Investigation of negative energy balance and postpartum anoestrus in an intensive dairy farm from the Chinese province of Heilongjiang

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Abstract

The aim of this investigation was to determine the relationship between postpartum anoestrus and negative energy balance in an intensive dairy farm from the Heilongjiang Province, China. At 14 to 21 d after parturition, 100 cows were randomly selected and their plasma indices, including β-hydroxybutyric acid, non-esterified fatty acid, and glucose were measured. Cows were assigned to a positive energy balance group (n = 37) and a negative energy balance group (n = 36) based on their β-hydroxybutyric acid concentrations (> 1.20 mmol/l). The two groups of cows were examined by B-mode ultrasonography and rectal examination from 60 to 90 d after parturition to identify the ovarian status of oestrous and anoestrous animals. The incidences of negative energy balance and positive energy balance were 49 and 57%, respectively, from14 to 21 d after parturition. From 60 to 90 d after parturition, 94.4% of the negative energy balance group were in anoestrus and 5.6% were in oestrus, while 62.2% of the positive energy balance group were in anoestrus and 37.8% were in oestrus. Furthermore, the proportion of inactive ovaries in the negative energy balance group was 61.8%. In conclusion, the negative energy balance is an important factor causing inactive ovaries in high-yielding dairy cows.

Dairy cows, postpartum anoestrus, negative energy balance

Anoestrus is defined as displaying no obvious signs of oestrus and no functional ovarian activity from 60 to 90 d after parturition in dairy cows which should gradually display signs of oestrus as the body and genitals recover to their normal conditions (Montiel and Ahuja 2005). Postpartum anoestrus in cows is usually classified into physiological and pathological anoestrus. The former consists mainly of a silent oestrus or no oestrous signs, and the latter is true anoestrus without the ovarian cycle and ovulation (Breukink and Wensing 1998). The latter includes (1) inactive ovaries, (2) persistent corpus luteum (Mwaanga and Janowski 2000); (3) corpus luteum cysts; or (4) ovarian cysts.

In recent decades, there has been a dramatic change in the breeding mode and scale of dairy farms in the Heilongjiang Province. With the improvement of milk yield (MY), the incidence of reproductive disturbances in cows gradually increased. Up to a 50% incidence of postpartum anoestrus occurs in high-yielding cows (López-Gatius et al. 2008). The high incidence of postpartum anoestrus is related to the negative energy balance (NEB), which may cause body weight loss after parturition, which reduces the secretion of the follicle-stimulating hormone, leading to high rates of anoestrus and low conception rates (Staples et al. 1990). The occurrence of anoestrus in high-yielding cows caused by the postpartum NEB has become a common reproductive disturbance. Therefore, an investigation of postpartum anoestrus was conducted at an intensive dairy farm in the Heilongjiang Province to reveal its incidence and aetiology, and to provide scientific data useful for preventing postpartum anoestrus in dairy cows in the future.

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Materials and Methods

Experimental animals

This experiment was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (National Academies 1985). All experimental animals were treated according to the International Guiding Principles for Biomedical Research Involving Animals (Pan 2010).

All cows were obtained from an intensive dairy farm located in the Heilongjiang Province. All diets were in accordance with the Chinese standards for cattle breeding (Ministry of Agriculture of the People's Republic of China 2004). These cows were fed total mixed ration diets in different periods as shown in Table 1. Cows were housed in intensive farming conditions with continuous access to fresh water and were milked × 3 daily. From 14 to 21 d after parturition, 100 cows were randomly selected based on their β-hydroxybutyric acid (BHBA), non-esterified fatty acid (NEFA) and glucose (GLU) plasma concentrations. If the plasma concentration of BHBA was more than 1.20 mmol/l, GLU was less than 2.80 mmol/l, and NEFA was more than 0.50 mmol/l, then the cow was assigned to the NEB group. If these criteria were not met, then the cows were assigned to the PEB group. Cows with NEB or PEB were grouped into oestrus and anoestrus subgroups during the 60 to 90 d after parturition by observations of oestrous signs, ovary rectal examinations, B-mode ultrasonography observations and the lack of another contributing disease.

Table 1. Composition of total mixed ration (TMR) and nutrition levels in different periods.

