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Effect of Hormonal Treatment during Early Postpartum Period on Uterine Involution, Steroid Hormone Levels and Ovarian Activity in Bulgarian Murrah Buffaloes

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A B S T R A C T

The aim of this study was to evaluate the effect of the hormonal treatment during early postpartum period on the uterine involution, the steroid hormone levels and the ovarian activity in Bulgarian Murrah buffaloes. The experiment was carried out with 20 clinically healthy animals in breeding season, randomly allotted in two groups: control group (non treated; n=10) and experimental group (hormonal treated; n=10) received 100 µg GnRH and 25 mg PGF2α seven days apart starting at day 14 after calving. Fertile bulls were ensured for all buffaloes. A transrectal ultrasonographic examination of the uterus and the ovaries and blood samples collection were performed at the days of the treatment. Serum estradiol-17β and progesterone levels were determined by ELISA. Ultrasound pregnancy check was done at day 30 and 60 postpartum. After data processing, mean cervical and gravid uterine horn diameters, involution rate of cervix and gravid uterine horn, percentage of the buffaloes bearing large follicle and/or corpus luteum, mean concentration of steroid hormones and pregnant buffaloes were calculated.

The present study demonstrated significant (P>0.05) decrease of the mean diameters of cervix and gravid uterine horn with increased follicular activity and mean concentration of estradiol-17β in treated compared to non-treated buffaloes between days 14 and 21 postpartum. The earlier uterine involution and resumption of ovarian activity in the experimental group corresponded with significant (P>0.05) enhancement of progesterone at day 21 and 20% pregnant animals after hormonal treatment. In conclusion, the application of GnRH-PGF2α protocol 7 days apart starting at day 14 postpartum resulted in accelerated uterine involution and earlier resumption of the ovarian activity and could be used for improvement of the reproduction in postpartum Bulgarian Murrah buffaloes.

Introduction

The prerequisites for successful reproductive performance in buffaloes are rapid uterine involution and early resumption of the ovarian activity after parturition (El-Wishy, 2007ab; Perera, 2011). Various investigations (Chaudhry *et al.*, 1990; Iqbal *et al.*, 2003; Ramoun *et al.*, 2006; Snel-Oliveira *et al.*, 2010; Atanasov *et al.*, 2012) report for a completed uterine involution in buffaloes between days 25 and 36 after calving. The rectal palpation and the ultrasonography are the most used methods for determination of the uterine involution and evaluation of the uterine involution rate, but the obtained results are still debatable. The regular estrous activity in postpartum buffaloes depended on the synchronized processes of hypothalamus, pituitary gland and ovaries and the capability of the dominant follicles to gain ovulatory capacity (Perea and Inskeep, 2008; Terzano *et al.*, 2012). The pattern of estrus cycle resumption in buffalo species showed a short luteal phases and sometimes low progesterone levels during the corpus luteum formation after first postpartum ovulation (Sharma and Kaker, 1990; Usmani *et al.*, 2001; Campanile *et al.*, 2010). A continued anovulatory periods after a short estrus cycle, more than three weeks have been described by Khattab *et al.*, (1990) and Ghoneim *et al.*, (1999). Yotov *et al.*, (2015) stated to differences in the development of the first dominant follicle and estradiol-17β concentrations during the time of dominant follicles formation in ovulating and non-ovulating buffaloes. According to (El-Wishy, 2007a) the start of ovarian activity after calving could be determined form reproductive potential of different buffalo breeds.

