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Original Research Article

ASSESSMENT THE SYNCHRONY BETWEEN UTERINE STATUS AND HORMONAL PROFILES IN MODIFIED OVSYNC PROTOCOLS IN RELATION TO FERTILITY IN BUFFALOES

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Abstract: A total of 36 pluriparous buffaloes were used to study the uterine morphometry in response to serum estradiol concentrations and associated estrogen receptors (ERs) expression in modified ovsync protocols. The buffaloes were assigned to St-ovsync, CIDR-sync and Insulin-sync (n=12 for each). The St-ovsync consisted of two IM injections of 20µg buserelin on the Day 0 (GnRH1) and on the Day 9 (GnRH2) and an IM injection of 500 µg of Cloprostenol sodium (Estrumate) on Day 7. Buffaloes in CIDR-sync and Insulin-sync protocols were treated as in the St-Ovsync protocol in addition to intravaginal insertion of CIDR from Day 0 to 7 in CIDR-sync and SC injection of Insulin at a dose of 0.25 IU/kg body weight on Days 7, 8 and 9 in Insulin-sync. Blood samples were collected and the uterine wall thickness (UWT) was simultaneously measured by transrectal ultrasonography on Days 0, 3, 5, 7, 8 and 9. Endometrial biopsies samples were collected from five buffaloes in each group to quantify the abundance of estrogen receptors. The UWT on Days 3 and 5 decreased significantly ($P < 0.05$) while serum P_4 concentration on Day 3 was significantly ($P < 0.05$) increased in eventually diagnosed pregnant (EDP) buffaloes in CIDR-sync compared with their counterparts in either St-Ovsync or Insulin-sync. On Day 9, although there was a decrease in serum E_2 concentration in CIDR-sync compared with either St-Ovsync or Insulin-sync in EDP buffaloes, there was an increase ($P < 0.05$) in the ER mRNA expression in CIDR-sync compared with St-Ovsync. It could be concluded that modifying the St-Ovsync by P_4 supplementation through intravaginal insertion of CIDR from Day 0 to 7 or by SC injection of insulin on the Days 7, 8 and 9, could modulate uterine morphometry such conductive to proper fertility response.

Key words: Buffaloes, ERα; uterine wall thickness; Ovsync

Introduction

Optimal uterine environment at the time of FTAI, especially in cows induced to ovulate immature follicles in ovsync programs, is critical to establish and maintain pregnancy (1). The variation in the fertility status of cows kept under the same nutritional and environmental conditions may result from the different response

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of the uterus to steroid hormones during the periovulation time (2). Adequate rise in the proestrous estradiol (E_2) following pre-exposure to progesterone (P_4) prepares the uterus for embryonic development and establishment of pregnancy (3, 1, 2, and 4). The growth and regression of endometrium are synchronized through changes in the circulating and/or local levels of E_2 and P_4 (5).

Endometrial thickness increases during proestrous and reach maximal thickness on the day before ovulation and decreases throughout diestrous (6, 7). Suboptimal uterine environment induced by low level of estrogen during proestrous and progesterone during diestrous decreases infertility in dairy cows (8). Infertility in cows induced to ovulate immature follicles especially in Ovsync-TAI program may be attributed to suboptimal uterine function caused by low steroids at the periovulation time (9, 10, and 1). Not only the availability of steroid hormones with optimal levels in the peripheral blood is adequate to produce the desired effect on the uterus but binding to their specific nuclear receptors is critical (11). The effect of steroid hormones on the uterus depends on tissue and cell specific expression of steroid receptors (12) which reach maximum levels around the time of heat (13, 14). E_2 modulates the expression of its receptors and their function at the cellular level in such way that increase the rate of mitosis and tissues edema (15). Ovsync-TAI programs either GnRHbased or estradiol-based, are used nowadays to improve the reproductive performance of dairy cows (16, 17). In estradiol – based TAI programs, the exogenous E_2, in addition to inducing GnRH/LH surges, it modulates uterine environment. However, the relatively small follicles that might be present prior to second GnRH in case of GnRH-based Ovsync-TAI program may not produce adequate amount of estradiol sufficient to modulate uterine environment such compatible to support pregnancy (1). The US-measured uterine wall thickness (UWT) and horn diameter could be utilized to test if the uterus has been exposed to adequate concentration of steroid hormones compatible with optimal fertility (18).

