

Thyroid Hormones Concentrations in Relation to Hormonal Estrous Induction, Laparoscopic Insemination and Pregnancy Out of Breeding Seasons

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Abstract: This study was aimed to determine serum concentrations of Thyroxine (T4) and Triiodothyronine (T3) after induction of estrous out of the breeding season during cold months. This work was carried out on 16 female local breed Iraqi does. Using 20 mg impregnated sponges with Medroxy Progesterone Acetate (MAP) for 13 days and an IM injection of 500 IU Pregnant Mare Serum Gonadotropine (PMSG) 24 h before sponges withdrawal for group A. And 400 IU PMSG for group B and 250 IU of human chorionic Gonadotropine (hCG) IM at the time of estrous appearance for group B. After 24-36 h from estrous onset each doe was inseminated laparoscopically with 1 mL of fresh diluted semen contain 100 millions of active fresh sperms directly to the uterine body. Blood samples were collected at the end of progesterone treatment, laparoscopic insemination and during pregnancy. The results were indicated all does showed signs of estrous 100% after 24-60 h with a mean time 46.9 ± 4.90 h after sponges removal. Estrous length was 37.1 ± 1.91 (24-72 h) for group A and $34.7 \pm 2-30$ h (24-60) for group B. Pregnancy diagnosis was performed by ultrasonography examination at 30, 60 and 90 days post insemination. At insemination time (during laparoscopy) the number of follicles were collected from the right and left ovaries in does and found it was ranged between 3-5 follicles of different sizes on each ovary. Pregnancy was found in 6 does 75% of group A of them 2 does had twine kids 25%, while 6 does 75% of the group B became pregnant and one doe had twinning kids 12.5%. No obstetrical problem was reported except adhesion and tearing of 2 sponges at the time of withdrawal. During the next seasons all does showed normal estrous and each had single kid. The overall average for serum T4 and T3 level during experimental period were 107.61 ± 3.36 and 2.03 ± 0.08 nmol/L, respectively, significantly higher thyroid hormones ($p < 0.05$) levels in progesterone, laparoscopic A.I and first month of gestation.

Keywords: Doe, estrous induction, laparoscopic insemination, out of breeding season, thyroid hormones, thyroxine, triiodothyronine

INTRODUCTION

Thyroid gland and thyroid hormones are considered crucial to productive and reproductive performance in domestic animals, hypothalamus-pituitary-thyroid system is important in animal reproductive and development in both sexes (Wakim *et al.*, 1993). Thyroid hormones play an important role in regulating the process of growth, lactation, reproduction and the general health (Jainu *et al.*, 2000). Which have an effect on reproductive function, fertility and fetal development (Chokis *et al.*, 2003). Thyroid hormones play a pivotal role in the mechanisms permitting the animals to live and breed in the surrounding environment, variation in nutrient requirement and availability, and to homeorhetic changes during different physiological stages (Todini, 2007).

Plasma T3 and T4 concentrations in the second half of pregnancy in doe was decreased (Todini *et al.*, 2007). Serum mean values of T4 during the last month of gestation was 51.7 ± 1.3 and 66.0 ± 1.3 ng/mL for the single and twin-carrying does (Salah, 1996). Mondal *et al.* (2006) reported the plasma T4 and T3 increased

during pregnancy to reach 81.8 ± 6.7 and 2.85 nmol/L at day 126. In adult non pregnant ewes the plasma concentration is 126.6 ± 1.14 ng/mL for T4, in does 120.8 ± 5.7 ng/mL (Etta, 1973). In adult non pregnant Iraqi does the serum T4 and T3 concentration are 90.1 ± 0.06 and 1.56 ± 0.01 nmol/L, respectively (Alwan, 1997). The objective of this work is to increase the knowledge of some endocrinology parameter in Iraqi goat, as limited information is available concerning this species. It is important to determine whether there is a variation in thyroid function rate that are related to the estrous induced out of the breeding season, as well as to improve the usage of sponges impregnated with 20 mg MAP, with a single injection of PMSG with or without hCG and one fixed time laparoscopically inseminations out of breeding season on goat fertility.

MATERIALS AND METHODS

The present work was conducted during cold months (December, January and February/2011, does fertility were followed up to May/2012 at the college of Veterinary medicine. Sixteen (16) fertile mature does were treated with sponges impregnated with 20 mg

MPA for 13 days (Intervet, International BV, Boxmeer, Holland), a day before sponges withdrawal (day 12), eight does (group A) were received an IM injection of 500 IU of PMSG (Folligon, Intervet, International Intervet, International). The other eight goats (group B) were injected with 400 IU of PMSG 24 h before sponges withdrawal and 250 h CG hormones at a time of estrous appearance.

