THE OVARY ACTIVITY IN COWS WITH AND WITHOUT RETAINED FETAL MEMBRANES

Santa Skuja , Vita Antāne
LLU Veterinārmedicīnas fakultāte, Latvija
LUA, Faculty of Veterinary Medicine, Latvia
santa.skuja@inbox.lv , vita.antane@llu,lv

ABSTRACT
The aim of the study was to find out the ovary activity in cattle with and without retained fetal membranes. Sixty cows of different age of Latvian black-and-white breed from two Joint Stock Companies were used in the present research. The animals were divided into two groups: control group (cows without retained fetal membranes - NRFM) of 15 cows and 45 cows with retained fetal membranes (RFM). Research was performed from September 2007 to January 2009. The level of progesterone in blood serum and rectal ultrasonography were used to establish the ovary activity in cows. The serum progesterone concentration was analyzed every two days for six weeks. The mean serum progesterone concentration during the first 14 days postpartum (PP) in cows with and without RFM was under 1ng/ml. Subsequently (day 18 to 32), progesterone concentration in cows with RFM increased from 1.24 ± 0.14ng/ml to 6.43 ± 0,76ng/ml but in NRFM (day 18 to30) the progesterone increased from 0.74 ± 0.11ng/ml to 3.40 ± 1.15ng/ml. On day 42 PP, the progesterone level was decreased in both groups of cows: in RFM group to 3.34 ± 0.32 ng/ml, in the NRFM group to 1.97 ± 0.68 ng/ml ($P<0.05$). When analysing the progesterone concentration in blood in individual animals during six weeks PP, it was found out that progesterone level was constantly low (<1ng/ml) in 32% of RFM cows and 20% of NRFM cows. The obtained results with ultrasonograph confirmed the ovary functional condition that was established according to the changes of the progesterone concentration in blood.
It was concluded that in cows with retained fetal membranes the progesterone level postpartum was higher and decreased later in comparison with control group cows that could be the reason for a delayed folliculogenesis and ovulation.

KEY WORDS: ovary, cows, ultrasonography, progesterone, retained fetal membrane.

INTRODUCTION
During the postpartum period, the uterus involution takes place and the ovary activity is renewed. Ten days postpartum, an antral follicle is found in ovaries indicating the beginning of a new follicular phase. In this time, the progesterone level in blood is low. In milking cows, the ovary activity and the increase of progesterone level in blood start a new in 18-25 days post partum (Senger, 2005).

In cows with retained fetal membranes and without retained fetal membranes the mean progesterone level just postpartum is similar and below 1ng/ml (Bosu et al., 1984; Chew et al., 1977). Two days later it increases and in cows with RFM reaches 3.4 ± 0.3 ng/ml, and in cows without RFM 2.3 ± 0.1 ng/ml (Chew et al., 1977). Kaczmarawski et al. (2006) reported, that in cows with RFM the progesterone concentration in blood 12-24 h postpartum is significantly higher (2,61 mmol/l) than in NRFM cows (1,59 mmol/l), and in cows with developed metritis the progesterone concentration decreases slower. Holt et al. (2010) has found the corpus luteum in the ovaries of cows with RFM

on the 27th day postpartum and the serum progesterone level 3.34 ng/ml, whereas in the control group cows the increase of progesterone level (4.66 ng/ml) and the corpus luteum are found on day 21st postpartum.

In animals with a delayed activity of ovaries, periparturient disorders such as dystocia, retained fetal membranes, metabolic disorders and metritis are observed (Thatcher et al., 2006). Könyves et al. (2009) report that retained fetal membranes result in a delayed renewal of the ovary activity and an increased interval between the parturition and the first ovulation. In cows with retained fetal membranes, bacterial number is increased in the uterus that reduces follicular activity in the ipsilateral ovary. Sheldon et al. (2002) in their research proved that the uterus bacterial contamination postpartum at the same time affected folliculogenesis but did not affect the ovarian follicle wave emergence.

