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EFFECT OF ALTERING DIETARY CATION-ANION DIFFERENCE, ENERGY AND PROTEIN INTAKE ON THE POSTPARTURIENT OVARIAN REBOUND

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ABSTRACT

The effect of nutrition on the occurrence of reproductive disorders differs according to the plane of nutrition, type and sources of nutrients, as well as the managemental status of the animals. This investigation was carried out on 70 pregnant Holstein Friesian cows aged 3-6 years old. The cows were divided into 2 groups. Group A, included 35 cows and were fed a properly formulated ration 28.00 Kg/ head /day for dry pregnant cow with a reduced dietary cation- anion difference (DCAD). Group B, included 35 cows and were fed 26.00 Kg formulated ration/ head/day for a dry pregnant cow with a slightly elevated DCAD. The cows in both groups were observed until parturition and after parturition they were examined ultrasonographically twice weekly for 9 weeks to follow up uterine involution and ovarian findings. Data concerning the postpartum reproductive performance (uterine involution, first estrus, open days, and conception rate) were recorded. Pregnancy diagnosis was done rectally 60 days post breeding using a proved fertile bull. Blood samples were taken twice weekly for 9 weeks, beginning 7 days postpartum for progesterone assay.

Serum progesterone level showed a significant rise in animals in group A than those in group B starting from the second week postpartum.

Animals in group A showed a significant ($p < 0.01$) reduction in days from calving to uterine involution (23.65±2.54 Vs. 37.67±4.65) as indicated by ultrasonographic examination, days to first estrus (58.87±6.54 Vs. 87.98±9.82) , number of services per conception (1.67±0.54 Vs. 3.21±0.87) and open days (87.76-9.75 Vs. 122.63±3.65). Group A showed a higher conceptuion rate than group B (80% Vs. 54.25%).

It could be concladed that careful adjustment of the rate of anionic salt supplementation in dry cow diets may support proper functioning of both Ca and energy regulatory system so that optimal health is achieved in the peripartum period.

INTRODUCTION

The effect of nutrition on the oecurrence of reproductive disorders differs according to the plane of nutrition, type and sources of nutrients as well as the managemental status of the animals.

Although the plane of nutrition and nutritional deficiency in addition to the managemental status of the animals play an important role in the reproductive performance. the ignorance of dairymen about the nutrition and management of their herds specially during late gestation period and early postpartum period may lead to the appearance of some reproductive disorders as abortion. stillbirth. vaginal prolapse. dystocia. retained placenta. metritis. cystic ovary. mastitis. claw affections and metabolic disorders as milk fever. Association among these reproductive disorders has been widely studied in dairy cattle **(Breden and Odegaard, 1996; Calavas et al., 1996 and Gant et al., 1998)**.

Besides tremendous changes in energy and protein flux around the time of calving. periparturn cows also experience large changes in mineral element dynamics. Daily body turnover of calcium changes from 10g in non lactating cows to greater than 30g in lactating cows **(Horst et al., 1997)**. Almost 70% of the affected multiparous cows in three commercial farms in Florida, Colorado and Wisconsin suffered from clinical or subclinical hypocalcaemia at calving. although only 80% exhibited clinical hypocalcaemia. i.e. milk fever **(Beede 1995)**. Because calcium has a role in smooth muscle function hypocalcaemia at calving is a predisposing factor for dystocia, prolapsed uterus. retained placenta and early metritis **(Grohn et al., 1989)**.

Decreasing the dietary cation-anion differenee (DCAD: milliequivalents [(Na+K) - (Cl+S)] / 100g of dry matter "DM") during the last 3-4 weeks before calving can have beneficial effects on systemic acid-base status, Ca metabolism, peripartum health and postpartum productive and reproductive performance **(Horst et al., 1994)**.

To reduce the severity of hypocalcaemia in peripartum cows, anionic salts are sometimes fed to cows during the dry period **(Horst et al., 1997)**. Anionic salts are defined as salts that are higher in negatively charged fixed anions Cl and S relative to the positively charged cations Na and K. These four ions are important components in the equation for dietary cation-anion difference (DCAD) **[Gant et al., 1998]**.

High crude protein (CP) intake has been implicated in increasing days to first ovulation due to interactions with reproductive health disorders **(Carroll et al., 1988)**.

