秋实

中国农业科学院兰州畜牧与兽药研究所
第三届中青年科技论文暨盛彤笙杯演讲比赛论文集

◎ 张继瑜　王学智　董鹏程　主编

中国农业科学技术出版社

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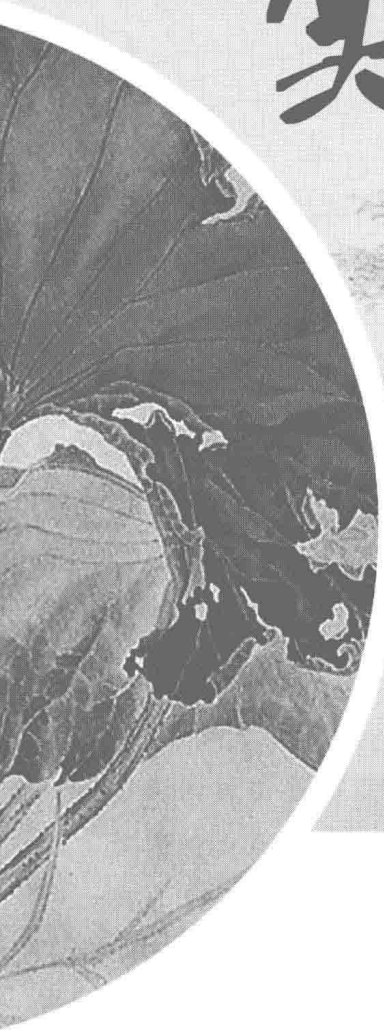

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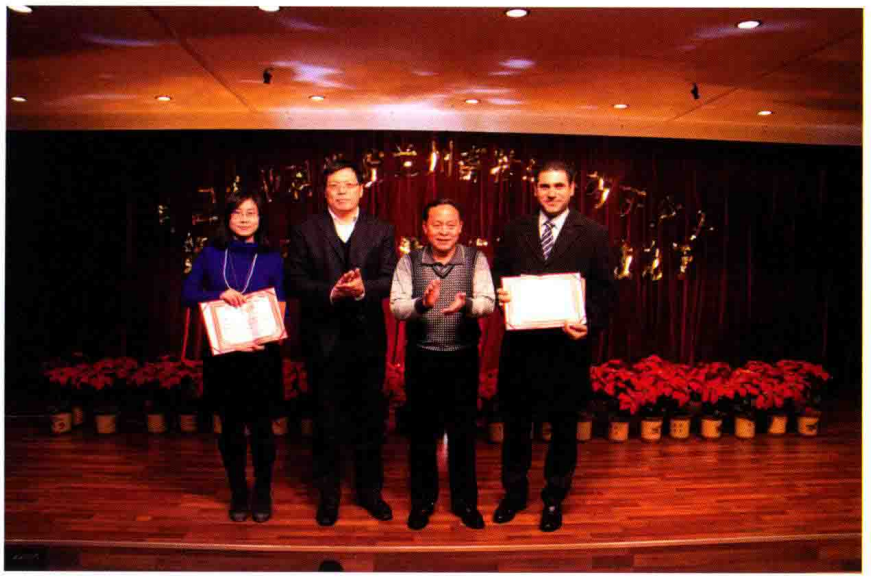

一等奖颁奖仪式

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序

今年，“第二届盛彤笙杯暨第三届青年科技论坛”如期顺利举办，本次论坛是研究所开展现代农业科研院所建设行动的重要活动之一，旨在培养青年科技人员的科研活力，激发年轻一代科技工作者献身农业科技的热情，提升科技创新能力，为青年人才展示自我、增进交流搭建平台。这也是继2008年建所50周年“第二届中青年科技论文暨盛彤笙杯演讲比赛”成功举办后的又一次青年科技交流盛会，本次活动有30位优秀青年学者和研究生提交了参赛作品，内容涵盖研究所牧草繁育、疾病防治、兽药创制、畜禽育种等学科领域，既有新的学术思想碰撞，又有最新的科研技术与成果的展示，经过专家遴选，13篇优秀论文推荐进行论坛演讲。演讲比赛精彩纷呈，全所科技人员欢聚一堂，选手们激烈角逐，评委们精彩点评，观众踊跃提问，为大家呈现了一场高水平的学术报告会。演讲赛最终评出一等奖2名、二等奖1名和三等奖3名。

