Animal Reproduction

Abstracts - 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE)

OPU - IVF and ET

Alpha-linolenic acid alleviates the detrimental effect of lipopolysaccharides during in vitro ovine oocyte development

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Administration of LPS in the culture medium detrimentally affected oocyte maturation and reduced cleavage, and embryo formation by orchestrating an inflammatory response (DOI: 10.1016/j.theriogenology.2020.07.034. Epub 2020 Aug 1). On the other hand, certain types of fatty acids such as n-3 fatty acids showed beneficial effects on oocyte developmental competence (DOI: 10.1007/s10815-015-0439-9. Epub 2015 Feb 26). It has been reported that alpha-linolenic acid (ALA) reverses the LPS-induced effects on developmental competence. Hence, the current study was designed to evaluate the impacts of the addition of ALA to the maturation medium to modulate the detrimental effect of LPS on ovine oocyte developmental competence in vitro.

Cumulus-oocyte complexes were matured in vitro in presence of BSA and different concentrations of ALA (0, 10, 50, 100, and 200 µmol $ml^{-1;}$ diluted using ethanol) either in the absence (Negative Control) or presence (Positive Control) of 1 µg ml^{-1} LPS. The concentration of LPS was the lowest level of LPS that negatively affected oocyte developmental competence; as revealed from our previous study. Following maturation in vitro, the oocytes were fertilized, and the presumptive zygotes were cultured in vitro. Rates of cleavage and blastocyst formation were recorded out of cultured oocytes and compared between the ALA-treated and the Control groups (10 replications were performed during the experiment). The logistic regression analysis was conducted to determine the association between the dependent variable. The strength of the association was estimated by an odds ratio measure.

We observed an improvement (*P<0.05*) in the proportion of cleaved oocytes in 10 (81.8%; n=117/143), 50 (77.9%; n=116/149), and 100 (79.2%; n=118/149) µmol ml^{-1} ALA groups as compared to the Positive Control (57.8%; n=85/147), however, such an improvement was not significant when 200 (68.5%; n=85/124) µmol ml^{-1} ALA group compared with the Positive Control (*P≥0.05*), as discussed in the above refrence/study from our group. The Negative Control produced more cleaved oocytes (77.1%; n=121/157) as compared with the Positive Control (*P<0.05*).

Our data showed ALA can improve the blastocyst formation in presence of LPS. We observed that 10 (37.8%; n=54/143), 50 (35.6%; n=53/149), and 100 (36.9%; n=55/149) µmol ml^{-1} of ALA produced more blastocyst (*P<0.05*) as compared with the Positive Control (21.8%; n=32/147); no differences (*P≥0.05*) were observed when ALA groups compared with the Negative Control (35.7%; n=56/157). Nevertheless, the addition of 200 µmol ml^{-1} ALA in presence of (1 µg ml^{-1}) LPS reduced the rate of blastocyst formation (15.3%; n=19/124) as compared to both Negative and Positive Control groups (*P<0.05*).

ALA potential for preventing LPS-induced effects seems to be presented at concentrations up to 100 µmol ml^{-1} and further enhancement of ALA concentration wouldn't be beneficial. The results from the current study demonstrate that ALA has the potential to mitigate the LPS deleterious effects on oocyte developmental competence.

Keywords: lipopolysaccharide, alpha-linolenic acid, oocyte

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OPU - IVF and ET

Can the hyaluronan-binding assay be used to evaluate the fertilizing capacity of dromedary camel spermatozoa?

