Research Article

Thyroidal and Progesterone Hormones in Cows with Ovarian Cysts and Effects of Therapy with Levothyroxine, KI, GnRH and Progesterone or Ovsynch

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Abstract | The plasma T3, T4, TSH and progesterone concentrations in cows with ovarian cysts (OC) (n=40) before and after treatment were compared with cows without ovarian cysts (n=8). Blood was collected from all affected cows before and 10 days after last treatment and plasma was separated and stored at -20^0 C till assay by enzyme immunoassay. Cows with OC were randomly treated with either SC injection of 2.5mg Levothyroxine (LV)every 48 hours for 4 occasions, oral feeding (10gm daily for 5 days) of potassium iodide (KI), GnRH (40 µg, IM injection Day 0) + Progesterone (750 mg, IM injection Day 0) + KI (GPI), or Ov-Synch protocol +Progesterone (OVP). The plasma progesterone concentrations before treatment were significantly higher in cows with OC compared to normal cows. Plasma progesterone concentration increased in GPI treated cows where as it decreased in the OVP treated cows significantly. The plasma triiodothyronine (T3) and plasma thyroxine (T4) were significantly lower (before treatment) in all treated cows with OC compared to normal control cows, whereas the thyroid stimulating hormone (TSH) was significantly higher (before treatment) in cows with OC. After treatment the plasma T3 and T4 increased significantly (P<0.01) and TSH decreased significantly (P<0.01) in levothyroxine treated cows. In cows treated with other treatments there were no changes in T3, T4, and TSH concentration. Out of 40 treated cows,50%,40%,10%, 0% cows conceived in the group OVP,GPI,LV and KI respectively. It was concluded that the treatment with levothyroxine resulted in positive alteration in thyroidal hormone without affecting pregnancy rates.

Keywords | Levothyroxine, Ovarian cyst, Progesterone, T3, T4, TSH

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | July 05, 2017; **Accepted** | August 18, 2017; **Published** | November 22, 2017
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Citation | Meena M, Purohit GN, Kumar D, Saraswat CS (2017). Thyroidal and progesterone hormones in cows with ovarian cysts and effects of therapy with levothyroxine, KI, GnRH and progesterone or Ovsynch. Adv. Anim. Vet. Sci. 5(12): 514-519.
DOI | http://dx.doi.org/10.17582/journal.aavs/2017/5.12.514.519
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Ovarian cysts (OC) represent one of the most common reproductive disorders affecting dairy herd fertility mainly during the postpartum period (Silvia et al., 2005; Vanholder et al., 2006; Purohit, 2008). OC result in prolongation of the calving interval, reduced milk production, increased culling rates (Bartlett et al., 1986; Fourichon et al., 2000) with significant economic losses to the dairy farmer (Scott and Dobson, 1997; Noble et al., 2000).

The etiology of OC continues to be poorly understood (Jeengar et al., 2014). One key hormonal characteristic of cows with OC is lack of, or aberrant luteinizing hormone (LH) surge, although they have increased plasma 17-β estradiol (E2) concentration (Ribadu et al., 2000; Todoroki and Kaneko, 2006). Another explanation is a relatively high level of pulsatile secretion of LH that promotes continued growth of the dominant follicle (DF) (Todoroki and Kaneko, 2006). More recently, it was mentioned that altered follicular dynamics and cellular differentiations in

OC are mediated through altered steroid receptor expression as well as changes in the expression of their regulators (Silvia et al., 2002). It was suggested that the differentiation of granulosa cells to express 3β-HSD (3Beta-Hydroxysteroid dehydrogenase) might be insufficient in cystic follicles and accordingly they fail to ovulate. The differences in frequencies of 3β-HSD-positive cells in the granulosa and theca interna layers between cystic and atretic follicles may be one of the reasons why regression is delayed in cystic follicles (Isobe et al., 2003).

A plausible explanation to the formation of OC appears to be the lack of regression of DF. One study showed that the granulosa and theca interna cells of the bovine cystic ovarian follicles had weak proliferative activity and low apoptotic frequency which implied that the cystic ovarian follicle grows slowly and then maintained a static condition without degeneration, which lead to long term persistence of the follicle (Isobe and Yoshimura, 2007). An increase in follicle stimulating hormone (FSH) secretion following a reduction in inhibin secretion was shown to trigger turnover of cystic follicles in cows with spontaneous follicular cysts (Kengaku etal., 2007). Similarly stress increased secretion of progesterone and cortisol from the adrenal gland that exerted inhibitory effect on the LH and FSH surges, suppressed ovulation and led to formation of OC.