Two gross factors have been linked to the number of days between calving and normal ovarian activity of healthy cows, amount of nutrients delivered for the cows and quantity of milk produced (**Whitmore et al., 1974**). Estimating energy balanee eombines these two factors into a possible assessment of the homeorrhetic state of the animal. Using 13 cows **Butler et al., (1981)** found that energy balance during the first 20 days of lactation was related inversely (-0.60) to days to normal ovulation.

The purpose of this study was to evaluate the efficiency of altering DCAD, energy and protein intake on some postpartum reproductive performance of dairy cows.

MATERIAL AND METHODS

Animals : This study was carried out on 70 pregnant Holstein Friesian cows in a private farm, Dakahlia Governorate, Egypt. These animals aged 3-6 years old and were kept in an open yard. All cows were received circular prophylactic treatment against internal and external parasites, in addition to vaccination against the endemic diseases. The eows were milked mechanically three times daily. The animals were allocated into two experimental groups.

Group A : included 35 cows and were fed a properly formulated ration 28.00kg/head/day for dry pregnant cow (table 1). The formulation of ration was done according to the National Research Council **(NRC, 1989)**.

Group B : included 35 cows and were fed 26.00kg formulated ration/head/day for dry pregnant cow (table 1).

The feeding ration analysis: All basal feed ingredients offered to the investigated animals were analyzed (table 2) according to **Willis (1960)**.

Dietary cation - anoin difference (DCAD): The inter relationship among dietary microminerals (DCAD) was evaluated in the basal diet of the dry and lactating cows according to **Byers (1992)**.

The cows under investigation were observed until parturition. After parturition all cows were examined ultrasonographieally twice weekly for 9 weeks to follow up uterine involution and ovarian findings. Ultrasongraphic examination was conducted by real-time B- and M-mode ultrasonography using linear rectal 5 MHz according to **Pierson and Ginther (1988)** until complete uterine involution was achieved. Ovaries were examined according to **Pierson and Ginther (1988)** using transrectal 7.5 MHz probe to identify only 2mm follicles which could be monitored clearly due to the resolntion of the used apparatus used (Ultrascan 50, Mitsubishi, Japan) which equipped with a printer (Sony, Japan).

Data concerning the postpartum reproductive performance (uterine involution, first postpartum estrus, days open, number of services per conception and conception rate) were recorded.

Pregnancy diagnosis was done reetally 60 days post breeding using a proved fertile bull.

Blood sampling: Blood samples were obtained from each cow by puncture of the jugular vein twice weekly for 9 weeks starting 7 days postpartum. The blood samples were centrifuged at 3000 r.p.m. for 20 minutes and only clear non-hemolysed sera were harvested and kept at $-20^{o}C$ until progesterone was measured aeeording to assay system deseribed by **Naber et al. (1999)**.

Statistical analysis : The results were represented as mean ± SD. Statistical analysis of the results was conducted according to **Snedecor and Cochran (1982)**.

RESULTS

The dietary components for dry pregnant cows as well as DCAD are presented in table 2. The table showed that the dietary concentrations of crude protein offered to cows was 11.34 and 14.97%, the net energy (Mcal/Kg) was 1.34 and 1.68, the calcium was 0.35 and 0.94% and the phosphorus was 0.29 and 0.43% for dry pregnant cows in groups A and B, respectively. The DCAD of diet offered to dry pregnant cows was 278 and 358 meq/Kg in groups A and B, respectively.

Table 3 showed serum progesterone level in A and B groups during 9 weeks postpartum. The results showed significant variations among the 2 groups starting from the 2nd week postpartum.

Table 4: presented the effect of DCAD, energy and protein intake on the postpartum reproductive performance. The postpartum reproductive performance showed a significant ($P<0.01$) variation between both groups. Cows in group A exhibited a significant ($P<0.01$) reduction in days from calving to uterine involution (Fig. 1 and 2), days to first estrus (Fig. 3), numbers of services per conception and open days. Moreover, the conception rate was greatly higher in cows of group A than in B (80% Vs. 54.29%).

DISCUSSION AND CONCLUSION

After calving and during the period of milk production and energy deficiency, the uterus, ovary, hypothalamus and pituitary of cows undergo a process of recovery and rebuilding for establishment of subsequent pregnancy. This period is characterized by hormonal maturation of the pituitary and hypothalamus **(Nett, 1987)**, morphological and histological changes in the uterus **(Marion and Gier, 1968)** and establishment of new follicular populations on the ovary leading to first ovulation **(Savio et al., 1990)**. Magnitude of energy deficiency (negative energy balance) seems to affect these processes of follicular growth and development leading to first ovulation. This may occur by a combination of events associated with negative energy balance including changes in circulating concentration of hormones and metabolites as well as interaction of higher brain centers with the hypothalamus and pituitary. The end result is that cows in most negative balance are most likely to remain anestrus **(Staples and Thatcher, 1990)**.