Early emergence of the ovary activity postpartum indicates an optimal calving-conception interval. Opsomer et al. (2000), Youngquist and Bierschwal (1985) report their research results that if the progesterone level increases before the 50th day postpartum, then can observe a regular estrus. On the contrary, the absence of estrus until the 50th, 60th day postpartum may prolong the calving-conception period, consequently causing great economic losses (Zduńczyk et al., 2002; Opsomer et al., 2000).

Domecq et al. (1997) report that the length of the dry period and body condition of cows are very important and later can affect the postpartum process (milk fever, retention of fetal membranes, metritis, abomasal displacement, lameness). The risk of retained placenta is greater for cows that are underconditioned at drying off (Markusfeld et al., 1997). On average, the dry period is 60 days. If this period is longer than 77 days, then the risk of development of delayed ovarian activity is 2.9 times greater (Opsomer et al., 2000).

A negative energy balance influences the formation of hypothalamic gonadotropin releasing hormone which, in its turn, influences folliculogenesis and ovulation (Vanholder et al., 2006; Wathes et al., 2007). The negative energy balance is connected with irregular oestrus that increases the interval to first service and reduces conception rates. If the cow is underconditioned, the follicular development and ovulation is reduced (Wathes et al., 2007; Staples et al., 1990). Feeding cows additionally with dietary fat in early postpartum period, the ovary activity and folliculogenesis is usually stimulated (Butler 2010).

The aim of the present research was to establish the ovary activity in cows with and without retained fetal membranes applying ultrasonography and progesterone level in blood serum.

MATERIAL AND METHODS

Sixty cows of different age of Latvian black-and-white breed from a Joint Stock Company "Agrofirma Tērvete" herd with 650 milking cows in Dobele region, and from "Daile Agro", Ltd. herd with 280 milking cows in Jelgava region were used in this research. Animal feed ratio was adequate to the standards of milking cows. On both farms the animal keeping, care and feeding were similar. In winter, on both farms animals were kept in the barn in tie-stalls and fed on corn, alfalfa, clover and grass crop silage, which was prepared in plastic rolls and mixed in the feed mixer before feeding and distributed by a feed mixer. Before silage feeding, concentrated mixed feed (barley+wheat) with microelements, rape seed oil-cake and salt was fed. The herd of "Agrofirma Tērvete" was also fed with beer distillery refuse together with the concentrated mixed feed. Several times a week, hay was fed on both farms. Molasses

was poured on the silage and hay. In summertime, cows of both farms were grazing twenty-four hours, and at the time of milking they were fed on concentrated mixed feed with microelements, salt and rape seed oil-cakes. On the farm of "Agrofirma Tērvete", animals were fed with beer distillery refuse also in summertime.
Research was carried out from September 2007 to January 2009.

Cows were divided into groups according to the process of the third stage of parturition (expulsion of fetal membranes) and applied treatment: control group where fetal membranes were expulled within 8-12 hours (n=15) and three groups of cows with retained fetal membranes:

1st group – cows whose fetal membranes were removed manually and cows treated (n=15);

2nd group – cows whose fetal membranes were not removed but were treated (n=15);

3rd group – cows whose fetal membranes were not removed and were not treated (n=15).

Cows with RFM were administered antibacterial and prostaglandin $F_{2\alpha}$ parenteral and intrauterin therapy.

Blood for the progesterone level estimation was sampled 48 hours after 4, 6, 14, 16, 18, 22, 24, 26, 28, 30, 32 un 42 days postpartum. The samples were collected in 7 ml sterile disposable vacuumteiner tubes without anticoagulant from the tail vein and were examined in the accredited laboratory „Central Laboratory", Ltd. complying with the standard analyses requirements by *Alecsis 2010* (Roche Diagnostics). Ultrasonography of ovaries was performed 14, 18, 22, 28 un 42 days postpartum by *Tringa Linear VET* (Esaote piemedical) ultrasonograph.

This diagnostic ultrasound scanner equipped with a linear-array, 7.5 MHz rectal transducer was used to monitor follicular size, ovulation and formation and regression corpus luteum. Ovarian follicles are fluid-filled structures surrounded by an inner layer of granulose cells and an outer layer of thecal cells. Fluids absorb rather than reflect ultrasound waves, it means that follicles appear as black circular structures surrounded by echogenic ovarian tissue. Corpus luteum appears as distinctly echogenic as within the ovarian stroma. Mostly corpus luteum appears as a solid tissue masses but may also contain fluid filled cavities.