The present experiment was start at November/2010, all does were detected for estrous, using fertile males, twice daily and Pregnancy diagnosis were also performed using Ultrasonic methods (Ultrasonography; equipment, prop 5-MHz; Wellid, China) just before sponges insertions. During and after progesterone treatment (sponges) the does were checked for estrus appearance by a buck. Laparoscopic (Laparoscope equipment, Carl storz company, Germany) Inseminations were performed 24-36 h after estrous onset using 1 mL diluted semen contains about 100 million fresh active sperm. During post insemination period all does were checked for estrous by bucks for 30 days, ultrasonography with prop 5 MHz were used for pregnancy diagnosis at days 30, 60 and 90 post inseminations. Daily checking was performed to pregnant does for any obstetrics or gynecological problems. Blood samples were collected at day 10 of progesterone treatment, 24 h following laproscopical insemination and at mid of the 1st, 2nd, 3rd, 4th and 5th months from pregnant does. Blood samples were centrifuged at 2500 rpm for 15 min and serum samples were stored in -2°C.

Serum T4 and T3 concentrations were determined by Radioimmuno Assay (RIA), kits purchased were from BioMeriux, Marcy-I Etoile, France). No cross reaction with revers Triiodothyronine (rT3) was observed.

Statistical analysis: Data were analyzed by using Complat Randomize Design (C.R.D.) by following model:

$$Y_{ij} = m + T_i + e_{ij}$$

where,

Y_{ij} = The hormonal level

m = Common mean

T_i = The effect of treatment ($i = 1-7$)

e_{ij} = Random error

Where used (SPSS, 2008) to calculate one way-ANOVA and to estimate the differences between treatments. Duncan Test was used (Duncan, 1955).

RESULTS AND DISCUSSION

Experimental animals did not show any signs of estrous during pre-treatment period. Estrous cycle,

ovulation did not occur as indicated by failure of corpus luteum formation and absence of progesterone rise thereafter. Similar results due to thyroidectomy or suppression of thyroid function in does have been shown to impair or even abolish normal ovarian function (Reddy *et al.*, 1996). Also, such results may be explained by the possibilities that impaired thyroid function may affected steroid feedback responses required for generation of pre-ovulatory, luteinizing hormone surge and the possibility of thyroid hormones required for normal ovarian function cannot be ruled out (Anderson and Barrell, 1998).

All does were not shown estrous behavior during progesterone treatment period These results agrees with suggestions of Kausar *et al.* (2009) that the progesterone has the ability to stop the estrous as long as it is secreted from intra vaginal sponge. Lead to blockage the gonadotropine release and subsequent estrous and ovulation in female treated with progesterone. Within 24-60 h after sponge with drawl all treated dose were showed typical estrous signs. This result indicated that 20 mg MAP is enough to stopped the release of gonodotropine. In Iraqi goat (Kashifalkitaa, 2003) reported that all does (100%) showed estrous after treatment with sponges' contained 40 mg MAP, during February.

Results of the present study indicated all treated does showed estrous 100% after 24-60 h with the mean time 46.9 ± 4.9 h after sponges removal. The estrous phase length was 37.1 ± 1.91 h for group A and 34.7 ± 2.3 h for groupB. The ovulation in doe was usually occurs 30-36 h after estrous onset (Smith, 2007). Smith (2007) and Motlomelo *et al.* (2002) claimed that the injection of PMSG 24 h before sponges withdrawal out of breeding season, the heat will come during 12-36 h later and the insemination will be done at 48 h of estrous. The present protocol was also, recommended by Hashemi *et al.* (2006) using out of breeding season regime and improved ovulation rate.

Semen deposition by methods used give 75% for both groups. Almost similar method and result was reported by Gomez-Brunet (2007) who suggested that these results could be due to one insemination time, insemination dose and method. The results of pregnancy indicated the using of fresh diluted semen is successful in laparoscopic surgical technique, as well, as the direct uterine insemination gave good result as compare to vaginal insemination.

Serum concentrations of thyroid hormones are represented in Table 1.

The mean values for T4 and T3 concentration shows a significant ($p < 0.05$) increases from days 10 of progesterone treatment (sponges), 24 h after laparoscopical insemination and at the mid of 1st month of pregnancy compared to values of thyroid hormones during the 2nd, 3rd, 4th and 5th months of pregnancy.