Butler et al., (1981) found that postpartum cows have first ovulation about 10 days after they have reached their most negative energy balance. Therefore, the sooner postpartum cows being consuming enough feed "so that they begin to return toward true energy balance" the sooner they will have their first ovulation and being cycling.

Villa-Gody et al., (1988) found that progesterone concentration of the first estrous cycle after calving were similar for cows experiencing different levels of negative energy balance. However, by the 2nd and 3rd estrous cycles, cows have greatest negative energy balance had also the lowest progesterone concentration.

Britt (1992) showed that there is no immediate effect of negative energy balance on progesterone secretion by the corpus luteum. At least 40-60 days are required before progesterone is reduced.

Fertility of cows that experince sever negative energy balance during early lactation may be compromised partially by reduced steroidogenic capacity of the corpus luteum that develops later in lactation.

Progesterone production by the corpus luteum indirectly causes ovulatory follicles to stop growing and gradually disappear, since continued growth of these follicles can not be supported by low concentrations of LH caused by progesterone suppression. This functional turnover of follicles ensures emergence of a new healthy follicle at time of corpus luteum regression and optimizes for good reproductive performance.

Under conditions of low serum progesterone concentrations, the dominant follicles does not undergo regression but instead continues to grow. In our investigation, the serum progesterone concentration rise and become greater than 1ng/ ml from the 4th week postpartum. This occurred only during the 7th week in group B. The increase in progesterone concentration in group A was followed by sustained increase during the next weeks whereas in group B the increase in progesterone level in weeks after the 7th week was undetectable and gradual. This indicates that even through the cows in group A have a lower energy balance and protein, they return earlier to cyclicity than cows in group B.

This may be attributed to the reduced DCAD. Decreasing the dietary cation-anion difference "DCAD" during the last 3 to 4 weeks before calving can have beneficial effects on systemic acid-base status, Ca metabolism, peripartum health and postpartum productive and reproductive performance **(Oetzal et al., 1988 and Tucker et al., 1991)**.

Also, **Oetzel et al., (1988)** found that dietary Ca intake did not affect serum concentration of inorganic calcium (iCa), but that higher dietary Ca concentration with negative DCAD lowered the incidence of hypocalcaemia.

Byers (1992) recommended that the reduced DCAD of diets for dry cows, reduced the incidence of milk fever. **Beede (1992) and Oetzel et al.,(1988)** reported that serum concentrations of ionized Ca for cows fed diets supplemented with anionic salts were higher than cows fed control diets, presumably because of the mobilization of Ca from the intestinal tract and its efficient absorption. These responses are claimed to eliminate clinical and subclinical hypocalcaemia in parturient cows and consequently to reduce the uterine involution period, days to first estrus and days open and hence to improve the conception rate & reducing the number of services required for each conception **(Beede 1992)**.

Thus it could be concluded that careful adjustment of the rate of anionic salt supplementation in dry cow diets support proper functioning of both Ca and energy regulatory systems so that obtimal health is achieved in the peripartum period.

Table (1): Composition of total mixed ration (TMR) for dry pregnant cows.

Ingredient (kg)	TMR (Kg) on feed basis	
	Group A	Group B
Forages:		
• Barseem	3.07	8.00
• Corn silage	13.06	10.23
• Wheat straw	7.52	--
• Barseem hay	--	1.00
Concentrates:		
• Corn grain	1.00	2.70
• Corn gluten	0.50	0.50
• Soy bean meal	1.08	1.02
• Linseed meal	0.75	0.46
• Cotton seed meal	--	0.46
• Whole cotton seed	0.50	0.46
• Molasses	0.50	0.90
• Protected fat (1)	--	0.09
• Lime stone	--	0.06
• Sodium dibasic Phosphate	--	0.06
• Sod. chloride		
• Sod. Bicarb.	--	0.02
• Vitamin premix (2)	--	0.02
• Micromineral premix (3)	0.01	0.01
	0.01	0.01
Total feed intake (Kg)	**28.00**	**26.00**

(1) Protected fat (magnabac): prepared by Norel Company, Spain.

