RESEARCH ARTICLE

Comparative Study of Placental Cotyledons of Gir Cows and Jaffrabadi Buffaloes

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Abstract

The present study was conducted to compare placental cotyledons' structural and endocrine function between Gir cows and Jaffrabadi buffalo cows. Placentas were collected from 10 Gir cattle(*Bos indicus*) and 10 Jaffrabadi buffaloes (*Bubalus bubalis*) at the time of parturition to study gross morphology of the placenta, histology of cotyledons, and *in-vitro* culture of cotyledon cells to estimate endocrine function. Observations revealed that in comparison to gir cows, the Jaffrabadi buffalo have a heavier placenta with more cotyledons and trophoblast cell density. The cotyledon cells of buffaloes secrete higher levels of progesterone and estradiol-17β as compared to cattle. The age of the animal was found negatively correlated with placental weight and total cotyledons in both species.

Keywords: Buffalo, Cattle, Cotyledon, Gir cow Gross morphology, Trophoblast.

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Introduction

Water buffalo (*Bubalus bubalis*) and zebu cattle (*Bos indicus*) represent different genera within the large family of Bovidae. Both have similarities in appearance but differences in their reproductive physiology. Cattle and buffalo both share a common type of placenta, namely the cotyledonary placenta, characterized by the presence of numerous smaller placentae. A placentome is consist of an oval or round-shaped cotyledon from the fetal side and a caruncle from the maternal side of the placenta. Despite the morphological similarities in the reproductive organs of both species, there are many physiological differences. Earlier, Schmidt (2005) attempted to determine whether differences in placentation could be responsible for abortions and other placenta-related pathologies. Both animals' productive and reproductive performance is negatively influenced by calving-related reproductive disorders, especially the retention of fetal membranes (El-Wishy, 2007). However, the retention of fetal membranes and genital prolapse is higher in the buffalo than cattle (Rabbani *et al*., 2010). Abd-Elnaeim *et al*. (2003) reported that the additional month of gestation in *Bubalus sp.* compared to *Bos sp.* results in the development of feto-maternal interdigitation. The consequence of this morphological difference results in a higher incidence of retention of placenta in *Bubalus sp.* (4.55%) compared to *Bos sp.* (1.25%) (Navarro, 2002). Differences in peripartum reproductive physiology and pathology might be due to differences in placental pattern and function. Hence, the objective of the present study was to study the comparative interrelationship of cotyledonary features and secretions between Gir cows and the Jaffrabadi buffaloes placentae.

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Materials and Methods

The full-term placenta was collected immediately after the parturition from twenty healthy gir cows and Jaffrabadi buffaloes (10 each) reared at Cattle Breeding Farm, Junagadh, under standard nutritional and managemental practice.

Gross Morphological Study

Weight of newborn calf, the weight of the full term expelled placenta, number of fetal cotyledons, and size of cotyledons were recorded in gir cows and Jaffrabadi buffalo. The

dimensions of the cotyledons were measured using a digital Vernier caliper (Padodara *et al.,* 2020[b]).

Cyto-Morphological Study

The placental cotyledons were collected in 10% neutral buffered formalin and kept for at least 24-48 hours for fixation. Further, these tissues were processed by the standard method of dehydration in graded alcohol, clearing in xylene, and embedding in paraffin. Tissue sections of 5-6 µm thicknesses were prepared and stained with Hematoxylin and Eosin (Luna, 1968; Padodara *et al.,* 2020[a]). The sections were observed under a light microscope, and the histological observations in the tissue were recorded. Micrometry was done by Carl Zeiss Zen 2 (blue edition) microscopic image analysis software and ImageJ software (Schneider *et al*., 2012).

Hormone Secretion from Cotyledonary Tissues

Placental cells were isolated from the cotyledons and *in-vitro* cultured in M-199 media. Briefly, fetal cotyledons were collected immediately in the phosphate buffer saline (PBS, supplemented with 100 IU/mL Penicillin G, 100 µg/ml Streptomycin and space 100 ug/mL Gentamicin) and transferred to the laboratory. Fetal cotyledon was cut and minced with scissors, and placental cells were dissociated with 0.125% trypsin in phosphate buffer saline (PBS) for 30 min at 37°C. The minced tissues and cells were centrifuged (450 g for 10 min) and resuspended into serum-free M-199 media (supplemented with Hank's salts and L-glutamine and antibiotics 1% penicillin/streptomycin 10,000 IU/ml). The tissue and cells were washed three times with serum-free M-199 medium and filtered with four layers of cheesecloth. The trophoblast cells were collected following centrifugation (450 g for 10 min) from the filtrate. The cells were seeded at a final density of 1 x 10^6 cells/ml in M-199 medium with 2% fetal bovine serum (FBS) in 6 well plates and incubated at 37°C for 24 h with 5% CO_2 and optimum relative humidity in a CO_2 incubator (modified method of Myers and Reimers (1988)). The supernatant culture medium was collected following incubation of 24 h and stored to estimate progesterone, estradiol-17β, and testosterone using standard ELISA kits (MyBioSource, Inc., USA).

