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NEW APPROACH FOR TREATMENT OF OVARIAN CYSTS IN DAIRY CATTLE

By

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ABSTRACT

The present study was performed to diagnose and treat ovarian cysts with either hormonal treatment or laparoscopic surgery. Thirty-four lactating dairy cows were divided after ultrasonographic examination into two main groups: Cows in group 1(with follicular cyst, n=20) were treated by administration of gonadotrophin releasing hormone (GnRH) at a one dose of 5 ml/im/cow. Fourteen cows were responded and assigned as group "1a" while the non-responders (6 animals) were assigned as group "1b". Cows in group 2 (with luteal cyst, n=14) were treated by the administration of two doses of prostaglandin $f_{2\alpha}$ each 2 ml/im/cow with11 days interval.Based on results of ultrasonographic examination, 10 cows responded and assigned as group '2a', and 4 were non-responding cows represent group '2b'. Animals belonging to 1b and 2b groups were treated with surgical laparoscopic interference via a left flank approach. The cysts were aspirated (follicular) or removed by bipolar cauterization (luteal) in standing cows with sedation and local anesthesia. Blood samples were collected from the jugular vein in plain vacuum tubes and centrifuged at 3000 rpm for 15 min. Serum was separated and stored at -20^{o}C till hormonal and cytokines assays.

The results obtained showed that values of TNF-α and IL-6 were significantly higher ($P<0.01$) in cystic cows with follicular and luteal cyst non-responded to hormonal treatment compared to those with cyclic ovarian cows. A high level of 17β-estradiol with concurrent low levels of progesterone showed in follicular cystic cows non-responded compared to responded cows. The significant drop in serum 17 β estradiol and high levels of progesterone in non-responded luteal cystic cows compared to responded cows. Significant decrease ($p<0.01$) in serum level of both insulin and IGF-1 in cows non-responded to hormonal treatment compared to responded ones.

The results revealed that percentage of conception rates at first AI in laparoscopic surgical treated cows was higher than hormonal treated cows. Based on the obtained results, measuring TNF-α, IL-6, insulin and IGF-I could predict the response to hormonal therapy.

In addition, it is recommended to apply the laparoscopic approach for the non-responding cases of follicular and luteal ovarian cysts.

Keyword:

ovarian-follicular- luteal-cyst-IL-6-TNF-α- insulin- IGF-I-GnRH-$PGF_{2\alpha}$-laparoscopic surgery.

INTRODUCTION

Ovarian cysts are one of the most important causes of infertility in dairy cows and are associated with considerable economic losses through their high incidence, increase in days to first service, and increase in days open **(Cattaneo *et al.,* 2014).** Ovarian cyst can be classified as two types, follicular and luteal cysts, the occurrence of follicular cyst ranges from 30-85% **(Lüttgenau *et al.,* 2016)** and 15-70% for luteal cyst **(Kumar *et al*., 2014).**Plasma steroid concentration (17 β estradiol and progesterone) are considered as an indicator of ovarian activity **(Picard-Hagen *et al.,* 2015)** and as a marker to predict response to hormonal treatment **(Roth *et al*.,2012).** Thus,estimation of hormones before and during treatment is helpful in studying ovarian function and response to treatment. Insulin and insulin-like growth factor-I (IGF-I) have been postulated as key mediators between nutritional status and ovarian function in cattle **(Khan *et al.,* 2011).** High insulin and IGF-I concentrations in the circulation are associated with a short interval from parturition to first ovulation **(Obese *et al.,* 2015).** In vitro and in vivo studies in bovines **Hein *et al*., (2015)** indicated that insulin and IGF-I stimulate estradiol synthesis in granulosa cells.

Thus, it can be hypothesized that any failure in function of the insulin and IGF-I system will lead to follicular regression and/or cyst formation.