Table 1: Peripheral plasma T4 and T3 hormones concentrations after hormonal treatment, laparoscopic insemination and pregnancy of Iraqi goats

Parameter	T3 (nmol/L)	T4 (nmol/L)	Correlations
Hormonal treatment (sponges removed)	2.23±0.08a	120.44±7.21a	0.900**
24 h after lapros insemination	2.19±0.17a	112.0±8.45a	0.912**
During pregnancy			
At the mid of 1 st month	2.38±0.23a	123.0±14.2a	0.980**
2 nd month	2.18±0.17a	115.77±8.53a	0.675**
3 rd month	1.78±0.08b	91.0±4.04b	0.782
4 th month	1.72±0.13b	96.0±8.82b	0.103
5 th month	1.84±0.10b	98.22±7.69b	-0.253
Total overall	2.035±0.06	107.61±3.36	0.708

Value represented Mean± S.E. small different letters denoted significant differences between treatment T3 and T4 ($p < 0.05$)

There was a significant correlation coefficients between serum T3 and T4 concentrations during the same period of sponges progesterone treatment, after laparoscopic insemination and first month of gestation. While there were no significant positive correlations during the 2nd, 3rd and 4th months of pregnancy. A negative non-significant correlation was observed only in total serum T3 and T4 concentrations in the 5th month of pregnancy (Table 1).

In present experiment exogenous hormone (vaginal sponges) considered as stress factors which interfere with the physiological states of animals caused increases in the metabolic rate, lead to increases in thyroid hormones peripheral serum concentration. Colavita and Malfatt (1989) reported during induced or spontaneous estrous in goat, arise in plasma total T4 during estrous while T3 concentrations were higher during luteal phase. In small ruminants, many particular conditions are well known to alter thyroid functions, including exogenous hormones or drugs intake, differences of experimental animals and conditions, as well as assay methods (Todini, 2007).

At insemination time the number of follicles was found to be ranged between 3-5 follicles of different size on each ovary. Deneff *et al.* (1981) mentioned that an increase of TSH secretion causes changes in follicular diameter.

After laparoscopic insemination, the higher T4 and T3 serum concentrations (Table 1) could be due to an increases in estrogen hormone level as a result of progesterone and PMSG treatment which increased number of different size follicles present on does ovaries lead to increases in estrogen secretion. Brent (1997) reported that an increase in thyroid hormones due to an increase in estrogen.

Such thyroid hormones secretion (Table 1) are probably associated with circadian rhythm of environmental temperature (cold stress), day light. Overlapping effect by season and the animal physiological state are expected (Dauncey, 1990). In winter T4 and T3 concentrations reach maximal levels due to response to cold stress to which the animals were exposed (Tata, 2007).

The higher thyroid hormones concentrations were measured during the first month of pregnancy (Table 1). Almost similar results reported by Okabe *et al.* (1993) and Yidiz *et al.* (2005). In ewe and goats highest level of the thyroid hormones during early pregnancy and decreased gradually to reach lowest value during, late pregnancy (Yidiz *et al.*, 2005). Numerous hormonal and metabolic demands occur during pregnancy as increase level of estrogen, progesterone, hcG hormones and such increase need for iodine, resulting in thyroid glands activity (Glinoe, 2001). During pregnancy the thyroid gland increased in size and enlargement of gland as early as the first trimester (Todini, 2007), also thyroid is stimulated by placental human chorionic Gonado tropine (hcG) (Savoid and Omrani, 2000). These changes are primarily due to estrogen-induced increase in the thyroxine-binding globulin concentration (Burrow *et al.*, 1994). Secretion of thyrotropic factors by the placenta enhanced responsiveness of pituitary TSH secretion to hypothalamus TRH and change in maternal thyroid hormones concentration and catabolism (Deleo *et al.*, 1998).

The present results indicated slightly decrease in thyroid hormones concentrations from the 2nd month toward the end of gestation but still higher than those reported by Mondal *et al.* (2006) and Salah (1996). In goat the plasma level of estrogen hormone increased steadily toward the end of gestation (Alwan *et al.*, 2010). Similar result reported in human pregnancy there is a progressive increase in serum Thyroid Binding protein (TBG) concentration (Fisher *et al.*, 1972) which is probably due to the high levels of oestrogen in human pregnancy (Osorio and Myant, 1962). The decreased in thyroid hormones could be due to an increase of hormones deionization by placenta and present results agreed with suggestion, placenta permeability depends upon the type of the placenta and the stage of the pregnancy (Alwan, 1987). More decreased in T4 hormones compared with T3 level may be due to considered T4 as precursor of T3 hormone through deionization of T4 hormone by placental deiodinase

enzymes (Erickson *et al.*, 1981). This study suggests that the passage of thyroid hormones through the placenta and the activity of placental inner ring deiodination of T4 and T3 increases in advancing pregnancy. Maternal thyroid hormones are necessary for fetal nervous, reproduction and cardiovascular and skeletal system, growth and development during fetal life (Sperelakis and Bank, 1996), also agreed with Thyroid hormones have been demonstrated for fetal development and adaptation to extra-uterine life (Thomas and Nathanielsz, 1983; Symonds *et al.*, 2003). Decrease in T4 secretion rate and T4 distribution space during pregnancy is considered to be part of homeorhetic adaptation to the condition (Riis and Madsen, 1985).

