

RETAINED FETAL MEMBRANES IN HOLSTEIN COWS: ECONOMICAL EVALUATION OF DIFFERENT THERAPEUTIC PROTOCOLS UNDER EGYPTIAN CONDITIONS

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Abstract

Post-partum affections are major causes of infertility in dairy cattle. The main objective of study was to evaluate the reproductive and productive parameters of Holstein cows suffering from retained fetal membranes (RFM) subjected to different therapeutic protocols. The cows were 4-8 years old at their 2nd to 6th calving season. Methods: Forty cows diagnosed with RFM were divided equally into 4 groups according to the assigned treatment protocol, group 1: manual removal of the RFM + 2gm intrauterine Oxytetracycline, group 2: injected 50 I.U/i.m Oxytocin, group 3: injected 500µg/i.m cloprostenol (PGF2α, analogue), group 4: injected of 2.5mg/i.m Carazolol (Beta-Blocker). A group of 10 cows was designed as control (fetal membranes were shed within the first 12 hours after parturition). Results showed that the least time needed to expel fetal membranes achieved by PGF2α treatment (18.20±1.22 hours). Cows treated with PGF2α showed significantly shorter days open (100 days) and calving interval (380 days) compared to other groups.

Key words: Infertility, Dairy cows, fetal membranes

INTRODUCTION

The post-partum period "also known as puerperium" is of a paramount value in the reproductive life of dairy cows. It starts with parturition and continues until uterine involution, resumption of ovarian activity, and normal estrous cycles resume. In normal post-partum phase, the uterine involution requires 25 to 50 days and it includes a reduction in uterine size, necrosis, shrinkage of the caruncles, and the regeneration of the endometrium (Sheldon et al. 2008). Elimination of bacterial contamination by means of phagocytic activity of uterine leucocytes is mandatory for successful uterine involution (Azawi 2008; Paisley et al. 1986). Uterine involution is affected by age, breed, nutrition, incidence of dystocia and other factors; therefore delayed uterine involution is not a specific indicator of uterine disease (Fonseca et al. 1983). The vaginal discharge during puerperium is called lochia; it is composed of mucus, blood, shreds of fetal membranes and caruncular tissue. Lochial discharge occurs between 2 to 10 days after parturition in dairy cows; by days 14 to 18 it disappears. Normal lochial discharge is usually brown or reddish brown then becomes paler in color between 7 to 14 days after calving and should have no unpleasant odor (Sheldon et al. 2006; Williams et al. 2005).

Aberrations of the post-partum period include retained fetal membranes "RFM", cystic ovaries metritis/endometritis, uterine prolapse, and pyometra are major causes of infertility in the post-partum period. Fetal membranes are usually expelled within 2 to 8 hours of parturition, its retention beyond 12 hours is considered pathological (Wetherill 1965). Retained placenta affects 5-10% of calvings and significantly increases the risk of metritis and endometritis as decomposing placental tissue provide favorable media for bacterial colonization (Eiler et al. 1997; LeBlanc 2008). There are number of common causes that predispose for retention of fetal membranes, including mechanical, nutritional; managerial and infectious factors. Dystocia, caesarean section, uterine torsion, abortion, stillbirth, and twin birth are mechanical causes of RFM. While nutritional causes may be due to deficiency of protein, selenium, iodine, vitamin A and E and calcium deficiency during pregnancy. Managerial causes of retained placenta include stress hereditary, inbreeding and obesity (Joosten et al. 1991). Infectious causes is associated with brucellosis, salmonellosis, leptospirosis, and listeriosis (Grohn and Rajala-Schultz 2000; Han and Kim 2005; LeBlanc et al. 2002). A large-scale analysis report stated that pregnancy rate was reduced by 4-10%, 2-3 extra days to first service in cows affected with RFM as compared to unaffected cows raised in northern hemisphere (Fourichon et al. 2000). Similar observations were reported in Egypt and some tropical areas (Gabr et al. 2005; Shiferaw et al. 2005; Swiefy 2003)

Diverse therapeutics has been employed for treatment of RFM. Manual removal, administration of intra-uterine and/or systemic antibiotics, injection of oxytocin, PGF2α and β2-receptor blockers; all assisted in

prevention/treatment of RFM (Bolinder et al. 1988; Frazer 2005; Garcia et al. 1992; Peters and Laven 1996). Because RFM negatively affect milk production and cow's fertility, effective treatment is crucial for improving puerperal performance of cows in order to raise their productiveness. The presented study aimed to compare different treatment protocols of Holstein cows diagnosed with RFM and its effect on reproductive and productive parameters in a scope of economical value.