“科技支撑发展，创新引领未来”。建所近六十年来，中国农业科学院兰州畜牧与兽药研究所坚持以研为本、以人为要，以推动我国现代畜牧业的发展为己任，紧紧围绕畜牧业生产中的重大科技问题，开展畜牧、兽药、兽医、中兽医、草业科学等科学领域的研究，为我国现代畜牧兽医科技创新、成果推广示范、畜牧兽医高层次人才培养和服务“三农”等做出了重要贡献。“十五”期间，研究所在农业部组织的“全国农业科研机构综合科研能力评估”中位列69。“十一五”以来，研究所通过全面改革、锐意创新和资源优化等使研究所的各项事业得到了蓬勃发展，2010年的“全国农业科研机构综合科研能力评估”中跃居44，在中国农业科学院排名11，甘肃省排名第1，研究所处在一个新的发展阶段。

站在新的起点上，面对新时期农业科技发展的新形势和新任务，按照建设世界一流研究所的总体要求，积极谋划和推进中国农业科学院创新工程试点工作。科技创新工程的实施不仅为青年科技人才成长提供了难得的机遇，也对年轻一代科研精神提出了新的更高的要求。我们举办“盛彤笙杯”青年科技论文演讲赛不仅是对我国著名的兽医学家、微生物学家、教育学家盛彤笙先生的缅怀，更重要的是激发青年一代传承

和发扬研究所的创始人“一代宗师一盛彤笙”的高贵的品格、渊博的学识、敬业精神和发展祖国兽医事业的历史担当，进一步弘扬研究所的科学精神，繁荣学术思想，展示科学风貌、树立创新理念。

创新是文明进步的不竭动力，是社会发展的智慧源泉。科技创新，人才是关键，建设国际一流的现代畜牧兽医科研院所需要有朝气、勇于探索的青年科技人才创新发展。近年来，中国农业科学院兰州畜牧与兽药研究所高度重视青年科技人才的培养，通过基本科研业务费项目对青年科技人员给予了稳定科研经费支持，为青年科技人才注入活力，一批优秀的青年科技人才渐露头角，在科研和技术创新方面发挥了积极的作用，日渐成为研究所科技事业发展的生力军和领头人。为此，我们期待着为研究所美好的明天而喝彩！

中国农业科学院兰州畜牧与兽药研究所所长

杨志强　研究员

2014. 8. 10

《秋实》编委会

目　录

Clinical investigations of nitric oxide, inhibin A and inhibin B and hormones profile of the infertility of dairy cows

（一氧化氮、抑制剂 A、B 和激素类对奶牛不育症临床研究）

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(Lanzhou Institute of Husbandry and Pharmaceutics Sciences of, CAAS, Lanzhou 730050, China)

Abstract: Unfortunately, there has been a decline in fertility in dairy cows over the last 10 ~ 40 years. The incidence of infertility of dairy cows has been correlated with changes in dairy cattle physiology and improvements in genetic progress, nutrition, and management practices, and important seek to identify physiological causes for this decrease in fertility.

To investigate clinically for the first time the level of the inhibin A and B and NO level in the infertile cows compare with normal cows taking in consideration the measurement of the hormones profile as attempt to illustrate the dependent parameters for treatment of the infertility in dairy cows in future.

Infertile and control cows used at follicular phase of estrus cycle, five hormone were measured in this study which are FSH, LH, E2, Testosterone, and prolactin using ELISA method. Inhibin A and B also measured by ELISA method. Nitric oxide in this study measured by Greiss reagent method.

The results showed difference concentrations of the hormone in which FSH illustrated higher concentration in the infertile cows (8.20 ± 2.10) ng/ml than the control cows (7.58 ± 2.22) ng/ml but no significant difference $P^{*} > 0.05$. while the LH and E2 showed significant decrease their concentrations in the infertile cows than the control cows $P < 0.05$, but the testosterone and prolactin were illustrated no significant difference $P > 0.05$ between the two group inspite of the increase the level of prolactin in the infertile caows compared to control cows. The both inhibins A and B showed markedly decrease concentration in the infertile cows, this decrease was significant $P < 0.05$ of both inhibins respectively. NO also showed clearly significant decrease $P > 0.05$ in the infertile cows than the control.