The concurrent evaluation of selected cytokines (IL- 6 and TNF-α) may be helpful to determine the processes that mediate the physiological development of the ovarian follicles **(Richards and Pangas, 2010)** and predict the occurrence of ovulation or its absence and development of the ovarian cyst **(Qiao and Feng, 2011, Sheldon, *et al.,* 2014).** Moreover, it appears that it is possible to differentiate follicular cysts from luteal cysts based on an evaluation of serum levels of cytokines in cows.

A more accurate understanding of the contribution of cytokines in these processes may facilitate the prevention of ovarian cysts in the future **(Smolikova *et al.,* 2012).**

Different approaches for treatment of ovarian cysts including cystic fluid aspiration **(Roth *et al.,* 2012),** and hormonal applications have been used in the treatment of ovarian cysts. Gonadotropin-releasing hormone (GnRH) have frequently been used for the treatment of follicular ovarian cysts and proved to be successful **(Taktaz *et al.,*2015).** Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the treatment of choice for luteal cysts **(Peters, 2005)** and has also been used as the initial treatment of bovine ovarian cysts **(Probo *et al.,* 2011).**

Laparoscopy has gained a leading role and appears to be the gold standard method for a quiet wide range of gynecologic procedures including cystic ovaries **(Hendrickson 2008, Daniilidis *et al.,* 2011).**

Although, the use of laparoscope as a diagnostic tool for infertility evaluation **(Arnold and Love, 2013)** but its use in bovines for infertility assessment is very meagre **(Sofi, 2018)**. In case of removal of the cystic content the cow will be deprived from the main source of estrogens along with other possible locally acting factors, which will permit a new follicle development and ovulation to occur.There were recorded attempts to aspirate follicular fluid from cysts guided by ultrasound monitoring **(Lievaar *et al.,* 2006; Amiridis, 2009)** but to the best of our knowledge there were no recorded trials for treatment of follicular and luteal cysts in cattle by the laparoscopic approach. In general, the primary advantages of laparoscopic surgeries are excellent intraoperative visibility, secure hemostasis, reduced surgical and postoperative morbidity, decreased postoperative discomfort,rapid and uncomplicated healing, reduced quantity of medication needed and shorter postoperative management.

The present study was conducted to find biomarkers to predict clinical response to traditional hormonal treatment of the cases follicular and luteal ovarian cysts and to evaluate treatment of the non-responders by laparoscopic intervention.

MATERIAL AND METHODS

Animals:

This study was conducted in El- Behaira Governorate, all cows were housed in free stalls, fed twice daily with a total mixed ration meeting the requirement for milk productions with ad libitum access to water. out of 200 Holstein dairy cows, 34 cows (aged 3-10 years) were found to have ovarian cysts and were divided after ultra-sonographic examination into two

main groups: Cows in group 1 (with follicular cysts, n=20) were treated by administration of gonadotrophin releasing hormone (GnRH, RECEPTAL®, MSD animal health) (each 1 ml contains 4 µg buserelin, Intervet international GmbH-Germany) at a one dose of 5 ml/im/cow. Fourteen cows were responded and assigned as group "1a" while the non-responders (6 animals) were assigned as group "1b". Cows in group 2 (with luteal cyst, n=14) were treated by the administration of two doses of prostaglandin $f_{2\alpha}$ (ESTRUMAT ®, MSD animal health) (synthetic prostaglandin analogue each 1 ml contains 250 µg Cloprostenol,Schering-Plough Animal Health), each 2 ml/im/cow with11 days interval. Based on results of ultrasonography examination, 10 cows were responded and assigned as group '2a', and 4 non-responding cows represent group '2b'.Animals belonging to 1b and 2b groups were treated with surgical laparoscopic interference via a left flank approach. Animals under investigation were in good body condition, healthy and have no history of other reproductive disorders or previous treatment with other hormones.

Sampling:

Blood samples were collected from the jugular vein in plain vacuum tubes and centrifuged at 3000 rpm for 15 min. Serum was separated and stored at -20°C till hormonal and cytokines assays.