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A practical technique to predict dromedary camel sperm fertilizing capability is needed. A hyaluronan binding assay (HBA) is available to predict *in vivo* fertility of human sperm samples and for sperm selection for intracytoplasmic sperm injection. However, it is not known if dromedary spermatozoa can bind to hyaluronan. The objective of this study was to assess the potential of the HBA or computer assisted sperm analysis (CASA) kinematics to predict the fertilizing capacity of dromedary camel spermatozoa. Two semen samples were collected from each of six males at the Camel Reproduction Center, Dubai, and were prepared for cryopreservation (Malo et al., Cryobiology 2017;74:141-147). All animal procedures were approved by the Animal Care and Use Committee (ACUC) of the Camel Reproduction Centre, United Arab Emirates. Aliquots of prepared semen were used for HBA using the Sperm Hyaluronan Binding Assay kit (Cooper Surgical, Denmark) before freezing and after thawing; CASA was made at the same time using a CEROS II® analyser (Hamilton Thorne; MA; USA). The fertilizing ability of thawed spermatozoa was determined using a sperm penetration assay (SPA) with goat oocytes, harvested from ovaries obtained from a local slaughterhouse (Malo et al., Reproduction in Domestic Animals 2017; 52: 1097-1103). Penetration rate (PEN), male pro-nucleus formation (PN) and number of spermatozoa penetrated per oocyte (SP/OC) were evaluated. Note: goat oocytes were used in a zona-free binding assay because of the scarcity of camel oocytes. This is a routine procedure in our laboratory for assessing sperm functionality. Dromedary camel spermatozoa bound to hyaluronan with no differences between males; mean PEN 46.07 ± 4.7%, mean PN 22.81 ± 5.3%, and mean SP/OC 1.70 ± 0.4. Of the CASA parameters in fresh sperm samples, only progressive motility and Straightness correlated with HBA (r = 0.65, P = 0.02; r = 0.69, p=0.01 respectively). There was no correlation between CASA parameters and HBA for post-thaw samples. In the SPA, dromedary camel sperm bound, penetrated, decondensed, and formed a pro-nucleus in goat oocytes. There was no correlation between HBA for fresh spermatozoa and SP/OC post-thaw (r = 0.65; p = 0.11), although there was a correlation between the fresh sperm HBA result and post-thaw PEN (r = 0.81, p = 0.03) as well as with PN (r = 0.8, p = 0.03). In conclusion, the HBA score for fresh dromedary camel spermatozoa may predict post-thaw IVF performance, but further investigation is needed, such as expanding to study to include sperm samples of known quality (poor, medium and good).

Keywords: dromedary camel spermatozoa, hyaluronan binding assay, goat oocyte penetration test

Acknowledgement

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OPU - IVF and ET

Niacin supplementation during oocyte maturation improves bovine in vitro fertilization rate after ICSI

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Intracytoplasmic sperm injection (ICSI) has effectively been used in both human and animal fertility laboratories. Reactive oxygen species production above its critical levels has been shown to deterimentally affect the oocyte competence. Optimal condition for the in vitro oocyte maturation is one critical step for the occurrence of normal fertilization. We hypothesized that the addition of nicotinic acid (niacin), a potent antioxidant, to the oocyte maturation media can improve the oocyte fertilization during ICSI. Therefore, the present experiment was designed to assess the effects of niacin treatment during in vitro maturation on the fertilization rates of the bovine oocytes after ICSI procedure. Bovine ovaries were collected from the local abattoir and then transported to the IVF laboratory in less than 2 hours. Then, good and excellent quality cumulus-oocytes complexes (COCs) were recovered and randomly divided in four groups: in group 1 (control), the COCs were matured and then fertilized (n=168) in a standard maturation medium (Azari et al., Veterinary Research Communication. 41, 49–56, 2017). In group 2, the COCs were matured in a standard maturation medium with no niacin and then fertilized (n=85) using a standard ICSI procedure (Ashibe et al., Theriogenology, 133, 71-78, 2019) under an inverted microscope (Olympus, IX71, Japan). In group 3, the COCs were matured in a standard maturation medium supplemented with 1mM niacin and then were fertilized (n=88) using a standard ICSI procedure as performed in group 2. A semen with high fertility was used in all replicates of IVF and ICSI (n=5). The motile spermatozoa were separated using a swim-up method. In group 4, the COCs were matured in a standard maturation medium with no niacin and then were chemically activated using calcium ionophore A23187 and ethanol 7% with no sperm injection (n=38) for the parthenogenetic division (Bevacqua et al., Theriogenology, 74, 922-931, 2010). Oocytes maturation protocol was basically similar in all groups by using TCM-199 medium supplemented with 10% FCS, 5 IU/mL hCG (Karma, Germany), 10 ng/ml EGF (Sigma, USA), and 0.1 IU/ml human FSH (Follitrope, South Korea). In all maturation media, 50µg/ml Gentamicin (Sigma, USA) was also added. Groups of 30–50 COCs were cultured for 24 h in a 500 µl culture media at 38.5°C in 5% CO_2. Assessment of oocyte fertilizzation was performed using aceto-orcein staining method. The percentage of normal fertilization rates among the groups were compared using chi-square test. The percentage of normally fertilized oocytes in group 1 (control IVF group) was higher than that of the group 2 (ICSI with no niacin) (59.6 vs. 39.0%, $p<0.05$). However, there was no difference in the percentage of normally fertilized oocytes when the ICSI procedure was performed on the matured oocytes supplemented with niacin (group 3) as compared to group 1 (50.9 vs. 59.6%, $p>0.05$). The percentage of fertilized oocytes in group 4 (the chemically activated oocytes) was lower (8.%) than those of the other experimental groups ($p<0.05$). The results of present study demonstrated that the addition of niacin to the maturation culture media can increase fertilization rate of bovine oocytes after ICSI.