Various experiments for improvement of the uterine involution and resumption of the ovarian activity by hormonal treatment during the early postpartum have been performed in buffaloes (Usmani *et al.*, 2001; Iqbal *et al.*, 2003; Khatri *et al.*, 2013; Kandiel *et al.*, 2013; Ingawale *et al.*, 2014). An accelerated uterine involution by prostaglandin and oxytocin treatment immediately after calving was observed by Khatri *et al.*, (2013). According to Kandiel *et al.*, (2013) the inclusion of GnRH-PGF2α-GnRH protocol on postpartum day 21 conducted to rapid uterine involution and ovarian activity resumption. Previous research (Yotov *et al.*, 2012) in Bulgarian Murrah buffaloes registered increased percentage of the buffaloes with corpus luteum after hormonal treatment by PGF2α at postpartum day 5, followed by GnRH-PGF2α treatment at days 21 and 28, respectively. Ingawale *et al.*, (2014) determined faster uterine involution and higher conception rate in Indian Murrah buffaloes after administration of GnRH or PGF2α two weeks after parturition.

Many of the aforementioned results are obtained by rectal or ultrasound examination of uterus only or ovaries, without steroid hormone assay. This study was designed to evaluate the effect of the hormonal treatment during early postpartum period on the uterine involution, the steroid hormone levels and the ovarian activity in Bulgarian Murrah buffaloes.

Material and Methods

The experiment was carried out with 20 animals, weighing 560-600 kg, average age 6.3±1.4 years, with normal parturition, without signs of endometritis during the experiment and a separation of the newborns from dams immediately after calving. All buffaloes were cultivated and fed uniformly with a mixed ration for lactation, controlled grazing seven hours daily and water ad

libitum. The average daily milk output was 7.8±0.6 l with milking twice daily. The study was conducted during the breeding season (June-December). The animals were randomly allotted in two groups: control group (non treated; n=10); experimental group (hormonal treated; n=10) received 100 µg GnRH and 25 mg PGF2α (Dinopros) on day 14 and 21 postpartum, respectively. Fertile bulls remained permanently in the control group while in the experimental group they were introduced after the prostaglandin injection.

A transrectal ultrasonographic examinations were performed at the days of the treatment by an ultrasound SonoScape A5 Vet (SonoScape Co. LTD, Shenzhen, China) with multifrequency (7-12 MHz) linear transducer. The vertical and horizontal diameters (mm) in the middle of the cervix and the part of the gravid uterine horn, located immediately before bifurcation were measured in cross-section projection by the in-built scale provided with the ultrasound. The average value between both measurements was accepted as a final value in evaluation of the uterine involution. The involution rate (mm per day) was calculated via method described by Usmani *et al.,* (2001). The evaluation of ovarian activity was based on the visualization of small, medium or large follicles (LF) and or corpus luteum (CL). Visualization of a large follicle in diameter ≥ 9 mm and/or newly formed corpus luteum was accepted as an indicator for ovarian activity resumption. Ultrasound pregnancy check was done on days 30 and 60 postpartum.

Blood samples were collected in vacutainers by jugular venipuncture at the same intervals as ultrasound examinations. Blood serum was separated by centrifugation at 3000 g for 15 minutes and stored in a sterile tube at – 200C until analysis. Serum estradiol-17β (pg/ml) and progesterone (ng/ml) levels were determined by an enzyme immunoassay (Estradiol and Progesterone ELISA Kits; Monobind Inc, Lake Forest, USA).

After data processing, cervical diameter (CD), gravid uterine horn diameter (GUHD), involution rate of cervix and gravid uterine horn, percentage of the buffaloes bearing large follicle and/or corpus luteum in one of the ovaries and percentage of pregnant buffaloes were calculated.

Statistical analysis was performed by statistical software Statistica version7.0 (Stat-Soft 1984-2000 Inc., Tulsa, OK, USA). The mean values were compared using of a non-parametric analysis for comparison of two means and proportions, using Student's t-criterion. Differences were considered significant in p-values ≤ 0.05.