Hormonal Assay:

All hormones were assayed using ELISA micro wells kits (Monobind Inc. Lakeforest, CA92630.USA): progesterone (P4) **(Bono *et al.* 1996),** estradiol (E17β) **(Kulick *et al.* 1999),** insulin like growth factor-I (IGF-I) **(Roth *et al.* 1996)** and insulin **Gerich (1988)**, respectively.

Measurements of cytokines in blood serum:

Serum concentrations of TNF-α and IL-6 were determined using commercially available kits (Bovine ELISA kits for TNF-α and USCN Life Science Inc., Houston, USA for IL-6).

All procedures were carried out according to **Kim *et al*. (2014).**

Surgical laparoscopic treatment:

Both animals of group 1b and group 2b were treated surgically according to **Farstvedt and Hendrickson (2005)**. Left flank approach was used to reach both ovaries.

A step that is important prerequisite to insure that there is sufficient intra-abdominal space for instrumentation. Food was hold for at least 36 hours before surgery and the ambient water was supplied. The cows were sedated with xylazine (Xyla-Ject 2% ADWIA) at a dose of 0.1-0.15 mg/kg B.W. and local anesthesia lidocaine 2% (Pharmacell) at the portal sites.

The port for the laparoscope (Hencke Sass Wolf - Germany) was introduced at the ventral angle of the left Para-lumber fossa approximately ten cm. cranioventral to the tuber coxae. Instruments were inserted through two portals approximately 20 cm and 30 cm ventral to the tuber coxae and after abdominal insufflation with carbon dioxide. The cystic ovary was grasped and local anesthetic was injected into the mesovarium and mesosalpinx, the follicular cystic fluid was suctioned and the luteal cyst were removed by bipolar laparoscopic cauterization (Hermann Medizintechnik GmbH electrosurgical system HHF300) and the cyst was carefully removed through an extended instrument portal.

The incisions were closed with single interrupted absorbable sutures in the musculature and single interrupted non-absorbable suture in the skin as done according to **Farstvedt and Hendrickson (2005).** A dose of long acting tetracycline i.m. dose was given (Terramycin LA (Fizer Co. Egypt): 1 ml/10kg body weight.

Pregnancy diagnosis:

All cows were checked for pregnancy by transrectal ultrasonography via a 5 MHz linear array transducer (CTS-900V, SIUI, Japan)on day 35±3 after artificial insemination(AI).

Conception rate was recoded as the proportion of inseminated cows that get pregnant at the time of pregnancy diagnosis.

Statistical analysis:

Data were presented as mean ± SEM (standard error of the mean). Independent-samples T test was carried out for the obtained data using SPSS program version 16.0 and $P \leq 0.01$ was considered as statistically significant.

RESULTS

As presented in Table 1, a high level ($p<0.01$) of 17β-estradiol with concurrent low levels of progesterone in the follicular ovarian cysts of non-responding cases as compared to responding cows. There was lower level ($p<0.01$) of estradiol with high levels of progesterone in non-responded luteal cystic cows compared to responded ones.The obtained results showed that values of TNF-α and IL-6 were more or less high in non-responded cows with follicular and luteal cyst compared to the responding ones (Table 1). There was a significant decline ($p<0.01$) in serum level of both insulin and IGF-I in cows that not responded to hormonal treatment compared to responded ones in both types of cystic ovaries.

Table (1): Hormonal profile and cytokines in serum of cows affected with follicular and luteal cysts either responded or not to hormonal treatment (means±0.05).

Parameters	Follicular cysts N=20		Luteal cysts N=14	
	Responded N=14	Non-responded N=6	Responded N=10	Non-responded N=4
P_4 (ng/ml)	**1.41±0.31^{a}**	**0.32±0.01^{b}**	**0.75±0.06 ^{B}**	**3.65±0.36 ^{A}**
E_2 (pg/ml)	**2.45±0.10 ^{b}**	**5.08±0.81^{a}**	**3.30±0.57 ^{A}**	**1.04±3.76 ^{B}**
Insulin(ng/ml)	**4.61±0.20 ^{a}**	**2.19±0.20 ^{b}**	**3.75±0.17 ^{A}**	**2.03±0.23 ^{B}**
IGF- (ng/ml)	**51.58±2.44^{a}**	**36.08±2.13^{b}**	**67.77±2.79^{A}**	**52.02±1.23^{B}**
TNF- (pg/ml)	**14.01±0.68^{b}**	**25.16±1.52^{a}**	**18.79±1.23^{B}**	**27.27±1.22^{A}**
IL-6 (pg/ml)	**252.09±2.15^{a}**	**276.93±2.14^{a}**	**354.66±1.72 ^{B}**	**462.72±1.13 ^{A}**