Keywords: niacin, ICSI, bovine

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OPU - IVF and ET

Pregnancy rates and parameters of an in vitro embryo production program between buffaloes and cattle in Colombia and Argentina

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Buffalo produce high quality milk and beef, most breeders use maternal models for breeding, implementing embryo transfer programs is a need due to the nature of the production system,. Establishing a baseline to set improvement objectives in the technique is necessary. This research compares the parameters and pregnancy rates in an *in-vitro* production program (IVEP) from cattle and buffaloes in two different Latin American countries. Data from an IVEP from 2012-2019 from 261 buffaloes (crossbred buffaloes, Murrah and Mediterranean) and 61 cattle (Brahman) were included in the analysis. The number of follicles, viable oocytes, cleaved oocytes, and blastocysts was recorded. Data from Colombia and Argentina were registered. Comparisons were performed using the Mann-Whitney Test. P< 0.05 was considered statistically significant, data were expressed as mean±s.d. Cattle embryos were considered as controls for buffalo. There are no statiscal differences (*P= 0,212)* in the number of total antral follicles observed between cattle 9.69±5.03 and buffaloes 8.70±6.62. It has been observed that the presence of a Corpus Luteum is significative for the number of viable oocytes (*P*< 0,001) compared to the presence of a dominant follicle (p=0.333) at the OPU. The number of viable oocytes, cleavage, blastocyst and pregnancy rate from buffalo and cattle was 8.00±5.91 vs 17.98±15,24, 4.12±3.89 vs 11.68±11.66, 1.31±1.79 vs 4.66±4.86, 26% vs 34% respectively (*P* < 0,05). Other authors have reported the differences between cattle and buffaloes in embryo production, low embryo yield and oocyte quality but very few try to discuss about the differences. The good results obtained in cattle as controls and the results from buffaloes show that the buffalo embryo production needs a careful review, of other aspects mainly related to oocyte quality, competence and the clinical conditions (health, ciclicity) of the animals used for the programs. Despite showing the feasibility of the implementation of the IVP-ET in buffaloes, the observed differences between buffalo and cattle demonstrate the need to gain a better understanding of the reproductive biology of the buffalo.

Keywords: IVPE, buffaloes, comparison, baseline

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OPU - IVF and ET

Follicular and systemic levels of IL-6, lipid metabolites, and oxidative stress index during the non-breeding season in mares