(2) Vitamin premix: prepared by Multivita Company to provide 6000 i.u. Vit. A., 1250 i.u. Vit. D3 and 20 mg vit E. provide.

(3) Micromineral premix: prepared by Multivita Company to provide 50 ppm Zn, 45ppm Mn, 40ppm Fe, 10ppm Cu, 0.6ppm I, 0.3 Se and 0 2 ppm Co.

Table (2): Dietary component and dietary cation-anion difference (DCAD) for dry pregnant cows.

Nutrient %	Group A	Group B
Crude protein (%dry matter)	11.34	14.97
Undegradable protein intake.	31.37	34.14
Degradable protein intake	66.88	65.02
Total digestable nutrients	60.45	69.67
Net energy (Meal/Kg)	1.32	1.68
Neutral detergent fiber	54.38	34.28
Acid detergent fiber	33.47	21.81
Non fibrous carbohydrates	22.22	38.11
Calcium	0.35	0.94
Phosphorus	0.29	0.43
Magnesium	0.21	0.26
Potassium	1.32	1.47
Sodium	0.11	0.25
Chloride	0.23	0.42
Sulphur	0.21	0.20
DCAD (meg/Kg)	**278**	**358**

Table (3). Effect of DCAD, energy and protein intake on serum progesterone level.

Weeks	Progesterone level (ng/ml)	
	Group A	Group B
1[st] w	0.518 ± 0.231	0.585 ± 0.275
2[nd] w	0.567 ± 0.243*	0.476 ± 0.223
3[rd] w	0.580 ± 0.240**	0.675 ± 0.228
4[th] w	1.047 ± 1.546*	0.623 ± 0.227
5[th] w	1.410 ± 1.364**	0.534 ± 0.201
6[th] w	2.251 ± 1.532**	0.819 ± 0.639
7[th] w	3.768 ± 1.559**	1.031 ± 1.126
8[th] w	2.469 ± 1.461**	1.432 ± 1.451
9[th] w	2.493 ± 1.301**	1.913 ± 1.300

* Significant variation at P< 0.05

** Significant variation at P< 00.1

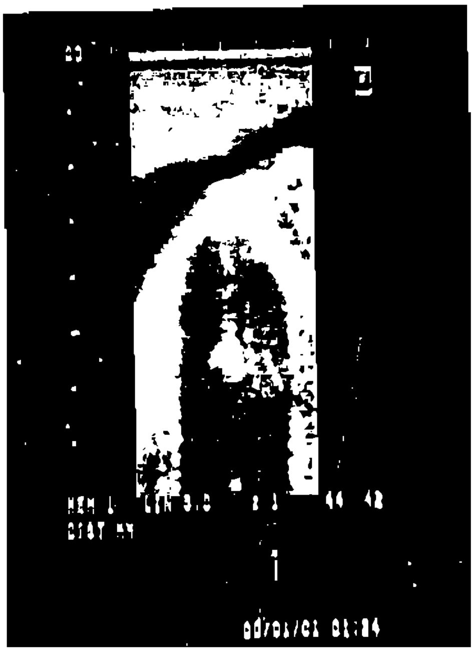
Table (4): Effect of DCAD, energy and protein intake on postpartum reproductive performance.

Parameter	Group A	Group B
Days from calving to uterine involution	23.65 ± 2.54**	37.67 ± 4.65
Days to first estrus	58.87 ± 6.54**	87.98 ± 9.82
Numbers of services per conception	1.67 ± 0.54**	3.21 ± 0.87
days Open	87.76 ± 9.75**	122.63 ± 3.65
Conception rate (%)	80.00 (n=28)	54.29 (n=19)

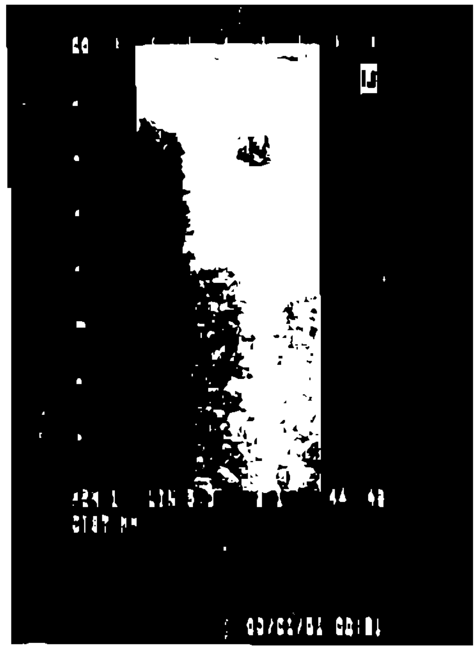
Data are expressed as Mean ± S. D.