Means with different alphabetic subscripts in the same row were significantly differ at (*P< 0.01*).

Data that is presented in (Table 2) revealed that percentage of conception rate at first AI in laparoscopic surgery higher than in groups treated with hormonal. Half of the cases treated by laparoscopic approach (either follicular or luteal cysts) respond well to the laparoscopic technique and get pregnant. The operation lasts for 30 - 40 minutes and no complications were noticed either during or after laparoscopy.

Table (2): Conception rate of cows as response to hormonal and surgical treatment.

Types of treatment	Follicular cyst		Luteal cyst	
Conception rate	**No**	**%**	**No**	**%**
Hormonal treatment	**6/14**	**42.9%**	**4/10**	**40%**
Surgical treatment (n = 10)	**3/6**	**50%**	**2/4**	**50%**

DISCUSSION

Ovarian cyst is an important ovarian dysfunction and a major cause of reproductive failure in dairy cattle **(Jeengar *et al.,* 2014).** So, the present study aimed to find predicting biomarkers for response of these cases to hormonal treatment as well as finding a new approach for the non-responders to hormonal therapy.The present study showed a high level of 17β-estradiol with concurrent low levels of progesterone in the non-responded follicular ovarian cysts compared to responded cows. These findings came in agreement with **Mimoune *et al.,* (2018) and Brodzki *et al.,* (2019)**. If a rapid method of completing the estradiol assay for cows within few hours is developed, unpromising treatments would be avoidable for follicular cysts in cows with abnormal estradiol concentrations on that day **(Mollo *et al*., 2012).**

However, in the present study,17 β estradiol level decreased with high level of progesterone in non-responded luteal cystic compared to responded cows. These results were similar to recent studies done by **Mimoune *et al.* (2018) and Brodzki *et al.* (2019).** As a result, suggested that low estradiol level can be predictor for impaired luteal function and lower likehood of pregnancy **(Rizzo *et al.,* 2011).**

High yielding dairy cows have, as a result of genetic selection and metabolic load, low concentrations of insulin and IGF-I, and there is considerable evidence that these may contribute to the development of cystic ovarian disease (COD) through an impairment of follicular cell proliferation and steroidogenesis **(Vanholder *et al.* 2006). Rodriguez *et al.* (2017)** found alterations of the IGF system, such that cystic follicles had greater expression of IGF receptor 1 (IGFR1) and IGF binding protein 4 (IGFBP4) than normal follicles.

In present study, both insulin and IGF-I levels were significantly low in non-responded cows compared to those had an early resumption of cyclicity after hormonal treatment.

The present results are similar to those of **Khan *et al.* (2011), Hein *et al.* (2015) and Mimoune *et al.* (2018)**. The low level of insulin not only reduce the production of estradiol, but also alters the follicle's ability to acquire LH receptors, which decreases the development and ovulation of follicles that lead to the persistence of follicles as anovulatory structures **(Obese *et al.,* 2015)**. Thus, IGF-I and insulin are important stimulators of follicle growth and low concentrations of one or both of the hormones may contribute to the formation of ovarian cyst**.** On the contrary, some studies reported high levels of IGF-I in ovarian cyst **(Probo *et al*, 2011).**