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The application of trans-vaginal OPU and ICSI is well est ablished for the commercial equine IVP. These assisted reproductive techniques are especially applied during the non-breeding season of the mare. Interestingly, no research data are available concerning the biochemical composition of the follicular fluid (FF) in small and medium-sized follicles routinely aspirated during OPU and how this is correlated with the serum composition. This study aimed to measure the FF concentrations of interleukin-6 (IL-6), total cholesterol (CHOL), triglycerides (TG), non-esterified fatty acids (NEFA), reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and oxidative stress index (OSI) in relation to follicle size, and to investigate a possible association with their systemic concentrations during the OPU-ICSI (non-breeding) season in mares. These parameters are representing the maternal inflammatory, metabolic, and oxidative stress status. At slaughterhouse, serum (n=12) and FF of small (SF; 5-10 mm in diameter, n=10), medium (MF; >10-20 mm in diameter, n=11), and large (LF; >20-30 mm in diameter, n=4) follicles were sampled from 12 apparently healthy mares. An enzymatic-colorimetric assay was used (CHOL2 and TRIGL kits; Roche Diagnostics, and NEFA F5 Kit; DiSys Diagnostic Systems, Germany) to assess the concentrations of CHOL, NEFA, and TG. For IL-6 estimation, equine kit (Nori® , Genorise Scientific, USA) was used. Concentrations of d-ROMs and BAP were identified using photometric Diacron® kits (Diacron International, Italy). One-way ANOVA was used to compare means between the different follicle categories, while t-test was conducted to compare between the systemic and follicular concentrations of each variable per follicle category. Pearson correlation coefficients were tested. P value <0.05 was considered significant. Concentrations of all variables did not show significant differences between follicle classes. Concentration of IL-6 did not differ significantly between serum (60.1±1.9 pg/mL), SF (72.8±9.5 pg/mL), MF (65.4±3.5 pg/mL), and LF (60.2±0.6 pg/mL). Concentrations of CHOL, TG, and NEFA, respectively were higher in serum (96.0±5.9 mg/dL, 45.2±6.1 mg/dL and 12.1±2.1 mg/dL) compared to SF (67.0±7.9 mg/dL, 19.3±1.9 mg/dL and 5.3±0.4) and MF (46.3±2.6 mg/dL, 15.5±1.3 mg/dL and 6.1±0.5). Concentrations of CHOL were higher in serum compared to LF (44.7±4.2 mg/dL, 16.7±1.6 mg/dL and 6.8±1.2 mg/dL). Concentrations of d-ROMs in serum (138.7±8.8 Carratelli units; UCARR) were markedly higher than SF (57.6±7.1 UCARR), MF (67.2±9.0 UCARR), and LF (42.0±11.2 UCARR). The higher concentration of BAP in serum (5571.6±619.9 µmol/L) was only significant when compared to MF (2521.8±464.4 µmol/L), but not to SF (4658.0±1116.1 µmol/L) and LF (2244.6±563.9 µmol/L). There was a strong positive association (r=0.8, P <0.01) between levels of IL-6 in serum and MF. Also, values of CHOL, d-ROMs, and OSI in serum were positively correlated with those measured in MF (P <0.05). Taken together, follicular size is not associated with alterations in the studied biochemical components during the non-breeding season of mares. There is a crosstalk between serum and follicular fluid composition. In addition, changes in the blood composition associated with maternal health (IL-6 and OSI) or diet (CHOL and NEFA) during the commercial OPU-ICSI season in mares may lead to an altered oocytes microenvironment, which may affect oocyte quality. More studies are recommended to check the influence of maternal health on the oocyte developmental capacity and subsequent embryo quality in mares.

Keywords: follicular fluid metabolism, maternal health, OPU/ICSI

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OPU - IVF and ET

Oocyte recovery and relationship between quality of oocytes and embryo production in zebu and crossbred dairy cattle