TMR	Dry period	Transition period	Lactation-peak period	Normal lactation period
Concentrated feed (kg)	2.02	8.50	11.32	4.68
Silage maize (kg)	17.37	18.50	32.60	35.46
Leymus chinensis (kg)	4.20	-	-	-
High-quality hay (kg)	-	3.75	-	-
Wet corn (kg)	-	-	4.00	-
Silage alfalfa (kg)	-	-	-	6.00
Net energy (Mcal/kg DM)	1.30	1.74	1.66	1.42
Dry matter (%)	42.90	55.60	40.85	34.73
CP (%)	12.89	6.00	16.51	14.64
Fat (%)	2.83	5.60	5.98	3.14
NDF (%)	49.21	39.1	30.97	35.55
ADF (%)	27.47	20.3	16.63	22.76
Ca (g)	71.71	180.00	179.40	179.80
P (g)	42.75	116.00	89.70	89.95

ADF - acid detergent fibre, NDF - neutral detergent fibre, CP - crude protein, Ca - calcium, P - phosphorus.
"-" means that no corresponding substance was added.

Cows with inactive ovaries were not in oestrus and rectal examinations revealed that there were not large follicles or corpus luteum (CL). B-mode ultrasonography also showed a lack of large ovarian follicles. The width and length of ovaries were approximately 13.2 mm and 20.3 mm, respectively.

Persistent CL resulted in no oestrus, and rectal examinations revealed that there were protuberant CLs in bilateral ovaries having increased volumes. B-mode ultrasonography showed echoes of intermediate density in the luteal tissue and strong echoes in the trabecula of the ovary.

Corpus luteum cysts were diagnosed by rectal examination. Affected ovaries were larger than normal size and shaped similarly to an egg with vesicles of approximately 20 mm in diameter, with soft walls that fluctuated when touched. Using B-mode ultrasonography, protuberant swollen vesicles were found on the surface of ovaries. The diameters of the cysts were from 20 to 30 mm without normal CL.

Follicular cysts were diagnosed by rectal examination. They were characterized by one or more protuberant follicles with thin and smooth walls on the surface of single or bilateral ovaries, which were soft and enlarged. The diameters of the follicles could increase to between 30 and 40 mm. Using B-mode ultrasonography, a fluid dark round or ovoid inerratic echoless area was seen at the same site as the ovary and lasted for more than 10 d. The diameter of the dark area was greater than that in the mature follicle. There were one or more large follicles with diameters larger than 25 mm, and the cyst wall was less than 3 mm thick.

Sample collection and clinical data

During the 14 to 21 d and 60 to 90 d periods after parturition, blood was collected from the selected cows via jugular veins. The blood (10 ml) of each cow was placed into an anticoagulant tube with 3 to 5 drops of heparin sodium, and blood and anticoagulant were mixed evenly. Then, the anticoagulant blood was centrifuged at 1,500 × *g* for 5 min and 600 µl of supernatant was placed into a 1.5 ml Eppendorf tube. Afterward, they were centrifuged at 12,000 × *g* for 5 min and 500 µl supernatant was placed into a 1.5 ml Eppendorf tube. Finally, the tubes were stored at -80 °C until further use.

Using the management software Afimilk (Afimilk Agricultural Cooperative Ltd, Afikim, Israel), basic information on the experimental cows was collected including age, parity, body condition score (BCS), MY, daily amount of exercise, electrical conductivity, health status, reproductive status, and treatments. Kits, including BHBA ELISA kits, NEFA ELISA kits and GLU ELISA kits, were purchased from Biosino (Bio-Technology and Science Inc., Beijing, China).

Statistical analysis

Experimental data was analysed using independent-sample *t*-tests by SPSS 17.0. The final experimental data were presented as mean values ± standard deviation (x ± SD). Different lowercase letters represent significant differences at a $P < 0.05$ level; different capital letters represent highly significant differences at a $P < 0.01$ level.

Results

Postpartum energy balance

As shown in Table 2, of the 100 experimental cows, which might have other diseases, 73 cows were selected. Based on the plasma BHBA, NEFA and GLU contents, these cows were assigned to PEB and NEB groups. There were 37 cows in the PEB group, which was 51% of the total number of selected cows, and 36 cows in the NEB group, which was 49% of that the total. Among the cows of each group, there was no significant difference in age, parity, BCS or MY, whereas a comparison between groups revealed that the plasma GLU content in the PEB group was highly significantly higher, while both NEFA and BHBA were highly significantly lower compared to the NEB group.

Table 2. Clinical data and biochemical indices in dairy cows with NEB and PEB from 14 to 21 d after parturition.

