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The Local Organizing Committee of the ISDAR 2015 has been solely responsible for the review of the abstracts contained herein. The abstracts were peer reviewed and edited by Mushtaq Ahmad and Muhammad Zahid Tahir

INVITED PRESENTATIONS

I-01 New Approaches in Estrus and Ovulation Synchronization Programs in Cattle and Buffaloes

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Reproductive performance in a dairy herd has a significant impact on production and profitability. Although artificial insemination (AI) and embryo transfer (ET) are an important tool to accelerate genetic gain and milk yield, the widespread use these reproductive biotechnologies are limited by factors which reduce the percentage of cows submitted for AI and ET such as a low efficiency of estrous detection and postpartum anestrus. The use of ovulation synchronization programs for AI and ET in dairy herds has been reported across multiple studies to consistently increase the service rate, decrease the interval from calving to first service and also to increase proportion of pregnant cows sooner after the end of the voluntary waiting period. The control of the emergence of follicular waves and of ovulation at predetermined times, without the need for estrus detection, has facilitated the management and improved the efficiency of AI and ET programs in dairy herds. Follicular wave development and ovulation can be synchronized by treatments with prostaglandin and GnRH or a combination of estradiol and progestogen/progesterone. Synchronization protocols dramatically improve reproductive success by offering the possibility of planning the application of assisted reproductive technologies and allowing producers to breed more cows in less time. Although there are similarities in estrous cycle length between cattle and buffaloes, reproductive differences between these genetic groups are well documented. For this reason, one cannot assume that outcomes obtained in cattle (*Bos taurus* or *Bos indicus*) would be the same as in buffalo. Conclusively, the estrus synchronization treatments may facilitate the application of assisted reproductive biotechnologies, thus allowing a more efficient genetic improvement and reproductive outcome in cattle and buffalo herds.

Key words: Animal reproduction, Dairy, Cattle, Buffalo

I-02 Role of Ultrasonography in Management of Dairy Cattle Reproduction and Disease Diagnosis

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Use of ultrasonography in bovine practice began in mid-80's and is now a routine practice for large farms in both developed and developing countries. Transrectal ultrasonography is no longer an adjunct to rectal palpation. It provides valuable information regarding dynamic changes in ovaries (follicle number, follicle size, corpus luteum changes) and uterus (echotexture, fluid accumulation, coiling) that are predictive of the stage of estrous cycle. Ultrasonography is particularly useful for early pregnancy diagnosis, assessing fetal health, twinning, and fetal sex diagnosis. Ultrasonography of testes and accessory sex glands (vesicular gland, prostate, bulbourethral glands) in male animals provides diagnostic and prognostic value for many disease conditions. Likewise, ultrasonography is useful for assessing the response to treatment for ovarian and uterine diseases (luteal cysts, follicular cysts, pyometra, mummified fetus, etc.). It is noteworthy that the use of ultrasonography is not associated with higher embryonic loss. Imaging the ovaries in cattle has led to an understanding of ovarian dynamics in this and other species, including humans. These advances have ushered the development of new protocols for ovarian synchronization, fixed-time artificial insemination, and superstimulation. Advances in Doppler imaging (Spectral, Color, Power), ultrasound biomicroscopy, 3-dimensional imaging, tissue stress analysis, and echotexture analysis will ultimately enable us to determine the health status of individual follicles and their contained egg and allow determination of the precise stage of the estrous cycle based on a single examination.

Key words: Ultrasonography, Reproductive diseases, Cattle reproduction

I-03 New Approaches of Estrus Synchronization to Improve Reproductive Performance in Dairy Buffaloes

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Buffaloes contribute more than 65% of total milk production in Pakistan. They are often blamed for having slow reproduction. Series of systematic experiments were conducted in our group at UVAS, Lahore to see if its reproduction can be enhanced through estrus and ovulation protocols. Our premier study showed that buffaloes can be successfully synchronized with optimum fertility using either PGF2 alpha alone (detected oestrus) or using (Ovynch protocol) during low breeding season, to calve during the period when milk availability is short (Warriach et al 2008). Then we moved on to synchronization with CIDR based protocols particularly during summer when most buffaloes are anoestrous. Administration of EB and GnRH after CIDR removal resulted in tighter synchrony (less variability) and improved fertility in anoestrous buffaloes (Naseer et al., 2010). This implied great potential in fertility improvement in anoestrous buffaloes. These results got confirmed in a study based on large number of buffaloes which indicated that administration of EB in conjunction with CIDR improves estrus intensity and pregnancy rate in multiparous buffaloes. Similarly, there was a trend ($P > 0.05$) of more synchronous timing of ovulation (62.9 ± 1.8 vs 72.4 ± 3.6 hrs) and higher ovulation rate (91% vs 64%) after EB administration (personal communication). Because ovulation studies in normal buffaloes are scarce, we determined it and found that timing of ovulation was 35.3 ± 0.21 hours from the onset of standing heat. More interestingly, highest pregnancy rate, 53% (15/28) was observed in buffaloes inseminated at 24 h, followed by 37% (10/27) at 12 h. These are exciting new findings which imply that AM-AM rule (24 hr) is more suitable for buffaloes (Riaz et al. 2014). In order to enhance ovulation and pregnancy rates we found that addition of eCG at the time of CIDR removal in FTAI program increased the estrus response, ovulation rate and pregnancy per AI in Nili-Ravi buffaloes (Naveed et al., 2014). On the question of reducing embryo loss in buffaloes we enhanced progesterone after AI on day 7 through administration of hCG. Results revealed that hCG successfully induced ovulation and improve overall conception rate (Husnain et al., 2013). In conclusion, various innovations in synchronization protocols indicate clearly that pregnancy per AI can be significantly enhanced, both breeding and low breeding season, in

buffaloes. This outcome is more pronounced when the body condition of buffaloes is good. However, its implications remain challenging for a small holder buffalo producer. (Acknowledgements: Author gratefully acknowledge financial assistance provided by the HEC Pak-US Project under S&T agreement).

Key words: Estrus synchronization, Hormones, Fixed time insemination

I-04 New Extension Approaches for Improving Small Dairy Holder in Pakistan

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Pakistan is a developing country whose economy primarily depends on agriculture. Livestock forms 55% share of agriculture in national GDP. Dairy farming forms the major segment of livestock industry in Pakistan. According to an estimate there are 9 million small holder dairy farmers in Pakistan. Livestock department is primarily focusing on providing veterinary care and treatment while meagre efforts are being done to increase the production of animals. Most of small holder dairy farmers are unaware of basic principles of livestock management. We launched a dairy extension program in 56 villages of Pakistan under the umbrella of Agriculture Sector Linkages Program (ASLP). We conducted monthly farmer meetings in selected villages to spread awareness on livestock health, management, nutrition and reproduction. We noticed that adoption of simple extension messages had a significant impact on the milk production of animals. For example, simply untying of animals resulted in 1.5 liter increase in milk production per animal per day. If we can achieve the same in whole lactating herd of the country, we can bring a revolution in total milk production. Similarly, high calf mortality is a big issue in Pakistan which can be easily dealt with by adopting simple calf rearing practices like provision of ad lib colostrum to calves. We experienced that individual visits of extension worker to farms on regular basis can help to develop trust and motivate dairy farmers to adopt the extension messages. Moreover, we observed that it is of utmost importance to adopt whole family approach in dairy extension because women and children are equally involved in small holder dairy production system. In this regard, monthly female farmer meetings and involvement of local schools in dairy extension proved to be quite useful. Lastly, information communication technology (ICT) can be used to spread mass awareness. Television, radio, social networking and text messaging on cell phones are effective tools to spread extension messages at mass level.

Key words: Communication, Extension, Small Dairy holder, Reproductive management

I-05 Recent Developments in Understanding and Approaches to Deal with Reproductive Disorders in Dairy Animals

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Dairying, an integral part of human livability, is undergoing fast structural changes, especially in Asia. Although the milk yield in dairy cattle has considerably increased over the last 50 years, there has also been a temporal increase in the incidence of infertility. With the invent of newer knowledge of animal reproductive process, and development and application of better diagnostic techniques, our abilities to understand, and to address the various reproductive disorders have also improved. The underlying causes for most of the reproductive failures lie in inappropriate and faulty animal management and nutritional inadequacies. Precise role of the various components of the nutrition is complex and yet remains to be fully understood. This information though is very vital to the development of appropriate feeding strategies for uncompromised harmonious health, production and reproductive processes. We have observed that dietary deficiency of polyunsaturated fatty acids affected the duration of oestrus in dairy cattle by altering the production of prostaglandins from endometrium. Further, the various feed supplementations with higher energy sources have not always been successful in preventing or curing infertility. Studies have revealed that increasing the proportion of proteins in animal diet, although improved milk yield but decreased the conception rates by altering the uterine environment. Some of the successful efforts in improving fertility included supplementary feeding of ionophores which increased circulatory glucose, decreased enteric methane production and improved fertility in dairy bovines. The oestrus synchronization protocols developed and tested in our laboratory have been successful to quite an extent in by-passing the problem of estrus detection and improving fertility. Studies have also suggested that changes in the duration of oestrus cycles, often associated with infertility in high yielding cows, may have occurred due to luteal deficiency owing to increased progesterone metabolism. Newer techniques have also been developed to facilitate stress free and uneventful relief in certain obstetrical conditions such as uterine torsion. Further studies are warranted on better understanding of climate change, and

various biotic and abiotic stressors vis-à-vis reproductive efficiency in dairy cattle.

Key words: Reproductive disorders, Reproductive efficiency, Fertility, Bovines

I-06 Management of Reproductive Disorders in Buffaloes and Cows

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Reproductive disorders occur frequently in dairy animals and severely affect both their reproductive efficiency and profitability. In Pakistan, dairy farming mainly based on buffaloes and cattle (Sahiwal, Cholistani and cross bred) population. Recently exotic animals (Friesian, Jersey and Friesian x Jersey) are imported to meet the increasing demand of milk production. Some of the most common reproductive disorders confronting dairy animals include anestrus, uterine torsion, metritis, prolapse, cystic ovarian degeneration (COD), retained fetal membrane (RFM), repeat breeding (RB) and abortion. Incidences of these disorders vary in different breeds and species. Like, anestrus is the major condition in dairy buffalo and in exotic cows repeat breeding and abortion are common disorders. Occurrences of these disorders increase in summer months. Most likely, inadequate feeding is the prime reason in the former case and lowered heat tolerance and poor management in the case of latter. Uterine torsion, prolapsed and dystocia are common disorders faced in buffaloes particularly of small farmers. Diagnosis generally based on physical and clinical examination and surgical procedures like caesarean section are relatively uncommon due lack of resources, expertise and post operative care. Likewise, the major problems of high yielding pure exotic dairy breeds are cystic ovarian degeneration, anestrus, repeat breeding and footrot. Nevertheless reproductive disorders are significantly lower in exotic cross bred cows. However, incidence of reproductive disorders can and are being reduced by avoiding summer calving in exotic breeds with the adaptation of balanced nutrition, and using estrus synchronization protocols with the application of best management practices. Moreover, extension and educational programmes focusing herd management and preventive veterinary practices are essential to improve the reproductive disorders in dairy animals.

Key words: Cystic ovarian degeneration, Infertility, Summer stress

I-07 Updates on Vaginal Prolapse in Dairy Animals

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Prolapse of vagina is the most common maternal gestational complication in dairy animals that includes outward protrusion of the vaginal floor, the lateral walls and a portion of the roof of the vagina through the vulvar lips. The basic cause appears to be the weakening or relaxation of the constrictor vestibuli muscle and atony of the vaginal musculature. The incidence of pre-partum cervico-vaginal prolapse has been higher (3-13%) in water buffalo. The incidence is higher in summer season and is more in stall fed animals. Postulated etiologies for a higher incidence include low levels of plasma copper, selenium and zinc, low levels of serum calcium and phosphorous and increased levels of plasma estradiol during late gestation. Less common etiological factors include vaginitis and urinary tract infection. Vaginal prolapse is more common between 8-10 months of pregnancy in buffalo. The protrusion of an ugly looking soiled mass is an obvious clue for diagnosis made by the owner. Except in extreme or severe cases, the prognosis is generally fair to good for the life of the animal, and the fetus, if treatment is prompt and aftercare is good. The vulvar truss is of practical value in pregnant dairy animals and is a frequently used method for prevention of recurrence of cervico vaginal prolapse. The truss made of leather, wide cloth and ropes are good. A wide variety of sutures have been suggested for retaining the prolapsed organ in dairy animals including the buhner suture, the bootlace suture, flessa sutures, and the interrupted horizontal mattress suture. Two techniques for surgical repair of vaginal and cervical prolapse include vaginopexy (Minchev's method) and cervicopexy (Winklers operation). Care of animals with prolapse include little of exercise, feeding of less bulky diets, reduction in estrogenic feeds and frequent watch of these animals. Recently a modified Buhner's technique, using sterile infusion set tubing as suture material, is suggested to be effective in retention of the mass in cattle.

Key words: Dairy animals, Vaginal prolapse, Incidence, Etiology, Treatment

I-08 Recent Advances in Bovine Semen Cryopreservation

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Cryopreservation is a long-term storage of living cells or tissues at extremely cold temperature. Cryopreservation of germplasm (semen, oocytes and embryos) plays an important role in the conservation of animal genetic diversity and exploitation of genetically superior animals. Frozen semen is extensively used in artificial insemination programs throughout the world. The success of semen cryopreservation largely depends upon species and breeds within species. During semen cryopreservation, sperm undergo various processes like dilution, cooling, and freezing and thawing. During these processes, sperm face the biophysical events, i.e. osmotic shock, solution effect and intracellular ice formation. These events affect sperm structures which ultimately result in cells' malfunctioning. Since the inception of semen freezing technology, cryobiologists have been trying to minimize sperm damage due to the biophysical events. Recently, the pre-freeze treatment of mammalian sperm with cholesterol-loaded cyclodextrin improved the post-freeze survival of sperm in various species. Generation of reactive oxygen species (ROS) during semen processing leads to lipid peroxidation of sperm plasma membrane and death. The addition of antioxidants in semen extender helps to neutralize the adverse effect of ROS on sperm health. Due to the bio-security risks associated with animal products, the scientists are eagerly looking for semen cryopreservation procedure without animal protein (egg yolk or milk). There is always a scope of improvement in semen cryopreservation technology. High quality of frozen-thaw semen ensures good fertility in the field.

Key words: Cryopreservation, Reactive oxygen species, Semen

I-09 Role of Early Onset of Puberty in Selection of Donor Bulls for Use in Artificial Insemination

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Pakistan has the best dairy buffalo breeds in the world (Nili-Ravi and Kundi). In spite of the fact that artificial insemination (AI) was introduced in the country in 1955, its benefits like in advanced dairy world, have so far failed to

show similar enhancement in Pakistan for buffalo productivity over the last 65 years. The main reason for this has been the use of donor bulls without exactly known genetic values. The semen production unit, Qadirabad (Sahiwal), since 1972 is maintaining a large number of donor buffalo bulls for production of semen doses to meet the insemination demands of the field. In 1980, a research project started investigating the state of fertility of the stock. The data analyzed as of normal bulls showed an average body weight of 511 ± 11.82 kg achieving first semen ejaculation by 35.22 ± 1.53 months of age. These averages for abnormally developing bulls maintained during the same period (1972-80) for body weight and age at first semen ejaculation were 548 ± 22.25 kg and 41.2 ± 2.62 months, respectively. During the conduct of this work, definite relationships between age and body weight, age and scrotal circumference (cm), scrotal circumference and semen production ability and sexual behavior indices and onset of puberty were established (Ahmad et al. 1983 and Arsalan et al. 1987). Following establishment of the selection criteria, two studies on normally developing young bulls raised under optimal feeding and management and observing/recording the various sexual behavior indices after 15 month of age till onset of puberty were undertaken. One such study was at semen production unit Qadirabad (Sahiwal) involving 13 young candidate bulls during 1981 to 1983. Five young bulls of this group donated semen before two years of age (from 21 to 23 month of age). The other study was undertaken at NARC, Islamabad during 1984-86 on 16 growing bulls. Six bulls of this group gave semen ejaculate before two years of age (from 16 to 24 months of age). These were remarkable findings and were worthy of follow up for selection of "Performance Tested Bulls" capable of yielding semen around two years of age. In early 2014, it was decided to make a "Calf Raising Center" operational at Buffalo Research Institute, Pattoki (District Kasur). As a first step, the existing status of bull raising for use in AI at different sites were evaluated by recording their age, body weight, scrotal circumference and age at first semen ejaculation. At BRI, a group of 18 young bulls brought from different sites and different owners was raised as a future donor bulls. Their ages, body weights (kg) and scrotal circumferences (cm) varied from 25 to 47 months, 250 to 413 kg and 17.5 to 25.0 cm respectively. These recorded values for different ages were much less than their qualifying standards. They were far more than two years of age, yet none of them was able to show the sign of onset of puberty. At SPU, Qadirabad, young bulls being raised under good managerial condition, 30 of them varying in age from 25 to 36 months had body weights and scrotal circumferences varying from 270-540 kg and 19 to 29 cm respectively. The second group of 11 bulls ranged between 36 to 47 months had body weights and scrotal circumferences varying from 395 to 553 kg and 22.5 to 29 cm respectively. None of these 41 growing bulls whose ages were much beyond two years had so far not yielded any

semen ejaculate. Among adult donor bull giving regular weekly semen ejaculates, showed that their ages at first semen ejaculation were 39 and 50 months (best bull and the oldest). Among four available progeny tested buffalo bulls, their ages at first semen ejaculation were 37, 37, 39 and 39 months. All these findings suggested that in years following 1987 till to-date, selection and rearing programs of breeding buffalo bulls failed to follow the targets of producing "Performance Test Bulls" capable of showing onset of puberty by around two years of age. This situation was causing less production of fertile semen doses and delaying the age of production of progeny tested bulls. Since August 2014, at CRC six candidate bull calves are being raised in four age groups under a uniform environment of nutrition, health cover and managerial practices. From birth onward their monthly body weights and thrice monthly scrotal circumferences are being recorded. After 15 month of age in group of eight they are taken out twice weekly in a special open paddock area to observe the expression of various sexual behavior indices (sexual interest (SI), mounting (M), penile protrusion (PP) and semen ejaculation (SE)). So far, four young bulls have expressed onset of puberty at 20, 21, 23 and 23 months of ages. Nine are showing partial penile protrusion and two have shown mounting behavior. These recording will continue till such time these bulls express the process of onset of puberty. The data thus obtained will be tabulated that at what age they completed the period of onset of puberty. This exercise will continue in a cyclic manner as a routine of the "Calf Raising Center". Those showing onset of puberty will leave CRC for SPU for further raising and progeny testing. New ones completing the age of 15 months will join the exercise for detection of arising signs of sexual behavior indices.

Key words: Young Buffalo Bulls; Onset of puberty; Calf Raising Center; Semen production Unit; Sexual Behavior Indices (Sexual Interest) (SI), Mounting; Penile Protrusion (PP) and Semen Ejaculation (SE)

I-10 Recent Advances in Cryobiology of Bubaline Semen

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Cryopreservation of bovine semen involves several critical steps that can affect sperm structure and functions. Plasmalemma is the primary site of injury, which occurs during the *freeze-thaw processes*, resulting in substantial loss of viable spermatozoa. Cryopreservation may also affect the sperm DNA stability resulting in decreased fertilization rates. Successful cryopreservation of bovine spermatozoa depends on several factors, like biochemical

makeup of semen, season of collection, composition of extenders (buffers and cryoprotectants), cooling/freezing and thawing rates. Therefore, this presentation details the recent advances in cryobiology of bovine semen to minimize the “adverse affects”.

