in the permeability of the blood-testis and the blood-ovarian barriers for thyroxine, and to relative total protein (TP) concentrations in FF. A positive correlation exists between $T_4$ levels in serum and $T_4$ in FF [25,26] but not between $T_4$ and corresponding $T_3$ levels (in horses; 25). The marked variation in $T_3$ levels is inexplicable and raises some questions as to why the FF level of $T_3$ differs from that in the peripheral blood, being in individual follicular fluids frequently higher. The free $T_3$ fraction in FF is similar or slightly below that in the serum of euthyroid patients [26,27].

### *3.1. The presence of thyroxine-monodeiodinase in FF*

Our measurements of thyroxine-monodeiodinase activity in follicular fluids from rabbits, mares and sows, revealed for the first time (1996–1998), the presence in the ovary of a 5′MD system able to generate $T_3$ from $T_4$ by outer ring deiodination. In mares the FF 5′MD (expressed in pmol $I^-$ liberated/(mg protein min)) was found to be in the range 0.60–1.60. Incubation of FF samples with 5′MD inhibitor propylthiouracil (PTU) reduced ovarian 5′MD activity by about 35%. This indicates the existence of a type II monodeiodinase PTU-resistant [25] which so far has been found in the tissues where it plays an essential role in paracrine regulation, e.g. in testes [23,24] and mammary gland [28].

In the ovary, the 5′MD coexists with a variety of thyroid hormone receptors (TR; TRα-1, TR-α2 and TR-β2) in oocytes, cumulus and granulosa cells [26,27,29,30]. During follicular growth, increased expression of TR, concomitantly with a greater binding $T_3$ ability by the granulose cells early in follicular maturation, was found [31].

Our preliminary experiments performed on seasonal polyestrous warmblooded mares (6–18 years of age), demonstrated a significant increase in 5′MD activity and $T_3$ concentration in FF during the ovulatory in contrast to the anovulatory season. The FF 5′MD activity was positively correlated with serum $T_4$ but not with serum $T_3$ levels [25]. No relation has been found between the concentrations of $T_3$ in FF and in the blood. However, a positive correlation exists between the FF $T_3$ and FF 5′MD activity in correlated samples (from the same mares). These new findings indicate that the ultimate $T_3$ content in FF may be partly a result of local $T_3$ generation (ovary-born $T_3$), supporting the view that ovary-born $T_3$ may act as a paracrine factor during folliculogenesis. In fact, the majority of present in vitro studies underline the importance of $T_3$ for folliculogenesis, namely its role as a biological amplifier of the stimulatory action of follicle-stimulating hormone on granulose cell functions [32–36].

### *3.2. What influences ovarian iodide uptake and ovarian $T_3$ generation?*

Old and current data indicate participation of the ovary in the iodine economy of the body as well as in the metabolism of the thyroidal thyroxine delivered from the blood.

The mechanism of the iodide uptake and iodine accumulation in ovarian cells may now be considered in the light of the activity of the ovarian sodium iodide symporter, relative to the stages of follicular growth and changes in a hormonal milieu. What role this iodide uptake and accumulation through the NIS plays in ovarian tissue remains unknown but an increasing body of evidence from non-thyroidal tissues (e.g. mammary gland) suggests its functional involvement in a variety of metabolic processes; antiproliferative, tissue defense and hyperplasia (for discussion see Cann et al. [37]).

The current view on the bioavailability of TH for tissues implies that the serum free $T_4$ and free $T_3$ fractions enter the target cells where the $T_4$, as pro-hormone, is converted into $T_3$ which interacts with thyroid hormone nuclear receptors. In the ovaries these free hormone fractions exist in the FF in concentrations within the normal range for serum [26,27]. Our findings indicate that in addition to the TH gained from the blood, free $T_3$ is generated within the ovary in quantities to meet the hormone requirements for the developing follicles. Existence of the ovarian 5′MD system, which converts thyroidal $T_4$ to ovary-born $T_3$, makes such a process possible. Furthermore, the presence of the 5′MD, makes the ovaries temporarily independent from variations in thyroid secretory activity or TH blood levels as the local generation of $T_3$ secures a sufficient supply of the hormone to have a direct action on thyroid hormone receptor(s).

