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Whether Peripartum Nutritional Supplementation Influence the Uterine Involution and Postpartum Fertility in Crossbred Cows

Theodore V.K., Panchal M.T., Dhami A.J., Hadiya K.K., Shah S.V., and Buhecha K.V.

Department of Animal Reproduction Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India

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Abstract The aim of this study was to investigate the effect of peripartum nutrients supplementation on uterine involution and postpartum fertility in crossbred cows. Twenty healthy advanced pregnant HF crossbred cows between 2-4 parity of identical body size were included in the study from two weeks prepartum to 8 weeks postpartum. They were equally divided into control (routine farm feeding-RFF) and treatment (RFF + bypass fat @ 100-200 g/h/day + ASMM @ 50 g/h/day) groups. Animals of both the groups were subjected to per rectal as well as ultrasonographic examinations at weekly intervals from day 7 till day 42 postpartum. On day 7 postpartum, gravid and non-gravid uterine horns in all animals were lying cranial and ventral to pelvic brim in abdominal cavity as large, soft, flabby water bag like structures. The cervical and uterine horn diameters and wall thickness showed a significant decreasing trend from day 7 to 28-35 postpartum with gaining tonicity and elasticity, yet the differences between groups were non-significant. The walls of the cervix and uterus appeared as bright hyperechoic structure, while lumens were found hypoechoic with bright hyperechoic spots. The middle uterine arteries were represented as dark circular anechoic structures and the caruncles as a bright hyperechoic structures. The diameter of artery reduced significantly ($p<0.05$) in the days postpartum. The gross involution of the uterus was observed to be completed by 24.50±1.14 and 23.80±1.14 days, while the complete uterine involution occurred by days 31.97±1.82 and 30.27±1.41 with occurrence of first estrus postpartum in 42.32±4.14 and 38.00±1.95 days ($p<0.05$) for control and nutrient supplemented groups, respectively. The service period (85 vs 100 days) and pregnancy rate (80 vs 60%) were non-significantly better in treatment than control group. Thus it can be asserted that the peripartum nutrient supplementation yielded no beneficial effect on uterine involution, but enhanced postpartum fertility in optimally fed and managed crossbred cows.
Keywords *Crossbred Cows; Nutritional Supplement; Uterine Involution; Clinical & Ultrasound Monitoring*

1. Introduction

The main factors which contribute to economic losses in dairy animal entrepreneur are delayed uterine involution, longer calving interval and short productive life. Fats in the diet can influence reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and by increasing precursors for the synthesis of reproductive hormones such as steroids and prostaglandins. Factors such as limited energy intake, lower body reserves and postpartum diseases can delay the uterine involution and thereby the ovarian recrudescence may also be hampered. The greatest change in the uterus occurs within a few days postpartum. Uterine involution and diameter of uterine horns can be monitored directly by palpation per rectum (Suthar and Kavani, 1992; Kindahl et al., 1999) or by using the transrectal ultrasonography (Sheldon et al., 2004; Parikh, 2009). Therefore, this investigation was planned to study the effect of incorporation of chelated minerals as well as bypass fat in the ration of transitional crossbred cows on their uterine involution and postpartum fertility.

2. Materials and Methods

2.1. Selection of Animals

The study was carried out at University Farm during November 2014 to May 2015 on 20 advanced pregnant HF crossbred cows of 2^{nd} to 4^{th} parity and of nearly identical body size from two weeks prepartum to 8 weeks postpartum. All the pregnant cows were maintained in well ventilated hygienic sheds and were stall fed as per feeding schedule followed on the farm, and had free access to drinking water. The term cows were isolated 60 days before calving in a separate shed. The cows approaching parturition were segregated in calving pen to monitor the calving events and separate the new born calves as the farm follows weaning system. The freshened cows were transferred to the lactating group of cows on the next day of normal calving and were machine milked twice daily.

2.2. Experimental Groups

The selected 20 cows were randomly and equally divided into two group, control and treatment and were managed as under.

Control Group (n=10): These cows were maintained on routine farm feeding schedule (green fodder, hay and compound concentrate mixture @ 18-20, 4-5 and 3.0-3.5 kg, respectively, with 50 g of mineral mixture, Amul brand) during last two months of pregnancy and postpartum. After calving the level of compound concentrate fed was @ 40 per cent of milk produced.