The statistical evaluation of data was performed by assessment of the mean values and standard error of the mean (SEm) in each monitored placenta of cattle and buffalo. The significance (p) of differences in the mean values between the species was calculated by paired "t" test (Snedecor and Cochran, 1994). The significance of the correlation coefficient evaluated the relationship between the monitored indicators.

Results and Discussion

The gross morphology, cytomorphology, and cotyledonary secretions observed of fetal cotyledons of Gir cows and Jaffrabadi buffaloes are shown in Table 1. The interrelationships among these attributes and cotyledonary features are presented in Table 2.

The gestation period, the weight of the expelled placenta, weight of newborn calf, and gross morphological observations, namely total number of cotyledons and diameter of cotyledon revealed a significant difference ($p < 0.05$) between Gir cow and Jaffarabadi buffalo. The difference in diameter and number of cotyledons between the species might be due to the difference in gestational length and physiological need. Buffaloes have a longer gestation period and heavier newborn calf than cattle; this might be the reason for larger and numerous cotyledons in Jaffrabadi buffalo. A fetus is largest in the last phase of gestation and requires more exchange of nutrients, gases, and waste through the placenta. The increased number and diameter of cotyledons might facilitate the extra exchange across the placenta in buffalo compared to cattle. A positive correlation between placental weight and calf birth weight was observed in both species, and this supports our hypothesis. Similar findings were observed in triple cross cattle (Padodara and Arya, 2014) and swamp buffaloes of Assam (Bhuyan *et al*., 2016).

The cytomorphological study did not show any significant difference in the length of tertiary villi, cellular and nuclear diameters of trophoblast epithelial cells (TEC), and trophoblast giant cells (TGC) between cattle and buffalo.

Table 1: Mean (± SEm) values of cotyledonary parameters of Gir cows and Jaffrabadi buffaloes (n = 10 each)

Sr. No.	*Parameters*	*Gir cow*	*Jaffrabadi buffalo*
1	Age (years)	6.35 ± 0.78	8.40 ± 0.48*
2	Gestation period (days)	286.40 ± 1.03	317.00 ± 2.48*
3	Calf weight (kgs)	22.30 ± 0.74	34.80 ± 0.79*
4	Weight of placenta (kgs)	2.85 ± 0.06	3.37 ± 0.10*
5	Total cotyledon (Numbers)	84.10 ± 2.99	102.45 ± 4.00*
6	Cotyledon diameter (cm)	3.12 ± 0.72	3.14 ± 0.72
7	Tertiary villi length (µm)	83.74 ± 3.59	85.36 ± 2.85
8	Diameter of TEC (µm)	7.23 ± 0.32	7.39 ± 0.20
9	Diameter of TGC (µm)	14.45 ± 0.38	15.23 ± 0.31
10	Nucleus diameter of TEC (µm)	3.74 ± 0.07	3.94 ± 0.11
11	Nucleus diameter of TGC (µm)	13.09 ± 0.12	13.28 ± 0.13
12	TEC (Numbers/mm^2)	262.50 ± 12.32	279.15 ± 8.41*
13	TGC (Numbers/mm^2)	87.35 ± 3.39	97.10 ± 2.93*
14	Cotyledonary P_4 (ng/mL)	1.75 ± 0.07	2.06 ± 0.06*
15	Cotyledonary E_2 (ng/mL)	16.19 ± 0.52	19.12 ± 0.44*
16	Cotyledonary Testosterone (ng/mL)	0.52 ± 0.03	0.56 ± 0.04

*Significant at $p < 0.05$ between the group, TEC – trophoblast epithelial cells, TGC – trophoblast giant cells, P_4 – progesterone, E_2 – estradiol-17β.

Table 2: Interrelationships between cotyledonary morphology, cytomorphology, and hormonal secretions of Gir cows and Jaffrabadi buffaloes

