Full Length Article

EFFECT OF OPU SESSION AT INDUCED AND RANDOM STAGES OF THE OVARIAN CYCLE ON OOCYTE YIELD AND *IN-VITRO* EMBRYO PRODUCTION IN ONGOLE AND GIR HEIFERS

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ABSTRACT

The present study was carried out to evaluate the effect of OPU session i.e OPU performed at known stage of cycle (OPU1) vs OPU performed at unknown stage of cycle (OPU2), on oocyte yield and in-vitro embryo production in two different Bos indicus breeds i.e., Ongole (n=6) and Gir (n=6) heifers, aged 2-4 years. OPU 1 was carried out on day 9 (luteal phase) of induced cycle and after a resting period of 3-4 weeks OPU2 was performed at random stage of cycle. A total of 24 OPU sessions were performed in 12 animals. The mean antral follicle count (AFC) (32.58±2.22 and 30.50±2.16), cumulus oocyte complexes (COC) recovery (24.58±2.50and 22±1.95), viable oocyte yield (21.25±2.20and 19.33±1.72) and embryos produced (5.91±0.9and 6.66±1.13) in OPU 1 and OPU 2 respectively,did not show significant variation (P>0.05). Similarly the breed of the oocyte donor had not shown any effect on OPU-IVEP parameters. The results suggest that the stage of estrous cycle did not impact the efficacy of OPU. Ongole and Gir heifers, both being Bos indicus category, performed uniformly in both OPU sessions.

Keywords: Bos indicus, OPU, COC recovery, in-vitro embryo production.

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INTRODUCTION

The technique of Ovum Pickup and *In-vitro* embryo production (OPU - IVEP), of late, has grown into a potential alternative to traditional embryo production methods (Bousquet *et al*., 1999; Kruip *et al.*, 1991) in view of its effective and enhanced utility in breed improvement programmes. OPU can be successfully applied to animals in

various physiological states as well as to older cows with reproductive disorders (Galli *et al.,* 2001), juvenile calves and pre-pubertal heifers (Presicce *et al.,* 1997, Majerus *et al.*, 1999 and Taneja *et al.*, 2000). It's a common practice to perform OPU at random stage of estrous cycle to aspirate follicles ≥3mm (Pontes *et al.,* 2011 and Santos *et al.*, 2016). However, the limitation with this methodology is that the follicles are at different stages of development with varying degree atresia due to presence of dominant follicle blocking the growth of subordinate follicles (Hendriksen *et al.,* 2000 and Ginther *et al.,* 2016) which may impact the developmental competence of oocytes recovered. On the contrary, reports suggest that COC recovered from follicles which are in growth phase show higher blastocyst rate compared to those recovered from follicles in dominant phase (Machatkova *et al.,* 2004). Earlier work on superovulation also confirmed that gonadotropin treatment initiated at the time of wave emergence before the selection of dominant follicle resulted in higher response and embryo production (Adams *et al.*, 1994). That is the reason why, superovulation treatments are usually initiated during mid luteal phase (day 8-12) coinciding with the time of emergence of second follicular wave. The present investigation was therefore attempted to study the efficacy of OPU conducted at the expected day of wave emergence (OPU1) vs. unknown stage of follicular growth (OPU2) with 3-4 week interval between the sessions, on oocyte yield and subsequent embryo production in heifers of two B.indicus breeds (Ongole and Gir).

MATERIALS AND METHODS

The present study was conducted at Livestock Research Station (LRS), Lam, Guntur, Andhra Pradesh during the period between August and December, 2024. All procedures were approved by animal ethics committee of the university (2024-SVVU-VGO043).

Reproductively sound Ongole heifers and Gir heifers aged 2 - 4 years and weighing between 250 and 300 kg body weight were randomly selected for the present study. The daily ration of each animal consisted of 2 Kg high protein feed containing 16-18% DCP and 70% TDN, 15-20 kg chopped fodder and 5-6 Kg paddy straw. The animals were kept under loose housing system with a large, open paddock for free movement.

The oocyte donors Ongole (n=06) and Gir heifers (n=06) were assigned to group 1 and group 2, respectively. On a random day of oestrous cycle Controlled Internal Drug Release (CIDR) intra-vaginal device (EAZI-Breed, Pfizer Animal Health, USA) impregnated with 1.38 g of progesterone device was inserted into anterior vagina and simultaneously 2ml preg heat equivalent to 2mg estradiaol benzoate (EB) (VHB Medi Science Limited, Uttarakhand) was administered intramuscularly (i.m). On day 7, CIDR vaginal implant was removed and simultaneously inj. Cloprostenol 500 µg (vetmate 2ml, Provimi animal nutrition India Pvt. Limited, Gujarat) i.m was administered. On day 8, EB (1mg) i.m was administered and on day 9 estrus detection was done.