Vascular dysfunction (Rizzo et al., 2009a), an imbalance between reactive oxygen species (ROS) and antioxidants (Rizzo et al., 2009b), alterations in ovarian sympathetic nerve activity (Rizzo et al., 2011), severe negative energy balance (NEB) during postpartum period, with consequent reduction in peripheral plasma concentrations of insulin-like growth factors (IGFs), insulin, glucose and leptin (Lucy, 2003; Block et al., 2011), changes in the expression of heat shock proteins (hsp) in the granulosa and theca cells of the follicular wall (Velázquez et al., 2011) are also considered contributing factors, for the onset of OC in dairy cows.

In humans thyroid disorders have been associated with ovarian hyper-stimulation syndrome (OHSS) (Vasseur et al., 2003; Shu et al., 2011) and with polycystic ovary syndrome (PCOS) (Jung et al., 2011). Both hypothyroidism (Shu et al., 2011) and hyperthyroidism (Jung et al., 2011) have been associated with OC in pre-menopausal women. Among domestic species, lactating dairy cows are typically hypothyroid. The peripheral deiodination of thyroxine (T4) to produce the more potent triiodothyronine (T3) is especially important to maintain function of many target cells and tissues (Husveth, 2011). Plasma T3, T4, and thyroid stimulatory hormone (TSH) were typically low during early lactation in dairy cows (Gueorgiev, 1999; Fiore et al., 2015) and it is this period when most OC form in dairy cows. TSH secretions from pituitary increase thyroidal uptake of iodine and stimulates the synthesis and release of T3 and T4. Thus deficiency of iodine might promote hypothyroidism (Patrick, 2009). Low serum T4 levels were observed in dairy cows with iodine deficiency (Anderson et al., 1988).

A few previous reports in cows with OC suggested that the oral feeding of potassium iodide (KI) to cows for 7 days resulted in recovery from OC (Bugalia and Kohli, 1981; Purohit et al., 2001), however such effects were not observed with oral feeding alone in a recent study (Pushp et al., 2016). The relationship of thyroid hormone and OC was postulated many years ago (Dzhambazov, 1976), however it was only recently that low TSH and T4 were reported in Holstein Frisian cows with OC in Italy (Mutinati et al., 2013). The effects of levothyroxine on therapy of OC in cows were however not reported. This study examined the plasma concentrations of T3, T4, TSH and progesterone in cows with and without OC and the effect of therapy with levothyroxine or GnRH based treatments.

MATERIALS AND METHODS

Cows presented to the department of Veterinary Gynecology and Obstetrics, College of Veterinary and Animal Science or belonging to farmer's dairy herds, Bikaner were included in the study. Cows with fluid filled or hard structures (>25 mm) on the ovaries on two examinations 10 days apart were considered to have OC as mentioned previously (Jeengar et al., 2014).

Blood was collected from all cows with OC (n=40) before and after treatment (10 days after last treatment) and plasma was separated and stored at -20^0 C till assay by enzyme immunoassay. The plasma T3, T4, TSH and progesterone was estimated by enzyme immunoassay using commercially available kits (Calbiotech, Spring valley, USA). Blood was also collected from cows without OC (control, n=8).

Cows with OC were randomly treated with either SC injection of 2.5mg Levothyroxine (LV), oral feeding (10 gm daily for 5 days) of potassium iodide (KI), GnRH (40 µg, IM injection Day 0) + Progesterone (750 mg, IM injection Day 0) + KI (GPI), or Ov-Synch protocol +Progesterone (OVP). Levothyroxine 2.5mg SC was administered at 8 am in the morning and repeated every 48 h for 4 occasions. (Levothyroxine 25 tablets of 100µg were dissolved in sterile distilled water and administered SC observing all aseptic precautions). The body weight of cows in the present study varied between 250-350 Kg and based on a previous suggestion of SC administration of 10µg/kg levothyroxine (EMEA, 1998) the present dose was used.

The prognosis was determined by hormonal profile changes. The treated animals were inseminated in the estrus following treatment and the conception rate was determined.

