World Academy of Science, Engineering and Technology
International Journal of Animal and Veterinary Sciences
Vol:7, No:12, 2013

Investigation the Difference of Several Hormones Correlated to Reproduction between Infertile and Fertile Dairy Cows

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Open Science Index, Animal and Veterinary Sciences Vol:7, No:12, 2013 publications.waset.org/9996696/pdf

Abstract**—**The object of this study was to investigate several hormones correlated to the reproduction and inhibin A, inhibin B and NO levels in the infertile dairy cows as attempt to illustrate the physiological causes of dairy cows infertility.

40 Holstein cow (21 infertile and 19 fertile) were used at estrous phase of the cycle, Hormones FSH, LH, E2, Testosterone, were measured using ELISA method. inhibin A and B also estimated by ELISA method, Nitric oxide was measured by Greiss reagent method.

The results showed different concentrations of the hormone in which FSH illustrated significantly higher concentration in the infertile cows than fertile cows (P<0.05). LH and E2 showed significant decrease in the infertile cows than the fertile cows (P<0.05), no significant difference appeared in testosterone concentrations in the fertile cows and infertile cows (P>0.05). The both inhibins A and B showed significant P<0.05 decrease concentrations in the infertile cows also NO showed clearly significant decrease P<0.05 in the infertile cows.

In conclusion, the present study approved the poorly ovarian activities and reproduction disturbance of infertile cows in spite of trigger estrous signs, the study confirmed a positive correlation between inhibins and NO to regulate the ovarian physiology. These inhibins represent effective markers of dairy cow infertility.

Keywords**—**Cows, Inhibin (A, B), Infertility, Nitric oxide (NO).

I. Introduction

REPRODUCTIVE performance is one of the most important factor affecting dairy farm profitability, because it directly or indirectly influences the amount of milk produced, reproductive culling rate, and the cost for breeding and calf sales [1]. Dairy cows should calve one time every year to maximize economic efficiency but their reproductive function had declined obviously in the past twenty years [2], [3]. The studies reported the genetic selection for high production traits and advances in management practice have dramatically increased average milk yield per cow; however, a decline in reproductive performance has also puzzled animal scientists [4].

The ovaries play the key roles in reproduction and any impairment in their functions can results in either sterility or infertility [5]. Postpartum infertility is caused by four factors: general infertility, lack of uterine involution, short estrous cycles and anestrus. The general infertility component is common to any estrous cycle and reduces potential fertility by 20 to 30%. Although, most postpartum follicular development occurs normally in a wave-like manner in normal cyclic cattle [6], recent studies have revealed that postpartum anovulatory anestrus in dairy cows due to the failure of a dominant follicle to ovulate [2].

Inhibin is a heterodimeric glycoprotein which has two isoforms consist of an α subunit and one of two β subunits (βA or βB); inhibin found in two kinds according to the β subunits which are: inhibin A (α /βA) and inhibin B (α /βB) [7], the ovary synthesizes inhibin dimmers (inhibin A and inhibin B) that have FSH-suppressing activity [8]. Previous study revealed that both subunits of inhibins secreted by granulose cells and theca cells of the large follicle, also the studies reported the α subunit of inhibin secreted by theca cells of the small follicles, 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 [9].

Nitric Oxide (NO) is the highly reactive free radical, nitric oxide 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 [10], the recent studies illustrated this molecule has diverse physiologic functions, including regulation of vascular resistance, participation in cellular injury, and signal transduction [11]. Evidence supports the involvement of NO in ovarian physiology indicated that NO is implicated in reproductive events such as ovulation, decidualization, and implantation [12]. During late pregnancy, NO is involved in the maintenance of a low vascular resistance, attenuating the action of vasoconstrictors [13].

The present study aims to investigate several hormones correlated to the reproduction and inhibin A, inhibin B and NO levels in the infertile dairy cows as attempt to illustrate the physiological causes of dairy cows infertility.