Groups	NEB	PEB
No.	36	37
Incidence (%)	49	51
Age	3.12 ± 1.22	3.23 ± 1.21
Parity	1.89 ± 1.04	2.02 ± 1.04
BCS	2.76 ± 0.24	2.74 ± 0.39
MY (kg/d)	36.18 ± 9.62	34.04 ± 7.94
GLU (mmol/l)	2.66 ± 0.52^{A}	3.25 ± 0.51^{B}
NEFA (mmol/l)	0.77 ± 0.32^{A}	0.47 ± 0.23^{B}
BHBA (mmol/l)	1.42 ± 0.49^{A}	0.61 ± 0.25^{B}

NEB - negative energy balance, PEB - positive energy balance, No. - number, BCS - body condition score, MY - milk yield, GLU - glucose, NEFA - non-esterified fatty acid, BHBA - β-hydroxybutyric acid. Different lowercase letters represent significant differences at $P < 0.05$; Different capital letters represent highly significant differences at $P < 0.01$.

Postpartum anoestrus

Following the selected cows from 60 to 90 d after parturition, their anoestrous status was identified by rectal examination and clinical data. As shown in Table 3, among the 36 cows in the NEB group, 2 were oestrous and 34 were anoestrous cows; whereas among the 37 cows in the PEB group, 14 were oestrous and 23 were anoestrous cows. Thus, the incidence of anoestrous cows in the NEB and PEB groups were 94.4 and 62.2 %, respectively.

Table 3. Incidence of anoestrus and oestrus in dairy cows with NEB and PEB from 60 to 90 d after parturition.

Groups	Subgroups	No.	Proportion between groups (%)	Proportion within subgroup (%)
NEB	Oestrus	2	2.70	5.60
	Anoestrus	34	46.60	94.40
PEB	Oestrus	14	19.20	37.80
	Anoestrus	23	31.50	62.20
	Total		73	100

NEB - negative energy balance, PEB - positive energy balance, No. - number.

Table 4. Clinical data and biochemical indices in anoestrous NEB and oestrous PEB dairy cows from 60 to 90 d after parturition.

Groups	Anoestrous NEB cows	Oestrous PEB cows
No.	34	14
Age	3.16 ± 1.24	3.62 ± 1.34
Parity	1.94 ± 1.04	2.36 ± 1.15
BCS	2.75 ± 0.24[a]	2.96 ± 0.32[b]
MY (kg/d)	30.81 ± 8.77	33.18 ± 8.62
GLU (mmol/l)	3.32 ± 0.31[a]	3.64 ± 0.15[b]
NEFA (mmol/l)	0.33 ± 0.24	0.22 ± 0.14
BHBA (mmol/l)	0.62 ± 0.18[a]	0.48 ± 0.15[b]

NEB - negative energy balance, PEB - positive energy balance, No. - number, BCS - body condition score, MY - milk yield, GLU - glucose, NEFA - non-esterified fatty acid, BHBA - β-hydroxybutyric acid. Different lowercase letters represent significant differences at $P < 0.05$; Different capital letters represent highly significant differences at $P < 0.01$.

As shown in Table 4, from 60 to 90 d after parturition, compared with the oestrous PEB cows, the age, BCS, parity and MY were not significantly different from those of anoestrous NEB cows, but the BCS was slightly lower. Compared with the oestrous PEB cows, the plasma NEFA content was not significantly different from that of the anoestrous NEB cows, but the GLU content was significantly lower and the BHBA content was significantly higher, although below 1.20 mmol/l.

Table 5. Incidence of anoestrus in dairy cows with NEB from 60 to 90 d after parturition.

Classification	No.	Incidence (%)
Inactive ovary	21	61.80
Persistent corpus luteum	7	20.60
Corpus luteum cyst	1	2.90
Follicular cysts	1	2.90
Other reasons	4	11.80
Total	34	100

No. - number

Classification of anoestrus

As shown in Table 5, the 34 anoestrus NEB cows had the type of anoestrus classified based on rectal examinations and B-mode ultrasonography. Only one cow had a CL cyst and one cow had a follicular cyst. The percentages of cows with an inactive ovary or persistent CL were 61.8 and 20.6%, respectively.

Discussion

During the breeding and production of cows, there are many common factors that can reduce the cows' reproductivity including genetic improvement, inadequate nutrition, poor reproductive management, an increased incidence of disease (Lucy 2001). It is also becoming gradually clear that good reproductive performance is dependent on the optimum nutrition of the cow. Due to the nutritional requirements of cows depending on the varying physiological state and specific nutritional demands (Boland et al. 2001; Overton and Waldron 2004), we should pay more attention to nutrition management. Dry matter intake and energy balance during the dry period and transition period have a vital influence on the reproductive efficiency of cows. They affect endocrine indices, metabolic hormones, and concentrations of key metabolic factors. Management of BCS is a critical component of cows' nutrition management (Roche 2007).