Table 1: The mean values of plasma T3, T4 and TSH in cows with ovarian cysts and without ovarian cysts (control)

Group	T3 (nmoL/L)		T4 (ng/mL)		TSH (µlU/mL)	
	Pre-treatment	Post treatment	Pre-treatment	Post treatment	Pre-treatment	Post treatment
LV	2.56±0.17^{aA}	3.17±0.13^{bB}	21.95±1.59^{aA}	34.46±1.31^{bB}	0.79±0.05^{bcA}	0.55±0.02^{aB}
KI	2.36±0.19^{aA}	2.76±0.20^{aA}	20.70±1.07^{aA}	22.01±1.30^{aA}	0.72±0.03^{bA}	0.69±0.06^{bA}
GPI	2.58±0.18^{aA}	2.75±0.18^{aA}	23.43±1.43^{aA}	24.89±1.23^{aA}	0.69±0.06^{bA}	0.68±0.05^{bA}
OVP	2.58±0.18^{aA}	2.59±0.15^{aA}	21.24±1.41^{aA}	21.71±1.19^{aA}	0.77±0.05^{cA}	0.74±0.05^{cA}
Control	3.04±0.10^{bB}	-	37.80±2.85^{bA}	-	0.52±0.04^{aA}	-

Values with different superscripted letters in a column (a, b, c) or row (A,B,C) are significantly different (P≤0.05 for T3 and P≤0.01 for T4 and TSH).

RESULTS

Plasma Progesterone

Mean plasma progesterone concentrations in cows with ovarian cysts (0.67±0.13 ng/ml, 0.64±0.08 ng/ml, 0.62±0.12 ng/ml and0.64±0.11 ng/ml in LV, KI, GPI and OVP groups respectively) at presentation were significantly higher (P<0.01) compared to normal cows (0.18±0.02 ng/mL). Overall out of 40 cows with OC 80% were follicular type cysts and 20% were luteal cysts. A high proportion (65.62%) of cows with follicular cysts had a plasma progesterone concentration between 0.1-0.5 ng/mL; whereas 34.38% (11/32) cows with follicular cysts revealed plasma progesterone concentrations from 0.51-1.0 ng/mL. All cows with luteal type of cysts had plasma progesterone concentrations above 1.0 ng/mL.

Plasma Triiodothyronine (T3), Thyroxine (T4) and Thyroid Stimulating Hormone (TSH)

The mean plasma T3 concentration was significantly lower (P≤0.05) in cows with ovarian cysts compared to cows without ovarian cysts. The mean plasma concentration of T3 in cows with ovarian cysts increased to reach normal levels subsequent to therapy with levothryoxine only. In all other treatments the mean plasma T3 concentration continued to be lower (Table 1).

The mean plasma T4 concentrations was significantly lower (P≤0.01) in cows with ovarian cysts compared to cows without ovarian cysts. The mean plasma concentration of T4 in cows with ovarian cysts increased to reach normal levels subsequent to therapy with levothryoxine only. However, the values were still lower compared to cows without ovarian cysts. In all other treatment groups the mean plasma T4 concentrations were not altered compared to values before treatment (Table 1).

The mean TSH concentrations were significantly higher (P≤0.01) in cows with ovarian cysts compared to cows without ovarian cysts. Significant decrease in mean plasma TSH concentration was observed after therapy with levothyroxine only. However, the values were still lower compared to cows without ovarian cysts. In all other treatment groups the mean plasma T4 concentrations were not altered compared to values before treatment (Table 1) or the control.

Estrus and Pregnancy

Of the total 10 cows treated in each group only 1, 0, 5 and 8 cows evidenced normal estrus in the group LV, KI, GPI and OVP treatments respectively. The overall pregnancy rates for the total number of cows that were treated was 10%, 0%, 30% and 50% respectively for the LV, KI, GPI and OVP treatments respectively.

DISCUSSION

The plasma progesterone concentrations associated with cystic ovaries in this study were similar to those in plasma or milk previously reported in cows with induced or spontaneously occurring cysts (Ribadu et al., 1994; Robinson et al., 2006; Roth et al., 2012; Mutinati et al., 2013; Pusph et al., 2016).