CONCLUSION

Levels of serum thyroid hormones were influenced by hormonal treatment, and pregnancy. Estrous could be induced in Iraqi goats successfully out of breeding season using 20 mg MAP impregnated sponges with PMSG alone or with hcG. Also Laparoscopic insemination had no effect on does fertility.

REFERENCES

- Alwan, A.F., 1987. The development of thyroid and adrenal function in fetal and newborn guinea pigs. Ph.D. Thesis, Physiology and Pharmacology Department, Southampton University, Southampton, UK, pp: 210-212.
- Alwan, A.F., 1997. Histological study of Iraqi caprine fetal thyroid gland development. *Veterinarian*, 1: 134-141.
- Alwan, A.F., F.A.B. Amine and N.S. Ibraheem, 2010. Blood progesterone and estrogen hormones levels during pregnancy and after birth in Iraqi sheep and goats. *Bas. J. Vet. Res.*, 2: 153-157.
- Anderoson, G.M. and G.K. Barrell, 1998. Out-of-season breeding in thyroidectomized red deer hinds. *Proc. New Zealand Soc. Anim. Prod.*, 58: 20-24.
- Brent, G.A., 1997. Maternal thyroid function: Interpretation of thyroid function test in pregnancy. *Clin. Obstet. Gynecol.*, 40: 3-15.
- Burrow, C.A., D.A. Fisher and P.R. Larsen, 1994. Maternal and fetal thyroid function. *N. Eng. J. Med.*, 16: 1072-1078.
- Chokis, N.Y., G.D. Jahnke, C.S. Hilaire and M. Shelby, 2003. Role of thyroid hormone in human and laboratory animal reproductive health. *Bir. Defe. Res.*, 68: 479-491.
- Colavita, G.P. and A. Malfatti, 1989. Hematic concentration of thyroid hormones T3 and T4 in goat at the beginning of the seasonal sexual activity. *Ital. Soc. Vet. Sci. (Italian)*, 43: 467-471.
- Dauncey, M.J., 1990. Thyroid hormones and thermogenesis. *Proc. Nutr. Soc.*, 49: 203-215.
- Deleo, V., A.L. Marca, D. Lanzetta and G. Morgante, 1998. Thyroid function in early pregnancy I: Stimulating hormone response to thyropin releasing hormone. *Gyneoco. Endocr.*, 12: 191-196.
- Denef, J.F., S. Haumont, C. Cornette and C. Beckers, 1981. Correlated functional and morphometric study of thyroid hyperplasia induced by iodine deficiency. *Endocr.*, 108: 2352-2358.
- Duncan, D.B., 1955. Multiple range and multiple F-tests. *Biometrix*, 11: 1-4.
- Erickson, V.J., R.R. Cavalier and L.L. Rosenberg, 1981. Phenolic and nonphenolic ring iodide iodothyronine deiodinase from rat thyroid gland. *Endocr.*, 108(4): 1257-1264.
- Etta, K.M., 1973. Comparative studies of the relationships between serum thyroxine and thyroxine binding-globulin. Ph.D. Thesis, Michigan-State University.
- Fisher, D.A., J.H. Dussault, A. Erenberg and R.W. Lam, 1972. T4 and T3 metabolism in maternal and fetal sheep. *Pediatr. Res.*, 6: 894-899.
- Glinoe, D., 2001. Pregnancy and iodine. *Thyroid*, 11: 471-481.
- Gomez-Brunet, A., 2007. Reproductive performance and progesterone secretion in estrus-induced Manchega ewes treated with hCG at time of IA. *Small Rumin. Res.*, 71: 117-122.
- Hashemi, M., M. Safdarian and M. Kafi, 2006. Estrous response to synchronization of estrus using different progesterone treatment outside the natural breeding in ewes. *Small Rumin. Res.*, 65: 279-283.
- Jainu, D.M.R., H. Wahid and E.S.E. Hafez, 2000. Sheep and Goats. In: Hafez, B. and E.S.E. Hafez (Eds.), *Reproduction in Farm Animals*. Lippincott Williams and Wilkins, Philadelphia, USA, pp: 172-181.
- Kashifalkitaa, H.F., 2003. A study of the effect of deep freezing with comparisons between AI and natural mating and hormone treatment on the conception rate of black Iraqi goats. M.Sc. Thesis, Baghdad University- Coll. Vet. Med. Dept. Obst. Surg.
- Kausar, R., S.A. Khanum, M. Hussain and M.S. Shah, 2009. Estrous synchronization with medroxy progesterone acetate impregnated sponges in goat (*Capra hircus*). *Pak. Vet. J.*, 1: 16-18.