Treatment Group (n=10): In addition to routine farm feeding, these cows were supplemented daily with extra 50 g of area-specific multi-minerals (developed by ANRS, AAU) and 100 g of bypass fat (Sunegry, Polchem) with compound concentrate mixture (Amul dan) for 2 weeks each before and after calving. The level of bypass fat was then increased as per the milk production @ 15 g per litre of milk produced until 60 days postpartum limiting to maximum of 200 g/day.

2.3. Clinical and Ultrasonographic Examinations

Per-rectal (using precalibrated hand) as well as ultrasonographic (using 5-10 MHz B mode transrectal transducer) evaluations of uterine and cervical involution were carried out along with monitoring of ovarian activity at weekly interval from day 7 till 42 postpartum as per Dobson-Hill (2009). All the animals were followed till 90 days postpartum for puerperal events and occurrence of postpartum first and fertile estrus. Animals detected in estrus after 60 days of calving were inseminated. For uterine involution following criteria were considered by giving the non-parametric scores to the parameters as per Scully et al. (2013). Uterine position was scored on a 0-3 scale [0= uterus and uterine horn returned to the previously non-gravid state and 1-3= uterine body and horns falling further over the pelvic brim i.e., between the pelvic brim and abdominal cavity], Size (Comparative size; >cervix 1; =

cervix 2; <cervix 3) of cervix, gravid uterine horn and non-gravid uterine horn, and Tone and consistency (low tonicity 1; moderate tonicity 2; good tonicity 3).

Cows were scanned trans-rectally using a linear array transducer, which was positioned dorsally and parallel over the uterus and sound beams were directed dorso-ventrally. Approximately 10 cm ahead from the gross bifurcation, the uterine diameter and thickness of uterine wall were measured as per the guidelines of Melendez et al. (2004) for both gravid and non-gravid horns. Two diameters of horns were evaluated; the first one from serosa to serosa to obtain the outer diameter of the uterine horn (Sheldon and Dobson, 2000) and the second from mucosa to mucosa to obtain the lumen diameter (Melendez et al., 2004). The difference between the first and the second measurements was divided by 2 to arrive at the estimate of the thickness of the uterine wall (Parikh, 2009). The diameters of middle uterine arteries of either side were measured by keeping the transducer transversely. Caruncles located approximately at the same position of scanning for uterus were measured for its length and width. The uterine and cervical involution in terms of diameters on different days and total time in animals of control and treatment groups were compared using ANOVA and 't' test.

3. Results and Discussion

3.1. Clinical Evaluation of Uterine Involution

On day 7 postpartum, gravid and non-gravid uterine horns were lying cranial and ventral to pelvic brim in abdominal cavity as large, soft, flabby water bag like structures without tonicity and elasticity in all the animals irrespective of groups and only non-gravid horn could be palpated to its full length. Morrow et al. (1969) also reported that the uterine horns were palpable cranial and ventral to the pelvis at days 4 to 7 postpartum in dairy cattle. By day 14 postpartum, the non-gravid horn and the cervix were found to be nearer to the pelvic brim than the gravid horn. By the end of the third, fourth and fifth week, the trend for position scores indicated that gravid horn involuted later as compared to the non-gravid horn. Practically, by the end of 6th week (42 days postpartum), the gravid and non-gravid horns as well as cervix were found to be located in the pelvic cavity with previous non-gravid state and concurred with Scully et al. (2013). Thus, based on the position scores, the uterine involution was observed to be completed by days 35 postpartum in all the animals with no apparent effect of nutrients supplementation.

By day 21 postpartum, both the gravid as well as non-gravid horns had their size comparatively smaller than the cervix in all 10 cows of treatment group, and in 9/10 animals of control group, and 70 and 80 % cows of control and treatment groups, respectively, had their genitalia located within the pelvic cavity. The remaining cows had their genitalia within the pelvis by 28th day postpartum and the horns appeared to be symmetrical. The uterine horns were palpated as smaller in size when compared to the cervix from 21 days onwards up to 42 days postpartum. These findings were supported by the observations of Gier and Marion (1968), that till about 25 days postpartum, the diameter of the uterus exceeded the diameter of the cervix.