A comparative assessment of progesterone parameters in cows with and without retained fetal membranes was carried out applying Microsoft Excel, performing ANOVA: single factor dispersion analysis, as well as calculating mean arithmetic indices. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The ovary activity is characterised by the changes of progesterone concentration of ovarian steroid hormone in milk and blood. Usually, the first serious increase of the progesterone level is observed on average around the 34th and 37th day postpartum. Nevertheless, due to modern dairy animal husbandry the raise of progesterone level is observed already around the 30th day postpartum (Opsomer et al., 2004; Wathes et al., 2007). In cows postpartum with retained fetal membranes the progesterone level in the blood serum is within the range from 0.10-0.60 ng/ml to 3.50ng/ml in some cases (Opsomer et al, 1999).

In this research the mean serum progesterone concentration during the first 14 days PP in cows with and without RFM was under 1ng/ml (Figure 1). Subsequently (day 18 to 32), progesterone concentration in cows with RFM increased from 1.24 ± 0.14ng/ml to 6.43 ± 0.76ng/ml but in NRFM (day 18 to30) the progesterone increased from 0.74 ±

0.11ng/ml to 3.40 ± 1.15ng/ml. The obtained results show that in cows with RFM luteal phases are longer because the progesterone concentration in the blood serum remains high even after the 30th day postpartum. Whereas in cows of NRFM group at this time a decrease of progesterone concentration is observed. Mateus et al. (2002) and Opsomer et al. (2000) in their investigations report that cows with some health problems have prolonged luteal phases.

Next time progesterone was established on the 42nd day postpartum, the progesterone level was decreased in both groups of cows: in RFM group to 3.34 ± 0.32 ng/ml, in NRFM group to 1.97 ± 0.68 ng/ml ($P<0.05$) (Figure1). The decrease of progesterone level at this time indicates onset of pro-estrus and estrus phase. Ultrasonography showed evidence that until the 14th day ovaries were inactive – small, with smooth surface and with 2mm large several follicles. Some cows had an antral follicles in ovaries. On the 18th day in most of the research groups cows there were observed one or two follicles of various sizes (5-8mm), but on the 22nd and 28th day corpora luteum of various sizes were observed. On day 42 regression of the corpus luteum and development of new follicles were observed. The obtained results confirm the ovary functional condition established by the changes of progesterone concentration in the blood serum.