Results and Discussion

The ultrasonographic results, connected with uterine involution and ovarian activity are presented in Table. 1. On Day 14 postpartum, the mean cervical diameters (22.6±2.1 mm and 23.7±8.7 mm) in control and experimental buffaloes (Fig.1A) no differed significantly (P>0.05). An absence of significance was determined for mean diameters (27.5±8.0 mm and 32.1±9.3 mm) of the gravid uterine horn in both groups (Fig.1B). Larges follicles were observed in 30% and 40% non-treated and treated animals, respectively (Fig.1C). At the same time corpus luteum was no detected. The mean concentrations of estradiol-17β and progesterone (17.1±6.9 pg/ml and 0.16±0.18 ng/ml) in the control group were comparable with the obtained (17.7±27 pg/ml and 0.36±0.53 ng/ml) in the experimental group (P>0.05) (Table. 2).

On Day 21 postpartum, the mean value for a cervical diameter in the control animals (22.2±2.9 mm) was close to the measured one in previous examination, but less ($P<0.05$) than calculated (16.3±3.7 mm) in hormonal treated buffaloes. Significant difference ($P<0.05$) between the mean diameters of gravid uterine horn (22.5±2.0 mm vs 19.2±3.1 mm) was also registered. The involution rates of cervix and gravid uterine horn after hormonal stimulation (1.06 mm and 1.84 mm) were high, while in lack of treatment they were low (0.06 mm and 0.71 mm). Percentage of the control animals presenting large follicle (30%) was lower than obtained 70% for the experimental group. A corpus luteum was observed in 10% and 30% non-treated and treated buffaloes, respectively (Fig.1D).

The hormonal investigation showed low mean value of estradiol-17β (16.7±9.8 pg/ml) in the control group and augmentation up to 61.2±88 pg/ml in the experimental group. However, the mean progesterone concentration (1.98±1.15 ng/ml) in hormonal treated buffaloes was greater ($P<0.05$) than thus (0.42±0.54 ng/ml) in non-treated animals. The pregnancy results showed 0% and 20% pregnant buffaloes (Fig.1E) in the first and the second group, respectively, until day sixty postpartum.

Duration of postpartum anoestrus has close relationship with intercalving interval in buffaloes (Ramoun *et al.*, 2006; El-Wishy, 2007b; Perera, 2011). The rapid uterine involution and the early resumption of ovarian activity are important factors conducting to shorter anoestral period and improvement of reproductive status (Chaudhry *et al.*, 1990; Hussein *et al.*, 2013). Different experiments (Shah *et al.*, 1990; Ramoun *et al.*, 2006; Ingawale *et al.*, 2014) for hormonal stimulation of the uterine involution and the follicular development after calving have performed, but clear standpoint in this area treatment is still not available.

The present results demonstrate positive effects of the hormonal treatment during the earliest postpartum on the involution of the cervix and the gravid uterine horn. Evidences for this assertion were significant ($P<0.05$) decrease of mean CD and GUHD in the experimental group between postpartum days 14 and 21 and absence of similar difference in the control group. In agreement with this result, Hussein *et al.*, (2013) reported significant ($P<0.05$) smaller cervical and previous gravid uterine horn diameters in pregnant than non-pregnant animals at day 28 postpartum. Close relationships between prostaglandin or gonadotropin treatment, uterine involution and ovarian function from calving to postpartum day 30 was registered by other authors (Iqbal *et al.*, 2003, Khatri *et al.*, 2013; Kandiel *et al.*, 2013).

The registered faster involution rates of cervix and gravid uterine horn in treated compare to non-treated Bulgarian Murrah buffaloes also supported the previous results and could be related to increased ovarian activity. This study indicated that GnRH administration on day 14 after calving results in earlier resumption of the ovarian activity that is in agreement with increased percentage of animals with LF during the second ultrasound examination. The injection of GnRH can eliminate the inadequate release of LH from pituitary gland in the early postpartum period (Shah *et al.*, 1990). It is connected with earlier restoration of the ovarian function, faster uterine involution with maturation and ovulation of the dominant follicle (Takkar *et al.*, 1999).

