FERTILITY AND PLASMA PROGESTERONE PROFILE IN REPEAT BREEDING COWS AND BUFFALOES IN OVSYNCH AND MID-CYCLE PGF$_2\alpha$ TREATMENT PROTOCOLS

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ABSTRACT

Twenty repeat breeding cows and buffaloes each were equally divided into two treatment groups, viz., Ovsynch and mid-cycle PGF$_2\alpha$ protocols. Ten each normal cyclic cows and buffaloes exhibiting estrus within 90 days postpartum and bred served as control. All the repeat breeding cows and buffaloes under both the protocols exhibited induced estrus within 72 to 96hrs from the time of PGF$_2\alpha$ injection. The first service conception rates at induced estrus obtained in repeat breeding cows and buffaloes under Ovsynch protocol were 50.00 and 40.00 %, and under mid-cycle PGF$_2\alpha$ treatment 40.00 and 50.00 %, respectively. The overall pregnancy rates of three cycles post-treatment with Ovsynch and mid-cycle PGF$_2\alpha$ protocol were 70.00 and 60.00 % in cows and 60.00 and 70.00 % in buffaloes, respectively. In normal cyclic control cows and buffaloes also the conception rates at first AI were 40.00 and 40.00 % and those of overall of three cycles 70.00 and 60.00 %, respectively. The mean plasma progesterone concentrations in cows on day 0, 7, 9(AI) of Ovsynch treatment protocol and on day 21 post-AI were 10.60±1.57, 9.51±2.34, 0.97±0.22 and 6.62±1.69 ng/ml, respectively. The corresponding values for buffaloes were 4.59±1.17, 5.69±1.14, 0.64±0.12 and 3.59±1.26 ng/ml. Similarly, the mean plasma progesterone concentrations in cows under mid-cycle PGF$_2\alpha$ protocol on day of PGF$_2\alpha$ injection, day of induced estrus/AI and day 21 post-AI were 5.61±1.66, 0.92±0.16 and 4.08±1.58 ng/ml, and in buffaloes 5.22±1.14, 0.37±0.62 and 4.29±1.27 ng/ml, respectively. The mean plasma P4 concentrations of normal cyclic cows and buffaloes on day of AI were 0.70±0.09 and 0.59±0.07 ng/ml and on day 21 post-AI 4.23±1.56 and 3.69±1.20 ng/ml, respectively. Conceived animals in each group had significantly higher plasma progesterone on day 21 post-AI as compared to values in non-conceived animals. In conclusion, both the Ovsynch and mid-cycle PGF$_2\alpha$ protocols can be effectively used to enhance pregnancy rate in repeat breeding cows and buffaloes.

INTRODUCTION

Numerous studies have shown that repeat breeding is still the most prevalent reproduction disorder in dairy cows and buffaloes despite technological advances in animal husbandry. The overall prevalence of repeat breeding has been documented as high as 28.31 % by Bhat et al.(2012).The recent reports demonstrated that the Ovsynch protocol resulted in increased conception rates by 21 per cent in repeat breeder dairy cows (Kasimanickam et al., 2005) and mid cycle PGF$_2\alpha$ treatment gave conception rate up to 70 per cent (Patel et al., 2014).In view of the above fact, the present study was aimed to study the relative efficacies of two hormonal therapeutic approaches in repeat breeding crossbred cows and buffaloes in terms of estrus response, conception rate and plasmas progesterone profile.

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MATERIALS AND METHODS

The current study was conducted on repeat breeding dairy animals selected from the villages of Amul as well as Panchamrut milk shed areas of Gujarat and at Livestock Research Station, NAU, Navsari from November 2014 to April 2015. Before the start of experiment, palpation of the reproductive tract per rectum was conducted and specific animals that had been bred three or more times and yet not conceived were diagnosed as repeat breeders. Twenty each repeat breeding cows and buffaloes and 10 each normal cyclic cows and buffaloes were selected for this study.