Key words: Buffalospem, Cryoprotectants, Thawing

I-11 Regulation of Puberty in Buffalo Bulls

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Puberty is hallmark of development with great physiological importance. This milestone is especially important in farm animals as it makes possible reproduction and production. Puberty is orchestrated by synchronized neural, endocrine and somatic signals. While the underpinning of puberty has been well studied in several dairy species surprisingly little is known about the pubertal dynamics in water buffalo which is a prime dairy animal in Asia. This talk focuses on somatic, morphologic, behavioral and endocrine determinants of puberty in male water buffalo based on our earlier work with this model and intends to give ideas for the future research in the area. Longitudinal (birth to puberty) and cross-sectional studies indicate that in Nili-Ravi male buffalo mean pubertal age, body weight and testicular volume are 22.8 ± 1.1 v months, 421 ± 19 kg and 188 ± 12 cm, respectively. The attainment of puberty is related to the time of first ejaculation with motile sperm. Correlations among age, body weight and testicular volume are >0.95 ($P < 0.01$). Increase in body weight and testicular size is markedly greater from 8-15 months of age than during any other period of development. A significant ($P < 0.05$) relationship is found between age at puberty and the time when a maximal increase in testicular growth rate is observed during the prepubertal period. Season of birth does not influence ($P > 0.05$) age, body weight or testicular volume at puberty. Mean serum testosterone concentrations remain low from birth to 12 months of age (0.3 ± 0.1 ng/ml). A marked rise in testosterone is observed at 14 months of age (2.7 ± 0.9 ng/ml) followed by a second peak at 18 months of age (3.3 ± 1.2 ng/ml). Increased androgenic activity leads to establishment of spermatogenesis, as reflected by appearance of significant number of spermatids, occurring by 18 months of age. A significant increase in sexual behavior score also manifests around this time. Serum estradiol levels increase during the early part of prepubertal development attaining a mean concentration of 27 ± 3 pg/ml at 5 months of age and remain more or less

unchanged until the age of 15 months. The levels decline thereafter and remain low throughout the rest of the pubertal development. Interestingly a pulsatile pattern of estradiol secretion is observed in neonate and juvenile males whereas the prepubertal animals show an absence of such a pattern. Collectively foregoing results suggest that in the buffalo testicular quiescence extends from 0 to 7 months of age, followed by a period of rapid testicular growth and considerable androgenic activity along with decreased estradiol tone. Enhanced testosterone secretion establishes spermatogenesis and induces sexual behavior. Lot needs to be studied regarding developmental gene expression in the buffalo hypothalamus that is the penultimate trigger of initiation of puberty.

Key words: Puberty, Hormones, Age, Testicular growth

I-12 Nutrition-reproduction Interactions in Buffaloes

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Buffalo rearing is very important for rural development in many countries. The buffalo production system, however, is a subsistence type in most of the countries. These buffaloes are generally kept only under roughage feed with an occasional concentrate supplement that put them under nutritional stress. Studies in rural buffaloes in Nepal using nutritional indices of body condition score (BCS) and some blood metabolic parameters indicated that buffaloes were under severe nutritional stress during the pre-monsoon fodder-scarce dry season of February to June. The effect on reproductive inability were related to sub-normal levels of some minerals and total protein in the blood. Also, the proportion of anestrus buffaloes with poor BCS was greater ($P < 0.01$) during the dry season as compared with the fodder-rich monsoon or post-monsoon season. Nutritional status also affected on the result of assisted reproduction in anestrus buffaloes. In silently ovulated buffaloes bearing CL in the ovary, treatment with PGF₂ was effective producing higher estrus detection and pregnancy rates as compared to the conventional treatment. However, BCS of the buffaloes before treatment affected ($P < 0.05$) pregnancy rate after treatment. Pregnancy rate was also adversely influenced by low serum concentrations of calcium ($P < 0.01$) and total protein. When the effect of progestin based CIDR co-synch protocol was compared between the group of anestrus buffaloes with BCS of >2.5 and <2.5 during the dry season, the first AI pregnancy rate was higher ($P < 0.05$) in buffaloes with >2.5 BCS than in <2.5 BCS buffaloes. Therefore, understanding of nutrition – reproduction interaction is important while implementing

modern techniques to improve reproductive efficiency of the buffalo.

Key words: Dry season, Reproductive performance, Fodders

I-13 Recent Advances in Nutrition- Reproduction Interactions in Dairy Animals

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Reproduction is the luxury function of mammalian bodies and has got the last priority in nutrients partitioning in dairy animals, after lactation and health. Appropriate intake of nutrients, associated with desirable concentrations of blood metabolites, especially glucose, is needed for a normal reproductive cyclicality. The interaction between reproduction and nutrition becomes critical during the postpartum periods in high yielding dairy animals when most of the nutrients are utilized for lactogenesis and little are left for supporting reproductive activities. The primary objective of rearing dairy animals has been the production of milk. Selective breeding has been in practice and lactation has been considered as the most important economic trait. The two traits of lactation and reproductive efficiency have been found to be associated with each other; hence the selection for milk yield also led to enhanced fertility. However, beyond certain levels of milk production the drain of nutrients from the circulating blood results in cessation of reproductive process. The declining fertility with the enhanced lactation yield during the last several decades has been found both under total mixed ration in the USA as well as pastoral feeding in the New Zealand. The postpartum initiation of lactation coincides with a decreased feed intake and the animals enter into a state of negative energy balance (NEBAL). The energy deficiency is met out by the process of lipolysis, leading to loss of body condition score (BCS). Pulsatile release of LH and response of the follicle to LH surge are hindered. Such a condition leads to a delayed ovulation and lowered conception rates. Plasma levels of insulin-like growth factor-I are directly related to energy status and is critical to ovarian follicular development. During the early NEBAL period, the ability of follicles to produce sufficient estradiol for ovulation seems to depend on the availability of insulin and IGF-I in serum and the changing energy balance profile. Leptin has been discovered as a product of white adipose tissues, reflecting the energy status of the body. Leptin concentrations are lower during a declining BCS and vice versa; being favorable during the rising concentrations. Hence leptin has been called as the permissive factor for

the reproductive process. The animals get out of NEBAL when the nutrients intake exceeds utilization and the BCS starts raising. It leads to resumption of ovarian cyclicality and conception. Poor nutrition and weight loss in cattle causes a decrease in blood progesterone concentrations. One possibility is that greater milk production in dairy cattle is negatively affecting blood progesterone concentrations and causing infertility in dairy cows. Lower plasma concentrations of progesterone have been reported in cows with higher milk yield. There are several potential mechanisms for decreased progesterone in high-producing cows. Progesterone concentrations in blood are determined by rates of secretion, metabolism and clearance through liver. In dairy buffaloes, crude protein intake (CPI) was positively correlated with the postpartum estrus and ovulation intervals (POI). CPI excess requirements was lower in animals which expressed oestrus than those which remained anoestrus. Prepartum metabolizable energy (ME) intake was higher in animals observed in oestrus than those remaining anoestrus. The animals resuming to oestrus had a narrow and almost constant CP/ME ratio, while the anoestrus animals had a widely fluctuating ratio, ranging from 10.7 to 13.1 g/MJ. CP/ME was related positively with POI. Increasing energy intake increased BCS ($r=0.16$) and duration of expulsion of placenta ($r=0.19$) and discharge of lochia ($r=0.24$) but decreased postpartum ovulation interval ($r=-0.27$, $p<0.01$). Reproductive process is regulated by the hypothalamus-pituitary gonadal axis and it functions only if the hypothalamus pituitary adrenal (or stress) axis is silent. Dairy cattle face the challenge of stress associated with higher metabolic rates, lactation, higher ambient temperatures and malnutrition. Oxidative stress results due to imbalance between the production of reactive oxygen species in higher quantities than their clearance through the antioxidant system. It causes damage to the cellular function, decreasing the production of hormones and gene products boosting the reproductive process. Antioxidants nutritional supplementation helps the dairy animals in maintaining reproductive activities.

Key words: Reproduction; nutrition, stress, fertility, ovarian cyclicality, protein, energy, antioxidants

I-14 Prospects of Nutrition and Reproduction in Dairy Cattle and Buffalo

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Improved reproduction is one of the main contributors to the profitability in the dairy production. Among the

several other factors, nutritional management has been found to play a vital role in achieving reproductive targets. It is generally accepted that nutritional management is the main limiting or controlling factor for reproduction in many types of domestic livestock. However, this relationship is complex and has given variable responses. Over the past few decades advancements in animal selection improved the animal productivity significantly with negative impact on reproductive traits. To date, the area of nutrition and reproduction in dairy cattle has been recognized and investigated extensively and reviewed. Studies suggest that reproductive efficiency and fertility can be directly or indirectly affected by the nutritional factors like energy, proteins, fats, minerals and vitamins. On the contrary to dairy cattle, buffalo reproduction is characterized by low fertility, longer calving interval, seasonal breeding, poor estrous behavior, delayed age of puberty and age at first calving in heifers. Main reason for poor reproduction in buffalo has been the poor nutrition. To date, limited published work is available on the effect of nutrition on reproduction in buffalo. The purpose of current paper is to review and highlight the nutritional factors that can possibly affect the reproduction, with specific emphasis on dietary components such as protein, energy, fat and vitamins or minerals in dairy cattle and buffalo.

Key words: Nutrition, Reproduction, Cattle, Buffalo

I-15 Transfer of Sperm and Embryo by Laparoscopic Method: A Technique to Improve Fertility in Small Ruminants

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Assisted reproductive technologies like artificial insemination (AI) and embryo transfer (ET) are being employed as rapid tools to extend productivity of livestock industry. The vaginal and trans-cervical methods of AI and ET are routinely practiced; however, the fertility rate in terms of pregnancy is low following these methods in small ruminants. In case of AI, the sperm have to travel farther to the point of fertilization; therefore, large numbers of sperm (100-300 million sperm) are required per insemination. Conversely, in sperm sorting business and genetically engineered sperm to produce transgenic animals, a very limited numbers of sperm are available for insemination. Similarly, the direct deposition of frozen thawed embryos is required for successful pregnancy. In this scenario laparoscopic method of sperm and embryo transfer is advantageous, since it is efficient (3-5 min are required per animal) and provide optimal pregnancy rate by using

minimum number of frozen thawed sperm. The overall pregnancy rates with laparoscopic AI and ET are reported upto 50-60 % and 45 % respectively. This pregnancy rate occasionally reached to 85% during breeding season. Furthermore, the laparoscopic AI is reported as the method of choice to get higher pregnancy rate in nulliparous sheep and goat. In conclusion the laparoscopic method of AI and ET has profoundly paved the way to improve fertility in small ruminants.

Key words: Laparoscopic AI, Embryo transfer, Small ruminants

I-16 Reproductive biotechnologies in dairy animals: from lab to field

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In Pakistan, the corporate dairies, though more inclined to exotic animals, but also claim for compromises due to climatic shifts. In contrast, the indigenous dairy animals are best accustomed to our environment but economically not suitable for corporate setup due to genetic diversity. To equilibrate the genetic variation, an efficient reproductive performance is required to reduce the generation interval. The reproductive efficiency could be improved by adopting the recent advances in biotechnologies. For field application, artificial insemination (AI) technique is most famous. For buffalo, the different insemination times (24h after heat signs) emphasize us further to investigate ovarian physiology in local animals. Parallel to AI for male genome, embryo transfer (ET) is being performed to harvest the genetic potential from female side. ET is not successful in our local animals due to poor super-ovulation response and is because of poor ovarian reserves. The efforts are being made to establish criteria for assessing ovarian reserves. The field applications of AI, ET and in-vitro produced embryos is being appreciated because of the availability of sexed progeny based on sexed semen. An *in-vitro* fertilization (IVF) technique has made it possible to harvest maximum number of embryos by ultrasound guided aspiration of oocytes (OPU) directly from ovaries. IVF also provides opportunities to use relatively low numbers of sperm to produce viable embryos. This allows for the utilization of high value semen and may provide significant opportunities when coupled with sexed semen. Another most attractive biotechnology is animal cloning. It has potential to produce carbon copies of elite genetics but its applications are limited to experimental purposes because of poor efficiency. Keeping in view the future prospects we need to establish our basic skills in this field of fourth generation

reproductive biotechnology. In conclusion, thereproductive biotechnologies are powerful tools for breed improvement but need further investigations related to indigenous dairy animals.

Key words: Embryo transfer, Ovum pick up, Artificial insemination

I-17 Reproductive Disorders in Dairy Animals

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Reproductive disorders affecting the fertility of main dairy animals is discussed in this paper. Data concerning reproductive disorders in cattle and buffalo have been gathered from different livestock farms and veterinary clinics in Pakistan. Repeat breeding, to combat anoestrus, uterine prolapse, retention of foetal membranes and dystokia have been recorded as major disorders affecting fertility of dairy animals. Measures have been suggested to combat these disorders and keep the fertility of dairy animals within acceptable limits.

Key words: Fetal membranes, Prolapse, Dairy animals

I-18 Recent Advances in Clinical Embryology

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Objective of this presentation is to review recent advances in clinical embryology. Since the first child, Louis Brown was born through *in vitro fertilization* (IVF) in 1979; selection of developmentally competent embryos has been the focus of clinicians. Embryo selection based on morphological evaluation has some clinical relevance but is highly subjective. In clinical embryology selection of developmentally competent embryos using pre-implantation genetic testing emerged in early 2000s. Implantation failures, recurrent pregnancy losses, and developmental defect like Down's syndrome (Trisomy 21) are attributed to embryo aneuploidy. More than 50% of the first trimester aborted fetuses are aneuploidy (Jacob 1992). Chromosomal abnormalities may range up to 60% in cleavage stage embryos from women younger than 35 years to 80% in women 41 years and older (Munne' et al; 2007). Genetic testing of embryos for chromosomal abnormalities and single gene defect helps to select a normal embryo. Time lapse imaging of *in vitro* growing embryos is emerging as a tool to select

developmentally competent embryos. A risk model of aneuploidy by studying the embryo morphokinetics has been reported (Campbell et al 2013). Fertility preservation in male has been in practice for many decades. However, female fertility preservation through oocyte cryopreservation for medical as well as social reasons is relatively a recent phenomenon. Liver births as a result of embryo produced from cryopreserved oocytes have been reported. A detailed account of selection of developmentally competent embryos using embryo genetic and morphokinetic techniques will be presented. Female fertility preservation by vitrification of oocytes will also be discussed.

Key words: IVF, Fertility, Genetic testing

ORAL PRESENTATIONS**O-01 Improving Conception Rate following Application of Fixed-Time Artificial Insemination Protocol in Buffalo during Non-Breeding Season**S. S. Ghuman^{1*} and D. S. Dhimi²*¹Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India²Veterinary Officer, Civil Veterinary Hospital, Badbar, Barnala-148106, India***ghuman_s@yahoo.co.in*

Small dairy farmers raising 1-3 bovines account to 3/4th of livestock wealth and 68% of milk production in India. Moreover, only 19.2% buffalo ownership in India is with large farmers. Thus, to have a major impact on total milk production in India, strategies need to focus on improving the reproductive potential of buffalo reared by small farmers. Applying fixed-time AI (FTAI) protocol in buffalo may provide a potential alternative for increasing their life time productive period. However, the major bottlenecks in wide application of FTAI at small farmer's doorstep are, a) poor conception rate during non-breeding season, and b) failure of non-conceived buffalo to return to estrus following FTAI. In addition, if a buffalo fails to conceive, a farmer has to bear a loss of \$4.2/d in terms of loss of milk and other management expenses. Thus, the small farmers can be convinced to adopt FTAI if a protocol with good conception rate is available. The present study during non-breeding season aimed at comparison of fertility outcome of two FTAI protocols in buffalo reared by small farmers. Group-I (Protocol cost: \$11.63): Buffalo (n=24) were subjected to Ovsynch protocol (d0 and d9, 20µg GnRH analogue each; d7 500µg PGF₂, both hormones by i.m. route; AI 16h after d9 GnRH). Group-II (Protocol cost: \$26.16): Buffalo (n=96) were administered (i.m.) 2mg estradiol benzoate on d0 and 500µg PGF₂ on d9. Sustained progesterone release device (1.38g progesterone) was placed intravaginally from d0 to d9. On d11, 20µg GnRH analogue and 500IU eCG were administered (i.m.). AI was done 16h after GnRH analogue administration. In both groups, pregnancy was confirmed by ultrasound aided diagnosis on d60 post-AI and the buffalo failing to conceive and returning to estrus were re-inseminated at observed spontaneous estrus without any additional hormonal treatment. Statistical analyses were performed using MINITAB release 13.2 statistical software. Numerical data differences were considered significant at P<0.05 using Chi-square (2) test. The conception rate following FTAI in group-I and group-II was 20.83% (n=5/24) and 53.50% (n=51/96; p<0.05), respectively. Also, the conception rate following re-insemination in

group-I and group-II was 25.00% (n=1/4) and 61.65% (n=16/26), respectively. Overall (1st AI and re-insemination) conception rate in group-I and group-II was 25.00% (n=6/24) and 69.80% (n=67/96; p<0.05), respectively. In summary, although estradiol/progesterone-based FTAI protocol is costlier compared to ovsynch, the former is economical due to much better conception rate in buffalo during non-breeding season.

Key words: Buffalo, Conception, Estradiol, FTAI, Season**O-02 Effect of Butylated Hydroxytoluene (BHT) Supplementation on Semen Quality at Different Stages of Cryopreservation of Beetal Buck Sperm**A. Rehman^{1*}, A. Sattar¹, A. Riaz¹, M. Ahmad¹, M. Ijaz², M. Shahzad¹, M. Ahmed¹ and M. U. Mehmood¹*¹Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan, ²Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan***drrehman@uvas.edu.pk*

Butylated hydroxytoluene (BHT) is a synthetic analogue of vitamin E and plays an important role as an antioxidant. The objectives of this study were two fold 1) to optimize the concentration of BHT in semen extender and 2) its effect during different stages of cryopreservation of Beetal buck sperm. A total of fourteen ejaculates were collected from two Beetal Bucks during seven successive weeks from January to March. After each collection the ejaculates from both bucks with motility 60 %, concentration > 2×10⁹sperm/mL and abnormality rate <10% were pooled and divided into five aliquots. The aliquots were extended (50×10⁶/0.5ml straw) at 37°C with Tris-citric acid fructose (TCF) having 15 % egg yolk and 5% glycerol, additionally supplemented with different concentrations of BHT (0, 0.5, 1.0, 1.5 or 2.0 mM). The extended samples were cooled to 4°C for 90 min, equilibrated at 4°C for 4 hrs, frozen in liquid nitrogen (LN₂) vapor (4 cm above the LN₂, for 7 min) and plunged into LN₂ for storage. Sperm evaluation assays, e.g., motility, viability, plasma membrane integrity (HOST), normal apical ridge (NAR) and DNA integrity (acridine orange) were evaluated at different stages of processing (e.g. after dilution, before freezing and post thawing) using phase contrast and fluorescent microscopes. Data were analyzed through ANOVA under CRD and significant differences were compared by using Duncan's Multiple Range Test. The addition of BHT has no significant effect after dilution and before freezing as all sperm evaluation assays were same (P>0.05) among groups. However, BHT, at an inclusion level of 1.0mM improved significantly (P<0.05) post thaw motility (52.14 ± 1.01), live % of

spermatozoa (61.29 ± 2.31), PMI (58.71 ± 2.37) and NAR (60.71 ± 1.01) as compared to control and other treatments groups. Post thaw DNA integrity of group 1mM and 0.5mM were same but significantly ($P < 0.05$) higher than control and other treatments groups. It is concluded that optimum concentration of BHT in TCF semen extender is 1.0 mM and it only improves post thaw sperm quality in Beetal Buck.

Key words: BHT, Beetal Buck, Cryopreservation

O-03 Repeat Breeding Problems in Dairy Cows of Bangladesh

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Repeat breeding (RB) is a large problem in the dairy cattle leading to great economic loss for the dairy producer due to more inseminations, increased calving interval and high culling rates. The objective of the present study was to evaluate the prevalence of RB in cows and to identify organisms in the uterine samples along with their antibiotic sensitivity tests. The data collected by using questionnaire and direct interview of the farmer. A total 1207 dairy cows of Rajshahi, Bangladesh were studied during the period from August, 2011-January, 2013. The overall prevalence of RB was 20.4%. Genotypes of cow had significant effect ($P < 0.05$) on prevalence of RB. Age also had significant effect ($P < 0.05$) on RB. The RB of cows were lowest in L×SL (3.0%), <4 years age (3.3%) and good body condition score (2.7%) groups. The RB of cows were found highest in L×F (8.5%), 6 to <8 years age (8.7%), 3rd parity (6.4%) and Artificial Insemination method (15.4%). A total 100 cows have been selected for uterine sample from repeat breeder cases at Rajshahi district. A significant number of bacteria were identified from diseases sample and they were *Fusobacterium* spp 14 (45.16%, $p=0.000$), *Actinomyces* spp 10 (32.25%, $p=0.003$), *Escherichia coli* 12 (38.70%), *Pseudomonas* spp 11 (35.48%) and *Haemophilus* spp 9 (29.0%, $p=0.012$). Amongst antibiotics, Azithromycin and Ciprofloxacin (99.38% and 93.20%, respectively) were highly sensitive. In contrast, Penicillin (51.85%) and Oxytetracycline (25.2%) were highly resistance. The present study suggested that genotype of L×SL, <4 year age, good body condition support significantly reduces the prevalence of RB in dairy cows. Also a lot of microorganisms identified which have very harmful effect on fertility status of dairy cows in this study area. Moreover, Azithromycin and Ciprofloxacin are very

effective drugs against the organisms of Repeat breeder cows.