Scoring the uterine status prior to the breeding time could be useful for predicting fertility in many species including human. Amongst the methods used to evaluate uterine status are cytology of uterine secretion, ultrasonographic (US) examination and uterine biopsy (19). The current study tests the hypothesis that if the US-measured UWT together with the uterine biopsy could be used to predict fertility of buffaloes subjected to modified ovsync programs. Souza et al (2014) (4) utilized endometrial thickness as a predictor of fertility in high producing dairy cows. They concluded that an endometrial thickness less than 7 mm is predictive to failure of conception in dairy cows. However, the associated steroid hormones profile together with estrogen receptors were quantified to correlate between hormonal profiles and uterine morphometry.

Thus, the current study was designed to match US-measured UWT with steroid hormones profiles together with estrogen receptors expression in the endometrial biopsy sample in an attempt to utilize UWT as a predicting measure of fertility following application of modified CIDR- and Insulin- ovsyncs in buffaloes.

Materials and methods

The animals

The study was performed in Mahallet Mousa Buffalo Research Station, affiliated to Animal Production Research Institute, present in Kafrelsheikh province in the northern of the Nile Delta, Egypt. The experiment was performedfrom April to October which coincides withlow breeding season in the Egyptian buffaloes.

A total of 36 pluriparous cyclic Murrah buffaloes having a parity of 2- 4 and an average body condition score of 2.75 to 3.50 (Scoringsystem was 1 = thin to 5 = fat) were used to carry out this study. Buffaloes were kept indoors throughout the year in yards where 50% of the yard area was sheltered. They hadfree access to water. They were milked twice daily and received a diet that covered both maintenance and production requirements according to the Recommendation of Animal Production Research Institute (APRI, 1997unpublished data).

All animals were cyclicand had healthy genital tract on the basis oftransrectal US scanning of the reproductive system. The cyclic activity was assessed onthe basis of detecting a corpus luteum in eitherof two transrectal US examinations of ovariesdone at 10 days' interval (from Day -10 to Day 0). Day 0 was the day of the first GnRH injection.

Experimental design

Buffaloes (N=36) were randomly assigned to three treatment protocols (12 each): standard ovsync (control group, St-Ovsync), modified CIDR-sync (CIDR–sync) and modified Insulin-sync (Insulin-sync). Each buffalo in the St-Ovsync group received IM injections of 20 µg buserelin acetate (GnRH agonist,5 ml Receptal®, Intervet Company, Holland) on the Day 0 (GnRH1), 500 µg of Cloprostenol sodium (PGF2α analogue, 2 ml Estrumate®, Coopers, Schering Plough Company, England) on the Day 7 and a 2nd dose of GnRH agonist similar to GnRH1, on the Day 9 (GnRH2). Buffaloes in the CIDR-syncgroup were treated as in St-ovsync in addition to intravaginal insertionof CIDR (Controlled Internal Drug Release, it contains 1.38 gm of progesterone, Pfizer Company, New Zealand) from the Day 0 to the Day 7. Buffaloes in Insulin-sync were also treated as St-Ovsync in addition to daily s/c injection of insulin (biphasic isophane insulin,0.25 IU / kg of B.W, Mixtard 30 HM®, Nova, Nordisk, Bagsvared, Denmark) on the Days 7, 8 and 9. Each 1 ml of Mixtard 30 HM® contain 30 IU of soluble and 70 IU of isophane biosynthetic human insulin. Buffaloes in all groupswere bred at 16 hr after the second GnRH treatment.

Ultrasonographic scanning

US examination was conducted by using portable ultrasound device (ULTRASCAN MODEL DP 30 VET, Shanghai International Holding Crop GmbH, Europe) equipped with multifrequency (3-10 MHz) linear probe. For transrectal scanning of the uterus, the probe while being carried in the palm of the hand was guided into the rectum. The uterus was identified then the probe was transversely placed on the base of each uterine horn at 3 cm cranial to the intercornual ligament. The UWT was measured by electronic calipers in the cross section of the frozen image. The UWT was measured as the distance between the edge of uterine lumen to the external edge of the perimetrium. The UWT was estimated for each uterine horn then the average value of the two horns was calculated for each cow. The UWT was measured on Days 0, 3, 5, 7, 8 and 9.