MATERIALS AND METHODS

This study was carried out on Holstein cows raised by dairy farm located at Cairo-Alexandria desert road. The age of cows was between 4 to 8 years old, at their 2nd to 6th calving seasons with good health conditions. All animals were subjected to the same environmental and managerial conditions and were observed closely before, during and after parturition. Cows were calved in calving pens hygienically and kept in individual yards for 10 days after parturition. The post-partum cows were kept with their calves to suckle colostrum during 30 minutes after parturition and for three days. Vaginal examination was done to detect fetal membranes and lochial discharge from first day until 6th week post-partum. Rectal examination of cows was also performed until complete involution of uterus and cervix is achieved. Both ovaries were examined rectally for detection of ovarian structures. All cows were visually observed (3times/day) for detection of estrus signs (confirmed by rectal palpation).

Experimental design:

Forty cows with history of birth help were diagnosed with RFM (more than 12 hours after parturition) divided equally into 4 groups according to the assigned treatment protocol:

Group 1: manual removal of the RFM in addition to intrauterine insertion of 2gm Oxytetracycline (Terramycin tablets, Pfizer).

Group 2: injected 50 I.U./i.m Oxytocin, (Oxytocin, Adwia)

Group 3: injected 500µg/i.m Cloprostenol " PGF2α, analogue" (Estrumate, Shering plough)

Group 4: injected of 2.5mg/i.m Carazolol (4-carbazolyloxy-3-Isopropylamine-2-propanol, Boehringer. Mannheim, Germany).

A control group: includes 10 cow dropped their fetal membranes within the first 12 hours after parturition and did not develop any post-partum clinical disorders.

• The following reproductive parameters were recorded:

- 1- Days open. 2- Days to first insemination.
- 3- Calving interval. 4- Services per conception.
- 5- First insemination conception rate. 6- Second insemination conception rate.

• The following productive parameters were recorded:

- 1- Days in milk. 2- Corrected milk yield (yield/ 305- days).
- 3- Return from milk. Price of one kg was (3 LE). 4- Return from calf.
- 5- Total return (return from calf & milk). 6-Return from manure

• The following costs was recorded:

1. Costs of medicaments.
2. Costs of artificial insemination price of one straw were (59.5 LE).
3. Veterinarian visit
4. Costs of Feeding
5. Net profit (total return- total cost).

Statistical analysis:

SPSS 13.0, Chicago, IL, USA (SPSS 2004) was used for statistical analysis. Data are represented in mean $\pm$ standard deviation values. Duncan's test was performed for comparing values between the groups. $P < 0.05$ was considered to be significant.

RESULTS**A) Response to treatment:**

The time elapsed from parturition to complete release of the fetal membranes in cows within the control group was 4.8 ± 1.12 hours. Cows with RFM took longer time (44.55 ± 4.19 hours) to release their fetal membranes, significant variation was found ($P < 0.05$) between the two groups (table 1). Within the treated groups; the mean time needed to expel fetal membranes for Oxytocin, Carazolol and $\text{PGF}_{2\alpha}$ treatments was 21 ± 2.24 , 21.50 ± 2.25 and 18.20 ± 1.22 hours respectively. While in cows treated with manual removal plus intrauterine Oxytetracycline the placenta was expelled after 44.27 ± 5.29 hours. The difference between the four groups (table 2) were significant ($P < 0.05$).