Infertility has adverse effects on the inhibin A, B and NO in dairy cows, there is relative correlation between the secretion of both inhibins and NO to regulate the hormones. The infertility increased with increasing the milk production.

Key words: dairy cow infertility; nitric oxide; inhibin A; inhibin B

1 Introduction

Dairy herd profitability is highly dependent on reproductive performance, since the latter directly af-

fects both milk production and the number of pregnant animals necessary to maintain herd size. Several parameters can be used to assess reproductive efficiency, emphasize the use of pregnancy rate as the single most important variable[1].

Fertility is defined as the ability of a cyclic animal to establish pregnancy and is an important economic trait that affects herd productivity in dairy cattle[2~3]. Unfortunately, there has been a decline in fertility in dairy cows over the last 10 ~ 40 years. Fertility, whether traditionally measured as conception rate (number of pregnant animals divided by the number of inseminated animals) or herd pregnancy rate (number of pregnant animals divided by the number of animals eligible to be bred), has declined in North America[4], Ireland[5], Spain[6], and the United Kingdom[7]. Other important reproductive measurements have changed during this time as well, including increases in days to first service, days to conception, and calving interval[8].

The incidence of infertility of dairy cows has been correlated with changes in dairy cattle physiology and improvements in genetic progress, nutrition, and management practices, and important seek to identify physiological causes for this decrease in fertility. The ovaries play the key roles in reproduction and any impairment in their functions can results in either sterility or infertility[9].

Reproductive inefficiency of dairy cattle causes great frustration for dairy producers [10]. Even under optimal conditions, the reproductive process is less than perfect because of the multiple factors involved in producing a live calf, to manage the complexities of the estrous cycle and the annual reproductive cycle, understanding of many interrelated physiological functions is critical, furthermore, reproductive efficiency involves successful management of not only the cows but also the people who milk, feed, house, inseminate, and care for them[11].

Inhibin is a heterodimeric glycoprotein that has two isoforms which consist of an α subunit and one of two β subunits (βA or βB).; inhibin A (α/βA) and inhibin B (α/βB)[12], the ovary synthesizes inhibin dimmers (inhibin A, a dimer of α and βA subunits; and inhibin B, a dimer of α and βB) that have FSH-suppressing activity[13]. Both subunits of inhibin secreted by granulose cells of the large follicle and theca cells, also the studies reported the α subunit of inhibin secreted by theca cells of the small follicles[14], other side, the previous studies demonstrated that inhibin B is high level in the early and mid-follicular phase but inhibin A showed increase in level in the late follicular and early luteal phase and this increase accompany with increase the LH surge secretion at the ovulation time.

Nitric Oxide (NO) is the highly reactive free radical, nitric oxide (NO), is synthesized from l-arginine by NO synthase (NOS), which catalyzes the mixed functional oxidation of a guanidino nitrogen atom of L-arginine to yield L-citrulline and NO[15], This molecule has diverse physiologic functions, including regulation of vascular resistance, participation in cellular injury, and signal transduction[16]. Evidence supports the involvement of NO in ovarian physiology[17]. NO is implicated in reproductive events such as ovulation, decidualization, and implantation[18]. During late pregnancy, NO is involved in the maintenance of a low vascular resistance, attenuating the action of vasoconstrictors[19].

The present study aimed to investigate clinically for the first time the level of the Inhibin A and B and NO level in the infertile cows compare with normal cows taking in consideration the measurement of the hormones profile as attempt to illustrate the dependent parameters for treatment of the infertility in dairy cows in future.

2 Materials and methods

Animals and sampling: the study was carried out in the lanzhou institute of husbandry and pharmaceutics sciences, CAAS, china in period from May 2012 to October 2012. A total number of cows were 50 Holstein cows include 26 infertile cows and 24 normal cows as control during the follicular phase days 17 ~ 19 of estrus cycle, these cows taken from three farms which are illustrated in (Fig) from different cities, the average of the offspring period of these infertile cows was eight months and 3. 2 month on normal cows respectively and the average age 5. 2 years on normal cows and 7. 1 years of infertile cows.

The blood samples taken from jugular vein in size of 10ml of each cow, then the blood kept in the sterile plane tube, then after, serum samples were aspirated by centrifugation the blood at 4℃. The serum of each cow divided into 4 tubes to avoid the repeated thawing during the measurement of parameters and kept at −20℃ until used.