Collection of endometrial tissues

On Day 9, endometrial tissue samples were obtained by an endometrial biopsy knife as described by Nielson (7). The endometrial tissues samples were removed by fine forceps and immersed in a microcentrifuge tube containing 30 mm of the lysis buffer supplemented with mercaptoethanol to quantify the expression of mRNAs of estrogen receptor alpha (ERα) gene in the endometrium.

RT-$_q$PCR assay for ERα gene expression

Total RNA was extracted from endometrial biopsy (30 mg) using RNA isolation kit (Thermo-scientific, fermentas Ko731) according to the manufacturer's instructions. The concentration of the total RNA was measured using Nanodrop Spectrophotometer. To obtain cDNA, the total RNA (1 µg) was reverse transcribed using reverse transcription kits (Thermo-scientific, fermentas # EP0451 according to the manufacturer's instructions).

Quantifying of mRNA for ERα in the three protocols was determined by quantative RTPCR using SYBR Green with GAPDH as an internal control reference. The isolated cDNA was amplified using 2x Maxima SYBER Green/Rox qPCR Master Mix (Thermo-scientific, USA, # K0221) and gene specific primers according to the manufacturer's instructions.

The primers for ERα gene were; 5’GAAGTGGGCATGATGAAAGG-3’ forward and AAGGTTGGCACGTCTCATGT reverse and for GAPDH gene were 5’- CCTGGAGAAACCTGCCAAGT-3’ forward and 5’GGTAGAAGAGTGAGTGTCGCT-3’ reverse. The primer for ERα receptor gene was

designed using web based tool (http://www.genome.wi.mit.edu/egiin/primer/primer3www.egi) on the basis of published buffalo sequences to ensure that the primer sequence is unique for the template sequence. Amplicon sequence identity was confirmed with NCBI Blast tool software (Blast http://www.Blast.ncbi.nlm.nih.gov/Blast.egi). The reactions were conducted in a final volume of 25µL using 12.5µL of 2x Maxima SYBER Green/ Rox qPCR Master MIX, 1µL of each primer (forward and reverse), 3µL of cDNA (10-20 ng/µL) and 7.5µL of water nuclease free. The protocol conditions included initial denaturation at 95ºc for 10 minutes and 40 cycles with denaturation at 95ºc for 15sec, annealing at 60ºc for 30sec and extension at 72ºc for 30 sec. The relative expression levels of target gene (ERα receptors) were calculated by using the – ΔΔCT method (20). The house keeping gene (GHPDH) was used as the normalizing reference gene. The cycle threshold (Ct) values calculated for target gene were normalized against reference gene. The St-ovsync (control) group was used as calibrator, while CIDR-sync and Insulin- were considered as test groups for both target and references genes. The mean cycle threshold (Ct) values were used to calculate ΔCT for both target and reference genes in each of test and control groups by the following equation:

ΔCT (test) = Ct (target in test groups) – Ct (ref in test groups)

ΔCT (calibrator) = Ct (target in control) – Ct (ref. in control).

Then ΔCt of the test genes were normalized to the ΔCt of the calibrator:

ΔΔCt = ΔCt (test) – ΔCt (calibrator).

The fold change of the relative gene expression was calculated as follows:

Fold change = $(2^{-\Delta\Delta Ct})$.

Blood sampling and hormonal assay:
Blood sampling

Blood samples were collected by jugular vein puncture on the Days 0, 3, 5 and 9. The samples were centrifuged at 3000 rpm for 15 min. The harvested sera were stored at - 20°C until estrogen and progesterone assays.

Serum progesterone assay

The serum P_4 concentrations were measured by radioimmunoassay using RIA kit (Beckman coulter RIA progesterone IMMUTECH, S.r.o Radiova 1-10227 Prague - Czech Republic) according to the manufacturer's instructions described in the catalog enclosed with the kit. The inter- and intra- assay coefficients of variations were 8.66 and 8.15 respectively. The average sensitivity was 9.58 pg/ml.

Serum estradiol assay

The serum E_2 concentrations were estimated by radioimmunoassay using estradiol kit (Beckman coulter RIA Estradiol; IMMUNTECH, s.r.o Radiova 1-10277 Prauge - Czech Republic) according to manufacturer's instructions described in the catalog enclosed with the kit. The inter- and intra- assay coefficients of variations were 14.5 and 14.4 respectively. The average sensitivity was 9.58 pg/ml.