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This study conceived to compare oocyte recovery within breeds of cattle [Gir (Zebu), Sahiwal (Zebu) and Holstein-Friesian Crossbred (HFCB)] and to investigate the relationship between quality of oocytes and embryo production in respective breeds. The study was conceived to reveal the outcome of ovum pick-up and *in vitro* embryo production (OPU-IVEP) in these breeds which is yet sparse. A total of 34 OPU sessions (11, 11 and 12, respectively) were performed in 4 Gir, 4 Sahiwal, and 8 HFCB donors, without hormonal stimulation. OPU-IVEP was performed according to Patel (2020). Before IVM, oocytes were graded as graded according to presence of layers of cumulus cells and homogeneity of ooplasm as described by Viana *et al.* (2004). Blastocyst rate was calculated by dividing the total no. of blastocysts (produced on day 7 & 8) by total no. of oocytes in IVC. The evaluation of stage and grade of embryos were performed as per IETS guidelines (Manual of the IETS, 4th Edition). All the media used for OPU-IVEP were from IVF Bioscience, UK. Sperm separation medium was from FUJIFILM Irvine Scientific, USA. Descriptive statistics were used to calculate the oocyte recovery per OPU, cleavage rate and blastocyst rate in different breeds and overall means were represented as Mean±SEM. Means were compared between different breeds using one-way ANOVA using GLM. All the analyses were performed using SigmaPlot 11. During the 34 OPU sessions, a total of 148, 248 and 301 oocytes (n=697) were recovered from Gir, Sahiwal and HFCB donors, respectively. Overall, significantly ($p<0.05$) higher percentage of Grade 3 (27.7±4.5) and Grade 4 (38.8±5.6) oocytes were recovered than Grade 1 (6.5±1.4) and Grade 2 (19.3±3.9) oocytes. A total no. of embryos produced after IVEP (n=222, blastocysts rate across breed-35.5%) were 48, 60 and 114 in Gir, Sahiwal and HFCB, respectively. The average no. of oocytes recovered per OPU (overall average 20.5±1.9) in Gir, Sahiwal and HFCB were 13.5±2.3, 22.5±3.2 and 25.1±3.3, respectively. The corresponding cleavage rates observed were 66.5±5.1, 53.0±4.5 and 83.4±2.2%. The average no. of blastocysts per OPU in Gir, Sahiwal and HFCB were 4.4±1.2, 5.4±0.8 and 9.5±1.6, respectively. In all three parameters, values were significantly higher in HFCB compared to Gir ($p<0.05$). Combined no. of oocytes in Grade 3 and Grade 4 had positive correlation with cleavage rate ($r=0.3$) and blastocyst rate ($r=0.4$, $p<0.05$). **In conclusion,** significantly higher no. of oocytes recovered per OPU, cleavage rate and no. of blastocysts per OPU were attained in HFCB than in Gir. If the oocytes of grades 3 and 4 are processed together with the oocytes of higher grades, there is a chance to produce blastocysts also from the oocytes of lower grades. This is important in the zebu donors in which the total no. of oocytes harvested is less than in the HFCB donors.

Keywords: oocytes grade, blastocyst, cattle breed

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OPU - IVF and ET

Optimizations of an ovum pick up protocol in Donkey.

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The Donkey is an animal classified as an endangered species, so the cryopreservation of oocytes would allow the creation of a germplasm bank that allows the preservation of its genetic material and the preservation of the species. The main process for obtaining oocytes in live females is Ovum pick up (OPU). This technique is widely used and established in horses, however, in Donkey, it is little used, so it is necessary to standardize an OPU protocol to obtain viable oocytes for cryopreservation. For this reason, the objective of our work is to standardize an OPU protocol in Donkey. To do this, first, a suitable sedation protocol was established during follicular aspiration. 4 different protocols were tested: Acepromazine 0.04 mg/kg + Xylazine 1 mg/kg; Detomidine 0.5mg/kg + Butorphanol 0.5mg/kg; Medetomidine 0.0035 mg/kg + Butorphanol 0.025 mg/kg and Acepromazine 0.02 mg/kg + Xylazine 0.44 mg/kg + Butorphanol 0.02 mg/kg. To check the best protocol, different stress hormones were measured before and after the OPUs, cortisol, C-reactive protein, lactate dehydrogenase (LDH) and creatinine kinase, choosing the most appropriate protocol that produced less stress and greater animal welfare. Before and after the OPUs, the levels of progesterone (P4) and estrogen (E2) were determined to check the effect of aspiration on follicular activity. Subsequently, the OPU (N=15 aspirations) were performed on a total of 9 donkeys, determining the number of follicles aspirated, number of oocytes retrieved and the number of mature follicles. To optimize the maturation process, two different culture media were tested; 1) DMEN/F-12 and 2) TCM-199; both enriched with 10% FBS + 25µg/ml gentamicin and 5mIU/ml FSH + 50ng/ml IGF-1. The oocytes obtained from each aspirated animal were divided between the 2 treatment groups. The degree of maturation was evaluated by the presence of the polar body after decumulation with hyaluronidase and the expansion of cumulus cells. In addition, viability was evaluated by staining with propidium iodide, as well as DNA integrity, cytoskeleton and mitotic spindle, using the anti-α-tubulin antibody linked to the fluorochrome Alexa fluor-488. Analyses were performed by fluorescence microscopy. The statistical analysis used to compare the different sedation protocols was a two-way ANOVA using the Bonferroni test. The statistical analysis used to compare the values of E2 and P4 in the different conditions was a one-way ANOVA using Tukey's multiple comparison test, in all statistical analysis $p<0.05$ was considered a significant difference. The results obtained indicated that the best sedation protocol was that of 0.5mg/kg detomidine + 0.5mg/kg Butorphanol, since it was the one that least increased stress hormones, this decrease being more significant in the case of cortisol ($p<0.01$) and creatinine kinase ($p<0.05$) with respect to the other treatments. Regarding the levels of P4 and E2, these increased after the OPUs, this increase being much greater in the case of P4 ($p<0.001$) compared to the baseline values. Of all the aspirations performed, a total of 259 follicles were aspirated, of which 178 oocytes (67.93%) were recovered and distributed equally for the two treatments, of all these, 127 matured (68.77%) after 34h of incubation. Of all these mature oocytes, 49 (38.6%) matured with TCM-199 medium and 78 (61.4%) with DMEN/F-12 being this difference significant ($p<0.05$). With DMEN/F-12, greater viability (80%) and greater integrity of the mitotic spindle (71%) were achieved tan TCM-199, Therefore, we can conclude that, with the results shown, DMEN/F12 is the best medium for the culture and maturation of oocytes obtained after OPU.