These findings have potential implications in situations which lead to a temporary, but marked, lowering of $T_4$ blood levels and/or the amount of $T_4$, as the pro-hormone for the ovarian 5′MD converting system. As is known from clinical and experimental studies, females made pharmacologically hypothyroid and then exposed to short-term ovarian hyperstimulation with gonadotropins, show various signs of ovarian dysfunction with characteristic cyst formation in some species. Mediators of the ovarian 5′MD activation or specific triggering factors for $T_4$ conversion and maintenance of optimal $T_3$ levels during early follicle growth have not been elucidated yet.

## Acknowledgements

The author would like to acknowledge the work of his colleagues, Prof. E. Brzezińska-Ślebodzińska, Drs. W. Lipczak and J. Ingarden, Ms. M. Szejnoga and E. Styczyńska, whose contribution at various stages of the research has been invaluable. I am also grateful to Prof. Geofrey Shaw for his language editing.

## References

[1] Bengtsson G, Ewaldsson B, Hansson E, Ullberg S. Distribution and fate of $^{131}$I in the mammalian ovary. Acta Endocrinol 1963;42:122–8.
[2] Elmer W. Fizjologia i patologia przemiany jodu. PAU: Kraków; 1936. p. 248–58 (English translation: Elmer W. Iodine metabolism and thyroid function. London: Oxford University Press; 1938).
[3] Newcomer WS, Peczely P, Pethes G. Transport of radioiodide between blood and ovary of Japanese quail. Domest Anim Endocrinol 1984;1:67–77.
[4] Thorsoe H. Inhibition of ovulation and changes in ovarian mucopolysaccharides induced by thyroidecromy in rabbits. Acta Endocrinol 1962;41:441–7.

[5] Leathem JM. Hormonal influences on the gonadotropin sensitive hypothyroid rat ovary. Anat Rec 1958;131:487–99.
[6] Lindsay AW, Vorhess ML, McGillivary MH. Multicystic ovaries in primary hypothyroidism. Obstet Gynecol 1983;61:433–6 [New York].
[7] Fitko R, Kucharski J, Szlezyngier B. The importance of thyroid hormone in experimental ovarian cyst formation in gilts. Anim Reprod Sci 1995;29:159–68.
[8] Ślebodziński A. Sex differences in the pattern of radioiodine excretion in the urine of intact stilboestrol-treated guinea pigs. Nature 1963;197:801–2.
[9] Ślebodziński A. Observations on the influence of stilboestrol on iodine economy of the body in intact guinea pigs. Acta Med Polona 1963;4:339–409.
[10] Ślebodziński A. Changes in ulitization of thyroid hormone and thyroxine distribution space in sheep treated with stilboestrol. J Endocrinol 1962;25:283–91.
[11] Robbins J, Rall JE. The interaction of thyroid hormones and protein in biological fluids. Recent Progr Hormone Res 1957;13:161–208.
[12] De-Silva M. Detection and measurement of thyroid stimulating hormone in human follicular fluid. J Reprod Med 1994;39:679–80.
[13] Yoshimura M, Hershman JM. Thyrotropic action of human chorionic gonadotropin. Thyroid 1995;5:425–34.
[14] Tanacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, et al. Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. Thyroid 2004;14:1012–9.
[15] Furnaletto TW, Nguyen LQ, Jameson JL. Estradiol increases proliferation and down-regulates the sodium /iodide symporter gene in FRTL-5 cells. Endocrinology 1999;140:5705–11.
[16] Spitzweg C, Joba W, Eisenmenger W, Heufelder AE. Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complementary deoxyribonucleic acids from salivary gland, mammary gland and gastric mucosa. J Clin Endocrinol Metab 1998;83:1746–51.
[17] Spitzweg C, Joba W, Schriever K, Goellner JR, Morris JC, Heufelder AE. Analysis of human sodium iodide symporter immunoreactivity in human exoceine glands. J Clin Endocrinol Metab 1999;84:4178–84.
[18] Perron B, Rodriquez AM, Leblanc G, Poucher G. Cloning a mouse sodium iodide symporter and its expression in the mammary gland and other tissues. J Endocrinol 2001;170:185–96.
[19] Smanik PA, Ryu K-Y, Theil KS, Mazzaferri EL, Jhiang SM. Expression, exon–intron organization, and chromosome mapping of the human sodium iodide symporter. Endocrinology 1997;138:3555–8.
[20] Chung J-K. Sodium iodide symporter: its role in nuclear medicine. J Nucl Med 2002;43:1188–200.
[21] Dai G, Levey O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. Nature 1996;379:458–60.
[22] Kosugi S, Sasaki N, Hai N, Sugawa H, Aoki N, Shigemasa C, et al. Establishment and characterization of a Chinese hamster ovary cell line, CHO-4J, stably expressing a number of $Na^+/I^-$ symporters. Biochem Biophys Res Commun 1996;227:94–101.
[23] Ślebodziński AB. The conversion of thyroxine to triiodothyronine. An essential link in paracrine regulation of testicular function. Poli J Endocrinol 1997;48(Suppl 3):136.
[24] Brzezińska-Ślebodzińska E, Ślebodziński AB, Kowalska K. Evidencer for the presence of 5′deiodinase in mammalian seminal plasma and for the increase in enzyme activity in the prepubertal testis. Int J Androl 2000;23:218–24.
[25] Ślebodziński AB, Ingarden J, Styczyńska E, Szejnoga M, Czwojdrak S. Triiodothyronine ($T_3$) as a paracrine factor in mares's follicular fluid. In: Proceedings of the 7th international symposium on equine reproduction. 1998. p. 61–2.
[26] Wakim AN, Polizotto SL, Buffo MJ, Marrero MA, Burholt DR. Thyroid hormone in human follicular fluid and thyroid hormone receptors in human granulosa cells. Fertil Steril 1993;59:1187–90.
[27] Zhang SS, Carrillo AJ, Darling DS. Expression of multiple thyroid hormone receptor mRNAs in human oocytes, cumulus cells, and granulose cells. Mol Hum Reprod 1997;3:555–62.
[28] Ślebodziński AB, Brzezińska-Ślebodzińska E, Styczyńska E, Szejnoga M. Presence of thyroxine deiodinases in mammary gland: possible modulation of the enzyme-deiodinating activity by somatotropin. Domest Anim Endocrinol 1999;17:161–9.