Table.1 Cervical and gravid uterine horn diameters, involution rate (IR) of cervix and gravid uterine horn, large follicles (LF) and corpora lutea (CL) in control and experimental buffaloes

Reproductive parameters	Cervical diameter (mm)			Gravid uterine horn diameter (mm)			Ovarian structures (%)			
Postpartum days	14 ден	21 ден	IR	14 ден	21 ден	IR	14 ден		21 ден	
Groups	Mean±SD	Mean±SD	mm/ day	Mean±SD	Mean±SD	mm/ day	LF	CL	LF	CL
Control (n=10)	22.6±2.1[a]	22.2±2.9[a]	0.06	27.5±8.0[a]	22.5±2.0[a]	0.71	30	0	30	10
Experimental (n=10)	23.7±8.7[a]	16.3±3.7[b]	1.06	32.1±9.3[a]	19.2±3.1[b]	1.84	40	0	70	30

Means within row and column for each parameter that have different superscripts differed at $P<0.05$

Table.2 Steroid hormone levels in control and experimental buffaloes

Parameter	Estardiol 17β (pg/ml)		Progesterone (ng/ml)	
Postpartum days	14	21	14	21
Groups	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control (n=10)	17.1±6.9[a]	16.7±9.8[a]	0.16±0.18[a]	0.42±0.54[a]
Experimental (n=10)	17.7±27[a]	61.2±48[a]	0.36±0.53[a]	1.98±1.15[b]

Means within row and column for each parameter that have different superscripts differed at $P<0.05$

Fig.1 Ultrasound images of cervix and gravid uterine horn, large follicle, corpus luteum and pregnany in postpartum Bulgarian Murrah buffaloes

In this respect Ingawale *et al.,* (2014) registered decrease in average days required for involution of the uterus and average days required for exhibition of first postpartum oestrus after GnRH or PGF2α administration at day 14 postpartum. On the other hand there was a tendency to raising of the estrogens levels after hormonal administration which can accelerate involution of the postpartum uterus. According to Shah *et al.,* (2007) the estrogens are responsible for the uterine contractions and for histological changes in fetal villi and maternal crypts. The lack of enhancement in the average estradiol-17β concentrations in non-treated group at day 21 supported abovementioned data.

Moreover, the used hormonal treatment led to successful ovulation in presence of follicles with ovulatory capacity in the ovaries. This is in agreement with detected corpora lutea in 30% of the treated buffaloes bearing large follicles during the GnRH injection and observation of only one CL in the control group. Similar result (thirty percentages ovarian response) was demonstrated by Shah *et al.,* (1990).

One of the most important proofs for favourable effect of GnRH-PGF2α protocol on the uterine involution and resumption of ovarian activity starting from day fourteen postpartum was the registration of more CL and pregnant animals in the treated group. Zain *et al.,* (2001) determine that seven days after GnRH injection, newly formed luteal structures are sensitive to PGF2α and GnRH-PGF2α treatment 7 days apart in different time after calving was used for optimization of reproductive efficiency in buffalo-cows (Yendraliza *et al.,* 2006). According to Ramoun *et al.,* (2006) GnRH-PGF2α-GnRH treatment between days 18 and 25 after calving concluded to enhancement of uterine involution and/or earlier initiation of post-partum cyclicity. Significant ($P<0.05$) higher average progesterone concentration in hormonal treatment group could be due to greater number of corpora lutea and/or increased luteal activity. Probably explanation for too low progesterone level in the non-treated group was functional insufficient of the CL in short luteal phase. Others investigations (Sharma and Kaker, 1990; Usmani *et al.,* 2001; Campanile *et al.,* 2010) in buffaloes also determined a short luteal phases with low progesterone levels after first postpartum ovulation.

In conclusion, administration of GnRH at day 14 postpartum followed by PGF2α injection seven days latter is connected with accelerate of the uterine involution and earlier resumption of the ovarian activity in clinically healthy Bulgarian Murrah buffaloes. This protocol could be used for shortening of a postpartum anoestrus and improvement of reproductive performance in buffaloes.

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