There were 21 cows with follicular cysts which evidenced plasma progesterone concentrations between 0.1-0.5 ng/ml, while 11 cows with follicular cysts had evidenced plasma progesterone concentrations between 0.51-1 ng/ml at presentation. Cows with plasma progesterone concentrations between 0.51-1 ng/ml at presentation probably had partially luteinized follicular cysts. Some studies have revealed that suprabasal progesterone (adrenal secretions) might be responsible for enhanced progesterone levels in animals with follicular cysts (Wagner et al., 1972; Patterson et al., 1995; Dobson et al., 2000).

Eight cows with luteal cysts evidenced plasma progesterone concentrations of greater than 1 ng/ml on the day of presentation. Similar findings have been recorded in many previous studies (Narayana and Honnappa, 1985; Farin et al., 1990; Douthwaite and Dobson 2000). In fact ovarian cysts have been classified on the basis of the plasma progesterone concentrations. While Farin et al. (1992) considered greater than 0.5 ng/ml concentrations as luteal cyst

whereas other workers (Halter et al., 2003; Nakao et al., 2003) considered concentrations of greater than 1.0 ng/ml as luteal cysts.

The concentration of T3 under different groups before therapy appeared to be significantly lower to that of control group with normalestrus (Mutinati et al., 2013). This significant difference in T3 value proves that hypothyroidism have some role to play in OC, as steroid–independent decline in LH pulse frequency has been mentioned to be dependent on presence of thyroid hormones (Anderson et al., 2002). A study using non-lactating cows showed that estrus behavior was unaffected by hypothyroidism induced by thyroidectomy (Stewart et al., 1993). When T3 concentrations were depressed by the induction of hypothyroid in lactating heifers, progesterone concentrations at day 14 of the cycle were significantly lower than those in control animals (Thrift et al., 1999a, Thrift et al., 199b). Thus, it may be hypothesized that low T3 was associated with low reproductive performance in cows during postpartum period. However, more studies are required. Following therapy with levothyroxine, value of T3 became significantly higher compared to that of other groups and reached near to control thus proving efficacy of treatment to bring back T3 values to that of normal ones as recorded in previous studies (Miller et al., 1965; Moraes et al., 1998).

Concentration of T4 in control cows in our observation correspond to the concentrations reported by other authors (Gueorgiev, 1999; Sinka et al., 2008; Djoković et al., 2010). The value of T4 observed under the different groups in the present study before treatment were similar in all groups but significantly lower compared to the control group as observed previously in cows with ovarian cysts (Mutinati et al., 2013). A significant (P≤0.01) increase in T4 concentration (21.95±1.59 to 28.62±1.37) was observed in LV group cows following therapy whereas other treatments had no effect.

Comparison of pre-treatment values of plasma TSH in group LV, group KI, group GPI, group OVP with untreated cows without ovarian cysts revealed significant differences. Concentration of TSH in control cows in our observation correspond to the concentrations reported by other authors (Gvozdic et al., 2008; Alameen and Abdelatif, 2012). The concentration of TSH under different groups before therapy appeared to be significantly higher compared to that of control group with normal estrus that means there is hypo function of the thyroid gland in cows with ovarian cysts as also recorded in a previous study in cows (Mutinati et al.,2013).The higher estrogen blood concentrations found in cows with OC compared to cyclic subjects, in turn, could contribute to a reduction in TSH pituitary synthesis, given the strict relationship between estrogens and the hypothalamic-pituitary-thyroid axis as inferred by Léan et al. (1977) and later confirmed by Böttner et al. (2006). Mutinati et al. (2013) have shown that cows with OC have reduced TSH concentrations.

In terms of resumption of normal estrus and subsequent pregnancy the administration of KI orally revealed the poorest results and OVP treatment the best however, more studies are needed to include levothyroxine along with OVP or GPI to reveal whether improvement in the marginal thyroid hormone concentrations can help in recovery from ovarian cysts in dairy cows.

CONCLUSION

It was concluded that thyroidal hormone levels are low in cows with OC, the treatment with levothyroxine resulted in positive alteration in thyroidal hormone without affecting pregnancy rates.

ACKNOWLEDGMENTS

The authors are highly thankful to the Dean College of Veterinary and Animal Sciences, Bikaner, Rajasthan, India for provision of the facilities required for conducting the study.

CONFLICTS OF INTEREST

The authors have no conflict of interest.

AUTHORS CONTRIBUTION

The present study is a part of work carried out for MVSc research by Mukesh Meena under the guidance of Prof G N Purohit.

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