Considering the day of estrus as reference point, heifers of both group 1 and group 2 were subjected to ultrasound examination of both the ovaries per-vaginally using a B-mode, 7.5 MHz micro convex transducer, recorded the antral follicle count (AFC) (follicle diameter of ≥3 mm) and performed ovum pick up (OPU 1), on day 9 of induced cycle. After a resting period of 3 to 4 weeks, all the heifers were again subjected to ultrasound examination of both ovaries to record AFC and 2nd OPU session (OPU 2) was conducted, at unknown stage of estrous cycle.

The OPU equipment include anultrasound scanner (My lab Gamma Vet, Esaote, Genova, Italy) equipped with a multi frequency (4-9 MHz) micro convex probe (SC 3123, Esaote) placed in a plastic vaginal probe carrier equipped with a needle, needle guide and aspiration line carrier (WTA, Brazil) which was connected to a vacuum pump set at 70-75mm Hg negative pressure and a foot operated pedal switch. The animal was restrained in an adjustable squeeze chute under epidural anaesthesia (3-5ml of 2% lignocaine hydrochloride) and the transvaginal probe fitted in the plastic probe carrier was advanced into the anterior vagina (fornix vagina). Then the ovary was positioned against the probe head to obtain clear image of the follicle on the ultrasound monitor and the number of follicles ≥3mm on both ovaries was recorded (antral follicle count). The aspiration needle was advanced into the antrum of the targeted follicle and the follicular fluid was aspirated into a conical tube containing pre warmed OPU recovery medium (IMV, France). A successful aspiration was confirmed by the disappearance of individual fluid - filled (non-echogenic and dark) follicle image on-screen display.

During each OPU session, all the visible follicles were aspirated and the recovered COCs were processed for in-vitro maturation (IVM), in-vitro fertilization (IVF) and in-vitro culture (IVC) as per the standard procedure (Vieira *et al*., 2014 and Sreemannarayana *et al.*, 2024).

The follicular aspirate was filtered through a mini oocyte filter (WTA, Brazil) and repeatedly washed with OPU recovery media. The filtered follicular aspirate was then transferred to a bottom grid petri dish and screened for COCs under zoom stereo microscope (SMZ 1000, Nikon, Japan) at 20x magnification. Under 80x magnification, the COCs were categorised as grade A, B, C, D and E and further classified into viable (A+B+C) and non-viable (grade D+E) based on oocyte integrity, homogeneity of cytoplasm and the number of cumulus cell layers surrounding the oocyte (Looney *et al.,* 1994).

Non-viable COCs were discarded and viable COCs were transferred to a four well IVM plate (NUNC© Thermo Scientific, USA) containing 500 µl pre-equilibrated maturation medium over layered with oil (Vitrogen – YVF Biotech, Brazil). The IVM plate containing COCs was then incubated in bench top incubator (6% CO2, 5% O2, 89% N2 38.8oC temperature and more than 90% RH) for 20-24 hours followed by examination of all COCs under stereo zoom

microscope at 80x magnification (Nikon, Japan), for cumulus cell expansion.

All the COCs were then transferred into a four-well IVF dish containing pre-equilibrated fertilization medium (Vitrogen – YVF Biotech, Brazil) and 10 µL of the sperm pellet, with a final concentration of $2x10^6$ sperm/ml, was added to the dish which was then placed in the bench top incubator. After 16-20 h of incubation, the presumptive zygotes were carefully denuded off the loose cumulus cells and sperms using a denudation pipette (Origio®, Denmark). The denuded oocytes were then transferred to a sterile four well IVC plate containing 500 µL in-vitro culture (IVC) medium overlaid by 300 µL of sterile oil (Vitrogen – YVF Biotech, Brazil). On day 7, IVC plate was examined under zoom stereo microscope at 80x magnification (Nikon, Japan) for different stages and grades of embryos as per the guidelines of International Embryo Technology Society (IETS).

The data was analysed using student's t-test and ANOVA.

RESULTS AND DISCUSSION

The results of OPU-IVEP session wise and breed wise were presented in Table 1.

The overall mean AFC was recorded be similar (P>0.05) in both OPU sessions (OPU1 - 32.58±2.22Vs. OPU 2 - 30.50±2.16) and there was no effect of breed on ovarian follicle population. It was also observed that AFC varied significantly between animals ranging from 20-46 at the expected time of wave emergence (OPU 1) and 16-41 at unknown stage of cycle (OPU2). Significant variation in AFC between animals was also reported by Burns *et al*., (2005) and B. indicus heifers had more follicle population than B. taurus heifers (Batista *et al.*, 2014). It was further demonstrated that AFC depletes with advancing age and young cum middle aged cows show higher follicle count than senescent cows (Malhi *et al.*, 2006 and Praveen, 2024).