II. Materials and Methods

A. Animals and Samples

The study was carried out in the Lanzhou Institute of Husbandry and Pharmaceutical Sciences, CAAS, in period from May to October 2012. 40 Holstein cows include 21

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Open Science Index, Animal and Veterinary Sciences Vol:7, No:12, 2013 publications.waset.org/9996696/pdf

infertile cows and 19 fertile cows as control during the estrous phase of estrus cycle to limit as possible as the hormones fluctuation were concerned in the present work, these cows taken from three farms as followings: 13 cows from Huachuang dairy cows farm of Lanzhou (6 infertile and 7 fertile); 14 cows from Qin Wangchuan dairy cows farm of Lanzhou experiment station (8 infertile and 6 fertile); and 13 cows from dairy cow farm of Yinchuan experiment station (8 infertile and 5 fertile), the infertile cows determined according to offspring period, so the average of the offspring period of these infertile cows was 8 months these cows were mounted by artificial insemination 4–5 times according the farms records and 3.2 months of fertile cows. The age averages were 5.2 years and 7.1 years of fertile and infertile cows respectively. 10ml of blood collected from jugular vein of each cow, then the blood transferred to the laboratory in ice to prevent hemolysis, serum aspirated by centrifugation the blood (3000 rpm for 10 minutes at 4°C) and kept at -20°C until use.

B. Measurement of Hormones

Four hormones were measured in this study using ELISA method which is Bovine Follicle-Stimulating Hormone (B-FSH), Bovine Luteinizing Hormone (B-LH), Bovine Estradiol (B-E2), and Testosterone using kits produced by Abnova Company, Taiwan. The assay procedure was done according to manufacturer's instructions presented with each kit. The results were recorded after construction a standard curve of each hormone.

C. Measurement of Inhibin A (INHA) and Inhibin B (INHB)

The concentrations 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 manufacturer's instructions. The results recorded after establish the standard curve obtained by ng/L.

D. 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). The assay protocol was done according to the manufacturer's instructions; briefly, prepare the standard dilutions to establish the standard curve, allow the sulfanilamide solution and NED solution to equilibrate to room temperature (15–30min). Add 50µl of each sample serum to each well in duplicate. Using the multichannel pipette, dispense 50µl of the sulfanilamide to each well. Incubate 5–10 minutes at room temperature, protected from light. Dispense 50µl of the NED solution then incubate at room temperature for 5–10 minutes also protected from the light, a purple/magenta color will begin to form immediately. Read the absorbance in the plate reader at 535nm; calculate nitrite concentrations of the samples after construction the standard reference curve.

E. Statistical Analysis

Microsoft Office Excel 2003 software used to analyze the data, which are presented as the mean ± SEM. Independent *t*-test was used to compare between the results of infertile cows and fertile cows. A value $P<0.05$ was considered to be statistically significant.

III. Results

A. Hormones Results

The results of the hormones profile revealed different concentrations that being significant in some and non significant of the other (Table I).

More clearly, FSH concentrations in the infertile cows revealed higher concentration than the fertile cows, the results were 9.13 ± 0.23 ng/ml and 6.87 ± 0.25 ng/ml respectively, this difference in the concentrations was significantly $P<0.05$.

While the LH concentrations decreased a clearly in the infertile cows compared with fertile cow so results were 12.25 ± 0.32 ng/ml and 17.60 ± 0.65 ng/ml respectively, the independent *t*-test of the statistic analysis in LH concentrations were statistically significant $P<0.05$.

Same to the LH, the estradiol E2 hormone was markedly decreased with significantly analysis $P<0.05$, the levels of E2 in the infertile and fertile cows were 49.70 ± 8.08 pg/ml and 82.53 ± 6.49 pg/ml respectively.

The results recorded that testosterone concentrations in the fertile cows 0.29 ± 0.07 pg/ml while the infertile cows was 0.27 ± 0.02 pg/ml, the statistical analysis of this difference showed it not significant $P>0.05$.

F. Inhibin A and Inhibin B Results

Both inhibins A and B results revealed nearly convergent concentrations of fertile cows but the opposite appeared in the infertile cows especially in inhibin B (Table I). More specific, inhibin A concentrations revealed markedly decrease in the infertile cows compared with fertile cows which were 33.75 ± 3.13 ng/L and 37.79 ± 5.15 ng/L respectively., this decrease was statistically significant $P<0.05$.

Whether inhibin B showed sharply decrease in the infertile compared with fertile cows so that results were 24.73 ± 0.8 ng/L and 47.60 ± 3.02 ng/L respectively, these decrease absolutely was significant because of $P<0.05$.