n = number of pregnant cows

** Significant variation at P< 0.01.

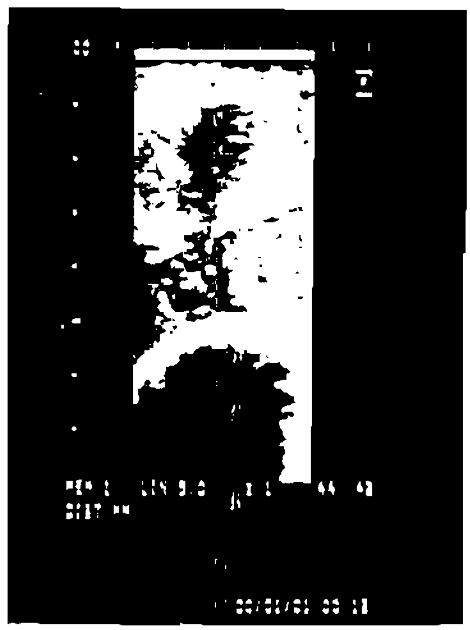


(A) First week postpatum.



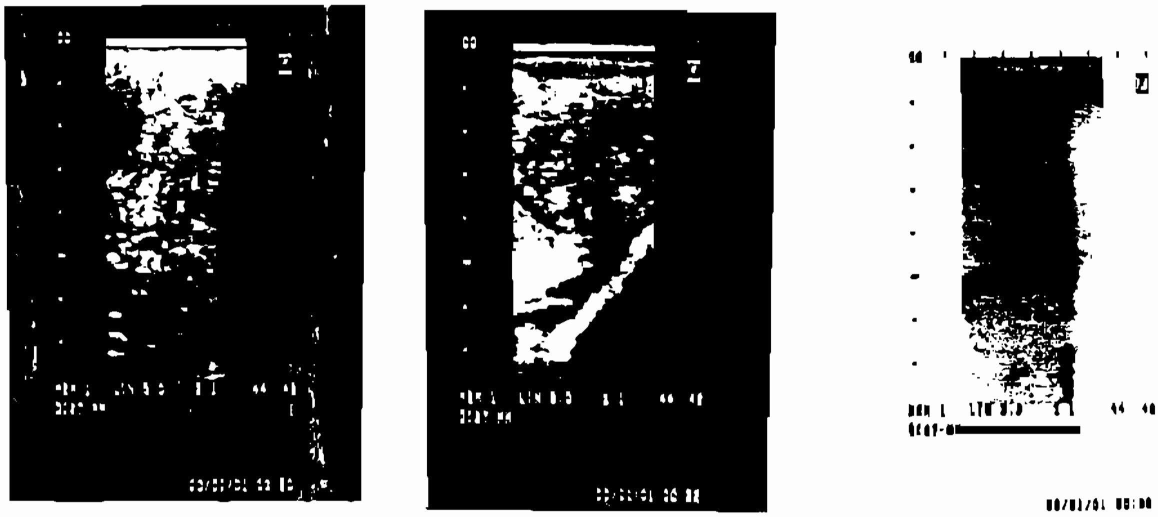
(B) Second week postpatum.

(C) Third week postpatum.