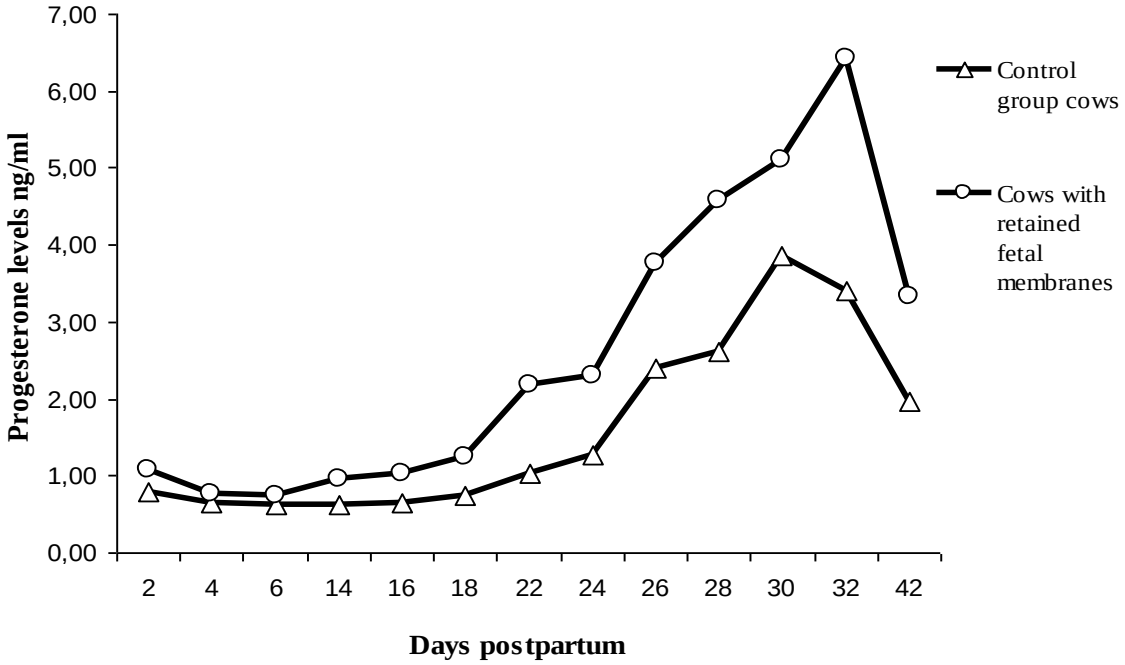


Figure 1. **Progesterone levels in cows with and without retained fetal membranes 45 cows with RFM, 15 cows without RFM**

Analyzing cows with RFM treated with different methods and NRFM cows, progesterone level was low in all the groups in the following tests till the day 18 (Fig. 2). On day 22 progesterone level was increased, especially in cows with RFM whose fetal membranes were not removed and cows treated – the second group (2.95 ± 1.18ng/ml). Greater progesterone level on day 28 was observed in cows with RFM whose fetal membranes were not removed and cows were not treated – the third group (5.80 ± 1.86ng/ml), but on day 32 in cows with RFM whose fetal membranes were removed manually and cows treated – the first group (7.78 ± 2.30ng/ml) and the third group (6.16 ± 2.28ng/ml). At the same time in the control group of cows the

progesterone concentration decreased. The difference was statistically significant between cows with RFM and control group on day 22, 28 and 32 ($P<0.05$) (Fig. 2).

On day 42 the progesterone concentration in all cow groups decreased significantly meaning that most of cows were coming in heat at that time (Fig. 2).

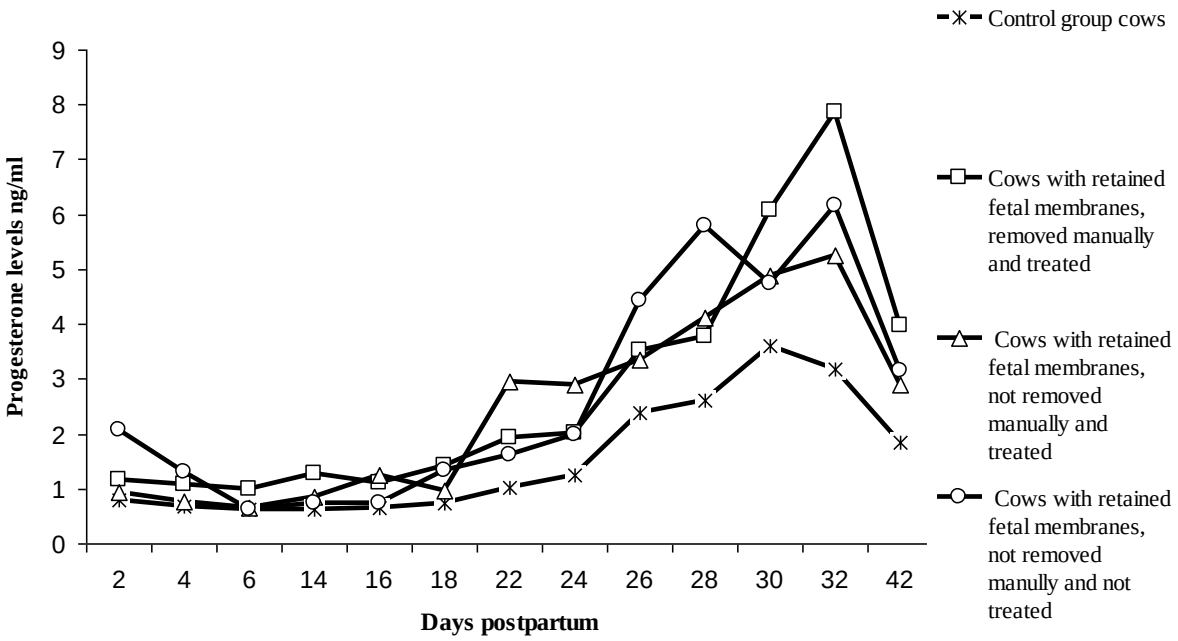
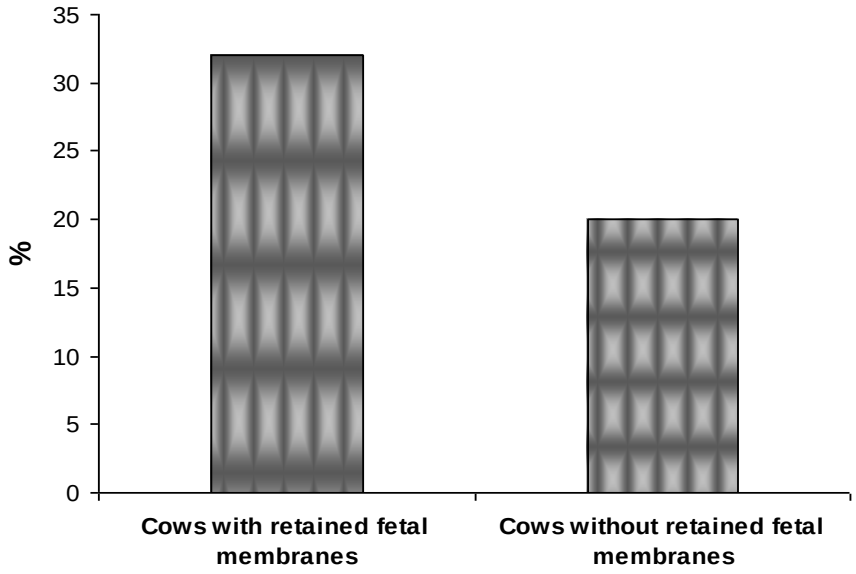