All repeat breeding animals identified were treated with s/c injection of ivermectin 100 mg (Inj. Neomec, 10 ml, 1 %, Intas pharmaceuticals Ltd., Ahmedabad),i/m injection of inorganic phosphorus (Inj. Alphos-40, 10 ml, Zoetis, Mumbai), IM injection of multivitamins AD3E (Inj. Intavita, 10 ml, IntasPharma), single shot i/m injection of 3.0 g enrofloxacin (Inj. Flobac SA, 30 ml, 10 per cent, Intaspharma). Animal owners were supplied with four multi-mineral boli (Garbhamin, Indian Immunologicals Ltd., Hyderabad) for PO use, one bolus on alternate day. The repeat breeder cows and buffaloes were then randomly allotted to the following treatment protocols.

Ten repeat breeding cows and buffaloes each with normal sized ovaries were put under Ovsynch protocol and were administered with i/m Inj. of Buserelin acetate 10 µg (Ovulanta, 2.5 ml, Vet Mankind, New Delhi) on day 0, Inj. $PGF_2\alpha$ 500 µg (Repregna, 2 ml, Vet Mankind) on day 7, and second i/m Inj. of Buserelin acetate was given on day 9, and fixed time AI (FTAI) was performed twice at 0 and 24 hrs later.

Another ten repeat breeding cows and buffaloes each having mid-cycle palpable CL were put under Mid-cycle $PGF_2\alpha$ and were given i/mInj. $PGF_2\alpha$ 500 µg (Repregna, 2 ml, Vet Mankind) and FTAIs were performed twice at 72 and 96 hrs later.

Ten each normal cyclic cows and buffaloes exhibiting estrus within 90 days postpartum and bred without any treatment served as control.

Jugular vein blood samples were collected in heparinized vacutainers two, three or four times as per the protocol, i.e. blood samples were collected just before treatment, on the day of PG injection in Ovsynch group, at inducted/spontaneous estrus and on day 21 post-AI, from 8 animals in each group. The plasma progesterone profile was estimated employing standard RIA technique of Kubasic *et al.* (1984).

The conception rates in animals under Ovsynch and Mid-cycle $PGF_2\alpha$ protocols and control group were compared by using Chi-square test. The data generated on plasma progesterone profile were analyzed using standard statistical procedure (ANOVA & 't' test) to compare variation within and between group and interpreted accordingly (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

All the repeat breeding cows and buffaloes (100 %) under Ovsynch protocol exhibited induced estrus within mean intervals of 81.17±0.63 and 78.73±1.10 hrs from the time of $PGF_2\alpha$ injection, and the corresponding values for mid-cycle $PGF_2\alpha$ protocol were 80.06±0.81 and 80.21±0.73 hrs, respectively. This observation with mid-cycle PG protocol is in line with the intervals reported earlier by Sathiamoorthy *et al.* (2007) and Patel *et al.* (2014). Khasatiya *et al.* (2008), however, observed this interval as 4.07 ± 0.53 days in five months postpartum suboestrus buffaloes.

The conception rates at induced estrus (first estrus) following Ovsynch protocol in cows and buffaloes were 50.00 and 40.00 %, in second cycle 20.00 and 33.33 %, and in third cycle 25.00 and 0.00 %, with the overall pregnancy rates of three cycles as 70.00 and 60.00 %, respectively. The conception rates in Mid-cycle PGF2α treated repeat breeder cows and buffaloes were found to be 40.00 and 50.00 % at induced estrus, 16.66 and 20.00 % in 2nd estrus and 20.00 and 25.00 % in 3rd estrus, with overall

pregnancy rates of 60.00 and 70.00 %, respectively. In normal cyclic control cows and buffaloes also the conception rates at first AI were 40.00 and 40.00 % and those of overall of three cycles 70.00 and 60.00 %, respectively. The conception rates in treated cows and buffaloes improved significantly and were statistically at par with control groups.