When cows have a NEB and are undergoing weight loss (Butler and Smith 1989; Wathes et al. 2007), the secretion of luteinizing hormone (LH) is reduced, which enhances the negative feedback of oestradiol, reduces the diameter of active follicles, and causes a high risk of anoestrus with a low successful conception rate (Garverick 1997; Dunn and Kaltenbach 1980). The time of the first oestrus cycle is closely related to the time of the NEB's nadir in cows after parturition (Butler 2001). Cows that quickly regain PEB after a NEB attain oestrus and conception earlier.

From 14 to 21 d after parturition, 73 of the 100 cows on the dairy farm were disease-free and selected for further study. Among the selected cows, the proportions of PEB and NEB were 51 and 49%, respectively. Thus, the NEB phenomenon was common on the dairy farm. From 14 to 21 d and from 60 to 90 d after parturition, 22 and 78% of the total number of cows were in oestrus and anoestrus, but 87.5% of the oestrous cows were in the PEB group and 94.4% of the anoestrous cows were in the NEB group. Thus, there was a significant correlation between NEB and postpartum anoestrus in cows. Additionally, the status of the energy balance after parturition had a great effect on oestrus in cows. Therefore, intensive dairy farms should monitor the status of the energy balance in high-yielding dairy cows after parturition and take effective actions to regulate and control nutrition, which could reduce the risk of NEB (LeBlanc 2010), ensure normal oestrus and increase the overall breeding efficiency.

From 60 to 90 d after parturition, the age, BCS, parity and MY of the anoestrous NEB cows were not significantly different from the oestrus PEB group, but the BCS was slightly lower. Thus, cows with low BCS were more likely to become anoestrous. Compared with the oestrus PEB cows, the plasma NEFA content was not significantly different from that of the anoestrus NEB cows, but the GLU content was significantly lower and the BHBA content was significantly higher, although below 1.20 mmol/l. Thus, from 60 to 90 d after parturition, cows with NEB basically regained normal energy status, but the earlier NEB could influence normal oestrus. After parturition, the MY of lactating cows gradually increases while the energy and nutrients required for lactation also gradually increase (Butler 2000). When lactating cows are in the state of NEB, to quickly meet the increased energy demand for lactation, cows must use their energy reserves. This negatively affects the cow's body condition, which decreases the secretion of LH, and the GLU and insulin concentrations in the plasma are also reduced (Veerkamp et al. 2003). Because of its influence on the follicle cell development, ovulation is prevented, leading to infertility. When lactating cows are in the state of NEB, there is the obvious physicochemical characteristic of a slightly lower GLU concentration. Because of the low energy intake and high energy output, the GLU concentration could not be kept constant in cows, leading to hypoglycaemia. Long-term hypoglycaemia could influence the secretion of gonadotrophin, leading to the occurrence of anoestrus, low pregnancy rates, and the hypofunction of the ovaries and uterus. Low GLU was related to poor fertility and could result in a lack of body energy, which had an effect on oestrus, ovulation, and reproduction-related hormone concentrations. Low GLU could also influence the development and maturation of oocytes, while NEB could influence the quality of the oocytes in cows (Beam and Butler 1999). The growth and development of follicles and ovulation of oocytes would be influenced by insufficient energy available.

Using B-mode ultrasonography and rectal examinations, inactive ovaries and persistent CL occurred in 35.5 and 20.6%, respectively, in anoestrous NEB cows. Only a single cow had CL cysts, and the same was true for follicular cysts. Inactive ovaries show no change in the ovary size, which is elastic, without follicles or CL. If the ovaries become small, hard and lack elasticity, then ovarian atrophy has occurred (Kumi-Diaka et al. 1981; Van Straten et al. 2008). When lactating cows are in a NEB state, the secretion of gonadotrophin releasing hormone and LH is restrained, leading to a shortage of these hormones (Canfield and Butler 1990). Therefore, the reactivity of ovaries to LH is reduced, restraining the development of dominant follicles, which leads to oestrus without ovulation or eliminates the cyclic activity (Butler 2003; Peter et al. 2009). However, specific physiological mechanisms of cows with NEB that result in inactive ovaries need to be studied further.

By investigating energy balance in oestrous and anoestrous cows, combined with clinical data and biochemical indices, the phenomenon of NEB-associated anoestrus after parturition was determined to be common in dairy farms and it severely influenced the health and reproductive performance of the farm's cows. The NEB was an important factor in the inactivation of cow ovaries. However, the specific physiological mechanisms should be explored further.

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