Serum insulin assay

The serum insulin concentrations were estimated using an IMMUNORADIOMETRIC kit (Insulin (e) IMRA kit; IMMUNOTECH, s.r.o Radiova 1-10227 praque- Czech Republic) according to the manufacturer's instructions described in the catalog enclosed with kit. The inter- and intra- assay coefficients of variations were 8.3% and 5.6% respectively. The average sensitivity was 4.55ng/ml.

Reproductive management

Buffaloes were inseminated at 16 h after the 2^{nd} GnRH treatment (FTAI) with frozen-thawed semen. On Day 30 post TAI, buffaloes were examined by transrectal US of their uteri for pregnancy diagnosis. Conception rates were calculated by dividing the number of buffaloes gets pregnant on the total number of buffaloes submitted to applied ovsync protocols TAI programs in the current study.

Statistical analysis

All data, except conception rates, were presented as means ± SEM. The statistical significance of differences was tested by the analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test using Graphpad prism ver. 6. 0 for Mac (Graphpad software. San Dieogo, USA). *Chi*-Square analysis was used to compare the conception rates among the three protocols.

Results

Ultra sound – Measured UWT

On Day 0, the US-measured UWT did not differ ($P > 0.05$) among St-ovsync, CIDR-sync and Insulin-sync in either eventually diagnosed pregnant (EDP) buffaloes or eventually diagnosed non pregnant (EDnP) buffaloes. It was observed that while the UWT decreased ($P<0.05$) in the CIDR-sync compared with either St-ovsync or Insulin-sync in EDP buffaloes, it did not differ among the 3 protocols in EDnP buffaloes on either of the 3rd or 5th day. Within each protocol, the UWT did not differ ($P > 0.05$) between EDP and EDnP buffaloes in all the investigated days (Table, 1).

Serum progesterone concentrations on the Days 0, 3 and 5

On the Days 0 and 5, the serum P_4 concentrations did not differ among the three protocols neither in EDP nor EDnP buffaloes. In contrary, on Day 3, it increased ($P < 0.05$) in EDP and EDnP buffaloes in CIDR-sync compared with either St-ovsync or Insulin-sync (Table 1).

The uterine wall thickness on Day 9

The UWT did not differ ($P < 0.05$) between either EDP or EDnP buffaloes on Day 9 as well as among EDnP buffaloes on Day 7. However, the UWT decreased ($P < 0.05$) in EDP buffaloes in CIDR-sync compared with either St-ovsync or Insulin-sync group on Day 7 (Table, 2).

Serum estradiol concentration on the Day 9

In EDP buffaloes, serum E_2 concentration on Day 9 showed a decrease ($P < 0.05$) in CIDR-sync compared with either Stovsync or Insulin-sync. In EDnP buffaloes, it showed nonsignificant ($P > 0.05$) variations among the three protocols.

Serum insulin concentration on Day 9

The serum insulin concentration on Day 9 increased ($P < 0.05$) in both EDP and EDnP buffaloes in Insulin-sync compared with either St-ovsync or CIDR-sync group (Table 2).

Estrogen receptor (ER) gene expression

The expression of *ERα* gene was upregulated ($P < 0.05$) in insulin-sync protocol compared with St-ovsync. Also the expression was upregulated in CIDR-sync compared with St-ovsync (Figure 1) regardless the buffaloes were EDP or EDnP. There was nonsignificant difference between insulin-sync and CIDRsync. Relative to the St-ovsync, the fold change of 2.81 and 2.45 were recorded for the upregulation of *ERα* gene expression in the Insulin-sync and CIDR-sync respectively (Figure 1).

The fertility response

Pregnancy diagnosis on Day 30 post- TAI revealed that 5/12 (41.66 %); 6/12 (50%) and 8/12 (66.67 %) buffaloes were diagnosed pregnant in St-ovsync, CIDR-sync and Insulin-sync protocols respectively (Figure 2).

Table 1: Ultrasound-measured UWT and serum P_4 concentrations in EDP and EDnP buffaloes in St-ovsync, CIDR-sync and Insulin-sync protocols on Days 0, 3 and 5