The results of the current study suggest that synchronisation of follicular wave and subsequent OPU at the expected time of second follicular wave emergence (day 9) had no positive impact on oocyte recovery (OPU1: 24.58±2.50 vs OPU2: 22.00±1.95), viable oocyte yield (OPU1: 21.25±2.20 vs OPU2: 19.33±1.72) and embryos produced (OPU1: 5.91±0.9vs OPU2: 6.66±1.13). Contrary to the present observation, Cavalieri *et al.* (2018) reported higher embryonic production rates in Nelore (*B. indicus*) cows subjected to OPU on day 5 of synchronized cycle in relation to unsynchronized cycles. Probably the AFC at the time of OPU is the key determinant of oocyte recovery and the subsequent development of embryos. In line with this hypothesis, Gobikrushanth *et al.* (2018) reported no significant variation in AFC between unknown stage of follicular growth (26.0 ± 1.0) and at expected day of wave emergence (23.0 ± 1.0).

The efficiency of OPU is affected by various technical and biological factors. Technical aspects that have been studied include operator experience (Scott *et al.*, 1994), scanner resolution and needle guidance system (Mullaart *et al.,* 1999), and vacuum pressure, needle diameter (Bols, 1997). Biological factors investigated include the donor animal herself (Ferret *et al.,* 2006; Merton *et al.*, 2008), origin of the oocytes (Karadjole *et al.,* 2007), hormonal pre-stimulation (Getz, 2004; Chaubal *et al.,* 2007), the timing and frequency of OPU sessions (Blondin *et al.,* 2002; Petyim *et al.*, 2003), synchronization of follicular wave, and the dominant follicle removal (Garcia *et al.,* 2000; Chaubal *et al*., 2006). Breed of the donor is also one of the key factors that influence the efficacy of OPU-IVEP. There was a common consensus across published findings that *B. indicus* breeds were superior to *B. tauru* breeds in the number of oocytes harvested and their subsequent developmental competence (Pontes *et al.,* 2010, Sales *et al.*, 2015, De Lima *et al.*, 2021). In the present study, since Ongole and Gir heifers, both belong to B.indicus genetic group, did not show much variation in the AFC, oocyte recovery and embryo development. Other factors like age and nutritional management were also similar to both heifer groups. In corroboration to the present findings, Rajkumar (2023) also did not record significant difference in COC recovery, viable oocyte yield and embryo development between parous Ongole and Gir cows.

The results of the present study suggest that OPU-IVEP variables were not affected by the stage of the estrous cycle and, follicular wave synchronisation and OPU at an expected day wave emergence has no beneficial effect in both Ongole and Gir heifers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Table.1. Effect of OPU session (OPU 1 vs OPU 2) on oocyte yield and *in-vitro* embryo production

Attribute	Treatment type	Group 1 (Ongole n=6)	Group 2 (Gir n=6)	Total
No. of OPU sessions	-	12	12	24
AFC	OPU 1	196	195	391
	OPU 2	196	170	366
Mean AFC	OPU 1	32.60±3.98^{a,A} (20-46)	32.50±2.40^{a,A} (27-43)	32.58±2.22^{A} (20-46)
	OPU 2	32.60±3.77a,A (16-41)	28.30±2.10^{a,A} (23-31)	30.50±2.16^{A} (16-41)
No. of COCs recovered	OPU 1	148	147	295
	OPU 2	140	124	264
Mean No. of COCs recovered	OPU 1	24.66±4.36^{a,A}	24.50±2.92^{a,A}	24.58±2.50^{A}
	OPU 2	23.33±3.55^{a,A}	20.66±1.83^{a,A}	22.00±1.95^{A}
Viable COCs	OPU 1	128	127	255
	OPU 2	131	101	232
Mean no. of viable COCs	OPU 1	21.33±3.84^{a,A}	21.16±2.58^{a,A}	21.25±2.20^{A}
	OPU 2	21.8±3.21^{a,A}	16.83±0.48^{a,A}	19.33±1.72^{A}
Embryos produced	OPU 1	44	27	71
	OPU 2	47	33	80
Mean no. of embryos produced	OPU 1	7.33±1.20^{a,A}	4.5±1.36^{a,A}	5.91±0.9^{A}
	OPU 2	7.83±1.47^{a,A}	5.5±2.02^{a,A}	6.66±1.13^{A}

Means bearing different superscripts (a…b) within a row differ significantly($P<0.05$)
Means bearing different superscripts (A…B) within a column within an attribute differ significantly ($P>0.05$).
Figures in parenthesis indicate range.

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