Key words: Repeat breeder, Cows, Antibiotics sensitivity test, Causal agents

O-04 Effect of Different Dietary Energy Levels on Dry Matter Intake, Milk Production, Milk Composition and Reproductive Hormonal Level of Early Lactating Dairy Cattle

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The aim of the study was to evaluate the effect of three different dietary energy levels on dry matter intake, Milk production, composition and reproductive hormonal level of early lactating Holstein Friesian cows. Nine multiparous early lactating (10 ± 5 days in milk) cows were selected on the basis of nearly the same body weight (410 ± 10) and milk production (12 ± 0.1). These cows were randomly divided into three groups with three animals in each group under a completely randomized design. Diets formulated were iso-nitrogenous with 16% CP contents and varying dietary ME levels: Group A meeting 100% NRC recommendations, Group B with 88% of NRC recommendations and group C containing 112% of the NRC recommendation for early lactating cows. The research trial was continued for a period of 75 days included adaptation period and data were analysed by using the GLM procedure (Proc GLM; SAS®, Version 8.02; SAS Institute Inc., Cary, NC) as described by steel and torrie (1981). Results for dry matter intake shows high value ($P < 0.05$) in or group C animals (13.78 ± 0.17 kg/day) in high energy 112% treatment, followed by group A (13.14 ± 0.12 kg/day) and less DMI was for B (12.68 ± 0.11 kg/day) animals. A higher ($P < 0.05$) milk yield (14.80 ± 0.10 lit/day) was reported in the treatment C compared to the group A and B (13.01 ± 0.10 kg/day). For milk composition 100 ml fresh sample of each cow was collected daily and analyzed by automatic milk analyzer (lacto scan) In milk composition high ($P < 0.05$) milk fats (3.91 ± 0.02) percentage was found at low energy diet B, followed by A (3.75 ± 0.01) and C (3.58 ± 0.03), whereas no statistical difference recorded between treatments for Milk protein, lactose and solid not fat. For blood serum hormonal determination 5 ml blood from all cows were collected after 15 days each and then analyzed. Serum progesterone hormones were significantly ($P < 0.05$) increased with increasing dietary energy High progesterone level of dairy cows were observed in group C (0.73 ng/ml) followed by group A and B having 0.21 and 0.16ng/ml and overall mean of

serum luteinizing profile was significantly higher (0.75ng/ml) in group C compared to group A and B is 0.17 and 0.16 ng/ml respectively with feeding of high energy ME (112%) diet. It is therefore concluded that feeding rations high in ME contents at 112 % of NRC recommendations improved DMI Milk yield and improved reproductive efficiency in term of hormonal level.

Key words: Dietary energy, DMI, Milk production, Milk composition, Reproductive Hormones

O-05 Investigations on Fertility Related Biomarkers in Water Buffalo Bull Semen to Reduce Male-Factor Losses during Peak Breeding Season

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A high fertility bull is a prerequisite for running any reproductive or breed improvement programme. With the wide spread application of artificial insemination in water buffalo, the demand for best males have increased considerably. Therefore, the present study was designed to assess the fertility related biomarkers in water buffalo bull semen to reduce male-factor losses. Semen was collected from five adult buffalo bulls of acceptable fertility with artificial vagina during peak breeding season. After initial evaluation, semen samples of each bull were split and diluted either in PBS-0.1% BSA for fresh analysis or in Tris-citric acid extender for cryopreservation. Fresh and frozen-thawed semen samples were analyzed by (i) Computer Aided Sperm Analyzer (CASA) for sperm motility parameters, velocity distribution and kinematics, (ii) Acridine Orange Assay for sperm DNA integrity, (iii) Neutral Comet Assay for sperm DNA fragmentation and (iv) Fluorescent Probes, propidium iodide and "5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl carbocyanine iodide" (JC-1) for sperm viability and mitochondrial transmembrane potential, respectively. The experiment was repeated six times. For *in vivo* fertility, data of at least 100 inseminations per bull were collected under controlled field conditions. Straight line velocity (VSL, 123.67±3.20 µm/s) of buffalo spermatozoa in fresh semen samples had a significant correlation with *in vivo* fertility (62.84 %; r=0.76, P<0.05), whereas tail length (TL, 68.55±3.11 µm) and olive moment (OM, 12.82±1.08 µm) of sperm DNA in fresh semen samples had a significant negative correlation with fertility (r=-0.49, P<0.05 and r=-0.72,

P<0.01, respectively). Sperm kinematics i.e., average path velocity (VAP, 71.97±1.38 µm/s), VSL (61.37±1.37 µm/s), straightness (STR, 84.37±0.80 %) and linearity (LIN, 56.45±1.27 %) of frozen-thawed semen samples were significantly correlated with *in vivo* fertility (r=0.52, P<0.05; r=0.67, P<0.01; r=0.67, P<0.01 and r=0.58, P<0.02, respectively). Similarly, % of viable spermatozoa with high mitochondrial transmembrane potential (V/HP, 29.34±1.24 %) in frozen-thawed semen samples were significantly correlated with *in vivo* fertility (r=0.89, P<0.01). Neutral comet assay parameters i.e., TL and OM in frozen-thawed semen samples had a significant negative correlation with fertility (TL, 78.44±0.98 µm, r=-0.63, P<0.01 and OM 17.27±0.56 µm, r=-0.50; P<0.04). The best predictive equation (R² adjusted=65.0%, P<0.01) of fertility obtained for fresh buffalo semen after step forward multiple regression analysis included two parameters i.e., TL and OM. The best predictive equation (R² adjusted=83.9%, P<0.001) of fertility for frozen-thawed buffalo semen included three parameters i.e., rapid subpopulation, VAP and VSL. The best single predictor of fertility for fresh and frozen-thawed buffalo semen was OM (R² adjusted=43.7%, P<0.04) and CASA progressive motility (R² adjusted = 43.1%, P<0.04), respectively. The present study identified the potential sperm quality parameters that could serve as biomarkers of fertility in water buffalo semen during peak breeding season. Moreover, the prognostic values of buffalo sperm quality parameters as predictors of *in vivo* fertility were better for frozen-thawed semen compared to fresh semen.

Key words: Buffalo spermatozoa, Viability, Mitochondrial transmembrane potential, *In vivo* fertility, CASA

O-06 Effects of Lycopene on Ram Sperm Parameters during Liquid Storage

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Lycopene addition of extender would be useful for liquid storage of ram semen. The aim of this study was to investigate the effects of three levels lycopene on ram sperm parameters during liquid storage at 5°C up to 72 h.

Five Merino rams (2-3 years of age) were housed at Selcuk University Faculty of Veterinary Medicine Research and Application Farm, Konya, and maintained under uniform feeding and housing conditions. A total number of 30 ejaculates (10 ejaculates for each ram) were collected from the rams with the aid of an artificial vagina twice a week, according to AI standard procedures. The ejaculates containing spermatozoa with >80% forward progressive motility and concentrations higher than 2×10^9 spermatozoa/ml were used in the study. Nine ejaculates for each ram were included in the study. Ejaculates were pooled at 37°C and pooled ejaculate was divided into four equal aliquots and diluted (37°C) with the Tris base extender, containing lycopene at three doses (0.5, 1.0 and 2.0 mM) and no additives (control), at a final concentration of approximately 2×10^8 sperm. Subjective sperm motility was determined at phase-contrast microscope, viability (SYBR14/PI), and mitochondrial activity (JC1-PI) were determined at fluorescent microscopy after 0, 24, 48 and 72 h of storage at 5°C. The study was replicated nine times. Results were expressed as the mean \pm SEM. Data for sperm motility, viability and mitochondrial activity were analyzed by analysis of variance, followed by Tukey's post-hoc test to determine significant differences between groups. Differences with values of $P < 0.05$ were considered to be statistically significant. Statistical analyses were performed by using SPSS 11.5 package program. The extender supplemented with 0.5 mM lycopene (51.97 ± 0.53) resulted in higher mitochondrial activity, in comparison to the control (47.17 ± 1.85) group at 72 h of storage. Lycopene at 0.5 mM (62.50 ± 1.11) led to higher sperm motility when compared to 2 mM lycopene (49.16 ± 4.72) group at 72 h of liquid storage ($P < 0.05$). Sperm viability was better in 2mM lycopene (78 ± 6.79) than the control, 0.5mM and 1mM lycopene groups (74.02 ± 3.43 , 76.96 ± 1.04 , 72.75 ± 0.66) respectively, with no significant differences ($P > 0.05$) at 24 h storage. Our data suggest that the lycopene can be added to Tris base extender for improving the ram sperm motility, viability and mitochondrial activity during the liquid storage.

Key words: Ram sperm, Lycopene, Liquid storage, Fluorescent staining.

O-07 Effect of Timing of AI on Pregnancy in Estrus Synchronized Nili-Ravi Buffaloes: A Preliminary Study

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The use of Controlled Internal Drug Releasing (CIDR) device has been reported to synchronize and induce estrus in cows and buffaloes. Fixed time artificial insemination (FTAI) is a value addition technique in CIDR-based synchronization programme and requires an ovulation control. Limited information is available on timing of AI following CIDR-GnRH synchronization protocol to achieve optimal fertility. In this background the objective of present study was to determine the effect of timing of AI on pregnancy rate in Nili-Ravi Buffalo synchronized with CIDR-GnRH protocol. The study was conducted during breeding season at a commercial dairy farm. Thirteen adult Nili-Ravi buffaloes having 400-450 Kg body weight, BCS (2.64 ± 0.06), parity ranges from 2 to 4 and more than 90 days postpartum were used in this study. Each buffalo was fitted with CIDR (1.38 g progesterone Pfizer Co, USA) on random stage of estrus cycle. On day 6 after CIDR insertion, luteolytic agent PGF2 (2 mL; i.m) was injected to all animals. CIDR device was removed on day 7 and GnRH (2 mL; i.m) was administered on day 8. Estrus detection was done through teasing and confirmed by visual observation. Buffaloes were then randomly allocated into three groups to get AI dose either at 12 h (n=4), 24 h (n=5), or 36 h (n=4) after the administration of GnRH. Follicular size of the largest follicle was measured from PG injection to 48 h after administration of GnRH (day 10) through ultrasonography at 12 h interval. Pregnancy diagnosis was conducted using ultrasonography on 35th days after insemination. Data for timing of ovulation and size of the ovulatory follicle was analyzed by using One way ANOVA and for estrus response ovulation and pregnancy per AI was analyzed by using Z-test for population proportion. Results revealed that estrus response was 100% (13/13). Overall mean size of dominant follicle just before ovulation, timing of ovulation after GnRH injection and ovulation rate were 13.2 ± 0.38 , 32.77 ± 1.46 h mm and 84% respectively. Pregnancy per AI was lower (25%, 1/4) when inseminated earlier (12 h), maximal (50%, 2/4) when bred at 24 h and zero (0%, 0/3) when inseminated later (36 h). It is concluded that buffaloes may be inseminated at 24 h GnRH administration following CIDR-GnRH synchronization protocol for achieving higher pregnancy.

Key words: Synchronization, CIDR, Buffalo, GnRH

O-08 Prevalence and Therapeutics of Iodine Deficiency as Abortifacient in Pregnant Goats

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Iodine deficiency is one of the major issues in pregnant goats in Lahore, Pakistan. Low levels of iodine result in decreased plasma Triiodothyronine (T₃) and Thyroxin (T₄) hormones leading to high risk of abortions, still births, and congenital anomalies in pregnant goats. The present study was carried out to 1) determine the prevalence of iodine deficiency in pregnant goats, and 2) evaluate the effectiveness of Thyroxin and mineral mixture in treating iodine deficient pregnant goats. Plasma samples from 300 pregnant goats were collected from Lahore metropolitan and subsequently tested through ELISA for T₃ and T₄ hormones. Overall 84 (28%) of the pregnant goats revealed significantly low (P<0.05) levels of plasma T₃ and T₄ hormones. Out of these 84 pregnant goats, 18 (21.4%) aborted. For therapeutic trials, 24 pregnant goats, having low levels of T₃ and T₄ hormones, were randomly divided into 3 groups of 8 viz. A, B and C. Goats in group A were treated orally with Thyroxin at 0.2mg/20kg/day while animals in group B were given mineral mixture at 50gm/animal/day orally. The members in group C served as positive control (untreated). The plasma levels of T₃ and T₄ were determined at day 0 (pre-treatment) and day 15 (post treatment) using ELISA. Data on prevalence was analyzed using Pearson Chi Square Test whereas T₃ and T₄ values in different treatment groups were analyzed by one way ANOVA. On day 0 (4.5 months pregnancy), plasma T₃ levels of goats in groups A, B and C were 51.71±12.18, 59.93±14.44 and 53.1±13.58ng/dL, respectively. Plasma T₄ levels at day 0 were 1.79±0.46, 1.94±0.77 and 1.70±0.69ug/dL in groups A, B and C, respectively. On day 15, plasma T₃ levels of goats in groups A and B (98.64±5.70 and 139.79±26.64ng/dL, respectively) were significantly increased (P<0.05) compared to group C. Similarly, plasma T₄ levels of goats in groups A and B (4.54±0.43 and 6.13±1.19ug/dL, respectively) were significantly increased (P<0.05) compared to group C. When compared T₃ and T₄ levels of goats in groups A and B, a significant increase (P<0.05) was observed in group B. At kidding, all the goats in groups A and B gave birth to normal kids without goiter or congenital anomaly/deformity. On the other hand, 3 of the goats in group C aborted during trial period and 5 of the goats gave birth to kids suffering from severe goiter. In conclusion, iodine deficiency is prevalent in pregnant

goats in Lahore and is associated with abortions and goiter. For treatment, mineral mixture is more effective than thyroxin in treating iodine deficiency in pregnant goats.

Key words: Goiter, Thyroxin, Mineral Mixture, ELISA

O-09 Efficiency of Fatty Acid-Free Defined System for In Vitro Maturation of Buffalo Oocytes

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The evaluation of medium for *in vitro* maturation of buffalo oocytes has progressed towards more defined conditions instead of use of complex medium with serum, for the studies evaluating effect of specific component in the medium. The requirement of a fatty-acid free defined medium is intensified if the effect of specific fatty-acid component of medium is to be investigated. The objective was to evaluate the effect of fatty-acid free-bovine serum albumin (FAF-BSA) alone or supplemented with epidermal growth factor (EGF) in the *in vitro* maturation medium on *in vitro* maturation of oocytes and further development of the embryos. The cumulus oocyte complexes (COCs) were collected from slaughterhouse ovaries by aspirating follicles, and kept in maturation medium for 24 h. Three types of maturation media were evaluated: TCM-199 supplemented with 0.02 IU/mL FSH, 1 µg/mL estradiol-17 (E2), 50 µg/mL gentamicin and 1) 10% fetal bovine serum (FBS), 2) 6 mg/ml FAF- BSA or 3) 6 mg/ml FAF- BSA + 10.0 ng/mL epidermal growth factor (EGF). After maturation, Tyrode's albumin lactate pyruvate (TALP) and Synthetic oviductal fluid (SOF) media were used for fertilization and further culture respectively. Data was recorded in percentages and analyzed by ANOVA at 5% level of significance. Least Significance Difference (LSD) test was used to compare treatment means. The percentage of COCs with expanded cumulus was higher (P < 0.05) in FBS and FAF-BSA+EGF supplemented maturation medium compared with FAF-BSA alone. No difference (P>0.05) was observed in meiotic resumption [Germinal-vesicle (GV), Germinal-vesicle breakdown (GVBD), Metaphase-I (MI)] and nuclear maturation (Metaphase-II (MII)] in all three types of maturation media. The percentage of embryos capable of crossing 8-16 cell block and reaching up to morula/blastocyst was higher (P

< 0.05) in FBS and FAF-BSA+EGF supplemented media compared with FAF-BSA alone. This study shows that lower development rates of FAF-BSA supplemented defined maturation medium were restored and enhanced by adding EGF and comparable with serum. This medium can be efficiently used in studies evaluating effect of specific fatty acid component in the medium.

Key words: *In vitro* maturation, Serum free medium, Defined medium, Epidermal growth factor

O-10 Effect of Skim Milk and Egg Yolk in Tris Based Extender on Liquid Storage of Buck Semen

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Artificial insemination is a major tool for wide spread use of high genetic potential of the male and use of liquid semen is less laborious and economical way of semen extension compared to frozen semen. The objective of present study was to compare the effect of Tris Citric acid Egg Yolk (TCEY: Tris 1.73gm, Citric Acid 1.93gm, Egg Yolk 20ml) and Skim Milk Tris Citric Acid (SMTC: Skim Milk 0.9gm, Tris 1.73gm, Citric Acid 1.93gm) semen extenders on buck semen parameters (motility, live sperm & membrane Integrity) at 4 °C from 0 to 96 hours of storage. Semen was collected from two Beetal bucks once in a week for four consecutive weeks (4 replicates) through artificial vagina. The ejaculates qualifying the minimum criterion (75% motility, 80% live sperm & 80% membrane integrity) were pooled and divided into two aliquots i.e. one diluted with TCEY and second with SMTC. After extension semen samples were cooled to 4° C and then incubated at 4° C for 96 hours. Motility, live ratio (Eosin and Nigrosine staining), HOS (Hyper Osmotic Swelling) & NAR (Normal Apical Ridge) were assessed at 12 hours interval from 0 to 96 hr of incubation. Semen samples were pre-warmed at 37°C before evaluation. Data was analyzed by repeated measure ANOVA in SAS Enterprise Guide 4.2. Sperm motility and HOS were higher (p 0.05) from 12 to 96 hours in SMTC compared to TCEY (17.5% ±3.22 vs. 3.75% ±2.39 ; 33.75% ±0.75 vs. 25.5% ±1.94 respectively at 96 hours). Moreover, live sperm and NAR were higher (p 0.05) from 0 to 24 hours in SMTC (72% ± 2.38 vs. 60.75% ±2.95; 70.25 ±1.93 vs. 55% ±1.87 respectively at 24 hours) than TCEY after 24 hours till 96 hours their difference was same. It is concluded that skim milk based extender can maintain higher sperm quality as compare to semen stored in egg yolk based extender during liquid

storage at 4°C for 72 hours for fruitful AI & maximum for 96 hours.

Key words: Buck semen, Tris Citrate, Skim Milk extender, liquid storage.

POSTER PRESENTATION**P-01 The Influence of Season on Surface Follicle Count and Oocyte Quality in Buffalo Ovaries**

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The influence of season was investigated on surface ovarian follicle and oocyte quality in buffalo. From an abattoir, buffalo ovaries were collected (n=142) during summer (July-August, n=66) and winter (December-January, n=76) season. Ovaries were examined for the surface follicles and oocyte quality. The surface follicles of each ovary were counted and measured using vernier's callipers. These were classified as small (<5 mm), medium (5-8 mm) and large (>8 mm) follicles. The medium and large surface follicles were aspirated using 18G needle attached with a 10 ml disposable syringe containing 0.5 ml cumulus-oocyte complex (COC) holding media (dPBS + 3-5% calf serum) at 37°C. Using SPSS-17, data was subjected to *t*-test. During winter season, average follicle count per ovary was higher than that in summer (7.61±0.37 vs 5.92±0.25; p<0.05). Between seasons, the percentage of small, medium and large size follicles was different (summer: 77.7, 15.3 and 6.9% vs winter: 73.4, 16.1 and 10.5%, respectively; p<0.05). During winter season as compared to summer, average oocyte recovery rate (2.07±0.12 vs 1.72±0.12; p<0.05) and the percentage of grade-A oocytes was high (43.7 vs 22.8%; p<0.05). During summer season, the recovery of grade-B oocytes was higher than that in winter (35.9 vs 20.9%; p<0.05). The microscopic examination of ovaries (10 each in summer and winter) revealed tertiary follicle with intact granulosa cell layers (9.05±0.32; layer thickness, 181.52±9.27µm) in winter and loosened granulosa cell layers (7.45±0.23; layer thickness, 209.69±6.90µm) in summer. Vacuolization was observed between the granulosa cell layers and antral fluid, as well as in between the granulosa cells in summer season and the vacuolization was absent in winter season. Also, many apoptotic bodies in ovarian granulosa cell layer were only observed in summer season. In summary, season has a significant influence on the number of follicles on ovarian surface, oocyte recovery rate and oocyte quality in buffalo.