Table (1): Time elapsed from parturition to complete release of the fetal membranes (hours):

Groups	Number of animals	Range of Placental expulsion	Mean $\pm$ S.E Placental expulsion
Control Group	10	2-10	4.8 ± 1.12 B
Groups 1-4	40	15- 48	44.55 ± 4.19 A

Table (2): The effect of different treatments on placental expulsion duration (hours)

Treatments	Number of animals	Cows passed their fetal membranes		Range of Placental expulsion	Mean $\pm$ S.E Placental expulsion
		No. Response	% of efficacy		
Group1	10	10	100%	24- 60	44.27 ± 5.29 A
Group 2	10	7	70%	18-21	21 ± 2.24 B
Group 3	10	9	95%	15-18	18.20 ± 1.22 C
Group 4	10	8	80%	15-24	21.50 ± 2.25 B

As shown in Table (3), the mean duration of lochial discharge of cows in control group was 10.50 ± 2.55 days following parturition. For cows with RFM the amount of discharged lochia was decreasing gradually until complete cessation at about day 21 following parturition with mean duration of 17.88 ± 4.88 days. The study also revealed that the mean interval from parturition to complete involution of the gravid horn, were 28.55 ± 2.55 and 42.50 ± 4.43 for cows within the control group, and cows with RFM respectively. The mean durations from parturition to the first post-partum heat were 45 ± 4.55 , 55 ± 5.77 days for cows within the control group, and cows within the treated group respectively. Average days open were 95 ± 5.99 days for cows within the control group and 109 ± 10.33 days for cows with RFM as depicted in Table (4)

Table (3): Duration of lochial discharge - duration elapsed from parturition to complete involution of the gravid horn (Days)

Groups	Number of animals	Range of lochial discharge	Mean $\pm$ S.E duration of lochial discharge	Range of elapsed time required for involution	Mean $\pm$ S.E of elapsed time required for involution
Control Group	10	5-18	10.50 $\pm$ 2.55B	25-35	28.55 $\pm$ 2.55B
Groups 1-4	40	10-21	17.88 $\pm$ 4.88A	37 – 47	42.50 $\pm$ 4.43A

Table (4): Days elapsed from parturition to first post-partum estrus- days from parturition to conception (Days open):

Groups	Number of animals	Days elapsed from parturition to first pp estrus	Mean $\pm$ S.E of days elapsed from parturition to first pp estrus	Range of days open	Mean $\pm$ S.E of days open
Control Group	10	30-65	45 $\pm$ 4.55B	80-110	95 $\pm$ 5.99B
Groups 1-4	40	35-78	55 $\pm$ 5.77A	100- 129	109 $\pm$ 10.33A

B) Reproductive parameters: results represented in table (5)

Cows treated with PGF2 α showed significantly lower days open (100 $\pm$ 10.12 days) than those treated with oxytocin, manual removal plus intrauterine oxytetracycline and carazolol. The days open in cows treated with oxytocin, manual removal plus intrauterine oxytetracycline and carazolol was 120, 129, 110 days respectively. Consequently, PGF2 α treated cows had significantly ($P < 0.05$) shorter calving interval (380 $\pm$ 7.77 days) than oxytocin, manual removal plus intrauterine oxytetracycline and Carazolol treated cows which were 400 $\pm$ 5.55, 409 $\pm$ 9.99, and 390 $\pm$ 8.55 days respectively. Furthermore, the 1st and the second services conception rate of cows treated with PGF2 α showed greater values (60%, 80%) than the corresponding values of oxytocin (30%, 60%), manual removal plus intrauterine oxytetracycline (30%, 70%) and Carazolol (40%, 60%) treated cows. As a result, PGF2 α treated cows required less services per conception (1.7) and had significant variation ($P < 0.05$) than oxytocin (2.7), manual removal plus intrauterine oxytetracycline (2.3) and Carazolol (2.5) treated cows.

C) Productive parameters: results represented in table (6)

PGF2 α treated cows produced milk for about 405 $\pm$ 14.30 days, therefore, return from milk was significantly ($P < 0.05$) higher in cows treated with PGF2 α (31590 $\pm$ 31.55 L.E), than those cows treated with other protocols. Return from milk of cows treated with manual removal plus intrauterine Oxytetracycline, Carazolol and oxytocin was 29640 $\pm$ 29.30 L.E, 28300 $\pm$ 22.44 L.E and 27200 $\pm$ 27.40 L.E respectively.