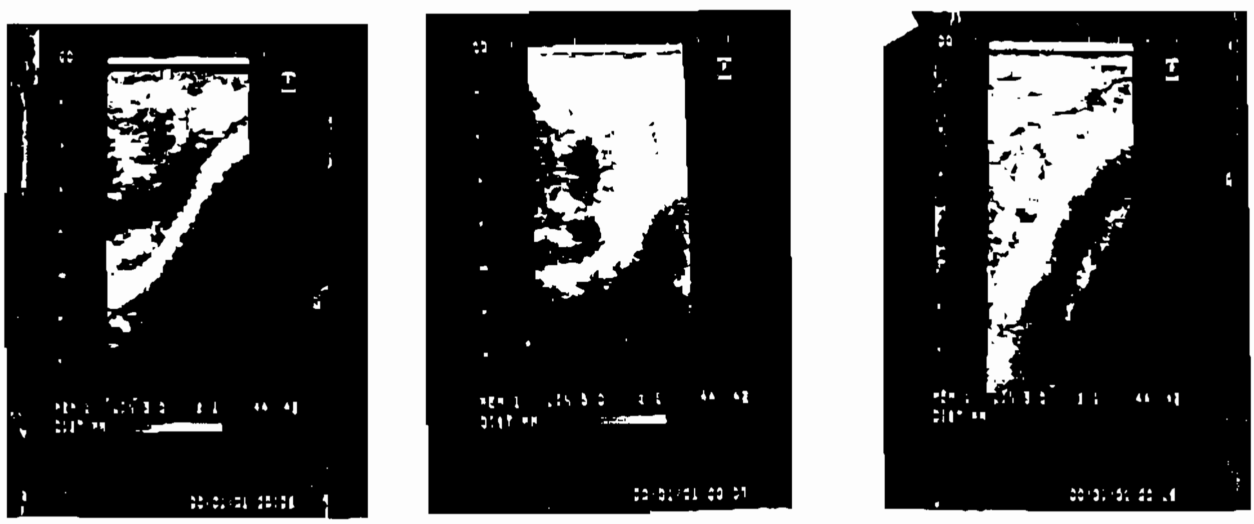


(D) Fourth week postpatum.

Figure 1 : Ultrasonographic images from calving till complete uterine involuation in cows of the group (A). Note the echogenicity of uterine wall and uterine contents.



(A) First week postpatum. **(B)** Second week postpatum. **(C)** Third week postpatum.



(D) Fourth week postpatum. **(E)** Fifth week postpatum. **(F)** Sixth week postpatum.

Figure 2 : Ultrasonographic images from calving till complete uterine involuation in cows of the group (B). Note the delayed decrease in uterine wall thickness than cows in group (A).

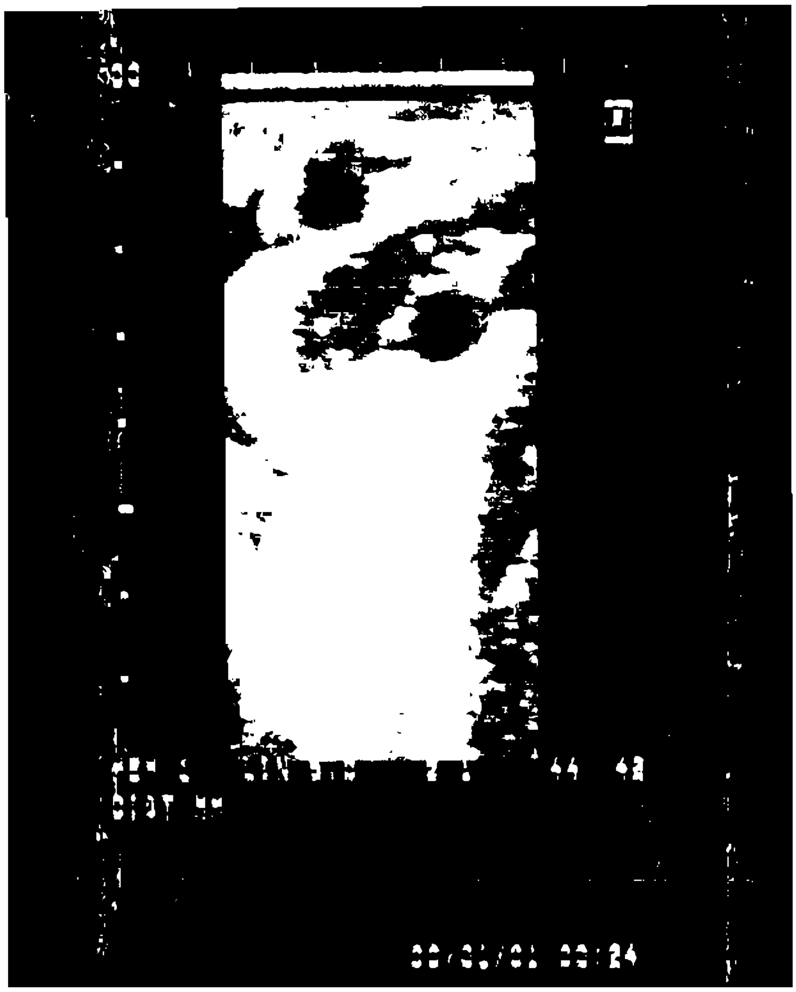


Figure 3 : Hypoechoic growing follicle upon the ovary few days before estrus in both groups.