- Mondal, A., M. Archana, M.C. Pathak and V.P. Varshney, 2006. Peripheral plasma thyroid hormone concentrations during pregnancy in black Bengal goats: Society for Endocrinology Annual Meeting, Endocri. Abstracts, 12: 100.
- Motlomelo, K.C., J.P.C. Grayling and L.M.J. Schwalbach, 2002. Synchronization of oestrus in goats: The use of different progesterone treatments. *Small Rumin. Res.*, 65: 279-283.
- Okabe, A.B., I.M. Elebanna, M.Y. Mekkawy, G.A. Hassan, F.D. Elnouty and M.H. Salem, 1993. Seasonal-changes in plasma thyroid-hormones, total lipids, cholesterol and serum transaminases during pregnancy and at parturition in barki and rahmani ewes. *Indian J. Ani. Sci.*, 63: 946-951.
- Osorio, C. and N.B. Myant, 1962. The binding of T4 by human fetal serum. *Clin. Sci.*, 23: 277-284.
- Reddy, I.J., V.P. Varshney, P.C. Sanwak, N. Agarwal and J.K. Pande, 1996. Peripheral plasma oestradiol-17B and progesterone levels in female goats induced to hypothyroidism. *Small Rumin. Res.*, 22: 149-154.
- Riis, P.M. and A. Madsen, 1985. Thyroxine concentrations and secretion rates in relation to pregnancy, lactation and energy balance in goats. *J. Endocr.*, 107: 421-427.
- Salah, M.S., 1996. Thyroid hormone levels during late pregnancy and early lactation in the Goats. *J. King Saud. Univ., Agric. Sci.*, 1: 87-96.
- Savoid, M. and G.H. Omrani, 2000. Thyroid function studies and goiter prevalence in normal pregnancy Iranian women. *Irn. J. Med. Sci.*, 3-4: 90-94.
- Smith, M.C., 2007. Clinical Reproductive Physiology and Endocrinology of Does. Youngquist, R.S. and W.R. Threfall (Eds.), 2nd Edn., Current Therapy in large Animal Theriogenology. USA, pp: 535-537.
- Sperelakis, N. and R. Bank, 1996. Essential of Physiology. 2nd Edn. Little Brown Co., Boston, pp: 575.
- SPSS, 2008. Statistical Package for Social Science Version, 16, 17. (Win/Mac/Linux) Users SPSS Inc-Chicago, USA, Retrieved from: <http://www.spss.com>.
- Symonds, M.E., A. Mostyn, S. Pearce, H. Budge and T. Stephenson, 2003. Endocrine and nutritional regulation of fetal adipose tissue development. *J. Endocr.*, 179: 293-299.
- Tata, J.R., 2007. Ahormone for all seasons. *Prespect. Biol. Med.*, 50(1): 89-103.
- Thomas, A.L. and P.W. Nathanielsz, 1983. The fetal thyroid current topic in experimental. *Endocrinol.*, 5: 97-116.
- Todini, L., 2007. Thyroid hormones in small ruminants: Effects of endogenous, environmental and nutritional factors. *Animal*, 1(7): 997-1008.
- Todini, L., A. Malfatti, A. Valbonesi, M. Trabalza-Marinucci and A. Debenedetti, 2007. Plasma total T3 and T4 concentrations in goats at different physiological stages, as effected by the energy intake. *Small Ruminant Res.*, 88: 285-290.
- Wakim, A.N., S.L. Polizotto, M.J. Buffo, M.A. Marrero and D.R. Burholt, 1993. Thyroid hormones in human follicular fluid and thyroid hormone receptors in human granulosa cell. *Fertil. Steril.*, 59: 1187-1190.
- Yidiz, A., E. Balikci and F. Gurdogan, 2005. Changes in some serum hormonal profiles during pregnancy in single-and twin-foetus-bearing Akkaraman sheep. *Med. Weter.*, 61: 1138-1141.