Table (5): Reproductive parameters of cows treated from retained fetal membranes:

Treatments	No.	Days open	Days to first	Calving	Services/	Conception rate
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	Cows		insemination	Interval (Days)	conception	1 st service conception Rate	two services conception Rate
Group 1	10	129±7.29A	87±7.88A	409±9.99A	2.3±0.33A	3/10 30%	7/10 70%
Group 2	10	120±7.20B	85±5.55AB	400±5.55B	2.7±0.22A	3/10 30%	6/10 60%
Group 3	10	100±10.12D	78±8.88C	380±7.77D	1.7±0.37B	6/10 60%	8/10 80%
Group 4	10	110±10.15C	80±8.83C	390±8.55C	2.5±0.55A	4/10 40%	6/10 60%

Table (6): Productive parameters of cows treated from retained fetal membranes:

Treatments	No. (cows)	Days in milk	Total milk produced	305- day milk yield (kg)	Return from milk (L.E)	Return from Calf (L.E)	Return from manure*	Total return (L.E)
Group 1	10	380 ±13.80B	9880 ±19.80B	7930 ±13.22A	29640 ±29.30B	2000 ±20.15A	750 ±15.14A	32390 ±31.55B
Group 2	10	350 ±15.30D	9100±19.50D	7930 ±14.55A	27200 ±27.40D	2000 ±21.12A	750 ±15.14A	29950 ±29.33D
Group 3	10	405 ±14.30A	10530±20.33A	7930 ±15.55A	31590 ±31.55A	2000 ±12.33A	750 ±15.14A	34340 ±34.55A
Group 4	10	365 ±13.55C	9490±20.14C	7930 ±16.14A	28300 ±22.44C	2000 ±12.14A	750 ±15.14A	31050 ±31.55C

*Return from manure Calculated according to the animal gave 30 m³ per year and the price of meter is 25 L.E.

D) Profits versus costs: results represented in table (7-8)

Statistically cows treated by manual removal + intrauterine Oxytetracycline showed significant higher total cost (18562.85±60.84 LE). The least insemination cost (101.15±5.55 L.E), and total cost (18425.15±45.22 LE) were estimated for cows treated by PGF2 α . Also, the results revealed that cows treated by PGF2 α showed significantly higher total return (34340±34.55 L.E), and consequently higher net profit (15914.85±19.55 L.E). The least net profit was estimated for cows treated by oxytocin (11474.35±24.75 L.E).

Table (7): The cost during treatment of retained fetal membranes:

Treatments	No. (cows)	Treatment cost (L.E)	Insemination cost (L.E)	Veterinary Visits (L.E)	Feed cost* (L.E)	Total costs (L.E)
Group 1	10	56 ±5.62A	136.85±6.85 C	120±1.15A	18250±22.50A	18562.85±60.84A
Group 2	10	5±1.53D	160.65±6.55 A	60±6.66B	18250±25.55A	18475.65±55.64B
Group 3	10	14±2.17C	101.15±5.55 D	60±6.55B	18250±25.18A	18425.15±45.22D
Group 4	10	25±3.55B	148.75±7.55 B	60±6.55B	18250±25.19A	18483.75±40.55C

* The average feed cost per head per day was 50 L.E

Table (8) Net profit from different treatments of retained fetal membranes:

Treatments	Total return (L.E.)	Total cost (L.E.)	Net profit (L.E.)
Group 1	32390±31.55B	18562.85±60.84A	13827.15±27.44B
Group 2	29950±29.33D	18475.65±55.64B	11474.35±24.75D
Group 3	34340±34.55A	18425.15±45.22D	15914.85±19.55A
Group 4	31050±31.55C	18483.75±40.55C	12566.25±25.66C

DISCUSSION

Reproductive performance is one of the major factors affecting profitability of a dairy herd. Reproductive performance is economically important in dairy cattle because it affects milk yield and culling rate. Poor reproductive performance due to post-partum reproductive disorders can reduce the number of born calves and milk production and increase the cost of nutrition, therapy and artificial insemination. Retained fetal membranes have adverse effect on reproductive performance as it represents a significant risk for metritis and endometritis. Parameters such as days open (the period between calving and the start of new pregnancy), days to first insemination, conception rate and calving interval are basic factors to measure reproductive performance.