Parameter	Synchronization protocols					
	St-Ovsync		CIDR-Sync		Insulin-Sync	
	EDP	EDnP	EDP	EDnP	EDP	EDnP
UWT (mm)						
Day 0	42 ± 0.02 [a]	42 ± 0.01 [d]	41 ± 0.02 [a]	41 ± 0.03 [d]	42 ± 0.01 [a]	45 ± 0.01 [d]
Day 3	45 ± 0.02 [a]	40 ± 0.01 [d]	34 ± 0.01 [b]	39 ± 0.02 [d]	41 ± 0.01 [a]	38 ± 0.02 [d]
Day 5	42 ± 0.03 [a]	42 ± 0.01 [d]	32 ± 0.01 [b]	38 ± 0.03 [d]	41 ± 0.01 [a]	45 ± 0.02 [d]
Serum P_4 concentration (ng/ml)						
Day 0	3.41 ± 0.83 [b]	1.91 ± 0.29 [e]	3.84 ± 0.66 [b]	2.55 ± 0.28 [e]	3.73 ± 0.98 [b]	2.00 ± 0.34 [e]
Day 3	3.58 ± 0.62 [b]	3.26 ± 0.67 [e]	6.11 ± 0.39 [a]	6.39 ± 1.01 [d]	3.49 ± 0.51 [b]	3.26 ± 0.52 [e]
Day 5	3.25 ± 0.15 [b]	4.05 ± 0.17 [e]	5.95 ± 1.39 [b]	5.14 ± 0.57 [e]	3.04 ± 0.64 [b]	3.98 ± 0.30 [e]

Within the same row, values carrying small letters from a to c in case of EDP and from (d to f) in case of EDnP buffaloes are different at P<0.05. Within the same treatment group, values carrying asterisk are different at P<0.05 between EDP and EDnP buffaloes.

Table 2: Ultrasound - measured UWT on Days 7, 8 and 9 and serum concentrations of E_2 and insulin on Day 9 in St-ovsync, CIDR-sync and Insulin-sync protocols

Parameter	Treatment protocol					
	St-Ovsync		CIDR-Sync		Insulin-Sync	
	EDP	EDnP	EDP	EDnP	EDP	EDnP
UWT (mm)						
Day7	42 ± 0.02 [a]	42 ± 0.01 [d]	33 ± 0.01 [b]	35 ± 0.3 [d]	43 ± 0.02 [a]	44± 0.05 [d]
Day 8	43 ± 0.04 [a]	44 ± 0.01 [d]	35 ± 0.02 [a]	36 ± 0.02 [d]	45 ± 0.02 [a*]	41 ± 0.04
Day 9	34 ± 0.02 [a*]	50 ± 0.03	34 ± 0.02 [b]	49 ± 0.04 [d]	43 ± 0.03 [a]	41 ± 0.04 [d]
Serum estradiol concentration (pg/ml)						
Day 9	17.3 ± 1.55 [a]	9.43 ± 1.23 [d]	12.1 ± 1.45 [b]	9.65 ± 2.11 [d]	22.26 ± 1.31 [a]	12.13 ± 1.90 [d]
Serum insulin concentration (ng/ml)						
Day 9	7.18 ± 1.85 [b]	5.79±2.56 [e]	6.00±0.72 [e]	6.00±0.72 [e]	32.13±2.64 [a*]	15.13±2.21 [d]

EDP = eventually diagnosed pregnant; EDnP = eventually diagnosed non-pregnant. Within the same row, values bearing different letters from a to b in case of EDP buffaloes and from d to e in case of EDnP buffaloes were different at (P<0.05). Within the same protocol, values bearing asterisk were different at P < 0.05 between EDP and EDnP buffaloes.

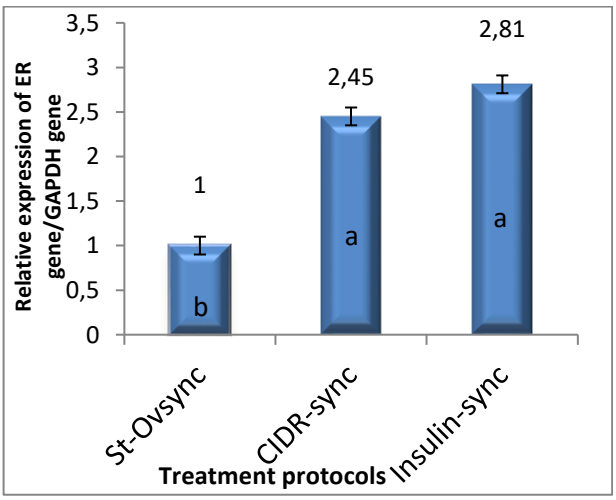

Figure 1: Oestrogen receptor alpha (*ERα*) gene expression in insulin-sync and CIDR-sync groups relative to St-ovsync shown as fold changes ($2^{-\Delta\Delta ct}$). Relative to the St-ovsync (fold change (Fc)=1), the fold changes of 2.81 and 2.45 represented the upregulation of *ERα* gene in Insulin-sync and CIDR-sync groups respectively