Expression of cytokines such as Interleukins (IL-6 and IL-8) and tumor necrosis factor–α (TNFα), all have roles in steroidogenesis and ovulation and differ between normal and cystic

follicles **(Stassi *et al.* 2017).**Our study revealed that serum levels of both TNF-α and IL-6 were significantly higher in cows with follicular and luteal cysts that were non-responders to hormonal treatment compared to those responded to hormonal treatment. The present results are in agreement with the previous findings **Baravalle *et al*. (2015) and Brodzki *et al.* (2019)**. The increased serum levels of both IL-6 and TNF-α in animal with cystic ovaries would be a consequence of a higher number of follicles releasing cytokines into the blood stream **(Bersinger *et al*., 2014).** Thus, cytokine detection can be used for characterization, prediction and regulation of the ovarian state, as well as for the treatment of ovarian disorders **(Stassi *et al.*, 2017).**

Significant differences were recorded in the percentage of conception rate at first AI, among the two groups treated with hormonal and surgical treatment in cows with follicular cyst and luteal cyst. No difference in percentage of conception rate between follicular and luteal cystic cows. These results are similar to those recoded by **Taktaz *et al.*, (2015); Marthold *et al.*, (2016) and Silviu *et al.*, (2018).**

There were no previous reports on surgical treatment of luteal cysts in cows to compare with our innovate results. Although many thin-walled cysts rupture accidentally during rectal palpation, it is nowadays widely believed that manual rupture of the cyst should be avoided, since adhesions which frequently follow the technique, may impair future fertility of the cow **(Peter, 1997).** Despite the variety of treatments used, a high proportion of animals with COD fail to be cured **(McLeod and Williams, 1991; Bartolome *et al.*, 2000; Douthwaite and Dobson, 2000).** Some authors suggested that emptying of the cystic content may benefit treatment in refractory cases **(Cairoli *et al.*, 2002; Cruz *et al.*, 2004).**

The use of an ultrasound scanner for cyst ablation **(Pieterse *et al.*, 1991)** requires additional equipment (a special transvaginal transducer with built-in needle guide) which is expensive and not routinely used by the practicing veterinarian. Some early attempts to treat cystic ovarian disease (COD) by aspiration of the cyst with a long needle passed either through the vaginal wall or the rump muscles **(Cited by Saratsis,1999)** were not adopted as being rather cumbersome and ineffective. In follicular and luteal ovarian cysts not responded to treatment, the surgical interference is considered the last choice to reserve the high quality cows for breeding. Today the operative laparoscopy is regarded as the gold standard for the surgical treatment of ovarian cysts in human **(Guglielmina *et al.*,1997)** and dogs **(Fayrer-Hosken *et al.*, 1992)** in cases which not responded to hormonal treatment by electric cauterization,

cutting and removal of the cyst. But until now this technique was not evaluated in cows. The laparoscopic method permitted clear diagnosis and visualization of the cystic structure, while electro cauterization completely controlled the bleeding. Our results of good response to laparoscopic aspiration of follicular cystic fluid are in agreement with previous results of dominant follicle aspiration **(Amiridis*etal.*,1999)**one could expect that estradiol concentration will be dramatically reduced soon after follicular cyst ablation. Estrogens have been shown to have a local preventive role in ovulation in rats and primates **(Dierschke *et al.*,1983; Hurtz *et al.*, 1986).** This being the case in cattle, after removal of the cystic content the cow will be deprived from the main source of estrogens along with other possible locally acting factors, which will permit a new follicle development and ovulation to occur. In the case of luteal cysts, the drainage caused regression of the cyst, which by that time acted as a functional but persisting corpus luteum. Suprabasal progesterone concentration blocks the LH surge but increases LH pulse frequency **(Duchens *et al.*, 1994).**

Thus,regression of the cyst led to restoration of the normal function of the hypothalamic-pituitary-ovarian axis that allowed the development and ovulation of a freshly selected dominant follicle.

In conclusion, the obtained results present the first report to describe a new technique of laparoscopy to treat ovarian follicular and luteal cysts in cows that are refractory to conventional hormonal treatment with no or negligible complications. In addition, measuring TNF-α, IL-6, insulin and IGF-I could predict the response to hormonal therapy.

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