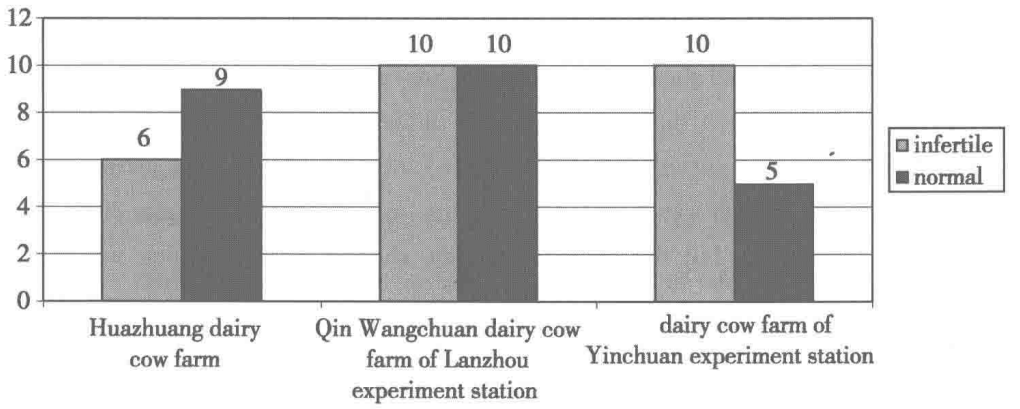

Fig Illustrate the farms and the numbers of the infertile and normal cows

2.1 Measurement of hormones

Five hormones were measured in this study using ELISA method which is Follicle-stimulating hormone (FSH), luteinizing hormone LH, Estradjon E2, and testosterone using kits produced by Abnova Company, Taiwan. The prolactin hormone PRL estimated using elisa kit purchased from (adlitteram diagnostic laboratories). The assay procedure was done according to manufacture's instructions presented with each kit. The results were recorded after construction standard curve of each hormone.

2.2 Measurement of Inhibin A (INHA) and Inhibin B (INHB)

Actually the levels of both INHA and INHB were determined using ELISA method using kit of bovine Inhibin A and B produced by AMEKO (shanghai yueyan biological technology Co., Ltd. -shanghai-china), 96 wells assay. The assay protocol was done according to manufacture's instructions. The results of the samples (serum) after establish the standard curve obtained by ng/L.

2.3 Measurement of the Nitric oxide (NO)

Nitric oxide in the present study was measured by Greiss reagent method using a kit presented by Promega Company (USA). According to the manufacture's instructions we prepared the standard dilutions to establish the standard curve, and the assay procedure as the following: allow the sulfanilamide solution and NED solution to equilibrate to room temperature (15 ~30min). Adds 50μl of each sample serum to each

wells in duplicate. Using the multichannel pipette, dispense 50μl of the sulfanilamide to each well. Incubate 5 ~ 10minutes at room temperature, protected from light. Dispense 50μl of the NED solution then incubate at room temperature for 5 ~ 10minutes alsó protected from the light, a purple/magenta color will begin to form immediately. Read the absorbance in the plate reader at 535 nm; calculate the results of nitrite concentrations of the samples after construction the standard reference curve.

2.4 Statistical analysis

The data are presented as the mean ± SD and these data analyzed using student *t*-test, to compare between the results of infertile cows and normal cows. A value pf $P > 0.05$ was considered to be statistically significant.

3 Results

3.1 Hormones results

The results of the hormones profile illustrate unstable levels that being significant some of them and non significant of the other hormones, all the results presented in Tab. 1.

Moreover, the results of FSH in the normal cows at follicular phase of estrous cycle was (7.58 ± 2.22) ng/ml, while the FSH concentration in the infertile cows revealed a little iccrease than that of the normal cows at the same period which was (8.20 ± 2.10) ng/ml, however, in spite of this difference concentrations between the two groups but the statistic analysis showed this difference was not significant $P > 0.05$.

While the LH level revealed a clear decreasing in the infertile cows compared with normal cow so results were (1.88 ± 1.15) ng/ml and (3.75 ± 1.27) ng/ml respectively, the independent t-test of the statistic analysis in LH levels revealed a significant decrease $P < 0.05$.