Figure 2. **Progesterone levels in the control group and three separate groups of cows with RFM**

When analysing the progesterone concentration in blood in individual animals during six weeks PP, it was found out that progesterone level was constantly low (<1ng/ml) in 32% of RFM cows and 20% of NRFM cows (Figure 3). At the same time ultrasonography showed small smooth ovaries. Opsomer et al. (2000), Werth et al. (1996) and Zduńczyk et al. (2002) report in their research if the progesterone level is consequently low during the first 50-60 days postpartum, then there is a delayed cyclicity.



Firgure 3. **Progesterone levels under 1ng/ml**

It should be pointed out that the average dry period of cow groups involved in the experiment did not exceed 77 days and the body condition 3.75 points (Table 1). Nevertheless, some cows of group with RFM (4% of cases) the dry period lasted from 77 to108 days and the body condition was 4.0 points.

Table 1

Dry period and body condition in cows with and without retained fetal membranes

Group of cows	Average dry period, days	Average body condition, points
Control group cows	66±4.84	3.66±0.05
Cows with retained fetal membranes	59±3.74	3.68±0.02

CONCLUSIONS

1. In total, in cows with retained fetal membranes the progesterone level postpartum was higher and decreased later in comparison with control group cows, that could be the reason for a delayed folliculogenesis and ovulation.
2. During six weeks postpartum in 32% of retained fetal membranes cows and 20% of control group cows had a low ovary functional activity (P_4 < 1ng/ml)
3. In cows with retained fetal membranes in postpartum period a longer luteal phase is observed.
4. The ovary activity estimated by the progesterone concentration in blood gives a conception that is obtained by establishing ultrasonography.