Keywords: Donkey. OPU, oocyte maturation

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OPU - IVF and ET

Comparative study of oocyte recovery and embryo production using OPU- IVP techniques in six indigenous cattle breeds of India

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In Vitro Embryo Production (IVP) is still an emerging technology under Indian context. The present experiment was undertaken to determine oocyte recovery rate and embryo production using In-Vitro Fertilization technique (IVF) in six indigenous cattle breeds of India. BAIF Development Research Foundation, Central Research Station, Uruli Kanchan, Pune, Maharashtra, initiated work for IVP and studied the performance of six zebu cattle breeds namely Gir (17 donors), Sahiwal (10 donors), Deoni (02 donors), Dangi (03 donors), Gaolao (05 donors), and Red Kandhari (05 donors). All these experimental donors were maintained under the same managemental practices with the same feeding regime. Data was generated from 901 OPU sessions performed during a study period from October 2019 to January 2022 for IVP. Ovum Pick Up (OPU) procedure was carried out once every 15 days irrespective of season. In all the OPU sessions during the experimental period, 20-gauge OPU needle was used, and the vacuum pump pressure was maintained in between 70 to 90 mm of Hg and temperature range maintained of vacuum pump was in between 37 to 38 ^{o}C. OPU was performed without using pre-stimulation protocols for the non-lactating donors. All OPU donors have lactation range from 1 to 4 and their average age ranged from 4 to 10 years. Throughout the study period, these donors were not inseminated to make them pregnant. Media used for IVP were of IVF Bioscience, UK and Vitrogen, Brazil both. Use of media was random for IVP. No separate records were maintained to study the embryo production within the breed using the two different types of media. All the recovered oocytes were further processed in laboratory for IVP with protocol of 20 to 22 hrs of maturation period, 16 to 18 hrs for fertilization and 6 to 7 days post fertilization of culture period for embryo production. The parameters studied were oocyte recovery and embryo production per OPU session.