Key words: Buffalo, Follicle, Oocyte Quality, Ovary, Season

P-02 Cysteine Supplementation Enhanced Post-Thaw Quality of Spermatozoa in Achai and Holstein Fresian Bulls

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Production of Reactive oxygen species (ROS) during the process of cryopreservation is associated with lipid peroxidation of the sperm membrane that adversely affects sperm motility and fertility. The objective of the current study was to establish the optimal concentration of L-Cysteine for cryopreservation of Achai and Holstein Fresian bull semen under the subtropical condition of Peshawar region. Experiments were executed on semen of six matured bulls, three Achai- the indigenous breed and three Holstein Fresian- exotic breed at Government Cattle Breeding and Dairy Farm Harichand Charsadda. Semen from the experimental bulls of either breed were collected with the help of artificial vagina maintained at 42°C and processed separately breed wise. Two consecutive ejaculates were collected from each bull at weekly interval for 3 weeks in April- May 2014. Immediately after collection, the ejaculates were subjected to gross and microscopic examination. Semen samples having more than 70% motility were selected for further processing. The semen was split into four aliquots in which tris-citric acid extender (TCA) supplemented with various concentrations of L-Cysteine was used to reach 0 mMolml⁻¹ (group I, control), 0.5 mMolml⁻¹ (group II), 1.0mMolml⁻¹ (group III) and 1.5mMolml⁻¹ (group IV). Diluted semen was cooled to 4°C for 2 h, equilibrated for 4 h at 4°C, filled in straws(0.5ml) at 4°C, kept in liquid nitrogen vapours for 10 min and then stored in the liquid nitrogen(-196 °C) for assessment. Post-thawed motility of frozen semen straws was assessed using standard procedure. Thawing of frozen semen straws was carried out at 37°C for 30 seconds in a hot-water bath. Sperm viability and acrosomal integrity were determined by dual staining procedure i.e Trypan-blue and giemsa stains. The supravital stain trypan-blue distinguished live and dead spermatozoa whereas giemsa stain was used to evaluate the integrity of the acrosomal membrane. Similarly plasma membrane integrity was assessed using the hypo-osmotic swelling (HOS) test. The data was statistically analyzed with two-way analysis of variance using SPSS version 16, Chicago, IL, USA. The group differences were compared by the Duncan's Multiple Range Test.

During current study, the progressive motility and viability of spermatozoa was significantly higher in 1.5mM or 1.0mM L-Cysteine supplemented groups in comparison with 0.5mM or 0.00 mM. Also current finding indicated that the number of HOST+ve spermatozoa at 1.5 or 1.0 mM was greater in comparison with lower concentration of 0.5 or 0.00mM L.Cysteine. Furthermore, significant difference for acrosomal integrity was recorded in semen samples treated with 1.5 mM L-Cysteine followed by 1mM, 0.5mM and control. The current study demonstrated that 1.0 or 1.5mMolml⁻¹ was the most favorable concentration of L-Cysteine to be added to the TCA extender for improving the quality of frozen-thawed Achai and Holstein Fresian bull semen.

Key words: L.Cystein, Achai, Holstein Frerian, Bull, semen, post thaw

P-03 Effects of Cysteamine on Ram Sperm Parameters during Liquid Storage

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The aim of this study was to investigate the effects three levels of cysteamine, on ram sperm parameters during liquid storage up to 72 h at 5 °C. Five Merino rams (2-3 years of age) were housed at Selcuk University Faculty of Veterinary Medicine Research and Application Farm, Konya, and maintained under uniform feeding and housing conditions. A total number of 30 ejaculates (10 ejaculates for each ram) were collected from the rams with the aid of an artificial vagina twice a week, according to AI standard procedures. The ejaculates containing spermatozoa with >80% forward progressive motility and concentrations higher than 2x10⁹ spermatozoa/ml were used in the study. Eight ejaculates for each ram were included in the study. Ejaculates were pooled at 37°C and pooled ejaculate was divided into four equal aliquots and diluted (37°C) with the Tris base extender, containing cysteamine at three doses (0.5, 1.0 and 2.0 mM) and no additives (control), at a final concentration of approximately 2 x 10⁸ sperm. Subjective sperm motility was determined at phase-contrast microscope, viability (SYBR14/PI), and mitochondrial activity (JC1-PI) were determined at fluorescent

microscopy after 0, 24, 48 and 72 h of storage at 5°C. The study was replicated eight times. Results were expressed as the mean±SEM. Data for sperm motility, viability and mitochondrial activity were analyzed by analysis of variance, followed by Tukey's post-hoc test to determine significant differences between groups. Differences with values of P<0.05 were considered to be statistically significant. Statistical analyses were performed by using SPSS 11.5 package program. The extender supplemented with 1mM cysteamine (78.33±2.10) resulted in higher motility, in comparison to the control group (70.83±1.53) for 48 h of storage (P<0.05). Sperm viability was better in 0.5 mM cysteamine (76.69±4.04) than control (69.08±3.88). For mitochondrial activity, 1 mM cysteamine gave the higher rate (73.51±4.39), compared to control (69.47±3.68), with no significant differences (P>0.05) up to 72 h of storage. This study highlights the protective effect of cysteamine (0.5 and 1 mM) on sperm quality during the liquid storage of ram semen.

Key words: Ram sperm, Cysteamine, Liquid storage. Fluorescent staining

P-04 Response of Cryopreserved Nili Ravi Buffalo Bull Semen to Gallic Acid Addition in Semen Extender

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In buffaloes, the ratio of fertility with cryopreserved semen as compared to cattle is lower as buffaloes are more prone to lipid peroxidation. Gallic acid (GA), a polyphenol, is known to carry excellent antioxidant properties. The objectives of this study were to improve buffalo bull semen quality with the use of GA as an antioxidant during the process of cryopreservation and to determine the optimal concentration of GA as an antioxidant for buffalo bull semen. For this study, four (4) healthy fertile Nili-Ravi buffalo bulls between 5-10 years of age, with ejaculates having 5-8 ml volume and >80% progressive motility were selected. GA with concentrations, 0, 15, 30, 45, 60 and 100µM was added to a 25 ml extended semen respectively and incubated prior to cryopreservation at 37°C for 5 min to allow GA to be absorbed in spermatozoa. The antioxidative effects of GA were evaluated indirectly in terms of spermatozoa motility, viability (Eosin-negrosin stain method), spermatozoa plasma membrane and acrosomal integrity

(Hypo-osmotic swelling test and Apical Ridge method respectively) and DNA status through Acridine orange staining assay. The data were analyzed using one way ANOVA, followed by Duncan multiple range test, $p < 0.05$ was considered significant. Results of this study showed that addition of GA to semen extender did not improve the buffalo bull spermatozoa quality parameters significantly as compared to the control group. However, it was observed that addition of GA at a concentration of 15 μM , improved the motility, viability and plasma membrane integrity ($p < 0.05$) of spermatozoa. Whereas, increasing concentration of GA from 30 μM to 100 μM , showed toxic effects. In conclusion, GA did not produce any significant protection against cryo-injury in buffalo bull semen.

Key words: Cryopreservation, Gallic Acid, Reactive oxygen species

P-05 Reducing Glycerol with Trehalose Supplementation Increases Survival of Bull Spermatozoa

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Glycerol is commonly used cryoprotectant for the preservation of spermatozoa. Apart from cryoprotective quality of glycerol, toxic effects on spermatozoa have been also reported. The addition of non-penetrating cryoprotactants like trehalose is reported to enhance the post-thaw semen quality in bovine, caprine, canine and Equine. Therefore, the present study was designed to minimize sperm damage by adding different concentrations of trehalose (0, 50, 100, 150, 200mM) in Tris-based egg yolk extender containing 3.5% either 7% glycerol. For this, semen was collected from three Sahiwal bulls (4-8 years of age) and was extended with different concentrations of glycerol and trehalose and cooled at 4°C for 2 h. The semen was filled in 0.5 ml straw ant kept on liquid nitrogen vapors for 7 minutes and finally stored in liquid nitrogen. The post thaw sperm motility, viability (eosin-nigrosin), membrane integrity (HOS solution), acrosome integrity (formaldehyde citrate) and DNA integrity (acridine orange) were carried out and statistically analyzed by two-way ANOVA. Reducing glycerol concentration from 7 to 3.5% (0mM trehalose; Control) declined overall post-thaw semen quality. The supplementation of 150mM trehalose (3.5% glycerol) and 100mM trehalose (7% glycerol) both groups were equal but gave significantly ($P < 0.05$) better post-thaw (plasma membrane, acrosome and DNA

integrity) sperm survival as compared to Contro group. The post thaw semen parameters in 100mM trehalose (7% glycerol) were significantly ($P < 0.05$) better as: motility ($57.00 \pm 1.22\%$ vs. $42.00 \pm 1.22\%$), viability ($67.60 \pm 0.93\%$ vs. $51.00 \pm 1.30\%$), plasma membrane integrity ($59.00 \pm 0.89\%$ vs. $41.40 \pm 1.17\%$), acrosome integrity ($55.00 \pm 0.71\%$ vs. $40.00 \pm 0.71\%$) and DNA integrity ($97.35 \pm 0.25\%$ vs. $96.51 \pm 0.37\%$) in comparison with Control. Significantly ($P < 0.05$) high DNA integrity was observed in all the treatment groups as compare to Control. Intact acrosome $45.20 \pm 0.86\%$, was observed higher in (3.5 % Glycerol; 0mM trehalose) as compare to $40.00 \pm 0.71\%$ in (7 % glycerol; 0mM trehalose), is the indication that glycerol may decrease the acrosome integrity. It is concluded that reducing 7% glycerol concentration to 3.5% supplemented with 150mM trehalose yelde better post-thaw parameters in frozen-thawed semen of cattle.

Key words: Trehalose, Cryopreservation, Spermatozoa, Sahiwal bull

P-06 Effect of Feeding TMR on Reproductive Performance of Non Descript Dairy Cattle

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The low reproductive performance of nondescript dairy cattle in Azad Jammu and Kashmir is because of poor genetic makeup and poor nutrition. Therefore, the present study was conducted to determine the effect of TMR feeding on fertility of dairy cows maintained at Livestock Development Research Centre Muzaffarabd. The seventeen multiparous cows (2-5 parity), with body condition score of (3.5 - 4) having the same reproductive status were divided into two groups. The control group (9 cows) was fed with the conventional ration consisting of wheat straw, cotton seed cake, wheat bran, and molasses. A non lactating cow weighing 250 kg body weight was fed with an allowance of ration to provide the quantity of the nutrients at a rate of Dry Matter 5.9 Kg, TDN 3.5 Kg, CP 0.62 Kg, Ca 0.02kg and P 0.01kg. The treatment group (8 cows) was fed only the TMR at the rate 2 % of the body weight of animal. TMR was containing crude protein 11.5 %, Fat 5%, TDN 58%, Crude Fiber 15.7% and Ash 10 %. The experiment was conducted from January 2014- July 2014 during the low breeding season. Fertility rate in term of number of services per conception was studied in both the groups. In control group 55.5 % cows showed the cyclicity where as 44.4% did not showed estrus or heat and remained silent during the

experimental period. Similarly numbers of services per conception were 3.5 in control groups. In treatment group 62.5 % cows showed the estrus where as 37.5 % remained silent. In treatment group the total numbers of services per conception were 1.5. The data was analyzed by chi square test that revealed that TMR feeding significantly ($P < 0.05$) increased the incidence of cyclicity in treatment group. The numbers of services per conceptions were significantly ($P < 0.05$) lower in treatment group compared to control group. Feeding of TMR improve the reproductive performance of nondescript dairy cattle.

Key words: TMR, Dairy Cows, Fertility, Low breeding Season

P-07 Serum Profiles of Certain Oxidants, Antioxidants, Reproductive and Stress Hormones in Buffaloes (*Bubalus Bubalis*)

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A preliminary study was conducted to determine certain oxidants, antioxidants and hormonal profiles in cyclic, acyclic and conceived buffaloes during low breeding season. Eight buffaloes at SB farm, Faisalabad, were synchronized using Ovsynch protocol and the buffaloes observing estrus were inseminated. The day of AI was specified as d 0. The blood sampling was started on d 0 up to 20 days, with 3-days gap between each sampling. Pregnancy diagnosis was made through rectal palpation after 2 months of AI. The levels of serum catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), total oxidant status (TOS), progesterone and cortisol were estimated. The estrus synchronization rate was 62.5%. The progesterone profile showed that two buffaloes were conceived; three observed cyclic and the 3 remained acyclic. The CAT activity was lower ($P < 0.05$) in cyclic and conceived buffaloes on d 0 than acyclic animals, whereas it was higher at mid-luteal phase in conceived animals. The SOD activity was considerably higher on d 0-8 (22.08 ± 5.98 to 30.38 ± 2.85 U ml⁻¹), then decreasing gradually till d 20 in all buffaloes. In acyclic buffaloes, the TOS was significantly higher ($P < 0.05$) on d 0-8 and d 16 than acyclic animals. The TAC was higher ($P < 0.05$) in conceived buffaloes at early luteal phase and in cyclic animals at mid-luteal phase. A parallel trend was observed in the levels of TOS and TAC in conceived buffaloes, both increasing and decreasing at the same time, with significantly higher levels at early-luteal phase

on d 4. The cortisol was higher ($P < 0.05$) in acyclic than cyclic and conceived buffaloes on d-0. It was concluded that cyclic, acyclic and conceived buffaloes exhibited different trends in the levels of oxidants, antioxidants and reproductive and stress hormones, indicating the relationship of oxidative stress with reproductive process. There is a need to further investigate this relationship to improve reproduction in buffaloes.

Key words: Buffalo, Hormones, Oxidants, Antioxidants, Reproduction

P-08 Effects of Different Equilibration Times on Cell Membrane and DNA Integrity of Sperm after Cryo-Reservation in Cattle Breeds

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Equilibration time in semen processing plays a vital role in semen qualitative and quantitative parameters. Nine mature bulls were selected in two ($n=2$) different breeds (Holstein- Friesian, Jersey) to find out the effect of different equilibration times on semen quality parameters. Artificial vagina with temperature 42°C was use to collect semen. Four semen samples were taken from each bull per week. Semen samples were extended in egg yolk extender (1:4), cooled, equilibrated for desired times and stored in liquid nitrogen. Semen was thawed at 37°C after 24 hours of storage and evaluated for sperm motility (subjective), seminal pH, dead sperm percentage, hypo osmotic swelling test (HOST), acrosomal integrity and DNA damage (Acridine orange test). Data was subjected to two way analysis of variance (Breed and equilibration time). Different equilibration times included were 2 hours (T1), 4 hours (T2) and 6 hours (T3) for each semen sample. Highest ($P < 0.0012$) sperm progressive motility ($33.45 \pm 0.31\%$) and sperm plasma membrane integrity ($70.95 \pm 0.33\%$) was observed for T2 in Jersey breed. Sperm DNA integrity decreased ($P < 0.0011$) linearly from T1 ($95.91 \pm 0.14\%$) in Jersey to T3 ($84.87 \pm 0.22\%$) in Holstein-Friesian. Seminal pH was significantly ($P < 0.0013$) high in T1 (6.82 ± 0.01) in Jersey, followed by T2 (6.47 ± 0.01) in Holstein-Friesian and T3 (5.90 ± 0.01) in Jersey and Holstein-Friesian. The dead sperm percentage was significantly ($P < 0.0011$) low in groups with semen sample treated with T2 ($52.37 \pm 0.56\%$) in Jersey compared to other group. Significantly ($P < 0.0011$)

high sperm individual motility percentage ($74.16 \pm 0.23\%$) and acrosomal integrity ($68.41 \pm 0.32\%$) was observed for T2 in Jersey breed. It is concluded that 4 hours equilibration time preserved cell membrane and DNA integrity of sperm and could be used for better post-thawed semen quality. Moreover, Jersey breed showed the best semen quality at different equilibration times.

Key words: Cattle breed, Semen quality, Sperm integrity, DNA damage

P-09 Relationship of Age, Breed and Libido with Semen Traits of Cattle Bulls

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The present study was conducted to find libido in different breeds in different age and their correlation with semen quality. Three mature cattle bulls from each of Friesian, Jersey, Achai, Cross (Friesian x Sahiwal) and Sahiwal in different age (1-3, 3-5 and above 5 years) were selected. For age grouping, data of all the breed was pooled. Libido was measured as 1. Time (seconds) the entering arena until mounting (P1) 2. Time (seconds) from mounting the teaser until developing of erection (P2) 3. Reaction time (seconds) (P3). Semen was collected twice a week and analyzed for semen volume, sperm concentration, sperm motility, dead sperm percentage (Eosin-negrosin dye) and hyposmotic swelling test (membrane integrity). Data was subjected to three factorial analyses (breed, age and libido) and Pearson's correlation. The results revealed that libido score in term of P1 (6.6 ± 0.46 Sec), P2 (1.88 ± 0.07 Sec) and P3 (9.57 ± 0.49 Sec) was significantly high ($P < 0.05$) in Jersey breed while cross bred bulls showed the lowest libido score. Jersey bulls showed significantly high ($P < 0.05$) semen characteristics including sperm concentration ($1560.0 \pm 59.00 \times 10^6$ /ml), membrane integrity ($77.42 \pm 0.97\%$) and livability ($83.32 \pm 8.32\%$). Libido calculated at different age groups showed that older animals having age above five years exhibited the best libido (P1, P2 and P3) compared to the younger animals. Similarly, semen characteristics including semen volume (6.50 ± 0.73 ml) and membrane integrity ($74.00 \pm 1.40\%$) were significantly high ($P < 0.05$) in older bulls (above 5 years) compared to the young animals with no significant effect ($P > 0.05$) on sperm concentration, motility and dead sperm percentage. Membrane integrity showed significantly high positive correlation with sperm concentration ($r = 0.52$) and significantly low negative

correlation with dead sperm percentage ($r = -0.28$). We concluded from the results that Jersey breed showed higher libido score and semen quality. From the results we concluded that libido and semen quality was higher in Jersey bulls compared to the other breeds. Moreover, libido and semen quality increased with increasing age (up to 8 years).

Key words: Cattle bulls, semen quality, libido, age, breed.

P-10 Cross Breeding Promotes Deterioration of Semen Quality in Cattle

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Nine normal mature bulls having average age was 3 ± 0.14 years were selected in three ($n=3$) different groups of Sahiwal, Holstein- Friesian and Cross-bred (Sahiwal x Holstein-Friesian) to compare the semen quality of pure and crossbred cattle bull. Semen samples were collected twice a week (total eight semen samples) by means of artificial vagina at 42°C and evaluated. Semen samples were individually diluted with extender (egg yolk extender) and tested for individual motility, progressive motility (under microscope), plasma membrane integrity (HOST), acrosomal and DNA integrity (Acridine orange test), seminal pH and dead sperm ratio (Eosin Negrosin dye) in fresh and thawed semen. Semen samples were processed and cryopreserved for 24 hours. Semen samples were thawed at 37°C and evaluated (manually) for above semen quality parameters in frozen thawed semen. Data was subjected to one way analysis of variance. High sperm individual motility ($85.12 \pm 0.51\%$), progressive motility ($20.25 \pm 0.47\%$), plasma membrane integrity ($82.25 \pm 0.43\%$), acrosomal integrity ($79.91 \pm 0.51\%$), and DNA integrity ($98.54 \pm 0.14\%$) were observed in fresh semen in Sahiwal bulls, which showed significant ($P < 0.01$) difference among the other cattle bulls. High dead sperm ratio ($51.87 \pm 0.50\%$) deterioration of plasma membrane integrity through HOST test ($38.29 \pm 0.45\%$) and DNA ($92.45 \pm 0.28\%$) was observed in frozen semen in Cross-bred bulls, which showed significant ($P < 0.01$) difference among the other cattle bulls. It is concluded that Sahiwal bulls showed best semen qualitative characteristics in fresh and frozen semen conditions followed by Holstein Friesian, while

Cross-bred bulls' semen samples showed the lowest semen quality in fresh and frozen conditions.

Key words: Cattle, Pure breed, Cross-bred, Semen quality

P-11 Determination of Prevalence of Reproductive Diseases in Small Ruminant by Retrospective Study at Northern Barind Tract in Bangladesh

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The retrospective study was done to identify the reproductive diseases in relation to age, sex and season of small ruminant. The survey conducted in the veterinary clinic and vaccination camp of the study area. Records of 265 clinical cases of small ruminant by closed questionnaires method under this study, from July 2012 to June 2013 were analyzed to assess the importance of existing reproductive diseases. The computer program SPSS were used to analyzed and chi square & F test were used for level of significance. The sex was significantly relation with various reproductive diseases and disorders. The female small ruminant was higher (84%) prevalence than male (15.5%). The maximum diseases frequency was 87 (32.8%) in anoestrus condition and lowest 5 (1.9%) in Prostatitis of small ruminants. The others diseases highest to lowest rate were 42 (15.8%), 27(10.2%), 21 (7.9%), 18 (6.8%), 15 (5.7%),14 (5.3%), 12 (4.5%) and 7 (2.6%) affected with abortion, retained placenta, destrocia, cervicitis, Urinary tract infection & mastitis, urolethiasis, repeat breeding and orchitis, respectively in small ruminants. The highest and lowest disease occurrence rate were anoestrus (20.4%) & repeat breeding (1.1%), anoestrus (7.9%) & prostatitis (0%) and anoestrus (4.5%) & cervicitis (0%), orcaitis (0%), urolethiasis (0%) and prostatitis (0%) was observed in kid (< 6mons), young (6 to 12 months) and old (>12months) ages of small ruminant. Similarly the seasons had significant effect (P<0.05) and the highest & lowest incidence rates of reproductive disorders were anoestrus (9.1%) & destrocia (0%), anoestrus (9.1%) & orcaitis (0%), and anoestrus (14.7%) & mastitis (0%), dystocia (0%), and prostatitis (0%), in summer, rainy and winter season. The most frequent reproductive diseases and disorder were of small ruminants were found in female, kids and rainy season at northern Barind tract in Bangladesh.