The present overall pregnancy rate of 70.00 per cent noted in cows under Ovsynch protocol is in line with 61.00 per cent reported by Geary et al. (2001), but is higher than 22.71 per cent noted by Derar et al. (2012).Tenhagen et al. (2004) reported slightly higher conception rate 81.80 per cent in dairy cattle. The pregnancy rate over the three services in buffaloes under Ovsynch protocol in the present study was found to be 60 per cent, which is in agreement with 60 per cent reported by Ali and Fahmy (2007) and 50 per cent by Biradar et al. (2014). However, lower conception rates of 33.33 and 18.00 per cent were reported by Paul and Prakash (2005) and Karen and Darwish (2010), respectively.

The first service conception rates of 40-50% found for mid-cycle PGF$_2\alpha$t reated repeat breeders are little better than the reports of Sathiamoorthy et al. (2007) in buffaloes and Patel et al. (2014) in crossbred cows. The present findings of 60.00 per cent overall pregnancy rate found in Mid-cycle PGF2α treated repeat breeder cows is similar with the conception rate recorded by Patel et al. (2010). The pregnancy rate of the three services found as 70.00 per cent following PGF$_2\alpha$ induced estrus in buffalo in the present study is lower than the pregnancy rate reported by Khasatiya et al. (2008).

The plasma progesterone values in Ovsynch protocol were significantly (P<0.05) higher on the day of initiation of treatment, day-7 and on day-21 post-AI as compared to day of AI. The P4 values were significantly (P<0.05) higher on day of treatment, and day 21 post-AI as compared to day of induced estrus/AI in mid-cycle PG treated groups. These higher plasma P$_4$ levels suggest that the animals had functional CL of mid-cycle on the ovaries when

treatment was initiated in both the groups. In the Ovsynch protocol the maintenance or rise in mean progesterone levels noted till day 7 of treatment might be due to persistence of CL and/or luteinization of some of the growing follicles and/or ovulation of dominant follicle and formation of accessory CL under the influence of first GnRH injection. However, single injection of PGF2α in both the groups, resulted in lysis of CL or luteal tissues thereby depressing the plasma progesterone and induction of folliculogenesis, estrus and ovulation in just next 48-96 hrs.

Gustafsson et al. (1986) opined that hormonal asynchrony starts before or early in oestrus presumably leading to improper hormonal sequential changes responsible for the elevated embryonic loss during the first 3 weeks after AI in repeat breeding animals. The present findings corroborated well with those of Patel et al. (2014) in repeat breeding cows using similar treatment protocols. Further, the mean plasma progesterone concentrations in conceived and non-conceived groups in all three treatment protocols were found to be statistically similar on day 0, 7 and even on day 9/10 (AI), but on day 21 post-AI, the conceived cows had significantly (P<0.01) higher mean plasma progesterone concentrations as compared to non-conceived ones in both the treatment groups, and even in normal cyclic Control group. The hormonal protocols significantly influenced the circulatory plasma progesterone concentrations from exogenous or endogenous origin.

Bugalia and Sharma (1990) observed basal plasma progesterone concentration at the time of insemination in repeat breeding cows (0.3 ± 0.5 ng/ml), as has been noted in the present study on repeat breeding buffaloes under mid-cycle PGF$_2\alpha$ injection group. Butani et al. (2011) reported the serum progesterone values at the time of estrus as 0.45 ± 0.05 in 53 repeat breeding buffaloes, which was in line with the present observations in repeat breeders buffaloes.

From the results, it can be inferred that the application of both Ovsynch protocol and mid cycle

$PGF_2\alpha$ injection are good tools for induction of timed estrus and ovulation and enhancement of pregnancy rate in repeat breeding cows and buffaloes.

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REFERENCES

Ali, A. and Fahmy, S. (2007). Ovarian dynamics and milk progesterone concentrations in cycling and non-cycling buffalo-cows (*Bubalusbubalis*) during Ovsynch program. *Theriogenology,* **68**: 23-28.

Bhat, F.A., Bhattacharyya, H.K. and Khan, M.Z. (2012). Studies on prevalence of repeat breeding in crossbred cattle of Kashmir valley. *Indian J. Anim. Res.,* **46**(3): 306-309.

Biradar, S., Tandle, M.K., Haribabu, Y., Usturge, S.M., Patil, N.A. and Suranagi, M.D. (2014). Study on efficacy of cosynch and ovsynch protocol on fertility in repeat breeder buffaloes. *Indian J. Advances in Plant Research,* **1**(5): 1-3.