	Age	*GP*	*Calf wt*	*Wt placenta*	*Total Coty.*	*Coty. Length*	*Villi length*	*TEC*	*TGC*	*TEC-nu*	*TGC-nu*	*TEC-no*	*TGC-no*	*P4*	*E2*	*T*
Correlations: Gir cows (n = 10)																
Age	1	-0.114	-0.186	-0.561*	-0.564*	0.005	0.100	0.135	-0.046	-0.593	0.145	-0.033	0.077	-0.220	0.158	-0.254
GP	0.464	1	0.264	0.167	-0.069	0.274	-0.083	-0.273	0.183	0.115	0.389	0.037	-0.351	0.008	-0.144	0.259
Calf wt	-0.148	-0.044	1	-0.009	0.070	0.084	0.074	-0.129	0.073	0.316	0.245	-0.682*	-0.380	-0.213	0.390	0.669*
Wt placenta	-0.783*	-0.442	0.172	1	0.071	-0.306	-0.213	-0.283	0.094	0.518*	0.074	0.089	0.056	0.206	-0.477	0.261
Total coty.	-0.572*	-0.325	0.036	0.692	1	-0.117	-0.053	-0.099	-0.059	0.137	-0.334	0.060	-0.218	-0.486	0.075	-0.447
Coty length	0.050	-0.005	-0.031	0.266	0.292	1	0.305	0.316	-0.169	-0.134	0.017	-0.332	-0.019	0.232	0.320	0.355
Villi length	-0.245	-0.143	-0.122	0.183	0.021	0.017	1	0.551*	0.086	-0.340	-0.237	0.277	0.030	0.118	0.350	0.491
TEC	0.188	0.300	0.077	-0.150	-0.501*	-0.176	-0.015	1	-0.368	-0.476*	-0.564*	-0.135	0.320	0.198	0.569	-0.054
TGC	0.051	0.139	0.323	-0.059	0.140	-0.150	0.235	-0.154	1	0.318	0.133	0.349	-0.654*	-0.161	-0.240	0.496
TEC - nu	-0.086	-0.250	0.150	0.188	0.340	-0.233	-0.159	-0.216	0.313	1	0.464	-0.142	-0.284	0.068	-0.452	0.607
TGC-nu	0.042	-0.243	0.162	-0.072	-0.089	-0.163	-0.399	-0.306	0.042	0.468*	1	0.072	0.074	-0.065	-0.138	0.288
TEC-no	-0.346	-0.213	0.740*	0.346	0.149	-0.234	-0.234	-0.066	0.291	0.433	0.362	1	0.223	-0.129	-0.361	-0.385
TGC-no	0.103	0.235	-0.310	-0.098	-0.133	0.336	-0.256	0.022	-0.447	-0.254	0.053	0.209	1	0.274	0.320	-0.292
P4	0.500	0.586	0.061	-0.392	-0.547	-0.245	0.273	0.264	0.482	-0.197	0.188	-0.048	-0.250	1	0.054	0.301
E2	0.076	0.025	0.035	0.018	-0.226	-0.146	0.209	-0.136	0.295	0.443	0.789*	0.062	-0.063	0.483	1	0.127
T	0.228	0.569	-0.358	-0.280	-0.507	-0.307	-0.114	0.516	-0.373	-0.182	0.037	0.000	0.590	0.176	0.359	1
Correlations: Jaffrabadi buffaloes (n = 10)																

*Significant at $p < 0.05$ level; GP–gestation period, Wt–weight, Coty–cotyledon, nu–nucleus, no–numbers, TEC–trophoblast epithelial cells, TGC–trophoblast giant cells, P_4–progesterone, E_2–estradiol-17β, T–testosterone.

However, the densities (number/mm^2) of TEC and TGC in the cotyledons were significantly ($p < 0.05$) higher in the Jaffrabadi buffalo than Gir cattle. TEC played an important role in the anchoring of the fetus and exchange of nutrients, gases, and waste products. In contrast, TGC was mainly involved in the secretion of a wide array of hormones to modulate maternal physiology. Higher density of TEC and TGC in the cotyledon of buffalo compared to cattle might suggest some clue in dissimilar reproductive physiology of the animals.

The progesterone (P_4) and estradiol-17β (E_2) secretions from the *in-vitro* cultured cotyledonary cells showed significantly ($p < 0.05$) higher values for Jaffrabadi buffaloes compared to Gir cows. However, there were no significant ($p > 0.05$) differences in the testosterone (T) levels between the two species. Earlier, there were no reports for the basal level of testosterone from full-term placental cells in bovine. However, a different level of testosterone secretion from placental tissues at a different time of gestation period in cattle has been reported. It demonstrated that the testosterone secretion in the third trimester was higher than in the first two trimesters (Khatri, 2011).

A significant negative ($p < 0.05$) correlation was observed between the age of the animal and the placenta's weight and the total number of cotyledon in both species. Advanced maternal age has been reported to compromise fetal growth and is associated with an increased risk of pregnancy complications in rats (Napso *et al.,* 2019). However, the intrauterine mechanisms involved are still mysterious. Reduction in cotyledon numbers with age advancement might affect fetal growth and maternal physiology due to reduced placental exchange and endocrine function. Surprisingly, we observed a significant ($p < 0.05$) negative correlation between newborn calf weight and the total number of TEC in Gir cows, whereas a significant ($p < 0.05$) positive correlation in Jaffrabadi buffaloes was observed. The reason for such contrast correlation in cattle and buffalo is obscure and needs further investigation. Fetal placental aromatase activity decreased after the release of the placenta, which may affect the number and activity of trophoblast cells (Gross *et al*., 1991). A significant ($p < 0.05$) positive correlation was observed between TGC nuclear diameter and estradiol-17β secretion by buffalo cotyledon cells. The trophoblast cells produce more estrogens that assist in parturition by softening the birth canal. Buffalo newborn calf was found to be heavier and larger than cattle in the present study, which might be the reason for more active TGCs and a higher level of progesterone and estrogen secretion by cotyledon cells in buffalo compared to cattle.

Conclusion

The Jaffrabadi buffaloes have heavier placenta with more cotyledons and higher trophoblast density in cotyledons that secrete more progesterone and estradiol-17β compared to those observed in Gir cows. Age of animals has a negative correlation with placental weight and total cotyledons.

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