Key words: Reproductive Diseases, Small Ruminant, Age, Sex and Seasons

P-12 Fertility Improvement in Cross-Bred Dairy Cows through Supplementation of Vitamin E as Antioxidant

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Heavy milk production along with environmental thermal stress causes stress to the dairy cows that result into poor fertility. This study was conducted to investigate the role of vitamin E in combating stress, reflected by general (cortisol), thermal (heat shock protein, HSP-70) and oxidative (malondialdehyde, MDA; Glutathione peroxidase, GPx; Super oxide dismutase, SOD) stress. The experiment was conducted on thirty six early lactating dairy cows, comprising indigenous (9 Sahiwal, 9 Achai), 9 cross-bred (Sahiwal x Holstein Frisian) and 9 Holstein Frisian located at livestock research and development station Surezai, district Peshawar, Pakistan. Vitamin E was supplemented to dairy cows in feed at a dose rate of 1000 IU/ cow/ day for 40 days. Cows were synchronized and sampling was conducted on day-zero (control) before Vitamin E supplementation, day-20 and day-40 of vitamin E supplementation during the diestrus phase of the estrus cycle. Results indicated that concentrations of SOD and GPx increased (P < 0.001) with vitamin E supplementation from day-0 to day-40. SOD and GPx activities varied (P < 0.05, P < 0.001, respectively) between breeds, more prominent in local and crossbred cows. The stress markers (plasma MDA, HSP-70 and serum cortisol) decreased (P < 0.001) with vitamin E supplementation. Crossbred and local cows showed higher response (P< 0.001) to vitamin E supplementation compared to HF in stress parameters. Progesterone concentration increased (P < 0.001) with vitamin E supplementation from day-0 to day-40. Progesterone concentrations increased more notably in Crossbred and HF (P< 0.05). However, highest value was observed for progesterone in Achaj cows. Vitamin E supplementation reduced the number of dominant follicles in all cows (P< 0.01) from day-0 to day-40. The effect of vitamin E on follicular growth was more intensive in crossbred than HF and local cows (P< 0.05). The stress markers cortisol was positively correlated with HSP-70 (r= 0.285, P= 0.003) and MDA (r= 0.225, P= 0.013). HSP-70 and

MDA were also positively correlated ($r= 0.371$, $P= 0.000$) with each other. Cortisol were negatively correlated with SOD ($r= -0.388$, $P= 0.000$) and GPx ($r= -0.330$, $P=0.000$). MDA and HSP-70 were also negatively correlated with SOD ($r= -0.399$, $P=0.000$ and $r= -0.355$, $P=0.000$) and GPx ($r= -0.447$, $P= 0.000$ and $r= -0.498$, $P= 0.000$) respectively. Progesterone was positively correlated with SOD ($r= 0.404$, $P=0.000$) and GPx ($r= 0.443$, $P=0.000$). The results of the present study reveals that vitamin E supplementation can increase reproductive performance in HF and crossbred cows suggesting regular feeding of antioxidant to these breeds.

Key words: Dairy, HSP-70, Stress, Fertility, Antioxidant

P-13 A Strategy to Improve Cryo-Survival and Fertility of Buffalo Bull Spermatozoa

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During cryo-processing of spermatozoa, cholesterol is an important component in the regulation of membrane fluidity. The objective of the present study was to determine the improvement in cryosurvival of spermatozoa by addition of cholesterol in Tris-citric acid (TCA) semen extender and also pregnancy rate in Buffaloes. Buffalo bulls were of Nili Ravi breed having 5-7 years of age. *Experiment-1:* Cholesterol was added to TCA semen extender in the form of cholesterol-loaded cyclodextrin (CLC). Extended semen was incubated with CLC at room temperature for 15 minutes before the addition of egg yolk and glycerol. Five replicates of each bull ($n=3$) were separately evaluated and further processed with at least 60 % sperm motility. Semen samples were diluted at 37° C in extender containing either 1 mg (Low), 2 mg (Medium), 3 mg (High) of CLC or without (Con) per ml containing 120 million spermatozoa. After extension at 37 °C, semen was cooled to 4°C in 90 min, equilibrated at 4°C for 2 hours, frozen in liquid nitrogen (LN₂) vapor (4 cm above the LN₂ for 7 min) and shifted to LN₂ for storage. Post thaw motility (PTM), live spermatozoa (LS), plasma membrane integrity (PMI) and normal apical ridge (NAR) were assessed using standard procedures. Analysis of variance revealed that mean PTM and LS values were significantly ($P < 0.05$) higher in Medium (44.33 ± 1.68 and 53.60 ± 2.03 %) as compared to Con (31.33 ± 1.98 and $41.20 \pm$

2.50 %) respectively. Mean PMI and NAR values were significantly ($P < 0.05$) higher in High and Medium groups as compared to Con. *Experiment-2:* Semen doses from Medium ($n=50$) and Con ($n=50$) were used for fertility trial in 100 Nili Ravi buffaloes in the field area of Muzaffarabad at > 90 days postpartum. Although non-significant ($P > 0.05$), the pregnancy rate was higher (64 %) in buffaloes inseminated with semen of medium group as compared to their Control (62%) counterparts It is concluded that addition of cholesterol in Medium concentration (2 mg CLC) to Buffalo bull semen can improve cryosurvival of buffalo bull spermatozoa but not fertility as compared to Con group.

Key words: CLC, Buffalo Bull, Spermatozoa

P-14 Studies on Uterine Microbial Flora of Sahiwal Cattle during Oestrus

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Conception rate of cattle various factors including bacterial pathogens affect the reproductive ability after calving resulting in subfertility. Post partum invasion of uterus by bacterial pathogens disturb uterine functionality leading to low fertility of the cows. Current study was envisaged to find out the microbial load in the uterine lumen of Sahiwal cattle at the time of estrus, just before artificial insemination. A total fifty (50) uterine samples of normal cyclic adult Sahiwal cows with no previous history of uterine disorder, kept at Livestock Production and Research Institute (LPRI), Bahadurnagar Farm, district Okara, were collected at the time of estrus prior to insemination using sterilized sampling device consisted of a rod, normally used for artificial insemination, having both the ends open. At one open end, there was a connection to a syringe which has normal saline in it and at the other end there was half gelatin capsule to prevent the rod from contamination while entering into the uterus. The uterine samples were cultured on nutrient and blood agar under aerobic and an-aerobic conditions and identification of bacteria was done on the basis of morphology, staining characteristics and biochemical reactions as per guidelines of Bergey's Manual of Determinative Bacteriology. Statistical analysis was done using SPSS 18.0 and frequency of bacterial isolates were calculated which showed isolation of total 11 bacterial species from the uterine samples. Out of these bacterial isolates, 28 (56%) isolates were *E. coli*, 22 (44%) were *Micrococcus spp.*, 19 (38%) were *Bacillus spp.*, 16 (32%)

were *Staphylococcus aureus*, 5 (10%) were *Staphylococcus epidermidis*, 10 (20%) were *Streptococcus spp.*, 2 (4%) were *Pseudomonas spp.*, 11 (22%) were *Citrobacter spp.*, 2 (4%) were *Salmonella spp.*, 5 (10%) were *Proteus spp.* and 3 (6%) were *Corynebacterium spp.* Statistical analysis showed that significant association ($p < 0.05$) of non pregnant Sahiwal cattle with isolation of *Streptococcus*. Other bacteria like *E. coli*, *Micrococcus*, *Bacillus*, *Staph aureus*, *S. epidemicus*, *Pseudomonas*, *Citrobacter*, *Salmonella*, *Proteus* and *Corynebacterium* were non significantly associated ($p > 0.05$) with pregnancy status of these animals when inseminated artificially indicating that *E. coli*, *Micrococcus spp.*, *Bacillus spp.*, *Staphylococcus aureus*, *S. epidemicus*, *Pseudomonas*, *Citrobacter*, *Salmonella*, *Proteus* and *Corynebacterium* are the normal microbial flora in the uterus of Sahiwal cattle while *Streptococcus spp.* are not normally present as uterine flora which could result in decrease pregnancy rate.

Key words: Uterine microflora, Conception rate, Uterine bacteriology, Sahiwal cattle.

P-15 Comparative Reproductive Performance of Beetal Goats in Accelerated and Annual Kidding Systems

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Beetal is a goat breed of Pakistan. This is popular for its milk and particularly meat production of tender quality. Fecundity is one of another valued economic trait in this breed. The present study was planned to evaluate the reproductive performance of accelerated kidding versus annual kidding system for a period of two years. A total of 50 Beetal goats were randomly divided in two groups having 25 animals each. Accelerated kidding system means to have more frequent services and kids per doe during a period of two years as compared to annual kidding system. The supplementary feeding was adopted for successful conception rate and judicious use of their natural breeding abilities. It was observed that accelerated kidding system is more profitable. The data were analyzed by complete randomized design (Steel et al., 1997) using proc GLM procedure of SAS.1995 (SAS institute, Cary, NC). The onset of estrus, kidding percentage and conception rate was 8, 1.33 and 10%, respectively. However, the number of kids born, single

and triplet births were augmented as 6, 1.94 and 20%, respectively in accelerated system. It was also found that twin births were reduced by 11.6% in accelerated system. Maximum 1.64 \pm 0.81 services per conception (SPC) were observed. The litter size (LS) was observed as 1.66 \pm 0.71 which was significant ($P < 0.05$) in accelerated kidding system as compared to maximum 1.19 \pm 0.40 (SPC) with 1.50 \pm 0.55 (LS) in annual kidding system. The shorter gestation length was also recorded in accelerated system. It is concluded that accelerated kidding system in this breed is economically doable. Further studies and research with larger data set is needed to explore reproductive performance in Beetal goats under accelerated kidding system and to validate the findings of current study.

Key words: Reproductive performance, Services per conception, Accelerated kidding system, Beetal goat, Pakistan

P-16 Use of Butylated Hydroxytoluene as an Antioxidant in the Buffalo Semen Extender

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Nili-Ravi breed of buffalo is one of the best breeds of the world. During freezing, the semen quality is damaged due to the production of Reactive Oxygen Species (ROS) from the plasma membrane of spermatozoa causing damage to the membrane integrity due to lipid peroxidation. The main objective of the present study was to determine the level of total antioxidants in the semen and to optimize the quantity of BHT in the semen extender of the Nili Ravi Buffalo bulls. In this study, semen from Nili-Ravi buffalo bulls (n=10) was collected twice per week in the month of October. The semen of each bull was diluted with Tris egg yolk citrate extender separately. Antioxidant BHT was added to the extended semen at the rate of 1.0mM, 1.5 mM and 2 mM (n=20 per bull per treatment) while Control was containing no BHT. The semen was visually analyzed with the help of phase contrast microscope for motility, vitality (Eosin/Nigrosin staining), plasma membrane (HOS assay) and acrosomal integrity (NAR), at three stages i.e. after dilution, before freezing and after freezing. The semen was frozen manually at SPU Qadir Abad. ABTS assay was used for the determination of total antioxidant capacity (TAC), in the Spectrophotometric Laboratory, GC University Lahore. The semen was transported in thermos having ice from SPU Qadir Abad to Lahore to limit ROS production during travelling. Data collected were presented as mean \pm SEM. Treatment groups were

compared using one way ANOVA and results were compared by using Duncan Multiple Range Test using SPSS (version 16.0); SPSS Chicago IL. Results of this study showed significant ($P<0.05$) improvement in sperm motility, vitality, plasma membrane and acrosomal integrity at 1.5 mM BHT concentration as compared to Control sample. Maximum TAC value (2.08 mM) was observed in the extended semen containing BHT and minimum TAC values (0.58 mM) in the post thaw semen sample with BHT. It is concluded that addition of antioxidants in the buffalo semen improves the semen quality before and after freezing. Fortification of buffalo semen extender with BHT (@ of 1-1.5 mM) improved post thaw semen quality. However, further study is required to analyze the fertility of buffaloes after AI with BHT fortified semen.

Key words: Butylated Hydroxytoluene, Buffalo Semen Extender, Nili-Ravi

P-17 Effects of Flaxseed Supplementation on Semen Quality and Quantity in Different Dairy Cattle Breeds

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Flaxseed is a good source of omega-3 fatty acids. The current study was designed to investigate the effects of dietary flaxseed as a source of omega-3 fatty acids on quality of liquid and frozen thawed semen in different dairy cattle bulls. Nine bulls of 3-5 years age, 3 each from Holstein Frisian (HF), Jersey and Cross-bred (HF x Sahiwal) were selected, and further divided into three treatment sub-groups, 1) Control group FL-0 was fed 6 kg concentrate, 6 kg wheat straw and 40 kg green fodder as basal diet, 2) FL-I bulls fed with 50 g of imported rumen protected flaxseed plus basal diet, 3) treatment FL-L bulls fed 50 g local non rumen protected flaxseed along with basal diet. Trial was carried out for a total of 11 weeks. Adaptation period was given to animals before the commencement of experiment. Semen collection was started from the third week of supplementation. Factorial design was used for statistical analysis to find out the effect of breed and flaxseed supplementation. Seminal volume did not vary among the treatment and control groups in all breeds ($P> 0.05$). Sperm concentration, mass motility, individual motility, viability and HOST of the fresh semen were higher ($P<0.05$) in the bulls fed with (FL-I) as compare to FL-0 and FL-L. Motility, viability and HOST of post-thawed semen were also higher ($P<0.05$) in the bulls fed with (FL-I) compared to

FL-0 and FL-L. Total Sperm output was significantly higher ($P<0.05$) in HF bull fed with rumen protected flaxseed (FL-I) compared with FL-L and FL-0 of the same breed. HF bulls of FL-I and FL-L group showed more favorable response to flaxseed supplementation than crossbred and Jersey of the same group. Total sperm output was correlated positively ($P<0.05$) with mass motility ($r=0.48$, $P= 0.001$), membrane integrity ($r=0.58$, $P= 0.000$), percent motility ($r=0.63$, $P= 0.051$) and seminal volume ($r= 0.64$, $P= 0.000$), whereas, seminal volume and concentration of spermatozoa correlated negatively ($r= -0.75$, $P= 0.000$). The results of the present study imply that feeding rumen protected flaxseed improve the quality of bull spermatozoa.

Key words:cattle Bull, Flaxseed, Semen quality, Dietary supplementation, Fertility

P-18 The Way to Preserve the Genome of Valuable Animal: Epididymis Gamete Retrieval in Slaughtered Ram and its Post-Thaw Evaluation

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Post-mortem spermatozoa recovery is an important technique for obtaining germplasm reserves from genetically valuable animals or endangered species. It can be used to dilute off a huge loss in any breed conservation program. The objective of the present study was to evaluate pre-freeze and post thaw sperm attributes collected from ram epididymis (n=05). Testes were collected from slaughter house during breeding season and transported at refrigeration temperature in normal saline. The evaluation was carried out within two hours of slaughtering. Sperms were obtained by slicing the tissue of cauda epididymis with a scalpel; fluid was collected and measured. The samples were carefully dissected free of blood clots and extraneous tissue. Semen samples were diluted with the Tris Citrate egg yolk extender. After extension, cooling and equilibration of semen for 2 hours at 4 °C, filling was done in 0.5 ml French straws to attain a final concentration of 100×10^6 spermatozoa per straw. Freezing of samples was carried out by keeping the straws at the level of 4 cm above liquid Nitrogen (LN) vapors for 7 min and finally frozen in LN through programmable biofreezer. The sperm attributes included percentages of sperm motility,

viability, normal morphology, plasma membrane integrity (PMI) and normal apical ridge (NAR). Results revealed that pre-freeze percentages of motility, viability, normal morphology, PMI and NAR were 65, 70, 20, 70 and 66%, being 30, 38, 25, 36 and 34 for post thaw spermatozoa, respectively. The post thaw sperm attributes were found to be within an acceptable limit. It is concluded that ram gametes attained from their epididymis can be preserved with acceptable quality. Further investigations can be directed towards gamete handling and addition of some supplements in extenders in order to improve its preservation.

Key words: Conservation, Epididymal Sperm, Ram

P-19 Effect of Varying Concentrations of Bovine Serum Albumin and Egg Yolk on Post-Thaw Quality of Beetal Buck Semen

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Bovine Serum Albumin (BSA) protects spermatozoa from harmful effects of lipid peroxidation upon its addition in semen extenders. The aim of present study was to optimize the concentration of BSA with varying concentration of egg yolk in semen extender through the evaluation of post thaw quality of Beetal buck semen. Two Beetal bucks were used for semen collection for 7 weeks. Semen from both the bucks were collected twice a week whereas once a week semen collection was used for this study and 2nd collection was used for another study going on in the Department. Ejaculates of both bucks having >70% motility, > 0.5 ml volume, >2.5×10⁹ per ml concentration and <10% abnormalities were pooled and divided into 9 aliquots. Tris Citrate Egg Yolk (TCEY) extender containing different concentrations of egg yolk (20, 15 and 10 %) was prepared. Extender with latter two concentrations also received four concentrations (0, 1, 2 and 3%) of BSA separately. Nine types of extenders were mixed with above said 9 aliquots of buck semen. After extension at 37°C, semen was cooled to 4°C in 90 min, equilibrated at 4°C for 4 hours, frozen in liquid nitrogen (LN₂) vapors (4 cm above the LN₂ for 10 min) and shifted to LN₂ for storage. Post thaw semen evaluation assays, *i.e.*, post-thaw motility (PTM%), live percentage (LP%), morphological abnormalities (MA%), plasma membrane integrity (PMI%), normal apical ridge (NAR%) and DNA integrity (DI%) were conducted after 24 hours of storage. Two-way ANOVA was performed

and significant differences between means were compared using Tukey's test. It was observed that mean ± SE values of PTM, LP, PMI, NAR and DI were significantly (P<0.05) higher in groups having 15 and 10 % egg yolk with 1 and 2 % BSA, respectively as compared to all other groups. In conclusion, this study indicated that the addition of 15% egg yolk with 1% BSA and 10% egg yolk with 2% BSA in Beetal buck semen may enhance its post thaw sperm quality.

Key words: Beetal Buck, Semen, BSA, Egg yolk

P-20 Effect of Different Levels of Concentrate Mixture for Attaining Early Puberty in Sahiwal Heifers

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The objective of the study was to evaluate effect of varying levels of concentrate on the growth and age at puberty in Sahiwal heifers. The research was conducted at livestock experimental station, Jahangirabad, Khanewal, Punjab, Pakistan. Sixty three Sahiwal female young stocks 405±23 days age were divided into three groups (21 FYS, with 7 FYS per replicate were offered green fodder (GF), GF+ Concentrate ration (CR) @0.5% of body weight (BW) and GF+CR@1% of BW. The feeding trial continued till age at which heifers on all treatments were expected to reach at age of puberty and got pregnant. The results showed a significant (P<0.01) difference in mean dry matter intake, average daily gain, body measurements and feed efficiency. The digestibility of nutrients was greater (P<0.05) in green fodder with 1 % of concentrate ration. Sahiwal heifers fed green fodder with 1 % of concentrate ration attained puberty earlier than those fed 0.5 % concentrate ration along with green fodder. Body height, body length and heart girth were found the highest (P<0.05) in heifers fed on green fodder with 1 % concentrate ration. Cost to gain ratio in heifers was Rs. 97.18, 90.60 and 93.03 on GF, GF+CR@0.5% and GF+CR@1%, respectively. The best performance in terms of increased dry matter intake, body condition score, feed efficiency, and weight gain and body measurements was observed in the heifers fed on green fodder along with starter @ 1 %. The cost to gain ratio was observed comparatively low in the heifers on the treatments supplemented with 1% concentrate. It is further suggested that the heifers be supplemented with concentrate @ 1 % of body weight to attain early maturity got pregnant.