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الملخص العربي

تأثير التغير فى الفارق الأنيونى والكاتيونى الغذائى والطاقة والبروتين المعطى على إستعادة المبيض لنشاطه بعد الولادة

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يختلف تأثير التغذية على حدوث بعض المشاكل التناسلية تبعاً لمستوى الغذاء، نوع ومصادر المواد الغذائية وكذلك تبعاً لحالة رعاية الحيوانات.

أجريت هذه الدراسة على ٧٠ بقرة فريزيان هوليشتين تتراوح أعمارها بين ثلاث - لست سنوات، وقد قسمت هذه الأبقار إلى مجموعتين. المجموعة (أ) وتتضمن ٣٥ بقرة وقد تم تغذيتها على عليقة كونت بدقة حيث تم إعطاء كل بقرة عشار جافة ٢٨كجم يومياً من العليقة المقلل بها الفرق الأنيونى والكاتيونى الغذائى.

المجموعة (ب) وتتضمن ٣٥ بقرة تم تغذيتها بإعطاء كل بقرة عشار جافة يومياً ٢٦كجم من العليقة ذات الزيادة القليلة فى الفرق الأنيونى والكاتيونى الغذائى. وتم ملاحظة الأبقار فى المجموعتين حتى الولادة، ثم بعد الولادة تم فحصها باستخدام جهاز الموجات فوق الصوتية مرتين إسبوعياً لمدة ٩ أسابيع حتى نتتبع عودة الرحم إلى حجمة قبل الولادة والتغيرات فى المبيض حتى يستعيد نشاطه، وتم رصد البيانات الخاصة بالكفاءة التناسلية بعد الولادة (عودة الرحم لحجمه الطبيعى ليصل تقريباً لمثل حجمه قبل الولادة - أول شبق بعد الولادة، عدد الأيام التى لم يحدث فيها إخصاب وحتى حدوثه - معدل الإخصاب)، وقد تم تشخيص الحمل بالفحص عن طريق المستقيم للأعضاء التناسلية ٦٠ يوم بعد التوثيب الطبيعى باستخدام عجل ثبت خصوبته

وتم أخذ عينات دم مرتين إسبوعياً لمدة ٩ أسابيع إبتداءً من اليوم السابع بعد الولادة وذلك لتقدير هرمون البروجيستيرون فى أمصال الحيوانات. وقد أظهر مستوى هرمون البروجستيرون إرتفاع معنوى فى أمصال أبقار المجموعة (أ) عن أبقار المجموعة (ب) وذلك بداية من الإسبوع الثانى بعد الولادة.

وقد أظهرت أبقار المجموعة (أ) إنخفاض معنوى (عند مستوى معنوية أقل من ١ر٠) فى عدد الأيام من الولادة وحتى عودة الرحم إلى حجمة السابق (٦٥ر٢٣ ± ٤٥ر٢ مقابل ٦٧ر٣٧ ± ٦٥ر٤ يوماً) كما إتضح ذلك بالفحص بجهاز الموجات فوق الصوتية. وكذلك إنخفاض معنوى (عند مستوى معنوية أقل من ١ر٠) فى عدد الأيام من الولادة حتى ظهور أول شبق على الأبقار (٨٧ر٥٨ ± ٥٤ر٦ مقابل ٩٨ر٨٧ ± ٨٢ر٩ يوماً)، وكذلك عدد التلقيحات المطلوبة لحدوث إخصاب (٦٧ر١ ± ٥٤ر٠ مقابل ٢١ر٣ ± ٨٧ر٠ تلقيحه) وعدد الأيام التى لم تخصب فيها الأبقار (٧٦ر٨٧ ± ٧٥ر٩ مقابل ٦٣ر١٢٢ ± ٦٥ر٣ يوماً). وقد أظهرت أبقار المجموعة (أ) معدل إخصاب مرتفعاً عن أبقار المجموعة (ب) (٨٠٪ مقابل ٢٥ر٥٤٪).

نستنتج من هذه الدراسة أن الضبط الدقيق لمعدل الأملاح الأيونية المزود بها عليقة الأبقار العشار الجافة يمكنها تدعيم الأداء الوظيفى الصحيح لكل من الكالسيوم ونظام الطاقة المنظم لتحسين الظروف الصحية للأبقار فى فترة ماقبل وبعد الولادة.