Bugalia, N.S. and Sharma, R.D. (1990). Variations in serum progesterone profiles in fertile and repeat breeder buffaloes *(Bubalusbubalis)* during late oestrus. *Indian J. Anim. Reprod.,* **11**: 140-141.

Butani, M.G., Dhami A.J. and Rajesh Kumar (2011). Comparative blood profile of progesterone, metabolites and minerals in anoestrus, suboestrus, repeat breeding and normal cyclic buffaloes. *Indian J. Field Vets.,* **7**(2): 20-24.

Derar, R., Hussein, H.A., Fahmy, S., El-Sherry, T.M. and Megahed, G. (2012). The effect of parity on the efficacy of ovulation synchronization (Ovsynch) protocol in buffalo (*Bubalusbubalis*). *Anim. Reprod.,* **9**(1): 38-43.

Geary, T.M., Whittier, J.C. Hallford, D.M. and Macneil, M.D. (2001). Calf removal improves conception to the Ovsynch and Co-synch protocols. *J. Anim. Sci.,* **79**: 1-4.

Gustafsson, H., Larson, K., Kindahl, H. and Madej, A. (1986). Sequential endocrine changes and behaviour during oestrus and metoestrus in repeat breeder and virgin heifers. *Anim. Reprod. Sci.,* **10**(4): 261-273.

Karen, M.A. and Darwish, S.A. (2010). Efficacy of Ovsynch protocol in cyclic and acyclic Egyptian buffaloes in summer. *Anim. Reprod. Sci.,* **119**: 17-23.

Kasimanickam, R., Cornwell, J.M. and Nebel, R.I. (2005). Fertility following fixed time AI or insemination at observed oestrus in Ovsynch and Heat-synch programs in lactating dairy cows. *Theriogenology,* **63**: 2550-2559.

Khasatiya, C.T., Kavani, F.S., Dhami, A.J., Derashri, H.J., Panchal, M.T. and Desai, P.M. (2008). Studies on puerperal events and reproductive efficiency following hormone therapy at day 42 postpartum in Surti buffaloes. *Int. J. Agri. Biol.,* **1**: 132-137.

Kubasic, N.P., Hallauer, G.D. and Brodows, R.G. (1984). Evaluation of direct solid-phase RIA for progesterone, useful for monitoring luteal function. *Clin. Chem.,* **30**(2): 284-286.

Patel, J.A., Dhami, A.J., Kavani, F.S., Panchal, M.T. and Ghodasara, D.J. (2010). Therapeutic management and changes in the endometrium of repeat breeding cows. *Indian J. Anim. Reprod.,* **31**(1): 1-5.

Patel, K.R., Dhami, A.J., Hadiya, K.K., Savalia, K.K., Killedar, A. and Patel, S.B. (2014). Effect of mid-cycle $PGF2\alpha$, and GnRH at AI on conception rates, plasma progesterone and biochemical profile in repeat breeding crossbred cows. *Indian J Field Vet.,* **9**(3): 5-11.

Paul, V. and Prakash, B.S. (2005). Efficacy of the Ovsynch protocol for synchronization of ovulation and fixed-time artificial insemination in Murrah buffaloes (*Bubalusbubalis*). *Theriogenology,* **64**: 1049-1060.

Sathiamoorthy, T., Parthasarathy, R., and Kathirchelvan, M. (2007). Efficacy of PGF_2 alpha, CIDR and Ovsynch treatment on oestrus induction and fertility in postpartum buffaloes. *Indian J. Anim. Reprod.,* **28**(1): 8-11.

Snedecor, G.W. and Cochran, W.G. (1994). *Statistical Methods.* 8th edn. Iowa State University Press, Ames, Iowa, USA.

Tenhagen, B.A., Surholt, R., Wittke, M., Vogel, C., Drilich, M. and Heuwieser, W. (2004). Use of Ovsynch in dairy herds-differences between primiparous and multiparous cows. *Anim. Reprod. Sci.,* **81**(1): 1-11.