[29] Wakim AN, Polizotto SL, Burholt DR. Alfa-1 and beta-1 thyroid hormone receptors on human granulose cells. Recent Prog Horm Res 1994;49:377–81.
[30] Wakim NG, Ramani N, Rao ChV. Triiodothyronine receptors in porcine granulose cells. Am J Obstet Gynecol 1987;156:237–40.
[31] Maruo T, Hiramatsu S, Otani T, Hayashi M, Mochizuki M. Increase in the expression of thyroid hormone receptors in porcine granulose cells in follicular maturation. Acta Endocrinol 1992;127:152–60.
[32] Loeken MR, Channing CP. Direct evidence for de-novo synthesis of LH receptors in cultured pig granulose cells in response to FSH. J Reprod Fertil 1985;73:343–51.
[33] Maruo T, Hayashi M, Matsuo H, Yamamoto T, Okada H, Mochizuki M. The role of thyroid hormone as a biological amplifier of the actions of follicle-stimulating hormone in the functional differentiation of cultured porcine granulose cells. Endocrinology 1987;121:1233–41.
[34] Kirkwood RN, Thacker PA, Rajkumar K. Effects of growth hormone and triiodoothyronine on insulin-induced progesterone production by granulose cells from repubertal gilts. Can J Anim Sci 1992;732:589–93.
[35] Goldman S, Dirnfeld M, Abramovici H, Kraiem Z. Triiodothyronine ($T_3$) modulates hCG-regulated progesterone secretion, cAMP accumulation and DNA content in cultured human luteinized granulosa cells. Mol Cell Endocrinol 1993;96:125–31.
[36] Gregoraszczuk EL, Skalka M. Thyroid hormone as a regulator of basal and human chorionic gonadotrophin-stimulated steroidogenesis by cultured porcine theca and granulosa cells isolated of different stages of the follicular phase. Reprod Fertil Dev 1996;8:961–7.
[37] Cann SA, Netten van JP, Glover DW. Iodide accumulation in extrathyroidal tissues. J Clin Endocrinol Metab 1999;84:281–3.