REFERENCES

1. Bosu, W.T.K., Liptrap R.M., Leslie K.E. Peripartal changes in plasma progesterone and 15-keto-13,14-dihydro-prostoglandin $F_{2\alpha}$ concentrations in Holstein cows with and without retained foetal membranes – Animal Reproduction Science. 1984; 7:6 497-510.
2. Butler, W.R. Dietary fatty acid supplementation and reproduction in dairy cows – Department of Animal Science, Cornell University. Materials from Summer School "Reproductive challenges facing today's dairy industry". May 24-28, 2010. Tartu, Estonia.
3. Chew, B.P., Keller H.F., Erb E.R., Malven P.V. Periparturient concentrations of prolactin, progesterone and the estrogens in blood plasma of cows retaining and not retaining fetal membranes – Journal of Animal Science. 1977; 44:6 1055-1060.
4. Domecq, J.J., Skidmore A.L., Lloyd J.W., Kaneene J.B. Relationship between body condition scores and milk yield in a large dairy herd of high yield in a large dairy herd of high yielding Holstein cows – Journal of Dairy Science. 72:2 233-239.
5. Holt, L.C., WWhittier W.D., Gwazdauskas F.C., Vinson E. Early postpartum reproductive profiles in Holstein cows with retained placenta and uterine discharges – Journal of Dairy Science. 1989; 72: 533-539.
6. Kaczmarowski, M., Malinowski E., Markiewicz H. Some hormonal and biochemical blood indices in cows with retained placenta and puerperal metritis - Bulletin of The Veterinary Institute in Pulawy. 2006; 50:89-92.
7. Könyves, L., Szenci O., Jurkovich V., Tegzes L., Tiriάn A., Solymosi N., Gyulay G., Brydl E. Risk assessment and consequences of retained placenta for uterine health, reproduction and milk yield in dairy cows – Acta Veterinaria Scandinavica. 2009; 78: 163-172/
8. Markusfeld, O., Galon N., Ezra E. Body condition score, health, yield and fertility in dairy cows – The Veterinary Record. 1997; 141:3 67-72.
9. Mateus, L., Lopes da Costa L., Bemardo F. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows – Reproduction in Domestic Animals. 2002; 37:1 31-35.
10. Opsomer, G., Coryn M., de Kruif A. Postpartum anoestrus in high yielding dairy cows – Vlaams Diergeneeskundig Tijdshrift. 2004; 73: 112-118.
11. Opsomer, G., Gröhn Y.T, Hertl J., Coryn M., Deluyker H., de Kruif A. Risk factor for post partum ovarian dysfunction in high producing dairy cows on Belgium: A field study – Theriogenology . 2000; 53:4 841-857.
12. Opsomer, G., Wensing Th., Leavens H., Coryn M., de Kruif A. Insulin resistance: the link between metabolic disorders and cystic ovarian disease in high yielding dairy cows? 1999; 56: 211-222.
13. Sheldon, M.I., Noakes D.E., Rycroft A.N., Pfeiffer D.U., Dobson H. Influence of uterine bacterial contamination after parturition on follicle growth and function in cattle – Reproduction. 2002; 123: 837-845.
14. Singer, P.L. Pathways to pregnancy and parturition. Second revised edition – Current Conceptions,Inc.USA. 2005 187-341.
15. Staples, C.R., Thatcher W.W., Clark J.H. Relationship between ovarian activity and energy status during the early postpartum period of high production dairy cows - Journal of Dairy Science. 1990; 73: 938-947.
16. Thacher, W.W., Bilby T.R., Bartolome J.A., Silvestre F., Staples C.R., Santos J.E.P. Strategies for improving fertility in the modern dairy cow – Theriogenology. 2006; 65:30-44.

17. Vanholder, T., Opsomer G., de Kruif A., Leroy J.L.M.R. Interactions between energy balance and ovarian activity in high yielding dairy cows early postpartum: a review - Vlaams Diergeneeskundig Tijdshrift. 2006; 75: 79-85.
18. Wathes, D.C., Fenwick M., Cheng Z., Bourne N., Llewellyn S., Morris D.G., Kenny D., Murphy J., Fitzpatrick R. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow – Theriogenology. 2007; 68S: S232-S241.
19. Youngquist R.S. and Bierschwal C.J. Clinical management of reproductive problems in dairy cows – Journal of Dairy Science. 1985; 68:2817-2826.
20. Zduńczyk, S., Mwaanga E.S., Małecki-Tepiciit J., Barański W., Janowski T. Plasma progesterone levels and clinical findings in dairy cows with post-partum anoestrus - Bulletin of The Veterinary Institute in Pulawy. 2002; 46: 79-86.