Key words: Concentrate Ration, Sahiwal Heifers, Growth Performance, Dry Matter Intake

P-21 Influence of Crude Proteins Levels on Milk Production, Blood Urea Nitrogen and Serum Progesterone Profile in Mid Lactating Jersey Cows

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An experiment was conducted to the influence of different crude protein (CP) levels on milk production and milk composition (fat, protein and lactose), blood urea nitrogen (BUN) and progesterone (P4) profile in mid-lactating Jersey cows. A total of nine cows at mid lactating stage (110±20 days) were randomly selected for the trial and divided into three groups. Each group was fed experimental diet having 13, 16 and 19% crude protein on dry matter (DM) basis respectively. All the animals were fed individually on maize fodder and wheat straw based ration ad-libitum along with 5kg of their respective experimental diets. The experiment was continued for 75 days including 15 days adaptation period and 60 days for data collection. Dry matter intake was not significantly ($p>0.05$) effected in all the groups but numerically high values were recorded for the group having high level of CP. Body weight gain was recorded significantly higher in group 13%CP (14.0±2.08) and group 16%CP (6.0±2.08) as compared to group 19% CP (-4.67±3.18) throughout the experimental period. Milk yield (kg/day) was significantly ($p<0.05$) higher in group 19%CP (11.8±0.23) compared to 13%CP (10.46±0.32) and 16%CP (11.23±0.34). Similar trend was recorded for milk fat percentage having mean values of 3.99, 3.85 and 3.75% fat in 19%CP, 16%CP and 13% CP group respectively. Mean percentage of milk protein and lactose were not significantly ($p>0.05$) effected in all the treated groups. Blood urea nitrogen was significantly ($p<0.05$) higher in group 19% CP followed by 16%CP and 13% CP groups having mean values 18.87, 15.93 and 12.07 mg/dl respectively. No significant difference was found for progesterone level in the serum having mean values of 3.02, 2.91 and 2.76 ng/ml for 13, 16 and 19% CP group respectively. It is concluded that performance of cows at mid-lactation can be maintained at lower dietary crude protein up to 13% in concentrates with maize fodder based ration reflected by optimum levels of milk yield, blood urea nitrogen and serum progesterone.

Key words: Crude Proteins, Milk Production, Blood Urea Nitrogen, Serum Progesterone Profile

P-22 Testicular Biometry, Scrotal Circumference, Serum Testosterone and Semen Characteristics in Achai and Jersey Bulls

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This study aimed to determine the relationship of scrotal circumference, age and body weight to testicular biometry and to establish criteria for Breeding Soundness Evaluation (BSE) of Achai (Indigenous breed) and Jersey (Exotic breed). Standard procedure using measuring tape was used to measure the scrotal circumference and testicular biometry of 8 Achai and 9 Jersey bulls of various ages. The greatest scrotal area of circumference was recorded for measurement. The length of testes was measured from dorsal to ventral side, width from right to left side of the testes and thickness of testes from anterior to posterior side. The volume of the testes were measured by $\frac{4}{3} \pi a b c$, in which a, b, c related to, thickness/2, width/2 and length/2 of the testes, respectively. The weight of the testes was calculated by multiplying volume with 1.038. The ejaculates were collected twice a week from each bull for 6 weeks starting from age groups of four different breeds that was 16-36 (n=3), 37-48 (n=3), 49 (n=3) month. Semen volume, concentration, motility and progressive motility were recorded through phase contrast microscope. Blood samples were collected at three times; at beginning, after 15 day and after 30 of the experimental period to measure testosterone level using ELISA. Data was statistically analyzed through analysis of variance and Pearson correlation using SPSS (version 16.0) statistical packages. Duncan Multiple Range Test was used to signify the age groups of bulls breed-wise separately. During current study, significant ($P<0.05$) increase has been observed in length, width and thickness of testes, scrotal circumference and body weight of both breed at adult age group (49 month). Furthermore, testosterone level was found significantly higher ($P<0.05$) in both indigenous and exotic breed. The result of the current study demonstrated that Scrotal Circumference, Testicular Weight, Sperm Volume, Motility, Progressive Motility and Sperm output were positive correlated with body weight. Therefore these indices could be used as practical indicators to select breeding bull during breeding soundness examination under existing management system.

Key words: Achai, Jersey, Testes Biometry, Scrotal circumference, Testosterone,

P-23 Dystocia due to Parapagus Monster in a Murrah Buffalo

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A Murrah buffalo in its 2nd lactation was presented to teaching veterinary clinical complex, LUVAS, Hisar, India with a history of a prolonged second stage of labor with forceful abdominal contractions and two forelimbs and head were protruding from the vulva. On vaginal examination it was found that the second rib cage was present, perpendicular to the birth canal in the uterus. Further detailed examination confirmed the presence of two tails in the birth canal. Because one portion of fetus along with both forelimbs and head were hanging outside the vulvar lips. Therefore on examination to right side of the fetus two tails were present in the uterus and other portion of fetus was present transversely. Hence it was confirmed that the fetus was a monster that was causing dystocia. Since forced extraction was not possible, therefore a fetotomy was performed under local anesthesia with 2% Lignocaine hydrochloride injecting 7-10 ml epidurally between 1st and 2nd inter-coccygeal space. The head of thygeson fetotome was placed dorsally at the lumber region whereas loop of wire was passed around the around the abdomen behind the last rib and a full-term dead fetal monster was extracted. The buffalo was treated with an injectable combination of 3.0 gm Ceftriaxone + 1.5 gm Salbactam (Polcef forte), plus an injection of Melonex (Meloxicam 0.5 mg/ kg b.wt), 30 ml intramuscularly, intra uterine bolus, Hyterus (combination of Nitrofurazone, Metranidazole, Povidone iodine, Urea as anantiseptic and proteolytic agent) and supportive fluid therapy for 5 days. The buffalo showed recovery without any complication. The development of conjoined twins was nearly complete. Two normal heads were present on two necks with normal eyes and ears. The twins were fused in the thoracic region. There were four forelimbs and an abnormal orientation of ribs. Two separate vertebral columns along the length of the animal ended with two tails. There was a single pelvis, and only two hind limbs were present. The calf had a single anus and vulva. Conjoint fetuses were attached with each other behind the xephoid region e.g. ventral abdomen as seen in photograph. Conclusion: Such type of cases can not be treated at field level and forced extraction should not be tried without knowing the etiology of the dystocia. The decision should be taken immediately without exhausting the animal.

Key words: Murrah buffalo, dystocia, parapagus monster, India

P-24 Effect of Cholesterol Loaded Cyclodextrin on Sperm Survival of Buffalo Bull during Stages of Cryopreservation

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Sperm cryopreservation results in loss of sperm structural and functional parameters. *In vitro* incorporation of cholesterol into mammalian sperm membrane increases sperm membrane fluidity at low temperatures. This is the first study sought to evaluate the effect of cholesterol loaded cyclodextrin (CLC) on buffalo sperm quality parameters at different stages of cryopreservation (i.e. after dilution, before freezing, and after thawing). Semen ejaculates from three buffalo bulls were pooled (Replicates = 6), treated either with CLC (2 and 4 mg per 120×10^6 spermatozoa) or without CLC (control), incubated for 15 minutes and diluted in tris-citric acid diluent. After dispensing in 0.5 ml straws, cooled to 4°C and frozen using standard procedure. Sperm motility, viability, plasma membrane integrity (PMI; hypo-osmotic swelling test), and normal apical ridge (NAR; formol citrate solution) were evaluated (%) after collection/fresh (F), after dilution (AD), before freezing (BF) and post-thawing (PT). Data were presented as mean \pm S.E.M and analyzed using analysis of variance taking replicate as random factor. Motility did not differ ($P > 0.05$) between control (C0), CLC 2 (C2) and CLC 4 (C4) groups from F, but reduced ($P < 0.05$) due to cooling in C0, C2 and C4 (58.33 ± 1.67 , 65.00 ± 0.60 , 65 ± 0.46 , respectively; $P > 0.05$ between groups). PT motility of C0 and C2 (43.44 ± 1.67 vs. 56.67 ± 1.67 , respectively; $P < 0.05$) was further reduced ($P < 0.05$), while C4 shows similar ($P > 0.05$) motility (58.33 ± 1.67) when compared to previous stages. In all groups (C0, C2, C4), sperm viability, PMI and NAR were same ($P > 0.05$) at AD. At thawing, sperm viability (57.33 ± 0.67 , 67.67 ± 0.33 , 71.67 ± 0.67 ; c0, c2 and c4 respectively), PMI (55.00 ± 1.00 , 67.00 ± 1.00 and 70.67 ± 0.67 ; C0, C2, C4 respectively) and NAR (56.00 ± 3.51 , 63.33 ± 0.33 and 67.33 ± 0.67 ; C0, C2 and C4 respectively) were reduced ($P < 0.05$). Both CLC treatments (C2 and C4) showed no difference ($P > 0.05$) in PMI from BF to PT, while PMI was reduced ($P < 0.05$) in control. The concentration of CLC affected post-thaw sperm quality in a dose-dependent manner. The main difference in sperm quality was observed in PT stage. It is concluded that CLC treatment for buffalo sperm cryopreservation significantly improves cell cryosurvival and minimizes sperm damage at PT stage.

Key words: Cholesterol Loaded Cyclodextrin, Buffalo Bull, Cryopreservation

P-25 Effect of Bypass Fat Supplementation on Productive and Reproductive Performance of High Yielding Murrah Buffaloes

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The study investigated the effect of dietary supplementation of bypass fat on productive and reproductive performance and blood biochemical profile of high yielding Murrah buffaloes (*Bubalus bubalis*). Twenty-four pregnant buffaloes with previous record of high productivity were divided in two homogenous groups of control and treatment. The animals of control were fed basal diet consisting of a concentrate mixture, available green fodder and wheat straw as per requirements, while the animals of treatment group were fed same ration supplemented with bypass fat starting from one month prior of the expected date of calving to whole lactation period of 305 days. There were no differences in the dry matter and nutrient intake as well as milk composition except milk fat. Similarly no difference in blood mineral content and haematological parameters was observed except percentage of monocytes. However, biochemical parameters such as enzymes alkaline phosphatase, creatinine phosphokinase, aspartate transaminase and amylase were significantly different while other parameters remained same. There was no response either positive or negative on the reproductive performance of the buffaloes statistically. Though supplementation of bypass fat statistically ($P < 0.05$) improved the birth weight of calves, as well as production of fat-corrected milk (6%) in high yielding buffaloes. It was concluded that bypass fat supplementation increased the fat-corrected milk production and birth weight of calves when supplemented one month prior to calving till mid-lactation without any adverse effect on reproductive performance of high yielding buffaloes.

Key words: Buffalo, Bypass fat, Reproduction and Production performance

P-26 Seasonal Pattern of Calving in Nili-Ravi Buffaloes

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Season of calving in buffalo shows trends in calving during different seasons. This study was conducted to explore seasonality of calving (calving frequency) in Nili-Ravi buffaloes. Data consisting of 17731 calving records of buffaloes maintained in three herds were analyzed. Herd 1, 2 and 3 contributed 7667(43.2%), 3965 (22.4%) and 6099(34.4%) records respectively in the total data set. Data were divided into four seasons viz. Spring (Feb-Apr), summer (May-Aug), autumn (Sep-Oct) and winter (Nov-Jan) considering the month of calving. The four seasons had 32.1% (n=5690), 20.5% (n=3637), 34.6% (n=6137) and 12.8% (n=2267) calving records, respectively. There were significant ($P < 0.05$) differences among frequencies of calving in different seasons. Most of the calves were produced in autumn (34.6%) followed by spring, summer and winter season. This distribution is helpful in making management decisions for providing feeding resources and other provisions for the buffaloes. This information is also helpful in making more appropriate breeding decisions. The results made it clear that buffaloes bred in winter (Nov-Jan) had high reproductive efficiency. It showed impact of season on reproductive efficiency of Nili-Ravi buffaloes.

Key words: Season, Reproductive efficiency, Nili-Ravi buffalo, Management

P-27 Distribution of Buffaloes Affected with Peri-Partum Reproductive Disorders, Maintained under Different Production Systems

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Present study was conducted to determine the distribution of reproductive disorders in riverine buffaloes. Data on

calving disorders of buffaloes were collected from three ecological zones. The data were put to descriptive statistical analysis. In southern- irrigated zone, the highest number of buffaloes suffering from calving related reproductive disorders were being maintained under rural subsistence small-holding followed by market-oriented small-holders and peri-urban commercial farms. There were significant differences among all production systems ($P<0.05$). In northern- irrigated zone the highest number of buffaloes who suffered from calving disorders was found in rural-subsistence small-holdings followed by peri-urban commercial farms, market-oriented small-holding and commercial farms. However, number of buffaloes which suffered from such disorders was significantly higher ($P<0.05$) under rural-subsistence small-holding as compared to market-oriented small holdings and commercial farms but non-significantly higher than peri-urban commercial farms. In arid-zone, significantly higher ($P<0.05$) percentage of buffaloes with calving disorders were found in rural-subsistence small-holdings as compared to those in market-oriented small-holdings, peri-urban commercial farms; the latter two did not differ ($P>0.05$). When pooled data were analyzed amongst with reference to production system, higher ($P<0.05$) percentage of buffaloes that suffered from calving disorders was found in arid-zone followed by southern-irrigated zone and northern-irrigated zone in rural-subsistence small-holding; the difference among three agro-ecological zones was significant ($P<0.05$). In market-oriented small-holdings, highest proportion of affected buffaloes was found in southern-irrigated zone followed by northern-irrigated zone and arid-zone; the difference among three zones was significant ($P<0.05$). In peri-urban commercial farms, highest percentage of affected buffaloes was present in northern-irrigated zone followed by southern-irrigated zone and arid-zone; difference among three zones was again significant ($P<0.05$). As regards the commercial farms, no significant difference was found in the three agro-ecological zones under study.

Key words: Reproductive disorders, buffalo, ecological zones, household surveys

P-28 Distribution of Buffaloes Affected with Peri-Partum Reproductive Disorders, with Reference to Feeding Pattern

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The present study was conducted to explore calving related reproductive disorders in Nili-Ravi buffaloes with reference to feeding regimes in Punjab, Pakistan. For data collection, the Punjab province was divided into three ecological zones (southern, northern and arid). In the southern irrigated zone, the highest ($P<0.05$) number of buffaloes suffered from calving related reproductive disorders was found in stall-fed buffaloes compared to open or grazing feeding pattern. In northern irrigated zone the highest number of buffaloes suffered from calving disorders which were kept in open or grazing feeding pattern ($P<0.05$) as compared to stall-fed buffaloes. In arid zone, the highest ($P<0.05$) number of buffaloes suffered from calving related reproductive disorders were those who were stall-fed compared to open or grazing feeding pattern. When the data in the three agro ecological zones of Punjab were compared for stall fed buffaloes, significantly lower ($P<0.05$) number of buffaloes with reproductive disorders was found in the northern irrigated zone as compared to the southern and the arid zone; the difference between the latter two was however non-significant ($P>0.05$). The number of buffaloes who were kept open or on grazing were significantly higher ($P<0.05$) in the northern irrigated zone as compared to the southern irrigated zone and the arid zone; the difference between the latter two was however non-significant ($P>0.05$). Pooled data showed that significantly higher number of buffaloes suffered from calving disorders was in stall fed feeding pattern as compared to those in open or on grazing ($P<0.05$).

Key words: Nili-Ravi Buffalo, Ecological zones, Calving disorders, Feeding pattern

P-29 Environmental Factors Affecting some Reproductive Traits in Holstein Friesian x Sahiwal Crossbred Cattle in Pakistan

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The objective of this study was to investigate the environmental effects on some reproductive traits in Holstein Friesian x Sahiwal crossbred cattle. Data on 2434 records of 533 Holstein Friesian x Sahiwal crossbred cattle maintained at two different Livestock Experiment Stations (LES, Rakh Dera Chahl and LES, Rakh Ghulaman) spanned over a period from 1995 to 2011 were analyzed by Mixed Model Least Squares Maximum Likelihood (LSMLMW) computer software. The overall least squares means for age at first calving, service period and calving interval were 1404.39±68.85 days, 215.96±20.73 days and 516.72 ± 23.13 days, respectively. The year of birth, sire breed (Sahiwal, Friesian, Cross bred) and dam breed (Sahiwal, Cross bred) exerted significant effect on age at first calving (AFC). The year of calving, season of calving and lactation number significantly affected the service period in crossbred cattle. The calving interval was significantly affected by year of calving and lactation number. The season of calving, herd and level of inheritance have non-significant effect on calving interval.

Key words: Environmental factors, Reproductive traits, Cross bred cows, Pakistan

P-30 Uterine Didelphys in Buffalo-A Case Study

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The basic aim of the study was to document a case study of uterine didelphys, a rare clinical condition in Nili-Ravi buffalo. Approximately 54 months old buffalo with the history of repeat breeding was brought to the Sihala slaughter house, Islamabad. During ante-mortem examination, trans-rectal palpation revealed the increased

cervical diameter with poorly described external os. Upon passing the AI rod, it was felt only in one side of narrow uterine body. After slaughtering, gross examination revealed the presence of two cervical openings. Upon dissection, it was revealed that the opening in separate uterine horn is due to presence of band of tissue, although uterine horn consistency was normal. Both ovaries were cyclic. As far as biometric measurements are concerned cervix was 7cm long with 7.5cm diameter. While ovaries were 2.5 cm and 3 cm long with 1cm diameter each. There was no common uterine body to both horns. The length of right horn and left horn were 15 cm and 16.5cm respectively. This is an interesting case which broadened the spectrum of causes of repeat breeding.

Key words: Uterine didelphys, Buffalo, Uterine horn, Nili-Ravi, Case study

P-31 Constraints Limiting the Conception Rates in Cows Inseminated with Frozen Semen

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Herd-level management factors and cow-level factors have a lot of importance for success of dairy farms. Economy of dairy farming largely depends on a high pregnancy rate after artificial insemination (AI) and high conception rate of dairy cows has a great importance to meet today's expectation of dairy owners to obtain one calf per cow per year. The study was undertaken to determine the effects of breed, age, parity, feeding practice, body condition score (BCS), interval between oestrus and AI, interval between calving and AI, and milk yield on first service conception rates in AI cows (n=308). The demographic factors were recorded by interviewing the farmers. All cows were examined for pregnancy diagnosis by per rectal palpation of genital tract at 60-80 days post-AI. The overall conception rate in cows was 52.6%. The conception rate was the highest (56.1%) in cows of 3-5 years of age and the lowest (47.1%) in cows of 2-3 years of age. The highest (57.4%) conception rate was in cows of parity 1-2 whereas the lowest (45.0%) was in cows of parity 0. The conception rate in cows fed with combination of green grass, straw and concentrate was significantly (P<0.05) higher (63.5%) than that with only straw fed counterpart (38.5%). The conception rate in cows with BCS 3-4 was significantly (P<0.05) higher (58.0%) than that of BCS 1.5-2 counterpart (35.0%). The conception rate in cows received insemination at 6-12 hours interval between oestrus and AI was significantly (P<0.05) higher (58.8%) than that of 13-24 hours counterpart (40.4%). The moderate yielding cows (>2-5 litres milk) showed the

highest (62.1%) conception rate. Feeding with green grass, straw and concentrate for gaining good BCS and AI within 6-12 hours of oestrus improve the first service conception rate in cows.

Key words: Conception, AI, BCS, Parity, Milk yield

P-32 Effect of GnRH Analogue Administration at the Time of A.I on Conception Rate in Nili Ravi Buffaloes

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Gonadotropin releasing hormone (GnRH) plays a major role in the growth of follicles and subsequent release of pre-ovulatory LH surge. This study was carried out to see the effect of GnRH analogue administration at the time of A.I on the conception rate in heifers expressing first estrus and buffaloes at different postpartum estrus. Multiparous buffaloes expressing the first post-partum estrus were placed in group 1 (n=20). Similarly multiparous buffaloes expressing estrus second (n=20), and third time (n=20) after previous A.I were placed in group 2 and group 3, respectively. Furthermore, 20 heifers expressing first estrus were placed in group 4. Each group was further divided into two sub-groups i.e. treatment and control, with 10 animals in each group. The buffaloes in each treatment group received single injection of GnRH analogue (Lecirelin, 50 mcg) at the time of A.I, whereas, control group did not receive any GnRH analogue at the time of A.I. Pregnancy tests were carried out on Day 45 after A.I through transrectal ultrasonography (Honda, Japan 7400, 7.5 M Hz). All the data was analyzed through SPSS version 13 and Chi square was applied to compare the results of treatment and control groups. Differences in conception rates between control and treatment sub-groups of groups 1 and 3 were non-significant (60% each). However, in group 2, conception rate was higher (P<0.05) in treatment sub-group (70%) as compared to control (60%). Similarly, in group 4 conception rate was significantly higher (P<0.05) in treatment sub-group (60%) as compared to control (40%). It can be concluded that single injection of GnRH analogue (Lecirelin, 50 mcg) at the time of A.I is beneficial in heifers expressing the estrus first time and adult buffaloes which are repeating the cycle 2nd time after previous A.I.

Key words: Buffaloes, Estrus, Heifers, GnRH, Conception.