The gross involution of the genitalia was observed to be completed approximately by 24.50±1.14 days in all the cows under control group, whereas the corresponding time taken by the cows under treatment group was 23.80±1.14 days. The increasing trend in the tonicity, elasticity and curling of horns was observed from day 7 postpartum to days 21 or 28 postpartum in all the animals with the highest score 3 on day 21 and 28 postpartum, being more pronounced in treatment group. On day 42 postpartum, the tonicity, elasticity and curling of the uterine horns were observed to be increased further as observed by Sutaria (2010) in Kankrej cows.

The observed reduction in size of organs and gradual increase in tonicity of genitalia from the day 7 to 42 postpartum in all animals under both the groups were in accordance with reports of Gier and

Marion (1968) and Jadhav (2005). However, Roberts (1971) opined that an increase in uterine tone occurs from days 10 to 14 coinciding with the onset of first postpartum estrus. The decrease in size of uterine horns was observed to be faster up to day 14 to 21 postpartum and thereafter it decreased very marginally in the present study as noted by Tennant et al. (1967) and Sutaria (2010). The present findings of gross uterine involution (23.80 to 24.50 days) observed are in close agreement with the earlier results of several researchers (Tiwari, 1999; Patel et al., 2005; Sutaria, 2010; Saut et al., 2011). The mean time recorded for uterine involution was, however, found to be comparatively lower than the values (30 to 45 days) reported by Guilbault et al. (1987), Sattar et al. (2007), Heppelmann et al. (2013) and Scully et al. (2013).

3.2. Ultrasonographic Evaluation of Uterine Involution

(a) *Echogenicity of Reproductive Tract*

The wall of the cervix appeared as bright hyperechoic structure, while its lumen was found to be hypoechoic with bright hyperechoic spots of cervical folds as recorded by Sutaria (2010) in Kankrej cattle. By day 7, the cervix was found to be reduced in diameter considerably with constant reformation of the folds evinced through echogenecity. The echogenicity of the cervix became even more pronounced with the hyperechoic cervical wall and the cervical folds being maintained in subsequent days postpartum. These findings were in accordance with the observations of Wehrend et al. (2003). Upon sonography of the uterine horns on the 7th day postpartum, the wall showed hyperechoic structures and the lumen was anechoic with some hyperechoic spots (Plate-I). On the later stages, the horns were seen as hyperechoic wall and hypoechoic lumen (Plate-II). By 42 days postpartum, the echogenicity of the uterine horns was much pronounced revealing the wall to be hyperechoic, whereas the lumen was hypoechoic (Plate-III). These findings concurred well with Gulvane (2005). The snowy appearance of the uterine lumen filled with lochia mixed with necrotic tissue debris was because of the differences in their echogenicity (Kamimura et al., 1993).

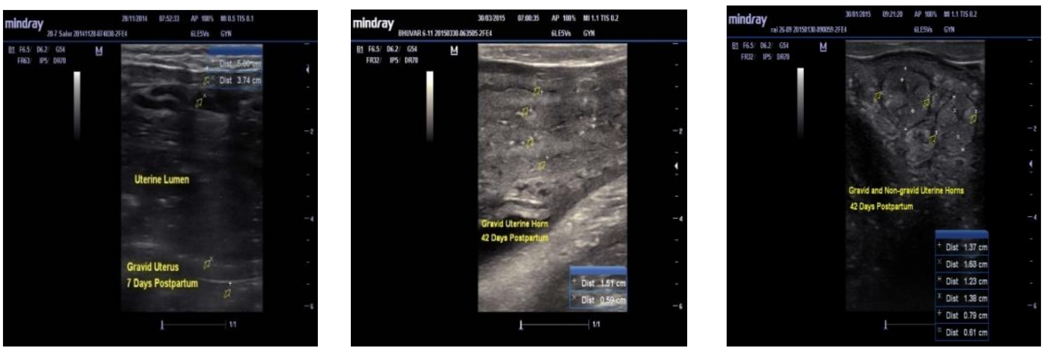

Plate I, II & III: *Ultrasonogram of Gravid uterine horn on day 7 and 42 postpartum*