Same to the LH, the estradiol E2hormone was markedly decrease with significantly analysis $P < 0.05$, the levels of E2 in the infertile and control cows were (56.85 ± 15.7) and (80.69 ± 37.42) pg/ml respectively.

No significant differences $P > 0.05$ illustrated in the levels of both Testosterone and Prolactin hormone in the two groups, the levels of these hormone obtained in Tab. 2 represented by mean ± SD.

Tab. 1 Illustrate the results of the hormones level of the infertile and normal cows' values

Hormone	Infertile cows results (mean ± SD)	Normal cows results (mean ± SD)
FSH (ng/ml)	8.20 ± 2.10	7.58 ± 2.22
LH (ng/ml)	1.88 ± 1.15	3.75 ± 1.27
E2 (pg/ml)	56.85 ± 15.7	80.69 ± 37.42
Testosterone (pg/ml)	0.25 ± 0.12	0.28 ± 0.30
PRL (ng/ml)	45.58 ± 37.25	36.42 ± 23.89

3.2 Inhibin A and inhibin B results

Both inhibins A and B results which secreted by the ovary (granulose and theca cells) which measured for the first time in the dairy cows specially in infertility field revealed a nearly convergent levels of nor-

mal cows (Tab. 2), but in the infertile cows revealed markedly decrease level especially in inhibin B which showed more decrease than inhibin A. more specific, Inhibin A levels in the normal and infertile cows were (47.67 ± 15.18) ng/L and (32.65 ± 14.37) ng/L respectively, the statistical analysis confirm its significantly $P < 0.05$.

Whether Inhibin B showed sharply decrease in the infertile compared with normal cows so that results were (24.94 ± 4.18) ng/L and (43.60 ± 14.36) ng/L respectively. These decrease absolutely was significant because of $P < 0.05$.

Tab. 2 Illustrate the results of Inhibin A and Inhibin B of the normal and infertile cows

Test	Normal cows (mean ± SD)	Infertile cows (mean ± SD)
Inhibin A	(47.67 ± 15.18) ng/L	32.65 ± 14.37
Inhibin B	43.60 ± 14.36	(24.94 ± 4.18) ng/L

3.3 Nirtic oxide results

Like the inhibins, Nitric oxide used in present study of first time especially its important role in to enhance the fertility but now was investigate NO in the infertility cows. Actually the results of our study illustrated the NO level decreased significantly $P < 0.05$ of the infertile cows compared with counterparts of the normal cows, the NO results of the two groups observed in details in Tab. 3.

Tab. 3 Illustrate the results of the nitric oxide NO uM of infertile and normal cows

cows	Nitric oxide NO uM (mean ± SD)
Normal cows	15.90 ± 7.22
Infertile cows	9.52 ± 5.21

4 Discussion

As we referred in the introduction there is decline in the fertility in dairy cows over the last 10 ~ 40 years in the whole world, to improve reproductive efficiency, the limiting factors must be identified. In general, detection estrus is the major limitation to achieving a pregnancy[20]. There are many factors that involved in trigger the estrus and estrus signs. The present study depends to detect and determined the most effective factors as clinical and researching parameters according to the hormones that involved in the physiology of the ovarian functions to control estrus cycle and drawing the ovulation that announced the estrus time in dairy cows, also the present study depend the measurement the regulatory factors of hormones which are inhibin A and B, these factors were measured in this study for the first time in dairy cows infertility, combined with vascular endothelial factor (nitric oxide NO).

The results of the present study avoid the ovarian atresia because the testosterone illustrated a normal level and no difference changes in the level of testosterone in the infertile and normal cows, because the previous studies confirmed the increasing level of testosterone might contributed to enhanced atresia in 1 or 2 ovaries[21]. More ever, the present study showed a little disturbance in the prolactin hormone which appeared higher concentration in the infertile cows compared with normal cows, it was reported that fertility

reduced in the lactating cows as compared to non-lactating cows so that milk yield has increased over time as fertility has decline[22], the sampling information in this study observed that the cows in one farm milking 3 times per day and the other two farms were milking two times per day therefore these finding explain why the prolactin increased in the infertile cow more than control cows.