The best average oocyte recovery resulted in Dangi (9.32 ± 0.75) followed by Gir (8.94 ± 0.36) and Sahiwal (7.15 ± 0.38) breed. In the remaining three breeds, the average oocyte recoveries were 6.91 ± 0.98, 6.74 ± 0.92 and 3.86 ± 0.61 per OPU session in Deoni, Gaolao and Red Kandhari breeds, respectively. In terms of embryo production, Gir breed produced on average 2.66 ± 0.14 embryos per OPU session, followed by 1.96 ± 0.15 in Sahiwal, then 1.90 ± 0.30 in Dangi breed. Results of oocyte recovery and IVP are significantly influenced by the breed ($P< 0.01$). One-way ANOVA and Duncan's multiple range test were used to identify the critical differences among the breeds. All results are depicted as mean ± standard deviation. With the present experiment we can infer that three breeds namely Dangi, Gir and Sahiwal have better performance with regard to oocyte recovery and embryo production as compared to remaining three breeds. Amongst all the six breeds, Red Kandhari cattle breed was a poor performer in terms of oocyte recovery (average 3.86 ± 0.61) and embryo production per OPU session (average 0.92 ± 0.24). It requires more data to conclude the performance of Zebu cattle in respect to OPU and in vitro embryo production under Indian conditions.

Keywords: In vitro Fertilization, Ova pick up, embryo

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OPU - IVF and ET

The effect of IGFBP-4 on IGF-2 stability in bovine cumulus-oocyte cells during in vitro maturation

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The insulin-like growth factor 2 (IGF-2) is essential for oocyte maturation, cumulus cell steroidogenesis and oocyte viability. Nonetheless, free IGF-2 demonstrated a short lifespan. Six high-affinity binding proteins (IGFBP-1, -6) regulate the biological functions of IGF-2 by prolonging its lifespan and regulating its bioavailability on the target cells. From the IGFBPs, especially IGFBP-4 inhibited IGF-2, and it has been linked with the appearance of follicle atresia. To gain an inside on the regulation of IGFBP-4 on IGF-2 during cumulus-oocyte cells (COCs) maturation, the *in vitro* maturation medium (TCM 199 based, supplemented with 1 mg/ml of fatty acid free bovine serum albumin, /Sigma-Aldrich, Taufkirchen, Germany/; 10 I.U./ml equine chorionic gonadotropin and 5 I.U./ml human chorionic gonadotropin /Suigonan® 80/40 I.U./ml lyophilizate and injection solution, MSD Animal Health, Unterschleissheim, Germany/) was supplemented with recombinant human IGF-2 (rhIGF-2; 50 ng/ml, R&D systems, Bio-techne, Abingdon, United Kingdom) alone or in combination with recombinant bovine IGFBP-4 (rbIGFBP-4; 2,000 ng/ml, InVivo Biotech Services, Hennigsdorf, Germany) in the presence or absence of COCs. Bovine COCs were collected from abattoir-derived ovaries. Groups of 25 COCs were randomly assigned to each experimental group and set to *in vitro* maturation for 24 h at 38°C, 5% CO_2. Three biological repetitions were performed. The IGFBP-4 binding capacity was evaluated by taking samples directly after medium preparation (0 h) and after 3, 6, and 24 h of *in vitro* maturation. Free IGF-2 concentrations were measured using a competitive radioimmunoassay (Mediagnost®, Reutlingen, Germany). The rhIGF-2 was bound to rbIGFBP-4 by 49.8 ± 24.6% directly at medium preparation (0 h), 74.7 ± 21.3% after 3 h, 55.4 ± 33.9% after 6 h, and 44.3 ± 40.5% remained bound after 24 h of incubation. Similarly occurred in the absence of COCs, IGF-2 was bound to IGFBP-4, 49.8 ± 24.6, 72.2 ± 23.5, 48.5 ± 38.1 and 32.5 ± 58.2% at 0, 3, 6, and 24 h respectively. The concentrations of rhIGF-2 added to the medium and incubated in the presence of COCs declined 13.2 ± 8.6, 16.0 ± 10.8 and 77.2 ± 6.2% after 3, 6 h, and 24 h incubation respectively. Contrary, the concentrations of rhIGF-2 without COCs remained stable throughout 24 h. We conclude that rbIGFBP-4 was able to bind rhIGF-2 with a maximum binding capacity around 3 h after incubation and decreased at the final stage of IVM. The binding capacity was not affected by the presence of COCs. Moreover, COCs used the free available rhIGF-2 in the medium. Finally, rhIGF-2 demonstrated to be stable for 24 h under

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