Plate IV, V & VI: *Ultrasonogram of Maternal caruncle and Middle uterine artery on day 7 & 14 postpartum*

The scanned uterine caruncles on day 7 postpartum were represented as a bright hyperechoic structures resembling mushroom, protruding in the anechoic uterine lumen, encircled with bright visible hyperechoic line (Plate-IV). The texture of the caruncles was similar afterwards but highly reduced in dimensions, i.e., in length and width. These findings corroborated with the reports of Gulvane (2005) and Jadhav (2005). However, Sutaria (2010) observed the caruncles as bright hyperechoic structure having hypoechoic spots in Kankrej cows. The echogenicity of middle uterine artery did not differ from day 7 to 14 postpartum and appeared as a black circular structure (Plate-V, VI) as noted by Sutaria (2010) and Heppelmann et al. (2013).

(b) *Diameter of Cervix and Its Wall Thickness*

The cervical diameter and wall thickness measurements revealed a significant reducing trend ($p<0.01$) from day 7 to 28, and thereafter non-significantly till day 42 postpartum in both the groups (Table 1). The day-wise variation observed in the mean diameter of the cervix between the groups was however non-significant. These observations corroborated well with the findings of Gier and Marion (1968), Guibault et al. (1987), Jadhav (2005), Sutaria (2010) and Kaewlamun et al. (2011) in pure, exotic, zebu and/or crossbred cows. The trend of reduction in cervical diameter and wall thickness throughout postpartum period was physiologically normal and similar, irrespective of groups, and indicated that the cervical involution was completed by day 28 postpartum, yet the fact is that the involution process was still proceeding as the thickness of the cervix was reducing till 42 days postpartum. These findings were in close agreement with Wehrend et al. (2003), Kasimanickam et al. (2004) and Sutaria (2010).

Table 1: *Mean ultrasonographic measurements of cervical diameter and wall thickness in postpartum cows under control and nutrient supplemented (treatment) groups*

Days post-partum	Cervical diameter (cm)		Pooled mean (n=20)	Cervical wall thickness (cm)		Pooled mean (n=20)
	Control (n=10)	Treatment (n=10)		Control (n=10)	Treatment (n=10)	
7	$5.13±0.05^{d}$	$5.44±0.10^{d}$	5.28±0.07	$0.79±0.02^{e}$	$0.82±0.02^{d}$	0.81±0.02
14	$4.48±0.14^{c}$	$4.53±0.11^{c}$	4.50±0.12	$0.70±0.03^{d}$	$0.68±0.01^{c}$	0.69±0.02
21	$3.51±0.16^{b}$	$3.82±0.11^{b}$	3.66±0.13	$0.60±0.03^{c}$	$0.56±0.05^{b}$	0.58±0.04
28	$3.15±0.02^{a}$	$3.19±0.04^{a}$	3.17±0.03	$0.52±0.02^{b}$	$0.52±0.03^{b}$	0.52±0.04
35	$2.99±0.08^{a}$	$3.09±0.04^{a}$	3.04±0.06	$0.43±0.01^{a}$	$0.43±0.01^{a}$	0.43±0.01
42	$2.88±0.09^{a}$	$3.06±0.03^{a}$	2.97±0.06	$0.43±0.01^{a}$	$0.40±0.01^{a}$	0.42±0.01

The means bearing different superscripts within column differ significantly ($p<0.01$) between the time intervals

(c) *Diameter and Wall Thickness of Uterine Horns*

The mean diameter and wall thickness of gravid and non-gravid uterine horns of the crossbred cows under both the groups reduced significantly ($p<0.01$) and progressively between days 7 and 28, but thereafter, the reduction was non-significant. The apparent variations in the day-wise mean diameters and thickness of gravid as well as non-gravid uterine horns between groups were found to be statistically non-significant (Table 2, 3). The mean time interval for complete involution of cervix was recorded to be 40.60±0.93 days for control group and 37.80±1.14 days for treatment group, and the uterine involution was completed by 31.50±2.39 and 30.80±1.55 days in respective groups. The time required for complete involution was slightly lower in treatment group, however the difference was non-significant. Thus, it can be surmised that the animals supplemented with peripartum nutrients did not have any beneficial/contributory effect on genital involution.