P-33 Effect of Exogenous Growth Hormone on Semen Quality, Hematological Variables and Serum Testosterone Concentrations in Nili-Ravi Buffalo Bulls

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In Pakistan, recombinant bovine somatotropin (rbST) is used to increase growth rate and milk production in beef and dairy animals, respectively. The aim of this study was to examine possible effects of rbST on semen quality, hematological variables and serum testosterone concentrations in adult Nili-Ravi buffalo bulls. Six adult Nili-Ravi buffalo bulls with clinically normal reproductive tract were divided into two equal groups. Bulls of the treatment group were injected S/C with rbST (Boostin-S, LG Life Sciences, Korea) @ 500 mg fortnightly for 10 weeks, while those of control group received equal volume of normal saline. Semen and blood collection was carried out during 10-week treatment period. Semen samples were collected twice per week and analyzed for physical quality parameters. A total of 120 semen samples, with 60 samples from three bulls of each group (20 samples per bull) were available. Blood samples were collected fortnightly and analyzed for red blood cell (RBC) count, hemoglobin (HB) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), white blood cell (WBC) count, neutrophils, lymphocytes and serum testosterone concentrations. Data were analyzed statistically, using t-test. Results indicated that rbST treatment of Nili-Ravi buffalo bulls significantly (P<0.05) increased ejaculatory volume (8.8±0.2 vs 7.7±0.2 ml), sperm motility (72.6±0.4 vs 67.4±0.7%), mass activity (3.35±0.07 vs 2.52±0.08), sperm concentration (982.2±67.8 vs 731.9±50.5 million/ml), live sperm (80.1±0.3 vs 75.8±0.5%) and serum testosterone (4.02±0.21 vs 2.37±0.07 ng/ml) compared to control bulls. Among hematological variables, lymphocytes were increased (P<0.05), MCV, WBC count and neutrophils decreased (P<0.05), while Hb, RBC count and PCV remained unchanged. However, values of these variables were within normal ranges for bulls. Body weight of these bulls could not be recorded. In conclusion, treatment of Nili-Ravi buffalo bulls with rbST improved semen quality and increased serum testosterone without adverse effects on the general health of bulls.

Key words: Semen quality, Hematology, Serum testosterone, rbST, Nili-Ravi bulls

P-34 Granulosa Cell Tumour of Ovary in Buffalo-A Case Study

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The present study reports a rare clinical condition of sex-cord originated granulosa cell tumor in Nili Ravi buffalo. Approximately 7 year old female buffalo with the anamnesis of repeat breeding and infertility was brought to Lahore Meat Processing Complex, Shahpur Kanjaran slaughter house, Lahore. Ante-mortem examination through transrectal palpation revealed the rounded, large, fluffy, firm mass having multiple compartments with variable consistency in the right ovary while the left ovary and the rest of the reproductive tract were normal on the basis of size and shape. This condition was suspected to be a tumor or cyst. During necropsy, gross examination revealed that 400 g weighed right ovary (15x7x9 cm) had irregular lobulations and bluish-red tinge with many irregular, follicular-appearing cysts. After incision, the ovarian surface appeared to be red and had friable necrotic tissue with several blood-clot having vascular spaces. The adjacent outer cysts contained dark or red-brown serous fluid. The left ovary (1.2x0.2x0.4 cm) contained neither follicle nor corpus luteum. Histopathology of the tissue sections showed the presence of ample necrotized tissue. The cells were slightly elongated with prominent hyperchromatic nuclei but with variable size and shape. Multiple sections of the tissue had many cells undergoing mitosis. Cell cytoplasm varied from scant to abundant. Based on the histopathology and clinical examination, this condition was confirmed as granulosa cell tumour. The present study highlighted a rare clinical cause of anestrus and infertility in Nili Ravi buffalo.

Key words: Granulosa cell, Sex-cord stromal tumour, Nili-Ravi buffalo

P-35 Comparative Evaluation of Rose Bengal Plate Test and ELISA for the Diagnosis of Brucellosis in Cattle at Private Dairy Farms of Punjab

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Brucellosis is mainly an important disease of cattle which is characterized by abortion and retention of placenta while in male animals it can lead to orchitis and infection of accessory sex glands. It is of major economic importance in the developing countries that have not a national brucellosis eradication program. Keeping in view the importance of brucellosis and following an outbreak in the area. The present study was designed to find the seroprevalence and comparative evaluation of Rose Bengal Plate Test (RBPT) and indirect Enzyme Linked Immunosorbant Assay (ELISA) for the diagnosis of brucellosis in cattle. For this purpose 204 serum samples (male= 4; female= 200) were collected from different private farms having this problem in their animals. The RBPT test was performed by Bengal test kit (Synbiotics, USA) according to company's instruction while an indirect ELISA kit (Svanova, Sweden) was used to identify *Brucella* antibodies in the serum samples by following the manufacturer's instruction. The data on seroprevalence was analyzed statistically by Chi-square test using Statistical Package for Social Sciences (SPSS). Overall seroprevalence of brucellosis in cattle was 15.2% (p= 0.050). Antibodies detected by RBPT in male and female animals were 0% (n=0) and 2.5% (n=05), while antibodies detection by ELISA was 50% (n=02) and 14.5% (n=29) respectively (p= 0.000). Study showed higher seroprevalence in female animals detected by both RBPT and ELISA, whereas ELISA detected more number of carrier animals as compared to RBPT showing that ELISA is relatively more efficient and can be used for mass screening of *B. abortus* carrier animals. During the study necessary control measures were adopted because of the zoonotic importance of this problem.

Key words: RBPT, ELISA, Seroprevalence, Brucellosis, Cattle

P-36 A Case Report; Fetal Mummification Accompanied with Viable Kids at Full Term in Teddy x Beetal Doe

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Fetal mummification has been reported in several species, including the cattle, sheep, goat, horse, pig, dog and cat. Fetal mummification accompanied with viable kids at full term is not frequently happened even in caprine, ovine and swine. A three year doe was presented at Theriogenology Clinic Ravi Campus-UVAS. She had delivered two live kids (one male and other female) last night. Owner brought her because something was hanging from vulva and he was confusing it with retained fetal membrane/prolapse. The Doe was purchased from flock three months ago. She has had two previous successful parturitions. Feeding history and temperature (102 F⁰) of the doe was normal. On physical examination it revealed that hanging material is dried and dead fetus (Fig. 1 and 2) and some of fetal parts were present in the vagina. The differential diagnosis was made with abortion, stillbirth, maceration and hydropes condition. It is diagnosed as mummified fetus. Fetal mummification occurs when the fetus dies due to genetic defects, torsion or compression of the umbilical cord, placental defects or infections during the second or third trimester of gestation after the formation of the placenta and substantial ossification has occurred but is retained in utero due to a high blood level of progesterone.

Key words: Fetal Mummification, Viable kids, Teddy x Beetal, Doe

P-37 Pinhole Technique – A Novel Way to Castrate Male Animals

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Castration is one of the most frequently performed surgical procedures in veterinary practice. All techniques of castration in animals have several principles in common: adequate restraint, safety, good anaesthesia, clean surgery, control of haemorrhage, and adequate postoperative drainage. Traditionally the ruminants are castrated by closed (Bloodless) techniques that include use of emasculatome (Burdizzo), elastic banding, elastrator, EZE bander or calicrate bander. Occasionally

the open techniques like short scrotum method, Newberry knife procedure and unilateral orchiectomy (for unilateral disorders) are also practiced. Pinhole castration an alternative simple, cheap, quick, requiring no special instruments and field applicable technique has recently been described. In a laterally recumbent animal, after infiltrating local anesthesia, *in situ* spermatic cord ligation is achieved by restraining the cord laterally within the scrotal sac and passing suture through a hypodermic needle inserted caudal to cranial at the neck of the scrotum and adjacent the medial margin of the restrained spermatic cord. The needle is removed leaving the suture in place and the spermatic cord repositioned medially. Then the needle is reinserted through the original holes and the suture passed back through the needle, which is withdrawn. The suture ends are tied ligating the spermatic cord, leaving the knot subcutaneously. Instead of the hypodermic needle, traumatic suture needle of a larger size may be used for passing the silk suture. The technique has given encouraging results in male animals of several species including ruminants. Modes of evaluation, various benefits and short comings of the procedure when compared to the conventional techniques will be discussed during the presentation.

Key words: Castration, Pinhole, Spermatic cord

P-38 Interventions during and Post AI to Improve Conception Rate in Repeat Breeder Sahiwal Cows

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The objective of present study was to know the effect of gonadotropin releasing hormone (GnRH) and recombinant bovine somatotropin (bST) on pregnancy rates in repeat breeder cattle. It was hypothesized that administration of GnRH and bST at insemination would enhance conceptus development and improve the fertility in repeat breeder cows. All experimental cows were clinically healthy and had at least three unsuccessful services before entering the study. Selected repeat

breeder cows with lactation number 2-5, body condition score 3 (0-5), suckled by calves were utilized. Estrus detection was performed twice daily (7:00 am and 6:00 pm) by visual examination to observe behavioural signs during the experiment. At natural estrus, a total of 196 lactating Sahiwal cows with a history of repeat breeding were divided into Treatment (n = 105) and Control (n = 91) group. Treatment group cows were given a single intramuscular injection of GnRH (Dalmarelin i.e. Lecirelin, FATRO Pharmaceutical Veterinary Industry, Italy) at the dose rate of 100 µg just before artificial insemination (day 0). The animals in the treated group were also injected subcutaneously with 500 mg of bovine somatotropin (Boostin-250 manufactured by LG Life Science Ltd.) at the time of AI and again 14 days later. Control group received a placebo injection (2 ml Saline Solution) at AI and again 14 days later. Frozen-thawed semen from a single bull was used and all cows in estrus were inseminated by the same technician. To determine serum progesterone concentrations, blood was withdrawn from 20 cows in each group twice weekly from day 0 to 42. The serum progesterone concentrations were measured by ELISA. The data for conception rate was analysed by least square analysis of variance where as progesterone concentrations were compared by split plot design. All experimental cows were examined for pregnancy by palpation per rectum of the uterus and its contents for detection of amniotic vesicle at 40 day after artificial insemination, and pregnant cows were re-examined 3 week later at 60 ± 1 day after artificial insemination. Pregnancy diagnosis was performed by single technician. Conception rates were significantly higher (P < 0.05) in cows treated with GnRH and bST (43%) in comparison to control cows (27%) at 60 ± 1 day after AI. The progesterone concentrations differed non-significantly (P > 0.05) between pregnant cows of treated and untreated group. The findings of present study demonstrated that GnRH at the time of artificial insemination whereas bST at AI and 14 days later improved conception rates in repeat breeder cattle.

Key words: Repeat breeder cow; GnRH; Somatotropin; Pregnancy rates; Progesterone

P-39 Ultra-Rapid Freezing Protocol Improves the Post-Thaw Quality and In Vivo Fertility of Water Buffalo Bull Spermatozoa

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Cryopreservation affects the structures and functions of spermatozoa and the major damage occurs during the

freezing process. Optimum freezing protocol can reduce the vulnerability of spermatozoa to cryoinjuries. Therefore, the aim of the present study was to compare the effect of three freezing protocols on post-thaw progressive motility (computer assisted sperm analyzer; CASA), velocity distribution (CASA), kinematics (CASA), viability (fluorescent probe-propidium iodide), mitochondrial transmembrane potential (fluorescent probe-5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl carbocyanine iodide, JC-1), DNA (acridine orange test) and acrosome (wet-mount) integrity, and *in vivo* fertility (pregnancy rate) of water buffalo bull (*Bubalus bubalis*) spermatozoa. Semen was collected in artificial vagina (~ 42°C) from three adult Nili-Ravi buffalo bulls. Qualifying semen ejaculates from individual bulls were diluted (37°C, Tris-citric acid extender), cooled (37°C to 4°C in 2 hr), equilibrated (4°C for 4 hr) and packed (4°C) in 0.54 ml straws. Freezing of straws was carried out either by (i) Static vapour protocol; nonprogrammable (5 cm above liquid nitrogen for 10 min, ~-2°C to -70°C and plunging in liquid nitrogen), or (ii) Rapid vapour protocol; programmable (4°C to -15°C @ 3°C per min, -15°C to -80°C @ 10°C per min, holding at -80°C for 1 min and plunging in liquid nitrogen), or (iii) Ultra-rapid vapour protocol; programmable (holding at 4°C for 2 min, 4°C to -20°C @ 10°C, -20°C to -100°C @ 30°C per min, holding at -100°C for 1 min and plunging in liquid nitrogen). The whole experiment was repeated three times. Three hundred inseminations (100 per freezing-protocol and 50 per bull) were made in buffaloes (2nd to 3rd lactation) on natural heat under controlled field conditions. Two-way analysis of variance revealed that post-thaw progressive motility (%), 23.05±1.89 vs. 16.48±1.70), rapid velocity (%), 30.66±1.96 vs. 22.45±2.14), curvilinear velocity (µm/s), 109.26±2.79 vs. 96.10±4.43), DNA (%), 96.33±1.21 vs. 91.66±1.87) and acrosome (%), 64.11±1.22 vs. 58.88±1.30) integrity, and percentages of viable sperm with increased mitochondrial transmembrane potential (28.11±0.59 vs. 23.83±0.43) were higher (P<0.05) with programmable ultra-rapid freezing than the other two protocols. The data on fertility were compared by Chi-square statistics. The *in vivo* fertility rate of buffalo bull spermatozoa cryopreserved with programmable ultra-rapid protocol was higher (68.0 vs. 55.1%; P<0.05) compared to other methods. In conclusion, ultra-rapid freezing protocol improves the post-thaw quality and *in vivo* fertility of water buffalo bull spermatozoa.

Key words: Freezing rates, CASA, Viability, Fertility, Water buffalo sperm

P-40 Evaluation of Sperm Quality Parameters of Fresh and Frozen-Thawed Water Buffalo Semen in Relation to Field Fertility during Peak Breeding Season

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Standard sperm quality parameters viz., visual motility and morphology have shown different associations with field fertility. Therefore, the objective of the present study was to determine if specific *in vitro* tests for sperm and acrosomal membranes could be used in predicting *in vivo* fertility of fresh and frozen-thawed buffalo semen during peak breeding season. Semen was collected from five adult buffalo bulls with artificial vagina (~ 42°C) during the months of October and November. Freshly ejaculated semen from each bull was split for dilution either in PBS-0.1% BSA for analysis or Tris-citric acid extender for freezing. Aliquots diluted in Tris-citric acid extender were cryopreserved by following the standard protocol for buffalo spermatozoa. Fresh and frozen-thawed samples were analyzed for visual motility (%), morphology (%) structurally and biochemically intact plasmalemma (%), supra-vital hypoosmotic swelling test and live sperm with intact acrosome (%), dual staining-Trypan blue and Giemsa stain). At least 100 inseminations per bull were made by using frozen-thawed semen under optimum field conditions. *In vivo* fertility of individual bull was determined through conception rate (%). Pearson's correlations suggested that visual motility (%) and percentage of viable sperm with intact acrosome in fresh semen were significantly associated with field fertility ($r=0.71$, $P<0.01$ and $r=0.62$, $P<0.01$, respectively). In frozen-thawed semen, visual motility (%), supravital plasma membrane integrity (%) and percentage of sperm with intact acrosome were significantly correlated with field fertility ($r=0.77$, $P<0.01$; $r=0.85$, $P<0.01$ and $r=0.91$, $P<0.01$, respectively), whereas percentage of nonviable sperm with damaged acrosome and percentage of sperm tail abnormalities were negatively correlated with field fertility ($r= -0.87$, $P<0.01$ and $r= -0.55$, $P<0.05$, respectively). The prognostic value to predict the field fertility by the equation of visual motility, supravital plasma membrane integrity and sperm with intact acrosome in fresh and frozen-thawed buffalo semen was highest (R^2 adjusted=55.8%, $P=0.02$ and R^2 adjusted=82.6%, $P=0.000$, respectively). The present

study confirms a strong association between *in vitro* tests to evaluate sperm and acrosomal membranes with field fertility in water buffalo. Furthermore, a multiple combined analysis of these parameters could be the best strategy to obtain a better prognostic value of the field fertility of water buffalo semen during peak breeding season.

Key words: Buffalo Spermatozoa, Viability; Plasma membrane integrity, Acrosome integrity, *In vivo* fertility

P-41 Duck Egg Yolk Plasma in Extender Improves the Freezability of Buffalo (*Bubalus bubalis*) Bull Spermatozoa

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The present study was designed to compare the effect of different concentrations of duck egg yolk plasma (DEYP; 10, 15 and 20 %) with control (whole chicken egg yolk, WCEY 20 % and whole duck egg yolk, WDEY 20 %) added to Tris-citric acid (TCA) based extender on visual motility (VMOT %), acrosome integrity (NAR %), supra-vital plasma membrane integrity (SV-PMI %), CASA (Computer assisted sperm analyzer) motility parameters (progressive, PROG %; rapid, RAP %; medium, MED %; slow, SLOW %) and kinematics (average path velocity, VAP $\mu\text{m}/\text{sec}$; straight line velocity, VSL $\mu\text{m}/\text{sec}$; curvilinear velocity, VCL $\mu\text{m}/\text{sec}$; amplitude of lateral head displacement, ALH μm ; beat cross frequency, BCF Hz) of frozen-thawed buffalo bull spermatozoa at time 1 (5 min incubation at 37°C) and 2 (45 min incubation at 37°C). For extraction of plasma, WDEY was diluted with 0.17 M NaCl solution (1:1) and stirred for up to 1 h at 4°C. After that the mixture was ultra-centrifuged (10,000 g for 45 min) twice at 4°C. The final supernatant obtained was termed as DEYP with an acceptable purity. Semen ejaculates from three buffalo bulls were collected at weekly intervals with artificial vagina (42°C) for a period of 3 weeks (3 replicates). Cryopreservation was carried out as per standard protocol for buffalo spermatozoa. Analysis of variance revealed that at time 1, sperm VMOT was significantly higher in extender containing 15 % DEYP than the controls (58.33 \pm 2.79 vs. 43.33 \pm 2.44). At time 2, sperm VMOT was significantly higher in extenders containing 15 and 20 % DEYP compared with the controls (33.75 \pm 5.91 vs. 17.08 \pm 3.65). Acrosome integrity and SV-PMI of buffalo

spermatozoa were significantly higher at time 1 and 2 in extender containing 15% DEYP than WCEY 20 % (control) (time 1 NAR, 62.67 ± 2.17 vs. 45.00 ± 2.86 ; time 2 NAR, 43.50 ± 3.36 vs. 26.83 ± 4.91 ; time 1 SV-PMI, 52.50 ± 1.34 vs. 38.50 ± 3.18 ; time 2 SV-PMI, 33.67 ± 2.85 vs. 15.00 ± 1.48). At time 1, mean percentages of PROG sperm was significantly higher in extenders containing 15 and 20 % DEYP than the controls (11.41 ± 2.04 vs. 3.95 ± 0.63). At time 2, sperm PROG motility was significantly higher in extenders containing 15 % DEYP compared with the controls (4.50 ± 1.83 vs. 0.67 ± 0.37). Mean percentages of RAP sperm were significantly higher at time 1 in extender containing 15% DEYP than controls (25.17 ± 4.34 vs. 7.29 ± 1.19). At time 2, mean percentages of RAP sperm were significantly higher in extenders containing 15 and 20 % DEYP than controls (6.73 ± 1.89 vs. 1.08 ± 0.56). At post thaw time 1 and 2, VAP and VCL of buffalo sperm were significantly higher in extender containing DEYP as compared with controls (time 1 VAP, 64.76 ± 2.73 vs. 50.71 ± 1.86 ; time 2 VAP, 49.07 ± 3.16 vs. 35.70 ± 3.02 ; time 1 VCL, 114.36 ± 6.36 vs. 84.54 ± 4.22 ; time 2 VCL, 87.03 ± 4.15 vs. 63.53 ± 7.41). Beat cross frequency (Hz) of buffalo sperm at both time 1 and 2 was significantly higher in extender containing DEYP as compared with control (WCEY 20 %) (time1, 28.33 ± 0.94 vs. 25.18 ± 1.54 ; time 2, 33.46 ± 1.14 vs. 22.38 ± 2.55). In conclusion, WCEY or WDEY can be replaced with 15 % DEYP in TCA based extender for cryopreservation of buffalo bull spermatozoa.

Key words: Acrosome integrity, Egg yolk Plasma, Bull spermatozoa.