The objective of the present study was to compare the effect of different therapeutic protocols employed for RFM taking into consideration the previous parameters and its reflection on profitability of the herd. The results showed that normally parturient cows expelled fetal membranes in significantly shorter time (4.8 ± 1.12 hours) than cows received birth help and diagnosed later with RFM, which took longer time (44.55 ± 4.19 hours) to release their fetal membranes. Moreover, cows with RFM characterized by longer days of lochial discharge (17.88 ± 4.88 Vs 10.50 ± 2.55), longer duration for involution of the gravid horn (42.50 ± 4.43 Vs 28.55 ± 2.55),

delayed exhibition of first post-partum estrus (55 ± 5.77 Vs 45 ± 4.55) compared to normally calved cows. These observations agreed with studies of (Badr 1992; Eiler et al. 1997; Gaafar et al. 2010; Williams et al. 2005).

The results revealed that, PGF 2α treatment achieved the shortest time (18.20 ± 1.22 hrs) needed for expulsion of RFM compared to the other treated groups. Similar findings have been reported by previous studies (Majeed et al. 2009; Noakes et al. 2003). Also, PGF 2α treated cows showed significantly shorter days open (100 ± 10.12 days) than those treated with other treatments; Van Werven and co-workers reported similar results (VanWerven et al. 1992). Consequently, PGF 2α treated cows had significantly ($P < 0.05$) shorter calving interval (380 ± 7.77 days) and lower services per conception (1.7 ± 0.37) and higher 1st service conception rate (60%) and two services (80%) conception rate than those observed in the other treated groups. These results are in accordance with findings of other studies (Kaya et al. 2012; Risco et al. 1994). Analysis of productive parameters showed that return from milk was significantly higher in cows treated with PGF 2α (31590 ± 31.55 L.E), than those cows treated with other protocols. Also, cows treated with PGF 2α showed significantly higher total return (34340 ± 34.55 L.E) and higher net profit (15914.85 ± 19.55 L.E).

The administration of ecboic drugs such as oxytocin and PGF 2α early in the post-partum period lead to the evacuation of the uterus by enhancing contractions, and supporting the process of involution (Bajcsy et al. 2006). In this study, treatment with oxytocin was not rewarding in comparison with PGF 2α . Failure of oxytocin to hasten expulsion of RFM was observed in previous studies (Palomares et al. 2010; Stevens and Dinsmore 1997). However, other studies reported contrasting results (El- Azab et al. 1988; Joosten et al. 1988; Miller and Lodge 1984), the authors showed that oxytocin assisted in prevention or treatment of cows with RFM. This conflict might be due to variation in oxytocin dosage, timing of injection after parturition or whether oxytocin was injected with or without estradiol. In the group treated with manual removal, cows had the longest days open (129 ± 7.29) and calving interval (409 ± 9.99). The reason for that is manual removal can cause micro- injuries of the endometrium (Bolinder et al. 1988) and therefore elongate the period required for uterine involution.

In the current study, days open in cows treated with Carazolol were 110 ± 10.15 following in order PGF 2α treated group (100 ± 10.12). On the other hand, calving interval (390 ± 8.55) and conception rate (40%, 60%) were comparable to those showed by oxytocin and manual removal treatments. previous studies reported the useful administration of B2-receptor blockers either prophylactically or as a treatment of RFM (EL-Amrawi 1992; Grunert 1983; Hammerl J. 1984; Holtz et al. 1990). The uterus contains β_2 receptors, which is primary involved in smooth muscle relaxation induced by sympathetic nerve stimulation (Gilbert and Schwark 1992). Epinephrine possesses an agonistic activity on β_2 - receptors, which arise due to pain or stress at delivery, obstetrical interferences, and uncomfortable surroundings of the cow and/or due to suckling. The use of β_2 - blockers after birth in cows can prevent or neutralize the effect of epinephrine (Labib et al. 1987; Parmigiani et al. 1995).

Treatment of RFM needs a comprehensive understanding of its patho-physiology and causes of occurrence. Recently, unusual therapeutic protocols such as ozonated foam administered into the uterus (Zobel and Tkalcic 2013) and oral administration of herbal tincture formulas (Cui et al. 2014a; Cui et al. 2014b) have showed substantial results, which open new alternatives for handling cases of RFM.

CONCLUSION

The present study demonstrated that treatment of Holstein cows raised under Egyptian managerial conditions using PGF 2α was the best effective and profitable therapeutic protocol. PGF 2α treated cows showed improved reproductive and productive parameters with higher economical return compared to cows treated with other protocols (manual removal, oxytocin and Carazolol).

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