Table 2: *Mean ultrasonographic measurements of gravid uterine horn diameter and wall thickness in crossbred cows under control and treatment groups*

Days post-partum	Gravid uterine horn diameter (cm)		Pooled mean (n=20)	Gravid uterine horn wall thickness (cm)		Pooled mean (n=20)
	Control (n=10)	Treatment (n=10)		Control (n=10)	Treatment (n=10)	
7	5.27±0.09[d]	5.52±0.11[d]	5.39±0.10	0.76±0.02[d]	0.69±0.01[d]	0.73±0.02
14	3.81±0.17[c]	3.38±0.07[c]	3.83±0.12	0.67±0.01[c]	0.63±0.11[c]	0.65±0.01
21	2.38±0.07[b]	2.61±0.13[b]	2.50±0.10	0.53±0.03[b]	0.55±0.03[b]	0.54±0.03
28	1.84±0.08[a]	1.80±0.06[a]	1.82±0.07	0.40±0.01[a]	0.41±0.02[a]	0.41±0.02
35	1.72±0.07[a]	1.63±0.05[a]	1.68±0.06	0.36±0.01[a]	0.37±0.01[a]	0.37±0.01
42	1.60±0.06[a]	1.56±0.04[a]	1.58±0.05	0.36±0.01[a]	0.37±0.01[a]	0.36±0.01

The means bearing different superscripts in column differ significantly ($p<0.01$) within the time intervals

Table 3: *Mean ultrasonographic measurements of non-gravid horn diameter and wall thickness in crossbred cows under control and treatment groups*

Days post-partum	Non-gravid uterine horn diameter (cm)		Pooled mean (n=20)	Non-gravid uterine horn thickness (cm)		Pooled mean (n=20)
	Control (n=10)	Treatment (n=10)		Control (n=10)	Treatment (n=10)	
7	4.27±0.08[d]	4.47±0.11[d]	4.37±0.10	0.71±0.01[c]	0.65±0.01[d]	0.68±0.01
14	3.18±0.11[d]	3.26±0.02[c]	3.22±0.07	0.67±0.01[c]	0.56±0.01[c]	0.62±0.01
21	2.18±0.07[c]	2.51±0.13[b]	2.35±0.10	0.51±0.03[b]	0.51±0.02[b]	0.51±0.02
28	1.81±0.08[a]	1.76±0.07[a]	1.79±0.07	0.38±0.01[a]	0.40±0.02[a]	0.39±0.01
35	1.69±0.07[a]	1.63±0.05[a]	1.66±0.06	0.36±0.01[a]	0.37±0.01[a]	0.36±0.01
42	1.60±0.07[a]	1.55±0.03[a]	1.58±0.05	0.30±0.01[a]	0.36±0.00[a]	0.35±0.01

The means bearing different superscripts in column differ significantly ($p<0.01$) within the time intervals

The uterine involution time recorded (31.62±1.62 days) through ultrasonography in the present study was in agreement with the findings (28-40 days) of Santos et al. (1994), Sheldon and Dobson (2000), Melendez et al. (2004), Zhang et al. (2010). However, it was slightly lower as compared to the values (40-45 days) reported by Kaewlamun et al. (2011), Heppelmann et al. (2013) and Scully et al. (2013). The present findings on the diameters of the uterine horns were comparatively lower than the findings of Jadhav (2005). The observed difference could be due to the breed variation. The reduction in the uterine wall thickness to complete uterine involution in our study was in agreement with the findings of Melendez et al. (2004) and Sutaria (2010). In the present study, the thicknesses of cervical and uterine walls continued to reduce for few more days even after the diameters of both the cervix and uterine horns were almost non-significantly different, indicating that the involution of cervix and uterus was not completed and it required few days more for complete involution as a mandatory change required for the next pregnancy to take place (Zemjanis, 1970).