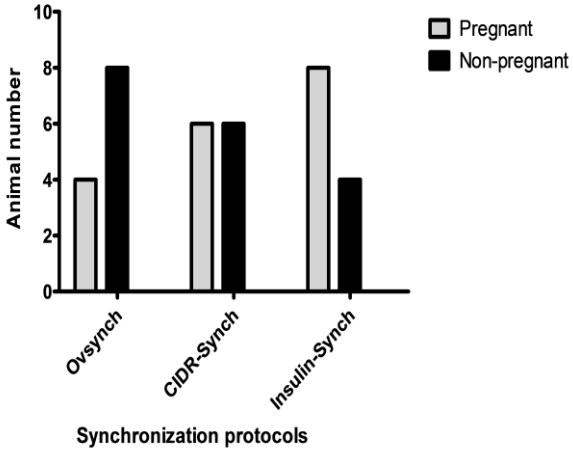

Figure 2: The conception rates on Day 30 post-TAI in buffaloes treated with St-ovsync, CIDR-sync and In sulin-sync. The conception rate was 5/12 (41.66 %); 6/12 (50 %) and 8/12 (66.67 %) in St-ovsync, CIDR- sync and Insulin-sync respectively

Discussion

The present work aimed to study the uterine morphometry in terms of us-measured UWT in relation to steroid hormones profiles and endometrial oestrogen receptors in modified ovsync programs in buffaloes. Since endometrial growth and regression are synchronized with ovarian function through changes in circulating and/or local levels of oestrogen and progesterone(21, 4), ultrasound measuring of the uterine wall thickness, simple and reproducible technique, can be used to evaluate steroid hormone induced uterine environment changes (22).

On the day 3 and 5, the decrease in the UWT in the buffaloes eventually diagnosed pregnant (EDP) in CIDR-Sync compared with either St-Ovsync or Insulin-sync may be attributed to the increase in the serum progesterone concentrations at $p < 0.05$ and $p > 0.05$ on the days 3 and 5 respectively. Taking into account the similarity between the diestrous phase in the estrous cycle and short luteal phase, 2-7 days, in an ovsync protocol (Ref), the reduced UWT, recorded on the days 3 and 5 in the current study, came in line with (23) who reported that the uterine horns had minimal thickness, minimal luminal fluid and maximal horn curl during the period extending from 3rd to 16th day of the oestrous cycle. Sajjan (2014) (23) reported that the thickness of the endometrium, (most responsive layer of the uterine wall to the changes in the serum steroid hormones concentrations) decreased from 5.47 to 5.06 mm with the increase in the serum P_4 concentration from 0.76 to 1.08ng/ml in cows. In the same respect, Jimenez-Krassel et al (2009) (24) attributed the decrease in the endometrial thickness to the increase in the circulating P_4 concentration

The non-significant variations in the UWT among EDP buffaloes on Day 0 as well as among EDnP buffaloes may be explained in the light of non-significant variations in the serum P_4 concentrations on the respective days.

On Day 9, matching the serum E_2 concentrations with UWT in case of EDP buffaloes revealed that the serum E_2 concentrations, being high, were nearly consistent with respective UWT in case of either ST-ovsync or Insulin-sync but not in CIDR-sync. Conversely in EDnP buffaloes, it was inconsistent with respective UWT in all of three groups. In the EDP buffaloes, then insignificant increase in the UWT in the insulin-sync protocol compared with either ovsync or CIDR- sync may be explained in the light of increase in the respective serum E_2 concentration in the insulin-sync compared with its counterpart value in case of either St-ovsync or CIDR – sync protocol. However, the increase in E_2 receptors in CIDR-sync compared with St– ovsync may increase the response of the endo- metrium, which is the main responder part of the uterine wall, to relatively low serum E_2 levels in CIDR- sync thereby increasing UWT that became comparable to its counterpart value in case EDP buffaloes in St-ovsync. In line with this explanation, Xia and Goff (25) reported that the number of endometrial receptors determines the sensitivity of the uterus to the steroid stimulation.