Continued to the milk production program, the present study showed low of plasma E2 concentration in the infertile cows than that control, this phenomenon agreed with previous study which approved that lactating cows have lower circulating progesterone and estradiol concentrations than heifers and dry cows[23]. Another interpretation of the disturbance of the hormones and inhibins in our study due to the milk production by the susceptibility to increase the heat stress in dairy cows due to the milking[24], and heat stress can compromise fertility throughout various reproductive processes such as oocyte developmental competence[25]. Indeed, follicular steroidogenesis, follicular dynamics and altered concentrations of FSH and inhibin become altered in response to heat stress[26]. The present study supported by the evidences above because the infertile cows showed higher level of prolactin than those normal cows which approved the correlation between the milk yields and induces infertility of dairy cow.

The present study clearly illustrated the difference in the levels of estradiol and inhibin between the two groups which decreased the concentration in the infertile cows; the previous studies confirm that the predominant secretion of inhibin in the circulation occurs during the follicular phase[27]. Plasma inhibin started to increase during the early follicular phase of animals whose ovaries contained several small antral follicles, whereas plasma estradiol showed a clear increase during the late follicular phase, a few preovulatory follicles were observed in the ovaries. These results support our hypothesis suggesting that inhibin is a chemical signal of the number of growing follicles in the ovary, and that estradiol is a signal of follicular maturation in the ovary in many other mammals[28~29].

The present study showed the higher FSH concentration in infertile cows and lower concentrations of both inhibin A, B, and estradiol whereas the opposite was observed in the normal cows, it was reported there is no relationship between the E2 and FSH concentrations during the luteal phase, also described the correlation between circulating inhibin A and plasma FSH levels and follicular dynamics throughout the pig estrous cycle. An increase in the concentration of plasma inhibin A occurred at the same time as the increase in the number of small follicles between the late luteal and follicular phases and during the early luteal phase[30]. More ever, it was indicated the increase of FSH at the late follicular phase referred to decrease the number of growing follicles on the ovary, in other hand the FSH stimulate the expression and secretion of inhibins from the granulose[31], our data showed high level of FSH and lower of inhibin A, B, and estradiol, to our knowledge this is the first report to describe the concentrations of inhibin A and B in infertility of dairy cows which indicated inhibin is effective marker that demonstrate the efficiency of the ovary of diary cows.

It was reported that inhibin B be higher concentration in early and decrease in the late follicular and early luteal phases[32] which indicate to be the key of regulation of FSH secretion and LH and referred to numbers of growing follicles and efficiency the endocrinology of ovaries, these finding support our finding which showed decrease the inhibin B in the infertile cows with increase FSH concentration compared with lower LH level than that normal cows.

Other side, the present study demonstrated the NO concentration in the infertile cows was lower than that of normal cows, this decreasing referred to many effects based on the major role of NO in ovary physiol-

ogy, so that our study agreed with previous studies that reported there is no correlation or effects of NO on the FSH secretion and the experiences confirm the inhibition of NO synthesis did not altered pulsatile FSH release[33], the same appeared in our study which illustrated low NO level with higher FSH concentration in the infertile cows compare the opposite in the normal cows respectively. The previous evidence supports the involvement of NO in ovarian physiology. NO is synthesized by the rat ovary and is postulated to participate in ovulation and atresia[34~35], Endothelial nitric oxide synthase protein was detected in granulosa and theca cells, as well as in blood vessels from primordial to antral follicles[36]. That means it's concentration relative to development of the follicles, also the control of ovarian vessel relaxation to accommodate the necessary change in blood flow, blood volume, and plasma exudation that accompany follicle rupture is likely to be the most important role of NO in the ovulation[37]. It is well known the role of the LH in the ovulation process, and there is large effect of NO on LH secretion, the previous findings indicated of inhibition of NO synthesis lead to decrease the plasma LH concentration and abolished LH pulse, this finding agreed and support with our results which showed low LH and NO concentrations in infertile cows compared to control cows, in addition the present study explain and approved there is correlation between the NO and inhibins (A, B) on the LH concentration also showed the adverse affect on infertility on these molecules.

In conclusion, the present study demonstrates the effect of infertility on the inhibin A, B and NO in dairy cows, also explains the role of these molecules to maintain the ovarian healthy and physiology, other one this study approved the new correlation between the inhibins and NO to regulate the hormones, and it referred to the effect of milk production to initiate the infertility in the dairy farms.

5 Acknowledgement

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