(d) ***Diameter of Middle Uterine Artery***

The mean diameters of middle uterine artery ipsilateral to the gravid uterine horn in cows of control group on day 7 and 14 postpartum were 1.31±0.03 and 1.08±0.02 cm, respectively, with the corresponding values of 1.33±0.02 and 1.13±0.03 cm in treatment group. Similarly, the values for artery of non-gravid side in cows of control group were 1.21±0.38 and 0.97±0.31 cm, and in treatment group 1.21±0.03 and 0.98±0.02 cm, respectively, the differences between days being significant ($p<0.01$) in both the groups for both the sides. The diminution of the middle uterine artery ipsilateral to both gravid and non-gravid horn was observed from day 7 to 14 postpartum for both the groups, with no significant difference between the groups. These findings concurred with the observations of Sutaria (2010) and Heppelmann et al. (2013), who also observed that the uterine artery on the non-gravid side reduced significantly ($p<0.05$) faster when compared to the gravid side.

(e) ***Length and Width of Caruncles***

The mean caruncular lengths in animals of control group on day 7 and 14 postpartum were found to be 3.10±0.15 and 1.50±0.05 cm ($p<0.01$), respectively. The corresponding values in animals of treatment group were 3.13±0.13 and 1.29±0.04 cm, respectively, with significant difference ($p<0.01$). The corresponding mean caruncular widths in animals of control group were 1.23±0.03 and 0.52±0.03 cm, and in treatment group 1.23±0.03 and 0.53±0.04 cm, respectively. A trend of significant ($p<0.01$) regressive changes was observed in the caruncular length and width from day 7 to 14 postpartum in animals under both the groups. However, the day-wise differences between the groups were non-significant. These findings were in agreement with Roberts (1971), Jadhav (2005) and Sutaria (2010). They all reported that the dissolution and sloughing of the caruncles was generally completed by 12 days postpartum and returned to nearly their original size by 2nd to 3rd week postpartum.

3.3. Postpartum Fertility

The reduced time required for the expulsion of placenta (3.83±0.21 vs 4.81±0.65 h) and marginally lesser mean involution time (30.27±1.41 vs 31.97±1.82 days) obtained in the cows under treatment group than the control group, with significantly shorter mean interval of first postpartum estrus (38.00±1.95 vs. 42.32±4.14 days) and service period (85.22±7.17 vs 100.67±5.60 days) and higher pregnancy rate (80 vs 60%) might be due to the probable positive or beneficial effect of nutrient supplementation in the form of chelated minerals and bypass fat. These findings are in agreement with the reports of Kaewlamun et al. (2011) and Khalil et al. (2012), who found that there was no effect of nutritional and bypass fat supplementation on the process of uterine involution, but had beneficial effect on postpartum fertility. However, McNamara et al. (2003) and Tyagi et al. (2010) reported that nutrient supplementation in the form of bypass fat in the diet of the cows had positive effect on the rate of uterine involution.

The present findings clearly indicated that there was a positive effect of peripartum nutrient supplementation in the cows so far as onset of postpartum ovarian activity is concerned, and concurred with Tyagi et al. (2010), Khalil et al. (2012) and Aungier et al. (2014). The earlier resumption of cyclicity in the crossbred cows under treatment group could be attributed to the effect of supplementation in the diet. Fats in the diet influence reproduction positively by altering both ovarian follicle and CL function via improved energy status and by increasing precursors (insulin and IGF-I) for the synthesis of reproductive hormones such as steroids and prostaglandins. The increase in insulin (Palmquist and Moser, 1981) plays a role in mediating increased follicular growth, either directly through its own receptor or indirectly by modulating granulosa cell IGF-I production which is required for follicle development (Rahbar et al., 2014). The fatty acid supplemented in the present study also increased the blood glucose concentration which had a positive effect on preovulatory follicles, by increase both in size and number (Lammoglia et al., 1997), with formation of larger corpora lutea having high progesterone values.

4. Conclusion

From the results of the study, it would be worth to surmise that the peripartum nutrient supplementation in the form of bypass fat and chelated minerals resulted into marginally shorter period of uterine involution, yet early onset of postpartum ovarian activity, with significantly ($p<0.05$) lower first postpartum estrus interval and service period as compared to control group of crossbred cows. Further, the regressive changes observed on per-rectal and ultrasonographic evaluation of genitalia reflected normal physiological puerperal events essential for new reproductive events in healthy dairy cows.

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