P-42 Effect of Various Concentrations of Catalase in Extender on Cryopreservation of Buffalo Bull Spermatozoa

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Catalase enzyme is usually distributed in mammalian seminal plasma, where it decomposes hydrogen peroxide into water and oxygen and enhances sperm survivability. The objectives of the present study were to determine the effect of catalase added at different concentrations (0, 100, 200 or 300 IU/ml) in Tris citric acid (TCA) based extender on visual motility, progressive motility (computer assisted sperm analyzer-CASA), motion characteristics (CASA), supra-vital plasma membrane integrity (supra-vital hypo-osmotic swelling test), acrosome (wet-mount) and DNA integrity (acridine orange assay) of buffalo bull spermatozoa at post dilution (PD) and post thawing (PT) stages of cryopreservation.

Semen was collected from four adult Nili-Ravi buffalo bulls with artificial vagina (42°C). Qualified semen samples from each bull were subdivided into four aliquots for dilution with TCA extender containing 0.0, 100, 200 or 300 IU/ml catalase. Activity of catalase was 12660 units/mg of dry weight. The experiment was repeated three times and data were analyzed by using ANOVA (general linear model). At PT, visual motility, CASA progressive motility, average path velocity and straight line velocity were significantly higher ($P < 0.05$) for catalase fortified treatment groups (61.25 ± 2.62 %, 21.05 ± 2.23 %, 75.48 ± 2.53 $\mu\text{m/s}$ and 62.01 ± 2.55 $\mu\text{m/s}$, respectively) as compared with control (46.25 ± 0.72 %, 13.15 ± 1.78 %, 62.72 ± 4.80 $\mu\text{m/s}$ and 51.57 ± 3.77 $\mu\text{m/s}$, respectively). Curvilinear velocity was significantly higher ($P < 0.05$) at PT with extender containing 300 IU/ml catalase as compared with control (112.44 ± 2.07 vs. 93.75 ± 6.67 $\mu\text{m/s}$). At PT stage of cryopreservation, mean supra-vital plasma membrane and acrosome integrity were significantly higher ($P < 0.05$) with extender containing 300 IU/ml catalase as compared with control (34.25 ± 6.55 vs. 20.59 ± 5.54 % and 68.84 ± 3.50 vs. 49.96 ± 2.56 %, respectively). Mean DNA integrity at PT was significantly higher for catalase fortified treatment groups (97.18 ± 0.11 %) as compared with control (93.78 ± 0.77 %). Therefore, it can be concluded that addition of catalase particularly at a concentration of 300 IU/ml in TCA extender improved the post-thaw motilities, motion characteristics, supra-vital plasmalemma, acrosome and DNA integrity of buffalo spermatozoa.

Key words: Buffalo sperm, Catalase, Cryopreservation, CASA, DNA integrity

P-43 Effects of Asphaltum on Libido, Serum Testosterone, Hematology and Biochemical Metabolites in Lohi Ram

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Asphaltum, also known as Salajit, is a pale-brown to blackish-brown exudation of variable consistency originated from layers of mountains rocks of Himalayan ranges. It is composed of 220 mineral and metal substances used in traditional Indian medical systems. This study was designed to investigate the effects of asphaltum on libido, serum testosterone, hematology and some biochemical metabolites in Lohi rams. For this purpose, 12 adult clinically normal and healthy Lohi Rams were selected and kept for a period of 9 weeks under same managemental conditions. These rams were divided randomly into three groups, A, B and C with four

rams in each group. Asphaltum was given direct orally in capsule to the rams of the groups A, B and C @ 0, 800 and 1600 mg, respectively, daily for a period of seven weeks. Blood was collected (once in a week) at the beginning of the experiment and from the 5th week of administration of asphaltum till the completion of the trial and was analyzed for hematology, serum testosterone and biochemical metabolites. After seven weeks of the treatment, the mean libido score, serum testosterone level, white blood cells, mean corpuscular volume, mean platelets volume, hematocrit, lymphocytes, granulocytes, minimum inhibitory dilution, albumin, globulin, total proteins and total cholesterol level were increased significantly ($P < 0.001$). Conversely, the mean values of red blood cells, red blood distribution width, platelets, hemoglobin, mean corpuscular hemoglobin concentration, lymphocytes percentage, granulocytes percentage and minimum inhibitory dilution percentage were non significant. However, the mean value of mean corpuscular hemoglobin decreased significantly. Conclusion: Asphaltum is an effective and potent libido and serum testosterone enhancer medicine. It also altered hematological parameter and serum biochemical metabolites in rams.

Key words: Asphaltum, Salajit, Lohi

P-44 Juvenile In-Vitro Embryo Transfer from 4- To 8-Week-Old Kundi Buffalo Calves

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The development of in vitro maturation and fertilization procedures, together with the ability to harvest relatively large numbers of oocytes, has made it possible to produce transferable grade embryos from young animals. The objectives of the present studies were to develop a robust and reliable protocol to improve the production of transferable grade embryos from 4- to 8-week-old Kundi buffalo calves. This study was conducted on the rationale that gonadotropin treatment protocols used in the past failed to maximise oocyte quality. Consequently, three experiments were conducted in Kundi Buffalo calves (4–8 week old) to examine: (1) the method of FSH administration (single versus multiple treatments), (2) the time of oocyte collection (48, 60 or 72 h after the first FSH treatment), (3) progesterone treatment and (4) eCG treatment and its timing on oocyte yield, cleavage rate and blastocyst formation rate. Ovaries were exposed by mid-ventral laparotomy conducted under general

anesthesia and all the oocytes were collected in a single session from 12 female calves. No factor significantly influenced oocyte yield, but all significantly influenced the rates of cleavage and/or blastocyst formation in one or more experiments. The preferred protocol consisted of the administration of 4 x 40 mg of FSH, no progesterone treatment and with 500 IU of eCG given at the time of the last FSH treatment. The optimal time of oocyte collection was 48 h after the first FSH treatment. A laboratory evaluation, involving calves, resulted in the production of 39.0–89 transferable embryos per donor calf. These JIVET preliminary results in Kundi buffalo calves are encouraging for improvement of its reproduction and production in Pakistan. At current levels of performance, the JIVET would be an attractive means of rapidly multiplying elite animals, although large-scale application is likely to depend on the identification of suitable gene markers and their use in genetic selection programs.

Key words: JIVET, Buffalo, Embryo, Oocyte, In vitro maturation, In vitro fertilization

P-45 Replacement for Commercial Dairy Farm: Intensive Breeding Program for Pre-pubertal Buffalo Heifers

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Efficient reproductive management is the key for profitable dairy enterprise. Replacement heifers are the back bone of the any commercial dairying. The objective of the study was to evaluate the effect of exogenous progesterone (P4) exposure on induction of cyclicity and pregnancy rate in pre-pubertal buffalo heifers. This study was conducted at commercial dairy farm in District Kasur-Pakistan during the breeding season (September to November). Buffalo heifers ($n=29$; 32 ± 1.2 mo; 303 ± 8.3 kg; mean \pm SEM) without luteal tissues on ovaries were considered pre-pubertal. These were divided randomly in two groups; control and treated. In control group (without P4 exposure, $n=13$); no treatment was given rather bull exposure. In treatment group (with P4 exposure, $n=16$); heifers were treated with CIDR (1.38 g progesterone Pfizer Co, USA) synchronization protocol and timed insemination 48 and 60 h after device removal. Bull was introduced from 15 to 30 days post insemination. 1st pregnancy test was performed at 40 days post AI with ultrasound. The heifers found pregnant at scan were separated. Heifers without CL were resynchronized with CIDR protocol as before and pregnancy determined at 40 days post AI. Pregnancy in control group was determined on 40 and 80 days similar

to treated heifers. The pregnancy rate (total number of pregnant heifers/treated) was compared through Chi-square in SPSS software. The overall pregnant heifers at 80 days were 63% (10/16) in treated and 16% (2/13) in control groups. It is concluded that the progesterone exposure can effectively induce the cyclicity in pre-pubertal buffalo heifers and provide the more replacement for dairy herds.

Key words: Progesterone, Breeding, Pre-pubertal, Buffalo heifer

P-46 Effect of Clomiphene Citrate and Human Chorionic Gonadotropin (hCG) on Ovulation Induction in Pre-Pubertal Sahiwal Heifers

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The aim of this pilot study was to evaluate the ovulation inducing effect of clomiphene citrate along with hCG in prepubertal Sahiwal heifers. Clomiphene citrate (an anti-estrogen) causes gonadotropin secretion from pituitary gland by removing the negative feedback effect of estrogen. This causes more FSH secretion which ultimately stimulates follicular growth and upon hCG administration (luteinizing hormone activity) ovulation occurs. Twelve prepubertal Sahiwal heifers were divided into experimental and control groups, with six animals in each group. The grouping of animals was made on the basis of plane ovary. The status of ovarian puberty was checked through rectal palpation and history of previous estrus signs. Experimental heifers were given clomiphene citrate at 300mg/heifer for 9 days orally, while heifers in control group were given placebo tablets. The dose of clomiphene citrate was selected on the basis of previous literature. On day 10, hCG (IVF-C 5000 iu (hCG) LG Life Sciences, Korea) was given to all heifers of both groups at 2500IU/heifer through intravenous route. Two base line blood samples were collected before start of the treatment at each rectal palpation examination for selection of animals. Subsequently, during and after treatment three blood samples per week (on alternate days) for 4 weeks were collected for measuring the progesterone (P4) in serum. The concentration of progesterone was measured through radioimmunity assay. For the comparison of variables, student t-test was applied. The P4 concentrations in five heifers (83.3%) in the experimental group with (P4 > 1 ng/ml) and one heifer (16.7%) in the control group along with corpus luteum on ovary showed ovulation. One heifer in control group ovulated that might be due to hCG. Mean progesterone concentration was significantly higher (P

<0.05) in treated heifers than control heifers. In conclusion, administration of clomiphene citrate and hCG can induce ovulation induction in pre-pubertal Sahiwal heifers.

Key words: Clomiphene citrate, Ovulation induction, Sahiwal heifers

P-47 Duration of CIDR Placement's Effect on Estrus Induction in Dairy Holstein Cows

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The aim of this study was to evaluate the effect of duration of CIDR device placement in vagina on estrus induction, corpus luteum status, plasma progesterone levels, follicle diameter, and pregnancy rate (PR) in Holstein dairy cows. Cows (n = 66) were randomly assigned by age, calving date and body condition score. In Group 1, CIDR was inserted on day 0 and cows were assigned to CIDR (n = 20) protocol starting with the injection of GnRH (10 µg buserelin, i.m.; Oviren). On day 7, CIDR was removed and PGF2 (10 mg, i.m.; Dinolytic) was applied (CIDR7). In Group 2, the same protocol was used except for CIDR (n = 23) removal on day 9 (CIDR9). In Group 3, cows were assigned to CIDR (n = 23) from day 0 to 14, and PGF2 (10 mg) was applied on day 30 (CIDR14). Artificial insemination was performed at predetermined fixed times (56 h, CIDR7 and CIDR9; 72 h, CIDR 14) and all cows were administered GnRH at FTAI. Ultrasonography (ibex pro, 7.5 MHz linear probe) was performed for follicular diameter during FTAI. CL status was also evaluated by ultrasonography on day 0 and 7 (CIDR7), day 0 and 9 (CIDR9), and day 0 and 14 (CIDR14), respectively. Blood samples were collected via jugular vein on the day of CIDR removal. P4 analysis was performed via Electrochemiluminescence immunoassay (ECLIA) method and ROCHE E170 device. Pregnancy rate was determined twice via transrectal ultrasonography between 40 and 60 days after FTAI. Follicle diameter was larger in CIDR7 and CIDR14 compared to CIDR9 (P<0.05) (ANOVA, SPSS 21). There was no effect of treatments on the diameter of corpus luteum and progesterone concentration among groups (P>0.05) (ANOVA, SPSS 21). Pregnancy rates (70%, 52% and 39%) were positively correlated with plasma progesterone levels (4.10 ng/ml, 4.83 ng/ml and 2.74 ng/ml) in CIDR7, CIDR9, CIDR14 respectively (SPSS 21). In this study, results suggest that CIDR7 (PR 70%, P4 4.10

ng/ml) would be a more effective protocol for fertility of Holstein dairy cows.

Key words: CIDR based, Estrus induction, Fixed-time artificial insemination, Pregnancy rate

P-48 Impact of Intensity of Biostimulation on Oestrus Incidence, Conception Rate in Postpartum Nili-Ravi Buffaloes during Peak Breeding Season

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Biostimulation (male effect) plays an important role in improving reproductive efficiency in cattle. There is some evidence to indicate that duration of daily pheromonal stimuli influences the time required for cows to respond to the biostimulatory effect of bulls. The study was planned to assess the impact of intensity of biostimulation on resumption of ovarian cyclicity and fertility in postpartum suckled buffalo kept under standard farm condition. The buffaloes given either 24 h physical exposure to mature fertile bull (BE), or partial, fence line exposure (BPFE) and no bull exposure (BN) for 60 days (n = 8 per group). Animals had calved (38.23±5.38d) before the experiment. Over 60 d study period, blood samples were collected once a week from 5 animals per group for Progesterone (P₄). Changes in plasma P₄ concentrations were used as criterion for resumption of ovarian activity. An increase in P₄ levels >1ng/ml in two consecutive samples was indication of ovarian activity. Oestrus detection was done twice daily in BE and BPFE by bull and manually in BN. *Animals showing one or more behavioural oestrus signs were considered in heat.* The results showed that a higher number of animals of BE showed one or more behavioral oestrus signs than BN group (100% vs. 62.5%; p<0.05, respectively). Number of buffalo showed standing heat in BE and BN were found as 100.0 and 50.0%, respectively. However BPFE (75.0%) group was not different (p>0.05) from BE or BN groups. Interval from the start of the treatment to resumption of ovarian activity was shorter (p<0.05) for BE (11±2.9d) and BPFE (20±2.6d) than for BN group (39±3.8d). Conception rate was higher (p<0.05) in BE (87.5%) than BN group (25 %) but not difference (p>0.05) with BPFE (50%). It was concluded that resumption of ovarian activity and conception rate in response to biostimulatory effect of bull depended upon exposure intensity in buffalo to a bull as these parameters were improved in animals having 24 h physical exposure for 60 days experiment period.

Key words: Buffalo, Biostimulation, Progesterone, Fertility rate

P-49 Estrus Synchronization does not Improve Fertility in Chronically Repeat Breeding Sahiwal Cows

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Repeat breeding can be caused by a number of factors, including sub fertile bulls, endocrine problems, malnutrition, sub-clinical infection of reproductive tract and poor management. The objective of the present study was to determine efficacy of three major estrus synchronization protocols (CIDR, Ovsynch and Single PG) as reproductive management tool in terms of estrus response, timing of ovulation, ovulation rate and pregnancy rate in chronically repeat breeding Sahiwal cows. Seventeen adult Sahiwal cows having 350-400 Kg body weight with BCS (3.5±0.0), parity ranges from 4 to 6 and had earlier received on an average eight inseminations but did not conceive, before the start of synchronization were selected. Cows were randomly divided into one of the following treatment groups: 1) CIDR (n=6), cows were inserted with an intra-vaginal device containing 1.38 g progesterone for seven days and administered PGF₂ on day 6 and fixed time insemination was performed at 48 and 72 h of CIDR removal. 2) Ovsynch (n=7), cows received an injection of GnRH on day 0, PGF₂ on day 7, second injection of GnRH on day 9 and timed AI at 12 and 24 h after last injection and 3) PG (n=4), cows received a single treatment of PGF₂ on random stage of estrous cycle and inseminated at fixed time (72 and 84 h after PGF₂ administration) with frozen thawed semen from bulls of known fertility. Estrus detection was performed by visual observation and teasing was performed twice daily for at least 30 min. Size of the largest follicle was measured from PG injection to ovulation by repeated ultrasonography after 12 hourly intervals. Pregnancy diagnosis was conducted using ultrasonography around 35 days after insemination. Data for timing of ovulation and size of the ovulatory follicle was analyzed by using One way ANOVA and for estrus response ovulation and pregnancy rate was analyzed by using Z-test for population proportion. Results revealed that estrus response was significantly higher (P=0.046) (100%; 6/6) in CIDR group as compared to PG (50%; 2/4), but not in Ovsynch (85%; 6/7). Size of ovulatory follicle before ovulation was significantly (P < 0.05) different 13.2±1.97, 13.6±0.17 and 9.4±0.00 mm among all groups

ie., CIDR, Ovsynch and PG, respectively. Mean ovulation time with reference to prostaglandin injection was significantly higher ($P=0.032$) among CIDR (87 ± 11.49 h) and Ovsynch (66 ± 12.00 h) than PG group (90 ± 0.00 h). Ovulation rate did not differ and was 66 (4/6), 71 (5/7), 25% (1/4) in CIDR, Ovsynch and PG groups respectively. Pregnancy rate was 0 (0/6), 20 (1/7) and 0% (0/4) in three groups. It is concluded that estrus synchronization does not enhance pregnancy rate of chronically repeat breeding Sahiwal cows.

Key words: Repeat breeding, Synchronization, Sahiwal cows, CIDR, Ovsynch, Prostaglandin

P-50 Predominance of Bovine Trichomoniasis in Multan District, Adjacent Areas, Pakistan

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Economic impact of Trichomoniasis is severe as calf crop can be abridged up to 40-50% in bovines' infection. It is the most common venereal disease which causes early embryonic loss, abortion, pyometra and overall reduces reproductive efficiency. For this purpose, a study was designed to rule out the predominance of Trichomoniasis in cattle and buffaloes in Multan District and adjacent areas. Initially, epidemiological Survey was made and samples (preputial flush $n=50$ and vaginal secretions $n=50$) were collected. Due to limited number of Inpouch™ TF kits (Bio Med USA), finally a total of eighty samples (preputial flush $n=40$ and vaginal secretions $n=40$) were inoculated into Inpouch™ TF (TF=Trichomonas fetus) media vertically in wide mouth jar and incubated at 37°C for six days. Out of eighty cultured samples, only four showed turbidity and observed under microscope at $\times 400$ first and then at $\times 1000$ for the presence or absence of flagellated protozoan. Results revealed that among 80 cultures, four become turbid and confirmed positive under microscopic examination. Out of eighty (cattle $n=69$ & buffaloes $n=11$), the entire four positive cultures were of cattle. Based upon these results, it can be speculated that 5% populations of animals in the study area were positive. Alternatively, it has need of further broad scale investigation through culture as well as more advance diagnostic techniques like Elisa and PCR.

Key words: Predominance, Bovines, Trichomoniasis, Multan, Pakistan

P-51 Effects of Ebselen on Ram Sperm Parameters during Liquid Storage

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The aim of this study was to investigate the effects three levels of ebselen, on ram sperm parameters during liquid storage up to 72 h at 5 °C. Five Merino rams (2-3 years of age) were housed at Selcuk University Faculty of Veterinary Medicine Research and Application Farm, Konya, and maintained under uniform feeding and housing conditions. A total number of 30 ejaculates (10 ejaculates for each ram) were collected from the rams with the aid of an artificial vagina twice a week, according to AI standard procedures. The ejaculates containing spermatozoa with >80% forward progressive motility and concentrations higher than 2×10^9 spermatozoa/ml were used in the study. Eight ejaculates for each ram were included in the study. Ejaculates were pooled at 37°C and pooled ejaculate was divided into four equal aliquots and diluted (37°C) with the Tris base extender, containing ebselen at three doses (10, 20 and 40 μM) and no additives (control), at a final concentration of approximately 2×10^8 sperm. Subjective sperm motility was determined at phase-contrast microscope, viability (SYBR-14/PI), and mitochondrial activity (JC-1/PI) were determined at fluorescent microscopy and DNA integrity was evaluated by COMET test after 0, 24, 48 and 72 h of storage at 5°C. The study was replicated eight times. Results were expressed as the mean \pm SEM. Data for sperm motility, viability, mitochondrial activity and DNA integrity were analyzed by analysis of variance, followed by Tukey's post-hoc test to determine significant differences between groups. Differences with values of $P < 0.05$ were considered to be statistically significant. Statistical analyses were performed by using SPSS 11.5 package program. The extender supplemented with 10 μM ebselen (93.39 ± 1.80) resulted in higher viability, in comparison to the control group (85.57 ± 1.27) for 0 h of storage ($P < 0.05$). Sperm motility was better in 40 μM ebselen (82.66 ± 1.20) than control (78.33 ± 1.05) and also 10 μM ebselen group (65.33 ± 1.14) higher DNA integrity than control (58.50 ± 3.10) group for 24 h of storage

($P < 0.05$). Ebselen 10 μM group motility (73.33 ± 1.05 ; 69.16 ± 1.05) and DNA integrity (58.00 ± 0.44 ; 54.33 ± 0.88) was better than control group respectively at 48 and 72 hour of storage ($P < 0.05$). This study highlights the protective effect of ebselen on sperm motility, viability,

and DNA integrity during the liquid storage of ram semen.

Key words: Ram sperm, Ebselen, Liquid storage, Fluorescent staining, DNA integrity

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