The increase in the concentration of serum E_2 in Insulin-sync compared with the other two groups may be due to stimulatory effect of insulin on the steroidogenic activity of the largest follicle. The exogenous insulin increase both follicular growth and steroidogenic activity in cows (26), buffaloes (27) and goats (28). In CIDR-sync, the lower serum E_2 concentration in EDP buffaloes on Day 9 compared with either St-ovsync or Insulin- sync may be attributed to the presence of persistent follicles that bypassed the effect of the first GnRH and continued to grow and produce E_2 at lower rates under the effect of low tonic level of LH induced by the negative feedback mechanism of P_4 released from CIDR until Day 7 (Day of CIDR removal). Some of these persistent follicles continued to produce E_2 at the previous lower level until Day 9. However, an interval of 48 h (from Day 7 to 9) is not sufficient for the persistent follicle to survive P_4-induced low tonic level of LH during the previous 7 days, thus their steroidogenic activity became compromised. In line with this explanation, Cerri et al (2010) (29) found that the tonic levels of LH were lower in cows having high serum P_4 concentrations on Day 5 of CIDR-sync protocol.

On the other hand, Cerri et al, (29) reported that the decreased concentrations of P_4 during synchronization protocol resulted in high basal LH concentrations, faster growth of dominant follicle (DF) and higher circulating E_2. In spontaneous estrous, a proestrous period of 3-4 days may be sufficient for resumption of proper tonic level of LH that stimulates proliferative and steroidogenic activity of the DF. In accordance with this suggestion, Bridges et al, (2010) (30) reported that reducing the duration of proestrous in cows, induced to ovulate small follicles, resulted in decreased serum preovulatory E_2 (30, 31). Nonetheless, Cerri et al. (29) found concentration was lesser from day 5 to 9 of CIDR-sync protocols in cows having high compared with those having low serum P_4 that the serum E_2 concentrations.

The benefit of studying the abundance of ERα in these three Ovsync protocols comes from the fact that the changes in the uterine morphometry in response to E_2 are an outcome of the interaction of the local/peripheral E_2 concentration with its receptors in the uterine tissues. Okumu et al. (11) reported that not only the availability of a steroid hormone in the peripheral circulation is adequate to produce the desired effect on the uterus but binding to their specific nuclear receptors is critical.

In the current study, the expression pattern of ERα in the endometrial biopsy is compatible with serum E_2 concentrations in either Insulin-sync or St-ovsync but not with CIDR- sync to some extent. The higher E_2 levels in case of Insulin-sync and St-ovsync came in agreement with Sunderland et al (1994) (32) who reported that the expression pattern of ERα is consistent with the circulating concentration of E_2 during oestrous cycle.

In the same respect, Clark et al and Mann et al (33, 34) detected the highest level of ERα and PRs mRNA at the time of oestrous and concluded that the stimulatory effect of E_2 on sex hormone receptors expression. Also Spencer and Bazer (35) reported that E_2 as well as those for P_4 and the functions at the cellular level to increase the rate of mitosis and tissue oedema (15), a finding which explained the increased UWT in either of Insulin-sync or St-ovsync compared with CIDR-sync. However, the reduced UWT in the CIDR-sync may result from the delayed recovery of UW from the effect of high P_4 concentration during period of CIDR insertion. However, the abundance of ERα was higher in CIDRsync compared with St-ovsync but the E_2 concentration was higher ($P > 0.05$) in St-ovsync compared with CIDR-sync. Although the E_2 concentration was lower ($P > 0.05$) in CIDR-sync compared with St-ovsync, the abun- dance of ERs in CIDR-sync was higher than St-Ovsync. This may be attributed to the pre exposure of the uterus in case of CIDR-sync to higher P_4 concentration during the period of CIDR insertion. This explanation may be supported by the results of Shimizu et al (36) who reported that E_2 and P_4 provoke transcriptome changes within the endometrium, with the response to E_2 being greater when the uterus was preexposed to higher P_4.

Conclusion

It is concluded that modifying the St-ovsync by including P_4 through CIDR-insertion from Day 0 to 7 or treatment with insulin on Days 7, 8 and 9 could modulate uterine morphometry, thereby improving fertility outcome of ovsync protocols. Further studies are required in the future to study the effects of the P_4 or insulin-induced transcriptome changes in the uterus of cows treated with modified P_4 or insulin-modified ovsyncs on the uterine histotrophs secretion that may explain its beneficial effects on the animal fertility.

Conflict of interest

The authors declare that they have no conflict of interest.

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