G. Nirtic Oxide Results

The results of NO concentrations of the fertile cows was 15.53 ± 1.42 uM, while the NO concentrations clearly decreased in the infertile cows which showed 9.76 ± 1.10 uM, the statistical analysis *t* test confirm a significant decrease $P<0.05$.

TABLE I
ILLUSTRATE THE RESULTS OF THE HORMONES, INHIBIN A, B AND NITRIC OXIDE CONCENTRATIONS OF THE INFERTILE AND NORMAL COWS (MEAN ± SEM)

Hormone	Infertile cow	Fertile cows
FSH (ng/ml)*	9.13 ± 0.23	6.87 ± 0.25
LH (ng/ml)*	12.25 ± 0.32	17.60 ± 0.65
E2 (pg/ml)*	49.70 ± 8.08	82.53 ± 6.49
Testosterone (pg/ml)	0.27 ± 0.02	0.29 ± 0.07
Inhibin A (ng/L)*	33.75 ± 3.13	37.79 ± 5.15
Inhibin B (ng/L)*	24.73 ± 0.8	47.60 ± 3.02
Nitric oxide NO (uM)*	9.76 ± 1.10	15.53 ± 1.42

* The difference between infertile and fertile cows is statistically significant for FSH, LH, E2, Inhibin A, Inhibin B, and NO (P<0.05).

IV. DISCUSSION

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 [14], [1]. The present study was designed to detect the most effective factors that involved in physiological ovarian functions; two parameters (inhibin A, B, and NO) were measured for the first time in field of infertility of dairy cows.

Our study confirmed there is no ovarian atresia lead to cause infertility or sterility of the infertile cows were participated in this study because of testosterone results illustrated a normal concentration in both infertile and fertile cows, according to the previous studies confirmed testosterone level increasing might contributed to enhanced atresia in 1 or 2 ovaries [15].

In general, both of estradiol and inhibins concentrations were decreased in the infertile cows, the previous studies approved the lactating cows have lower circulating progesterone and estradiol concentrations than heifers and dry cows due to increase the susceptibility to the heat stress because of milking, and heat stress can compromise fertility throughout various reproductive processes such as oocyte developmental competence [16], [17]. It was reported that inhibin is a chemical signal of the numbers of growing follicles in the ovary, and that estradiol is a signal of follicular maturation in the ovary in many other mammals [18]. The higher FSH concentration in infertile cows and lower concentrations of both inhibin A, B, and estradiol whereas the opposite were observed in the fertile cows in the present study, explained by Garrett et al. [19] who described the correlation between circulating inhibin A and plasma FSH levels and follicular dynamics throughout the 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. 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 [20], our data showed high level of FSH and lower of inhibin A, B and estradiol indicated inhibin is effective marker that demonstrate the efficiency of the dairy cows ovaries.

Other side, the present study demonstrated the NO concentrations of infertile cows were lower than the concentrations of fertile cows, this decrease referred to effects based on the role of NO in ovarian physiology, our study agreed with previous studies which 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 [21], endothelial nitric oxide synthase protein was detected in granulosa and theca cells, as well as in blood vessels from primordial to antral follicles [22], this means its concentration relative to development of the follicles, also the control of ovarian blood vessels relaxation to accommodate the necessary change in blood flow, blood volume, and plasma exudation that accompanies follicle rupture is likely to be the most important role of NO in the ovulation [23], that approved the lower concentration of NO in infertile cows due to the low number of developing follicles. 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, LH is brought about by pulsatile release of LHRH that is driven in turn by pulsatile release of norepinephrine and the latter acts on α1-adrenergic receptors to induced LHRH release from terminals of LHRH-secreting neurons in juxtaposition to hypophyseal portal capillaries in the median eminence, NO block lead completely the norepinephrine induced LHRH release, leading to decrease LH secretion and LH pusatile. These findings support our results which showed low LH accompanied with NO concentrations in infertile cows with the same effect inhibins on LH secretion and both are affected adversely in the infertile cows.

In conclusion, the present study approved the poorly ovarian activities and reproduction disturbance of infertile cows in spite of trigger estrous signs, the study confirmed a positive correlation between inhibins and NO to regulate the ovarian physiology. Inhibins represent effective markers of dairy cow infertility.

ACKNOWLEDGMENT

This research is supported by grants from NSFC (31072162) and China Agriculture Research System (CARS-37), special thanks the managers and employees of the three farms we mentioned above.

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